

Development and Application of Quantitative MRI Methods for Assessing White Matter Integrity in the Mouse Brain

by

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Abstract

Healthy white matter in the brain and spinal cord is composed primarily of myelinated axons and glial cells. Myelinated axons transfer information between the peripheral nervous system and the central nervous system (CNS) as well as between centres within the CNS. Demyelination, a hallmark of neurodegenerative autoimmune diseases such as multiple sclerosis (MS), can cause nerve damage and degrade signal propagation. Magnetic resonance imaging (MRI) methods thought to assess myelin integrity and the structural integrity of axons are improving both the diagnosis and understanding of white matter diseases such as MS. Current methods, however, are sensitive to many different pathologies, making the interpretation of individual MRI results difficult. For this dissertation, several quantitative MRI methods were developed and compared, including single component T_1 and T_2 relaxometry, multicomponent T_2 relaxometry, diffusion tensor imaging (DTI), and quantitative magnetization transfer imaging (qMTI). These methods were tested on agarose gels, fixed rat spinal cords, healthy control mice, and the cuprizone mouse model of demyelination. Quantitative MRI measurements were correlated to ultrastructural measurements of white matter to determine the influence myelin content and axonal structure have on different MRI methods. Cellular distributions measured in electron micrographs of the corpus callosum correlated strongly to several different quantitative MRI metrics. The largest Spearman correlation coefficient varied depending on cellular type: longitudinal relaxation rates (R_A/T_1) vs. the myelinated axon fraction ($\rho = 0.90/-0.90$), the qMTI-derived bound pool fraction (f) vs. the myelin sheath fraction ($\rho = 0.93$), and the DTI-derived axial diffusivity ($\lambda_{\parallel}$) vs. the non-myelinated cell fraction ($\rho = 0.92$). Using Pearson's correlation coefficient, f was strongly correlated to the myelin sheath fraction ($r = 0.98$) with a linear equation predicting myelin content ($5.37f - 0.25$). Of the calculated MRI metrics, f was the strongest indicator

of myelin content while longitudinal relaxation rates and diffusivity measurements were the strongest indicators of changes in tissue structure. Multiparametric MRI measurements of relaxation, diffusion, and magnetization transfer give a more complete picture of white matter integrity.

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In the summer of 2006 I performed research for the first time as an undergraduate student with my supervisor, Dr. Melanie Martin. After a summer tracking mice in a Morris water maze and imaging them in an MRI, I was hooked. I would like to thank Melanie, for first taking me on as an unknown undergraduate student and for the years of guidance, support, and advice she has given me since. I would also like to thank my committee members: Dr. Kathleen Gough, Dr. Scott King, and Dr. Michael Gericke; for their guidance and support in our progress report meetings.

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For Sonya

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Chapter 1

Introduction

Demyelinating autoimmune diseases such as multiple sclerosis (MS) attack myelin in the brain and spinal cord, causing inflammation and often damaging both myelin sheaths and the underlying axons. When this happens, the usual flow of nerve impulses is interrupted or distorted. MS affects more than one million people worldwide, with increasing prevalence in Canada and the province of Manitoba within [160].

Given its ability to detect lesions prior to clinical symptoms, magnetic resonance imaging (MRI) has played an important role in the diagnosis and management of MS. MRI has been shown to strengthen clinical diagnoses and is a powerful tool that can both improve our understanding of MS pathophysiology and help monitor the effects of therapies on individual patients [207]. Despite the demonstrated efficacy of conventional MRI such as T_1 -weighted and T_2 -weighted imaging [155], correlations between conventional MRI findings and clinical status are weak [66]. While conventional methods can visualize some MS lesions; often axonal degeneration, demyelination, gliosis and other diffusive white matter damage occurs where no MR-visible lesion is present [6]. As a solution to these limitations, several functional and quantitative MRI methods have been proposed as complementary biomarkers for MS, including measurements of brain atrophy, magnetic resonance spectroscopy, changes in the blood-oxygen level with functional MRI, structural changes with diffusion tensor imaging (DTI), and indirect measurements of myelin itself with magnetization transfer imaging (MTI) or multicomponent T_2 analysis (mc T_2) [66, 12]. In particular, DTI, MTI and mc T_2 show promise in understanding changes in white matter integrity associated with MS.

1.1 Magnetic Resonance Imaging of White Matter

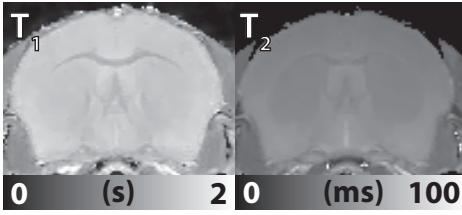
The brain can be roughly partitioned into regions of grey matter, white matter and cerebral ventricles containing cerebrospinal fluid (CSF) [197]. Grey matter contains accumulations of neuronal cell bodies (responsible for information processing) and neuropil while healthy white matter is composed primarily of myelinated axons emanating from the neuronal cell bodies and interspersed with glial cells [197].

Myelinated axons in white matter generate and propagate nerve impulses between different neuronal cell bodies in the brain. The corpus callosum is the largest white matter structure in the human brain and contains $\approx 2 \times 10^8$ axons in directionally coherent tracts connecting the two cerebral hemispheres of the brain [1]. In general, an axon's diameter is proportional to the number of neurofilaments and the thickness of its myelin sheath [102]. In human brains, median axonal diameters range from $0.6 \mu\text{m}$ in the genu of the corpus callosum to $1.0 \mu\text{m}$ in the splenium [1]. At 90 days of age, the mean diameter of myelinated axons is $(0.42 \pm 0.03) \mu\text{m}$ in the body of the mouse corpus callosum [235]. Myelin sheaths are a compacted membrane formed and maintained by a type of glial cell called the oligodendrocyte (which individually can myelinate several axonal tracts). Oligodendrocytes are aligned in rows between myelinated fibres in white matter, but can also be found (along with myelin) in grey matter [217]. Trapezoidal in shape and spirally wrapped around axon fibres, myelin sheaths help control axonal development as well as isolate segments electrically to enable saltatory conduction between the nodes of Ranvier [102]. The myelin membrane is characterized by a high ratio of lipids to proteins (70:30 compared with 35:65 in most other membranes) and is approximately 40% water, whereas grey matter is 80% water [22].

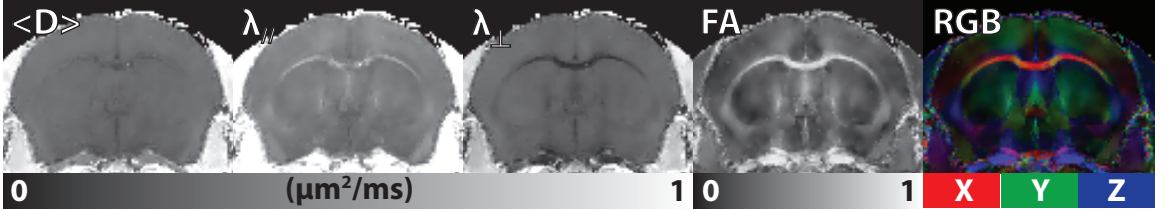
MRI, as described in Chapter 2, relies on the magnetization of hydrogen nuclei, when placed in a strong magnetic field, to create signals and images that are sensitive to different types of tissue structure and content. The abundance of mobile water in tissue (and the hydrogen nuclei within) is the source of MRI's predominantly soft tissue signal. Due to differences in water content and tissue structure, MRI methods also yield excellent contrast between grey matter, white matter, and CSF in the brain. The signal from hydrogen nuclei can be manipulated by a variety of pulse sequences to sensitize the image to the proton

density, longitudinal relaxation times (T_1), and transverse (T_2) relaxation times of the magnetization (e.g. conventional proton density, T_1 -weighted, and T_2 -weighted imaging). Quantitative MRI methods described in Chapter 3, combine several different weighted MR images to estimate the relaxation times of the hydrogen nuclei (e.g. T_1 and T_2 relaxometry), diffusivity of water in tissue (e.g. diffusion tensor imaging, DTI), and properties of water bound in macromolecular structures such as myelin (e.g. magnetization transfer imaging, MTI).

T_1 and T_2 Relaxometry



Diffusion Tensor Imaging



Magnetization Transfer Imaging

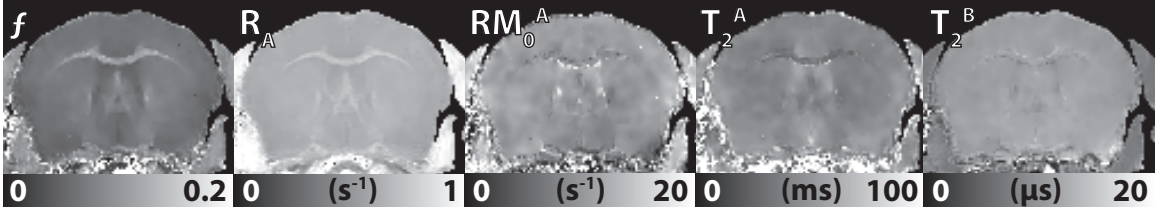


Figure 1.1: Quantitative MRI maps in the same slice of a freshly excised healthy mouse brain. The corpus callosum is the large, dark feature in the middle of the T_1 map and is visible, with varying contrast, in all of the different quantitative MRI maps.

Each of the quantitative MRI methods described in Chapter 3 model different aspects of the complicated motion, relaxation and exchange of magnetization in tissue. Quantitative MRI maps taken in the same slice of a freshly excised, healthy mouse brain are shown in Figure 1.1. Visually, we can see that different MRI metrics are sensitive to different features in the the white matter of the corpus callosum and the surrounding grey matter.

For example, the T_1 relaxation time is sensitive to changes in the water content, tissue lattice, and water mobility and is generally shorter in healthy white matter compared to grey matter [173]. T_2 in healthy white matter can be decomposed into a short- T_2 component attributed to water trapped between layers in the myelin sheath and an intermediate- T_2 component attributed to water in the intra- and extra-cellular compartments of tissue [266]. Like T_1 , the single-component T_2 relaxation time tends to be shorter in healthy white matter and the myelin water fraction (MWF), derived from the relative signal amplitude of the short- T_2 component, is related to myelin content [263, 135, 138].

Using the DTI method, mean diffusivity ($\langle D \rangle$) reveals changes in the extracellular space and overall water diffusivity, while axial diffusivity ($\lambda_{\parallel}$), radial diffusivity ($\lambda_{\perp}$), and fractional anisotropy (FA) reveal changes in the anisotropy of water diffusing in tissue. In healthy white matter with directionally coherent axonal tracts such as the corpus callosum, $\lambda_{\parallel}$ and FA are larger and $\lambda_{\perp}$ is smaller than the surrounding grey matter. The RGB color map represents both the directionality and anisotropy of diffusion, with bright red representing highly anisotropic diffusion primarily along the x-direction, green along the y-direction, and blue along the z-direction.

Finally, quantitative MTI parameters such as the bound pool fraction (f) are closely related to the macromolecules in myelin, yielding larger f values in healthy white matter, while R_A and T_2^A are related to the rate of longitudinal relaxation and transverse relaxation time in the liquid pool and are similar to the $R_1 = 1/T_1$ relaxation rate and the single component T_2 relaxation time already mentioned. RM_0^A represents the rate of magnetization transfer between water bound in myelin and the surrounding liquid pool while T_2^B represents the transverse relaxation time in the bound pool. Both are somewhat sensitive to noise and fitting method [194], but RM_0^A tends to be slightly smaller and T_2^B slightly larger in healthy white matter compared to the surrounding grey matter [220]. The magnetization transfer ratio (MTR) is related to f , but is dependent on the chosen pulse sequence and field strength and will vary with changes in RM_0^A and R_A .

Chapter 4 details the development of the quantitative MR imaging and analysis methods. The methods were validated using a water phantom, agarose gel, fixed rat spinal cords, healthy control mice, and the cuprizone-fed mouse model of demyelination.

Although individual quantitative MRI methods give additional information about tissue structure and content, they cannot measure individual tissue pathologies. Part of this is due to limitations of resolution. A $(100 \times 100 \times 100) \mu\text{m}^3$ voxel in the healthy white matter of a mouse brain can contain hundreds of myelinated axons along with various support cells. Also, MRI can only measure tissue structure and content indirectly via methods sensitized to the mobility, compartmentalization, and magnetization transfer of water, among others. To overcome some of the limitations of individual MRI methods, a multiparametric approach combining several quantitative MRI methods could give a more complete picture of white matter integrity.

1.2 Multiparametric MRI of a Demyelinating Model

Cuprizone is a toxic model of demyelination, causing cell death of oligodendrocytes followed by extensive demyelination [245] with only moderate inflammation and no neuronal loss [104]. C57BL/6 mice are fed 0.2–0.4% cuprizone (w/w) at 8 weeks of age. After 6 weeks of feeding, extensive demyelination is observed predominantly in the corpus callosum and superior cerebellar peduncles. Chronic demyelination occurs if the cuprizone diet is maintained for an extended period, however, if a normal diet is resumed at 6-weeks, the brain is mostly remyelinated after 6 weeks of recovery [161]. The cuprizone mouse model, thus, provides a good analogue for demyelinating/remyelinating diseases such as MS.

In the cuprizone mouse model of demyelination, myelin sheaths separate from the axon and eventually disappear (Figure 1.2). Demyelination is often accompanied by several other tissue changes, such as axonal degeneration, gliosis, edema, and increased extracellular space. Several groups have used different quantitative MRI methods for detecting demyelination and assessing white matter integrity. These methods include DTI [223, 171, 224, 240, 122, 35, 277], MTI [220, 198, 273, 248, 49, 211, 58], and multicomponent T_2 analysis [166, 135, 138, 157, 266, 156, 128]. Some quantitative MRI parameters, such f or MWF, appear to be strong indicators of myelin content and myelin integrity in white matter, while others, such as $\lambda_{\parallel}$, $\lambda_{\perp}$, or FA, give more information about the structural integrity of white matter. Each quantitative MRI metric is sensitive to different

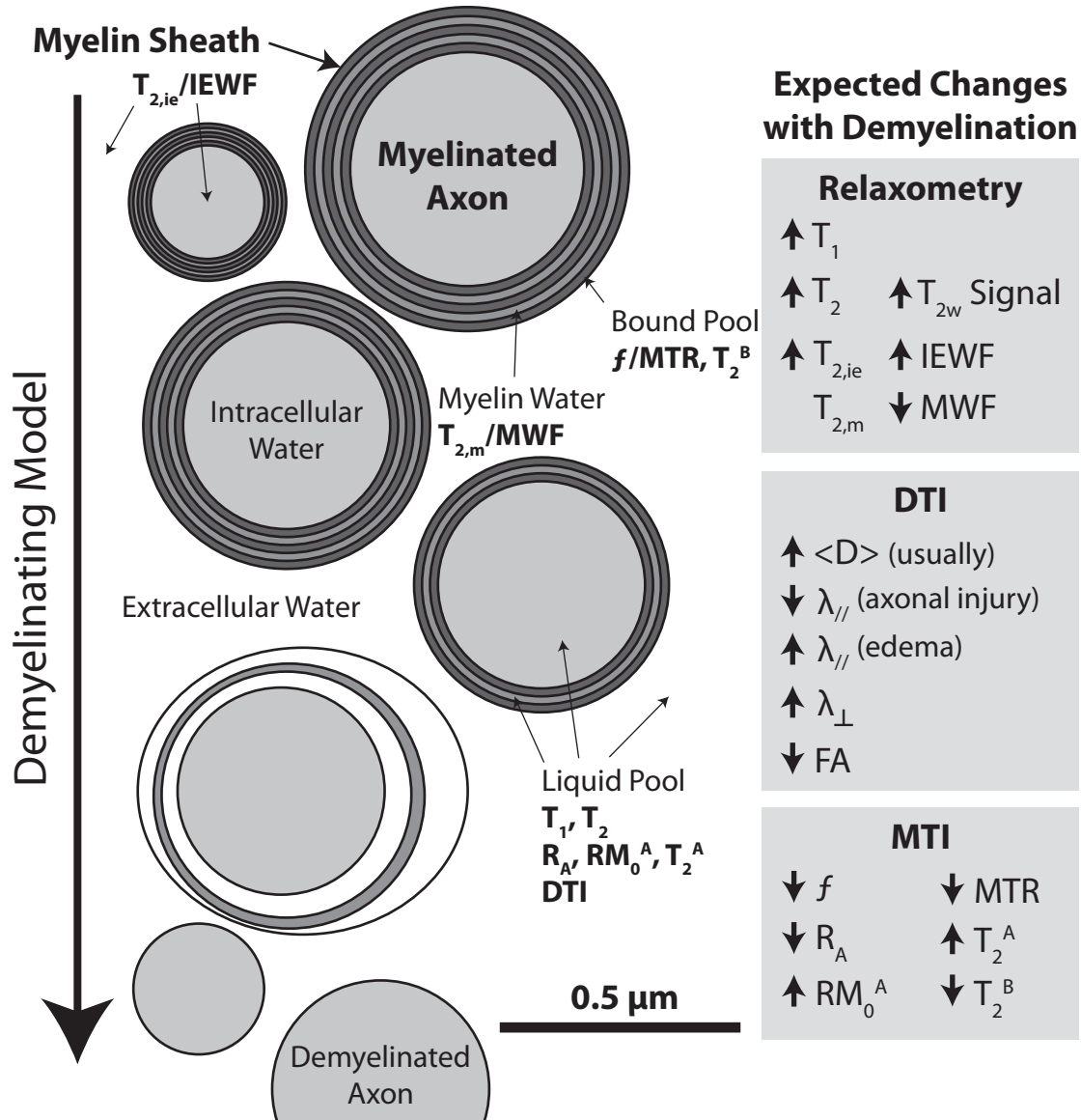


Figure 1.2: Cross-section of demyelinating axons with expected changes in various MRI parameters. Scale bar is based on mean axon diameter measurements in the healthy corpus callosum from Table 5.3.

aspects of myelinated and demyelinated axons in white matter. Varying sensitivity to the different compartments and diffusivity of water lead to the trends illustrated in Figure 1.2.

In order to correlate changes in quantitative MRI metrics to the underlying white matter integrity, ultrastructural features such as axonal diameter, myelin sheath thickness, and the distribution of myelinated axons can be measured using electron microscopy. Electron microscopy yields exquisite details about white matter integrity in both control and cuprizone-fed mice (Figure 1.3). Correlations between electron microscopy and quantitative

MRI metrics show the relationship between measurements on the axonal scale ($< 1 \mu\text{m}$) and changes observed on the MRI voxel scale ($\approx 100 \mu\text{m}$).

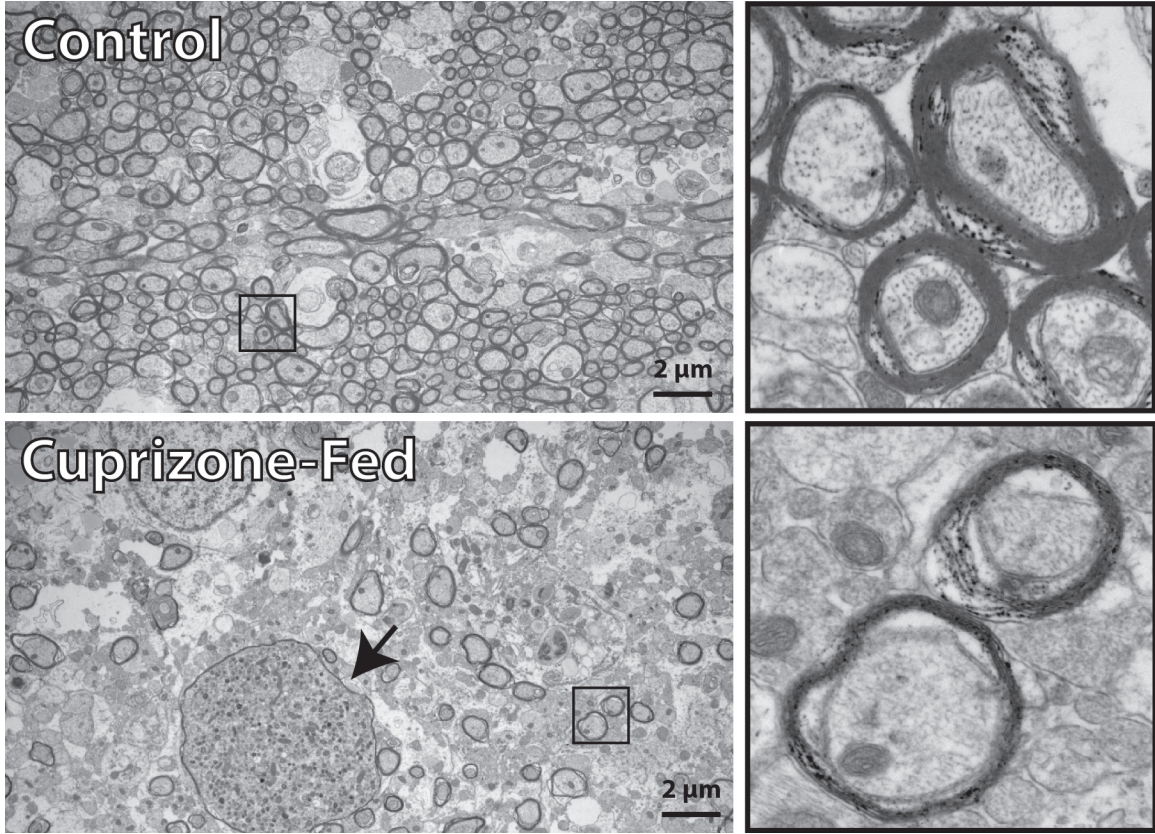


Figure 1.3: Cross-sections of individual axons in electron microscopy images of the corpus callosum in control mice and mice fed 0.4% cuprizone for 6 weeks. Myelinated axons in the control mouse are surrounded by a thick layer of myelin membranes. Axons in the cuprizone-fed mice are predominantly demyelinated, with thinner myelin sheaths in the axons that remain myelinated. Other pathological features, such as large, swollen axons (arrow) can also be seen in the cuprizone-fed mouse.

In Chapter 5, the cuprizone mouse model was used to study how ultrastructural tissue changes due to demyelination correlate with different MRI methods. *In vivo* mice were imaged every week from 0 to 6 weeks with conventional T_2 -weighted images and MTR maps, revealing weekly changes in the corpus callosum of the cuprizone-fed mice. After 6 weeks of 0.4% cuprizone treatment, quantitative MRI methods, including T_1 and T_2 relaxometry, DTI, and quantitative MTI, were applied immediately after sacrifice. Ultrastructural examination was performed with electron microscopy to measure changes in tissue pathology. In addition, quantitative MRI metrics were correlated with each other to understand better

the influence of varying tissue features on paired MRI metrics.

A multiparametric approach combining several quantitative MRI methods gives a more complete picture of white matter integrity. Understanding how these metrics vary with changes in white matter integrity can improve our understanding of demyelinating diseases such as MS. The MRI and analysis methods developed for this dissertation can be applied to a broad range of future studies.

Chapter 2

Nuclear Magnetic Resonance: Signal and Image

Magnetic resonance imaging relies on the behaviour of nuclear spins when placed in a strong magnetic field. While a complete survey of magnetic resonance imaging won't be provided here, it is useful to build up a basic theoretical framework to understand better the quantitative MRI parameters described later in this dissertation. For a more thorough discussion of the theoretical underpinnings of MRI, several excellent books are available [38, 150, 87, 24, 148].

2.1 Nuclear Magnetic Resonance

2.1.1 Spin-1/2 Particles

Nucleons (protons and neutrons) have an intrinsic angular momentum called spin. Specifically, nucleons are spin-1/2 particles, with two possible eigenstates along the z-axis, denoted as $|1/2\rangle$ (spin up) and $|-1/2\rangle$ (spin down). Spin-1/2 particles are not restricted to these two eigenstates, but can exist in a superposition of states, described by the normalized, superposition coefficients, $c_{1/2}$ and $c_{-1/2}$:

$$|\Psi\rangle = c_{1/2} |1/2\rangle + c_{-1/2} |-1/2\rangle \quad (2.1)$$

In the nuclei of atoms, spin up nucleons pair with antiparallel spin down nucleons, resulting in a total angular momentum (or overall spin) of $I = 0$ for even-Z, even-N nuclei

[130]. Nuclei with unpaired protons or neutrons, however, have a non-zero I which in turn contributes to an intrinsic magnetic moment, μ .

The hydrogen nucleus (^1H) is composed of just one unpaired proton and has a total angular momentum of $I = \frac{1}{2}$. The spin angular momentum can take a range of values represented by the magnetic quantum number, $m = -I, -I + 1, \dots, I - 1, I$. For ^1H , there are just two possible eigenstates: $m = +\frac{1}{2}$ and $m = -\frac{1}{2}$. In matrix representation, this can be represented as:

$$\left| +\frac{1}{2} \right\rangle = \begin{bmatrix} 1 \\ 0 \end{bmatrix} \quad (2.2)$$

$$\left| -\frac{1}{2} \right\rangle = \begin{bmatrix} 0 \\ 1 \end{bmatrix} \quad (2.3)$$

where the angular momentum operator along the z-axis, I_z , is a 2x2 matrix:

$$I_z = \frac{1}{2} \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix} \quad (2.4)$$

Realizing the basis vectors are orthogonal, the expectation value of I_z in the arbitrary state is:

$$\langle \Psi | I_z | \Psi \rangle = \sum_m |c_m|^2 m \quad (2.5)$$

For a large number of identically prepared nuclei, the sum of the possible eigenvalues are weighted by the normalized probabilities $|c_m|^2$.

2.1.2 Spin-1/2 Particles in a Magnetic Field

In the absence of an external magnetic field, the two eigenstates of spin-1/2 particles are of equal energy. However, if an external magnetic field is applied, the energy levels split.

The nuclear magnetic dipole moment, μ , has a magnitude of:

$$\mu = \gamma \hbar \sqrt{I(I+1)} \quad (2.6)$$

In the presence of an external magnetic field, the z-component of $\boldsymbol{\mu}$ has a discrete value

$$\mu_z = \gamma \hbar m \quad (2.7)$$

where γ is a constant of proportionality known as the gyromagnetic ratio ($\gamma/2\pi = 42.58$ MHz/T for ^1H). The x- and y-components of $\boldsymbol{\mu}$, however, are randomly oriented. We can define the transverse components of $\boldsymbol{\mu}$ as [150]:

$$\begin{aligned} \mu_x &= |\boldsymbol{\mu}_{xy}| \cos \xi \\ \mu_y &= |\boldsymbol{\mu}_{xy}| \sin \xi \end{aligned} \quad (2.8)$$

where ξ is a random variable uniformly distributed from 0 to 2π and $|\boldsymbol{\mu}_{xy}|$ is

$$|\boldsymbol{\mu}_{xy}| = \sqrt{\mu^2 - \mu_z^2} = \gamma \hbar \sqrt{I(I+1) - m^2} \quad (2.9)$$

For ^1H , $I = 1/2$ and $m = \pm 1/2$, yielding the three components of $\boldsymbol{\mu}$:

$$\begin{aligned} \mu_x &= \frac{\gamma \hbar}{\sqrt{2}} \cos \xi \\ \mu_y &= \frac{\gamma \hbar}{\sqrt{2}} \sin \xi \\ \mu_z &= \pm \frac{\gamma \hbar}{2} \end{aligned} \quad (2.10)$$

The interaction energy of a magnetic dipole $\boldsymbol{\mu}$ in a magnetic field $\mathbf{B}_0 = B_0 \hat{\mathbf{z}}$ is

$$E = -\boldsymbol{\mu} \cdot \mathbf{B}_0. \quad (2.11)$$

For $m = \pm \frac{1}{2}$, E becomes:

$$E = \pm \frac{\gamma \hbar B_0}{2} \quad (2.12)$$

This splitting of energy levels in a magnetic field is known as the Zeeman effect, and results in two energy levels with an energy difference:

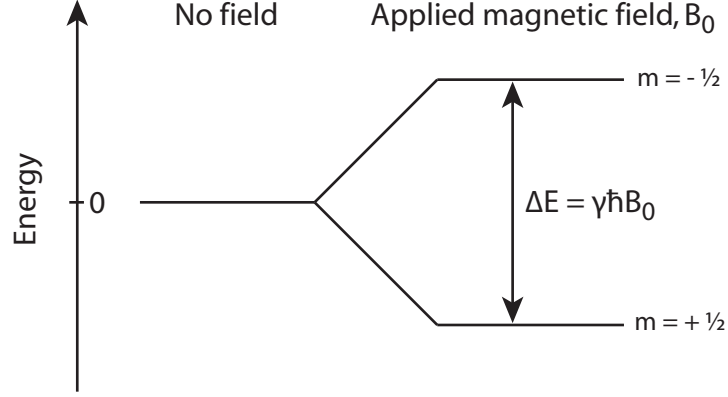


Figure 2.1: Energy levels for a nucleus with $I = \frac{1}{2}$

$$\Delta E = \gamma \hbar B_0 \quad (2.13)$$

which can be visualized in an energy level diagram (Figure 2.1).

It is important to realize that we are always dealing with an ensemble of nuclei, with a large population of nuclei in each energy level. A population difference will arise in thermal equilibrium according to the Boltzmann probability factor [38]. For two energy levels separated by $\hbar\gamma B_0$, the ensemble averaged probability, $\overline{|c_m|^2}$, becomes:

$$\overline{|c_m|^2} = \overline{|c_{\pm\frac{1}{2}}|^2} = \frac{e^{\pm \frac{\hbar\gamma B_0}{2k_B T}}}{e^{-\frac{\hbar\gamma B_0}{2k_B T}} + e^{+\frac{\hbar\gamma B_0}{2k_B T}}} \quad (2.14)$$

At room temperature, $\hbar\gamma B_0 \ll k_B T$, and we can write:

$$\overline{|c_{\pm\frac{1}{2}}|^2} = \frac{1}{2} \left[1 \pm \frac{\hbar\gamma B_0}{2k_B T} \right] \quad (2.15)$$

2.1.3 Larmor Frequency

Semiclassically, when placed in a magnetic field, ^1H nuclei experience a torque which aligns them with the direction of the magnetic field. This, in turn, results in a precessional motion oriented around the magnetic field at a characteristic rate called the Larmor frequency, ω_0 , given by,

$$\omega_0 = \gamma B_0 \quad (2.16)$$

with cyclic frequency, $f_0 = \omega_0/2\pi$. The MRI used in our experiments has a magnetic field $B_0 = 7$ T which results in a Larmor frequency $f_0 = 300$ MHz. In the time-dependent wavefunction, the evolution of the state through time is identical to a clockwise rotation of the state about the z-axis by an angle $\gamma B_0 t$ [38].

$$|\Psi(t)\rangle = e^{-iHt/\hbar} |\Psi(0)\rangle = e^{-i\gamma B_0 I_z t} |\Psi(0)\rangle = e^{-i\omega_0 I_z t} |\Psi(0)\rangle \quad (2.17)$$

where H is the Hamiltonian (or energy) operator and I_z is the spin angular momentum operator with the resultant eigenvalue m .

According to the Boltzmann distribution, a slight majority of the nuclei will precess about the direction of $\mathbf{B}_0$ in the spin-up position (the lower energy state). If, however, we briefly apply an oscillating magnetic field (also known as a radiofrequency (RF) pulse) perpendicular to $\mathbf{B}_0$ at the resonant Larmor frequency (equivalent to the transition energy), some of the lower energy nuclei will absorb the energy and flip from $m = \frac{1}{2}$ to $m = -\frac{1}{2}$ (Figure 2.2). This absorption can occur until the populations of the two energy states become equal. At this point, the spin system is saturated, and no further absorption occurs.

It is worth noting that the energies associated with spin are much smaller than the thermal energies of particles, thus the vast majority of spins will exist in a superposition of states with random orientations (not just the two eigenstates). In fact, while the properties of spin in individual particles are quantum in nature, magnetic resonance imaging, which depends on a very large ensemble of spins described by the net magnetization vector, $\mathbf{M}$, is classical in nature, and more easily described by classical mechanics [92].

In 1946, Felix Bloch and Edward Purcell independently demonstrated the application of RF fields to nuclei suspended in a constant magnetic field [31, 196]. Their observations of the nuclear magnetic moment via nuclear magnetic resonance earned them both the 1952 Nobel Prize in Physics.

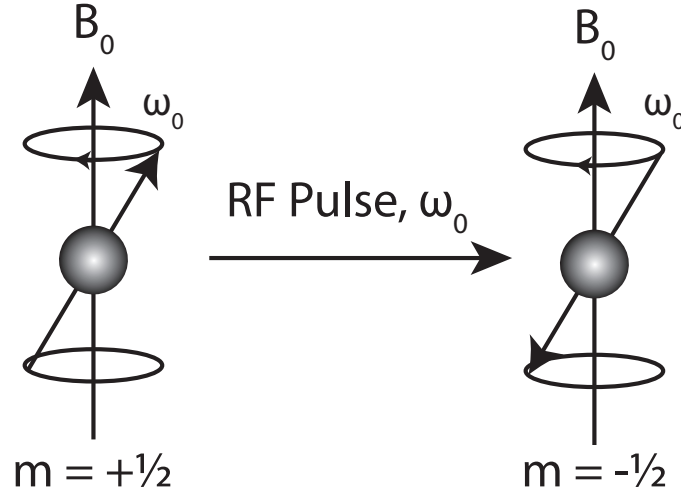


Figure 2.2: Precession of nucleus about B_0 at spin $m = +\frac{1}{2}$ and $m = -\frac{1}{2}$

2.1.4 Net Magnetization Vector

The cumulative effect of all of the magnetic moments of the nuclei is the net magnetization vector, $\mathbf{M}$ defined as the magnetic moment per unit volume (Figure 2.3).

$$\mathbf{M} = \sum_{n=1}^N \boldsymbol{\mu}_n \quad (2.18)$$

where N is the number of spins in the unit volume. In the presence of a static magnetic field, the transverse components of the magnetic moments are randomly oriented (Equation (2.10)), resulting in a transverse magnetization, $M_{xy} = 0$. The longitudinal components of the magnetic moments, however, are additive, with the number of spin-up nuclei slightly larger than the number of spin-down nuclei. This results in a net magnetization vector pointing along the direction of the applied magnetic field. Based on the Zeeman splitting of eigenstates described in Equation (2.15), the difference in distribution of spin-up and spin-down states is:

$$N \left(\overline{c_{+\frac{1}{2}}^2} - \overline{c_{-\frac{1}{2}}^2} \right) = N \frac{\hbar \gamma B_0}{2k_B T} \quad (2.19)$$

For $B_0 = 7T$ and $T = 300K$, this amounts to a fractional population difference,

$$\frac{N_+ - N_-}{N} = \frac{\gamma \hbar B_0}{2k_b T} = \frac{(42.58 \times 10^6 \text{ Hz/T}) (6.6 \times 10^{-34} \text{ J} \cdot \text{s}) (7 \text{ T})}{2 (1.38 \times 10^{-23} \text{ J/K}) (300 \text{ K})} = 24 \times 10^{-6} \quad (2.20)$$

Immediately, you can see the relatively small magnetization available to MRI (only 24 in a million spin-1/2 nuclei contribute to the signal at 7 T) and the benefits of increasing the magnetic field (net magnetization scales linearly with magnetic field strength).

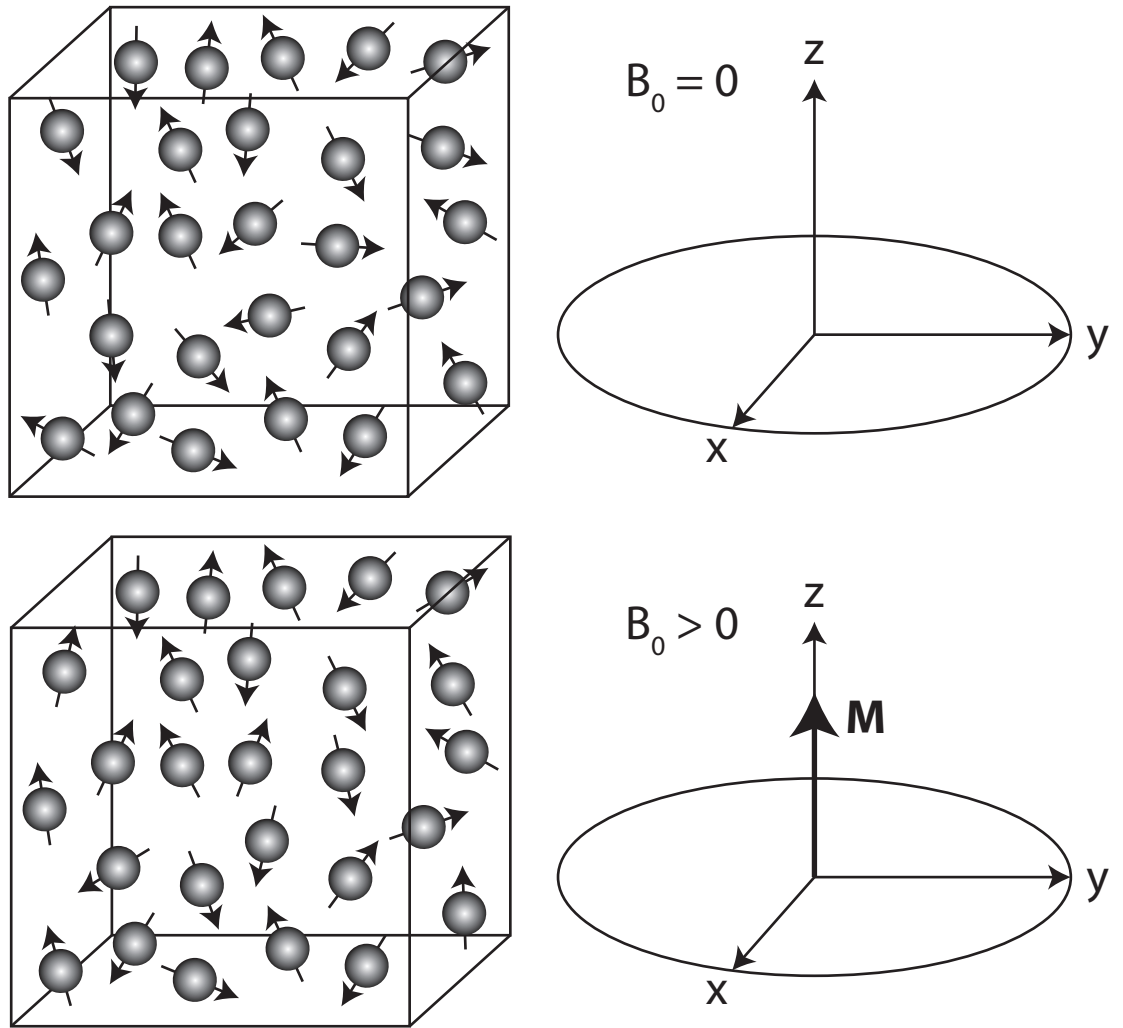


Figure 2.3: Ensemble of spin-1/2 particles contributes to the net magnetization vector $\mathbf{M}$. In the absence of a magnetic field, $\mathbf{M} = 0$. In the presence of a magnetic field, $\mathbf{M} > 0$ and points along the direction of the magnetic field.

2.1.5 Bloch Equation

The time-dependent nature of the magnetization vector in the presence of a magnetic field is described by the Bloch equation [31]:

$$\frac{d}{dt}\mathbf{M}(t) = \gamma\mathbf{M} \times \mathbf{B} - \frac{M_x\hat{\mathbf{x}} + M_y\hat{\mathbf{y}}}{T_2} - \frac{(M_z - M_0)\hat{\mathbf{z}}}{T_1} \quad (2.21)$$

where $\mathbf{M} = M_0\hat{\mathbf{z}}$ at thermal equilibrium and T_1 and T_2 are time constants related to longitudinal and transverse relaxation processes respectively. In the absence of an applied $\mathbf{B}_1$ magnetic field, $\mathbf{B} = B_0\hat{\mathbf{z}}$. During the application of an RF pulse, however, $\mathbf{B}$ includes a $\mathbf{B}_1$ magnetic field that, in turn, influences $\mathbf{M}$. The application of a resonant RF pulse and the subsequent relaxation of the magnetization vector can be described by the Bloch equation.

2.1.6 RF Pulses

An RF field applied perpendicular to $\mathbf{B}_0$ at a frequency, ω_{rf} , is described by the function, $\mathbf{B}_1(t)$

$$\mathbf{B}_1(t) = B_1^e(t) [\cos(\omega_{rf}t + \phi)\hat{\mathbf{x}} - \sin(\omega_{rf}t + \phi)\hat{\mathbf{y}}] \quad (2.22)$$

where $B_1^e(t)$ is a function describing the pulse envelope, ω_{rf} is the frequency of the applied RF pulse, and ϕ is the initial phase angle [150]. In complex notation, this is reduced to

$$B_1(t) = B_1^e(t) e^{-i(\omega_{rf}t + \phi)} \quad (2.23)$$

The envelope function describes the shape and duration of the RF pulse. In the rotating frame of reference, $\mathbf{B}_1$ reduces to

$$B_{1rot}(t) = B_1(t) e^{i\omega_{rf}t} = B_1^e(t) \quad (2.24)$$

where we assume $\phi = 0$.

Common shapes for RF pulse envelopes include rectangular, Gaussian, sinc, and Her-

mite. A rectangular (or block) pulse can be defined as

$$B_1^e(t) = B_1 \Pi\left(\frac{t - \tau_p/2}{\tau_p}\right) \quad (2.25)$$

where τ_p is the pulse width. A sinc pulse can be defined as

$$B_1^e(t) = \begin{cases} B_1 \text{sinc}[\pi \Delta f (t - \tau_p/2)], & 0 \leq t \leq \tau_p \\ 0, & t > \tau_p \end{cases} \quad (2.26)$$

In particular, after a Fourier transform, sinc pulses are characterized by a rectangular pulse in the frequency domain,

$$p(f) = \Pi\left(\frac{f - f_c}{\Delta f}\right) e^{i2\pi(f - f_c)\tau_p/2} \quad (2.27)$$

where Δf is a frequency bandwidth centered on the Larmor frequency, f_c . A constant $p(f)$ in the frequency domain becomes more important when we want to select a range of frequency-encoded volumes for magnetic resonance imaging (as in Section 2.3.1).

2.1.7 RF Pulses and the Net Magnetization

An RF pulse applied at the Larmor frequency ($\omega_{rf} = \omega_0$), will tip the magnetization vector toward the transverse plane. In relation to the magnetization vector, we can assume the time we apply the RF pulse (τ_p) is much less than the T_1 or T_2 relaxation times. This simplifies Equation (2.21) to

$$\frac{d}{dt} \mathbf{M}(t) = \gamma \mathbf{M} \times \mathbf{B} \quad (2.28)$$

In the rotating frame, this becomes:

$$\frac{\partial \mathbf{M}_{rot}}{\partial t} = \gamma \mathbf{M}_{rot} \times \mathbf{B}_{eff} \quad (2.29)$$

where

$$\frac{\partial \mathbf{M}_{rot}}{\partial t} = \frac{d\mathbf{M}}{dt} - \boldsymbol{\omega} \times \mathbf{M}_{rot} \quad (2.30)$$

and

$$\mathbf{B}_{eff} = \mathbf{B}_{rot} + \frac{\boldsymbol{\omega}}{\gamma} = (B_0 \hat{\mathbf{z}}' + \mathbf{B}_{1rot}) + \frac{\omega_{rf}}{\gamma} \hat{\mathbf{z}}'. \quad (2.31)$$

For $\phi = 0$,

$$\mathbf{B}_{1rot} = B_1^e(t) \hat{\mathbf{x}}' \quad (2.32)$$

Since $\omega_{rf} = \omega_0$ and $B_0 = \omega_0/\gamma$, the z-components cancel, leaving us with

$$\mathbf{B}_{eff} = B_1^e(t) \hat{\mathbf{x}}' \quad (2.33)$$

and the equation of motion for the magnetization vector in the rotating frame becomes

$$\frac{\partial \mathbf{M}_{rot}}{\partial t} = \gamma \mathbf{M}_{rot} \times B_1^e(t) \hat{\mathbf{x}}' \quad (2.34)$$

For initial conditions $M_{x'}(0) = M_{y'}(0) = 0$ and $M_{z'}(0) = M_0$ this has the solution

$$\mathbf{M}_{rot} = 0 \hat{\mathbf{x}}' + M_0 \sin(\alpha) \hat{\mathbf{y}}' + M_0 \cos(\alpha) \hat{\mathbf{z}}' \quad (2.35)$$

Equation (2.35) describes how the magnetization vector is tipped toward the transverse plane due to the application of an RF pulse. The tipping angle, α , is dependent on the envelope function of the RF pulse ($B_1^e(t)$) and the length of time (τ_p) it is applied,

$$\alpha = \int_0^{\tau_p} \gamma B_1^e(t) dt \quad (2.36)$$

If B_1^e is constant in time (i.e. a rectangular pulse),

$$\alpha = \gamma B_1^e \tau \quad (2.37)$$

RF pulses are often characterized by the angle α at which they tip $\mathbf{M}$. Common pulses

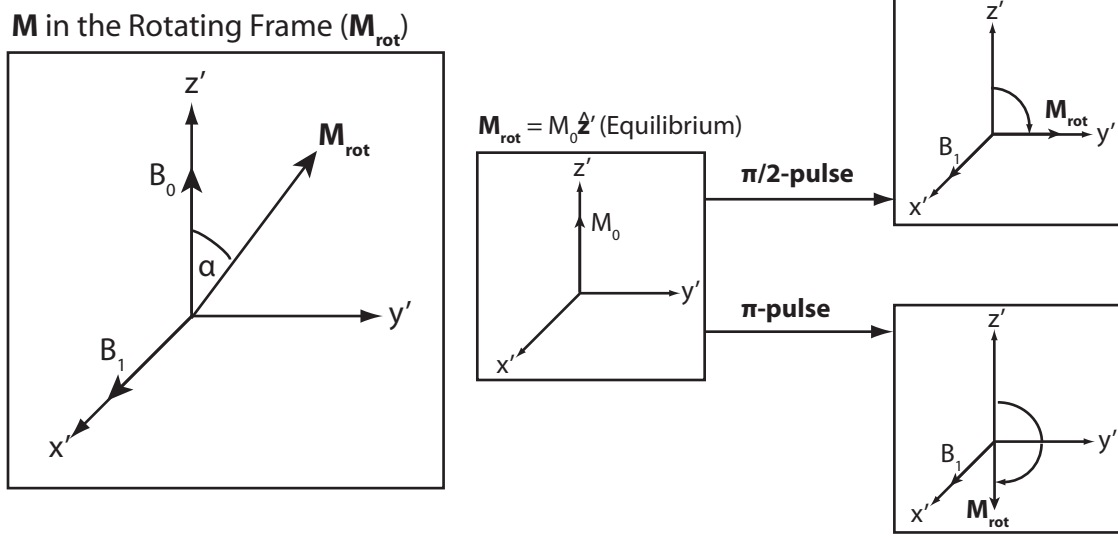


Figure 2.4: Net magnetization vector $\mathbf{M}_{rot}$ in the rotating frame (left) and the application of $\pi/2$ and π RF pulses to $\mathbf{M}_{rot}$ at equilibrium (right).

include a $\pi/2$ -pulse and a π -pulse (Figure 2.4). The signal detected from the relaxation of nuclei is composed entirely of the transverse component of the magnetization (i.e. $S \propto M_{xy}$). Thus, a $\pi/2$ -pulse results in maximum signal ($M_{xy} = M_0 \sin(\pi/2) = M_0$) and a π pulse results in minimum signal ($M_{xy} = M_0 \sin(\pi) = 0$).

2.1.8 Relaxation

In the previous section, we saw that an RF pulse applied at the Larmor frequency will tip the magnetization toward the transverse plane. When the pulse is turned off, the system returns to equilibrium by a process known as relaxation. After an RF pulse, B_1 returns to zero and $\mathbf{B} = B_0 \hat{z}$. In Equation (2.35), the components of the magnetization vector immediately after an RF pulse were

$$\begin{aligned} M_x^0 &= 0 \\ M_y^0 &= M_0 \sin(\alpha) \\ M_z^0 &= M_0 \cos(\alpha) \end{aligned} \tag{2.38}$$

We can solve Equation (2.21) using these initial conditions and the Larmor relation, $\omega_0 = \gamma B_0$

$$\begin{aligned} M_x(t) &= M_y^0 \sin(\omega_0 t) e^{-t/T_2} \\ M_y(t) &= M_y^0 \cos(\omega_0 t) e^{-t/T_2} \\ M_z(t) &= M_0 + (M_z^0 - M_0) e^{-t/T_1} \end{aligned} \quad (2.39)$$

After applying a $\pi/2$ -pulse, $M_y^0 = M_0$ and $M_z^0 = 0$. As the system returns to equilibrium, $M_z \rightarrow M_0$ and $M_{xy} \rightarrow 0$ based on the following equations:

$$\begin{aligned} M_x(t) &= M_0 \sin(\omega_0 t) e^{-t/T_2} \\ M_y(t) &= M_0 \cos(\omega_0 t) e^{-t/T_2} \\ M_z(t) &= M_0 (1 - e^{-t/T_1}) \end{aligned} \quad (2.40)$$

The transverse magnetization can be defined as $M_{xy} = M_x(t) + iM_y(t)$. We can then combine $M_x(t)$ and $M_y(t)$ into a single equation

$$M_{xy}(t) = M_0 e^{i\omega_0 t} e^{-t/T_2} \quad (2.41)$$

In the lab frame, the trajectory of the magnetization vector is described by exponential growth and decay mediated by the T_1 and T_2 time constants as well as oscillations around the z-component at the Larmor frequency (see Figure 2.5). Multiplying Equation (2.41) by $e^{-i\omega_0 t}$ demodulates the transverse magnetization to give us (in the rotating frame)

$$M_{xy}(t) = M_0 e^{-t/T_2} \quad (2.42)$$

Relaxation of the magnetization vector is defined by two independent time constants: T_1 and T_2 . T_1 and T_2 relaxation are attributed to the thermal motion of molecules creating fluctuating magnetic fields for individual nuclear spins [148]. For spin-1/2 particles, this can include the following mechanisms:

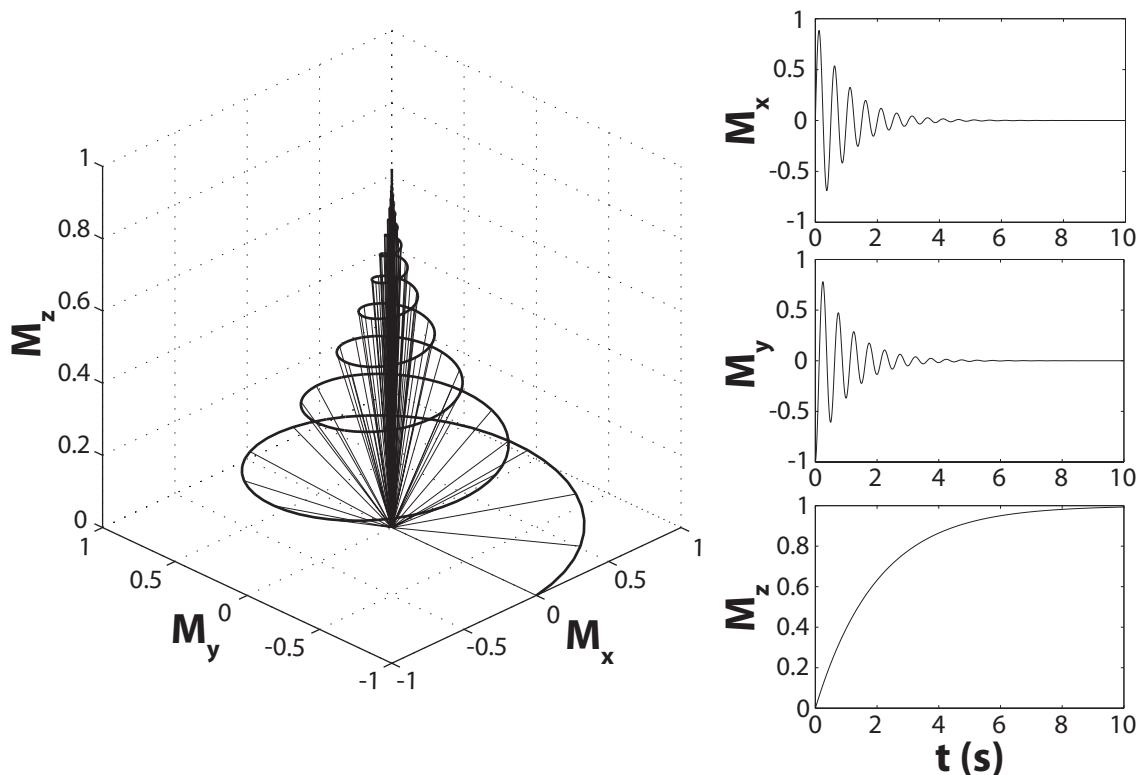


Figure 2.5: Trajectory of the magnetization vector in the lab frame after a $\pi/2$ RF pulse. $M_x(t)$ and $M_y(t)$ oscillate at the Larmor frequency, ω_0 , and decay according to the T_2 relaxation time. $M_z(t)$ grows according to the T_1 relaxation time. For clarity, $T_1 = 2$ s, $T_2 = 1$ s, and $\omega_0 = 4\pi$ rad/s (much less than the typical Larmor frequency).

1. Direct dipole-dipole coupling between nuclear spins in the same molecule creates a varying magnetic field between two spins as the molecule tumbles (e.g. the two ^1H protons in H_2O).
2. The B_0 field induces currents in the electron clouds of molecules which, in turn, generate small local magnetic fields. This leads to slightly different magnetic fields in different types of molecules, called the chemical shift effect. Tumbling molecules with chemical shift anisotropy lead to small fluctuations in the total field. This effect increases in importance at higher magnetic fields.
3. The rotational motion of molecules leads to small local magnetic fields due to spin-rotation.

Of these, dipole-dipole coupling and chemical shift anisotropy are the dominant relaxation

mechanisms [148]. The “fat” shift is the most well-known example of the chemical shift effect, where protons in CH_2 have a resonant frequency approximately 3.35 ppm higher than the resonant frequency of protons in H_2O [150]. At 7 T (300 MHz), protons in fat will have a resonant frequency approximately 1005 Hz higher than protons in water.

Spin-lattice (longitudinal) relaxation time, T_1

M_z returns to equilibrium by a process known as spin-lattice relaxation. It involves the return of spin populations to their Boltzman distribution values [148] via an exchange of energy between the spin system and the surrounding thermal reservoir, known as the ‘lattice’ [38]. The decay time constant describing how M_z reaches equilibrium, T_1 is known as the spin-lattice or longitudinal relaxation time constant. After a $\pi/2$ -pulse, it is characterized by the equation:

$$M_z = M_0(1 - e^{-t/T_1}) \quad (2.43)$$

Spin-spin (transverse) relaxation time, T_2

M_{xy} returns to equilibrium by a process known as spin-spin relaxation. It involves a return to equilibrium within the spin system itself, as spin is transferred amongst excited nuclei. The decay time constant describing how M_{xy} reaches equilibrium, T_2 , is known as the spin-spin or transverse relaxation time constant. After a $\pi/2$ -pulse, it is characterized by the equation:

$$M_{xy} = M_0 e^{-t/T_2} \quad (2.44)$$

Relaxation in the transverse plane is sensitive to dephasing of nuclear spins caused by local field inhomogeneities. Up until now, we assumed $\mathbf{M}$ was a single vector precessing at the Larmor frequency ω_0 . In reality, local field inhomogeneities and chemical shift effects lead to a magnetization composed of several vectors with different resonant frequencies. These vectors (also known as isochromats) contribute to a total magnetization vector, $\mathbf{M}^*$. $\mathbf{M}^*$ will decay in the transverse plane according to a time constant, T_2^* , defined as

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \quad (2.45)$$

where T_2' represents factors such as local field inhomogeneities that cause the magnetization to dephase faster than anticipated. As we will see later, the true T_2 -value can still be measured by reversing the effects of T_2' . It is important to note, however, that dephasing due to the random motion of spins is irreversible and inherent to the true T_2 -value [38]. In general, we are more interested in the true T_2 since it is characterized by different tissues, whereas T_2^* is influenced by inconsistencies in the magnetic field.

T_1 vs. T_2

Although the theoretical upper limit of T_2 is $T_2 \leq 2T_1$, in most cases, $T_2 \leq T_1$ [148]. At room temperature, T_1 is typically 0.1 to 10 seconds for protons in dielectric materials. Typical T_2 values range from $10\mu\text{s}$ to 10s [38]. T_1 and T_2 are also a function of field strength. In the mouse brain at $B_0 = 7.0$ T, $T_1 \approx 1500 - 1750$ ms and $T_2 \approx 34 - 41$ ms [85]. The difference between T_1 and T_2 in different tissue leads to variations in the signal contrast depending on the chosen imaging parameters.

2.2 Magnetic Resonance Signal

2.2.1 Signal Detection

According to the Faraday law of induction and principles of reciprocity, a time-varying magnetization, $\mathbf{M}(\mathbf{r}, t)$, will induce a voltage in a receiver coil [150]:

$$V(t) \propto -\frac{\partial}{\partial t} \int_{\text{object}} \mathbf{B}(\mathbf{r}) \cdot \mathbf{M}(\mathbf{r}, t) d\mathbf{r} \quad (2.46)$$

where $\mathbf{r}$ is the position vector for the magnetic field of the receive coil per unit current, $\mathbf{B}(\mathbf{r})$, and the time-varying magnetization, $\mathbf{M}(\mathbf{r}, t)$, inside the coil. To accomodate later signal processing stages, the voltage is demodulated to a lower frequency. Quadrature detection yields a complex signal:

$$S(t) = S_1(t) + iS_2(t) \quad (2.47)$$

Assuming a homogeneous magnetic field in the receiver coil, the signal can be written as

$$S(t) \propto \int_{object} M_{xy}(\mathbf{r}, 0) e^{-i\omega(\mathbf{r})t} d\mathbf{r} \quad (2.48)$$

where $\omega(\mathbf{r})$ is the frequency in the rotating frame (see [150] for a full derivation of this result). By assuming the spatial density of hydrogen nuclei, $\rho(\mathbf{r})$ is proportional to the longitudinal magnetization at thermal equilibrium

$$\rho(\mathbf{r}) \propto M_0(\mathbf{r}). \quad (2.49)$$

the transverse magnetization after a $\pi/2$ -pulse becomes

$$M_{xy}(\mathbf{r}, 0) \propto \rho(\mathbf{r}) \quad (2.50)$$

which gives us an expression of signal related to the spatial distribution of ^1H nuclei and their frequencies

$$S(t) \propto \int \rho(\mathbf{r}) e^{-i\omega(\mathbf{r})t} d\mathbf{r} \quad (2.51)$$

2.2.2 Free Induction Decay

After an α -pulse, the oscillating and decaying signal can be described as

$$S(t) \propto \sin(\alpha) \int \rho(\mathbf{r}) e^{-t/T_2(\mathbf{r})} e^{-i\omega(\mathbf{r})t} d\mathbf{r}. \quad (2.52)$$

The detected signal is called a free induction decay (FID) and is the foundation of all subsequent pulse sequences. After a $\pi/2$ -pulse, a homogenous sample with a single resonant frequency, ω_0 , will reduce to

$$S(t) \propto M_0 e^{-t/T_2} e^{-i\omega_0 t} \quad (2.53)$$

See Figure 2.6 for an example of an FID signal.

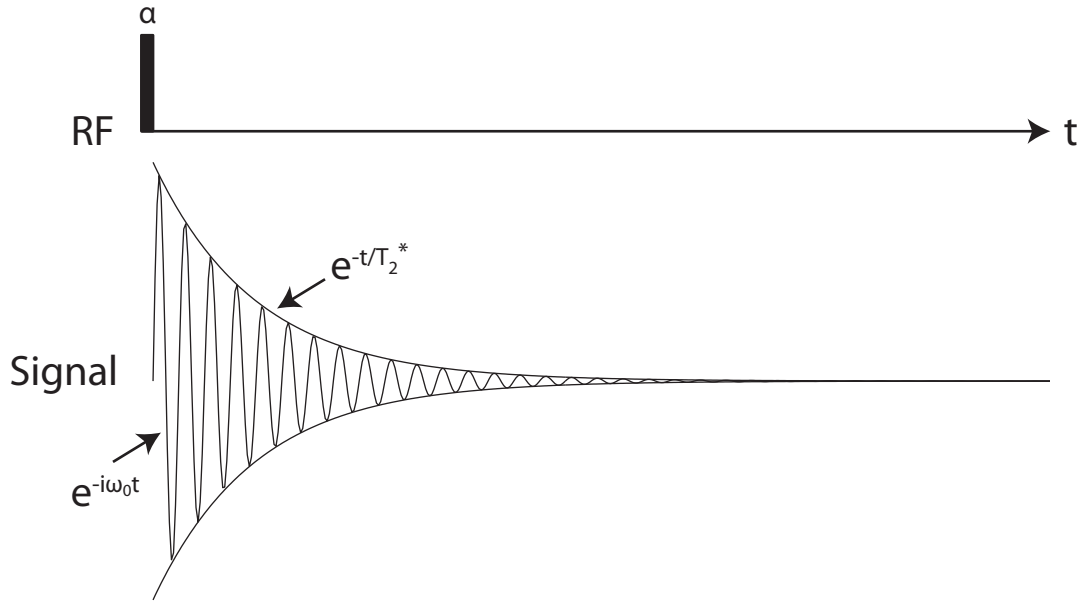


Figure 2.6: FID signal after an α -pulse. In the presence of local field inhomogeneities, the exponential decay is approximated by the T_2^* time constant. ω_0 is small for visual clarity.

2.2.3 Gradients

In the presence of a strong homogeneous magnetic field, $\mathbf{B}_0$, ^1H nuclei in water will precess at the same Larmor frequency, ω_0 . In order to spatially localize the signal, a linearly varying magnetic field independent of $\mathbf{B}_0$ is applied. For example, to $\mathbf{B} = B_0 \hat{\mathbf{z}}$ we can apply a magnetic field that varies linearly as a function of x , $\mathbf{G}_x = G_x x \hat{\mathbf{z}}$ where G_x is the gradient of the magnetic field [208] defined as

$$G_x = \frac{\partial B_z}{\partial x} \quad (2.54)$$

The total magnetic field becomes (see Figure 2.7)



Figure 2.7: Spatially-independent magnetic field, $\mathbf{B} = B_0\hat{\mathbf{z}}$ (left) and spatially-dependent magnetic field, $\mathbf{B}_z(x) = (B_0 + G_x x)\hat{\mathbf{z}}$ (right).

$$\mathbf{B}_z(x) = (B_0 + G_x x)\hat{\mathbf{z}} \quad (2.55)$$

and the Larmor frequency becomes spatially dependent,

$$\omega(x) = \gamma B_z(x) = \gamma(B_0 + G_x x). \quad (2.56)$$

Gradients that vary linearly as functions of y and z are defined as

$$\begin{aligned} \mathbf{G}_y &= G_y \hat{\mathbf{z}} \\ \mathbf{G}_z &= G_z \hat{\mathbf{z}} \end{aligned} \quad (2.57)$$

In high-field MRI, the B_0 field is much stronger than the gradient strength in the z-direction, so it is usually safe to ignore magnetic fields in the x- and y-direction produced by the gradient coils.

Pulse Sequence Diagrams

Gradient and RF pulses can be represented in a pulse sequence diagram. For our purposes, RF pulses will be represented as black bars, although the envelope defining them will generally not be rectangular. The effective flip angle will be subscripted by the initial phase

angle, where x implies initial phase along the x -axis and y implies initial phase along the y -axis. If relevant, applied gradients will be defined by their direction (G_x , G_y , and G_z or G_{read} , G_{phase} , and G_{slice}). A rectangular gradient pulse produces a magnetic field with a constant slope proportional to its height and a duration proportional to its length. If noteworthy, the time-dependent signal will also be included.

2.2.4 Gradient Echo

A gradient echo sequence measures the T_2^* -weighted signal of the FID. After a negative linear gradient ($-G_x$) is applied for time, τ along the x -direction, the signal in Equation (2.52) is dephased relative to the gradient strength

$$S(\tau) \propto M_0 e^{-\tau/T_2^*} \int e^{i\gamma G_x x \tau} dx \quad (2.58)$$

If immediately afterwards, a positive linear gradient ($+G_x$) is applied for the same amount of time and at the same gradient strength, the signal lost due to forced dephasing is recovered

$$S(2\tau) \propto M_0 e^{-2\tau/T_2^*} \int e^{i(\gamma G_x x \tau - \gamma G_x x \tau)} dx = M_0 e^{-2\tau/T_2^*} \quad (2.59)$$

The detected signal at $t = 2\tau$, called the gradient echo, has an amplitude weighted by the T_2^* time constant (see Figure 2.8).

2.2.5 Spin Echo

In order to measure the true T_2 -weighted signal, a spin echo sequence is employed. The reversal of factors contributing to T_2' was first proposed by Hahn in 1950 [89, 90]. It involves a $\pi/2$ -pulse followed by a π -pulse at $t = \tau$. At $t = 2\tau$, a spin echo is formed with an amplitude weighted by the T_2 time constant (see Figure 2.9).

$$S(2\tau) \propto M_0 e^{-2\tau/T_2}. \quad (2.60)$$

The spin echo sequence can be understood by plotting the evolution of three isochromats

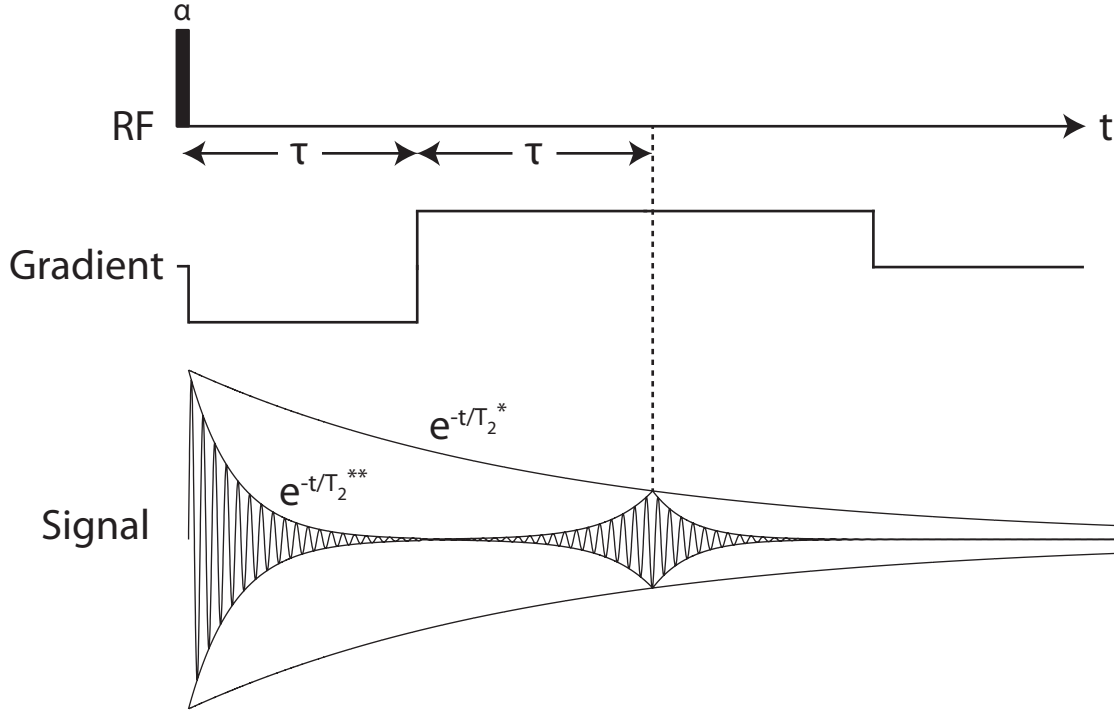


Figure 2.8: Gradient echo pulse sequence. Gradient dephasing and rephasing is characterized by the T_2^{**} time constant. Detected signal at $t = 2\tau$ has an amplitude proportional to $e^{-2\tau/T_2^*}$.

($\mathbf{M}_-$, $\mathbf{M}_0$, and $\mathbf{M}_+$) with three different frequencies: $\omega_- < \omega_0 < \omega_+$. In a homogeneous sample, ω_- and ω_+ arise from static local field inhomogeneities. After a $\pi/2$ -pulse (Fig. 2.10a), the three isochromats will dephase as $\mathbf{M}_+$ precesses slightly faster than $\mathbf{M}_0$ and $\mathbf{M}_-$ precesses slightly slower than $\mathbf{M}_0$ (Fig. 2.10b), resulting in T_2^* decay. At $t = \tau$, a π_y -pulse flips the isochromats into the opposite x-y plane, and the isochromats precessing away from the $\mathbf{M}_0$ start precessing towards it (Fig. 2.10c), resulting in a T_2^* -weighted growth. The isochromats rephase at $t = 2\tau$ (also called the echo time, T_E) with a signal amplitude weighted by the T_2 time constant (Fig. 2.10d).

2.2.6 Inversion Recovery

An inversion recovery sequence sensitizes the signal to the T_1 component. It can be used to eliminate signal from components with a specific T_1 value (such as fat) or for measuring

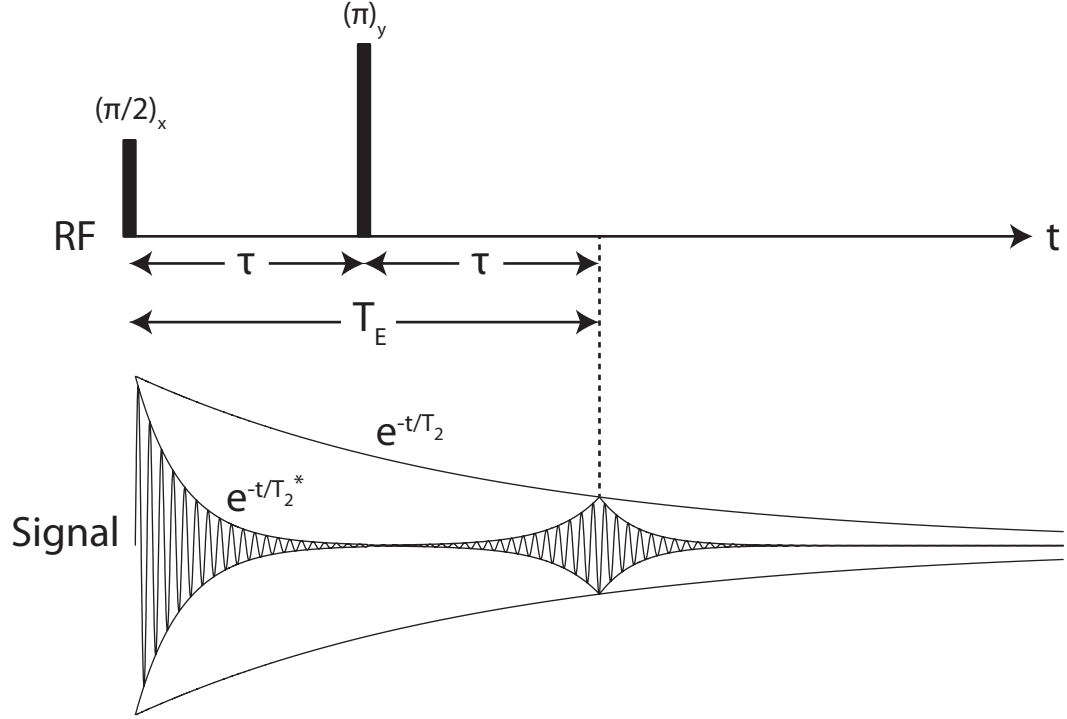


Figure 2.9: Spin echo pulse sequence. Signal dephasing and rephasing is characterized by the T_2^* time constant. Detected signal at $t = 2\tau$ has an amplitude proportional to $e^{-2\tau/T_2}$

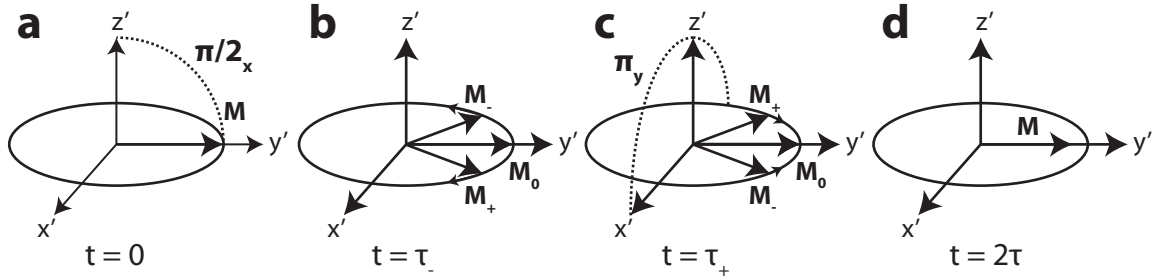


Figure 2.10: Isochromats (M_- , M_0 , and M_+) in the ω_0 rotating frame immediately after a $\pi/2_x$ -pulse (a), dephased before (b) and after (c) a π_y -pulse at $t = \tau$, and rephased at $t = 2\tau$ (d).

the T_1 time constant.

In the inversion recovery pulse sequence, a π -pulse is followed by a $\pi/2$ -pulse at an inversion time T_I (Figure 2.11). After a π -pulse, the longitudinal magnetization goes to

$$M_{z'}(0) = -M_0 \quad (2.61)$$

Prior to the $\pi/2$ -pulse, the magnetization regrows as

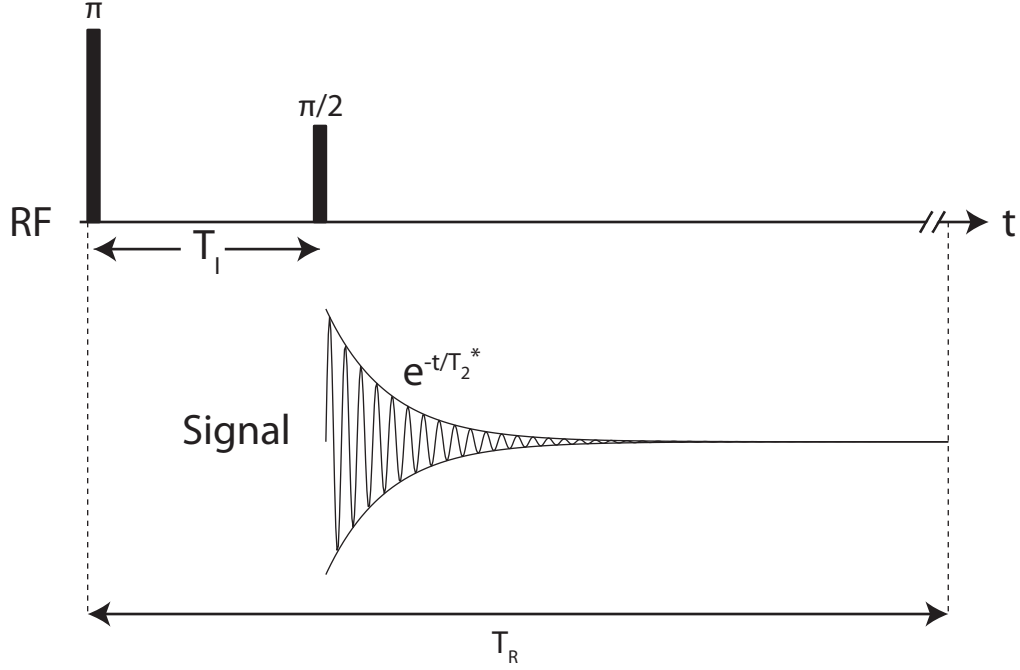


Figure 2.11: Inversion recovery pulse sequence.

$$M_{z'}(t) = M_0 \left(1 - 2e^{-t/T_1}\right) \text{ for } 0 < t < T_I \quad (2.62)$$

The $\pi/2$ -pulse at $t = T_I$ tips the magnetization into the transverse plane, creating an FID with signal [87]

$$S(t) \propto \left| M_0 \left(1 - 2e^{-T_I/T_1}\right) \right| e^{-(t-T_I)/T_2^*} \text{ for } t > T_I. \quad (2.63)$$

At $T_I = T_1 \ln 2$, $S(T_I)$ goes to zero

$$S(T_I = T_1 \ln 2) \propto \left| M_0 \left(1 - 2e^{-T_1 \ln 2 / T_1}\right) \right| = 0 \quad (2.64)$$

Thus, by varying T_I , the T_1 value can be determined.

2.3 Magnetic Resonance Imaging

In order to create a magnetic resonance image, we need to spatially localize the signal. We can do this by applying a sequence of gradient magnetic fields in the orthogonal read,

phase, and slice directions (called G_x , G_y , and G_z or G_{read} , G_{phase} , and G_{slice}). These three orthogonal gradient fields are assigned the role of frequency encoding, phase encoding, and slice selection, respectively. Although for simplicity we often use x, y, and z to describe their direction, gradient fields are usually a combination of all three directions to selectively excite and spatially encode a volume of space.

The application of magnetic gradient fields to spatial localization in MRI was first outlined by Paul Lauterbur in 1973 [139]. Together with Sir Peter Mansfield, he received the 2003 Nobel Prize in Medicine for his discoveries.

2.3.1 Slice Selection

Applying a gradient field that varies linearly in z (G_z) creates a varying Larmor frequency along the z-direction,

$$\omega(z) = \omega_0 + \gamma G_z z \quad (2.65)$$

or in cyclic frequency,

$$f(z) = f_0 + \frac{\gamma}{2\pi} G_z z. \quad (2.66)$$

Previously, we saw that the sinc pulse (Equation (2.26)) yielded a rectangular pulse in the frequency domain (Equation (2.27)). If we define the centre of our slice as z_0 and the desired slice thickness as Δz , the central frequency is

$$f_c = f_0 + \frac{\gamma}{2\pi} G_z z_0. \quad (2.67)$$

with bandwidth

$$\Delta f = \frac{\gamma}{2\pi} G_z \Delta z \quad (2.68)$$

The sinc pulse (Equation (2.27)) can now be defined spatially along the z-direction

$$p(z) = \prod \left(\frac{z - z_0}{\Delta z} \right) e^{i\gamma G_z (z - f_0) \tau_p / 2} \quad (2.69)$$

Using these equations, by increasing the bandwidth or decreasing the gradient strength, we can increase the slice thickness; and by decreasing the bandwidth or increasing the gradient strength, we can decrease the slice thickness (see Fig. 2.12b).

After the slice has been selected, a linear phase-shift is introduced. This can be corrected by applying the gradient $-G_z$ for time $t = \tau_p/2$ to rewind the phase angle, $\phi(t)$ to zero (see Fig. 2.12a)

$$\phi(t) = \begin{cases} \gamma G_z (z - f_0) \tau_p / 2, & t = \tau_p \\ \gamma G_z (z - f_0) \tau_p / 2 - \gamma G_z (z - f_0) \tau_p / 2 = 0, & t = \tau_p + \tau_p / 2 \end{cases} \quad (2.70)$$

A sinc pulse only yields a perfectly rectangular excitation profile if $t = \infty$. Truncating the sinc pulse to $t = \tau_p$ results in a nonuniform excitation profile and prominent side lobes outside the desired frequency bandwidth. This can be partially corrected by using apodizing window functions [24]. In practice, to reduce the sidelobe amplitudes or improve RF power efficiency, other pulse shapes such as Gaussian or Hermite might be preferable.

2.3.2 k-Space

We now want to spatially localize the signals in the x and y planes of the slice. Before we do that, it is useful to introduce the concept of **k**-space.

In the rotating frame, we can define the Larmor frequency as a function of the position vector, $\mathbf{r}$

$$\omega(\mathbf{r}) = \mathbf{G} \cdot \mathbf{r} \quad (2.71)$$

where the gradient vector, $\mathbf{G}$ is

$$\mathbf{G} = G_x \hat{\mathbf{x}} + G_y \hat{\mathbf{y}} + G_z \hat{\mathbf{z}}. \quad (2.72)$$

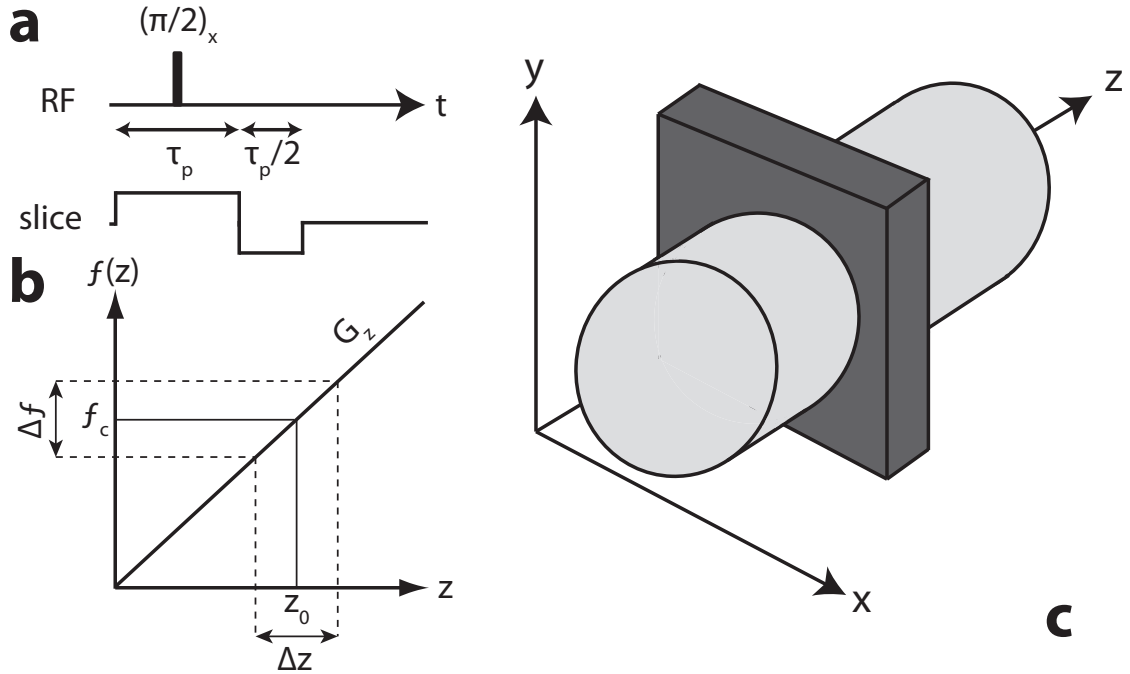


Figure 2.12: (a) Slice selection with a $\pi/2_x$ sinc pulse and G_z gradient field. (b) Bandwidth and gradient strength determine the slice thickness. (c) Slice selection results in the magnetization being tipped into the transverse plane in a specific volume of the sample (dark grey slice).

Note that while $\mathbf{G}$ describes how the gradient components affect the magnetic field along the $\hat{x}$, $\hat{y}$, and $\hat{z}$ directions, the gradient fields themselves still all point in the $\hat{z}$ direction. The equation for signal (Equation (2.51)) becomes

$$S(t) \propto \int \rho(\mathbf{r}) e^{i\gamma \mathbf{G} \cdot \mathbf{r} t} d\mathbf{r} \quad (2.73)$$

where $\rho(\mathbf{r})$ is the proton density. This has the form of a Fourier transformation. To make this more clear, define the reciprocal space vector, $\mathbf{k}$:

$$\mathbf{k} = \frac{\gamma \mathbf{G} t}{2\pi} \quad (2.74)$$

This results in the Fourier transform pair:

$$\begin{aligned} S(\mathbf{k}) &\propto \int \rho(\mathbf{r}) e^{i2\pi \mathbf{k} \cdot \mathbf{r}} d\mathbf{r} \\ \rho(\mathbf{r}) &\propto \int S(\mathbf{k}) e^{-i2\pi \mathbf{k} \cdot \mathbf{r}} d\mathbf{k} \end{aligned} \tag{2.75}$$

Thus, the signal $S(\mathbf{k})$ detected in the time domain can be Fourier-transformed into the spatial domain function, $\rho(\mathbf{r})$. We can acquire data in $\mathbf{k}$ -space by varying $\mathbf{G}$ and t . The center of the $\mathbf{k}$ -space image contains information about gross form and tissue contrast, whereas the edges contain information about fine details in the image [26]. In two-dimensions, $\rho(\mathbf{r})$ becomes $\rho(x, y)$ and $S(\mathbf{k})$ becomes [150]

$$S(k_x, k_y) \propto \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \rho(x, y) e^{-i2\pi(k_x x + k_y y)} dx dy. \tag{2.76}$$

We can use a phase-encoding method to rectilinearly traverse the k_x and k_y components of $\mathbf{k}$ -space in [Equation \(2.76\)](#).

2.3.3 Phase Encoding

Applying a G_y gradient pulse varies the phase of the nuclei along the y-direction of the selected slice ([Figure 2.13](#)). If G_y is applied for time τ_{phase} , k_y becomes

$$k_y(t = \tau_{phase}) = \frac{\gamma G_y \tau_{phase}}{2\pi} \tag{2.77}$$

To sample k_y at N_y equally spaced positions, we need to apply the gradient pulse, G_y at N_y equally incremented amplitudes. If we define the step-wise gradient amplitude, G_{yn} to have N_y values ranging from $-G_y$ to $+G_y$

$$G_{yn} = G_y \left(\frac{2n}{N_y - 1} - 1 \right) \text{ for } n = 0 \dots N_y - 1 \tag{2.78}$$

the N_y values of k_{yn} become

$$k_{yn} = \frac{\gamma G_{yn} \tau_{phase}}{2\pi} = \frac{\gamma G_y \tau_{phase}}{2\pi} \left(\frac{2n}{N_y - 1} - 1 \right) \text{ for } n = 0 \dots N_y - 1 \quad (2.79)$$

After each phase encoding step, the system is allowed to return to equilibrium for a time T_R (called the repetition time). The acquisition time to acquire N_y phase-encoded steps is $N_y T_R$. Whereas previously we assumed a single signal acquisition, the repetition of a pulse sequence introduces a dependency on the longitudinal magnetization, resulting in a T_1 -weighted component influencing the signal. This can be accounted for by increasing the T_R to properly equilibrate the longitudinal magnetization or, given time constraints, performing several dummy scans prior to acquisition to achieve a steady-state of the spin system.

2.3.4 Frequency Encoding

After a phase encoding gradient, we can sample the signal along the x-direction using a frequency encoding gradient, G_x . Prior to signal acquisition, $-G_x$ is applied for $t = \tau_{read}/2$, rewinding k_x to the position

$$k_{x0} = -\frac{\gamma G_x \tau_{read}}{4\pi} \quad (2.80)$$

After this gradient pulse, called a readout dephaser, G_x is applied for a time τ_{read} . To sample k_x at N_x equally spaced positions, we need to acquire the signal at N_x equal time increments. If we define the step-wise readout time, τ_n to have N_x values ranging from 0 to τ_{read}

$$\tau_n = \tau_{read} \left(\frac{n}{N_x - 1} \right) \text{ for } n = 0 \dots N_x - 1 \quad (2.81)$$

the N_x values of k_{xn} become

$$k_{xn} = \frac{\gamma G_x \tau_n}{4\pi} + k_{x0} = \frac{\gamma G_x \tau_{read}}{4\pi} \left(\frac{2n}{N_x - 1} - 1 \right) \text{ for } n = 0 \dots N_x - 1 \quad (2.82)$$

This has an obvious symmetry with [Equation \(2.79\)](#). If $G_x \cdot \tau_{read}/2 = G_y \cdot \tau_{phase}$ and $N_x = N_y$, k_{xn} and k_{yn} will traverse the same range of values in the k_x and k_y directions,

respectively.

We can acquire all of the k_{xn} values during a single frequency encoding gradient by simultaneously sampling the signal in τ_{read}/N_x increments. Thus, frequency encoding gradients are often called read or readout gradients. By applying an incremental phase-encoding gradient (G_y) followed by a frequency-encoding gradient (G_x) in conjunction with signal acquisition, $\mathbf{k}$ -space can be traversed in a total acquisition time of $N_y T_R$.

After a slice selective gradient and RF pulse, Figure 2.13 shows how phase encoding gradients and frequency encoding gradients can spatially localize signal in a simple 3x3 grid. After spatial encoding, each square (called a voxel) has a frequency (ω) and phase (ϕ) that defines its location (top right). After acquiring the signal, $S(t, \phi)$ at $N_y = 3$ phase-encoding steps ($\phi = \phi_-, 0$, and ϕ_+), the resultant signal in $\mathbf{k}$ -space ($S(k_x, k_y)$) can be Fourier transformed into an image space representation of the original sample ($\rho(x, y)$).

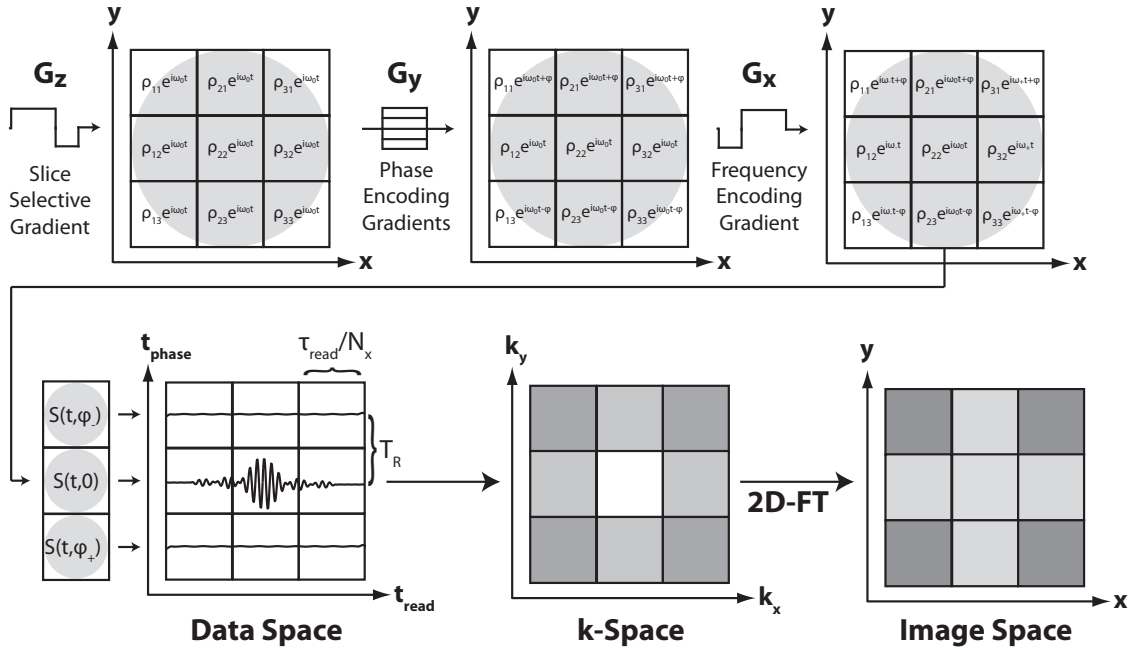


Figure 2.13: Spatial encoding of signal using slice selective (G_z), phase encoding (G_y), and frequency encoding (G_x) gradients. Assume the sample (light grey) is homogeneous with Larmor frequency ω_0 . ρ_{xy} represents the proton density in each voxel. The range of frequencies created by G_x is $\omega = \omega_0 -$, ω_0 , and $\omega_0 +$. The range of phases created by G_y is $\phi = \phi_-$, 0 , and ϕ_+ . A two-dimensional Fourier transform of $\mathbf{k}$ -space yields an image space representation of the sample.

The $\mathbf{k}$ -space formalism is a powerful tool for constructing complicated imaging schemes. Although we only discussed a simple rectilinear trajectory, variations using radial and spiral trajectories can also be applied.

2.3.5 Spatially Encoded Gradient Echo

Applying the spatial encoding gradients in sequence results in a spatially encoded version of the gradient echo pulse sequence (2.2.4). Slice and read gradient pulses are fixed amplitude pulses. The phase gradient pulse has varying amplitude, and is represented by a series of horizontal lines on the pulse. Echo time (T_E) is the time between the application of the flip angle, α , and the peak of the echo signal. Often, a spoiler gradient with varying amplitude is applied along the slice direction to minimize residual transverse magnetization after signal acquisition[34]. If we assume that $T_R \gg T_2$, the measured signal at $t = T_E$ is [150]

$$S(T_E) \propto M_z^0 \frac{(1 - e^{-T_R/T_1})}{1 - \cos\alpha e^{-T_R/T_1}} \sin\alpha e^{-T_E/T_2^*}. \quad (2.83)$$

Thus, by changing α , T_E , or T_R , we can influence the relative amplitude of the signal and its T_1 - and T_2^* -weighting. Specifically, T_E will influence the T_2^* -weighting of the image while α and T_R will influence the T_1 -weighting of the image. See Figure 2.14 for a pulse diagram of the spatially encoded gradient echo sequence.

2.3.6 Spatially Encoded Spin Echo

To spatially encode a spin echo sequence, slice encoding gradients are applied during the $\pi/2$ -pulse and, usually, the π -pulse. A positive readout dephaser gradient placed before the π -pulse, $+G_x$ is applied for $t = \tau_{read}/2$, incrementing k_x to the position

$$k_{x0} = +\frac{\gamma G_x \tau_{read}}{4\pi} \quad (2.84)$$

The π -pulse flips the magnetization vector such that $k_x = -k_{x0}$ and $k_y = -k_y$. Using the same sequence of phase-encoding gradients results in the k_y coordinates being traversed in the opposite direction, from $-k_y$ to $+k_y$ (albeit, with no real consequence on the final $\mathbf{k}$ -

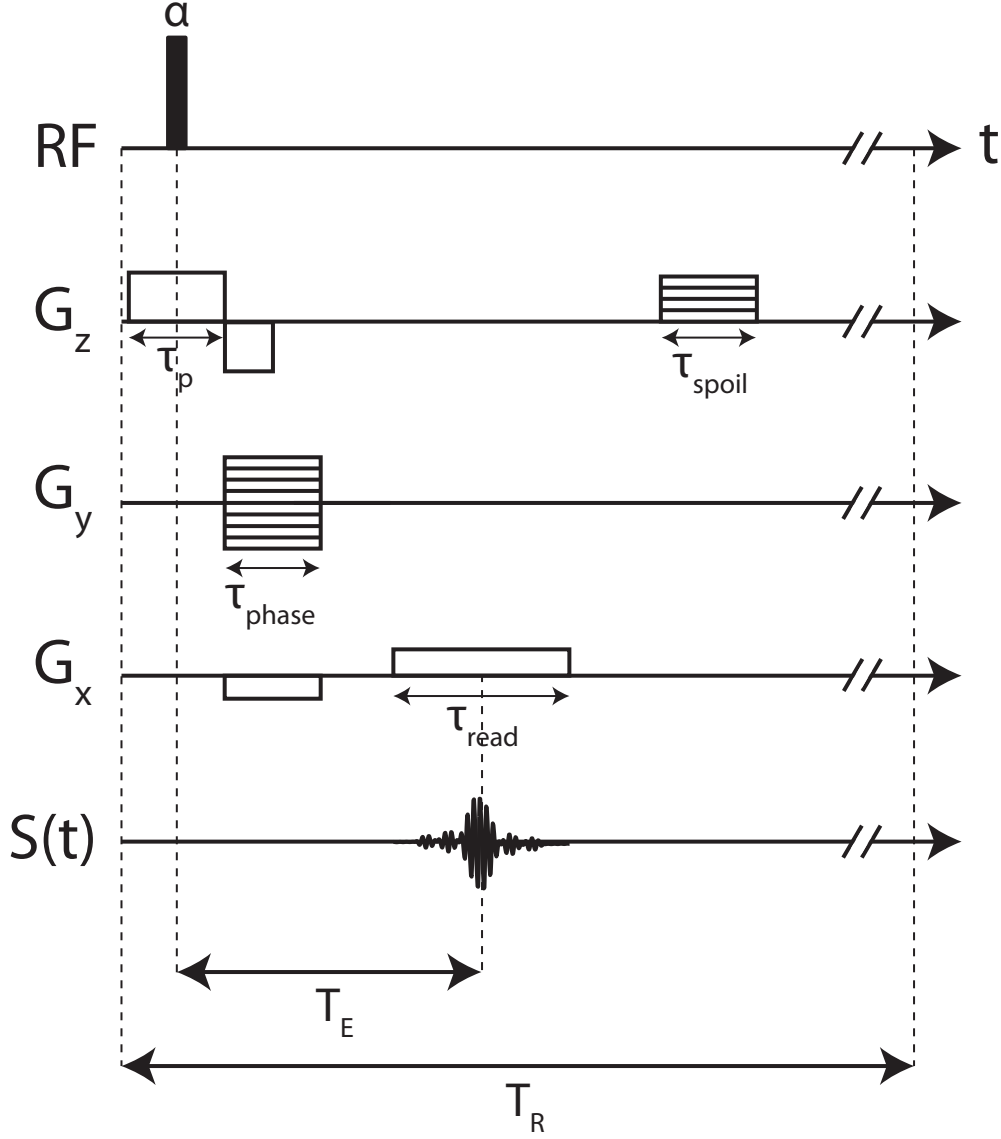


Figure 2.14: Gradient echo pulse sequence diagram with spatial-encoding gradients.

space image other than it being flipped in the y -direction). The measured signal at $t = T_E$ is [150]

$$S(T_E) \propto M_z^0 \left(1 - 2e^{-(T_R - T_E/2)/T_1} + e^{-T_R/T_1} \right) e^{-T_E/T_2}. \quad (2.85)$$

For $T_E \ll T_R$, this becomes

$$S(T_E) \propto M_z^0 \left(1 - e^{-T_R/T_1} \right) e^{-T_E/T_2} \quad (2.86)$$

Table 2.1: Spin echo image contrast

Contrast	T_E	T_R
T_1 -weighted	short	optimal
T_2 -weighted	optimal	long
ρ -weighted	short	long

Thus, changing T_E will influence the T_2 -weighting of the image while T_R will influence the T_1 -weighting of the image. The effects of T_E and T_R on image contrast are summarized in Table 2.1. See Figure 2.15 for a pulse diagram of the spatially encoded spin echo sequence.

2.3.7 Spatially Encoded Inversion Recovery

In order to spatially encode the inversion recovery sequence (2.2.6), a spin echo inversion recovery pulse sequence can be used. If another π -pulse is applied at $t = T_I + \tau$, a spin echo will form at $t = T_I + 2\tau$. Signal measured at this time will have a T_1 -weighting influenced by both the T_I and T_R values and a T_2 -weighting influenced by $T_E = 2\tau$. For $T_E \ll T_R$, the signal at $t = T_I + T_E$ becomes [150]

$$S(T_I + T_E) \propto M_z^0 \left(1 - 2e^{-T_I/T_1} + e^{-T_R/T_1} \right) e^{-T_E/T_2} \quad (2.87)$$

For a single T_1 component, the signal goes to zero at

$$T_I = T_1 \ln \left(\frac{2}{1 + e^{-T_R/T_1}} \right) \quad (2.88)$$

This property can be exploited to eliminate signal from components with a specific T_1 value (such as fat) or for measuring the T_1 time constant. See Figure 2.16 for a pulse diagram of the spatially encoded spin echo inversion recovery sequence.

2.3.8 Image Reconstruction

Fourier transformation of $S(k_x, k_y)$ (Equation (2.76)) yields a representation of the image in the image-space, $\rho(x, y)$. The acquired $\mathbf{k}$ -space image is discrete, necessitating a discrete

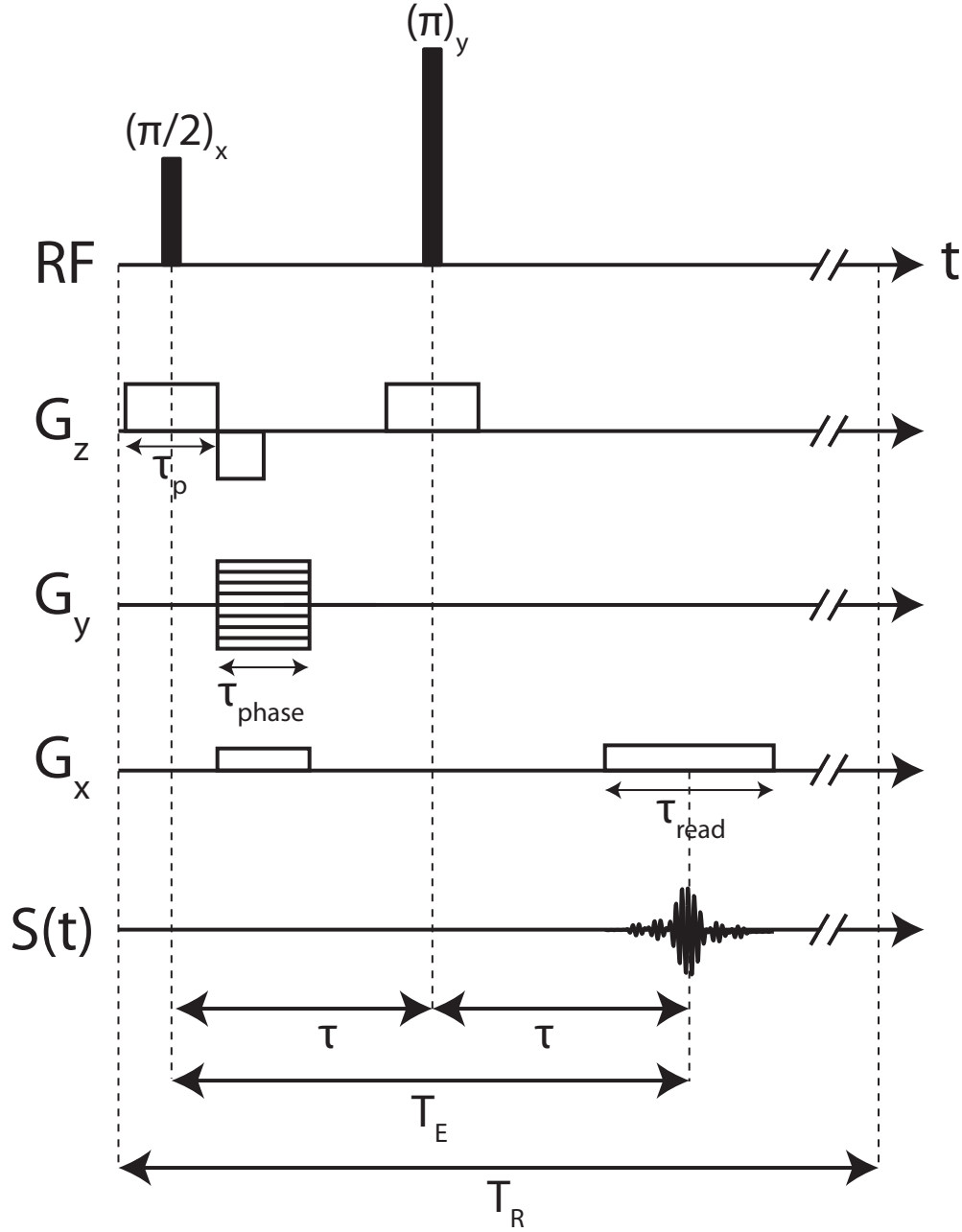


Figure 2.15: Spin echo pulse sequence diagram with spatial-encoding gradients.

version of the inverse Fourier transform. In one-dimension, $S(k_x)$ can be written as

$$S(k_{xn}) \propto \int_{-\infty}^{\infty} \rho(x) e^{-i2\pi k_{xn}x} dx \quad (2.89)$$

where Δk_x is the step-size of $k_{xn} = n\Delta k_x$. $S(k_{xn})$ becomes

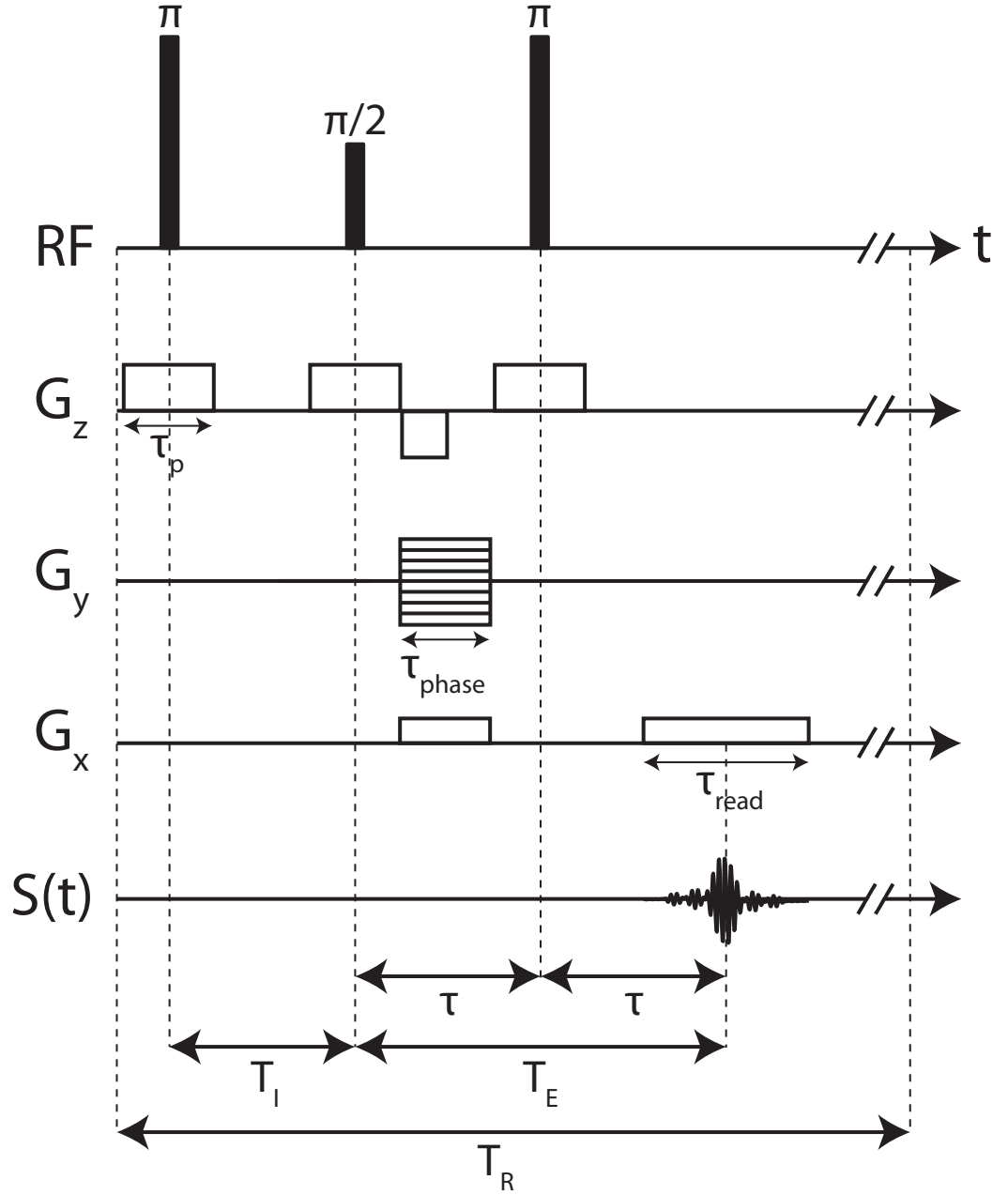


Figure 2.16: Spin echo inversion recovery pulse sequence diagram with spatial-encoding gradients.

$$S[n] \propto \int_{-\infty}^{\infty} \rho(x) e^{-i2\pi n \Delta k x} dx. \quad (2.90)$$

$\rho(x)$ can be reconstructed using a truncated Fourier series [150]

$$\rho(x) \propto \Delta k_x \sum_{n=-N_x/2}^{N_x/2-1} S[n] e^{i2\pi n \Delta k_x x} \text{ for } |x| < \frac{1}{\Delta k_x} \quad (2.91)$$

The step-size in image-space (corresponding to the voxel size) is

$$\Delta x = \frac{1}{N_x \Delta k_x}. \quad (2.92)$$

If we define the x-position of each pixel as $x_m = m \Delta x$, $\rho(x_m)$ becomes

$$\rho(x_m) = \rho[m] \propto \sum_{n=-N_x/2}^{N_x/2-1} S[n] e^{i2\pi n m / N_x} \text{ for } -N_x/2 \leq m \leq N_x/2. \quad (2.93)$$

$\rho(x_m)$ can be calculated quickly using the inverse fast Fourier transform (iFFT) algorithm [47]. The FFT algorithm requires that both N_x and N_y equal 2^n , where n is a positive integer (i.e. $N = 2, 4, 8, 16, 32, 64, 128, 256$, etc.). See [Figure 2.17](#) for MR images in both k -space and image-space.

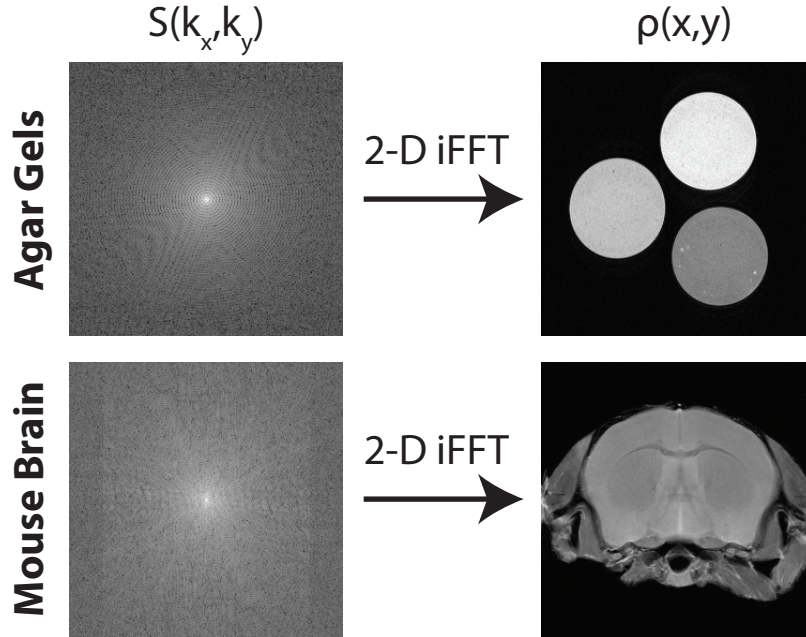


Figure 2.17: 256x256 MR images of agar gels and a mouse brain in k -space, $S(k_x, k_y)$ and image-space, $\rho(x, y)$ after an inverse fast Fourier transformation (iFFT).

Chapter 3

Quantitative MRI Methods

In the previous chapter, we saw how individual spin-1/2 particles, when placed in a magnetic field, can be manipulated to create a magnetic resonance image. In building up the framework that describes MRI, the T_1 and T_2 relaxation time constants were introduced. Since both T_1 and T_2 are characteristic of the tissues being studied, it would be useful to be able to measure them quantitatively. Methods for measuring T_1 and T_2 are discussed in [Section 3.1](#). In discussing the T_2 time constant, we mentioned the random motion of particles that contributes to the spin-spin relaxation. With the application of gradient magnetic fields, we can encode the translational motion of spins to measure their average random motion. This average motion (also known as Brownian motion) leads to the characteristic diffusion of water in different types of tissue. Methods for measuring diffusion and the diffusion tensor are discussed in [Section 3.2](#). Although hydrogen nuclei in water generally have a T_2 on the order of milliseconds, hydrogen nuclei bound in macromolecules and membranes tend to have a relaxation time too short to be accessible using regular spin echo methods. In order to probe this hidden bound pool, we can measure their magnetization indirectly using magnetization transfer imaging ([Section 3.3](#)). Finally, approaches combining two or more quantitative MRI methods are discussed in [Section 3.4](#).

3.1 Relaxometry

The term “relaxometry”, although originally used in reference to measurements of the field dependence of relaxation times, will be used here in reference to any measurement of relaxation times in MRI. Previously, we saw that simple spin-systems can be characterized by

a T_1 time constant that describes longitudinal (or spin-lattice) magnetization relaxation, and a T_2 time constant that describes transverse (or spin-spin) magnetization relaxation. In more complex environments such as tissue, there might be several different spin-systems in a voxel, with different T_1 and T_2 relaxation rates. Some of these times can be measured directly using multi-component analysis of T_1 and T_2 relaxation datasets. Extremely short T_2 components tied up in fat and other macromolecules can be measured using ultrashort spin echo methods or indirectly using magnetization transfer imaging (see [Section 3.3](#)). The methods used in this dissertation for acquiring and analyzing T_1 , T_2 , and multi-component T_2 datasets are discussed below. Specificity to healthy tissue and tissue pathology are also discussed.

3.1.1 Measuring T_1

The T_1 relaxation time is mediated by the lattice environment in which the spin system exists. Although only the saturation recovery method was used in this dissertation to calculate T_1 , it is worth reviewing the three most intuitive ways to determine T_1 based on the saturation recovery, gradient echo, and spin echo inversion recovery pulse sequences, respectively.

1. Saturation Recovery

In the spin echo signal equation for $T_E \ll T_R$ ([Equation \(2.86\)](#)), varying the repetition time, T_R , while keeping the echo time, T_E , constant results in the signal varying according to the T_1 time constant. Thus, we can simplify the signal equation to

$$S(T_R) = S_0 \left(1 - e^{-T_R/T_1}\right). \quad (3.1)$$

If we acquire several images with varying repetition times, we can fit the change in signal to [Equation \(3.1\)](#), yielding a saturation recovery curve ([Figure 3.1](#)). In the normalized equation, $S_0 = 1$ and $S(T_R) \rightarrow 1$ as $T_R \rightarrow \infty$.

The saturation recovery method is sensitive to inaccurate $\pi/2$ -pulses, although if the error in flip angle is constant, the correct T_1 can still be determined. It is also slow when

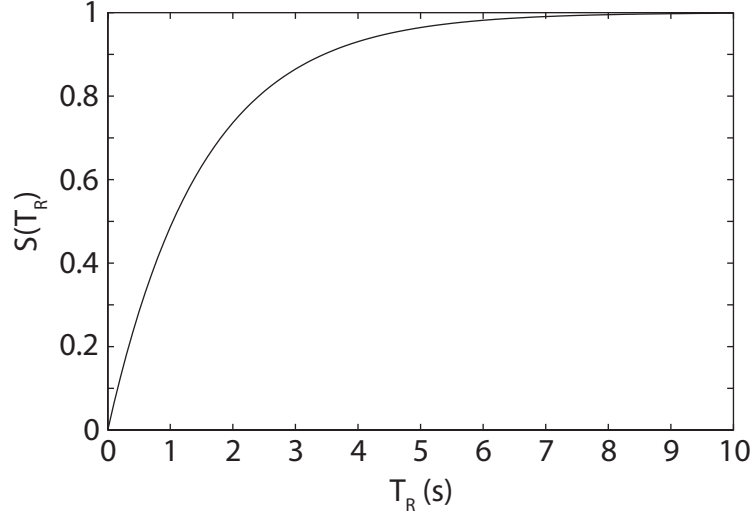


Figure 3.1: Saturation recovery curve for $S_0 = 1$, $T_1 = 1.5$ s and T_R values ranging from 0 to 10 s.

compared to the gradient echo method. However, it allows multiple spin echos to be acquired, facilitating concurrent acquisition of the T_2 relaxation time.

2. Spoiled Gradient Echo

In the spoiled gradient echo signal equation (Equation (2.83)), varying the flip angle, α while keeping T_R and T_E constant results in a signal varying according to the T_1 time constant. The signal equation becomes

$$S(\alpha) = S_0 \frac{\sin \alpha}{1 - \cos \alpha e^{-T_R/T_1}} \quad (3.2)$$

If we acquire several images with varying flip angles, we can fit the change in signal to Equation (3.2), yielding a spoiled gradient echo curve (Figure 3.2).

The spoiled gradient echo method requires accurate α pulses, but it can be acquired faster than the saturation recovery method. There are several factors which influence the accuracy of this method, including slice selection, off-resonance effects, and incomplete spoiling due to short repetition times.

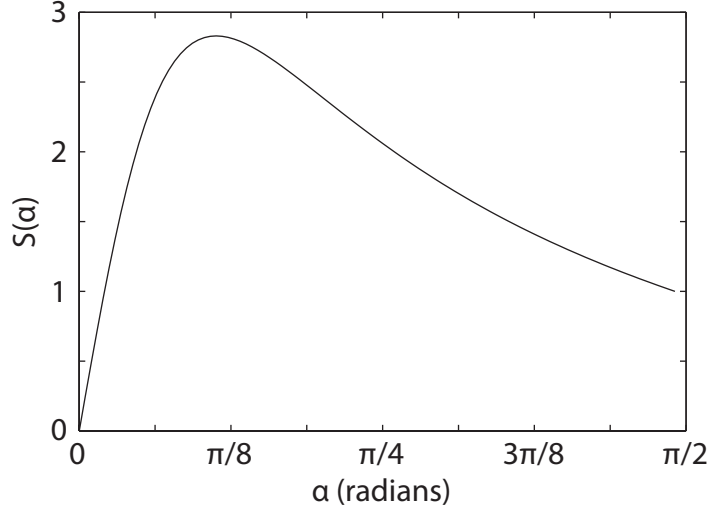


Figure 3.2: Spoiled gradient echo curve for $S_0 = 1$, $T_1 = 1.5$ s, $T_R = 100$ ms and α values ranging from 0 to $\pi/2$ s.

3. Spin Echo Inversion Recovery

In the inversion recovery signal equation for $T_E \ll T_R$ (Equation (2.87)), varying the inversion time, T_I while keeping T_R and T_E constant results in a signal varying according to the T_1 time constant. The signal equation becomes

$$S(T_I) = S_0 \left(1 - 2e^{-T_I/T_1} + e^{-T_R/T_1} \right). \quad (3.3)$$

If we acquire several images with varying inversion times, we can fit the change in signal to Equation (3.3), yielding an inversion recovery curve (Figure 3.3).

The inversion recovery method is considered the gold standard for measuring T_1 in spectroscopy [48], however it is slow and doesn't allow for simultaneous T_2 measurements.

There are several other methods for measuring T_1 (e.g. Look and Locker [153] or DESPOT1 [46, 52]). However, for the purposes of this dissertation, only a saturation recovery method was used so that simultaneous T_1 and T_2 measurements could be made.

3.1.2 T_1 in Tissue

The T_1 relaxation time is related to the magnetic interaction of spins with the lattice of surrounding molecular structures. This interaction is akin to friction, where the T_1

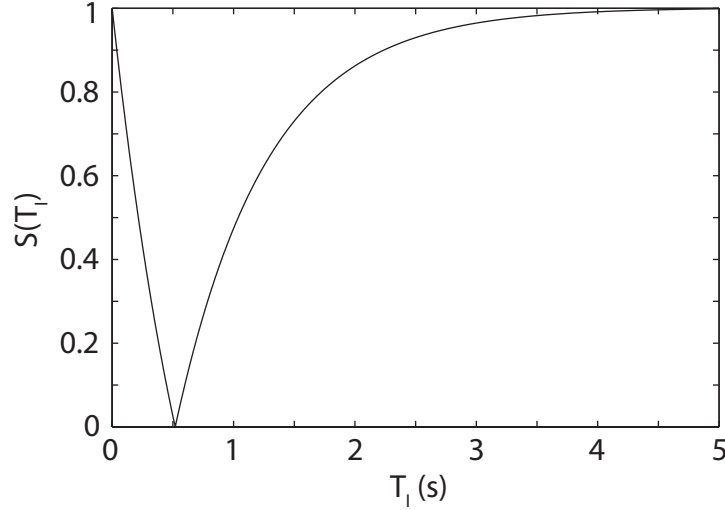


Figure 3.3: Inversion recovery curve for $S_0 = 1$, $T_1 = 1.5$ s, $T_R = 10$ s and T_I values ranging from 0 to 5 s.

depends on both the distribution of the lattice and the molecular tumbling of the water. Water in macromolecules is constrained by a large lattice and has limited mobility. Free water has increased mobility but no structured lattice to transfer its energy. In both cases, T_1 times are relatively long. In soft tissue, water mobility and the surrounding lattice of macromolecules conspire to increase the longitudinal relaxation rate ($R_1 = 1/T_1$) and shorten the T_1 relaxation time. In this context, we expect longer T_1 times for tissue in which the lattice size has decreased and the water mobility has increased (e.g. demyelinated white matter, inflammation, or axonal loss). In contrast, we expect shorter T_1 times in healthy myelinated white matter and pathological tissue with iron deposition or increased macrophage activity.

T_1 is generally longer in grey matter than white matter. Shorter T_1 times have been attributed to the presence of myelin in healthy white matter which, in the adult human brain, accounts for approximately one-quarter the wet mass and one-half the dry mass in myelinated white matter [126]. At the lipid-water interfaces of myelin, there is a rapid exchange of magnetization between the lipids in myelin and the surrounding free water [126].

In damaged or diseased white matter, there are many factors which may increase T_1 relaxation constant, including demyelination, inflammation, gliosis, edema, and axonal and

neuronal loss [173]. There are also factors which may shorten T_1 , such as iron deposition or macrophage activity [184, 23]. Thus, while lipids in myelin may play a large role in shortening T_1 times in healthy tissue, T_1 in damaged tissue has less specificity.

Guilfoyle *et al.* conducted *in vivo* T_1 measurements at 7 T, and found $T_1 = (1503 \pm 56)$ ms in the corpus callosum (white matter), $T_1 = (1632 \pm 52)$ ms in the retrosplenial granular cortex (grey matter), and $T_1 = (1767 \pm 70)$ ms in the hippocampus (grey matter) of 6-week old healthy mouse brains [85].

The existence of different tissue compartments suggests that we should be able to measure multiple components of T_1 . However, the slow longitudinal relaxation rate relative to the rate of diffusion and magnetization exchange between compartments makes detecting multicomponent T_1 difficult [132, 142, 230].

3.1.3 Measuring T_2

We can take the spin echo pulse sequence (Figure 2.15) and apply additional π -pulses at $t = m\tau$ for $m = 3, 5, 7, \dots$. The resultant series of spin echo images at $t = n\tau$ for $n = 2, 4, 6, \dots$ will be weighted by the T_2 time constant. The multiple spin echo signal (Figure 3.4) decays according to the spin echo signal equation (Equation (2.86)). For constant T_R , this becomes

$$S(n\tau) = S_0 e^{-n\tau/T_2} + C \text{ for } n = 2, 4, 6, 8, \dots \quad (3.4)$$

where C represents a baseline value due to the Rician noise threshold at low signal.

In 1954, Carr and Purcell extended Hahn's spin echo pulse sequence to acquire multiple spin echoes [39], however their measurements had low reproducibility. For an imperfect π -pulse, some of the magnetization remains along the longitudinal axis. After a subsequent π -pulse, a stimulated echo will form due to this stored magnetization. In the original pulse sequence, the $\pi/2_x$ - and π_x -pulse were both applied along the same axis. This introduces a cumulative error due to stimulated echoes that rotates the magnetic moment vectors out of the transverse plane [27]. As a solution, Meiboom and Gill proposed a phase-shifted π_y -pulse [163], thus recovering the magnetization with every second imperfect π_y -pulse.

See [Figure 3.4](#) for what is now known as the Carr-Purcell Meiboom-Gill (CPMG) pulse sequence.

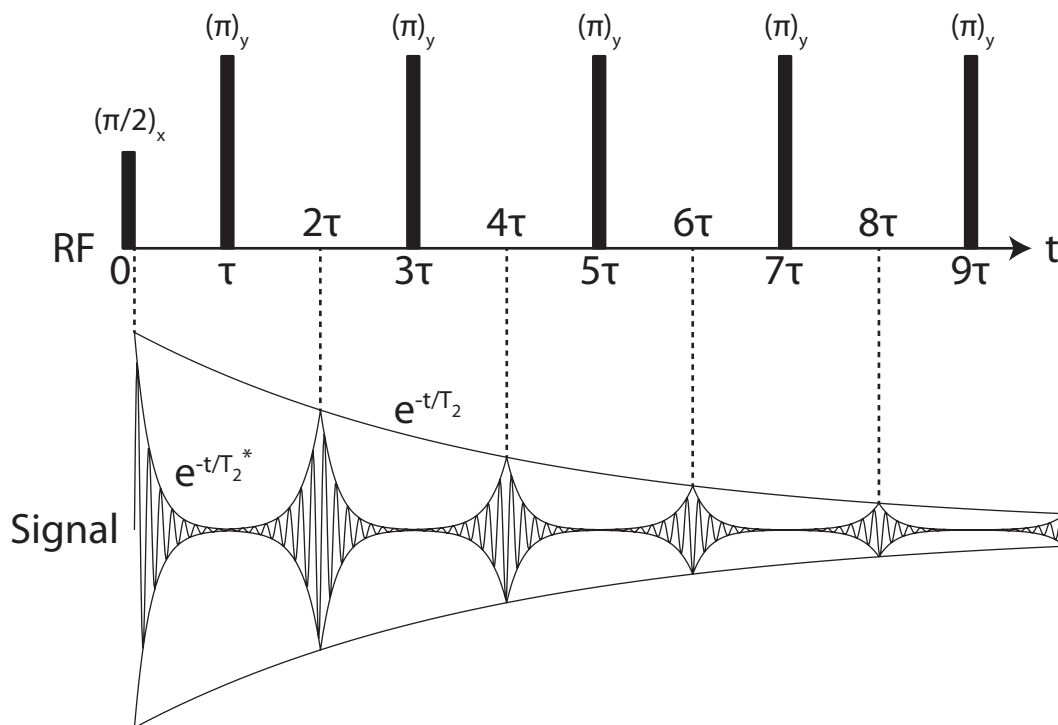


Figure 3.4: Multiple spin echo signal.

We can calculate the single component T_2 decay curve ([Figure 3.5](#)) by fitting the signals from a CPMG pulse sequence to [Equation \(3.4\)](#).

Additional Considerations for Measuring T_2

Besides phase-shifting the π -pulse, stimulated echoes can be reduced by using composite refocusing pulses, replacing the π_y -pulse with a series of pulses, such as a $\pi/2_x - \pi_y - \pi/2_x$ chain of block pulses [\[70\]](#).

Slice-selective shaped pulses and non-selective hard pulses will excite protons outside of the slice. To suppress signal from outside the slice and reduce stimulated echos, alternating sign and decreasing amplitude spoiler gradients that bracket each successive π -pulse can be used [\[193\]](#).

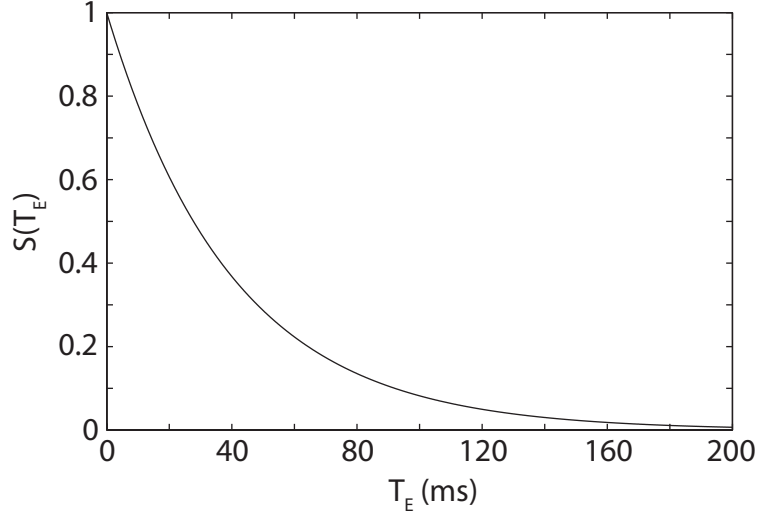


Figure 3.5: Single component T_2 decay curve for $T_2 = 40$ ms and T_E values from 0 to 200 ms.

Magnetization transfer effects can also reduce the detected signal. RF pulses that are on-resonance for the slice being imaged will be off-resonance in adjacent slices. These off-resonance RF pulses can saturate the magnetization in macromolecular protons. This saturated magnetization is then transferred to the on-resonance liquid pool, decreasing the available magnetization for imaging [258]. The easiest way to reduce signal loss due to magnetization transfer is to limit the acquisition to a single-slice. As an aside, we can model the behaviour of magnetization transfer to understand better the influence of macromolecular protons (see [Section 3.3](#)).

3.1.4 Multicomponent T_2 Analysis

A single component T_2 decay curve is often an over-simplification in tissue. In reality, the signal measured in a voxel of tissue might consist of several compartments with different concentrations and T_2 components. For example, in a voxel containing myelin water, intracellular water, and extracellular water, we could represent the measured signal as

$$S(T_E) = S_m e^{-T_E/T_{2,m}} + S_{ie} e^{-T_E/T_{2,ie}} \quad (3.5)$$

Where $T_{2,m}$ and $T_{2,ie}$ are the T_2 components of the myelin water and intra-/extra-cellular water (typically similar in value) and S_m and S_{ie} are there respective signal amplitudes. The detected signal decay curve for $T_{2,m} = 15$ ms and $T_{2,ie} = 50$ ms is shown in [Figure 3.6](#).

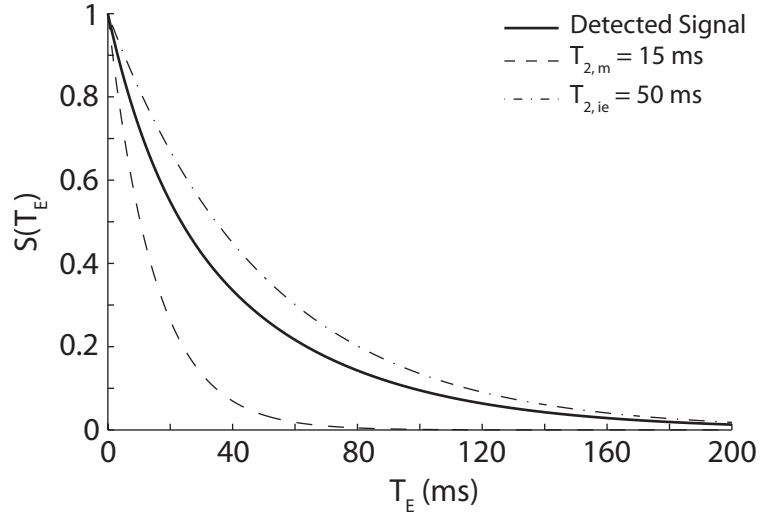


Figure 3.6: Multicomponent T_2 decay curve for $T_{2,m} = 15$ ms and $T_{2,ie} = 50$ ms. Relative signal contributing to total detected signal was $S_m = 0.3$ and $S_{ie} = 0.7$.

In order to extract these compartments, multicomponent analysis of the T_2 decay curve is applied. We can describe the T_2 -decay curve using M T_2 time coefficients (T_{2j}) with corresponding relative amplitudes (s_j). Given N spin echo measurements at echo times, T_{Ei} , the decay curve, y_i can be expressed as:

$$y_i = \sum_{j=1}^M s_j e^{-T_{Ei}/T_{2j}}. \quad (3.6)$$

If we define the components of an $M \times N$ matrix, A to be

$$a_{ij} = e^{-T_{Ei}/T_{2j}} \quad (3.7)$$

then [Equation \(3.6\)](#) can be expressed as $\mathbf{y} = \mathbf{A}\mathbf{s}$, or in matrix notation,

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ \vdots \\ y_M \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1N} \\ a_{21} & a_{22} & \cdots & a_{2N} \\ a_{31} & a_{32} & \cdots & a_{3N} \\ \vdots & \vdots & \vdots & \vdots \\ a_{M1} & a_{M2} & \cdots & a_{MN} \end{pmatrix} \begin{pmatrix} s_1 \\ s_2 \\ s_3 \\ \vdots \\ s_N \end{pmatrix} \quad (3.8)$$

In order to account for the Rician noise threshold, a baseline signal value, C , needs to be incorporated as well [113].

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ \vdots \\ y_M \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} & \cdots & a_{1N} & 1 \\ a_{21} & a_{22} & \cdots & a_{2N} & 1 \\ a_{31} & a_{32} & \cdots & a_{3N} & 1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ a_{M1} & a_{M2} & \cdots & a_{MN} & 1 \end{pmatrix} \begin{pmatrix} s_1 \\ s_2 \\ \vdots \\ s_N \\ C \end{pmatrix} \quad (3.9)$$

Non-Negative Least Squares Solution

A non-negative least squares algorithm (NNLS) can be used to solve for the s_j values in (3.9) [265]. This creates a fixed number of discrete T_2 amplitudes corresponding to a minimization of the χ^2_{min} -value of the estimated decay curve, $A\mathbf{s}$ and detected signal, $\mathbf{y}$

$$\chi^2_{min} = \sum_{i=1}^N \frac{([A\mathbf{s}]_i - y_i)^2}{\sigma_i^2} \quad (3.10)$$

where σ_i is the standard deviation of the noise for each component of the detected signal.

If the differences between $[A\mathbf{s}]_i$ and y_i are solely due to noise, then $([A\mathbf{s}]_i - y_i)^2 = \sigma_i^2$ and

$$\chi^2_{min} = \sum_{i=1}^N \frac{\sigma_i^2}{\sigma_i^2} = N \quad (3.11)$$

The non-regularized NNLS method is sufficient for the analysis of a region of interest, where T_2 components are first fitted to individual voxels and then analyzed as a distribution of voxel values [29].

Regularized Nonnegative Least Squares Solution

When calculating the decay curve based on the mean signal from a region of interest, it is desirable to create a continuous T_2 distribution as opposed to a few discrete T_2 components. To do this, the NNLS algorithm is regularized to create a smooth distribution. We can define the regularized signal as

$$\mathbf{y}_{reg} = A\mathbf{s} + \mu H\mathbf{s} \quad (3.12)$$

where μ is the regularization parameter that determines the broadening of the T_2 distribution and H is an $M \times N$ identity matrix [113],

$$H = \begin{pmatrix} 1 & 0 & 0 & \cdots & 0 \\ 0 & 1 & 0 & \cdots & 0 \\ 0 & 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & 1 \end{pmatrix} \quad (3.13)$$

The χ^2 -value for the regularized NNLS solution becomes,

$$\chi^2 = \sum_{i=1}^N \frac{([A\mathbf{s} + \mu H\mathbf{s}]_i - y_i)^2}{\sigma_i^2}. \quad (3.14)$$

To smooth the T_2 distribution, we can find a value for $\mu > 0$ according to a misfit of the χ^2 -value relative to the non-regularized χ_{min}^2 -value. A value for μ is typically found for $1.02\chi_{min}^2 \leq \chi^2 \leq 1.025\chi_{min}^2$. We can solve for μ by minimizing the error function,

$$\Xi = \left(\chi^2 - 1.025\chi_{min}^2 \right)^2 \quad (3.15)$$

The results of a non-regularized NNLS and regularized NNLS solution are shown in [Figure 3.7](#).

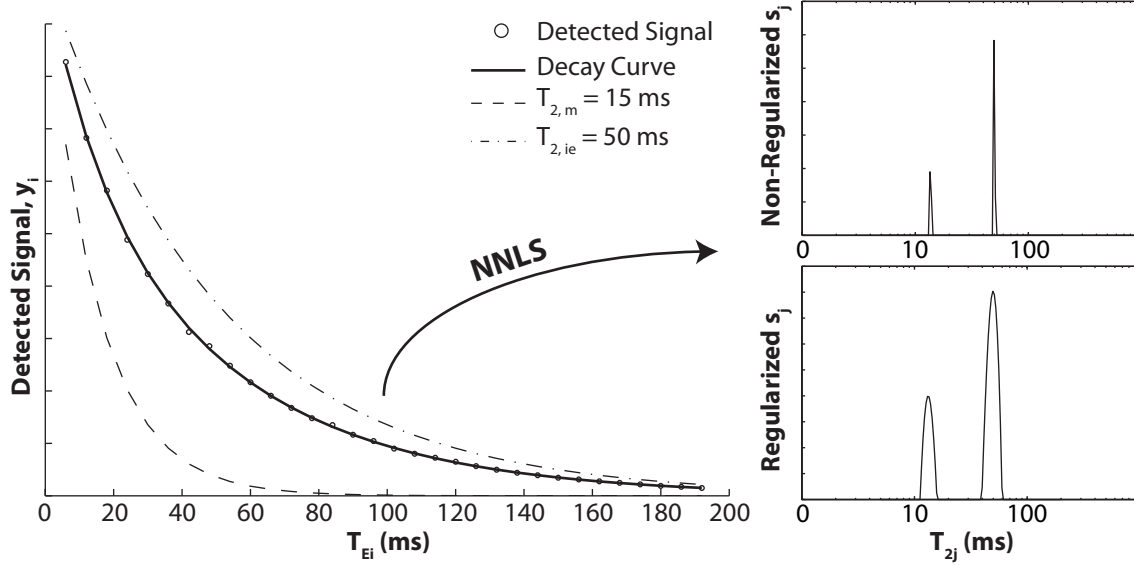


Figure 3.7: Multicomponent T_2 decay curve for $T_{2,m} = 15$ ms and $T_{2,ie} = 50$ ms at echo times from 6 to 192 ms and a 6 ms echo time spacing. Relative signal contributing to total detected signal was $S_m = 0.3$ and $S_{ie} = 0.7$. Random Rician noise was added to the signal, y_i for a signal-to-noise ratio of 100. Non-regularized and regularized NNLS solutions are shown to the right. T_{2j} has $M = 120$ bins logarithmically spaced from 5 to 200 ms. Regularized NNLS was performed with $\chi^2 = 1.025\chi_{min}^2$.

Noise, Echo Spacing, and Reproducibility

Noise and echo spacing can significantly affect the accuracy of multicomponent T_2 (mcT_2) distributions [82, 115, 60, 29]. Based on a typical T_2 acquisition with 32 echoes and $T_E = 10$ ms, Graham *et al.* determined a minimum SNR of 700 is required to identify the short- T_2 component associated with myelin water [82]. The influence of noise can be reduced by the application of spatial filters, such as the anisotropic diffusion filter [113, 115] or by calculating metrics using a histogram of non-regularized NNLS T_2 distributions calculated from individual voxels [29]. Optimal echo time spacing can also improve the SNR and determination of the short- T_2 component, with the optimal T_E decreasing as the short- T_2 component decreases [60].

The reproducibility of mcT_2 measurements is an important consideration. Vavasour *et al.* imaged 5 healthy controls 5 times over the course of a year [259]. They found significant variability between subjects. This variability was attributed to biology and not signal noise, suggesting that mcT_2 studies might require large sample sizes and significant differences

in biology to differentiate groups. In a similar study, Levesque *et al.* found moderate reproducibility in healthy subjects, suggesting a minimum of 32 patients for longitudinal studies [146].

Multicomponent T_2 Metrics

Using a histogram of non-regularized NNLS T_2 distributions or a single, regularized NNLS T_2 distribution, we can estimate the number of distinct compartments in the tissue based on the number of peaks and calculate the water fractions and geometric means of the associated T_2 values. In healthy white matter, decomposition of the decay curve identifies two components with T_2 values of 10–20 ms and 50–100 ms, respectively [266]. In our example, there are two peaks: one at $T_2 = 15$ ms, associated with water in the myelin bilayers, and one at $T_2 = 50$ ms, associated with water in the intracellular and extracellular water environments. Based on the width of the distributions, we might associate the myelin water distribution with all T_{2j} -values ranging from $T_{2jmin} = 5$ ms to $T_{2jmax} = 30$ ms. Likewise, we might associate the intra-/extracellular water distribution with all T_{2j} -vales range from $T_{2jmin} = 30$ ms to $T_{2jmax} = 100$ ms. We can then define the water fraction as,

$$WF = \frac{\sum_{j=jmin}^{jmax} s_j}{\sum_{j=1}^M s_j} \quad (3.16)$$

and the geometric mean of the T_{2j} values as [27],

$$gmT_2 = \exp \left[\frac{\sum_{j=jmin}^{jmax} s_j \log T_{2j}}{\sum_{j=jmin}^{jmax} s_j} \right] \quad (3.17)$$

The water fraction of the short- T_2 distribution is often called the myelin water fraction (MWF). We can call the water fraction of the intermediate- T_2 distribution the intra-/extracellular water fraction (IEWF). The geometric means of the short- and intermediate- T_2 distributions will be called T_{2m} and T_{2ie} respectively.

3.1.5 T_2 in Tissue

The short- T_2 distribution has been linked to myelin water in a number of studies [166, 135, 138, 157, 266, 156, 128]. Three studies from the same group compared post mortem samples of MS brains. Samples were stained with luxol fast blue (LFB), which is sensitive to myelin, and subsequently imaged with an mc T_2 sequence [166]. LFB-stained images registered to MWF maps demonstrated strong correlation between optical density of the LFB stain (related to myelin content) and the MWF at 1.5 T ($R^2 = 0.67$) (0.45 – 0.92) [135] and 7 T ($R^2 = 0.78$ (0.56 – 0.95)) [138]. In both studies, correlations included regions of grey matter, normal appearing white matter (NAWM), and lesions.

In vivo human studies have had similar findings. The MWF in healthy human brains is larger in white matter than grey matter, with the difference attributed primarily to myelin content [157, 266, 156]. Likewise, white matter in healthy human spinal cords has larger MWF values than white matter in the brain (brain MWF = 11.3% [266, 178]) that varies based on the level (cervical MWF = $(21.8 \pm 2.1)\%$, thoracic MWF = $(24.3 \pm 3.6)\%$, and lumbar MWF = $(11.4 \pm 6.4)\%$) [165]. In demyelinating diseases such as MS, lesions form due to inflammation, demyelination, and axonal damage. Reduced MWF has been observed in both lesions and normal appearing white matter of MS patients [134, 178, 127, 250]. Also, the presence of a long- T_2 component ($200 \text{ ms} < T_{2l} < 800 \text{ ms}$) may suggest vacuolation or increased extracellular water in MS lesions and NAWM [137].

In a study of normal and injured rat sciatic nerves, Webb *et al.* found a similar correlation between histological measurements of myelin content (light microscopy of 1- μm thick cross-sections stained with toluidine blue) and the MWF [263]. While the short- T_2 component was found to be a good estimate of total myelin content, it was unable to distinguish intact myelin from myelin debris. Short- T_2 components have been found in rat spinal cords (MWF = $(32 \pm 3)\%$) [129], trigeminal nerves (MWF = $(19 \pm 3)\%$) [55], sciatic nerves (MWF = $(28 \pm 4)\%$) [263, 229, 177], and glioblastoma (glioblastoma MWF = $(6.8 \pm 6.2)\%$ compared to grey matter MWF ≈ 0) [57]. Short- T_2 was also observed in mouse spinal cords (MWF = $(31 \pm 1)\%$) [162], the corpus callosum of guinea pigs (MWF = $(28 \pm 6)\%$) [72], bovine optic nerves (MWF = $(32 \pm 3)\%$) [228], and the sciatic nerve of African clawed toads

(MWF = $(27 \pm 7)\%$) [56].

We can understand the T_2 value found from a monoexponential fit as a combination of the different T_2 components relative to the size of their respective water fractions. In the case of healthy white matter, MWF is relatively large, and the mean T_2 value will be relatively small compared to the surrounding grey matter. In a T_2 -weighted image, this leads to darker regions of voxels corresponding to white matter tracts. In the case of demyelination (decreased MWF) or increased extracellular water (increased IEWF), the mean T_2 value will increase. In a T_2 -weighted image, this leads to bright region of voxels corresponding to an MS lesion.

Ideally, multicomponent T_2 will reveal the underlying tissue compartments. However, magnetization exchange due to diffusional motion might limit its specificity, particularly in short- T_2 compartments that are relatively small and have relatively fast exchange. In a compartmental study of T_1 and T_2 in rat brain and trigeminal nerve, Does and Gore found three distinct $T_1 - T_2$ components in the trigeminal nerve but only mono-exponential $T_1 - T_2$ components in the corpus callosum where, given the myelin content, a short- T_2 component would be expected [55]. This can be attributed to relatively fast exchange between intra- and extracellular water. In a study of different white matter regions in rat spinal cord, Dula *et al.* also found differing myelin water fractions despite similar myelin content in histology [61]. These discrepancies were attributed to smaller myelin water compartments and larger intercompartmental water exchange, both proportional to average fiber thickness. As intercompartmental water exchange increases, T_2 components shift to shorter T_2 values and measured myelin water fractions decrease. Thus, white matter with smaller axon diameters and thin myelin sheathes may underestimate the myelin content and T_2 times [61]. In a follow-up study, numerical simulations and *in vivo* measurements of the rat spinal cord demonstrated that regional differences in exchange, mediated by differences in axon radii and myelin sheath thicknesses, sufficiently accounted for variations in measured MWF [93].

The influence of intercompartmental exchange on T_2 is controversial. While the two studies mentioned above determined intercompartmental exchange had a large effect in rat spinal cord [61, 93], Kalantari *et al.* determined intercompartmental exchange only has a

a small effect in human brain white matter ($< 15\%$) [120]. Despite similarities in MWF measurements, lower myelin content in the human corpus callosum relative to the rat spinal cord might lead to different exchange properties and help explain their different conclusions [93, 120]. Nevertheless, it is reasonable to hypothesize that myelin water measurements in the mouse corpus callosum, with axon diameters and myelin thicknesses much smaller than those found in the rat spinal cord, will be even more strongly influenced by intercompartmental exchange. This may also explain mono-exponential $T_1 - T_2$ components previously observed in the rat corpus callosum [55].

3.2 Diffusion Tensor Imaging

Although hinted at in the discussion of T_2 , the influence of molecular self-diffusion has yet to be addressed explicitly. We will now cover how diffusion can be measured directly with MRI and how it can be interpreted in relation to the structure of different tissues.

3.2.1 Molecular Self-Diffusion

Molecules in a liquid undergo random motion at thermal equilibrium, called Brownian motion. Brownian motion is responsible for molecular diffusion. Unrestricted diffusion of a molecule can be described by its mean-squared displacement [62], which in one-dimension is:

$$\langle x(t_1)x(t_2) \rangle = 2D(t_2 - t_1) = 2D\Delta \quad (3.18)$$

where the proportionality constant, D , is called the diffusion coefficient and has dimensions of distance squared per unit time (e.g., mm^2/s or $\mu m^2/ms$). t_1 is the initial time, t_2 is the final time, and $\Delta = t_2 - t_1$ is the diffusion time over which diffusion is measured. In three-dimensions, the position vector is $\mathbf{r} = [x \ y \ z]$ and the mean-squared displacement becomes:

$$\langle \mathbf{r}(t_1) \mathbf{r}(t_2) \rangle = 2\Delta \begin{pmatrix} \langle x_1 x_2 \rangle & \langle x_1 y_2 \rangle & \langle x_1 z_2 \rangle \\ \langle y_1 x_2 \rangle & \langle y_1 y_2 \rangle & \langle y_1 z_2 \rangle \\ \langle z_1 x_2 \rangle & \langle z_1 y_2 \rangle & \langle z_1 z_2 \rangle \end{pmatrix} = 2\Delta \underbrace{\begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix}}_{\mathbf{D}} \quad (3.19)$$

$\mathbf{D}$ is called the diffusion tensor. If diffusion is unrestricted (as in bulk water), all of the non-diagonal terms in $\mathbf{D}$ go to zero, and $D_{xx} = D_{yy} = D_{zz}$. This, however, is not the case for restricted diffusion. In biological tissues, diffusion of water molecules is affected by the presence of macromolecules, organelles, cell membrane, and other cellular and subcellular structures [24]. These obstacles can severely restrict the molecular displacement. By measuring the diffusion coefficient in multiple directions, we can construct a diffusion tensor, $\mathbf{D}$ and deduce the underlying cellular structure of the tissue.

In the case of axonal bundles, water moves more freely parallel to the axonal bundles. Thus, in a voxel of axons pointing in primarily the same direction, we expect the diffusion coefficient, D , to be largest in that direction. This is known as anisotropic diffusion. In a voxel that contains non-directional cells, water is equally likely to diffuse in any direction, thus resulting in isotropic diffusion. See Figure 3.8.

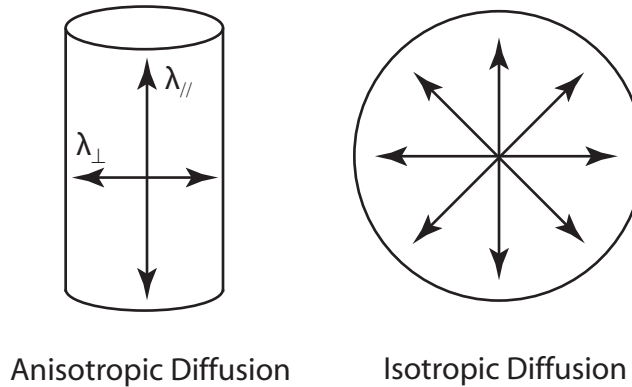


Figure 3.8: Anisotropic diffusion in axonal bundles is characterized by larger axial diffusion ($\lambda_{\parallel}$) than radial diffusion ($\lambda_{\perp}$). Isotropic diffusion is equivalent in all directions.

3.2.2 Bloch-Torrey Equation

Previously, the Bloch equation was solved assuming no diffusive motion [Equation \(2.21\)](#). In 1956, Torrey introduced a diffusivity coefficient, D to account for the self-diffusion of molecules at thermal equilibrium [\[247\]](#):

$$\frac{\partial}{\partial t} \mathbf{M}(\mathbf{r}, t) = \gamma \mathbf{M} \times \mathbf{B} - \frac{M_x \hat{\mathbf{x}} + M_y \hat{\mathbf{y}}}{T_2} - \frac{(M_z - M_0) \hat{\mathbf{z}}}{T_1} + D \nabla^2 \mathbf{M}. \quad (3.20)$$

If we neglect relaxation and consider just the transverse magnetization, this reduces to [\[247\]](#)

$$\frac{d}{dt} \mathbf{M}(\mathbf{r}, t) = \gamma \mathbf{M} \times \mathbf{B} + D \nabla^2 \mathbf{M}. \quad (3.21)$$

We can define the time-dependent magnetic field along the z-direction as $\mathbf{B} = \mathbf{r} \cdot \mathbf{G}(t) \hat{\mathbf{z}}$. After a $\pi/2$ -pulse, the equation for the transverse magnetization becomes [\[232\]](#),

$$\frac{\partial}{\partial t} M_{xy}(\mathbf{r}, t) = -i\gamma (\mathbf{r} \cdot \mathbf{G}) M_{xy} + D \nabla^2 M_{xy}. \quad (3.22)$$

For $D = 0$ (no self-diffusion), this has the solution

$$M_{xy}(\mathbf{r}, t) = A e^{-i\gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt'}. \quad (3.23)$$

If we assume that diffusion is time-dependent but spatially invariant, $A \rightarrow A(t)$ where $A(t)$ represents the effect of diffusion on the transverse magnetization. Substituting [Equation \(3.23\)](#) into [Equation \(3.22\)](#) yields a differential equation for $A(t)$

$$\frac{\partial}{\partial t} A(t) = e^{i\gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt'} \left(A(t) e^{-i\gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt'} \right) \quad (3.24)$$

Applying the natural logarithm to both sides and solving for $\ln A(t)$ gives us an equation representing signal loss due to diffusion [\[87\]](#)

$$\ln \left(\frac{A(t)}{A(0)} \right) = -\gamma^2 \left[\int_0^t \left(\int_0^{t'} \mathbf{G}(t'') dt'' \right) \cdot \left(\int_0^{t'} \mathbf{G}(t'') dt'' \right) dt' \right] D \quad (3.25)$$

If we define the k-space vector, $\mathbf{k}(t')$ to be

$$\mathbf{k}(t') = \gamma \int_0^{t'} \mathbf{G}(t'') dt'' \quad (3.26)$$

Equation (3.25) becomes

$$\ln \left(\frac{A(t)}{A(0)} \right) = - \left(\int_0^t \mathbf{k}(t') \cdot \mathbf{k}(t') dt' \right) D. \quad (3.27)$$

This is further simplified by defining the diffusion-weighting factor, b

$$b(t) = \int_0^t \mathbf{k}(t') \cdot \mathbf{k}(t') dt'. \quad (3.28)$$

If we now equate the time-dependent diffusion-weighted signal, $S(t)$ to $A(t)$ and the signal without diffusion-weighting, S_0 to $A(0)$, we are left with the familiar expression for a diffusion-weighted signal,

$$S(t) = S_0 e^{-b(t)D} \quad (3.29)$$

Based on this equation, we can see that the application of time-dependent gradient magnetic fields (encoded in b) will influence the signal proportional to the diffusivity constant, D .

3.2.3 Pulsed Gradient Spin Echo (PGSE) Signal

Measuring diffusion with a pulsed gradient spin-echo (PGSE) was first proposed by Stejskal and Tanner in 1965 [232]. The PGSE (or Stejskal-Tenner) sequence applies two motion-sensitizing gradient pulses before and after the π -pulse in a spin echo sequence. S_0 in Equation (3.29) is proportional to the spatially encoded spin echo signal (Equation (2.86)) and the diffusion-weighted signal becomes:

$$S(T_E) = M_z^0 \left(1 - e^{-T_R/T_1} \right) e^{-T_E/T_2} e^{-b(T_E)D} \quad (3.30)$$

Varying the diffusion-weighting factor, b while keeping T_R and T_E constant results in a series of diffusion-weighted images that vary solely due to b and D . In this case, we can treat everything preceding e^{-bD} as a constant, so that the signal equation is, once

again, Equation (3.29). Signal attenuation is a function of the diffusion-weighting factor, b . Thus, as b is varied, the observed signal changes and the diffusion coefficient, D , can be determined.

3.2.4 PGSE Sequence with Rectangular Gradients

Figure 3.9 illustrates a PGSE pulse sequence with rectangular gradient pulses equally spaced on either side of the π -pulse. The diffusion-weighting gradients are described by their gradient amplitudes (g), gradient pulse durations (δ), and the time interval between gradient pulses (Δ). The first gradient pulse creates a spatially dependent phase shift along the z -direction. The second gradient pulse induces a spatially dependent phase shift on the reversed magnetization M , refocusing the phases. For static molecules, the second gradient will refocus the phases perfectly and there will be no change in the spin echo signal at $t = T_E$. For molecules undergoing Brownian motion, some of the particles will have moved between pulses. Refocusing will be imperfect and the introduced phase difference will cause a loss in signal.

The net phase difference caused by the pulse sequence, $\Delta\Phi$ is given by:

$$\Delta\Phi = \gamma g \delta (\epsilon_2 - \epsilon_1) \quad (3.31)$$

where ϵ_1 and ϵ_2 represent random displacements due to diffusion between the first and second gradients [244]. For individual static spins, $\epsilon_1 = \epsilon_2$ and $\Delta\Phi = 0$. For individual diffusing spins, $\epsilon_1 \neq \epsilon_2$ and $\Delta\Phi \neq 0$. In an ensemble of diffusing spins, the phase accumulation is zero but the signal is attenuated due to the random phase shifts of the individual spins. In an ensemble of spins experiencing just coherent motion (e.g. flow), there is a phase shift but no signal attenuation.

The rectangular gradient pulses in Figure 3.9 can be described by $G(t)$,

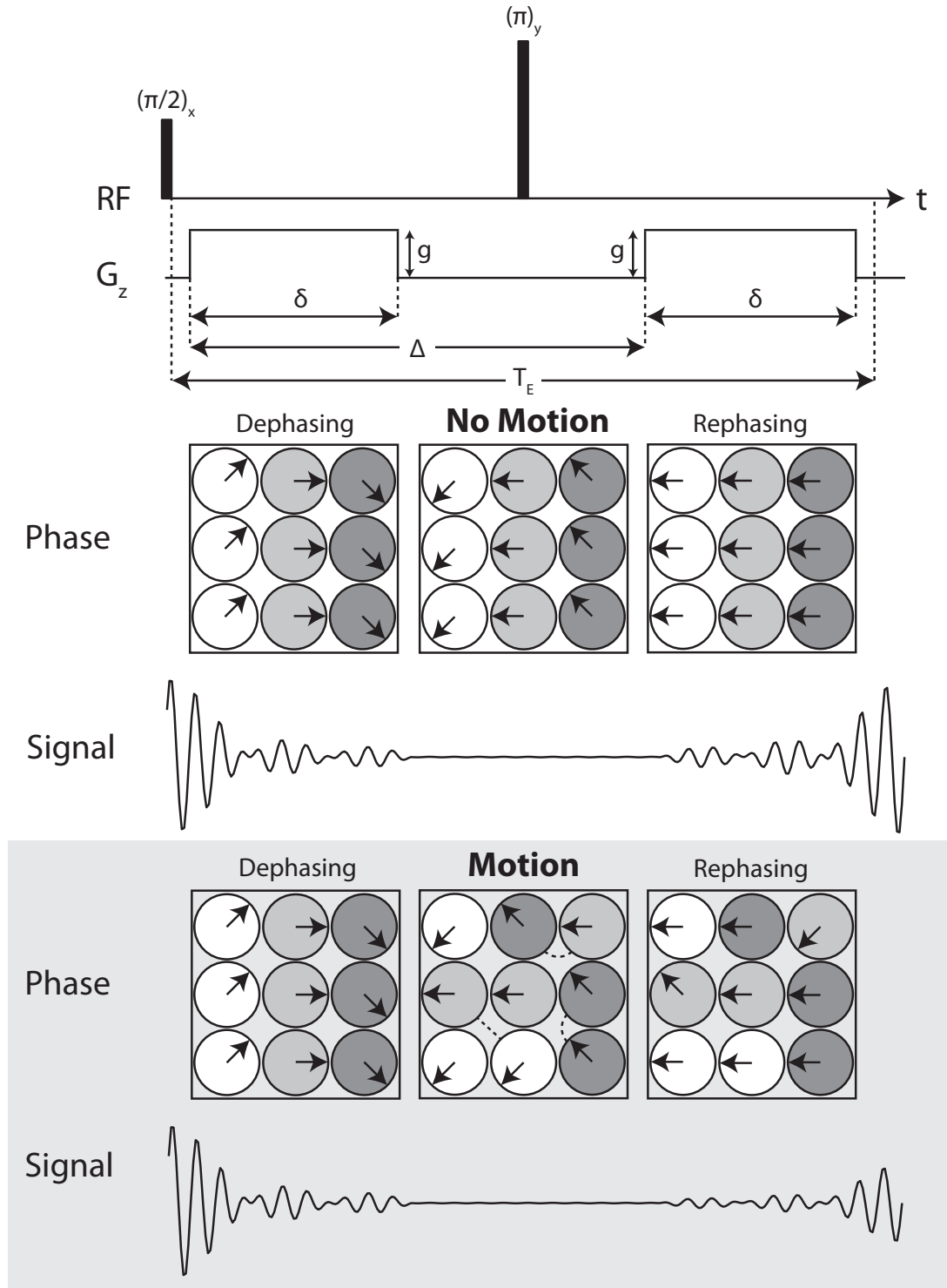


Figure 3.9: Pulsed gradient spin echo pulse sequence. Phase for a simple 3x3 system of magnetization vectors is represented by the arrows. The second gradient rephases the magnetization vectors, yielding a diffusion-weighted spin echo signal. Signal attenuation is proportional to the amount of diffusive motion that occurs between the two gradients.

$$\mathbf{G}(t) = \begin{cases} -g\hat{\mathbf{z}} & \text{for } 0 < t < \delta \\ 0 & \text{for } \delta < t < \Delta \\ +g\hat{\mathbf{z}} & \text{for } \Delta < t < \delta + \Delta \\ 0 & \text{otherwise} \end{cases}. \quad (3.32)$$

The corresponding k-space vector, $\mathbf{k}(t)$, becomes

$$\mathbf{k}(t) = \begin{cases} -gt\hat{\mathbf{z}} & \text{for } 0 < t < \delta \\ -g\delta\hat{\mathbf{z}} & \text{for } \delta < t < \Delta \\ -g(\delta + \Delta - t)\hat{\mathbf{z}} & \text{for } \Delta < t < \delta + \Delta \\ 0 & \text{otherwise} \end{cases} \quad (3.33)$$

yielding a diffusion-weighting factor, $b(T_E)$

$$b = \int_0^{T_E} \mathbf{k}(t) \cdot \mathbf{k}(t) dt = \gamma^2 \int_0^{\Delta+\delta} k^2(t) dt = \gamma^2 g^2 \left(\int_0^\delta t^2 dt + \int_\delta^\Delta \delta^2 dt + \int_\Delta^{\Delta+\delta} (\delta + \Delta - t)^2 dt \right). \quad (3.34)$$

After simplification, $b(T_E)$ becomes

$$b = \gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right). \quad (3.35)$$

Alternatively, trapezoidal gradient pulses with a ramp rise time, ϵ will give a b-value of [15]

$$b = \gamma^2 g^2 \left[\delta^2 \left(\Delta - \frac{\delta}{3} \right) + \frac{\epsilon^3}{30} - \frac{\delta\epsilon^2}{6} \right]. \quad (3.36)$$

Imaging gradients on either side of the π -pulse will also contribute to the effective b-value. For example, the slice-selective gradient applied during the π -pulse in [Figure 2.15](#) will increase the effective diffusion weighting by $g_{\text{slice}}\hat{\mathbf{z}}$. Using [Equation \(2.68\)](#), a slice $\Delta z = 1\text{mm}$ thick can be refocused using a $\Delta f = 3420\text{Hz}$ Hermite pulse, requiring a gradient strength of $g_{\text{slice}} = 0.0803T/m$. Applied for $500\ \mu\text{s}$ on either side of the π -pulse (with δ and Δ both equal to $500\ \mu\text{s}$), g_{slice} will increase the b-value by $0.038\ \text{s/mm}^2$. In reality, g_{slice} will rarely be exactly along the z-direction and will vary depending on the precise

slice desired in a subject. The acquisition software calculates effective b-values based on a b-value defined by the user plus any imaging gradients applied for the desired slice.

3.2.5 Calculating the Apparent Diffusion Coefficient

To determine D in the z-direction, we can obtain multiple images using the PGSE pulse sequence while varying the gradient pulse strengths in the z-direction. From equations (3.29) and (3.28), it follows that

$$\ln(S_i) = -b_i D + \ln(S_0) \quad (3.37)$$

Plotting $\ln(S_i)$ versus b_i yields a straight line with y-intercept $\ln(S_0)$ and slope $-D$ (Figure 3.10). S_0 is the expected signal with no gradients applied. D represents the apparent diffusivity of the sample in the same direction as $\mathbf{G}$.

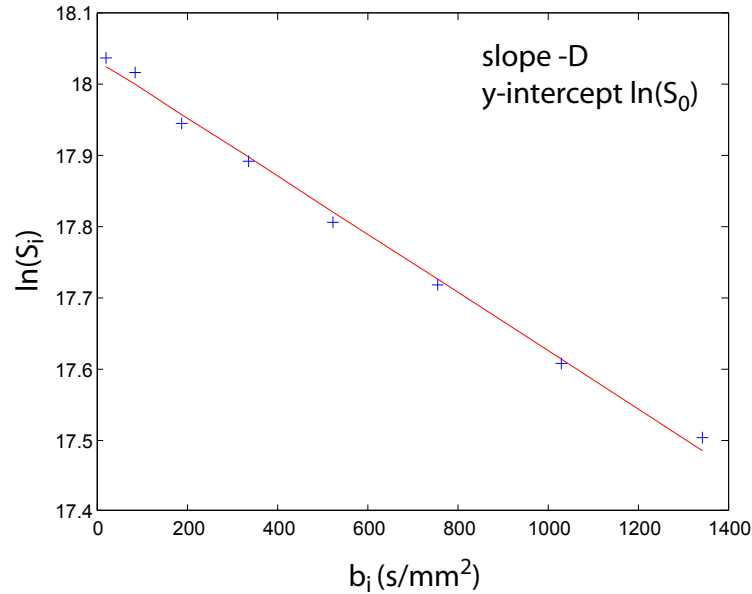


Figure 3.10: Plot of $\ln(S_i)$ vs. b_i for 8 different gradient strengths.

If we are only interested in the apparent diffusivity along a specific direction, we can solve for D by acquiring one image without any diffusion-weighting gradients ($b = 0 \Rightarrow \ln(S_1) = \ln(S_0)$) and one image with diffusion-weighting gradients applied along the direction of interest ($b > 0 \Rightarrow \ln(S_2) = \ln(S_0) - bD$), assuming the decay curve is monoexponential. In

this simple scenario, D is then

$$D = \frac{[\ln(S_1) - \ln(S_2)]}{b} \quad (3.38)$$

We can extend this to calculate the mean apparent diffusivity in a voxel by acquiring one image at $b = 0$ (S_0) and three images at $b > 0$ with gradients applied in three orthogonal directions (e.g. x , y , and z). If we calculate D in this manner along three orthogonal directions (D_x , D_y , and D_z), we can calculate the mean apparent diffusivity in a voxel:

$$\langle D \rangle = (D_x + D_y + D_z) / 3 \quad (3.39)$$

Mean diffusivity has been a particularly effective measure in the early diagnosis of stroke, where $\langle D \rangle$ is significantly lower in the region of a stroke [262]. However, it only describes the magnitude of the apparent diffusivity with no information about its directionality. In order to describe both the magnitude and directionality of diffusion, a tensor model is necessary [15].

Diffusion-weighted imaging with MRI was first demonstrated by Le Bihan *et al* in 1986 [140]. It is important to note that diffusivity in tissue is not truly Gaussian in nature, thus the diffusion coefficients are reported as “apparent”, where the Gaussian model is assumed. The unrestricted diffusion in bulk water is very different from diffusion taking place in tissue, which can be composed of multiple compartments with different diffusion coefficients, geometries, cell membrane permeabilities, T_2 relaxation rates, and extracellular tortuosities. Models that don’t assume Gaussian diffusion are briefly discussed in Section 3.2.9.

3.2.6 Determining the Effective Diffusion Tensor

The Diffusion Tensor and $\mathbf{b}$ -Matrix

In 1994, Basser introduced a technique for determining the effective diffusion tensor with MRI [15]. By obtaining diffusion-weighted images in 6 or more gradient directions, we can construct a 3-dimensional representation of diffusion in a voxel – the apparent diffusion

tensor,

$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix}. \quad (3.40)$$

The diffusion-encoding gradients can be described in a 3x3 matrix, $\mathbf{b}$, constructed from b (Equation (3.28)) and the normalized gradient directions $[g_x \ g_y \ g_z]$.

$$\mathbf{b} = b \begin{bmatrix} g_x \\ g_y \\ g_z \end{bmatrix} \begin{bmatrix} g_x & g_y & g_z \end{bmatrix} = b \begin{pmatrix} g_x^2 & g_x g_y & g_x g_z \\ g_y g_x & g_y^2 & g_y g_z \\ g_z g_x & g_z g_y & g_z^2 \end{pmatrix} = \begin{pmatrix} b_{xx} & b_{xy} & b_{xz} \\ b_{yx} & b_{yy} & b_{yz} \\ b_{zx} & b_{zy} & b_{zz} \end{pmatrix} \quad (3.41)$$

If we assume diffusion is the same either way along a gradient direction, $\mathbf{D}$ and $\mathbf{b}$ are symmetric and we can equate their off-diagonal terms:

$$\begin{aligned} D_{xy} &= D_{yx} & D_{xz} &= D_{zx} & D_{yz} &= D_{zy} \\ b_{xy} &= b_{yx} & b_{xz} &= b_{zx} & b_{yz} &= b_{zy} \end{aligned} \quad (3.42)$$

This reduces the diffusion tensor to 6 unknown parameters: D_{xx} , D_{yy} , D_{zz} , D_{xy} , D_{xz} , and D_{yz} . The diffusion-weighted signal equation (Equation (3.37)) becomes,

$$\ln(S_i) = -\mathbf{b}_i : \mathbf{D} + \ln(S_0) \quad (3.43)$$

where $\mathbf{b}_i : \mathbf{D}$ is the double dot product of $\mathbf{b}_i$ and $\mathbf{D}$,

$$\mathbf{b}_i : \mathbf{D} = b_{xxi}D_{xx} + b_{yyi}D_{yy} + b_{zzi}D_{zz} + 2b_{xyi}D_{xy} + 2b_{xzi}D_{xz} + 2b_{yzi}D_{yz} \quad (3.44)$$

Solving for the diffusion tensor and $\ln(S_0)$ requires at least 7 independent measurements: one measurement with $b = 0$ for $\ln(S_0)$ and at least 6 measurements with $b > 0$ along at least 6 independent gradient directions.

Linear Least-Squares (LS) Fit

The linear least-squares fit of Equation (3.43) can be written as a matrix equation for $N \geq 7$ diffusion-weighted experiments,

$$\underbrace{\begin{pmatrix} \ln(S_1) \\ \ln(S_2) \\ \ln(S_3) \\ \ln(S_4) \\ \ln(S_5) \\ \vdots \\ \ln(S_N) \end{pmatrix}}_{\mathbf{S}} = - \underbrace{\begin{pmatrix} b_{xx1} & b_{yy1} & b_{zz1} & 2b_{xy1} & 2b_{xz1} & 2b_{yz1} & 1 \\ b_{xx2} & b_{yy2} & b_{zz2} & 2b_{xy2} & 2b_{xz2} & 2b_{yz2} & 1 \\ b_{xx3} & b_{yy3} & b_{zz3} & 2b_{xy3} & 2b_{xz3} & 2b_{yz3} & 1 \\ b_{xx4} & b_{yy4} & b_{zz4} & 2b_{xy4} & 2b_{xz4} & 2b_{yz4} & 1 \\ b_{xx5} & b_{yy5} & b_{zz5} & 2b_{xy5} & 2b_{xz5} & 2b_{yz5} & 1 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ b_{xxN} & b_{yyN} & b_{zzN} & 2b_{xyN} & 2b_{xzN} & 2b_{yzN} & 1 \end{pmatrix}}_{\mathbf{B}} \underbrace{\begin{pmatrix} D_{xx} \\ D_{yy} \\ D_{zz} \\ D_{xy} \\ D_{xz} \\ D_{yz} \\ \ln(S_0) \end{pmatrix}}_{\mathbf{d}} \quad (3.45)$$

$\mathbf{S}$ is the vector containing the logarithms of the measured signal intensities. $\mathbf{d}$ is the vector containing the diffusion tensor components and $\ln(S_0)$. If $\mathbf{B}$ is symmetric, we can find it's inverse and solve for $\mathbf{d}$

$$\mathbf{d} = \mathbf{B}^{-1} \mathbf{S} \quad (3.46)$$

If $\mathbf{B}$ is not symmetric, we can solve for $\mathbf{d}$ by first calculating the Moore-Penrose pseudoinverse of $\mathbf{B}$, $\mathbf{B}^+$

$$\mathbf{B}^+ = [\mathbf{B}^T \mathbf{B}]^{-1} \mathbf{B}^T. \quad (3.47)$$

The solution for $\mathbf{d}$ is then

$$\mathbf{d} = \mathbf{B}^+ \mathbf{S} \quad (3.48)$$

Weighted Linear Least-Squares (WLS) Fit

In the presence of noise, the logarithm of the signal introduces an uncertainty proportional to $1/S_i$ [123]. In order to account for this uncertainty, a weighted linear-least squares fit

gives more weight to high S_i values. A diagonal $N \times N$ covariance matrix containing signal strength and relative noise can be introduced [15],

$$\mathbf{\Sigma}^{-1} = \begin{pmatrix} S_1^2/\sigma_1^2 & 0 & \cdots & 0 \\ 0 & S_2^2/\sigma_2^2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & S_N^2/\sigma_N^2 \end{pmatrix} \quad (3.49)$$

where σ_i is the standard deviation of a noise region in each image. The solution for $\mathbf{d}$ is then [15],

$$\mathbf{d} = \left(\mathbf{B}^T \mathbf{\Sigma}^{-1} \mathbf{B} \right)^{-1} \left(\mathbf{B}^T \mathbf{\Sigma}^{-1} \right) \mathbf{S} \quad (3.50)$$

Non-Linear Least-Squares (NLS) Fit

If we use the weighted linear least-squares solution for $\mathbf{d}$ as a starting point, we can further refine $\mathbf{d}$ by applying a non-linear least-squares fit. This accounts for the fact that symmetric noise in S_i is assymetric in $\ln(S_i)$. A solution for $\mathbf{d}$ is found by minimizing the sum of squares difference between the measured and estimated signals,

$$\min \sum_{i=1}^N \left(S_i - S_0 e^{-\mathbf{b}_i : \mathbf{D}} \right)^2 \quad (3.51)$$

Constrained Solutions for Positive Definiteness

In the presence of noise or high anisotropy, sometimes the generated diffusion tensor will yield negative eigenvalues or a fractional anisotropy greater than 1, both of which are physically impossible. To address this, diffusion tensors that are not positive definite can be modified using the Cholesky decomposition to ensure physical values [125].

The diffusion tensor is positive definite if it meets the following three criteria [124]:

1. $D_{xx} > 0$
2. $(D_{xx}D_{yy} - D_{xy}^2) > 0$

$$3. \det(\mathbf{D}) > 0$$

If $\mathbf{D}$ is not positive definite, it can be made so using the Cholesky parameterization. In order to create a positive definite matrix, the Cholesky parameterization creates an upper triangular matrix with non-zero diagonal elements [124],

$$\mathbf{R} = \begin{pmatrix} R_0 & R_3 & R_5 \\ 0 & R_1 & R_4 \\ 0 & 0 & R_2 \end{pmatrix}. \quad (3.52)$$

Using this parameterization, $\mathbf{D}_p = \mathbf{R}^T \mathbf{R}$ becomes a positive definite matrix. In order to make $\mathbf{D}$ positive definite, we can search for a matrix $\mathbf{E}$ using the modified Cholesky decomposition such that $\mathbf{D}_p = \mathbf{D} + \mathbf{E}$ [75]. In comparison with linear least squares fitting, Koay *et al.* demonstrated that non-linear least squares fitting resulted in fewer non-positive definite diffusion tensors [124]. In addition, constraining the diffusion tensors to be positive definite resulted in lower mean squared errors when estimating fractional anisotropy and mean diffusivity [124].

Gradient Directions and Noise

In order to improve the noise performance and accuracy of diffusion tensor images, more than 6 gradient directions can be used. There are several papers that analyze the influence of different gradient encoding schemes on accuracy [216, 97, 17, 116]. These schemes range from 6 directions to over 100 directions. One measure of an encoding scheme's relative performance is the condition number, $\kappa = \|\mathbf{B}\| \|\mathbf{B}^{-1}\|$, where $\|\mathbf{B}\|$ is the matrix norm of the $\mathbf{B}$ matrix. The condition number provides a bound for how much noise in the diffusion-weighted images can propagate to noise in the diffusion tensor [17]. That is,

$$\frac{\text{relative error in } \mathbf{D}}{\text{relative error in } \mathbf{S}} \leq \kappa \quad (3.53)$$

Generally, a small condition number implies less noise propagation. In one study, the condition number for the tetrahedral encoding scheme was 9.1479 [216]. For the tetra-orthogonal encoding scheme (used in most of our DTI experiments), the condition number

Table 3.1: Tetra-orthogonal gradient encoding scheme

$$(1,0,0) \quad (0,1,0) \quad (0,0,1) \quad \frac{1}{\sqrt{3}}(1,1,1) \quad \frac{1}{\sqrt{3}}(-1,-1,1) \quad \frac{1}{\sqrt{3}}(-1,1,-1) \quad \frac{1}{\sqrt{3}}(1,-1,-1)$$

was 1.5275.

In a Monte Carlo study comparing the noise performance of multiple gradient encoding schemes, Jones proposed that 30 or more evenly distributed gradient encoding directions was preferable for robust determination of mean diffusivity, fractional anisotropy, and tensor orientation [116]. For measures of just anisotropy, he deemed 20 directions sufficient. Fiber tractography, which requires higher angular resolution, benefits from as many encoding directions as possible. In our case, high-resolution mouse brain images necessitated a PGSE sequence (unlike the much faster echo-planar imaging (EPI) schemes commonly used in human studies). Slower acquisition and multiple averages limited the number of diffusion-weighted images that could be acquired with sufficient SNR and in a timely fashion. The white matter structures studied in this paper (primarily the corpus callosum) had a predictable geometry that didn't vary between subjects or treatments and the same slice was selected in each mouse. In addition, we only calculated scalar metrics of anisotropy and didn't require the higher angular resolution useful for fiber tractography. For all of these reasons, a tetra-orthogonal gradient encoding scheme was chosen as a suitable compromise.

The tetra-orthogonal gradient encoding scheme consists of 7 normalized gradient directions (Table 3.1). Visually, we can see the difference between the tetra-orthogonal gradient encoding scheme and the Jones 30 gradient encoding scheme, with 30 gradient vectors equally distributed using Coulomb repulsion of positively charged particles on a sphere [118] Figure 3.11.

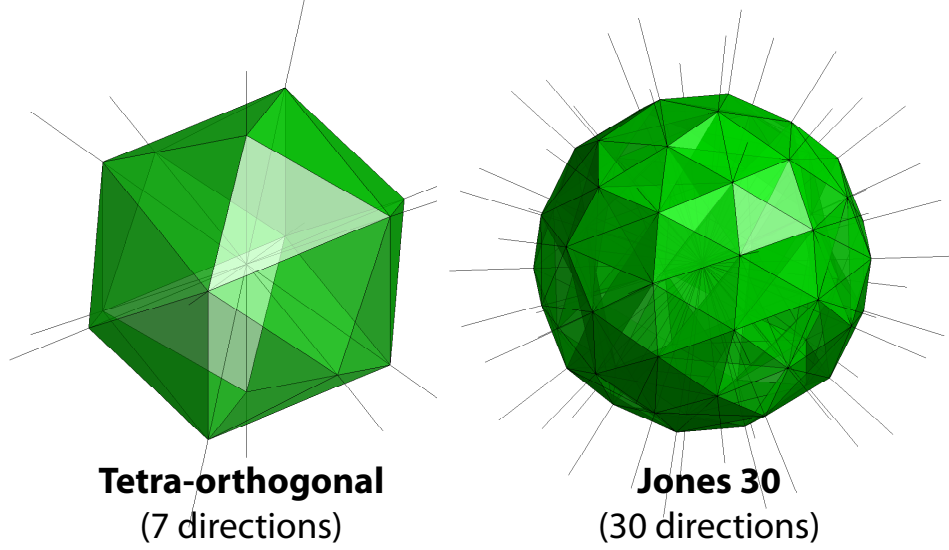


Figure 3.11: Comparison of tetra-orthogonal and Jones 30 gradient encoding schemes.

For the tetra-orthogonal gradient encoding scheme, the signal equation becomes,

$$\begin{pmatrix} \ln(S_1) \\ \ln(S_2) \\ \ln(S_3) \\ \ln(S_4) \\ \ln(S_5) \\ \ln(S_6) \\ \ln(S_7) \\ \ln(S_8) \end{pmatrix} = \begin{pmatrix} 0 & 0 & b_1 & 0 & 0 & 0 & 0 & 1 \\ -b_2 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & -b_3 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & -b_4 & 0 & 0 & 0 & 0 & 1 \\ -b_5/3 & -b_5/3 & -b_5/3 & -2b_5/3 & -2b_5/3 & -2b_5/3 & 1 \\ -b_6/3 & -b_6/3 & -b_6/3 & -2b_6/3 & 2b_6/3 & 2b_6/3 & 1 \\ -b_7/3 & -b_7/3 & -b_7/3 & 2b_7/3 & 2b_7/3 & -2b_7/3 & 1 \\ -b_8/3 & -b_8/3 & -b_8/3 & 2b_8/3 & -2b_8/3 & 2b_8/3 & 1 \end{pmatrix} \begin{pmatrix} D_{xx} \\ D_{yy} \\ D_{zz} \\ D_{xy} \\ D_{xz} \\ D_{yz} \\ \ln(S_0) \end{pmatrix}. \quad (3.54)$$

Note that the first image, S_1 , is acquired with no diffusion-weighting gradients, however there is still a small b-value, b_1 along the z-direction due to the imaging gradients (slice selection and spoiling). S_2 to S_8 represent images encoded with high b-values (b_2 to $b_8 > 800 \text{ s}^2/\text{mm}$) encoded along the 7 gradient directions.

3.2.7 Diffusion Tensor Metrics

Each voxel in the acquired image has a representative diffusion tensor. After diagonalization, the diffusion tensor reveals information about the directionality and anisotropy of the apparent diffusivity in the voxel element.

Eigenvalues and Eigenvectors of D

Diagonalization of D yields the eigenvalues (λ_1 , λ_2 , and λ_3) and the corresponding eigenvectors (e_1 , e_2 , and e_3). The eigenvalues are sorted so that λ_1 has the largest value. D becomes

$$D = \begin{pmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{pmatrix} \quad (3.55)$$

Ellipsoid Representation of D

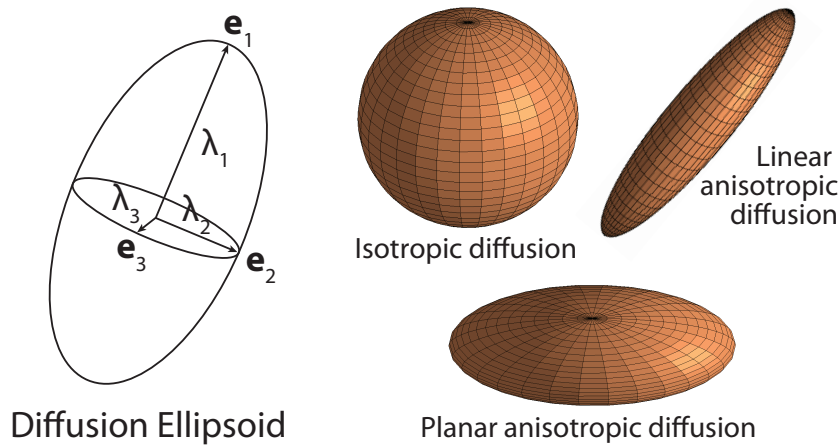


Figure 3.12: **Left:** Diffusion ellipsoid. **Right:** Ellipsoid representations of linear anisotropy, planar anisotropy, and spherical isotropy.

D can be represented as an ellipsoid [Figure 3.12](#). The magnitude of the major axis is proportional to λ_1 with the minor axes proportional to λ_2 and λ_3 . Directionality of the ellipsoid is determined by the corresponding eigenvectors. For $\lambda_1 \approx \lambda_2 \approx \lambda_3$, the ellipsoid is spherical and diffusion is isotropic. For $\lambda_1 \approx \lambda_2 \gg \lambda_3$, the ellipsoid is oblate and diffusion

is planar anisotropic. For $\lambda_1 \gg \lambda_2 \approx \lambda_3$, the ellipsoid is prolate and diffusion is linear anisotropic.

Scalar Metrics

Scalar metrics derived from the eigenvalues of $\mathbf{D}$ are rotationally invariant. This independence of coordinate system is useful for comparisons within the same subject and for comparisons between different subjects. There are numerous detailed papers on variant and invariant metrics derived from $\mathbf{D}$ [10, 11, 191, 264, 91]. Scalar metrics analyzed in this paper include mean diffusivity, axial diffusivity, radial diffusivity, and fractional anisotropy.

Mean Diffusivity

$$\langle D \rangle = \frac{\text{Trace}(\mathbf{D})}{3} = \frac{D_{xx} + D_{yy} + D_{zz}}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (3.56)$$

Mean diffusivity is the average diffusion in a voxel. $\langle D \rangle$ differentiates between cerebral-spinal fluid (high values) and brain tissue (low values), but it fails to differentiate between different kinds of healthy brain tissue [91]. This uniformity across normal white and grey matter in the brain makes diseased regions with increased or decreased $\langle D \rangle$ more conspicuous [14].

Axial ($\lambda_{\parallel}$) and Radial ($\lambda_{\perp}$) Diffusivity

$$\lambda_{\parallel} = \lambda_1 \quad (3.57)$$

$$\lambda_{\perp} = \frac{\lambda_2 + \lambda_3}{2} \quad (3.58)$$

Axial diffusivity, $\lambda_{\parallel}$ indicates the amount of diffusion running parallel to the major axis of the ellipsoid (λ_1). Radial diffusivity, $\lambda_{\perp}$ indicates the amount of diffusion running perpendicular to the major axis of the ellipsoid (λ_2 and λ_3). In healthy white matter with ordered and myelinated axonal bundles, $\lambda_{\perp}$ will be substantially smaller than $\lambda_{\parallel}$ [167].

Fractional Anisotropy (FA)

$$FA = \sqrt{\frac{3}{2} \frac{\sqrt{(\lambda_1 - \langle D \rangle)^2 + (\lambda_2 - \langle D \rangle)^2 + (\lambda_3 - \langle D \rangle)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}} \quad (3.59)$$

Fractional anisotropy (FA) is based on the standard deviation of the eigenvalues and characterizes the anisotropy of the diffusion ellipsoid. FA ranges from 0 to 1 – a higher number implies more anisotropy, with maximum anisotropy at $FA = 1$. While relative anisotropy (another anisotropic metric) is linear over a wide range of diffusion anisotropy, FA is more sensitive at low values of anisotropy and has become the most widely reported measure of anisotropy [91, 167]. FA does not distinguish the shape of the diffusion ellipsoid, but provide useful information nonetheless. In healthy white matter with ordered and myelinated axonal bundles, FA is high due to the directional structure of the axonal bundles. FA is low in grey matter, where directionality is limited.

Eigenvector Colour Maps

We can represent the directionality of diffusion with an eigenvector colour map. Eigenvectors define the orientation of the diffusion tensor, with $\mathbf{e}_1$ representing the direction of the major axis. In order to visualize the direction of diffusion, we assign an (R,G,B) colour value to the (x,y,z) components of $\mathbf{e}_1$, where (x,y,z) refers to the reference frame of the sample. Furthermore, we can weight the assigned colour value with the FA value for that coordinate. Thus,

$$R = \text{abs}(e_{1x})FA \quad G = \text{abs}(e_{1y})FA \quad B = \text{abs}(e_{1z})FA \quad (3.60)$$

With this scheme, voxels that are predominantly red, green, or blue represent diffusion in the x, y, or z directions, respectively. Grey or brown pixels represent isotropic regions. The intensity of the colour becomes a measure of FA, with bright coloured regions implying higher fractional anisotropy. With this method, one image can represent both the directionality and anisotropy of diffusion in a region of tissue.

3.2.8 DTI in Tissue

In 1990, Moseley *et al.* demonstrated that DWI detected cerebral ischemia in cats earlier than T_2 -weighted imaging [170]. The decrease in mean diffusivity due to cerebral ischemia was eventually attributed to water moving from extracellular compartments to intracellular compartments (cells swelling) along with an increase in extracellular tortuosity [176]. It is useful to describe diffusion in tissue as water diffusing in intracellular and extracellular compartments and moving between compartments according to the permeability of the cellular membranes [227]. This is the basis of countless diffusion simulations and often the starting point for interpreting DTI metrics in different tissues and pathologies.

When sampled with multiple b-values, the diffusion-weighted signal can be fit to a biexponential model (much like the T_2 relaxation curve) to isolate slow and fast diffusion components (D_{slow} and D_{fast}). Previously, $D_{slow} \approx 0.01 \times 10^{-3} mm^2 s^{-1}$ has been assigned to intracellular water and $D_{fast} \approx 3.9 \times 10^{-3} mm^2 s^{-1}$ to extracellular water [175]. These associations, however, appear to be wrong, with intracellular diffusivity in the axoplasm very similar to extracellular diffusivity ($\approx 1.3 - 1.6 \times 10^{-3} mm^2 s^{-1}$) [19]. Rather, after experimental evidence and computational models, diffusivity in tissue appears to be largely due to the relative volume of different compartments, extracellular tortuosity (determined by geometry), and intracellular micro-streaming [176, 18].

In general, mean diffusivity increases with increased extracellular space and tissue water. Increased $\langle D \rangle$ has been attributed variously to edema, inflammation, axonal loss, tissue necrosis, and demyelination [3]. Conversely, mean diffusivity will decrease with cellular swelling, as in ischemia [170, 262], or increased cellularity [3]. Whether increasing or decreasing diffusivity, white matter damage will generally decrease the structural coherence and directional uniformity of axonal bundles. Thus, regardless of their trend in white matter, changes in mean diffusivity are generally accompanied by decreased fractional anisotropy.

Fractional anisotropy has been used in numerous studies as an assessment of white matter structural integrity and connectivity. If radial diffusivity increases or axial diffusivity decreases, FA will decrease. FA won't show changes when radial and axial diffusivity both increase or decrease in equal amounts. In general, though, decreased FA has been observed

in many different neurological disorders, including but not limited to, multiple sclerosis [98], Alzheimer’s disease [242], schizophrenia, epilepsy, neoplasia, and hydrocephalus (see various DTI reviews [106, 237, 3] for additional references).

Given that FA decreases when radial diffusivity increases and/or axial diffusivity decreases, analyzing radial and axial diffusivity might give more information about white matter pathology. Although demyelination in the corpus callosum has been linked to increased radial diffusivity [223, 171, 224, 240, 122, 35, 277], other studies have found only marginal decreases in the anisotropy of demyelinated axonal fibres, suggesting factors such as axonal degeneration and other physiological changes might be contributing to changes in anisotropy [14, 171]. Rather than having a large effect on the membrane permeability, it is likely that demyelination, along with decreasing axonal density, changes the internal geometry of white matter tracts, thus altering the radial diffusivity by affecting the extracellular tortuosity and relative volumes of the different compartments. In contrast with radial diffusivity, decreased axial diffusivity might be a more specific marker of axonal injury [240, 50, 35, 128, 36, 277], where axonal debris and cellular swelling appears to inhibit diffusion parallel to the axonal bundles.

3.2.9 Limitations of DTI

Fiber tractography relies on the direction and magnitude of diffusion tensors to trace white matter tracts in the brain [16, 168, 13, 117, 143, 96]. While yielding beautiful images of white matter tracts, fiber tractography also reveals limitations in the diffusion tensor model. In particular, voxels that contain crossing or diverging fibers are more challenging to trace through, especially when represented by the single primary direction and magnitude of a diffusion tensor. In these regions, a more sophisticated representation of diffusion in tissue might be necessary.

The accuracy of DTI is inherently limited by three basic assumptions [167]:

1. **Diffusion of water reflects tissue structure.** In reality, diffusion is influenced by a variety of cellular structures and membrane permeabilities. Measured diffusion will be a function of diffusion time and represent an averaging over a voxel several orders

of magnitude larger than individual cells and axons.

2. **Diffusion-weighted signals accurately sample diffusion in a voxel.** Due to time constraints, gradient encoding will be along a limited number of gradient directions with a limited number of diffusion-weighting parameters (i.e. gradient strength, duration, shape, and separation). In addition, there are a variety of influences that may distort our measurements: hardware constraints; inaccurate tuning, shimming, and RF pulse calibrations; errors specific to the pulse sequence (e.g. EPI is particularly sensitive to eddy currents and field inhomogeneities, leading to geometric distortions); noise and subject motion; and subvoxel variations in T_1 , T_2 , magnetization transfer and temperature (to name a few).
3. **Diffusion in a voxel is accurately represented by the diffusion tensor.** In diffusion tensor imaging, all of the diffusion-weighted signals are fit to six parameters. Increasing the number of measurements improves the accuracy of the diffusion tensor fit, but doesn't increase the information. The diffusion tensor model assumes homogeneous tissue structure in a voxel. This, however, is not the case in most situations: white matter contains axonal fibers as well as support cells such as oligodendrocytes and microglia; as well, axonal fibers can cross or diverge inside individual voxels (so-called "kissing" fibers). DTI might overestimate or underestimate actual anisotropy at the subvoxel level.

Beyond increasing the spatial resolution, the first assumption is somewhat fixed. Diffusion imaging can only image the random motion of water in the presence of tissue structures. One method that can measure the underlying macromolecular structure, magnetization transfer imaging, is discussed in [Section 3.3](#).

The second assumption is improved by using better hardware and acquisition methods in conjunction with more gradient-encoding directions (yielding higher angular resolution) and varying gradient parameters (yielding higher resolution of diffusion along individual directions). Several groups have explored the benefits of high-angular resolution diffusion imaging (HARDI) [182, 5]. Varying the diffusion time (i.e. the gradient duration and separation) may provide insight into different structures as short-diffusion times will reduce

the number of barriers experienced by individual water molecules and long-diffusion times will increase the number of barriers experienced [208, 171, 222]. More sophisticated gradient pulse shapes such as the oscillating gradient spin echo sequence can be used to probe very short diffusion times [208, 209, 185, 2]. Increasing the number of acquisitions will increase the imaging time and may lead to other distortions (changes in temperature or relaxation properties over time). *In vivo* imaging in particular is severely limited by how long a human can stay in the magnet or an animal can stay under anesthetic. Subject motion also plays a larger role as imaging time increases. Faster imaging methods and better hardware can improve acquisition time, but may have other challenges (such as geometric distortions in high-field EPI).

The third assumption can be addressed, in part, by analytical and numerical simulations of diffusion in tissue which reveal the underlying structures and molecular behaviour that might contribute to the measured signal in a voxel.

Analytical solutions exist for one dimensional systems with permeable barriers [195, 236], two-dimensional systems with square [190] and cylindrical [255] cell geometries, and three-dimensional cubic cell lattices [241]. Myelinated axons can be roughly approximated by a central cylinder representing the axon surrounded by a larger cylinder representing the myelin sheath. These cylindrical axons can be modelled in a square or hexagonal array with different permeabilities and diffusivities assigned to the individual components (axon, myelin sheath, and extracellular fluid) [213]. Stanisiz *et al.* proposed a model with prolate ellipsoids and spheres representing axons and oligodendrocytes respectively [227]. Although limited to simple geometries, analytical solutions have the distinct advantage of speed and a relatively small parameter space.

Numerical simulations allow more complicated geometries to be explored. Three-dimensional lattices of cubic and cylindrical cells have been modelled with Monte Carlo simulations of diffusing particles [241, 68]. More complicated geometries could be explored by introducing random variations in the spacing and size of the cylindrical axons. Alternatively, geometries can be obtained directly from digitized histological images of myelinated and demyelinated axons, after which Monte Carlo simulations are performed [151, 45].

The third assumption can also be addressed by more sophisticated fitting models for the

data. Given its inherently non-Gaussian nature, diffusion in tissue might be better characterized by a non-Gaussian fit. Diffusion kurtosis imaging addresses this by introducing a shape parameter, κ that represents the deviation from a Gaussian form (called the excess kurtosis) [111]. Spherical deconvolution [112] and multiple-tensor fitting [4] fit to multiple fiber orientations in each voxel. More complicated (and computationally intense) fits can be achieved using diffusion spectrum imaging or q-space imaging, where an analytical form, such as the Gaussian distribution, is not assumed [251, 185, 8, 271].

Despite the limitations of the diffusion tensor model, it is still a powerful indicator of white matter structural integrity. We can account for some of its limitations by using DTI to analyze white matter tracts with axonal bundles ordered in a common direction, such as the corpus callosum or spinal cord. It is also important to recognize what DTI metrics can tell us about tissue pathology (overall structural integrity and directionality of fiber tracts) and what they cannot (relative myelin content and myelin sheath integrity). Although not reported in this dissertation, work I have done in our lab (and elsewhere) continues to look at correlations between simulations of diffusion in white matter and actual DTI measurements. Both the limitations and strengths of DTI motivated the search for complementary MRI metrics in this dissertation.

3.3 Magnetization Transfer Imaging

Traditional MRI methods, such as the spin echo or gradient echo sequence, can only detect mobile protons (liquid pool) with T_2 relaxation times sufficiently long enough to allow for some remaining signal after spatial encoding gradients are applied (i.e. $T_2 > 1$ ms). However, less mobile protons found in macromolecules and membranes (bound pool) have a $T_2 \ll 1$ ms, making them essentially invisible to traditional methods. The RF absorption lineshape of macromolecular spins is much broader than that of mobile spins. An appropriately chosen off-resonance RF pulse will selectively saturate the bound pool, which will then transfer its magnetization via spin exchange (dipole-dipole interactions across space) to the liquid pool (see [Figure 3.13](#)). Magnetization transfer imaging (MTI) relies on this spin exchange to indirectly image semi-solid tissue such as myelin [100].

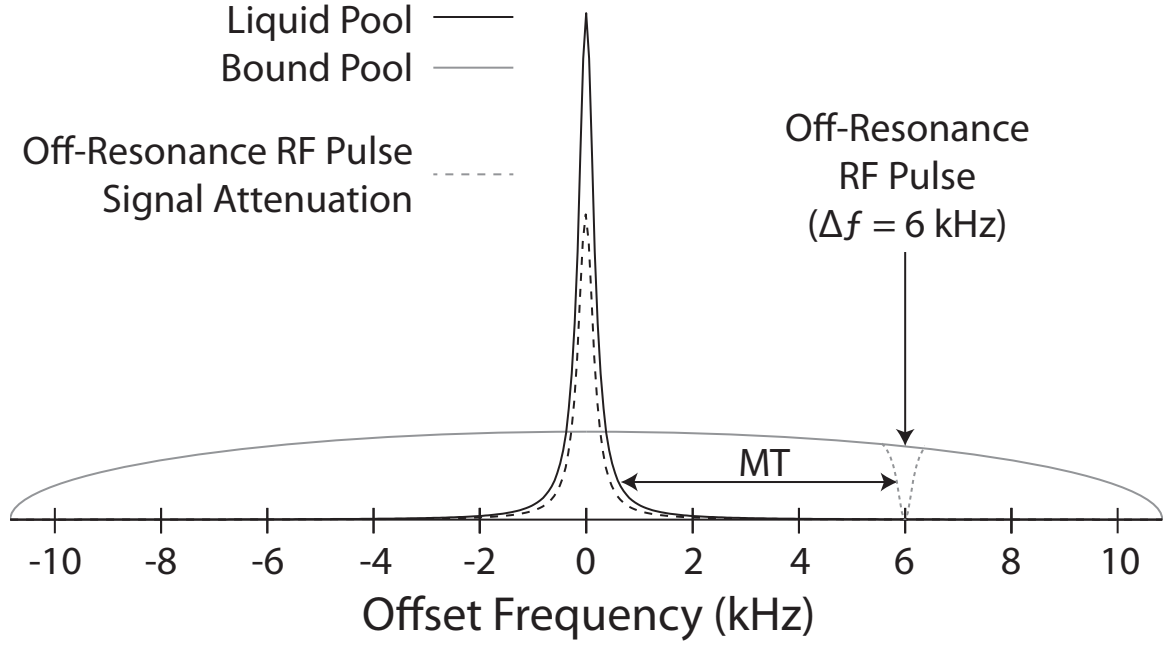


Figure 3.13: Schematic of magnetization transfer (MT) from RF saturated bound pool ($\Delta f = 6$ kHz) to liquid pool. 0 is equivalent to the Larmor frequency of the liquid pool, ω_0 . Signal attenuation in the liquid pool is represented by the dashed lineshape.

3.3.1 Pulsed Magnetization Transfer Imaging

A proton density image can be acquired using a spoiled gradient echo imaging sequence (Figure 2.8) with the signal amplitude determined by the subject-dependent T_1 and T_2^* relaxation times and the operator-controlled flip angle, T_R , and T_E (Equation (2.83)). Minimizing the T_R and reducing the flip angle, α to reduce T_1 -weighting, combination with the spoiler gradients, allows us to acquire a fast proton density image, commonly known as the Fast Low Angle Shot (FLASH) method [88].

An off-resonance RF pulse can be characterized by its offset frequency, Δf (in Hz) and the angular frequency it induces in the precession, ω_1 (rad/s). ω_1 can be determined using its pulse envelope (Gaussian, in our case), duration (τ_{MT}), and peak amplitude (B_1). Applying an off-resonance RF pulse prior to spoiled gradient echo sequence will result in an image with magnetization transfer contrast (MTC) proportional to the imaging parameters and magnetization transfer properties of the tissue (Figure 3.14).

Optimal imaging parameters (T_R and α) and MT preparation pulse parameters (ω_1 and

Δf) can be determined to find the optimal contrast between white and grey matter (or some other tissue of interest) [81]. In general, reducing T_R increases the magnetization transfer effect [81]. Optimal flip angle will be dependent on both T_R and the MT preparation pulse. Δf is usually chosen to reduce direct saturation of the liquid pool while also minimizing the required power. Typical values for Δf are between 1 and 6 kHz. Spoiled gradient echo image acquisition methods are preferable to fast spin echo methods, which both reduce magnetization transfer because of the echo train length and introduce unwanted MT contrast with repeated slice-selective refocusing pulses [81].

A simple metric for magnetization transfer is the magnetization transfer ratio (MTR). In order to calculate an MTR map, two images are acquired: a proton-density image (M_0) and an RF saturated image (M_{sat}).

$$MTR = \frac{M_0 - M_{sat}}{M_0} = 1 - \frac{M_{sat}}{M_0} = 1 - \left(\frac{M_{dir}}{M_0} + \frac{M_{MT}}{M_0} \right) \quad (3.61)$$

The MTR map will include both signal loss due to direct saturation of the liquid pool (M_{dir}) and true magnetization transfer (M_{MT}), but it can still be a good indicator of a shrinking bound pool due to demyelination [276]. MTR is also dependent on the T_1 relaxation time and is intrinsically tied to the imaging and MT pulse parameters used during acquisition, making it more of a semi-quantitative method, but useful in a study where the imaging parameters are held constant [100].

In order to estimate the size of the bound pool, a model describing the liquid and bound pool in a magnetization transfer experiment is required.

3.3.2 Two-Pool Model of Magnetization Transfer

Magnetization transfer can be interpreted quantitatively using a two-pool model (Figure 3.15). At equilibrium, the liquid pool (A) has a magnetization of M_0^A and the smaller bound pool (B) has a magnetization of M_0^B . At any time, magnetizations are either longitudinal (white) or transverse/saturated (grey) in each pool. Given the broader absorption lineshape of pool B, an off-resonance RF pulse will increase R_{rfB} more than R_{rfA} , saturat-

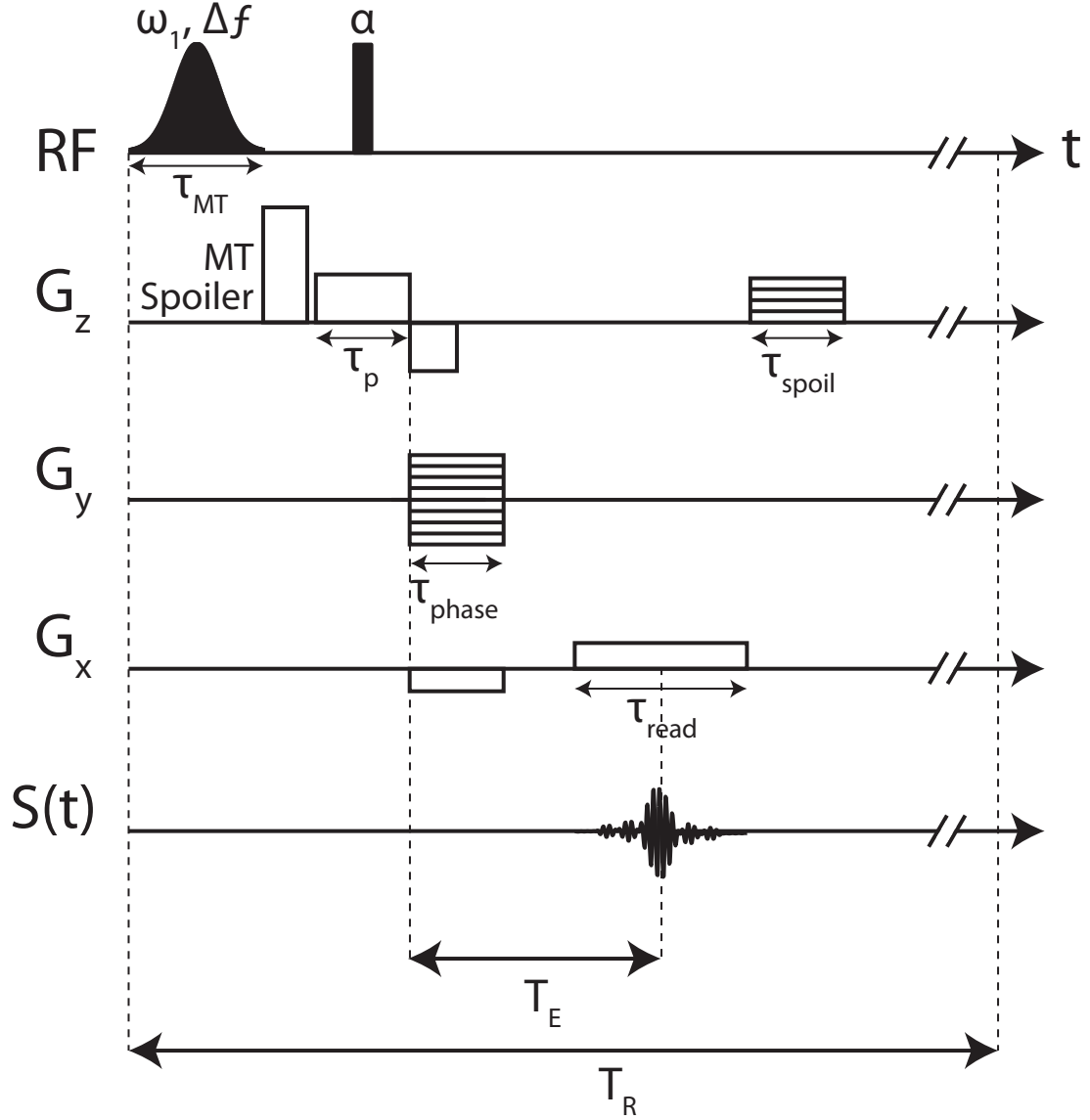


Figure 3.14: Spoiled gradient echo sequence with MT preparation pulse defined by its pulse envelope, duration (τ_{MT}), amplitude, and offset frequency (Δf). A spoiler gradient (MT Spoiler) prior to the α -pulse eliminates any residual transverse magnetization prior to imaging.

ing pool B. After RF irradiation, the pools relax longitudinally with rates $R_A = 1/T_1^A$ and $R_B = 1/T_1^B$ and also exchange magnetization with rate R [99]. Assuming time-dependent behaviour akin to the Bloch equation, this coupled system can be represented by differential equations for each component of the liquid pool magnetization, $\mathbf{M}^A$ and the bound pool magnetization, $\mathbf{M}^B$ [37, 268, 99]:

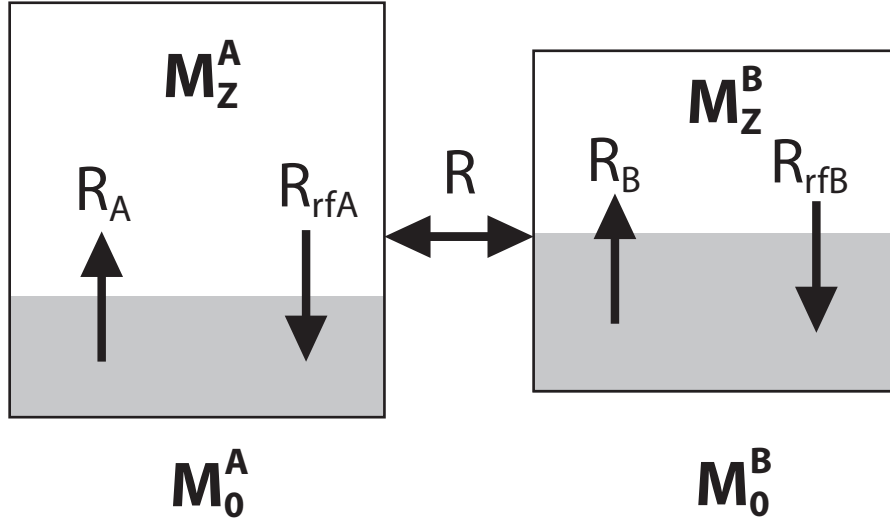


Figure 3.15: Two pool model of magnetization transfer. The liquid pool (A) has a magnetization M_0^A normalized to unity ($M_0^A = 1$) and the smaller bound pool (B) has a magnetization M_0^B . At any time, spins are either longitudinal (white) or saturated (grey) in each pool. Given the broader absorption lineshape of the bound pool, an off-resonance RF pulse will primarily increase the saturation in pool B. After RF irradiation, the pools relax longitudinally with rates R_A and R_B and also exchange spins with rate R .

$$\frac{dM_x^A}{dt} = -\frac{M_x^A}{T_2^A} - 2\pi\Delta f M_y^A \quad (3.62)$$

$$\frac{dM_x^B}{dt} = -\frac{M_x^B}{T_2^B} - 2\pi\Delta f M_y^B \quad (3.63)$$

$$\frac{dM_y^A}{dt} = -\frac{M_y^A}{T_2^A} + 2\pi\Delta f M_x^A - \omega_1 M_z^A \quad (3.64)$$

$$\frac{dM_y^B}{dt} = -\frac{M_y^B}{T_2^B} + 2\pi\Delta f M_x^B - \omega_1 M_z^B \quad (3.65)$$

$$\frac{dM_z^A}{dt} = R_A (M_0^A - M_z^A) - R M_0^B M_z^A + R M_0^A M_z^B - \omega_1 M_y^A \quad (3.66)$$

$$\frac{dM_z^B}{dt} = R_B (M_0^B - M_z^B) - R M_0^A M_z^B + R M_0^B M_z^A - \omega_1 M_y^B \quad (3.67)$$

where T_2^A and T_2^B are the transverse relaxation times for the liquid and bound pools, respectively.

In a steady state, Equations (3.62) to (3.67) are all equal to zero. $M_x^{A,B}$ becomes,

$$M_x^{A,B} = -2\pi\Delta f M_y^{A,B} T_2^{A,B}. \quad (3.68)$$

Plugging $M_x^{A,B}$ into $M_y^{A,B}$ gives:

$$M_y^{A,B} = \frac{-\omega_1 M_z^{A,B} T_2^{A,B}}{1 + (2\pi\Delta f T_2^{A,B})^2}. \quad (3.69)$$

Solving for M_z^A and plugging in M_y^A gives,

$$M_z^A = \frac{R_A M_0^A + R M_0^A M_z^B}{R_A + R M_0^B + R_{rfA}}. \quad (3.70)$$

and M_z^B becomes,

$$M_z^B = \frac{R_B M_0^B + R M_0^B M_z^A}{R_B + R M_0^A + R_{rfB}} \quad (3.71)$$

where

$$R_{rfA} = \frac{\omega_1^2 T_2^A}{1 + (2\pi\Delta f T_2^A)^2} \text{ and } R_{rfB} = \frac{\omega_1^2 T_2^B}{1 + (2\pi\Delta f T_2^B)^2}. \quad (3.72)$$

Plugging Equation (3.71) into Equation (3.70) gives an expression for M_z^A/M_0^A

$$\frac{M_z^A}{M_0^A} = \frac{R_A R_B + R_A R M_0^A + R_A R_{rfB} + R R_B M_0^B}{(R_A + R M_0^B + R_{rfA})(R_B + R M_0^A + R_{rfB}) - R^2 M_0^A M_0^B} \quad (3.73)$$

Before proceeding any further, let's look at some of the equations at hand. The signal detected immediately following an RF saturation pulse is equivalent to M_z^A and M_0^A is equivalent to the signal from a proton density image without an applied RF saturation pulse. The normalized signal intensity, M_z^A/M_0^A is equivalent to the ratio used to describe the magnetization transfer ratio, M_{sat}/M_0 and assuming some magnetization transfer occurred, will always be ≤ 1 . Assuming that the proton density in the liquid pool doesn't change,

M_0^A is often normalized to unity ($M_0^A = 1$) [99, 81]. We will, however, keep M_0^A in the equation as per Ramani *et al.* to maintain dimensionality [198]. Because T_2^B is very short, the transverse coherence between the two pools is limited, and the transverse magnetization exchange doesn't need to be modelled explicitly [99].

The RF absorption rates of the liquid and bound pool (R_{rfA} and R_{rfB}) are both described by a Lorentzian lineshape. This comes out directly from the differential equations and, as we've seen from the previous Bloch equations, is a good approximation for the liquid pool, where motional narrowing is prevalent [169]. The Lorentzian lineshape, however, is inadequate when describing the broad RF absorption lineshape of the bound pool. As an alternative, Gaussian [99] and Super-Lorentzian [169] lineshapes have been proposed as better models for RF absorption in the bound pool. Gaussian lineshapes tend to give the best fit for rigid systems, such as agar gels [99, 220], and Super-Lorentzian lineshapes tend to give the best fit for partially ordered systems, such as biological tissue [169, 248]. While numerical estimates have been used to create a flexible RF absorption lineshape [149], the Gaussian and Super-Lorentzian lineshapes are more practical for numerical model fitting [100].

We can define the RF absorption rate as

$$R_{rf} = \omega_1^2 g(2\pi\Delta f) \quad (3.74)$$

where $g(2\pi\Delta f)$ is the absorption lineshape. The three lineshapes used to model the RF absorption rate are:

$$\textbf{Lorentzian: } g(2\pi\Delta f) = \frac{T_2}{1 - (2\pi\Delta f T_2)^2} \quad (3.75)$$

$$\textbf{Gaussian: } g(2\pi\Delta f) = \sqrt{\frac{\pi}{2}} T_2 e^{-\frac{(2\pi\Delta f T_2)^2}{2}} \quad (3.76)$$

$$\textbf{Super-Lorentzian: } g(2\pi\Delta f) = \sqrt{2\pi} T_2 \int_0^\infty d\theta \frac{\sin\theta}{|3\cos^2\theta - 1|} e^{-2\left[\frac{2\pi\Delta f T_2}{|3\cos^2\theta - 1|}\right]^2} \quad (3.77)$$

Figure 3.16 demonstrates the much broader Gaussian and Super-Lorentzian lineshapes at typical T_2 values for agar and brain tissue when compared to the Lorentzian lineshape at a typical T_2 value for the liquid pool.

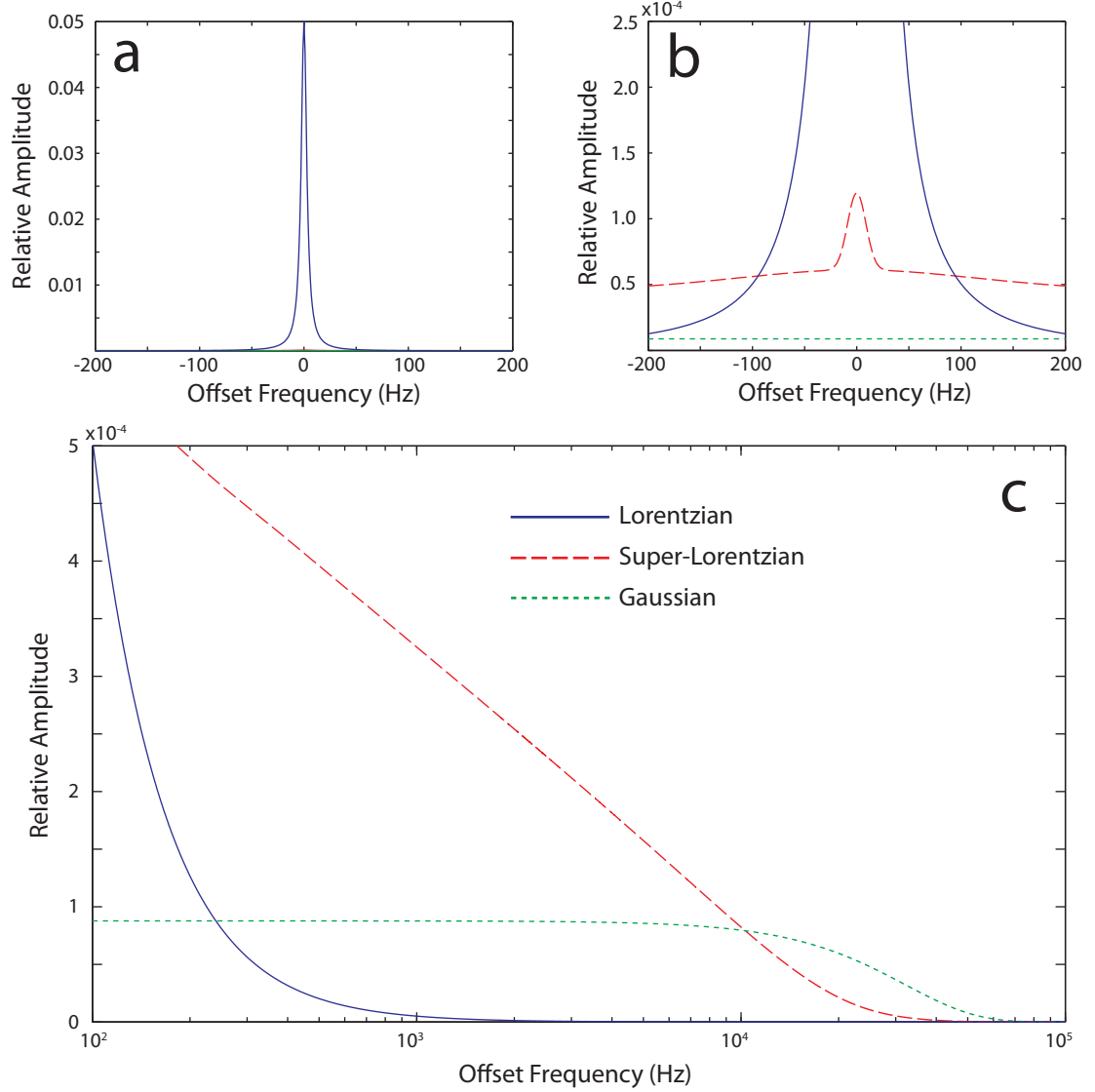


Figure 3.16: Full view (a), detail (b), and logarithmic scale (c) representations of the Lorentzian (solid blue line), Super-Lorentzian (dashed red line), and Gaussian (dotted green line) lineshapes with equal amplitudes. $T_2 = 50$ ms and relative amplitude approaches zero at $\Delta f \approx 2$ kHz for the Lorentzian lineshape, $T_2 = 22 \mu\text{s}$ and relative amplitude approaches zero at $\Delta f \approx 60$ kHz for the Gaussian lineshape, and $T_2 = 7 \mu\text{s}$ and relative amplitude approaches zero at $\Delta f \approx 40$ kHz for the Super-Lorentzian lineshape (values typical for agar gel and *ex vivo* brain tissue).

At $\Delta f = 1000$ Hz, almost no RF absorption occurs in the Lorentzian lineshape when

compared to the Gaussian or Super-Lorentzian lineshapes. For $\Delta f \geq 1000$ Hz and $T_2^A \approx 50$ ms, $(2\pi\Delta f T_2^A)^2 \gg 1$ and the Lorentzian form of R_{rfA} becomes

$$R_{rfA} \approx \left(\frac{\omega_1}{2\pi\Delta f} \right)^2 \frac{1}{T_2^A} \quad (3.78)$$

After substituting R_{rfA} and simplifying Equation (3.73), the normalized signal intensity becomes,

$$\frac{M_z^A}{M_0^A} = g \left(\frac{R_B \left[\frac{RM_0^B}{R_A} \right] + R_{rfB} + R_B + RM_0^A}{\left[\frac{RM_0^A M_0^B}{R_A} \right] (R_B + R_{rfB}) + \left(1 + \left[\frac{\omega_1}{2\pi\Delta f} \right]^2 \left[\frac{1}{R_A T_2^A} \right] \right) (R_B + R_{rfB} + RM_0^A)} \right) \quad (3.79)$$

where g is a scaling factor to account for small, scanner dependent differences between the acquisition of M_z^A and M_0^A , such as variations in the gain of the RF amplifier [198].

If we define the bound pool fraction as $f = M_0^B / (M_0^A + M_0^B)$, M_0^B can be replaced with,

$$M_0^B = \frac{f}{1-f} \quad (3.80)$$

and the normalized signal intensity becomes, [198]:

$$\frac{M_z^A}{M_0^A} = g \left(\frac{R_B \left[\frac{RM_0^A f}{R_A(1-f)} \right] + R_{rfB} + R_B + RM_0^A}{\left[\frac{RM_0^A f}{R_A(1-f)} \right] (R_B + R_{rfB}) + \left(1 + \left[\frac{\omega_1}{2\pi\Delta f} \right]^2 \left[\frac{1}{R_A T_2^A} \right] \right) (R_B + R_{rfB} + RM_0^A)} \right) \quad (3.81)$$

By fitting the observed signal at various values of Δf and ω_1 to Equation (3.81), 6 unique parameters can be determined: R_B , RM_0^A , $f/R_A(1-f)$, T_2^B , $1/R_A T_2^A$, and g . The fit is insensitive to R_B , thus R_B is fixed arbitrarily to 1 s^{-1} as recommended by several groups [99, 219, 198].

Figure 3.17 shows the relative signal vs. offset frequency at different saturation amplitudes. Also in Figure 3.17, if we set $RM_0^A = 0$, we can see the effect direct saturation of

the liquid pool has on the relative signal. We can see how the signal loss due to both true magnetization transfer (M_{MT}) and direct saturation of the liquid pool (M_{dir}) contributes to the measured MTR value (see Equation (3.61)). As the offset frequency increases, M_{dir} decreases and is essentially zero at $\Delta f = 6000$ Hz.

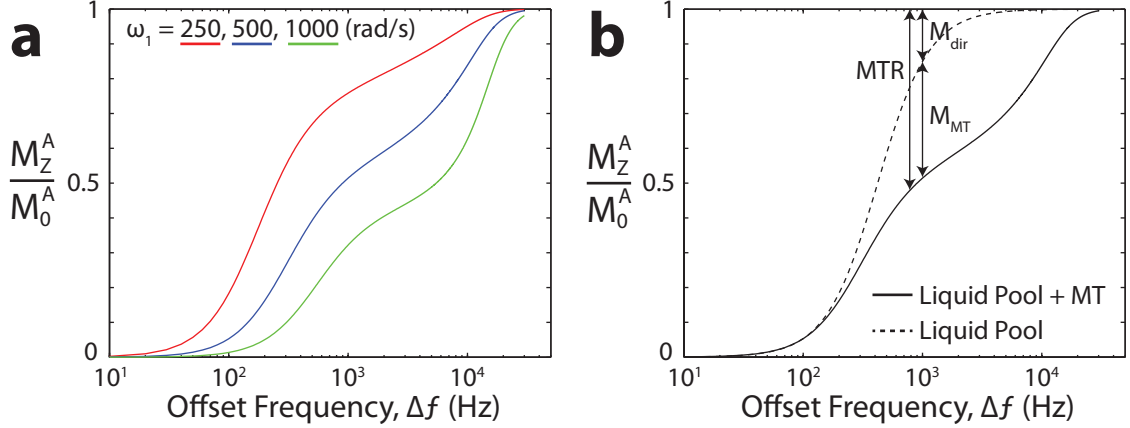


Figure 3.17: Two pool model of magnetization transfer. (a) Relative signal (M_z^A/M_0^A) vs. offset frequency ($\Delta f = 10 - 30,000$ Hz) at different saturation amplitudes ($\omega_1 = 250, 500$, and 1000 rad/s). Curves were generated using typical qMTI parameters found in a healthy mouse corpus callosum. (b) If we set $RM_0^A = 0$, we can see the effect direct saturation of the liquid pool has on the relative signal for $\omega_1 = 500$ rad/s (dashed line). The signal loss due to both true magnetization transfer (M_{MT}) and direct saturation of the liquid pool (M_{dir}) contributes to the measured MTR value (see Equation (3.61)).

Modelling Pulsed Magnetization Transfer

Equation (3.81) assumes a continuous RF saturation pulse resulting in the steady state condition. In Henkelman *et al.*, this was accomplished with a 5 s off-resonance RF pulse immediately followed by a $\pi/2$ -pulse and signal acquisition during the free induction decay [99]. This is impractical for magnetization transfer imaging due to time constraints and RF heating of tissue. As a compromise, pulsed magnetization transfer imaging uses a short, shaped (usually Gaussian) off-resonance RF pulse prior to imaging with a spoiled gradient echo sequence.

In order to model the pulsed magnetization transfer signal, the original, time-dependent differential equations can be solved numerically [80, 194] or by using a continuous wave approximation of the RF saturation to modify Equation (3.81) [219, 273, 198]. Sled and Pike

approximated the effect of the MT pulse on the liquid pool as an instantaneous saturation of the longitudinal magnetization and on the bound pool as an off-resonance rectangular pulse [219]. Assuming that the MT pulses will be sufficiently offset to have negligible effect on the liquid pool, Yarnykh neglected the direct saturation of the liquid pool and modeled the MT saturation as a rectangular pulse acting solely on the bound pool [273]. Alternatively, Ramani *et al.* calculated a continuous wave power equivalent of the short MT pulse amplitude (ω_{1CWPE}) that could be used to directly fit Equation (3.81). Although Sled and Pike’s solution appears to model direct saturation better at $\Delta f < 1000$ Hz [194], Ramani’s solution appears to perform slightly better at low SNR [41], is simpler to implement, and is accurate at the offset frequencies used in our experiments ($\Delta f > 1000$ Hz). Compared to a model with minimum assumption, Portnoy and Stanisiz concluded that all pulsed MT approximations were robust when estimating f and T_2^B , while Ramani *et al.*’s method underestimates RM_0^A by $\approx 15\%$ and overestimates T_2^A by $\approx 12\%$ [194]. Both T_2^A and, in particular, RM_0^A , have been shown to have large variability in numerous experiments [194, 219, 198, 253].

Using Ramani’s method, ω_{1CWPE} is defined as [198, 248, 250],

$$\omega_{1CWPE} = \gamma \sqrt{\frac{p_2 \theta^2}{p_1^2 T_R' \gamma^2 \tau_{sat}}} \quad (3.82)$$

where T_R' is equal to the sequence repetition time, T_R , divided by the number of slices collected per T_R . p_1 is the ratio of the area of a shaped pulse to the area of a rectangular pulse of the same duration, τ_{sat} and amplitude, B_{1sat} (also called the integral ratio). The area of a continuous Gaussian pulse envelope is,

$$\int_{-\tau_{sat}/2}^{+\tau_{sat}/2} B_{1sat}^e(t) dt = \int_{-\tau_{sat}/2}^{+\tau_{sat}/2} B_{1sat} e^{-\frac{t^2}{2\sigma^2}} dt \quad (3.83)$$

where σ is defined so that the pulse is 1% of its amplitude at $t = \pm \tau_{sat}/2$. The Gaussian pulse envelope is described and implemented by Bruker as $N = 256$ normalized discrete pulses of amplitude, y_i (Figure 3.18). The integral ratio is then

$$p_1 = \frac{B_{1sat} \sum_{i=1}^N y_i \frac{\tau_{sat}}{N}}{B_{1sat} \tau_{sat}} \quad (3.84)$$

where the area of the rectangular pulse is simply $B_{1sat}\tau_{sat}$. p_2 is the ratio of the square of the shaped pulse amplitude to the square of the rectangular pulse amplitude,

$$p_2 = \frac{B_{1sat}^2 \sum_{i=1}^N y_i^2 \tau_{sat}}{B_{1sat}^2 \tau_{sat}} \quad (3.85)$$

θ is the effective flip angle of the MT pulse. Bruker defines the MT pulse by its amplitude (B_{1sat} in units of μT), the flip angle is then

$$\theta = \gamma B_{1sat} \tau_{sat} p_1 \quad (3.86)$$

and

$$\omega_{1CWPE} = \gamma B_{1sat} \sqrt{\frac{p_2 \tau_{sat}}{T_R'}}. \quad (3.87)$$

In our experiments, a typical MT pulse sequence would have the following parameters: $B_{1sat} = 10 \mu T$, $\tau_{sat} = 10.25$ ms, $T_R' = 70$ ms, and $p_2 = 0.2987$, yielding $\omega_{1CWPE} = 559.5$ rad/s. Replacing ω_1 with ω_{1CWPE} in Equation (3.81), we can fit for the 6 unknown parameters that define the two-pool model of magnetization transfer.

In general, pulsed MT experiments tend to overestimate R with higher uncertainty in comparison to continuous wave MT experiments [194]. In comparison to a numerical fit of pulsed MT, Ramani's method underestimated R by 15% and overestimated T_2^A by 12%, however, f and T_2^B were very close to the true parameter values [194]. In addition to pulsed saturation (PS), alternative methods for measuring quantitative MT parameters have been proposed using either selective inversion recovery (SIR) [79, 78, 181, 58] or balanced steady-state free precession (SSFP) [76, 77, 71]. Each method has advantages: PS and SSFP have faster acquisition times, SIR is less sensitive to high field artifacts, and SSFP and SIR measure R with less variability; and disadvantages: PS requires additional measurements of T_1 , SSFP and SIR can't measure T_2^B and measure T_2^A with high variability, and SSFP is more sensitive to high field artifacts. A full comparison between these three MT methods would be of interest, but is beyond the scope of this dissertation.

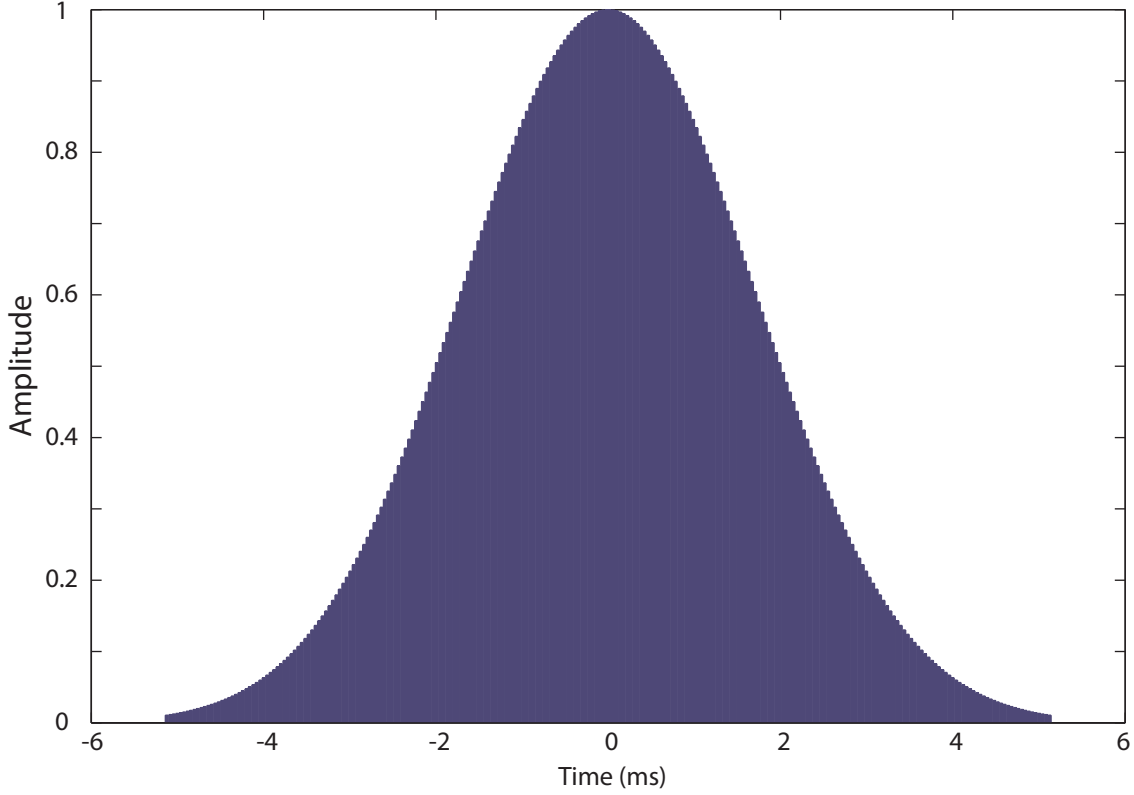


Figure 3.18: Discrete, 256-element, 10.25 ms Gaussian pulse. Used to calculate the continuous wave power equivalent flip angle.

Estimating R_A and f

In order to calculate f , an estimate of R_A is required. Starting with the coupled longitudinal equations without off-resonance irradiation, Henkelman *et al.* solved for R_A [99].

Incorporating f as per Ramani *et al.* [198], R_A becomes,

$$R_A = \frac{R_{1obs}}{1 + \left(\frac{RM_0^A f}{(1-f) R_A} (R_B - R_{1obs}) \right)} \quad (3.88)$$

where $R_{1obs} = 1/T_{1obs}$, the observed longitudinal relaxation rate of the liquid pool. This necessitates a separate measurement of T_1 , which can be accomplished using one of the methods outlined in Section 3.1. Having already found $f/R_A(1-f)$ in Equation (3.81), the bound pool fraction, f can be calculated:

$$f = \frac{\left(\frac{f}{R_A(1-f)}\right) R_A}{\left(\frac{f}{R_A(1-f)}\right) + 1} \quad (3.89)$$

3.3.3 qMTI Metrics

Longitudinal Relaxation Rates of the Liquid (R_A) and Bound (R_B) Pools

As with T_1 measurements, R_A and R_B are dependent on both the magnetic field strength and their respective lattice environments. We can expect R_A to decrease as the lattice becomes more sparse and the molecular mobility increases (as in demyelination). While R_B can't be reliably found using the two pool model, the restricted water environment in macromolecules suggests that R_B would be lower than R_A in healthy tissue. At the time of writing, no MT method has reliably measured R_B , and as such, R_B is set arbitrarily to 1 s^{-1} .

Transverse Relaxation Times of the Liquid (T_2^A) and Bound (T_2^B) Pools

As with T_2 measurements, T_2^A and T_2^B are sensitive to dephasing of nuclear spins caused by local field inhomogeneities.

Spins in the liquid pool will dephase faster and T_2 will be smaller in restricted environments, such as water trapped in the myelin bilayers. In the two-pool model, T_2^A should be comparable to the monoexponential T_2 found in [Section 3.1](#), although in the case of multiple water compartments, T_2^A may represent different weightings of the multi-component T_2 values [\[41\]](#). A T_2 value specific to myelin water could potentially be found using a four-pool model of magnetization transfer, which includes separate pools for myelin water, intra-/extracellular water, myelin macromolecules, and non-myelin macromolecules [\[28, 145\]](#), however, it is somewhat unwieldy to acquire the necessary number of MT images. T_2^A will also be sensitive to intercompartmental water exchange.

T_2^B is much shorter than T_2^A (of the order of μs vs. ms). It defines the width of the bound pool lineshape, and as such, will vary depending on whether a Gaussian or Super-Lorentzian lineshape is used. It represents the time that spins trapped in macromolecules

take to dephase and may be sensitive to changes in the macromolecular environment.

Magnetization Transfer Rate, RM_0^A and the Magnetization Transfer Ratio

The magnetization transfer rate from the bound pool to the liquid pool, RM_0^A (also called k_m , k_{mf} , or just R) defines the rate at which magnetization transfer occurs following an MT saturation pulse. It is dependent on both the size of the liquid and bound pools and the magnetization exchange that occurs between the two. Sometimes, the magnetization transfer rate from the liquid to the bound pool, RM_0^B (also called k , k_f , or k_{fm}) is reported instead. Using the definition of the bound pool fraction, it is easy to show that

$$RM_0^B = RM_0^A \frac{f}{1-f} \quad (3.90)$$

The magnetization transfer ratio (MTR) is dependent on two competing rates. Larger magnetization exchange rates (RM_0^A or RM_0^B) will increase the MTR. Conversely, larger longitudinal relaxation rates (R_A) will allow the saturated spins to return to equilibrium more quickly, decreasing the MTR. To show the true MT, Henkelman *et al.* proposed a ratio between the two rates (RM_0^B/R_A or RM_0^A/R_A) [100]. Plotted against MTR, it shows the inherent dependency of MTR on longitudinal relaxation and field strength. As field strength increases, RM_0^A remains constant [99], but R_A decreases and, as a result, MTR increases. In a simple model [212],

$$MTR \propto RT_1 f \quad (3.91)$$

Bound Pool Fraction, f

The bound pool fraction, f (also called the BPF or f_B) represents the relative size of the bound pool to the combined bound and liquid pools.

$$f = \frac{M_0^B}{M_0^A + M_0^B} \quad (3.92)$$

An alternative bound pool fraction has been defined as $F = M_0^B/M_0^A$ (also called the pool size ratio (PSR) or M_0^B). F is related to f by the simple equation,

$$F = \frac{f}{1 - f} \quad (3.93)$$

When T_1 hasn't been measured, R_A can't be determined and the apparent bound proton fraction, f^* is sometimes used as an approximation to f [203, 200]

$$f^* = \frac{f}{R_A(1 - f)} \quad (3.94)$$

3.3.4 qMTI in Tissue

Decreased MTR has been used in numerous studies as an indicator of demyelination in multiple sclerosis [83, 67, 189, 254, 206, 44]. MTR can provide lesion contrast where T_1 -weighted or T_2 -weighted MRI fails. In patients with MS, the peak-height decreases in histograms of whole-brain volumetric MTR values [254, 65]. In addition, changes in T_2 -weighted lesion volumes and whole-brain MTR histograms might predict the clinical evolution of MS [206]. Large decreases in MTR have been associated with demyelinated lesions, whereas slightly decreased [83] or slightly increased [59] MTR has been associated with lesions that exhibit edema but not demyelination. Normal appearing white matter also exhibits slightly decreased MTR [59]. In a longitudinal study of cuprizone-fed mice, MTR was strongly correlated with myelin content ($R = 0.79$) [276], although in another animal study, MTR was also sensitive to inflammation [72]. While MTR can provide valuable information about lesion evolution, care needs to be taken when interpreting MTR as solely a marker for myelin. Vavasour *et al.* showed a strong correlation between water content and MTR ($R = -0.65$), suggesting inflammation and edema might also alter MTR [260]. This is immediately obvious when inspecting the two pool model, where the MTR is connected to both the size of the liquid pool (M_0^A) and the rate of longitudinal relaxation (R_A). MTR also varies significantly based on the chosen imaging parameters and field strength, necessitating a qMTI method to effectively compare values between studies.

Metrics derived from the two-pool model give a more complete representation of the bound/liquid pools and their magnetization transfer rates.

In general, f is lower in healthy grey matter when compared to healthy white matter

[81, 220, 198, 273, 248, 221, 274, 58] with variations between different healthy WM regions associated with myelin content [221]. Decreased f appears to be the most consistent qMTI characteristic of MS lesions and is often associated with myelin loss [220, 198, 273, 248, 49, 211, 58]. f will also decrease as the liquid pool size increases or due to other changes in the bound pool, such as axonal loss, gliosis, or increased cellularity [273]. These observations have been confirmed by histological comparisons, where f had a strong correlation with myelin content and axonal density in studies of postmortem MS brain [211]. Underhill *et al.* also found strong correlation between f and myelin content in an *in vivo* rat brain study, but they only found weak correlation with axonal density [253]. Ou *et al.* found f to be specific to myelin content in the optic nerves of control and shiverer mice, but not to axonal degeneration. Additional care needs to be taken when interpreting qMTI parameters found in fixed tissue, as fixation has been found to increase f by $\approx 50\%$ when compared to unfixed tissue [212], although correlation to myelin content remains strong [212, 61].

T_2^A should be approximately equivalent to the T_2 value found with a monoexponential CPMG experiment. As such, it will be influenced by the same tissue characteristics described in Section 3.1.5. We can expect T_2^A to be lower in healthy WM when compared to GM and higher in MS lesions, however, without much specificity to different tissue pathologies. T_2^B is slightly lower in GM and MS lesions when compared to healthy WM [220, 273, 248, 49], however the variations are small between tissue and also dependent on the lineshape used to model the bound pool (usually Super-Lorentzian, but sometimes Gaussian [198, 248]).

RM_0^A can also vary depending on tissue, however it has large variability in the pulsed saturation experiment [219, 194, 253], possibly due to sensitivity in the fitting model and timing of the MT pulse sequence [221]. RM_0^A was found to be higher in healthy WM when compared to GM and MS lesions [220, 221, 274, 252, 61, 58]. Conversely, in a healthy ferret, RM_0^A was lower in WM when compared to GM [78]. RM_0^A was also slightly lower in the spinal cord [194] and optic nerve [181] of control mice when compared to a mouse model of dysmyelination. SIR-based experiments, which might provide a more direct measure of the magnetization transfer rate, yield values almost half that found with pulsed saturation experiments [78, 58].

In summary, f appears to be the most specific qMTI metric with respect to myelin content. While RM_0^A and T_2^B may provide additional insight into tissue pathology, they suffer from high variability and low tissue contrast, respectively.

3.4 Hybrid and Multiparametric Approaches

Using two or more quantitative MRI methods, we can find complementary information about tissue pathology. When looking at studies that combine multiple quantitative MRI methods, it is useful to distinguish between hybrid methods, which combine two quantitative methods into a single pulse sequence, and multiparametric approaches, which compare two or more quantitative MRI methods acquired using separate pulse sequences.

3.4.1 Hybrid Methods

Several hybrid methods have been proposed to better characterize different tissue compartments and understand the interplay of different quantitative MRI methods.

T_1 and T_2

Simultaneous T_1 and T_2 measurements can be acquired to better distinguish T_1 - T_2 components in tissue [55, 53]. In particular, Does and Gore observed three distinct T_1 - T_2 compartments in trigeminal nerve which could be individually eliminated using an inversion recovery preparation [55]. Multi-exponential T_1 is more readily observed using integrated T_1 - T_2 measurements and could be another useful indicator of myelin and intra-/extracellular water compartments.

Magnetization Transfer in Multicomponent T_2

Magnetization transfer prepared T_2 components can be acquired by applying an MT saturation pulse prior to a CPMG sequence [94, 228, 258]. The magnetization transfer ratio can then be measured in both the short and long T_2 compartments. In essence, this splits the liquid pool in the two-pool model of MT into two liquid pools represented by the short- and long- T_2 compartments. As expected, the short- T_2 compartment associated with

myelin water has a significantly larger MTR than the long- T_2 compartment [258]. Stanis *et al.* showed that magnetization transfer was approximately nine times larger in the short- T_2 component of bovine optic nerve, suggesting that magnetization transfer is mediated primarily through the myelin water [228]. In another study of porcine and bovine white matter, the MT curves of the two T_2 components were nearly identical [94]. In that experiment, it was suggested that exchange between the two T_2 compartments was on a timescale characteristic of the MT experiment [94]. In fact, magnetization transfer is particularly sensitive to repetition time, thus short- T_R imaging methods such as the spoiled gradient echo method are recommended [81]. In order to measure the MT in different T_2 compartments adequately, MT saturation pulses might need to be interleaved in between the π -pulses of a CPMG sequence.

Magnetization Transfer and Diffusion

Magnetization transfer prepared diffusion-weighted imaging can be used to measure the diffusion of water saturated due to an off-resonance MT pulse [204, 9]. If myelin water is in fact significantly more affected by magnetization transfer [228], then an MT saturated diffusion measurement will be more significantly affected due to saturation in the myelin water compartment. Avram *et al.* found significantly higher fractional anisotropy in white matter using an MT-prepared DTI pulse sequence and proposed this was reflective of the myelin water trapped in the myelin sheathes [9]. Although an interesting approach, correlation between the short- T_2 myelin water fraction and the magnetization transfer ratio are weak [162]. Further work needs to be done to show how myelin water interacts with the bound pool.

Diffusion in Multicomponent T_2

A PGSE sequence can be combined with a CPMG sequence to acquire diffusion-weighted multicomponent T_2 measurements [256, 20, 226, 187, 7]. Differing diffusion in short- and long- T_2 compartments might give additional information about the underlying tissue structure [226]. Unlike Avram *et al.*'s results for MT-prepared DTI [9], two studies found larger anisotropy in the long- T_2 component associated with intra-/extracellular water in bovine

optic nerve [226] and frog sciatic nerve [187]. These results suggest that diffusion anisotropy is mediated more by the restricted diffusion in and around fiber tracts than due to restricted diffusion in the myelin water itself. In contrast, Beaulieu *et al.* found ADC and anisotropy to be relatively constant across different T_2 components [20] and Andrews *et al.* found higher anisotropy in the myelin water compartment [7], making the correct interpretation of multicomponent T_2 diffusion controversial.

Low SNR is a common problem for all hybrid methods, as signal attenuation increases when multiple quantitative methods are combined. In order to achieve sufficient signal, long imaging times are required. In addition, unforeseen interactions between different pulses and gradients might alter the acquired images and the resultant quantitative measurements. Given these confounding factors and the varying results of different hybrid methods, a multiparametric approach might yield more consistent complementary information about tissue pathology.

3.4.2 Multiparametric Approaches

qMTI and Multicomponent T_2

Comparisons of T_2 measurements to qMTI metrics show conflicting results. Using a discriminant function analysis, Merkler *et al.* demonstrated that *in vivo* myelin status could be predicted using a combination of T_1 -weighted, T_2 -weighted, and MT images [164]. McCreary *et al.* suggested MWF reflects the time course of myelin histology more closely than MTR when imaging demyelination and remyelination in murine spinal cord [162]. Conversely, Levesque *et al.* suggested qMTI parameters (f and T_2^A) were more sensitive to MS recovery and less variable than MWF, reflecting quick resolution of inflammation followed by slower partial remyelination [145].

Correlation of qMTI and T_2 metrics will be different in fixed tissue, where slower inter-compartmental exchange might increase the apparent MWF while cross-linking of macromolecules will shorten T_1 and increase f [212]. Given the dependence of MTR on both T_1 and f , fixation might have unpredictable results. For example, MTR was reduced after fixation in a study by Schmierer *et al.* [212].

Weak correlation between MWF and MTR or f [257, 249, 61, 146] might arise due to differing sensitivities to pathological processes: MWF appears to be more sensitive to regional variations in axonal diameter and intercompartmental exchange rates [61], while MTR and f are influenced by macromolecules other than myelin and by inflammation, which increase the bound and liquid pool sizes, respectively [72, 249]. Weak correlation suggests that MWF and f are sensitive to different aspects of tissue pathology, and thus measuring both would be beneficial. Correlation appears to increase when using a voxel-based correlation in specific white matter tracts, where variations in the liquid pool size and intercompartmental exchange are relatively small ($R = 0.18$ to 0.56) [61].

DTI and Multicomponent T_2

In a study by Kolind *et al.*, MS lesions with a long- T_2 component ($200 \text{ ms} < LT_2 < 800 \text{ ms}$) had strong negative correlations between myelin water content (related to MWF) and $\langle D \rangle$, $\lambda_{\perp}$, and $\lambda_{\parallel}$ ($R^2 = 0.61, 0.54$, and 0.60 , respectively). In the same lesions, strong positive correlations were also found between the long T_2 fraction, and $\langle D \rangle$, $\lambda_{\perp}$, and $\lambda_{\parallel}$ ($R^2 = 0.64, 0.69$, and 0.42 , respectively). Both T_2 metrics had weak correlations with FA and weak correlations with all DTI metrics in lesions without a long- T_2 component [127]. Previously, it had been shown that long- T_2 lesions were accompanied by increased T_1 , T_2 , and water content values and decreased MTR, suggesting greater tissue destruction and possibly inflammation or edema [137]. Thus, increased $\langle D \rangle$, $\lambda_{\perp}$, and $\lambda_{\parallel}$ in long- T_2 lesions is not entirely unexpected [127].

In a study of healthy human brains, Mädler *et al.* found varying correlations when comparing MWF and DTI metrics in GM and WM structures [159]. Correlation of MWF and FA was moderate at 1.5 T ($R^2 = 0.39$), but stronger at 3 T ($R^2 = 0.56$). Correlation of MWF and $\lambda_{\perp}$ was stronger at both field strengths ($R^2 = 0.65$ and $R^2 = 0.77$, respectively). Using a voxel-based correlation for an entire slice of the axial midbrain, correlation between FA and MWF was stronger still ($R^2 = 0.75$), as were voxel-base correlations in various white matter structures ($R^2 = 0.78$ in frontal WM, $R^2 = 0.81$ in central WM, and $R^2 = 0.80$ in posterior WM) [159]. Mädler concluded that, while diffusion anisotropy appears to be correlated with the MWF, this correlation is dependent on the tissue structure analyzed

[159]. For example, Liu *et al.* found weak correlations between MWF and $\langle D \rangle$, $\lambda_{\perp}$, $\lambda_{\parallel}$, and FA ($R = -0.27, -0.35, 0.22$, and 0.40 , respectively) when comparing all subjects and regions in the human corpus callosum [152].

MWF is sensitive to water trapped in the myelin bilayers (albeit, moderated by intercompartmental exchange). DTI metrics are sensitive to water movement in the intra-/extracellular water compartments and are strongly influenced by fibre organization, independent of myelin content. Given their shared sensitivity to the liquid pool and its relation to tissue structure, moderate correlation is not surprising when analyzing a broad spectrum of tissue pathologies. However, strong correlation appears to depend on the type of tissue pathology and tissue structures included in the analysis [159].

DTI and qMTI

Correlation between MTR and $\langle D \rangle$ varies, with some groups finding moderate correlation in all MS lesions ($R = -0.7$ [42]) or only in severely damaged MS lesions ($R = -0.758$ [179]). In normal appearing white matter (NAWM) of MS patients, correlations between MTR and DTI metrics were insignificant [108, 86], however, when all white matter regions were considered (lesions, NAWM next to lesions, and NAWM remote from lesions), there was a strong correlation between FA and MTR ($R = 0.89$) and $\langle D \rangle$ and MTR ($R = -0.99$) [86]. Looking at the profiles of MTR and DTI metrics along the corticospinal tract in MS, Reich *et al.* found tract asymmetry between individual patients, between metrics, and between patients and controls [201]. In general, $\langle D \rangle$, $\lambda_{\perp}$, and $\lambda_{\parallel}$ were increased, FA was unchanged, and MTR was decreased in patients with MS [201]. MTR is dependent on f , longitudinal relaxation, and exchange. Increased intercompartmental exchange, which is influenced by both diffusion and membrane permeability, might explain the larger correlations observed in MS lesions.

Ou *et al.* found moderate correlation between average values of F and $\lambda_{\perp}$ in the corpus callosum of control and shiverer mice ($R = -0.57$) but not in control and shiverer mice separately [180]. In a study comparing injured and uninjured optic nerves in shiverer and control mice, Ou *et al.* found $\lambda_{\perp}$ and F were significantly different between shiverer and control mice, but not between injured and uninjured nerves [181]. Conversely, $\lambda_{\parallel}$ and

relative anisotropy were significantly different between injured and uninjured nerves, but not between shiverer and control mice. These results appear to confirm the specificity of f and $\lambda_{\perp}$ to myelin integrity and $\lambda_{\parallel}$ to axonal integrity. It is important to note that fixed tissue was used in both studies, which has been shown to substantially increase f and decrease diffusivity in tissue (although anisotropy remains relatively constant post-fixation) [239, 212].

In a study of healthy human brains, Underhill *et al.* found that correlation between qMTI and DTI metrics was poor in white matter but strong between qMTI metrics (f , RM_0^B , and R_{1obs}) and DTI metrics (FA, $\langle D \rangle$, and $\lambda_{\perp}$) in grey matter ($|R| = 0.59$ to 0.92) [252]. When comparing all tissues, correlation was also strong between the same qMTI metrics and FA, $\lambda_{\parallel}$, and $\lambda_{\perp}$ ($|R| = 0.48$ to 0.75) [252]. In a study of the motor pyramidal system in patients with benign MS, F and RM_0^B were significantly reduced, but T_2^B and FA were not [225]. No correlation was found between FA and any of the qMTI metrics [225].

In another study of healthy human brains, Stikov *et al.* found weak voxel-based correlations between f and FA in all white matter regions ($R = 0.12$ to 0.19), but in white matter tracts with high directional coherence, such as the corpus callosum, correlations appeared to increase ($R = 0.19$ to 0.42) as did correlations across mean values in the major white matter tracts ($R = 0.65$) [234]. Stikov *et al.* went on to estimate the axon diameter: fiber diameter ratio (g) in directionally coherent white matter. Using a simulation of diffusion in typical fiber caliber distributions, they found a relationship between the fiber volume fraction (FVF) and FA, and, based on the relationship between the myelin volume fraction (MVF) and f reported by Dula *et al.* [61], they calculated the g -ratio [234]:

$$g = \sqrt{1 - \frac{MV F}{FV F}}. \quad (3.95)$$

Based on their tissue model, Stikov *et al.* conclude that FA and f covary primarily due to variations in the fiber volume fraction. Differences arise due to variations in the directional coherence of fibers (affecting diffusion anisotropy but not f) and myelin density (affecting f more than it affects diffusion). In addition, both FA and f appeared to be unaffected by variations in the fiber caliber distribution [234].

Conclusion

Weak correlations between f and MWF and between f and diffusion anisotropy suggest that they provide complementary information about tissue pathology. Correlations between MWF and DTI metrics were generally stronger, suggesting more shared underlying influences, such as intercompartmental water exchange and myelin integrity. The interrelationship between quantitative MRI methods and tissue pathology is the focus of the remaining chapters of this dissertation. Chapter 4 details quantitative MRI results in agarose gels, excised rat spinal cords, and fixed mouse brains. Chapter 5 combines a multiparametric MRI study with electron microscopy in the cuprizone mouse model of demyelination.

Chapter 4

Quantitative MRI of Agar, Fixed Rat Spinal Cords, and the Cuprizone Mouse Model of Demyelination

During the development of the MR imaging and analysis methods, several phantoms and tissues were imaged. Agarose gel (agar) possesses similar characteristics to tissue, including a liquid and bound pool that can be fitted to the two-pool model of magnetization transfer [99, 219]. Fixed rat spinal cords feature directionally coherent white matter tracts. Alongside a water phantom, measurements were used to test the DTI methods and assess the stability of the MRI. In addition, T_1 and T_2 measurements could be compared to previous studies conducted at 7 T. When fed to C57BL/6 mice, the copper chelator cuprizone causes cell death of oligodendrocytes and subsequent demyelination. It is a predictable model of demyelination that has been used in numerous studies, with applications to white matter diseases such as multiple sclerosis and schizophrenia. Cuprizone-fed C57BL/6 mice were imaged at various stages of demyelination, both *in vivo* and *ex vivo*, culminating in a longitudinal and quantitative MRI study with comparisons to electron microscopy in Chapter 5. Methods for acquiring and analyzing multiple quantitative MRI datasets in the cuprizone mouse model are detailed in Section 4.3.

4.1 Agar

Agar is a useful analogue to tissue when validating the two-pool model of magnetization transfer. Quantitative MTI measurements in 2%, 4%, and 8% (w/w) agar gels were compared to previous continuous wave [99] and pulsed magnetization transfer [220] measurements.

4.1.1 Sample Preparation and MRI

2%, 4%, and 8% (w/w) agar gels were prepared using agar powder (Sigma-Aldrich) and distilled water and poured into small sample tubes [21]. After cooling, the sample tubes were placed in a custom-built 38 mm inductively coupled quadrature RF volume coil (NRC Institute for Biodiagnostics, Winnipeg, MB, Canada). All MR experiments were performed on a 7 T 21 cm Bruker Avance III NMR system with a BGA12-S actively shielded gradient system and Paravision 5.

A 1 mm thick slice was placed perpendicular to the three sample tubes with an FOV of 4x4 cm and a 256x256 matrix size for an in-plane resolution of 156x156 μm^2 . A proton density image, M_0 was acquired using a Fast Low Angle Shot (FLASH) sequence [88] ($T_E/T_R = 6/70$ ms, 10° flip angle). Eighteen magnetization transfer images were acquired with the same sequence preceded by a magnetization transfer saturation pulse (Gaussian-shaped RF pulse, 10.25 ms long) with peak RF amplitudes of 5, 10, and 20 μT and frequency offsets at each power of 1000, 2000, 4000, 6000, 10000, and 30000 Hz. MTI datasets were acquired with 32 averages for a total acquisition time of 3 hours. Combined T_1 and T_2 relaxometry data were acquired using a series of RARE acquisitions (effective $T_E = 11, 33, 55, 77$, and 99 ms; $T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5$, and 95.5 ms; RARE factor = 2; 16 averages; 4h 39m acquisition time).

4.1.2 Image Analysis

Circular regions of interest were defined in the 2%, 4% and 8% agar images. A threshold mask was created from the M_0 image to remove bubbles and applied to all images prior to fitting. A Gaussian lineshape was used to represent the bound pool lineshape, because it

gives the best fit for rigid systems such as agar gels [99, 220]. The SNR in each image was calculated according to Equation (4.17).

T_1 maps were generated by fitting RARE images (effective $T_E = 11$ ms; $T_R = 4895.5$, 2895.5, 1395.5, 695.5, 295.5, and 95.5 ms) to the saturation recovery curve (see Equation (3.1) and Appendix C.3.1). Single-component T_2 maps were generated by fitting RARE images (effective $T_E = 11$, 33, 55, 77, and 99 ms; $T_R = 4895.5$ ms) to the spin echo decay curve (see Equation (3.4) and Appendix C.3.2).

Magnetization transfer images were normalized to M_0 and fit to the two-pool model of magnetization transfer (Equation (3.81)) [99] with continuous wave pulse equivalent approximations (Equation (3.87)) [198] and a Gaussian absorption lineshape for the bound pool (Equation (3.76)). For the peak RF amplitudes of 5, 10, and 20 μT , $\omega_{1CWPE} = 279.7$, 559.5, and 1119 rad/s (Equation (3.87)) [198]. Relevant metrics derived from the two-pool model include the magnetization transfer rate between the free and bound pools (RM_0^A), longitudinal relaxation rates of the free and bound pools (R_A and R_B), transverse relaxation times of the free and bound pools (T_2^A and T_2^B), and the bound pool fraction (f). R_B was fixed arbitrarily to 1 s^{-1} as recommended by several groups [99, 219, 198]. R_A was calculated using the T_1 map (Equation (3.88)) [99, 198]. Using the fitted value of $f/R_A(1-f)$ in Equation (3.81), the bound pool fraction, f was calculated using R_A and Equation (3.89).

To show goodness of fit between the two-pool model and signal, a χ^2/ν map and mean χ^2/ν values were calculated as per Equation (4.18), where ν is the degrees of freedom (19 images - 6 model parameters = 13).

4.1.3 Results and Discussion

SNR was larger than 100 in most images (Table 4.1), ensuring that the influence of noise would be minimal [41].

In all three preparations, f and R_A were slightly lower than previously reported values measured using continuous wave [99] and pulsed magnetization transfer measurements [220]

Table 4.1: SNR measurements in qMTI images based on signal in 2%, 4%, and 8% agar regions of interest.

		Offset Frequency (kHz)					
	RF Power	1	2	4	6	10	30
2% Agar	5 μ T	188	218	212	205	196	225
	10 μ T	176	205	193	193	218	212
	20 μ T	156	186	183	198	212	229
	M_0	193					
4% Agar	5 μ T	167	198	194	189	181	209
	10 μ T	143	178	172	175	198	197
	20 μ T	111	147	154	169	186	211
	M_0	179					
8% Agar	5 μ T	138	169	168	165	158	184
	10 μ T	108	144	144	148	169	173
	20 μ T	74	108	120	135	152	184
	M_0	158					

Table 4.2: qMTI metrics in 2%, 4%, and 8% agar. RM_0^B and F reported in the original references were changed to RM_0^A and f according to equations (3.90) and (3.93), respectively. Values reported as mean (standard deviation).

Metric	Reference	2% Agar	4% Agar	8% Agar
f	Our results	0.0042 (0.0008)	0.0080 (0.0010)	0.0152 (0.0017)
	[99]	0.0051 (0.0010)	0.0109 (0.0020)	0.0215 (0.0040)
	[220]	0.0066 (0.0004)	0.0120 (0.0020)	0.0253 (0.0020)
R_A (s ⁻¹)	Our results	0.450 (0.006)	0.504 (0.009)	0.624 (0.009)
	[99]	0.51 (0.07)	0.7 (0.1)	1.08 (0.16)
	[220]	0.419 (0.02)	0.483 (0.1)	0.76 (0.04)
RM_0^A (s ⁻¹)	Our results	27 (11)	26 (7)	26 (6)
	[99]	176 (20)	164 (18)	177 (23)
	[220]	111 (8)	109 (17)	107 (12)
T_2^A (ms)	Our results	157 (12)	69 (4)	32 (3)
	[99]	63 (8)	32 (4)	16 (2)
	[220]	56.4 (3)	33.7 (2)	16.8 (0.9)
T_2^B (μ s)	Our results	25.6 (2.4)	24.2 (1.7)	23.2 (1.6)
	[99]	12.9 (0.1)	12.9 (0.1)	12.9 (0.1)
	[220]	14.3 (0.5)	14.1 (0.3)	14.1 (0.3)
T_2 (ms)	Our results	90.9 (6.7)	46.6 (2.1)	26.8 (2.1)
	[220]	70.8 (3)	40.1 (1)	18.4 (0.5)
χ^2/ν		3.3 (1.0)	4.6 (1.3)	5.0 (1.3)

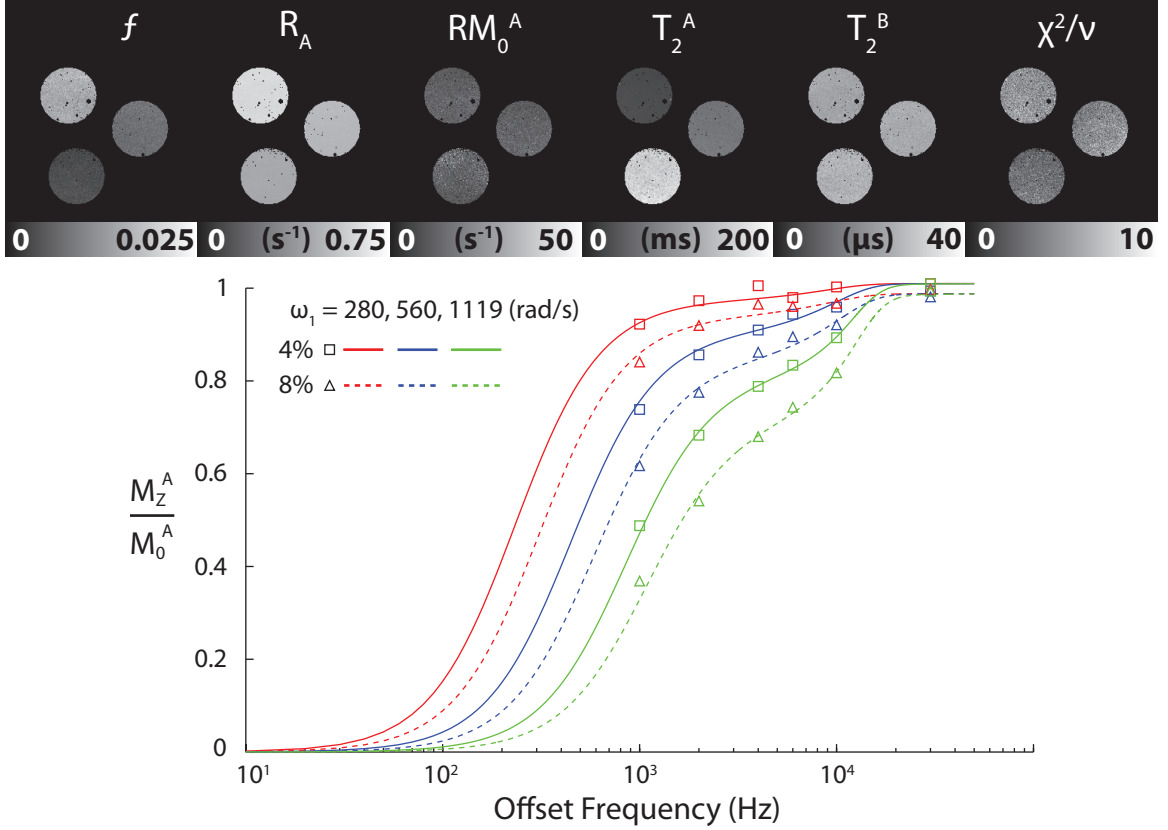


Figure 4.1: Thresholded quantitative MTI maps along with the χ^2/ν map. 8% agar is on the top, 4% on the right, and 2% on the bottom. f , R_A , and T_2^A differentiate different concentrations of agar. Quantitative MT curves are shown below for a voxel in the 4% agar (solid line/squares) and 8% agar (dashed line/triangles).

(Table 4.2). These discrepancies can be attributed to differences in acquisition and agar preparation. However, RM_0^A was 4 to 6 times smaller and T_2^A and T_2^B were double the values found by Henkelman *et al.* [99] and Sled and Pike [220]. The T_2 value measured independently was smaller than T_2^A but larger than the T_2 value reported by Sled and Pike [220]. Larger T_2 values can be attributed to stimulated echoes in the RARE sequence contributing to an overestimation of T_2 . Both of the referenced studies were performed at 1.5 T, however, T_2 only decreases slightly with increasing field strength while RM_0^A is relatively constant [100]. In addition, Ramani *et al.*'s pulse approximation has been shown to underestimate RM_0^A and overestimate T_2^A , but only by 12–15% [194].

Close inspection of the two-pool model equations and code didn't reveal any discrepancies, however, it was discovered that the fit doesn't change when the ω_{1CWPPE} values are

scaled by a constant factor, while the estimated parameters change substantially. Inspection of the ω_{1CWPE} approximations didn't reveal any differences from Ramani *et al.* [198], and the calculated flip angle of the MT pulse (Equation (3.86)) exactly matched the one calculated by the scanner software.

To test the assumption that the ω_{1CWPE} values were underestimated, the two-pool model was fit to average signal values in 8% agar MTI data while scaling the ω_{1CWPE} values from $k = 0.5 \dots 5$. Some parameters went to infinity or zero at $k < 0.5$ and approached a constant at $k = 5$. Plots of the qMTI parameters vs. k show the effects of varying ω_{1CWPE} (Figure 4.2). Importantly, as ω_{1CWPE} was scaled, the fitted curves remained unchanged. Between $k = 2$ and 3, RM_0^A approached the values found previously in agar, however other parameters were significantly smaller (f) or significantly larger (T_2^A) than Henkelman [99] *et al.* or Sled and Pike [220] (Table 4.3). R_A and T_2^B were relatively constant with changing ω_{1CWPE} . Given the offset frequencies and relative saturation strengths of an MTI dataset, R_A and T_2^B parameters could potentially be determined without knowing the actual ω_{1CWPE} values.

Table 4.3: Comparison of qMTI parameters fit to the average signal values in 8% agar MTI data while scaling ω_{1CWPE} by a factor, k . At $k = 1$, values correspond to those determined in Table 4.2.

k	f	R_A (s ⁻¹)	RM_0^A (s ⁻¹)	T_2^A (ms)	T_2^B (μ s)
0.5	0.0654	0.605	5	8	23.4
1	0.0150	0.624	25	32	23.3
1.5	0.0066	0.627	58	71	23.2
2	0.0037	0.628	104	127	23.2
2.5	0.0023	0.629	164	199	23.2
3	0.0016	0.629	237	286	23.2

While optimal offset frequencies and saturation strengths have been studied previously [40, 41, 147], the effects of signal noise on parameter estimates has only been addressed briefly [41], and to our knowledge, no study has assessed the impact of inaccurate saturation strengths. This study shows that a constant scale factor applied to ω_1 can have a significant effect on some two-pool model parameters. The effects of non-linear and random variations

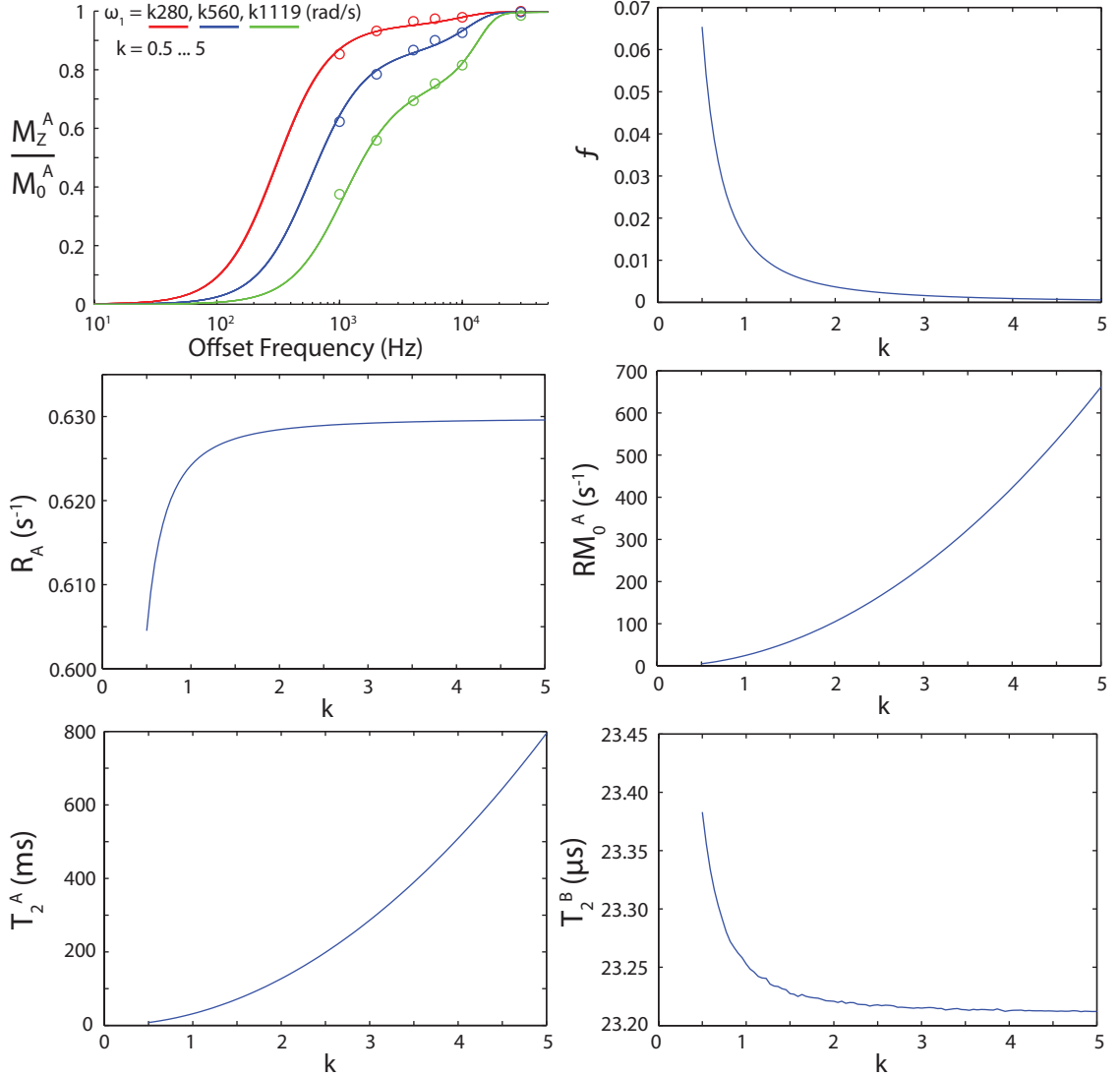


Figure 4.2: Quantitative MT curves (top left) for 8% agar while scaling ω_{1CWPE} by a factor of $k = 0.5 \dots 5$. MT curves don't change, but model parameters do. Specifically, f goes from 0.07 to 0, RM_0^A goes from 0 to almost 700 s^{-1} , and T_2^A goes from 0 to almost 800 ms. R_A and T_2^B are relatively constant, but precipitously drop or rise, respectively, as k goes to 0.

in ω_{1CWPE} might merit further investigation.

Although RM_0^A , T_2^A , and T_2^B did not match the previously reported values, they followed the same trends. T_2^A decreased linearly as agar concentration increased and both RM_0^A and T_2^B remained relatively constant. Both RM_0^A and T_2^A have been shown to have large variability in tissue [194, 221, 198, 253] while T_2^B shows little contrast between different tissues [220, 273, 248, 49]. The most consistent and relevant metric, f , increased linearly

with agar concentration and was close to the values reported previously [99, 220].

4.2 Rat Spinal Cord

Excised spinal cord has long, directionally coherent white matter tracts. As such, it provides an excellent starting point for verifying DTI measurements. Two excised rat spinal cords were imaged adjacent to 0.1 M phosphate-buffered saline (PBS) solution contained in NMR glass. Measurements presented here were made with varying gradient spacing (Δ) and gradient strength (g) but a constant gradient duration (δ) and b-value. Additional measurements were made where the b-value was allowed to vary with either gradient spacing or gradient strength. These measurements are not presented here, but are currently being compared to a Monte Carlo simulation of diffusion in order to understand better the influence DTI acquisition parameters have on measurements of diffusivity. For the purposes of this study, the results will focus solely on the fixed b/δ and varying Δ/g measurements.

4.2.1 Methods

Sample Preparation

Two healthy adult rats were sacrificed using transcardiac perfusion. Under anesthetic, PBS solution was injected into the left ventricle of the heart for 10 minutes, followed by 4% paraformaldehyde (PFA) in 0.1 M PBS (pH 7.6). Spinal cords were excised and fixed in 4% PFA for one week. Prior to imaging, spinal cords were washed in 0.1 M PBS for 48 hours to remove any excess fixative. After washing, two thoracic spinal cord sections were wrapped together with Teflon tape along with an NMR glass containing 0.1 M PBS solution capped on both ends with paraffin wax. Teflon tape was wrapped around both ends of the sample to hold it firmly in a 50 mL plastic sample tube. The tube was filled with room-temperature FOMBLIN®Perfluoropolyether Y04 grade fluid (Solvay Solexis) to eliminate external proton signal and susceptibility artifacts and maintain moisture in the samples. The sample holder was left at room temperature for several hours to allow the sample temperature to equilibrate.

MRI

The sample tube was placed in a custom-built 33 mm inductively coupled quadrature RF volume coil (NRC Institute for Biodiagnostics, Winnipeg, MB, Canada). All MR experiments were performed on a 7 T 21 cm Bruker Avance III NMR system with a BGA12-S actively shielded gradient system and Paravision 5.

Three 1 mm thick slices, spaced 3 mm apart, were placed perpendicular to the two spinal cords and the PBS sample. All acquisitions had an FOV of 3x3 cm and a 256x256 matrix size for an in-plane resolution of 117x117 μm^2 .

T_1 and T_2 relaxometry data were acquired using a series of RARE acquisitions (effective $T_E = 11, 33, 55, 77$, and 99 ms; $T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5$, and 95.5 ms; RARE factor = 2; 4 averages; 70 minute acquisition time) before and after the DTI experiments.

DTI data were acquired with a PGSE sequence using a 6-direction icosahedral gradient-encoding scheme. In order to accommodate a large range of diffusion times and reduce the effect of T_2 relaxation, T_E was held constant at 75 ms. $T_R = 1500$ ms and gradient pulse duration, $\delta = 4.5$ ms. The low b-value was 1.23 s/mm² and the high b-value ranged from 979 to 1002 s/mm² dependent on gradient direction. Fat suppression was turned on to reduce chemical shift artifacts and the readout bandwidth was relatively low (12.5 kHz) to improve SNR. Eight different gradient separation times were logarithmically spaced between 8 and 56.5 ms ($\Delta = 8, 10.5, 14, 18.5, 24.5, 32.5, 42.5$, and 56.5 ms). At $\Delta = 8$ ms, the gradient strength was 78.3% of $g_{max} = 450$ mT/m to achieve $b = 1000$ s/mm², but only 25.8% of g_{max} at $\Delta = 56.5$ ms. The effective diffusion times are $\Delta - \delta/3 = 6.5, 9, 12.5, 17, 23, 31, 41$, and 55 ms. Each DTI experiment was acquired with 8 averages for a total acquisition time of 6 hours x 8 experiments = 48 hours.

Image Analysis

Using a custom-built MATLAB tool (Appendix C.1.2), all images were registered to the low b-value image from the first experiment ($\Delta = 8$ ms) using a rigid affine transformation matrix determined automatically by maximizing the 2D correlation coefficient between the

two images [192]. An anisotropic diffusion filter was subsequently applied to improve the apparent SNR [188, 73]. The filter was applied with 3 iterations and a gradient modulus threshold equal to three times the standard deviation of the noise in each image [115].

T_1 maps were generated by fitting RARE images (effective $T_E = 11\text{ms}$; $T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5$, and 95.5 ms) to the saturation recovery curve (Equation (3.1)). T_2 maps were generated by fitting RARE images (effective $T_E = 11, 33, 55, 77$, and 99 ms; $T_R = 4895.5$ ms) to the spin echo decay curve (Equation (3.4)). Diffusion tensors were determined using a non-linear least squares fit and a modified Cholesky decomposition to ensure positive definiteness [125]. After diagonalization, DTI metric maps were calculated: fractional anisotropy (FA), mean diffusivity ($\langle D \rangle$), axial diffusivity ($\lambda_{\parallel}$), and radial diffusivity ($\lambda_{\perp}$). Regions of interest were defined in the PBS sample and in the left, right, ventral, and dorsal regions of the white matter and in the left and right regions of the grey matter of the spinal cords in all three slices. DTI metrics in each region were plotted against diffusion time.

One-way ANOVA was used to test whether DTI metrics in each ROI were significantly different at different diffusion times. One-way ANOVA was also used to test whether DTI metrics at each diffusion time were significantly different between regions of interest. Both tests were followed by Tukey's post-hoc test. Paired samples T-tests were used to compare filtered and non-filtered DTI results in each region of interest.

4.2.2 Results & Discussion

Filtered and Non-Filtered Results

The anisotropic diffusion filter was used to smooth the noise in individual diffusion-weighted images while maintaining anatomical features. It has previously been shown to improve the noise performance in multicomponent T_2 analysis [113, 107] and DTI [183]. Filtered and non-filtered DTI metrics were significantly different in each region of interest.

Table 4.4: Non-filtered vs. filtered DTI metrics in fixed rat spine and PBS. Values reported as mean (standard error of the mean) from all diffusion times and slices (n = 48 for rat spine ROIs and n = 24 for PBS ROIs). Pearson correlation coefficient (R), mean difference, and percent difference also reported.

Metric	ROI: Reference	WM		Right		Ventral		Dorsal		GM		Right		PBS	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	Non-Filtered	0.471 (0.006)	0.475 (0.006)	0.479 (0.006)	0.479 (0.006)	0.482 (0.005)	0.482 (0.005)	0.388 (0.005)	0.388 (0.005)	0.394 (0.003)	0.394 (0.003)	2.108 (0.004)	2.108 (0.004)	2.108 (0.016)	2.108 (0.016)
	Filtered	0.442 (0.006)	0.446 (0.006)	0.454 (0.005)	0.454 (0.005)	0.454 (0.005)	0.454 (0.005)	0.388 (0.005)	0.388 (0.005)	0.390 (0.003)	0.390 (0.003)	2.108 (0.004)	2.108 (0.004)	2.108 (0.016)	2.108 (0.016)
	Mean Difference	-0.030*	-0.030*	-0.025*	-0.025*	-0.029*	-0.029*	0.000	0.000	0.003*	0.003*	-0.001*	-0.001*	-0.001*	-0.001*
	% Difference	6%	6%	5%	5%	6%	6%	0%	0%	1%	1%	0%	0%	0%	0%
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	Non-Filtered	1.060 (0.012)	1.060 (0.012)	1.070 (0.012)	1.070 (0.012)	1.065 (0.011)	1.065 (0.011)	0.620 (0.006)	0.620 (0.006)	0.642 (0.006)	0.642 (0.006)	2.016 (0.005)	2.016 (0.005)	2.016 (0.014)	2.016 (0.014)
	Filtered	1.043 (0.015)	1.041 (0.015)	1.048 (0.012)	1.048 (0.012)	1.048 (0.012)	1.048 (0.012)	0.614 (0.006)	0.614 (0.006)	0.644 (0.006)	0.644 (0.006)	2.200 (0.006)	2.200 (0.006)	2.200 (0.019)	2.200 (0.019)
	Mean Difference	-0.017	-0.019*	-0.023*	-0.023*	-0.017	-0.017	-0.006*	-0.006*	0.002	0.002	0.184*	0.184*	0.184*	0.184*
	% Difference	2%	2%	2%	2%	2%	2%	1%	1%	0%	0%	9%	9%	9%	9%
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	Non-Filtered	0.176 (0.004)	0.183 (0.004)	0.183 (0.004)	0.183 (0.004)	0.191 (0.004)	0.191 (0.004)	0.272 (0.005)	0.272 (0.005)	0.269 (0.004)	0.269 (0.004)	2.292 (0.005)	2.292 (0.005)	2.292 (0.023)	2.292 (0.023)
	Filtered	0.141 (0.004)	0.148 (0.004)	0.157 (0.003)	0.157 (0.003)	0.157 (0.005)	0.157 (0.005)	0.275 (0.005)	0.275 (0.005)	0.264 (0.004)	0.264 (0.004)	2.062 (0.005)	2.062 (0.005)	2.062 (0.015)	2.062 (0.015)
	Mean Difference	-0.036*	-0.035*	-0.027*	-0.027*	-0.034*	-0.034*	0.002*	0.002*	0.004*	0.004*	-0.231*	-0.231*	-0.231*	-0.231*
	% Difference	23%	21%	16%	16%	20%	20%	1%	1%	2%	2%	11%	11%	11%	11%
FA	Non-Filtered	0.820 (0.003)	0.817 (0.003)	0.814 (0.004)	0.814 (0.004)	0.809 (0.005)	0.809 (0.005)	0.510 (0.005)	0.510 (0.005)	0.542 (0.009)	0.542 (0.009)	0.090 (0.009)	0.090 (0.009)	0.090 (0.003)	0.090 (0.003)
	Filtered	0.855 (0.004)	0.848 (0.004)	0.838 (0.004)	0.838 (0.004)	0.838 (0.005)	0.838 (0.005)	0.499 (0.005)	0.499 (0.005)	0.532 (0.010)	0.532 (0.010)	0.046 (0.010)	0.046 (0.010)	0.046 (0.001)	0.046 (0.001)
	Mean Difference	0.035*	0.032*	0.023*	0.023*	0.029*	0.029*	-0.011*	-0.011*	-0.010*	-0.010*	-0.043*	-0.043*	-0.043*	-0.043*
	% Difference	4%	4%	3%	3%	4%	4%	2%	2%	2%	2%	64%	64%	64%	64%
R		0.839*	0.836*	0.863*	0.863*	0.266	0.266	0.979*	0.979*	0.991*	0.991*	0.695*	0.695*	0.695*	0.695*

* p < 0.05 for paired samples T-test or Pearson correlation coefficient

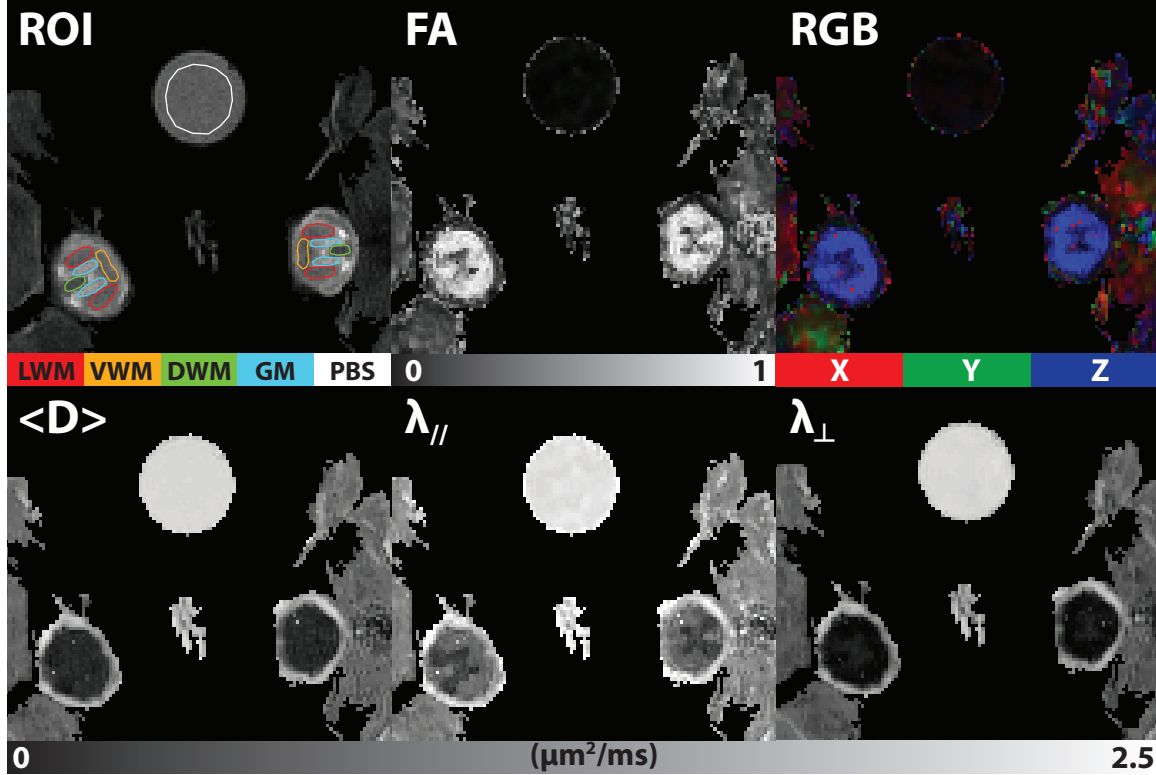


Figure 4.3: DTI ROI and metric maps in rat spinal cord and PBS phantom.

PBS Phantom

Measuring anisotropy in a water phantom (or PBS, in our case) provides a good assessment of the quality of DTI estimates. The PBS phantom had low anisotropy in all experiments. Given the isotropic nature of diffusion in water, a perfect measurement would yield $FA = 0$ and $\lambda_{\parallel} = \lambda_{\perp}$ for each diffusion time, regardless of differences in temperature. Limitations of the method and equipment result in noise, however, yielding an average fractional anisotropy across all experiments and slices of $FA = 0.047 \pm 0.006$. The apparent SNR in the high b-value images of PBS ranged from 17 to 20. In other words, noise contributes to approximately 5% of the signal intensity in each diffusion-weighted image. In a Monte Carlo study of noise in DTI, Jones and Bassar demonstrate that a large part of this apparent anisotropy is probably due to RF noise [119]. At a known $FA = 0.7$, $b = 1000 \text{ s/mm}^2$, and $SNR = 20$, the estimated FA was ≈ 0.75 , or ≈ 0.05 larger than the known value [119]. Experimentally, decreased SNR has been shown to increase FA but not significantly change $\langle D \rangle$ [64]. A similar study found FA values ranging from 0.034 to 0.179 in a water phantom,

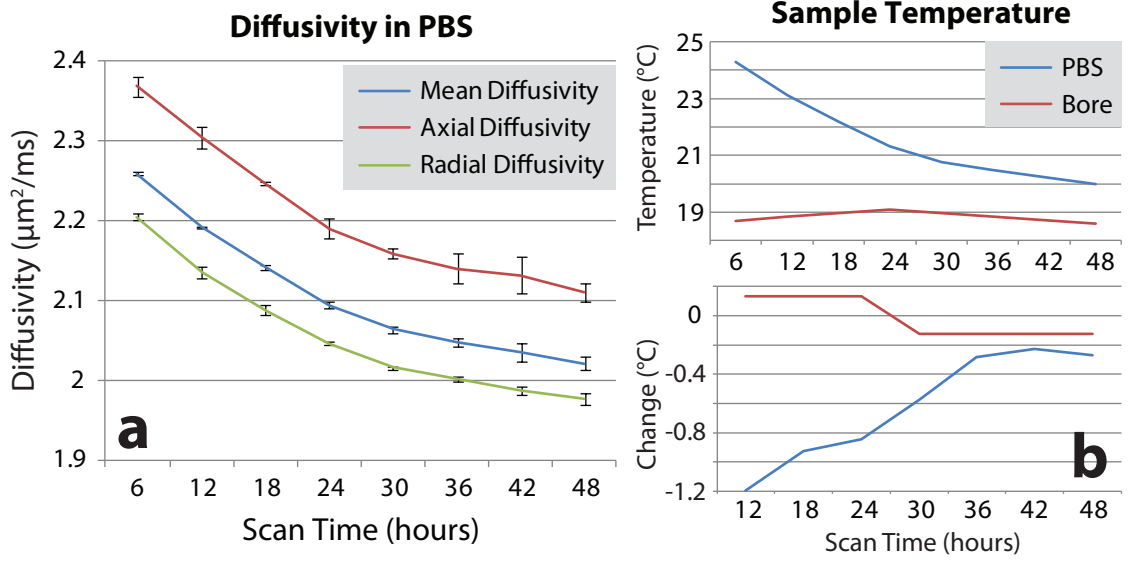


Figure 4.4: (a) Diffusivity decreases with time in the PBS phantom. Difference between $\lambda_{\parallel}$ and $\lambda_{\perp}$ represents small anisotropy attributable to RF noise. (b) Relating the diffusivity of PBS to temperature, we can see the change in temperature with time as the sample temperature equilibrates to the bore temperature. The largest change is at the start, where the measurements at 6 and 12 hours represent a temperature change of ≈ 0.2 °C/hour.

dependent on the number of averages and gradient encoding scheme used [158].

The diffusivity measurements decreased in each successive PBS experiment (Figure 4.4), but the fractional anisotropy stayed relatively constant (Figure 4.5). This can be attributed to a slow downward drift in temperature. The temperature dependence of self-diffusion in free water can be described by the power-law equation [105]:

$$D = D_0 \left[\frac{T}{T_S} - 1 \right]^{\gamma} \quad (4.1)$$

where $D_0 = (16.35 \pm 0.02) \mu\text{m}^2/\text{s}$, $T_S = (215.05 \pm 1.20) \text{ K}$ and $\gamma = 2.063 \pm 0.051$ are accurate fits in the range from +5 to +55 °C [105]. At room temperature (20 °C), this gives $D = 2.023 \mu\text{m}^2/\text{s}$. We can calculate the temperature of water based on its diffusivity by solving for T:

$$T = T_S \left[\left(\frac{D}{D_0} \right) + 1 \right] \quad (4.2)$$

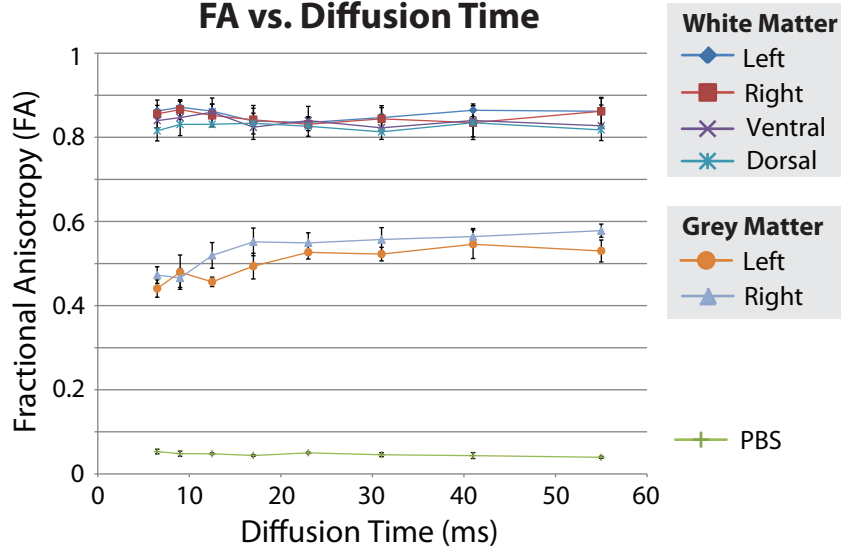


Figure 4.5: Fractional anisotropy vs. diffusion time in fixed rat spine and a PBS phantom. Error bars = $\pm$ standard deviation.

Given the diffusion measurements of PBS during the experiment, we can estimate the average sample temperature during each 6-hour long DTI experiment. The sample temperature goes from 24.3 °C in the first experiment to 20.0 °C in the final experiment, but never reaches the bore temperature of ≈ 19 °C.

The bore temperature was relatively constant throughout the experiment: 18.7 °C before the first DTI acquisition, 19.1 °C after 24 hours, and 18.6 °C after the last acquisition. Prior to the first DTI acquisition, the sample had been in the system for approximately 6 hours, and it was assumed (possibly incorrectly) the sample had gone from room temperature ($\approx 24^\circ\text{C}$) to the bore temperature ($\approx 19^\circ\text{C}$).

Rat Spinal Cord

To account for the temperature drift observed in the PBS phantom, diffusivity measurements were scaled to $T = 20$ °C using a correction factor derived from the estimated mean diffusivity (Equation (4.1)), $D(T = 20^\circ\text{C})$ and the corresponding D_{PBS} measured in each experiment:

$$D_{corrected} = \left(\frac{D(T = 20^\circ\text{C})}{D_{PBS}} \right) \cdot D_{exp} \quad (4.3)$$

Although the temperature dependence of diffusivity in tissue is more complicated than in free water [243] and the tissue changes over a 48 hour period are unknown, a correction was needed and this was our best approximation.

After correcting for the temperature shift, axial diffusivity showed no significant changes in grey and white matter with diffusion times ranging from 6.5 to 55 ms (Figure 4.6). Radial diffusivity didn't change significantly in white matter but did change significantly in grey matter (ANOVA results), decreasing by 29% when comparing measurements at $\Delta = 56.5$ ms to measurements at $\Delta = 8$ ms. Similarly, fractional anisotropy didn't change significantly in white matter but did change significantly in grey matter (ANOVA results), increasing by 34% when comparing measurements at $\Delta = 56.5$ ms to measurements at $\Delta = 8$ ms. One interpretation of these results is that the barriers that restrict diffusion are further apart in the grey matter, such that longer diffusion times allow more restricted diffusion behaviour to be observed.

Table 4.6 compares the DTI metrics to similar measurements made in excised rat spinal cord by Refs. [158, 63, 128]. In particular, results for the dorsal white matter were all close to those previously acquired in fixed cervical spinal cord with similar acquisition parameters to our own [128]. It is important to recognize that acquisition parameters such as gradient encoding schemes and diffusion times can influence the results and could explain differences. Resolution and SNR directly influence the calculated DTI metrics. In particular, larger voxels will tend to average out differences in white matter tracts and may have more partial volume effects, where several different types of tissue are included in a single voxel. This will tend to decrease the observed anisotropy, as seen in [63]. Likewise, decreased SNR increases the observed fractional anisotropy in water [64]. In our DTI maps calculated using filtered diffusion-weighted images (to reduce the adverse effects of noise), FA was decreased in the PBS phantom, increased in white matter, and stayed relatively constant in grey matter compared to non-filtered DTI maps (Table 4.4). The anisotropic diffusion filter is explored in more detail in Section 4.3.4. Finally, increased temperature increases the self-diffusion of water as does the state of the tissue (*in vivo* diffusivity [158] > fixed *ex vivo* diffusivity

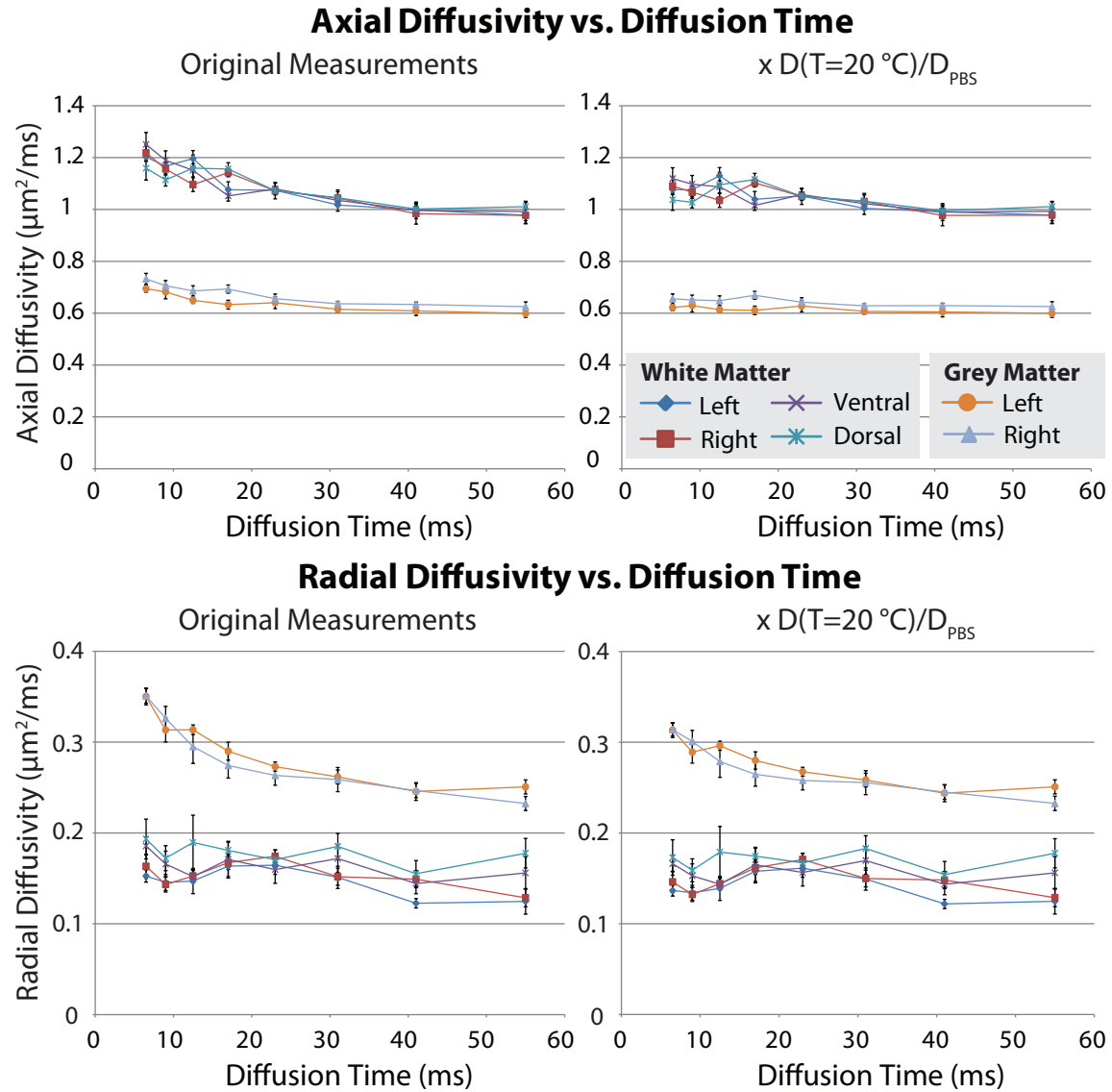


Figure 4.6: Axial and radial diffusivity vs. diffusion time in excised rat spinal cord. Original measurements (left) and measurements normalized to mean diffusivity of water at 20°C . Error bars = $\pm$ standard deviation.

Table 4.5: DTI metrics in fixed rat spine at diffusion times from 6.5 to 55 ms. Values reported as mean (standard error of the mean).

Metric	ROI: Δ (ms)	WM		Right	Ventral	Dorsal	GM		Right				
		Left	Right				Left	Right					
$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	8	0.450	(0.010)	0.461	(0.014)	0.484	(0.019)	0.461	(0.020)	0.416	(0.003)	0.428	(0.004)
	10.5	0.448	(0.015)	0.444	(0.013)	0.468	(0.013)	0.448	(0.007)	0.403	(0.004)	0.418	(0.011)
	14	0.469	(0.029)	0.441	(0.007)	0.458	(0.010)	0.484	(0.034)	0.402	(0.005)	0.402	(0.011)
	18.5	0.452	(0.019)	0.475	(0.010)	0.448	(0.013)	0.488	(0.009)	0.390	(0.005)	0.399	(0.006)
	24.5	0.458	(0.013)	0.464	(0.016)	0.457	(0.015)	0.462	(0.009)	0.387	(0.009)	0.386	(0.006)
	32.5	0.434	(0.018)	0.443	(0.009)	0.454	(0.015)	0.466	(0.014)	0.375	(0.005)	0.380	(0.006)
	42.5	0.412	(0.006)	0.424	(0.010)	0.426	(0.015)	0.434	(0.012)	0.364	(0.005)	0.373	(0.006)
	56.5	0.409	(0.018)	0.412	(0.010)	0.435	(0.018)	0.456	(0.015)	0.367	(0.007)	0.364	(0.009)
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	8	1.076	(0.023)	1.090	(0.034)	1.119	(0.042)	1.037	(0.041)	0.621	(0.012)	0.655	(0.019)
	10.5	1.076	(0.034)	1.066	(0.032)	1.097	(0.034)	1.028	(0.021)	0.630	(0.025)	0.651	(0.018)
	14	1.130	(0.071)	1.035	(0.023)	1.087	(0.024)	1.095	(0.048)	0.614	(0.011)	0.648	(0.019)
	18.5	1.039	(0.037)	1.102	(0.028)	1.016	(0.019)	1.116	(0.023)	0.611	(0.016)	0.669	(0.015)
	24.5	1.052	(0.025)	1.050	(0.036)	1.057	(0.022)	1.051	(0.031)	0.627	(0.022)	0.642	(0.018)
	32.5	1.004	(0.034)	1.031	(0.025)	1.022	(0.018)	1.032	(0.024)	0.607	(0.011)	0.628	(0.008)
	42.5	0.991	(0.021)	0.977	(0.039)	0.990	(0.033)	0.995	(0.019)	0.605	(0.019)	0.629	(0.009)
	56.5	0.978	(0.043)	0.978	(0.032)	0.993	(0.037)	1.011	(0.020)	0.599	(0.016)	0.626	(0.019)
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	8	0.137	(0.006)	0.146	(0.012)	0.166	(0.009)	0.173	(0.020)	0.313	(0.008)	0.314	(0.007)
	10.5	0.134	(0.008)	0.132	(0.008)	0.153	(0.013)	0.159	(0.013)	0.289	(0.012)	0.301	(0.012)
	14	0.139	(0.013)	0.144	(0.007)	0.143	(0.007)	0.179	(0.028)	0.296	(0.005)	0.279	(0.017)
	18.5	0.158	(0.013)	0.162	(0.007)	0.165	(0.018)	0.175	(0.010)	0.280	(0.009)	0.265	(0.013)
	24.5	0.161	(0.010)	0.171	(0.007)	0.156	(0.015)	0.167	(0.011)	0.267	(0.005)	0.258	(0.011)
	32.5	0.149	(0.012)	0.150	(0.009)	0.170	(0.015)	0.183	(0.014)	0.258	(0.008)	0.255	(0.013)
	42.5	0.122	(0.005)	0.148	(0.009)	0.143	(0.011)	0.154	(0.015)	0.244	(0.009)	0.245	(0.008)
	56.5	0.125	(0.014)	0.129	(0.010)	0.156	(0.018)	0.178	(0.016)	0.251	(0.008)	0.233	(0.008)
FA	8	0.862	(0.006)	0.855	(0.014)	0.840	(0.004)	0.815	(0.020)	0.441	(0.021)	0.473	(0.020)
	10.5	0.872	(0.006)	0.866	(0.010)	0.847	(0.016)	0.831	(0.017)	0.479	(0.041)	0.466	(0.023)
	14	0.862	(0.013)	0.852	(0.011)	0.860	(0.008)	0.831	(0.014)	0.456	(0.011)	0.519	(0.031)
	18.5	0.839	(0.012)	0.842	(0.007)	0.824	(0.021)	0.833	(0.012)	0.494	(0.031)	0.551	(0.033)
	24.5	0.835	(0.006)	0.831	(0.006)	0.840	(0.014)	0.826	(0.015)	0.527	(0.016)	0.549	(0.024)
	32.5	0.847	(0.010)	0.844	(0.013)	0.823	(0.013)	0.813	(0.011)	0.522	(0.016)	0.557	(0.028)
	42.5	0.864	(0.006)	0.835	(0.016)	0.840	(0.014)	0.835	(0.016)	0.546	(0.034)	0.564	(0.019)
	56.5	0.862	(0.013)	0.862	(0.013)	0.827	(0.020)	0.818	(0.014)	0.530	(0.026)	0.578	(0.016)

Table 4.6: Mean values for temperature corrected DTI metrics: $\langle D \rangle$, $\lambda_{\parallel}$, $\lambda_{\perp}$, and FA of fixed rat spinal cord with comparisons to Refs. [158, 63, 128]

Metric	Reference	Vivo	ROI: Region	WM				GM	
				Left	Right	Ventral	Dorsal	Left	Right
$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	Our results ¹	Ex	Thoracic	0.450	0.461	0.484	0.461	0.416	0.428
		Ex	Thoracic	0.409	0.412	0.435	0.456	0.367	0.364
	[158] ²	In	Thoracic	0.77	0.80	0.85	0.82	0.75	0.75
		Ex	Thoracic	0.36	0.36	0.37	0.36	0.40	0.40
	[63] ³	In	Thoracic	0.394	0.400	0.414	0.404	0.421	0.421
		Ex	Cervical	-	-	-	0.36-0.45	-	-
$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	Our results ¹	Ex	Thoracic	1.076	1.090	1.119	1.037	0.621	0.655
		Ex	Thoracic	0.978	0.978	0.993	1.011	0.599	0.626
	[158] ²	In	Thoracic	-	-	-	-	-	-
		Ex	Thoracic	-	-	-	-	-	-
	[63] ³	In	Thoracic	0.699	0.721	0.724	0.731	0.706	0.706
		Ex	Cervical	-	-	-	0.92-1.06	-	-
$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	Our results ¹	Ex	Thoracic	0.137	0.146	0.166	0.173	0.313	0.314
		Ex	Thoracic	0.125	0.129	0.156	0.178	0.251	0.233
	[158] ²	In	Thoracic	-	-	-	-	-	-
		Ex	Thoracic	-	-	-	-	-	-
	[63] ³	In	Thoracic	0.241	0.240	0.259	0.241	0.278	0.278
		Ex	Cervical	-	-	-	0.11-0.17	-	-
FA	Our results ¹	Ex	Thoracic	0.862	0.855	0.840	0.815	0.441	0.473
		Ex	Thoracic	0.862	0.862	0.827	0.818	0.530	0.578
	[158] ²	In	Thoracic	0.88	0.89	0.83	0.89	0.41	0.40
		Ex	Thoracic	0.68	0.68	0.64	0.67	0.29	0.28
	[63] ³	In	Thoracic	0.624	0.636	0.604	0.634	0.573	0.573
		Ex	Cervical	-	-	-	0.81-0.93	-	-

¹ See Section 4.2.1 for DTI method. Results given for diffusion times of 6.5 and 55 ms, respectively.² 7 T MRI, EPI, 21.3 ms diffusion time, $b = 842 \text{ s/mm}^2$, 21 gradient directions, 20/37 °C ex/in vivo, $T_E/T_R = 40/1000 \text{ ms}$, FOV = 30 mm, 128x128 matrix size, $195 \mu\text{m}^2$ in-plane resolution, 2 mm slice thickness.³ 9.4 T MRI, PGSE, 14 ms diffusion time, $b = 1000 \text{ s/mm}^2$, 6 gradient directions, 37 °C in vivo, $T_E/T_R = 31/500 \text{ ms}$, FOV = 38.4 mm, 128x128 matrix size, $300 \mu\text{m}^2$ in-plane resolution, 2 mm slice thickness.⁴ 7 T MRI, PGSE, 6.9 ms diffusion time, $b = 750 \text{ s/mm}^2$, 6 gradient directions, 20 °C ex vivo, $T_E/T_R = 21.3/2000 \text{ ms}$, FOV = 12.8 mm, 128x128 matrix size, $100 \mu\text{m}^2$ in-plane resolution, 1 mm slice thickness.

[158, 128]).

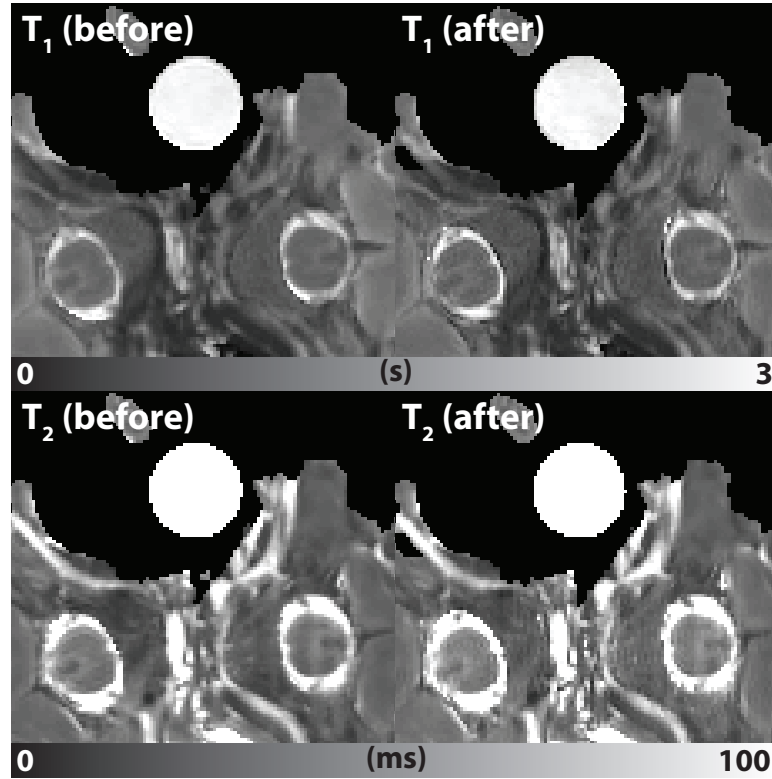


Figure 4.7: T_1 and T_2 maps before and after DTI experiments in the rat spine and PBS phantom.

Figure 4.7 shows T_1 and T_2 maps collected before and after the DTI experiments, 48 hours apart. Table 4.7 summarizes the T_1 and T_2 values. The T_1 values acquired before and after the DTI experiments were significantly different from each other in all regions. T_2 values acquired before and after the DTI experiments were also significantly different and had a larger percent change after 48 hours of imaging. These changes are attributable to the temperature change and Larmor frequency drift in the MRI. Both T_1 and T_2 are temperature dependent [174].

Acquiring data for Chapter 5, a series of MRI experiments were performed at approximately the same time on freshly excised *ex vivo* mouse brains. Linear regression suggests a Larmor frequency shift of approximately -14 Hz/day in the Bruker 7 T magnet (see Figure 4.8). Given a readout bandwidth of 12.5 kHz and matrix size of 256x256, each voxel has a frequency bandwidth of ≈ 49 kHz. Over the course of 48 hours, the MR image will

shift approximately half the voxel resolution, or $\approx 60 \mu\text{m}$, along the readout direction. In reality, the affine transformation matrix needed to shift the last set of DTI images by at least one pixel to properly register the images. Other effects, such as small variations in the shimming gradients and small movement in the sample itself are likely contributors.

Because the RF attenuation to produce $\pi/2$ - and π -pulses was determined automatically at the start of the study, Larmor frequency drift will result in imperfect π -pulses, which in turn, can lead to stimulated echoes and inaccurate T_2 measurements. Although the RARE sequence used here alleviates some of these effects by alternating the phase of the π -pulse and applying spoiler gradients, T_2 measurements are still particularly sensitive to noise and inhomogeneities in the RF and static fields [193]. To alleviate some of these issues, Poon *et al.* developed a CPMG sequence with non-slice-selective composite block pulses surrounded by alternating sign and decreasing amplitude spoiler gradients [193]. While this sequence was adapted in our lab as well, echo images occasionally had substantial signal loss, possibly due to imperfections in the gradient spoiling. Because of this, the CPMG sequence included with Paravision 5.0 was used for all subsequent acquisitions (described in Section 4.3.2). Improvements to the T_2 data acquisition should substantially improve the consistency of our measurements and allow for more advanced multicomponent T_2 analysis. Further development of these techniques, however, was beyond the scope of this dissertation.

Using the relaxometry dataset acquired prior to the DTI experiments, T_1 time constants (Table 4.7) ranged from (1110 ± 80) ms to (1220 ± 50) ms in white matter, (1260 ± 20) ms to (1330 ± 50) ms in grey matter, and was (2870 ± 60) ms in the PBS phantom. These values are lower than freshly excised rat spine measurements taken in a 9.4 T MRI at 37 °C [25], where T_1 was (2280 ± 230) ms in grey matter and (1970 ± 210) ms in white matter. Conversely, they are higher than *in vivo* rat spine measurements taken at 2 T, where T_1 was 1021 ms and 1089 ms in grey and white matter, respectively [172]. This is not surprising, because T_1 has previously been shown to decrease with temperature [174, 131], fixation [212], and magnetic field strength [205]. The trend of higher T_1 values in grey matter was reflected in our data as well.

T_2 values (Table 4.7) were similar to those found in other studies of *in vivo* and fixed rat

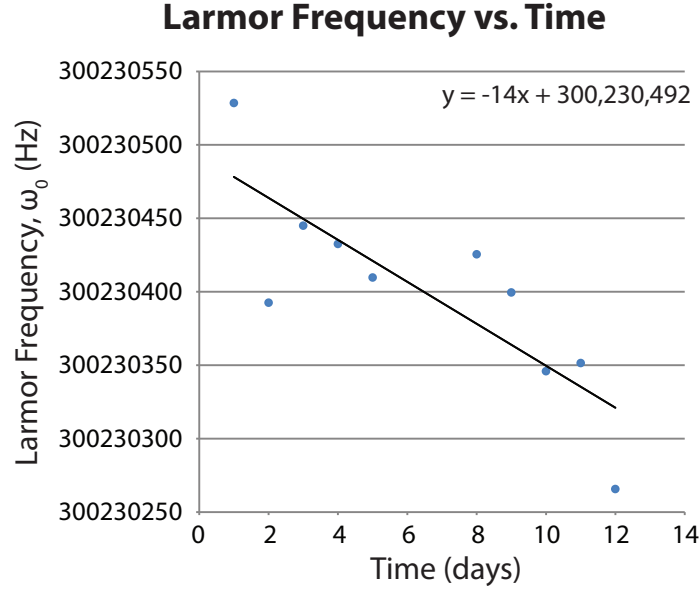


Figure 4.8: Larmor frequency vs. time in a series of *ex vivo* mouse brain experiments. Linear regression fit has a slope of -14 Hz/day.

spinal cord measured at 7 T. Franconi *et al.* measured *in vivo* T_2 values of (57.0 ± 1.6) ms and (43.2 ± 1.0) ms in white and grey matter of rat cervical spinal cord, respectively [69]. In fixed rat cervical spinal cords analyzed with multicomponent T_2 analysis, Kozlowksi *et al.* found the medium T_2 component values, $T_{2,ie}$, was (42.72 ± 1.43) ms in dorsal white matter, (45.75 ± 2.59) ms in lateral + ventral white matter, and (35.36 ± 1.78) ms in grey matter [129]. At a bore temperature similar to our own ($\approx 18^\circ\text{C}$), Dula *et al.* found $T_{2,ie}$ values ranging from (43.9 ± 3.9) ms to (60.9 ± 4.5) ms in different dorsal, ventral, and lateral white matter regions and (48.4 ± 4.5) ms in grey matter [61]. Our values, although they assume a single T_2 component, fall within the ranges they observed.

In conclusion, T_1 , T_2 , and DTI measurements made in a PBS phantom and fixed rat spine were consistent with those found previously. Drift in temperature and Larmor frequency need to be considered when conducting lengthy MRI studies.

Table 4.7: T_1 and T_2 relaxation constants were determined before and after the DTI experiments. Mean values from each rat spine and the PBS phantom are shown.

	ROI:		WM		Right		Ventral		Dorsal		GM		PBS		
			Left		1	2	1	2	1	2	Left	Right			
	Spine:		1	2	1	2	1	2	1	2	1	2			
T ₁ (ms)	Before		1219.1	1152.3	1222.7	1198.8	1219.1	1200.7	1128.1	1113.9	1311.0	1262.1	1329.5	1273.3	2865.7
	After		1196.1	1139.1	1187.3	1156.9	1219.7	1143.5	1107.1	1115.0	1326.0	1259.8	1321.9	1250.7	2862.2
	Mean Difference		-23.0	-13.2*	-35.4*	-41.9*	0.6	-57.2*	-21.0	1.1	15.0	-2.3	-7.5	-22.6*	-3.5
	% Difference		2%	1%	3%	4%	0%	5%	2%	0%	1%	0%	1%	2%	0%
	R		0.645*	0.683*	0.624*	0.571*	0.754*	0.402*	0.651*	0.825*	0.485	0.549*	0.240	0.897*	0.322*
T ₂ (ms)	Before		49.1	41.3	43.3	48.9	46.9	45.4	39.7	39.7	49.4	47.7	49.6	49.5	339.8
	After		52.2	46.7	46.8	53.1	51.4	52.1	46.0	47.4	53.2	53.8	54.2	54.8	418.7
	Mean Difference		3.1*	5.4*	3.5*	4.1*	4.5*	6.7*	6.3*	7.7*	3.8*	6.1*	4.6*	5.3*	78.9*
	% Difference		6%	12%	8%	8%	9%	14%	15%	18%	7%	12%	9%	10%	21%
	R		0.716*	0.870*	0.634*	0.902*	0.628*	0.769*	0.899*	0.858*	0.694*	0.917*	0.625*	0.759*	0.932*

* p < 0.05 for paired samples T-test or Pearson correlation coefficient

4.3 Cuprizone Mouse Model of Demyelination

Cuprizone is a toxic model of demyelination, causing cell death of oligodendrocytes followed by extensive demyelination [245] with only moderate inflammation and no neuronal loss [104]. After 6 weeks of feeding, extensive demyelination is observed predominantly in the corpus callosum and superior cerebellar peduncles. Chronic demyelination occurs if the cuprizone diet is maintained for an extended period, however, if a normal diet is resumed at 6-weeks, the brain is mostly remyelinated after 6 weeks of recovery [161]. As an analogue for demyelinating diseases such as MS, the cuprizone mouse model is useful for developing multiparametric MRI methods capable of distinguishing healthy and diseased white matter.

Two separate sets of control and cuprizone-fed mice were imaged during and after a 6-week cuprizone treatment. The study conducted on the second set of mice is detailed in Chapter 5. Method development and comparisons relating to both studies are detailed in this section.

The first cuprizone study (CPZ1) consisted of eight female C57BL/6 mice. The cuprizone-fed group ($n = 4$) ate 0.4% cuprizone (w/w) mixed into milled rodent chow and pressed into pellets and the control group ($n = 4$) ate regular chow (both *ad libitum*). After 6 weeks of feeding, mice were perfused with 0.1 M PBS followed by 4% PFA. Heads were first fixed for 24 hours in 4% PFA, after which all tissue external to the skull was removed. 48 hours prior to *ex vivo* imaging, brains were transferred to PBS to wash out the PFA. After *ex vivo* imaging, brains were transferred back into 4% PFA prior to histological preparation. The experimental protocol was approved by the University of Manitoba Animal Care Committee who adhere to the guidelines and principles created by the Canadian Council on Animal Care.

The second cuprizone study (CPZ2) consisted of ten C57BL/6 mice and is detailed in Chapter 5. After 6 weeks of cuprizone treatment and *in vivo* MRI, the second study differs primarily in the circumstances of *ex vivo* MRI (performed on unfixed brains the same day of sacrifice) and the method of histology (quantitative measurements with electron microscopy).

4.3.1 *In Vivo* MRI Methods

Mouse Setup

In the CPZ1 study, mice underwent weekly *in vivo* T_2 -weighted and magnetization transfer imaging from 2–6 weeks after start of cuprizone treatment.

In the CPZ2 study, the cuprizone-fed group was imaged immediately prior to the start of the cuprizone treatment (week-0) and every week after until 6 weeks of treatment had been completed. Control mice were imaged the following day of each week.

Mice were anaesthetised using 5% isoflurane in O_2/N_2O and maintained at 1.5% isoflurane in O_2/N_2O with a nose cone. Respiration and external skin temperature on the abdomen were monitored during imaging using an MR-compatible small animal monitoring and gating system (SA Instruments, Inc., Stony Brook, NY). External skin temperature was maintained at 37° with a heating circulator bath (Thermo Scientific Haake, Karlsruhe, Germany). Mouse heads were held in place with a tooth bar inside a custom-built 24 mm diameter, 300 MHz inductively coupled quadrature RF volume coil (NRC Institute for Biodiagnostics, Winnipeg, MB, Canada). The entire apparatus was placed inside a Bruker BGA12-S actively shielded gradient system with integrated shim coils (Bruker BioSpin). All MR experiments were performed on a 7 T 21 cm Bruker Avance III NMR system with Paravision 5.0 (Bruker BioSpin).

After mouse placement, the MRI is setup in the following order:

1. RF coil is tuned and matched to the mouse head.
2. Magnetic field inside the coil is made homogeneous by adjusting the 1st and 2nd order gradients (shimming). After automatic shimming, a B_0 fieldmap is acquired and the shimming gradients are re-calculated using Bruker's MapShim function.
3. The Larmor frequency, ω_0 is found at peak signal strength.
4. Appropriate RF attenuation is determined for creating $\pi/2$ - and π -pulses.
5. Receiver gain is adjusted dependent on pulse sequence used.

6. After an initial pilot scan to ensure proper placement of the mouse, a series of scout scans is performed to consistently orient the coronal slices relative to the centerline of the brain.
7. With square coronal and sagittal scans down the centerline of the brain, the slice(s) required for an experiment can be placed.

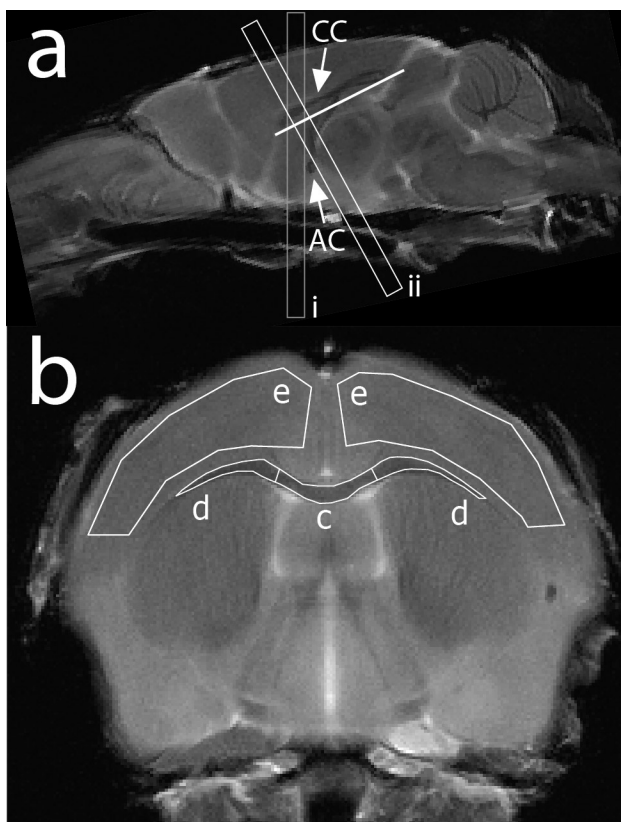


Figure 4.9: (a) Based on a T_2 -weighted sagittal image along the centerline of the brain, a coronal slice (i) was placed 0.5 mm anterior to the anterior commissure (AC) in the rostral region of the corpus callosum. A second slice (ii) was then placed perpendicular to the corpus callosum (CC) and intersecting the region of the corpus callosum contained in the first slice. This second slice was the basis of all subsequent *in vivo* and *ex vivo* image acquisitions. This procedure allowed for very consistent slice placement in each mouse (as evidenced by the longitudinal images) and reduced volume-averaging effects in the corpus callosum that would skew any quantitative analysis. (b) Regions of interest include the (c) corpus callosum, (d) external capsule, and (e) cerebral cortex.

In order to reduce volume averaging effects, coronal slices were placed in each mouse perpendicular to the rostral region of the corpus callosum (Figure 4.9). T_2 -weighted images

and MTI were acquired with the same coronal 0.75 mm thick slice with a $(2.5 \text{ cm})^2$ field-of-view (FOV) and 256x256 matrix size resulting in a $98 \times 98 \times 750 \text{ } \mu\text{m}^3$ voxel resolution.

T_2 -Weighted Imaging

T_2 -weighted images were acquired using a spatially encoded CPMG sequence (imagine [Figure 3.4](#) with different spatial encoding for each π -pulse), also known as a Rapid Acquisition with Relaxation Enhancement (RARE) sequence [101]. In the RARE sequence, each echo is spatially encoded with a different phase-encoding gradient strength, increasing the speed of acquisition by the number of echoes acquired, or RARE factor, per repetition time. The effective echo time (T_E) is defined as the time elapsed until the occurrence of the echo with the least phase encoding (closest to the middle of k -space). The echoes are phase-encoded such that the effective echo time is half the RARE factor times the echo spacing. While some spatial blurring occurs in this technique, RARE imaging is superior to traditional spin echo sequences for imaging tissue with relatively long T_2 as it can acquire similar signal amplitudes in a shorter amount of time [101].

Coronal T_2 -weighted images were acquired using a RARE sequence with effective $T_E = 80 \text{ ms}$, $T_R = 1640 \text{ ms}$, RARE factor = 8, echo spacing 20 ms, bandwidth 20 kHz, 12 averages, and a 10 minute acquisition time.

Magnetization Transfer Imaging

Proton density and magnetization transfer contrast images were acquired using a spoiled gradient echo sequence with low flip angle (FLASH) sequence [88]. In order to calculate MTR maps, FLASH images ($T_E/T_R = 6/70 \text{ ms}$, 10° flip angle, 48 averages, 2x14 minute acquisition time) were acquired both with (M_{sat}) and without (M_0) an MT saturation pulse (Gaussian-shaped RF pulse, 10.25 ms long). A $10 \text{ } \mu\text{T}$ RF saturation amplitude with a frequency offset of 6000 Hz was chosen to optimize the MTR, as described below.

The ideal offset frequency and amplitude for the MT saturation pulse can be found with quantitative MTI data (from Chapter 5). We can define three criteria for the MTR:

1. Maximize tissue contrast between healthy white and grey matter.

2. Maximize the correlation between MTR and the bound pool fraction, f .
3. Avoid direct saturation of the liquid pool.

With these criteria in mind, the percent difference and correlation with qMTI parameters (f , T_1 , and RM_0^A) were plotted for MTR maps calculated at six offset frequencies (1, 2, 4, 6, 10, and 30 kHz) and three amplitudes (5, 10, and 20 μ T) ((a) and (b) in [Figure 4.10](#)).

Based on the percent difference between average MTR in white matter and grey matter, the optimal contrast in healthy control mice is found at $\Delta f \approx 6000$ Hz for all RF saturation amplitudes (5, 10 and 20 μ T). Although there is less contrast in the cuprizone-fed mice, it appears to be largest for the 5 μ T RF pulse at $\Delta f = 6000$ Hz ([Figure 4.10a](#)).

Because we want MTR to represent the bound pool size, a strong correlation with f is preferable. $R^2 = 0.65$ at 10 μ T and $\Delta f = 2000$ Hz and is ≥ 0.6 for all Δf from 1000 to 6000 Hz ([Figure 4.10b](#)).

The offset frequency needs to be large enough that signal loss due to direct saturation of the liquid pool is small. Using typical qMTI parameters for a healthy mouse corpus callosum, the two-pool model of signal from the liquid pool was plotted at 5, 10, and 20 μ T against offset frequency ([Figure 4.10c](#)). Signal loss due to direct saturation of the liquid pool was $\approx 1\%$ at $\Delta f = 2340$ Hz for 5 μ T, 4680 Hz for 10 μ T, and 9370 Hz for 20 μ T.

Based on [Figure 4.10](#), an RF amplitude of 10 μ T and offset frequency, $\Delta f = 6000$ Hz was chosen to maximize contrast, minimize direct saturation, and strongly correlate with the bound pool fraction.

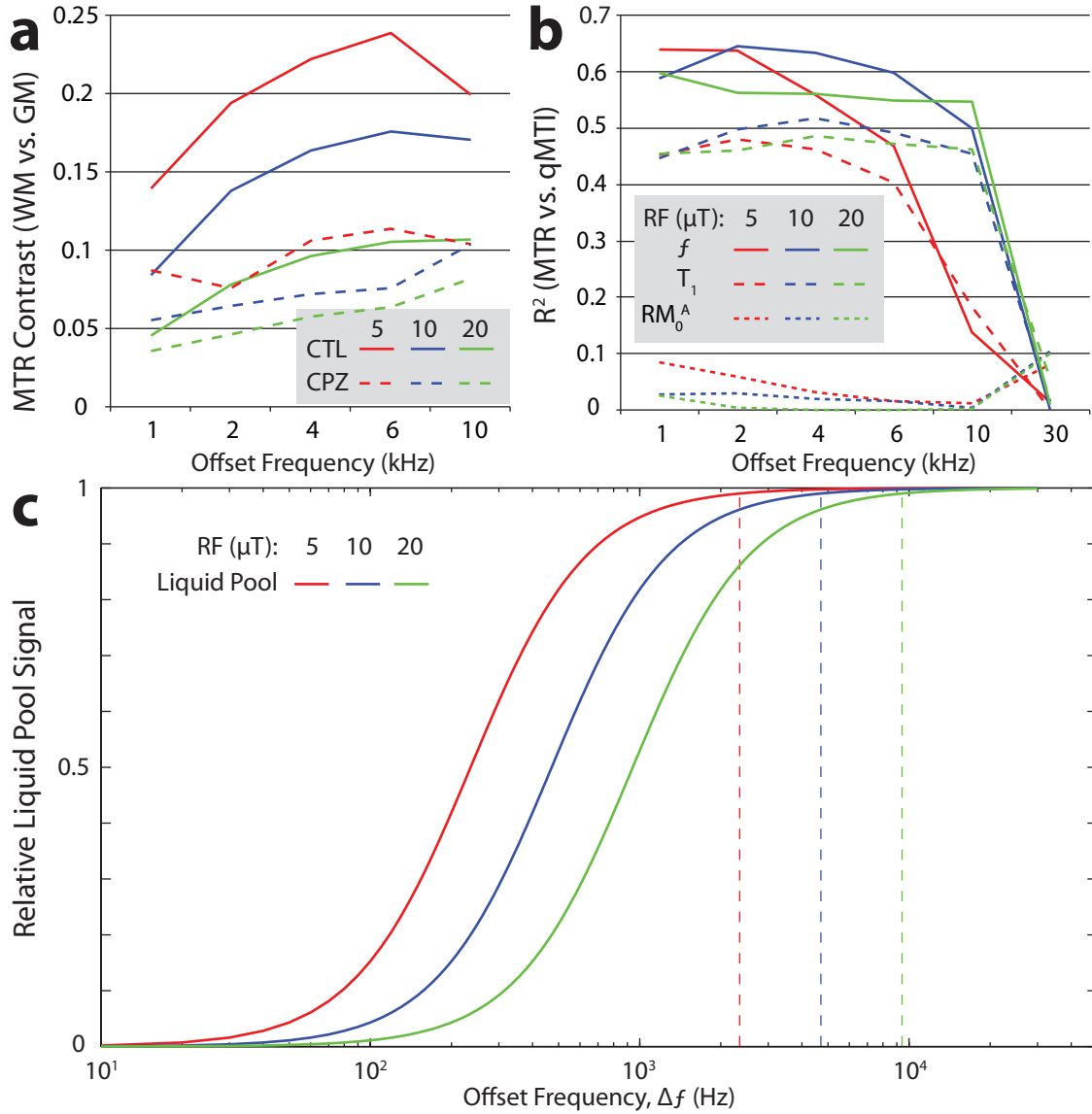


Figure 4.10: (a) Calculating the percent difference between average MTR in white matter (WM) and grey matter (GM), the optimal contrast in healthy control (CTL) mice is found at $\Delta f \approx 6000\text{Hz}$ for all RF saturation amplitudes (5, 10 and 20 μT). Although there is less contrast in the cuprizone-fed (CPZ) mice, it appears to be largest for the 5 μT RF pulse at $\Delta f = 6000\text{ Hz}$. (b) Using all white matter voxels in cuprizone-fed and control mice, the Spearman correlation coefficient, R^2 was found for MTR values at different offset frequencies and RF amplitudes vs. f , T_1 , and RM_0^A (the main influences on MTR). Since we want MTR to represent the bound pool size, a strong correlation with f is preferable. $R^2 = 0.65$ at 10 μT and $\Delta f = 2000\text{ Hz}$ and is ≥ 0.6 for all Δf from 1000 to 6000 Hz. (c) Two-pool model of signal from the liquid pool relative to M_0 using typical qMTI parameters for a healthy mouse corpus callosum. The offset frequency needs to be large enough that signal loss due to direct saturation of the liquid pool is small. Liquid pool signal is $\approx 99\%$ at $\Delta f = 2340\text{ Hz}$ for 5 μT , 4680 Hz for 10 μT , and 9370 Hz for 20 μT (dashed lines).

***In vivo* MRI Analysis**

T_2 -weighted and M_0 images were aligned to the accompanying M_{sat} image using an affine transformation matrix determined by maximizing the 2D correlation coefficient and/or mutual information of the two images, with manual adjustments if needed, using a custom-built MATLAB GUI (detailed in Appendix C.1.2). T_2 -weighted images were normalized to the mean CSF signal in the third ventricle and MTR maps were calculated using the M_0 and M_{sat} images (Equation (3.61)).

Normalized T_2 -Weighted and MTR Results

Comparison of normalized T_2 -weighted image (Figure 4.11) and MTR maps (Figure 4.12), shows significant differences in the effect of cuprizone treatment on both the corpus callosum and external capsule. In the CPZ2 study, both regions were significantly more hyperintense in the T_2 -weighted images and had significantly lower MTR values after 6 weeks of 0.4% cuprizone treatment. It was determined that the two studies could not be combined, despite identical imaging parameters.

Although cuprizone was prepared identically between the two studies, CPZ1 was performed in 2009 and CPZ2 was performed in 2011 with different batches of cuprizone feed. Cuprizone is sensitive to heat, which may be produced when creating pressed pellets. Some studies suggest using powdered chow for this reason [218]. Increased heat can decrease the efficacy of treatment, explaining the less severe results in both studies relative to previous experiments at 0.4%. Rodent feed with cuprizone already added is commercially available (Harlan Laboratories), and may address some of the inconsistencies between batches of cuprizone feed. Based on our results, caution is advised when comparing mice fed cuprizone from two different studies, as subtleties in preparation may influence its effect on mice.

Recent studies have commented on the variable effects due to the cuprizone treatment [109, 218], however, in our experience, the effect of cuprizone was remarkably homogenous when looking at images in each study on its own. For example, see Figure B.2 for T_2 -weighted images and B.4 in Appendix B for MTR maps in all 5 cuprizone-fed mice in

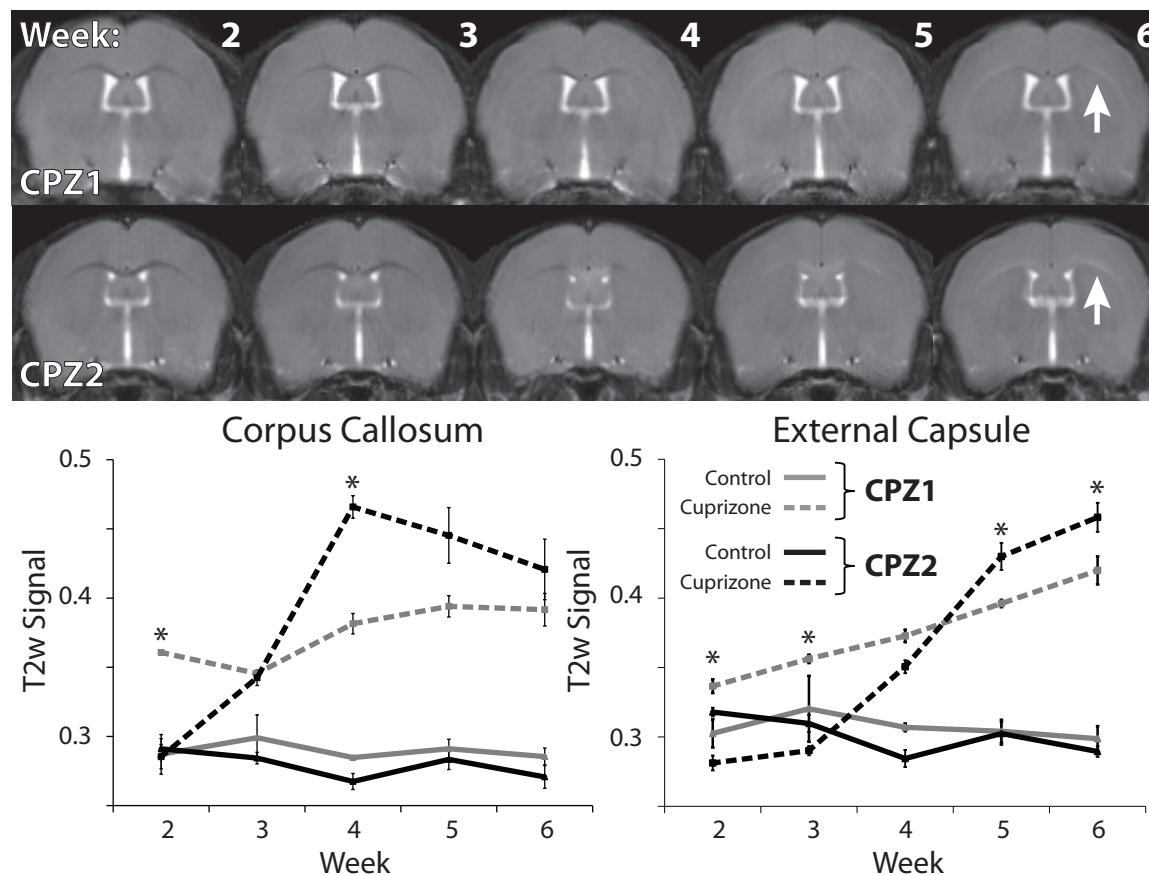


Figure 4.11: Longitudinal *in vivo* T_2 -weighted images in 0.4% cuprizone-fed mice from two separate studies (CPZ1 and CPZ2) spanning two to six weeks of treatment. Error bars are standard error of the mean. T_2 -weighted signal normalized to the CSF was significantly different between the two studies in both the corpus callosum and external capsule ($*p < 0.05$ using an independent samples T-test).

the CPZ2 study spanning all 6 weeks. Despite being well-characterized in the literature, pathohistology is advised when assessing the efficacy of different MRI metrics.

4.3.2 *Ex Vivo* MRI Methods

Tissue Preparation

CPZ1 Study: After 6 weeks of feeding, mice were intracardially perfused with 0.1 M PBS followed by 4% PFA. Heads were fixed for 24 hours in 4% PFA, after which all tissue external to the skull was removed. 48 hours prior to *ex vivo* imaging, brains were transferred to PBS to wash out the PFA. After *ex vivo* imaging, brains were transferred back into 4%

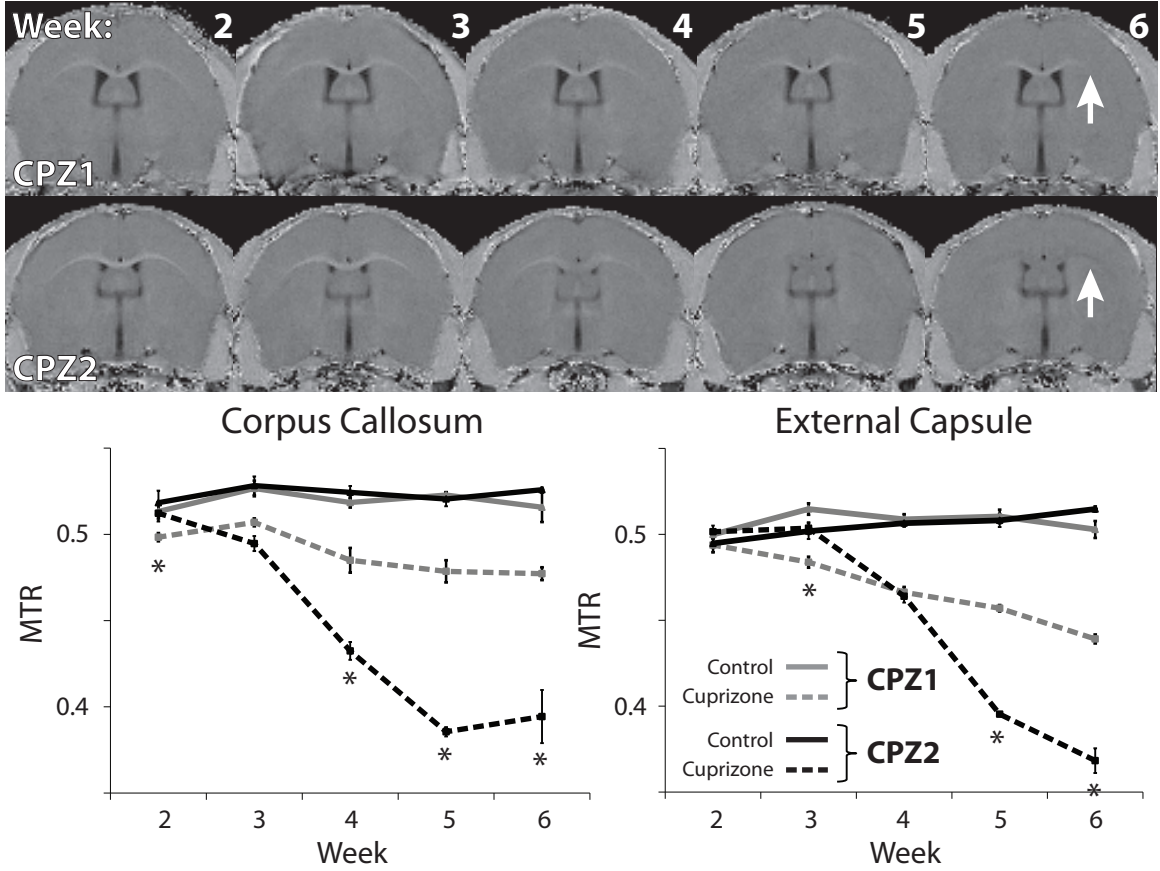


Figure 4.12: Longitudinal *in vivo* MTR maps in 0.4% cuprizone-fed mice from two separate studies (CPZ1 and CPZ2) spanning two to six weeks of treatment. Error bars are standard error of the mean. MTR values were significantly different between the two studies in both the corpus callosum and external capsule ($*p < 0.05$ using an independent samples T-test).

PFA prior to histological preparation. One of the cuprizone-fed DTI acquisitions could not be completed due to an equipment malfunction and subsequent downtime.

CPZ2 Study: Cuprizone-fed mice were sacrificed and imaged over a 5 day period encompassing the sixth week of cuprizone treatment. Control mice were sacrificed and imaged over a 5 day period in the following week. On each day, *in vivo* imaging was performed for one mouse prior to that mouse being sacrificed. Under deep anaesthesia, intracardial perfusion was performed with 10 mL of 0.1 M phosphate buffered saline (PBS) for ≈ 2 minutes followed by 0.5% glutaraldehyde and 2% paraformaldehyde (PFA) for ≈ 10 minutes. This was followed by another 10 mL of 0.1 M PBS to flush out any remaining fixative. All tissue external to the skull was removed and the mouse head was stored in PBS for 1–2 hours prior to overnight imaging.

In both studies, brains (in skull) were secured in a custom-built acrylic brain holder and immersed in room temperature FOMBLIN Perfluoropolyether Y04 grade fluid (Solvay Solexis) to maintain hydration and eliminate external proton signal and susceptibility artifacts. The sample holder was left at room temperature for several hours to allow the sample temperature to equilibrate. During overnight imaging, ambient bore temperature was maintained at 18° C. In 4.2, it was shown that sample temperature was likely higher than 18° and was shown to drift ≈ 0.2 °C/hour between 6 and 12 hours of imaging. Although this temperature change may contribute to a small error in the DTI and relaxometry measurements, it should be consistent across samples.

Quantitative MRI Methods

All MRI datasets were acquired using the same coronal slice shown in Figure 4.9 with a $(1.25 \text{ cm})^2$ FOV, 0.75 mm slice thickness, and 128x128 matrix size resulting in a $98 \times 98 \times 750 \text{ } \mu\text{m}^3$ voxel resolution. Combined T_1 and T_2 relaxometry data were acquired using a series of RARE acquisitions (effective $T_E = 11, 33, 55, 77, \text{ and } 99 \text{ ms}$; $T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5, \text{ and } 95.5 \text{ ms}$; RARE factor = 2; 8 averages; 71 minute acquisition time). In the CPZ2 study, an additional T_2 relaxometry dataset was acquired using a CPMG sequence with 32 slice-selective π -pulses (3.105 ms Mao-shaped pulse envelope) and 10 ms echo spacing ($T_E = 10, 20, 30 \dots 320 \text{ ms}$, $T_R = 2500 \text{ ms}$, 24 averages, 96 minute acquisition time). MTI data were acquired using the same FLASH sequence as *in vivo* with 1 M_0 image and 18 M_{sat} images acquired with peak RF amplitudes of 5, 10, and 20 μT and frequency offsets at each power of 1000, 2000, 4000, 6000, 10000, and 30000 Hz (64 averages each, 19x9.5 minute acquisition time). DTI data were acquired with a PGSE sequence using a 7-direction tetra-orthogonal gradient-encoding scheme. The low b-value was 2.7 s/mm^2 and the high b-value ranged from 968 to 1034 s/mm^2 dependent on gradient direction with gradient pulse duration, $\delta = 6 \text{ ms}$ and gradient pulse spacing, $\Delta = 14 \text{ ms}$ ($T_E/T_R = 26/1500 \text{ ms}$, 12 averages, 307 minute acquisition time).

MR Image Registration and Filtering

T_1 , T_2 , diffusion-weighted and magnetization transfer images were aligned to the low b-value diffusion-weighted image using the same method applied to the *in vivo* images. When registering images acquired with the same type of pulse sequence (i.e. gradient echo used for magnetization transfer images and spin echo used for T_1 , T_2 and diffusion tensor images), it was sufficient to only adjust the translation and rotation parameters of the affine transformation matrix. In the case of spin echo images being registered to gradient echo images (and vice versa), the scaling parameters of the affine transformation matrix need to be adjusted as well. In the case of diffusion-weighted images, b-matrices associated with each image had to be rotated to reflect rotations applied during registration to maintain accurate orientational information [144]. Prior to calculating quantitative maps, *ex vivo* images were filtered with an anisotropic diffusion filter to improve SNR [188, 73]. The filter was applied with 5 iterations and a gradient modulus threshold equal to three times the standard deviation of the noise in each echo [115].

Image Analysis

T_1 maps were generated by fitting RARE images (effective $T_E = 11$ ms; $T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5$, and 95.5 ms) to the saturation recovery curve (see Equation (3.1) and Appendix C.3.1). Single component T_2 maps were generated by fitting RARE images (effective $T_E = 11, 33, 55, 77$, and 99 ms; $T_R = 4895.5$ ms) and CPMG images ($T_E = 10, 20, \dots, 160$ ms — SNR was too small after $T_E = 160$ ms) to the spin echo decay curve (see Equation (3.4) and Appendix C.3.2). Using the CPMG dataset, multicomponent T_2 values were generated using a non-negative least squares algorithm with 120 logarithmically spaced signal amplitudes and T_{2j} values from 5–500 ms (see Section 3.1.4 [265]). Regularized and non-regularized T_2 maps generated using filtered T_2 -weighted images were visually similar to each other. Based on this and Bjarnason *et al.*'s recommendation of voxel-based, non-regularized analysis of regions of interest [29], non-regularized solutions were used in all subsequent analysis. The distribution of signal amplitudes vs. T_{2j} values were visually analyzed in each region of interest to identify peaks corresponding to different

water components. Myelin water fractions (MWF) and intra-/extracellular water fractions (IEWF) were calculated for T_{2j} values ranging from 5 to 20 ms and 20 to 100 ms, respectively [129]. Geometric means were calculated for the same range of T_{2j} values, $T_{2,m}$ and $T_{2,ie}$.

Magnetization transfer images were normalized to M_0 and fitted to the two-pool model of magnetization transfer (Equation (3.81)) [99] with continuous wave pulse equivalent approximations (Equation (3.87)) [198] and a super-Lorentzian absorption lineshape for the bound pool (Equation (3.77)). For the peak RF amplitudes of 5, 10, and 20 μT , $\omega_{1CWPE} = 279.7, 559.5, \text{ and } 1119 \text{ rad/s}$ (Equation (3.87)) [198]. Relevant metrics derived from the two-pool model include the magnetization transfer rate between the free and bound pools (RM_0^A), longitudinal relaxation rates of the free and bound pools (R_A and R_B), transverse relaxation times of the free and bound pools (T_2^A and T_2^B), and the bound pool fraction (f). R_B was fixed arbitrarily to 1 s^{-1} as recommended by several groups [99, 219, 198]. R_A was calculated using the T_1 map (Equation (3.88)) [99, 198]. Using the fitted value of $f/R_A(1-f)$ in Equation (3.81), the bound pool fraction, f can be calculated using R_A and Equation (3.89).

Diffusion tensors were determined using a non-linear least squares fit (Equation (3.51)) and a modified Cholesky decomposition to ensure positive definiteness [125]. The eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and eigenvectors ($\mathbf{e}_1, \mathbf{e}_2, \mathbf{e}_3$) of the diagonalized diffusion tensor represent the magnitude and directionality, respectively, of the three orthogonal diffusion directions. Diffusion tensor metrics include mean diffusivity ($\langle D \rangle = (\lambda_1 + \lambda_2 + \lambda_3)/3$), axial diffusivity ($\lambda_{\parallel} = \lambda_1$), radial diffusivity ($\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$), and fractional anisotropy (FA) (Equation (3.59)). An RGB colour map was constructed from the x, y, and z components of the first eigenvector $\mathbf{e}_1 = (e_{1x}, e_{1y}, e_{1z})$ (Equation (3.60)). Red represents diffusion in the x-direction, green in the y-direction, and blue in the z-direction, with the voxel brightness weighted by its fractional anisotropy.

With reference to a mouse atlas [186], ROIs representing the corpus callosum (average number of voxels, $n = 40 \pm 6$), external capsule ($n = 66 \pm 10$), and cerebral cortex ($n = 690 \pm 60$) were manually defined in the M_{sat} image and applied to analysis of all registered *in vivo* MRI methods (Figure 4.9). The M_{sat} image was chosen since the corpus callosum stood out relative to the surrounding tissue throughout the progression of the cuprizone treatment.

This was particularly important in weeks 4 through 6, when the corpus callosum in the T_2 -weighted and proton density images became harder to discern. Similar ROIs for the corpus callosum ($n = 48 \pm 7$) external capsule ($n = 74 \pm 9$) and cerebral cortex ($n = 650 \pm 90$) were defined in the DTI RGB colour maps (which also maintained excellent contrast in the corpus callosum of the cuprizone-fed mice) and were applied to the analysis of all registered *ex vivo* MRI methods.

4.3.3 Image Registration

Once an unscaled image is loaded in MATLAB, a series of manipulations can take place. In the case of multiparametric analysis on the same slice, all of the images can be aligned to a single image. In order to facilitate image registration, an image registration tool was written in MATLAB to allow visualization and registration of images. In two dimensions, transformation matrices defining translation (A_t), scaling (A_s), shearing (A_{sh}), and rotation (A_r), can be combined to form an affine transformation matrix (A). The translation matrix is defined as,

$$A_t = \begin{pmatrix} 1 & 0 & t_x \\ 0 & 1 & t_y \\ 0 & 0 & 1 \end{pmatrix} \quad (4.4)$$

where t_x and t_y define the translation along the x and y axes, respectively. The scaling matrix is defined as,

$$A_s = \begin{pmatrix} s_x & 0 & 0 \\ 0 & s_y & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (4.5)$$

where s_x and s_y define the scaling factors along the x and y axes, respectively. The shearing matrix is defined as,

$$A_{sh} = \begin{pmatrix} 1 & sh_y & 0 \\ sh_x & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (4.6)$$

where sh_x and sh_y define the shearing factors along the x and y axes, respectively. The rotation matrix is defined as,

$$A_r = \begin{pmatrix} \cos\theta & \sin\theta & 0 \\ -\sin\theta & \cos\theta & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (4.7)$$

where θ is the angle of rotation. Finally, the complete affine transformation matrix is defined as

$$A = A_t A_s A_{sh} A_r. \quad (4.8)$$

The original position of a voxel, defined by x and y , is transformed into a new position, defined by x' and y' ,

$$\begin{pmatrix} x' \\ y' \\ 1 \end{pmatrix} = A \begin{pmatrix} x \\ y \\ 1 \end{pmatrix} \quad (4.9)$$

The transformed voxels are subsequently combined into an aligned image using bicubic interpolation.

To search for the optimal affine transformation parameters, `fminsearchbnd` was used [54], a constrained version of `fminsearch` which uses the Nelder-Mead simplex search method to search for minimal solutions to multivariable functions [133]. `fminsearchbnd` performed better than `fminsearch` by reducing the parameter space to a reasonable set of boundaries. A function defining the common information between the two images needs to be defined. In the case of the image registration program detailed in Appendix C.1.2, three different functions were tested. If we define the reference and target images as A_{mn} and

B_{mn} , respectively, the functions are:

i. Absolute Difference

The sum of the absolute difference of values in A_{mn} and B_{mn} is,

$$d = \sum_m \sum_n |A_{mn} - B_{mn}| \quad (4.10)$$

and the two images are assumed to be aligned when d is minimized.

ii. 2-D Correlation Coefficient

The 2-D correlation coefficient is defined as,

$$r = \frac{\sum_m \sum_n (A_{mn} - \bar{A})(B_{mn} - \bar{B})}{\sqrt{\left(\sum_m \sum_n (A_{mn} - \bar{A})^2\right) \left(\sum_m \sum_n (B_{mn} - \bar{B})^2\right)}} \quad (4.11)$$

where $\bar{A}$ and $\bar{B}$ are the mean values of A_{mn} and B_{mn} , respectively. r is larger when A_{mn} and B_{mn} are more strongly correlated. The two images are assumed to be aligned when $-r$ is minimized.

iii. Mutual Information

128-bin distributions of grey values were created individually for A_{mn} and B_{mn} (p_i^A and p_i^B where $i = 1 \dots 128$). 128 distributions from B_{mn} were also created corresponding to the location of grey values in each bin of p_{Ai} , resulting in a 128x128 distribution matrix called the joint distribution, p_{ij}^{AB} . The dispersion of the joint distribution can be described by the Shannon entropy of the joint distribution[192],

$$h = - \sum_i \sum_j p_{ij}^{AB} \log(p_{ij}^{AB}). \quad (4.12)$$

It is low when there are a few sharply defined peaks and large when all values are relatively constant. The two images are assumed to be aligned when h is minimized. The mutual information, which is related to the Shannon entropy, is defined as,

$$m = \sum_i \sum_j p_{ij}^{AB} \log \left(\frac{p_{ij}^{AB}}{p_i^A p_j^B} \right). \quad (4.13)$$

Mutual information takes into account the entropy of the individual images as well as the joint entropy, thus avoiding some of the misregistrations that can occur with the Shannon entropy. It can be interpreted as measuring the distance between the joint distribution p_{ij}^{AB} and the independent distributions $p_i^A p_j^B$ [192]. When m is low, the images are independent of each other. When m is high, the images are dependent. The two images are assumed to be aligned when $-m$ is minimized.

Minimization of $-r$ or $-m$ was best for aligning images acquired with different imaging parameters or methods. Minimizing d was adequate for aligning images that had similar imaging parameters and signal intensities. In practice, the 2-D correlation coefficient was used most frequently, as it was faster than the mutual information method and performed as good if not better in situations where more than translation and rotation was required (such as registration of gradient echo images acquired for MTI to spin echo images acquired for T_2 -weighted, relaxometry and DTI datasets). In rare cases (usually *in vivo* images that had small motion between acquisitions), affine transformation parameters had to be set manually to properly align the images. After manual adjustments, the affine transformation parameters could then be further refined with one of the automatic methods.

By first registering images with high SNR (e.g. first echo image of a relaxometry dataset, M_0 image in qMTI, or low b-value image in DTI), good starting parameters were found, which subsequently helped when registering images with lower SNR (e.g. later echoes in the echo train or RF-saturated images in the qMTI dataset). The MATLAB tool used for image registration is described in Appendix C.1.2.

4.3.4 Noise and Filtering in Quantitative MRI

Besides motion, quantitative MRI methods are sensitive to noise. In this section, the noise in different MR images is characterized. This is followed by the application of an anisotropic diffusion filter to alleviate the effects of noise in fitting images to various quantitative models. Mean values are compared in filtered and non-filtered metrics. Finally, the goodness-of-fit

was evaluated with χ^2 values for filtered and non-filtered fits.

Noise in the MRI Data

The Fourier-transform of the $\mathbf{k}$ -space dataset creates a complex image-space dataset. When creating an image, the magnitude of each voxel is the the magnitude of the Fourier-transformed complex signal, $S = \sqrt{A^2 + B^2}$. Because the components of the complex signal (A and B) have noise following the Gaussian probability distribution,

$$P_{Gaussian}(x) = \frac{1}{\sqrt{2\pi}\sigma^2} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (4.14)$$

the magnitude, $S = \sqrt{A^2 + B^2}$ has noise following the Rician probability distribution,

$$P_{Rician}(x) = \frac{x}{\sigma^2} e^{-(x^2 + \mu^2)/2\sigma^2} I_0\left(\frac{x\mu}{\sigma^2}\right) \quad (4.15)$$

The variance due to noise, σ^2 , can be calculated using the standard deviation, σ_M measured in a region outside of the sample composed of only signal noise. In order to account for the Rician distribution at zero signal, the measured standard deviation, σ_M is divided by a factor of $\sqrt{2 - \pi/2}$ [84]. The variance is,

$$\sigma^2 = \frac{\sigma_M^2}{(2 - \pi/2)} \quad (4.16)$$

and the signal-to-noise ratio (SNR) is,

$$SNR = \frac{S}{\sigma} = \sqrt{2 - \pi/2} \left(\frac{S}{\sigma_M} \right) = 0.655 \left(\frac{S}{\sigma_M} \right) \quad (4.17)$$

Numerous papers have explored the effects of noise on different quantitative MRI methods. Multicomponent T_2 relaxometry appears to have a large SNR requirement, ranging from 500 [263] to 700 [82]. However, a more modest SNR of $\approx 200 - 300$ in the first echo image was used in other studies that detected myelin water components in human brains [157, 156, 146, 29].

For accurate determination of DTI metrics, diffusion-weighted images tend to require

Table 4.8: SNR values in quantitative MRI datasets from 5 freshly excised control mice. Mean signal is calculated in the corpus callosum and cerebral cortex for comparison. Values reported as mean (standard deviation).

T_1											
T_R (ms):		4895.5	2895.5	1395.5	695.5	295.5	95.5				
Corpus Callosum		87 (8)	78 (12)	63 (10)	41 (2)	21.5 (1.8)	6.8 (0.5)				
Cerebral Cortex		97 (8)	85 (12)	66 (10)	42 (2)	22.2 (1.8)	7.0 (0.5)				
T_2 (5 echoes)											
T_E (ms):		11	33	55	77	99					
Corpus Callosum		89 (8)	59 (6)	43 (5)	30 (2)	24 (2)					
Cerebral Cortex		98 (9)	62 (6)	42 (5)	28 (3)	22 (3)					
T_2 (16 echoes)											
T_E (ms):		10	20	30	40	50	60	70	80		
Corpus Callosum		128 (19)	99 (19)	83 (13)	66 (7)	57 (2)	43 (3)	35 (3)	28.4 (1.8)		
Cerebral Cortex		137 (20)	105 (19)	86 (12)	67 (7)	56 (2)	41 (3)	32 (2)	25.6 (1.7)		
T_E (ms):		90	100	110	120	130	140	150	160		
Corpus Callosum		21.6 (1.3)	19.1 (0.7)	14.9 (0.4)	13.1 (0.7)	11.1 (0.5)	9.9 (0.7)	8.1 (0.4)	6.8 (0.8)		
Cerebral Cortex		19.0 (1.6)	16.6 (0.8)	12.8 (1.1)	11.0 (0.4)	9.4 (1.0)	8.0 (0.3)	6.6 (0.5)	5.6 (0.7)		
DTI											
b-value (s/mm ²):		3	1003	1003	1005	1004	1004	1034	968		
Corpus Callosum		60.5 (1.9)	54.2 (3.7)	42.1 (2.8)	55.4 (1.9)	49.9 (5.4)	49.7 (0.5)	48.4 (0.8)	48.5 (1.9)		
Cerebral Cortex		64.2 (3.6)	46.1 (3.4)	48.6 (1.8)	46.1 (2.0)	47.5 (5.8)	46.6 (0.3)	46.1 (1.2)	45.1 (2.7)		
MTI											
RF Power		Offset Frequency (kHz)		1	2	4	6	10	30		
Corpus Callosum		M_0	56 (6)	43 (4)	47 (6)	49 (5)	51 (6)	58 (6)			
		5 μ T	40 (3)	31 (3)	33 (4)	36 (5)	40 (6)	49 (9)			
		10 μ T	28 (3)	21 (3)	23 (4)	26 (3)	31 (4)	48 (8)			
		20 μ T	17 (3)								
Cerebral Cortex		M_0	61 (7)	51 (5)	55 (7)	56 (6)	58 (7)	64 (6)			
		5 μ T	47 (4)	39 (4)	40 (5)	43 (6)	47 (7)	55 (10)			
		10 μ T	34 (4)	27 (4)	29 (5)	32 (4)	37 (4)	54 (9)			
		20 μ T	22 (4)								

an SNR > 20 [191, 116, 64]. Measuring DTI, however, is complicated by numerous factors such as field strength, gradient encoding schemes, gradient strength, gradient spacing, and tissue structure. When given the choice of increased SNR vs. additional gradient encoding directions, increasing the number of gradient encoding directions is usually suggested for robust determination of DTI metrics [216, 116]. Signal and, by extension, SNR, will vary substantially in different diffusion-weighted images because they map out the underlying magnitude and direction of water diffusing in the tissue.

The noise performance of quantitative MTI is also complicated by variations in the acquisition schemes [40, 147] and fitting methods [194, 41]. Both T_2^A and, in particular, $RM0^A$, have been shown to have large variability in numerous experiments [194, 219, 198, 253], and may be more sensitive to noise propagation. At SNR < 120 , Ramani *et al.*'s method appears to be more robust than Sled and Pike's when estimating $RM0^A$ and T_2^A , however T_2^A is consistently overestimated [41]. SNR ranging from 40 to 100 is typical in other qMTI studies [41].

SNR was calculated in different quantitative MRI datasets using the mean signal in the corpus callosum and cerebral cortex (Table 4.8). All methods achieved a sufficient SNR, with the possible exception of T_2 datasets for multicomponent analysis. Even so, to improve the effective SNR, an anisotropic diffusion filter was applied to the images prior to model fitting.

Anisotropic Diffusion Filter

The anisotropic diffusion algorithm is a locally adaptive filter that improves SNR while still preserving features within the image itself [188, 73]. It has previously been demonstrated to improve quantitative analysis of multicomponent T_2 [115, 107] and DTI [183] data. Prior to calculating quantitative maps, *ex vivo* images were filtered with an anisotropic diffusion filter [188, 73]. The filter was applied with 5 iterations and a gradient modulus threshold equal to three times the standard deviation of the noise in each echo [115].

Quantitative maps were generated using both filtered and non-filtered images of the 5 control mouse from the CPZ2 study. Filtered images resulted in smoother quantitative maps with fewer random variations due to noise. Mean values were calculated in each region

of interest and compared using an independent samples T-test. Figure 4.13 shows T_1 , T_2 , DTI, and qMTI fits for filtered and non-filtered signal from a single voxel in the corpus callosum of a control mouse.

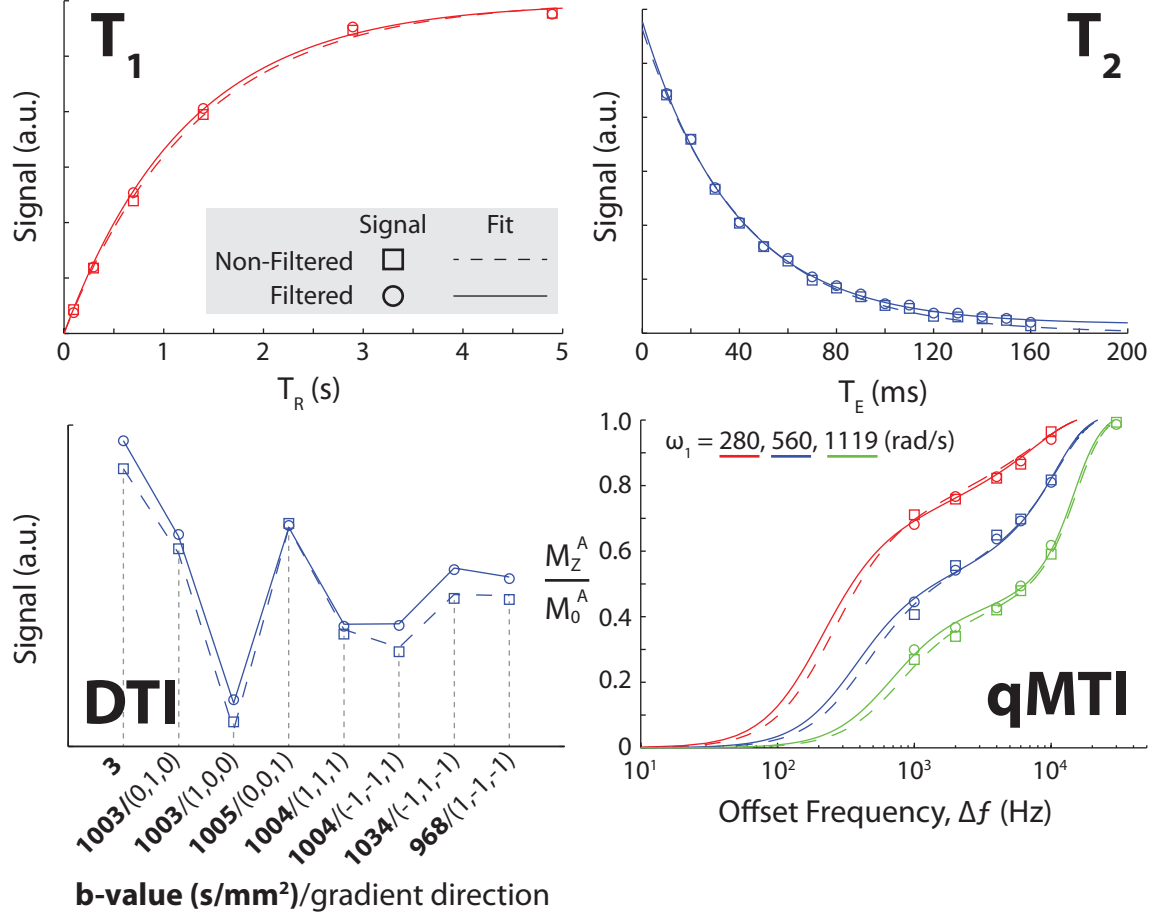


Figure 4.13: T_1 , T_2 , DTI, and qMTI fits for filtered (circle) and non-filtered (square) signals from a single voxel in the corpus callosum of a control mouse. Filtered and non-filtered fits are represented as solid and dashed lines, respectively.

Comparison of mean values in quantitative maps suggests that filtering did not significantly alter the estimated values in most regions of interest and quantitative methods. In the DTI maps, FA was significantly larger in the non-filtered maps, with a percent difference of 5% in the corpus callosum and 15% in the external capsule ($p < 0.05$). FA has previously been shown to be sensitive to noise and in cases of high noise or anisotropy, benefits from a constrained non-linear least squares fit (3.2.6). Thus, larger FA in non-filtered white matter regions is not surprising (Figure 4.14).

The quantitative MTI maps, RM_0^A and T_2^A , previously shown to be sensitive to noise, were significantly more mottled in the non-filtered brains (Figure 4.15).

While T_1 was significantly larger in the external capsule of the non-filtered map when compared to the filtered map, the percent difference was only 1.1% (Figure 4.16). This was reflected in the R_A map as well, which is derived in part from T_1 .

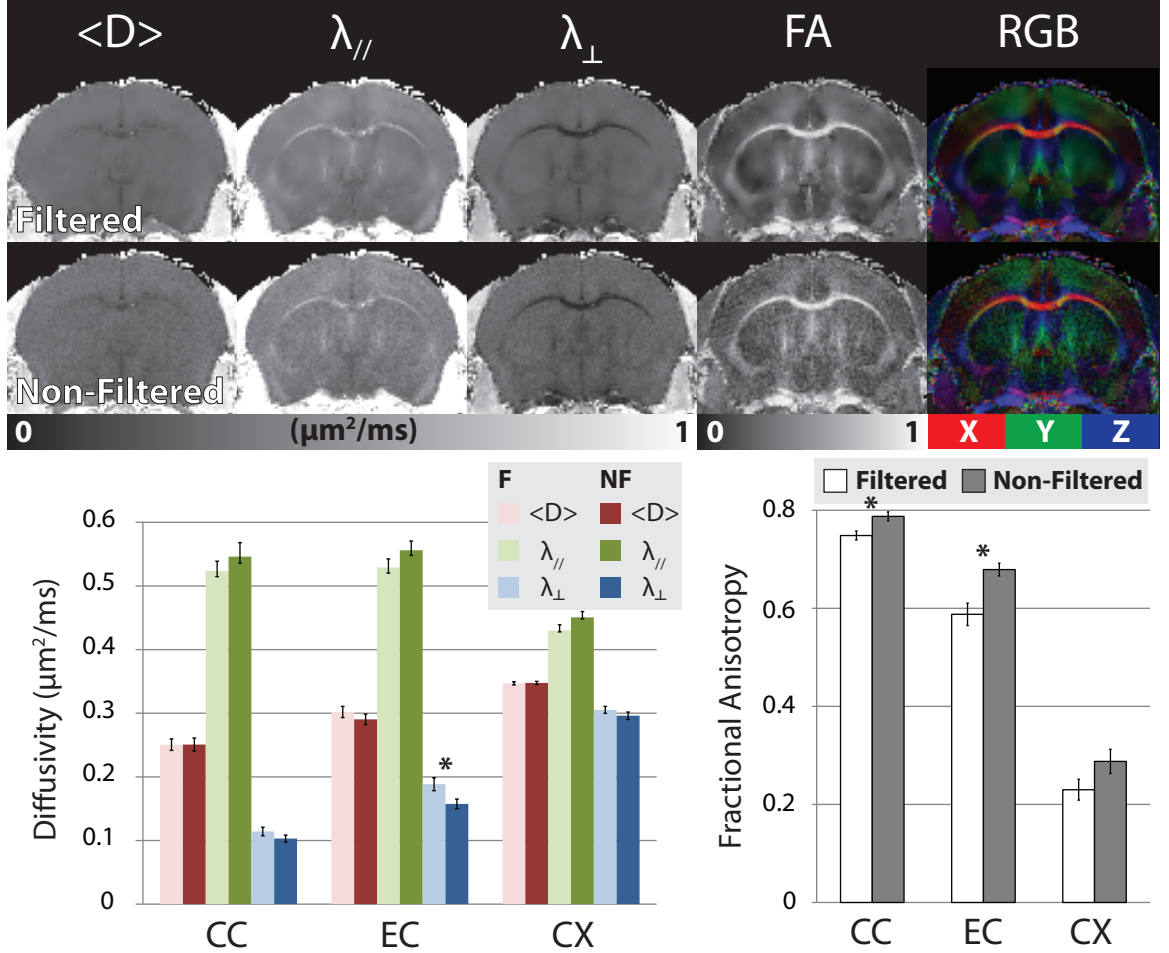


Figure 4.14: Comparison of DTI maps and metrics in filtered and non-filtered mouse brains. Error bars are standard error of the mean. Fractional anisotropy was significantly higher in the corpus callosum and external capsule of non-filtered mouse brains, but most metrics were similar to each other. $*p < 0.05$ when comparing filtered and non-filtered mouse brains using an independent samples T-test.

χ^2 values were calculated for each voxel using the filtered and non-filtered images and their respective quantitative models,

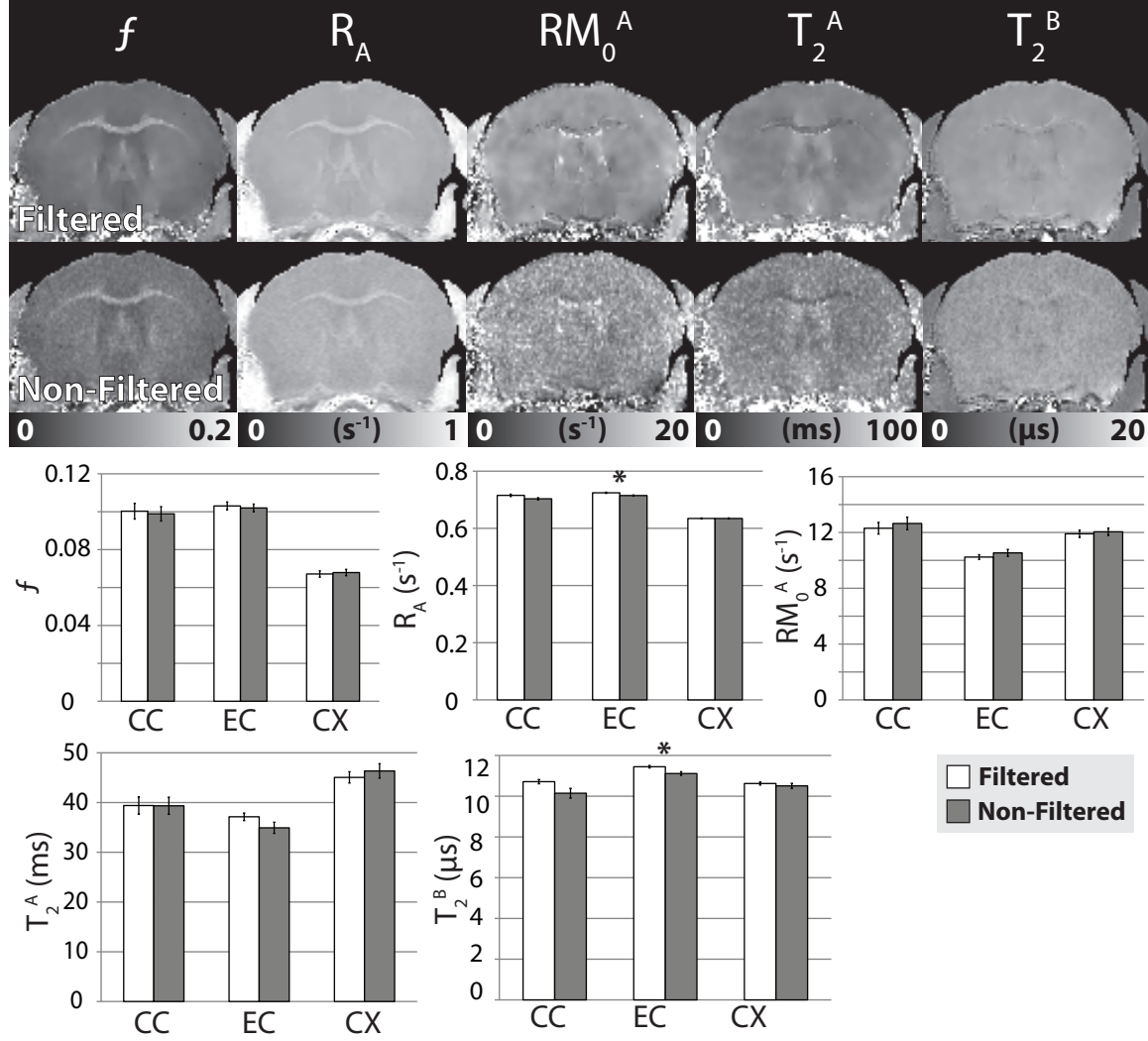


Figure 4.15: Comparison of qMTI maps and metrics in filtered and non-filtered mouse brains. Error bars are standard error of the mean. In most cases, magnetization transfer metrics were not significantly different. RM_0^A and T_2^A , previously shown to be sensitive to noise, are significantly more mottled in the non-filtered brains. $*p < 0.05$ when comparing filtered and non-filtered mouse brains using an independent samples T-test.

$$\chi^2 = \sum_{i=1}^n \frac{(S_i - S_i^M)^2}{\sigma_i^2} \quad (4.18)$$

where n is the number of images used in the fit, S_i is the signal in each image, S_i^M is the signal predicted by the model, and σ_i^2 is standard deviation of the noise in each image, corrected to reflect the Rician distribution. The normalized χ^2 can be calculated as χ^2/ν where the degrees of freedom, ν , is the number of images minus the number of parameters

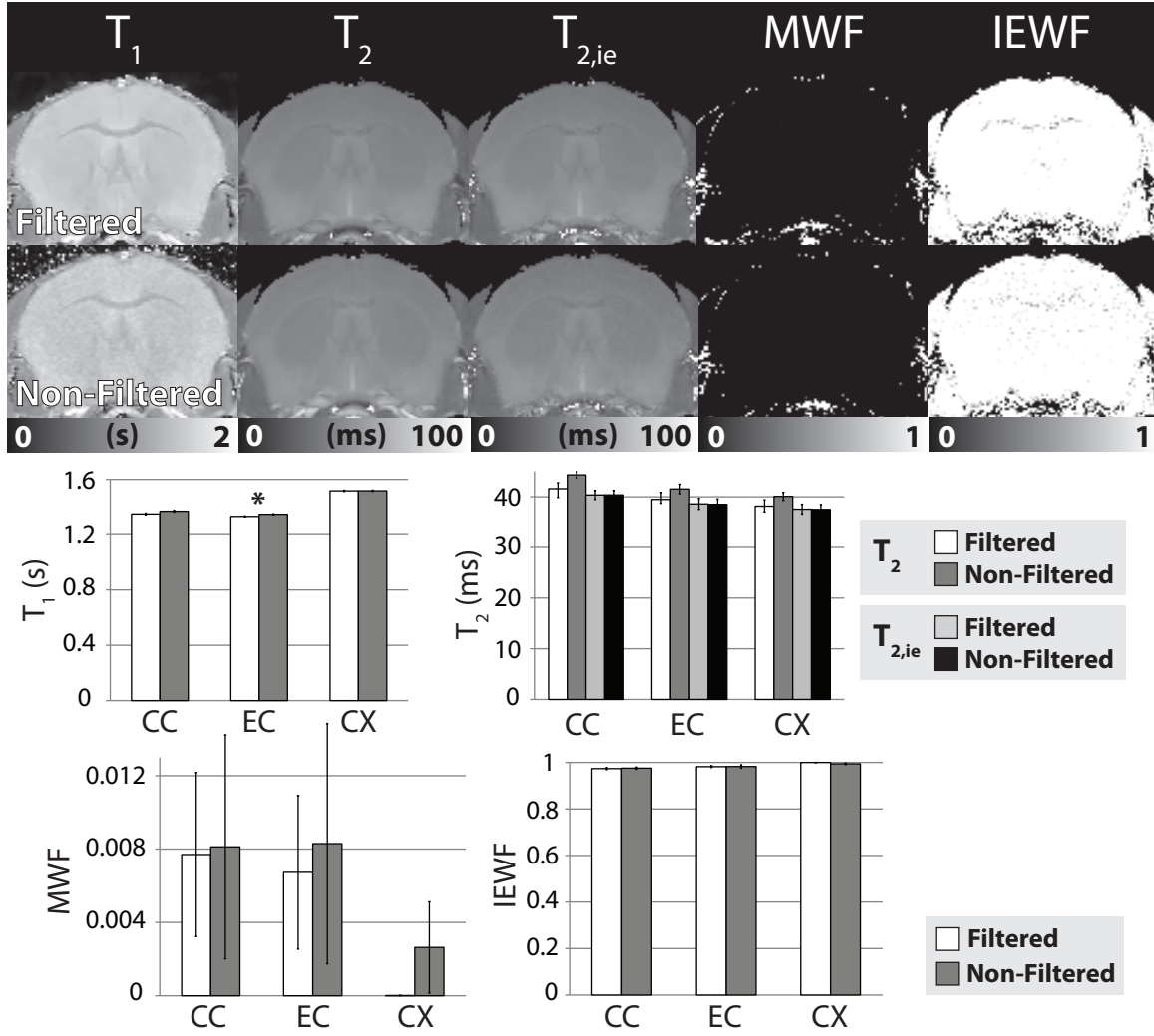


Figure 4.16: Comparison of T_1 and T_2 maps and metrics in filtered and non-filtered mouse brains. Error bars are standard error of the mean. In most cases, relaxometry metrics were not significantly different. * $p < 0.05$ using an independent samples T-test.

used in the quantitative model. Ideally, $\chi^2/\nu = 1$. $\chi^2/\nu > 1$ suggests there are discrepancies between the observed signals and the quantitative model. In the case of noisy MRI data, $\chi^2/\nu > 1$ values frequently occur, however, we can use strategies such as filtering to improve the goodness of fit.

χ^2/ν maps for filtered and non-filtered MRI in a control mouse are shown in Figure 4.17. χ^2/ν values are visibly smaller in the filtered maps, although where the corpus callosum transitions with surrounding tissue, the χ^2/ν appear to be larger. This is most evident in the qMTI χ^2/ν map, where larger χ^2/ν values clearly outline the corpus callosum.

Mean χ^2/ν values were compared in the corpus callosum and cerebral cortex of 5 freshly excised control mouse brains. In general, χ^2/ν was improved when using filtered images to generate quantitative MRI maps (Table 4.9), with significantly lower values particularly in the cerebral cortex.

Given the the visual improvements in quantitative maps, small variations between filtered and non-filtered results, and lower χ^2/ν values, the anisotropic diffusion filter is a viable solution for improving the apparent SNR and goodness-of-fit in all of the quantitative MRI methods presented in this dissertation.

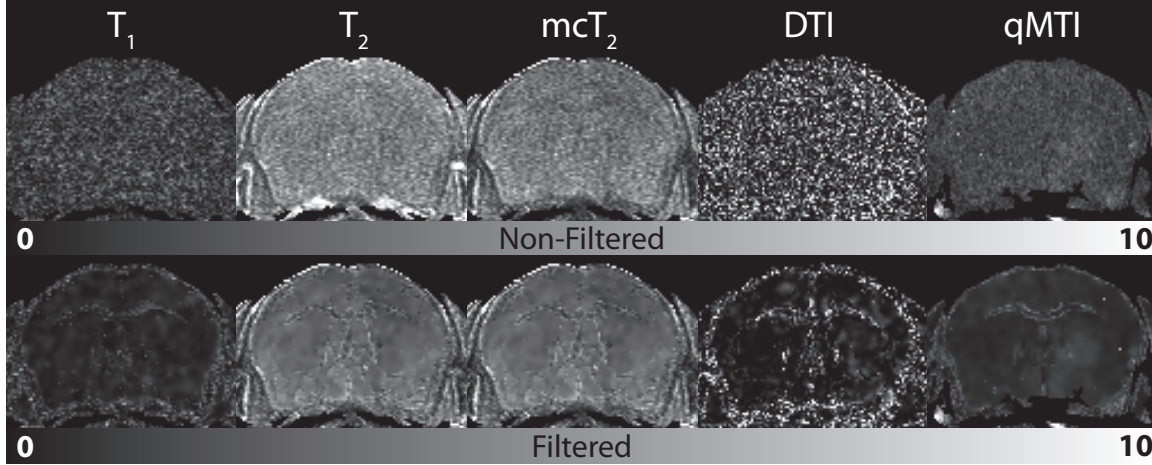


Figure 4.17: χ^2/ν maps for filtered and non-filtered MRI in a control mouse. Maps are scaled from 0 (black) to 10 (white).

Table 4.9: χ^2/ν values for filtered and non-filtered images were compared in the corpus callosum and cerebral cortex of 5 freshly excised control mouse brains. n is the number of images and ν the degrees of freedom. Values reported as mean (standard error of the mean).

Method	n	ν	χ^2/ν Values			
			Corpus Callosum		Cerebral Cortex	
			Non-Filtered	Filtered	Non-Filtered	Filtered
T_1	6	4	1.43 (0.05)	0.90 (0.05)*	1.37 (0.03)	0.48 (0.03)*
T_2	16	13	4.04 (0.80)	2.25 (0.73)	3.98 (0.76)	1.75 (0.43)**
mcT_2	16	13	1.76 (0.30)	1.45 (0.38)	2.22 (0.37)	1.27 (0.37)
DTI	8	1	1.99 (0.36)	0.85 (0.24)**	1.69 (0.12)	0.76 (0.08)*
qMTI	19	13	1.50 (0.11)	1.22 (0.16)	1.56 (0.07)	0.71 (0.06)*

* $p < 0.001$ and ** $p < 0.05$ when comparing filtered and non-filtered χ^2/ν values using an independent samples T-test.

4.3.5 ROI- and Voxel-Based Analysis

After registering and filtering individual MR images, quantitative models are typically fitted to signals averaged over an entire ROI (ROI-based analysis) or fitted to signals in individual voxels (voxel-based analysis). One advantage of voxel-based analysis is that you can generate parameter maps to spatially represent various quantitative parameters. Mean quantitative parameters can be calculated in different regions of interest from these parameter maps with error equal to the standard deviation. In addition, voxel-based quantitative maps can be correlated to understand better the interplay of different quantitative metrics. The disadvantage, of course, is that individual voxels are inherently more noisy, possibly affecting the quality of the quantitative fit, especially when dealing with complicated quantitative methods such as multi-component T_2 and quantitative MTI. As such, ROI-based analysis is often reported in the literature [115], due mostly to the SNR advantages of averaged signal values, but also likely due to computational advantages of doing a single ROI-based fit vs. doing several thousand voxel-based fits.

Only voxel-based fits are reported in this dissertation. Mean and standard deviation in a region of interest are calculated using the voxel-based quantitative maps. Mean values from the same ROI in control or cuprizone-fed mice are reported as the mean $\pm$ standard error of the mean.

4.3.6 Quantitative MRI in Fresh and Fixed Mouse Brains

T_1 , T_2 , and MTR are significantly decreased and f is significantly increased following fixation of MS brain tissue [212]. This can be attributed to both the presence of formalin and the cross-linking of macromolecules causing a shift of protons to the bound pool [212]. As well, $\langle D \rangle$, $\lambda_{\parallel}$, and $\lambda_{\perp}$ are significantly reduced in normal mouse brain [238], damaged mouse optic nerve [239], and MS brain tissue [212] after fixation. Only part of this reduction is explained by differences in tissue temperature (18°C *ex vivo* vs. 37°C *in vivo*) [238]. Relative measures of anisotropy such as FA are relatively unchanged after fixation [212, 238, 239]. While T_2 values can be somewhat restored by washing the tissue in PBS, T_1

Table 4.10: Comparison of qMRI values in fresh and fixed mouse tissue along with the percent difference (%). Values reported as mean (standard error of the mean).

	Metric	Corpus Callosum		(%)	External Capsule		(%)	Cerebral Cortex		(%)
		Fresh	Fixed		Fresh	Fixed		Fresh	Fixed	
Relaxometry	T_1 (s)	1.350	1.111*	-19.4	1.332	1.072*	-21.7	1.517	1.179*†	-25.1
	T_2 (ms)	41.6	39.7	-4.7	39.5	37.2	-5.9	38.2	38.4	0.6
DTI	$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	0.251	0.176**	-35.2	0.302	0.212*	-35.0	0.347	0.318	-8.8
	$\lambda_{\parallel}$ ($\mu\text{m}^2/\text{ms}$)	0.524	0.359*	-37.3	0.529	0.376*	-33.9	0.430	0.391	-9.6
	$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.114	0.084	-30.4	0.189	0.130**	-36.6	0.305	0.281	-8.2
	FA	0.749	0.752	0.4	0.588	0.613	4.1	0.230	0.226	-2.0
MTI	f	0.100	0.114***	13.1	0.103	0.118**	13.4	0.067	0.075***	10.6
	R_A (s^{-1})	0.715	0.891*	21.9	0.724	0.929*	24.7	0.635	0.841*†	27.9
	RM_0^A (s^{-1})	12.31	12.71	3.3	10.24	12.86***†	22.7	11.91	14.02**	16.3
	T_2^A (ms)	39.4	47.6***	18.9	37.1	43.3	15.3	45.1	58.4*	25.8
	T_2^B (μs)	10.71	10.15***	-5.4	11.45	9.85*	-15.0	10.62	9.33*	-13.0

* $p < 0.001$, ** $p < 0.01$, and *** $p < 0.05$ when comparing fresh and fixed mouse brains using an independent samples T-test.
[†] equal variances not assumed

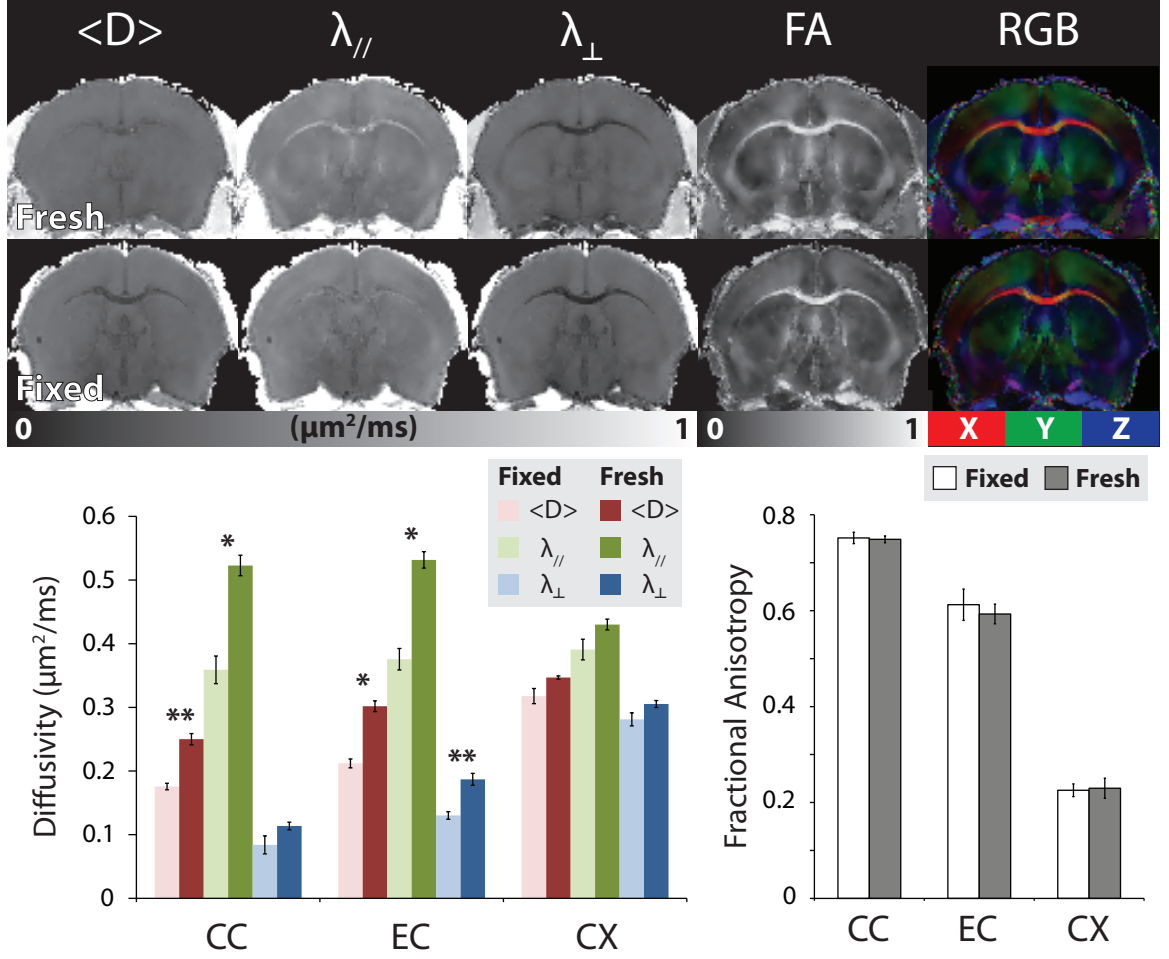


Figure 4.18: Comparison of DTI maps and metrics in fixed and fresh mouse brains. Error bars are standard error of the mean. Diffusivity measurements were significantly lower in the corpus callosum and external capsule of fixed mouse brains, but fractional anisotropy was not. * $p < 0.001$, ** $p < 0.01$ when comparing fresh and fixed mouse brains using an independent samples T-test.

and diffusivity remain significantly reduced due to irreversible changes in membrane permeability and compartment size [243, 214]. Nevertheless, correlation remains strong between myelin content and f in both fixed and unfixed MS brain tissue [212]. Likewise, despite a significant decrease in diffusion metrics, the relative changes are directly proportional to their *in vivo* counterparts in healthy mouse brains [238].

Our results reflect those found previously. Of the DTI metrics, $\langle D \rangle$, $\lambda_{||}$, and $\lambda_{\perp}$ were significantly smaller in fixed white matter, while fractional anisotropy was relatively unchanged (Table 4.10 and Figure 4.18). In Figure 4.18, differences in contrast are most notable in the maps of $\langle D \rangle$ and $\lambda_{||}$, with the white matter tract substantially clearer in the

fixed map of $\langle D \rangle$ and in the fresh map of $\lambda_{\parallel}$.

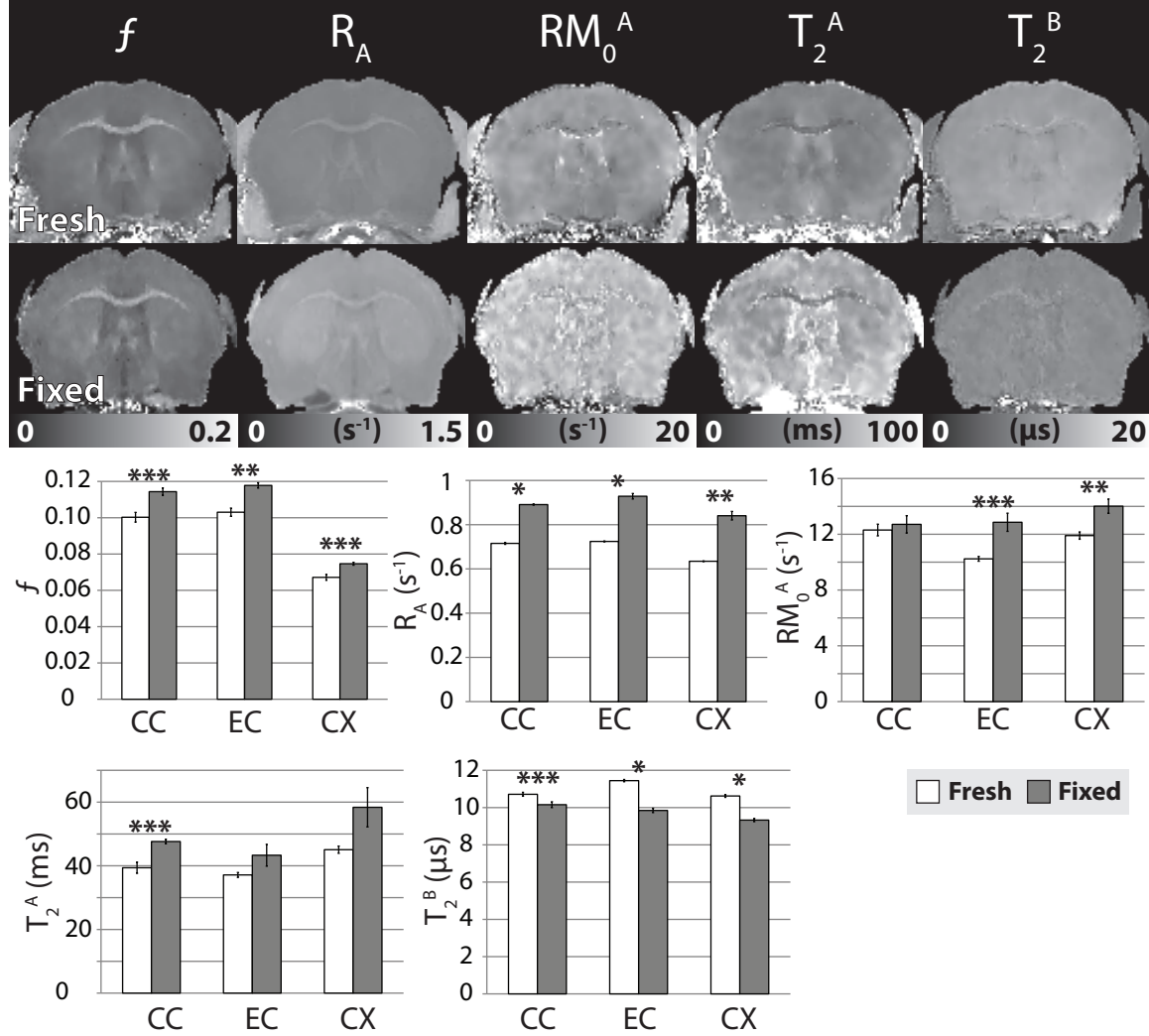


Figure 4.19: Comparison of qMTI maps and metrics in fixed and fresh mouse brains. Error bars are standard error of the mean. Magnetization transfer measurements were significantly larger in the corpus callosum and external capsule of fixed mouse brains, with the exception of T_2^B , which was significantly lower. RM_0^A and T_2^A , previously shown to be sensitive to noise, are more mottled in the fixed brains. * $p < 0.001$, ** $p < 0.01$, and *** $p < 0.05$ when comparing fresh and fixed mouse brains using an independent samples T-test.

In the qMTI maps, f , R_A , and RM_0^A were significantly larger in most regions of the fixed mouse brains, but only in the corpus callosum when comparing T_2^A (Table 4.10 and Figure 4.19). T_2^B was significantly lower in all regions of the fixed mouse brains. While maps of f and T_2^A had similar contrast, R_A , RM_0^A and T_2^B had less discernible white matter tracts in the fixed mouse brain.

T_1 was significantly lower in all regions of the fixed mouse brains (Table 4.10 and Figure 4.20). T_2 was slightly lower in the fixed white matter, but not significantly. As has been observed previously, washing the tissue with PBS for 48 hours prior to imaging mostly restored the T_2 [214].

Given the varying effects of fixation on different quantitative methods, MRI of freshly excised mouse brains might be more reflective of *in vivo* changes in cuprizone-fed mice. Removing the influence of fixation can also improve the comparison of multiple quantitative metrics between control and cuprizone-fed mice. Because we are interested in how different MRI methods correlate with each other and with tissue structure and content, measurements in freshly excised mouse brains appear to be superior to measurements in fixed mouse brains. Delaying the fixation may have resulted in small, dark spots in some of the electron microscopy images in Chapter 5, although with no detriment to image analysis.

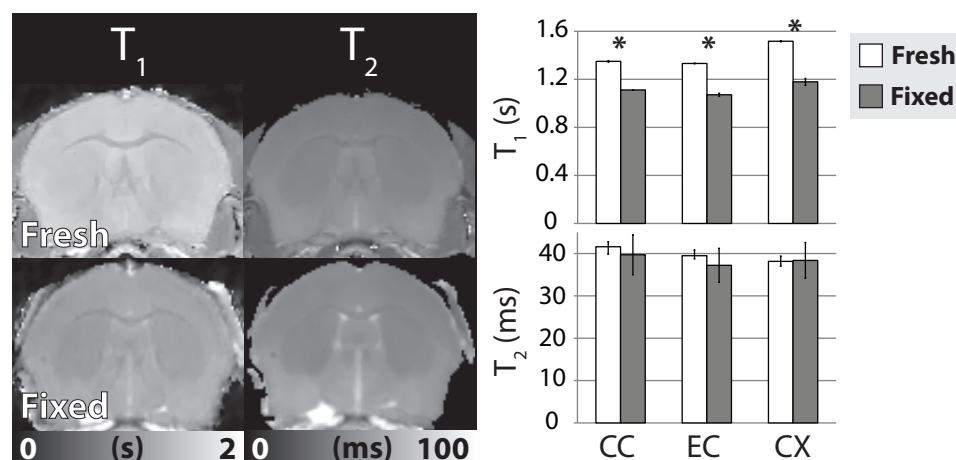


Figure 4.20: Comparison of T_1 and T_2 maps and metrics in fixed and fresh mouse brains. Error bars are standard error of the mean. T_1 was significantly smaller in the fixed mouse brains. T_2 was not significantly different, but had a large variability in both fresh and fixed mouse brains. * $p < 0.001$ using an independent samples T-test.

4.3.7 Histopathology

In the CPZ1 study, brains were removed from the skull and post-fixed overnight in 4% PFA followed by 30% sucrose at 4 °C for 24–48 hours. Serial 30 μm coronal sections were cut using a sliding microtome and collected in plates containing 0.01 M PBS. To evaluate

demyelination, sections were stained with Luxol fast blue-periodic acid Schiff (LFB-PAS) [278].

LFB-PAS Staining

After 6 weeks of 0.4% (w/w) cuprizone treatment, mice showed obvious demyelination in the corpus callosum (Figure 4.21). While some of the myelin sheath remained in the corpus callosum, myelin tracts disappeared completely in the external capsule, with profound cell proliferation. These results mirror the *in vivo* MRI results, where the external capsule changed more significantly than the corpus callosum in the longitudinal T_2 -weighted images (Figure 4.11) and MTR maps (Figure 4.12) of the CPZ1 study.

LFB-PAS staining might overestimate the degree of organized myelination because lipid debris from degraded myelin still gives a positive signal [161]. In order to quantitatively analyze ultrastructural features in the cuprizone model, electron microscopy was utilized in Chapter 5.

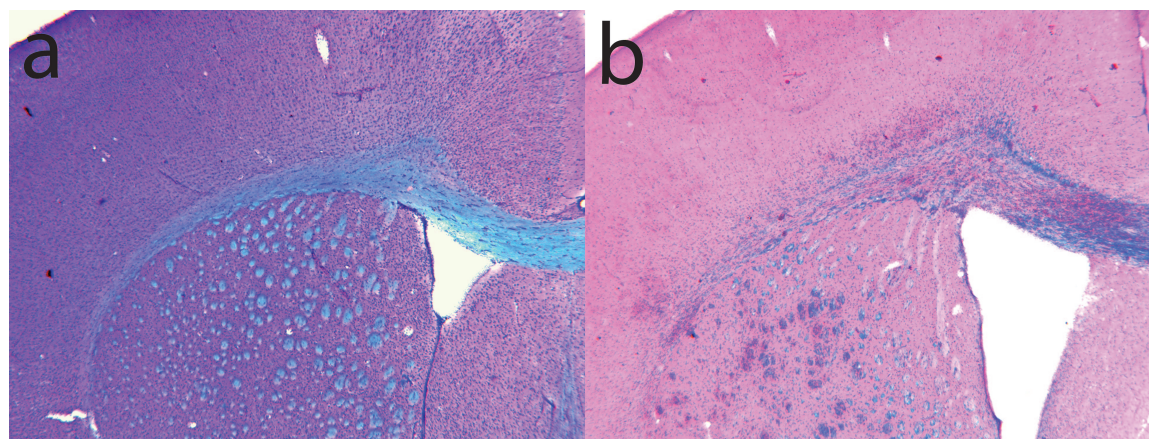


Figure 4.21: LFB-PAS at 6 weeks in a control (a) and 0.4% cuprizone-fed (b) mouse. Corpus callosum in the control is evenly myelinated (blue) with nuclei lined up along the fibers. In the cuprizone-fed mouse, some of the myelin sheath remains in the medial region of the corpus callosum while in the lateral region of the corpus callosum, myelin tracts disappear (pink) with profound cell proliferation (purple dots).

4.3.8 Conclusion

Based on the MRI protocols and methods described in this chapter, a study combining weekly *in vivo* MRI measurements (T_2 -weighted and magnetization transfer imaging) and quantitative MRI measurements in freshly excised mouse brains (including T_1 and T_2 relaxation, DTI, and qMTI) was performed on the cuprizone-fed mouse model in conjunction with electron microscopy to quantify and correlate ultrastructural changes in the corpus callosum due to demyelination. This is the CPZ2 study described in this chapter and further explored in Chapter 5.

Chapter 5

Quantitative MRI and Ultrastructural Examination of the Cuprizone Model of Demyelination

5.1 Introduction

While traditional MRI methods, such as T_1 - and T_2 -weighted imaging, have been used extensively to monitor demyelinating lesions in MS, they are unable to distinguish inflammation, demyelination and reactive glial changes in both lesions and normal appearing white matter (NAWM) [6]. Quantitative MRI methods such as diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), and multicomponent T_2 imaging (mc T_2) provide more information about tissue pathology. DTI is sensitive to changes in water mobility and anisotropy, reflecting tissue structure, while MTI and mc T_2 are sensitive to myelin content and myelin integrity, respectively [66, 12]. A multiparametric approach combining two or more quantitative MRI methods can give more insight into tissue pathology.

In mc T_2 analysis, myelin water fractions (MWF) derived from the short T_2 -component correspond to the distribution of myelin [166, 72, 263, 228, 229, 177, 129, 138] while longer intermediate T_2 -components are thought to indicate inflammation [228, 229]. Multicomponent T_2 gives more structural information than quantitative MTI (qMTI) [200], but sensitivity to water exchange and tissue geometry [200, 145] also reduces its specificity to myelin content. mc T_2 does not distinguish intact myelin from myelin debris [263, 129], requires a relatively high SNR [177], and has larger inter-subject variability than qMTI [250].

The magnetization transfer ratio (MTR) and bound pool fraction (f) are MTI metrics

representative of the bound pool. MTR and f both decrease with demyelination [177], but f is a more specific marker of myelin content [211, 212, 181, 61] and is relatively insensitive to axonal injury and variations in axonal density [181, 61]. MTR is strongly influenced by the pulse sequence, longitudinal relaxation, and magnetization transfer rates; and is difficult to compare between studies.

DTI metrics are sensitive to tissue structure and orientation. Previously, decreased axial diffusivity ($\lambda_{\parallel}$) has been associated with axonal damage [253, 224] and increased radial diffusivity ($\lambda_{\perp}$) has been associated with demyelination [177, 224, 36, 270, 240, 18]. However, there are several confounding influences which might reduce the specificity of DTI metrics. Diffusion anisotropy decreases with fiber crossing and bending [18]. As well, radial diffusivity is influenced by axonal damage and inflammation [36, 270] and does not correlate strongly with myelin loss or astrogliosis [270]. Axial diffusivity does not reflect axonal atrophy [270].

Given the varying influence tissue pathology can have on different quantitative MRI metrics, a multiparametric approach might be necessary to separate pathological processes. To this end, several studies have performed pairwise comparisons of quantitative MRI methods: mcT_2 to qMTI, mcT_2 to DTI, and qMTI to DTI.

Comparisons of mcT_2 to qMTI show conflicting results. McCreary *et al.* suggested MWF reflects the time course of demyelination more closely than MTR [162], while Levesque *et al.* suggested qMTI parameters (f and the T_2 value of the liquid pool, T_2^A) were more sensitive to MS recovery and less variable than MWF, reflecting quick resolution of inflammation followed by slower partial remyelination [146]. Poor correlation between MWF and MTR or f [250, 61, 146] arises due to differing sensitivities to pathological processes such as regional variations in axonal diameter, intercompartmental exchange rates [61], and inflammation [250]. Myelin content, which has been shown to influence both methods, may play a stronger role in cases where stronger correlation exists.

Comparisons of mcT_2 to DTI metrics demonstrate slightly stronger correlations. In a study by Kolind *et al.*, lesions with a long- T_2 component had strong negative correlations between MWF and mean, axial, and radial diffusivity but not fractional anisotropy (FA) and weak correlations in lesions without a long- T_2 component [127]. Long- T_2 lesions are

accompanied by increased T_1 , T_2 , and water content values and decreased MTR, suggesting greater tissue destruction and possibly inflammation or edema [136]. Madler *et al.* found strong correlation between MWF and radial diffusivity and fractional anisotropy [159]. Because both mcT_2 and DTI are sensitive to tissue geometry, these results are not surprising.

Since we were unable to observe a myelin water component in the mouse corpus callosum, comparisons to relaxometry results were limited to T_1 and the T_2 component of intra-/extracellular water, $T_{2,ie}$.

In general, comparisons of MTI to DTI metrics yield weaker correlations, suggesting the two methods might provide complementary information. Correlation between MTR and mean diffusivity ($\langle D \rangle$) is poor except in severely damaged MS lesions [179]. Moderate correlation was found between f and $\lambda_{\perp}$ in average values of white matter (combination of control/shiverer mouse datasets) but not in control and shiverer mice separately [181]. Correlation between f and $FA/\lambda_{\perp}/\lambda_{\parallel}$ was poor in healthy white matter but strong between f and $FA/\lambda_{\perp}$ in grey matter [252]. However, correlation between f and FA appears to increase in white matter tracts with high directional coherence, such as the corpus callosum (CC) [234].

A few studies have used multiple MRI methods to study the cuprizone model of demyelination with promising results. Merkler *et al.* [164] acquired T_1 -weighted, T_2 -weighted, and MT images after 6 weeks of cuprizone treatment and again following 6 weeks of remyelination. A combination of two or more MRI contrasts improved the ability to predict myelinated, demyelinated, and remyelinated tissue using discriminant function analysis with the strongest prediction strength (95%) resulting from a combination of all three contrasts [164]. After 4 weeks of cuprizone treatment, Wu *et al.* [267] found hyperintense T_2 -weighted signal and increased T_2 relaxation times along with decreased axial diffusivity and increased radial diffusivity, most significantly in the caudal region of the corpus callosum. After 12 weeks of cuprizone treatment, Harsan *et al.* [95] also found increased T_2 -weighted signal and radial diffusivity in both the rostral and caudal regions of the corpus callosum, but no significant difference in axial diffusivity compared to controls.

While there are studies that look at the cuprizone mouse model with individual [224, 270, 240, 275, 246, 276] and multiparametric [164, 267, 95, 277] MRI methods, to date, there

are no multi-contrast (T_2 -weighted and MTI) weekly longitudinal studies and no multiparametric quantitative MRI studies (T_1 , mc T_2 , DTI and qMTI) focused on the cuprizone mouse model. Conventional T_2 -weighted images of the corpus callosum become hyperintense with continued cuprizone treatment [164, 267, 95, 275, 246] with greater changes in the caudal regions of the corpus callosum relative to the rostral regions at 4 weeks treatment [267] and significantly larger ventricles after 12 weeks of treatment [95]. Magnetization transfer ratios (MTR) are significantly lower in the corpus callosum after 4 to 6 weeks of cuprizone treatment [164, 276] with strong correlation between MTR and myelin basic protein (MBP) immunoreactivity [276] and moderate correlation between MTR and myelination measurements made with electron microscopy [164]. Several DTI studies of 0.2% cuprizone-fed mice have found significantly lower FA and higher $\lambda_{\perp}$ in the *in vivo* and *ex vivo* corpus callosum [224, 240, 95, 270, 277, 261]. Significantly lower $\lambda_{\parallel}$ was also observed *in vivo* after 4 to 6 weeks of treatment, indicative of axonal injury [222, 270, 277, 261], but not *ex vivo* [224, 277].

This study characterizes the cuprizone mouse model in the rostral corpus callosum and external capsule using weekly longitudinal measurements and multiparametric MRI analysis, including previously unapplied multicomponent T_2 and quantitative MTI analysis. We also used the cuprizone mouse model to measure the influence of different tissue features on various quantitative MRI metrics using ultrastructural examination after 6 weeks of cuprizone treatment along with correlations between quantitative MRI methods.

In vivo T_2 -weighted and magnetization transfer images were acquired weekly in control and cuprizone-fed mice. These are reflective of MRI methods commonly used in a clinical setting and were chosen, in part, due to constraints on imaging time. After sacrifice, DTI, qMTI, T_1/T_2 relaxometry and electron microscopy were used to analyze *ex vivo* tissue after 6 weeks of cuprizone delivery. Correlations between *in vivo* longitudinal MRI, *ex vivo* quantitative MRI, and electron microscopy measurements were made, with a focus on the rostral corpus callosum and external capsule. Using both longitudinal and multiparametric MRI analysis, new and complementary information about tissue pathology in the cuprizone mouse model was found.

Table 5.1: Timeline of cuprizone treatment, *in vivo* MRI (*in*), *ex vivo* MRI (*ex*), and electron microscopy (EM).

		MRI								
Week:		0	1	2	3	4	5	6	7	
Control (n = 5)		<i>in</i> [*]	<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in/ex</i>	EM
0.4% Cuprizone (n = 5)		<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in</i>	<i>in/ex</i> ^{**}		EM

* *In vivo* MRI includes T_2 -weighted and MTI.

** *Ex vivo* MRI includes T_1/T_2 relaxometry measurements, qMTI, and DTI.

5.2 Methods

5.2.1 Cuprizone Mouse Model

Ten female C57BL/6 mice (University of Manitoba breeding colony) were housed in the University of Manitoba animal facility with a 12 h dark/12 h light cycle. Cuprizone (Sigma-Aldrich) was mixed into milled rodent chow with a final concentration of 0.4% (w/w) and pressed into pellets. Starting at 8 weeks of age, the control group (n = 5) ate regular chow and the cuprizone-fed group (n = 5) ate chow containing cuprizone (both ad libitum). The experimental protocol was approved by the University of Manitoba Animal Care Committee who adhere to the guidelines and principles created by the Canadian Council on Animal Care.

5.2.2 MRI

In vivo imaging and analysis of the mice is described in Section 4.3.1 for the CPZ2 Study. Tissue was prepared and *ex vivo* imaging and analysis was performed as described in Section 4.3.2 for the CPZ2 Study.

5.2.3 Electron Microscopy

After *ex vivo* imaging, mouse heads were washed in PBS to remove any excess FOMBLIN. The top of the skull was removed and brains (in skull) were fixed in 2% glutaraldehyde and 2% PFA for at least 3 days. After fixation, brains were removed from the skull and a 1 mm

thick slice was removed corresponding to the oblique coronal slice in [Figure 4.9](#). From this slice, the corpus callosum was dissected using a dissecting microscope. Elongated cubes of corpus callosum were further fixed in phosphate buffered 4% glutaraldehyde followed by 1% osmium tetroxide overnight, dehydrated in alcohol then acetone, and embedded in Epon resin. Semithin sections (1 μm) were stained with toluidine blue. Ultrathin sections (90 nm) from the corpus callosum were contrasted with uranyl acetate and lead citrate then viewed and photographed with a JEOL 1010 transmission electron microscope equipped with digital camera. An experienced neuropathologist took representative photomicrographs. The differences between control and cuprizone-fed mice were obvious and therefore blinding was not possible.

In order to count the cellular features, a $2 \times 2 \mu\text{m}^2$ grid was overlaid on two 5000x electron microscopy images per mouse with 180 points/image (360 points/mouse). At each point, tissue was classified as myelinated axons (A), myelin sheaths (M), non-myelinated material (C), and extracellular space (E). The non-myelinated material included demyelinated/unmyelinated axons, nuclei, glial processes, lipid droplets, damaged axons, and apoptotic cells, with the last four abnormalities occurring only in cuprizone-fed mice. The fraction of A, M, C, and E was calculated for control and cuprizone-fed mice.

On two 15000x images per mouse, the narrowest axon diameter (to compensate for possible oblique sections) and myelin sheath thickness was manually measured in all visibly myelinated axons. Axon diameters were split into three equal-sized bins based on the distribution in the control mice ($< 0.3\mu\text{m}$, $0.3\text{--}0.45 \mu\text{m}$, and $> 0.45\mu\text{m}$). The same was done for the myelin sheath thickness ($< 0.06\mu\text{m}$, $0.06\text{--}0.075 \mu\text{m}$, and $> 0.075\mu\text{m}$). Based on these measurements, the number of myelinated axons per $100 \mu\text{m}^2$ was calculated. As well, the M/A ratio (myelin sheath thickness/axon diameter) and g-ratio (axon diameter/fiber diameter) were calculated for each mouse, where,

$$g = \frac{A}{2M + A} = \frac{1}{2M/A + 1} \quad (5.1)$$

is inversely proportion to the M/A ratio.

5.2.4 Statistical Analysis

For *in vivo* T₂-weighted images and MTR maps, two-way repeated measures ANOVA was performed with one within-subjects factor (week) and one between-subjects factor (treatment) along with Mauchly’s test of sphericity. In the case of significant differences between-subjects, independent samples T-tests were performed to test for significant weekly differences between treatments. One-way ANOVA was used to measure the significance of weekly changes in both control and cuprizone-fed mice followed by Tukey’s post-hoc test.

For *ex vivo* T₁, T₂, DTI, qMTI, and EM measurements, independent samples T-tests along with Levene’s test for equality of variances were performed to test differences between control and cuprizone-fed mice.

In all *in vivo* and *ex vivo* MRI maps, Spearman’s rank correlation coefficients were calculated using both mean values (ROI-based analysis) and individual voxel values (voxel-based analysis) in the corpus callosum, external capsule, and cerebral cortex of control, cuprizone-fed and combined (control+cuprizone-fed) datasets. In addition, Spearman correlation coefficients were calculated between mean MRI values in the corpus callosum and electron microscopy measurements.

The Spearman’s rank correlation coefficient finds the Pearson correlation coefficient of the rank of the two variables, denoted as x_i and y_i :

$$\rho = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \quad (5.2)$$

Unlike the Pearson correlation coefficient, which uses the actual variable values, Spearman’s rank correlation coefficient assumes a monotonic relationship between the two variables, but doesn’t assume normality, homoscedasticity, or linearity. Because we cannot assume the relationship between the various MRI metrics and EM measurements are linear, this is a suitable test of correlation. In the case of f vs. the myelin sheath fraction, both values are strongly related to the bound water content. In this case, a linear relationship was assumed and Pearson’s correlation coefficient (r) was calculated along with the linear regression.

5.3 Results

5.3.1 Cuprizone Diet

The cuprizone-fed mice weighed significantly less than the control mice at 3 weeks of treatment ((16.5 ± 1.0) g vs. (21.1 ± 1.3) g) but did not change significantly over the following three weeks (21.7% lighter at 3 weeks vs. 23.7% lighter at 6 weeks). None of the mice showed signs of lethargy or increased mortality previously reported at 0.4% cuprizone [103].

5.3.2 *In Vivo* MRI

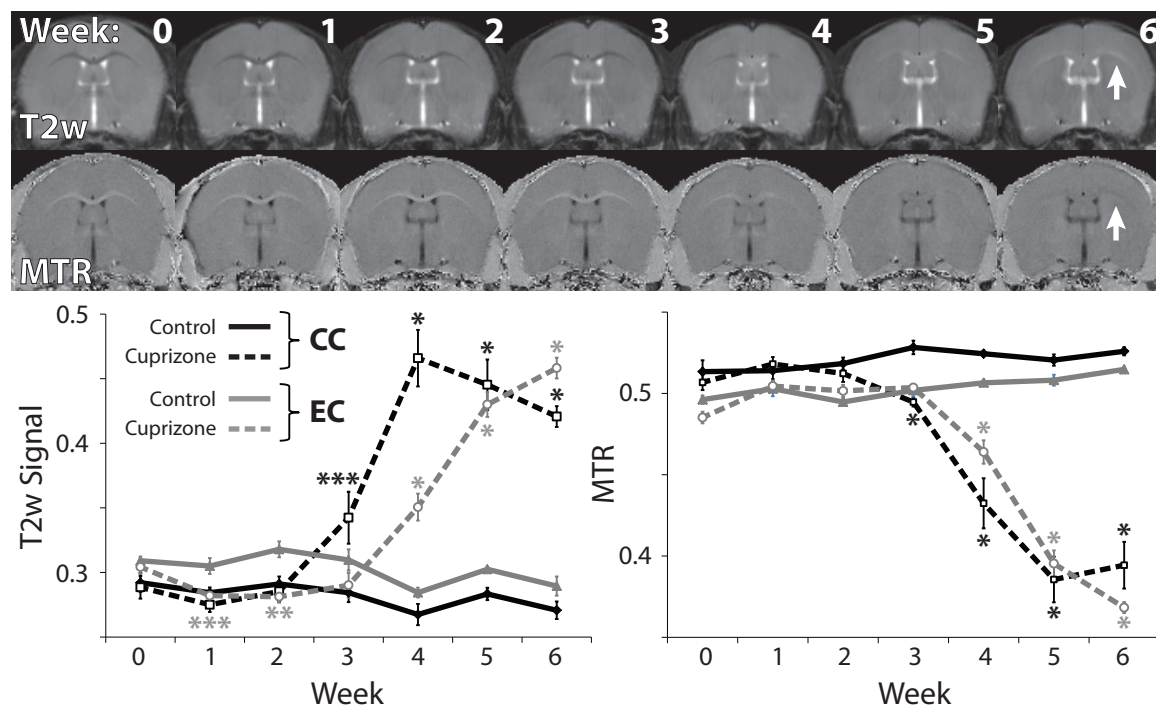


Figure 5.1: Normalized T_2 -weighted (T2w) and MTR maps from 0 to 6 weeks in a cuprizone-fed mouse. Signal inversion in the T_2 -weighted image and decreased MTR is obvious in the white matter regions (arrows). Mean $\pm$ SEM values are presented for normalized T_2 -weighted signal and MTR in the corpus callosum (CC) and external capsule (EC) of control and cuprizone-fed mice. $*p < 0.001$, $**p < 0.01$, $***p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.

As cuprizone treatment progressed, contrast inversion was evident in the corpus callosum and external capsule of the cuprizone-fed mice [Figure 5.1](#). In the normalized T_2 -weighted im-

ages, two-way repeated measures ANOVA showed significant differences in signal intensity between treatment in the corpus callosum and external capsule ($p < 0.001$). Independent samples T-tests verified differences between treatment were significant starting at week 3 in the corpus callosum and week 4 in the external capsule ($p < 0.001$ for both). Two-way repeated measures ANOVA also indicated significant weekly changes and significant interaction between week and treatment ($p < 0.001$ for both) in the corpus callosum (sphericity not assumed) and external capsule (sphericity assumed). One-way ANOVA confirmed significant week-to-week changes occurred in the cuprizone-fed corpus callosum and external capsule ($p < 0.001$ for both). In the corpus callosum, Tukey's post-hoc test confirmed significant differences between the first 3 weeks of treatment and the last 3 weeks of treatment ($p < 0.01$), with week 4 significantly larger than week 3 and weeks 4–6 significantly larger than all of the preceding weeks. Similar results were found in the external capsule except for week 4, which was significantly different from all weeks, both before and after ($p < 0.01$).

Lower MTR values were evident in the corpus callosum of the cuprizone-fed mice when compared to control mice [Figure 5.1](#). Two-way repeated measures ANOVA showed significant differences in MTR between treatment in the corpus callosum and external capsule ($p < 0.001$). Independent samples T-tests verified differences between treatment were significant starting at week 3 in the corpus callosum ($p < 0.001$) and week 4 in the external capsule ($p < 0.001$). Two-way repeated measures ANOVA also indicated significant weekly changes and significant interaction between week and treatment ($p < 0.001$ for both) in the corpus callosum (sphericity not assumed) and external capsule (sphericity assumed). One-way ANOVA confirmed significant week-to-week changes occurred in the cuprizone-fed corpus callosum and external capsule ($p < 0.001$ for both). In the corpus callosum, Tukey's post-hoc test confirmed significant differences between the first 3 weeks of treatment and the last 3 weeks of treatment ($p < 0.01$), with week 4 significantly smaller than week 3 ($p < 0.01$) and significantly larger than week 5 ($p < 0.05$) and weeks 5–6 significantly smaller than all of the preceding weeks. Similar results were found in the external capsule except for weeks 4, 5 and 6, which were each significantly smaller than the preceding weeks and significantly larger than the subsequent weeks ($p < 0.01$).

Both methods were sensitive to the effects of the cuprizone treatment. Normalized T_2 -weighted images and MTR maps detected changes in the corpus callosum at 3 weeks. T_2 -weighted signal in the corpus callosum peaked at week 4, and decreased at weeks 5 and 6. This was in direct contrast to the normalized T_2 -weighted signal and MTR values in the external capsule, which both continued to increase/decrease throughout the treatment. The MTR values in the corpus callosum followed the external capsule more closely, peaking one week later at week 5.

5.3.3 *In Vivo* T2-Weighted and MTR Correlations

Sample size was too small to test correlation significantly between mean values from ROIs in control and cuprizone-fed mice on a weekly basis. ROI-based correlations across all weeks in control, cuprizone-fed, and combined datasets (light grey lines in [Figure 5.2](#)) reflect their voxel-based counterparts, with the strongest correlations in the cuprizone-fed mice and weakest in the control mice, corresponding to the breadth of tissue pathologies experienced throughout the cuprizone treatment. Larger correlations in the external capsule vs. the corpus callosum in control mice reflects greater breadth of tissue characteristics in the external capsule, possibly due in part to partial volume averaging with grey matter in the narrower external capsule.

The progression of the cuprizone treatment can be tracked by looking at the 95% confidence ellipses in the scatter plots ([Figure 5.2](#)). From 0–2 weeks in the corpus callosum and 0–3 weeks in the external capsule, the confidence ellipses are similar to the confidence ellipse for all control mice (solid dark grey line). After 2 or 3 weeks, the confidence ellipses of the cuprizone-fed mice start to move away from the control mice, although the correlation coefficients don't change significantly. Correlation of the combined dataset (solid black line) reflects the progression of the cuprizone treatment, with changes in the corpus callosum preceding changes in the external capsule, reflected by increasingly negative correlation coefficients starting at weeks 3 and 4, respectively. Weekly correlations were similar in both the control and cuprizone-fed mice until week 6, when correlations decreased dramatically in the cuprizone-fed mice. The *in vivo* normalized T_2 -weighted and MTR values obtained

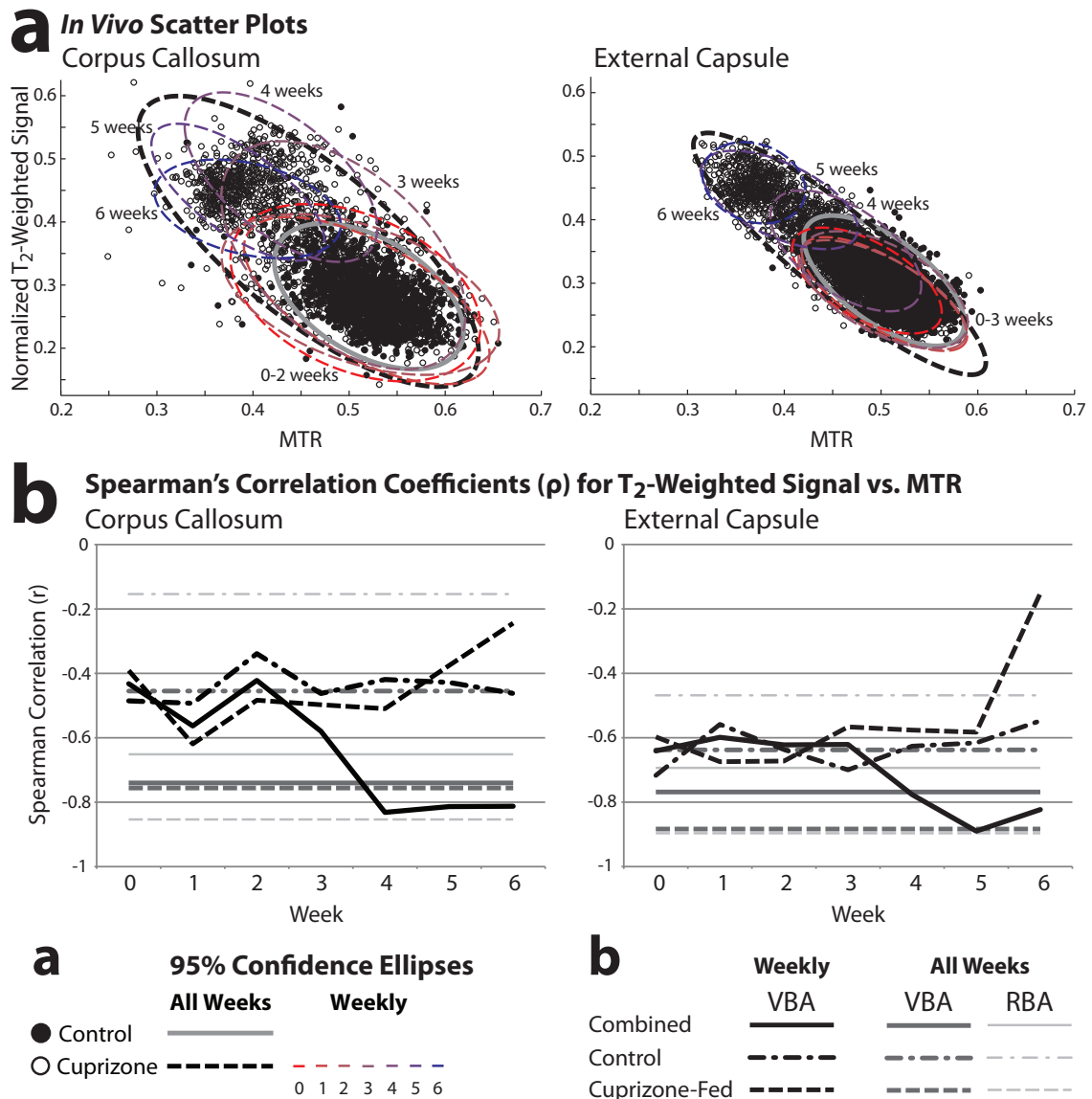


Figure 5.2: (a) Scatter plots of normalized T_2 -weighted and MTR voxel values in the corpus callosum and external capsule. 95% confidence ellipses outline regions encompassing all weeks for control (solid grey line) and cuprizone-fed (dashed black line) mice. In addition, 95% confidence ellipses outline regions in each week for control (not shown, but all were similar to the solid grey line) and cuprizone-fed mice (dashed line from red (week-0) to blue (week-6), see scale for exact colour-coding). (b) Voxel-based Spearman correlation coefficients (r) of normalized T_2 -weighted and MTR voxel values from 0 to 6 weeks in the control, cuprizone-fed, and combined datasets. Correlation stays relatively constant in control and cuprizone-fed mice until the sixth week, when correlation dramatically decreases in the cuprizone-fed mouse. Correlation of the combined dataset reflects the progression of the cuprizone treatment, with changes in the corpus callosum preceding changes in the external capsule.

prior to perfusion are given in Table 5.2.

5.3.4 Relaxometry

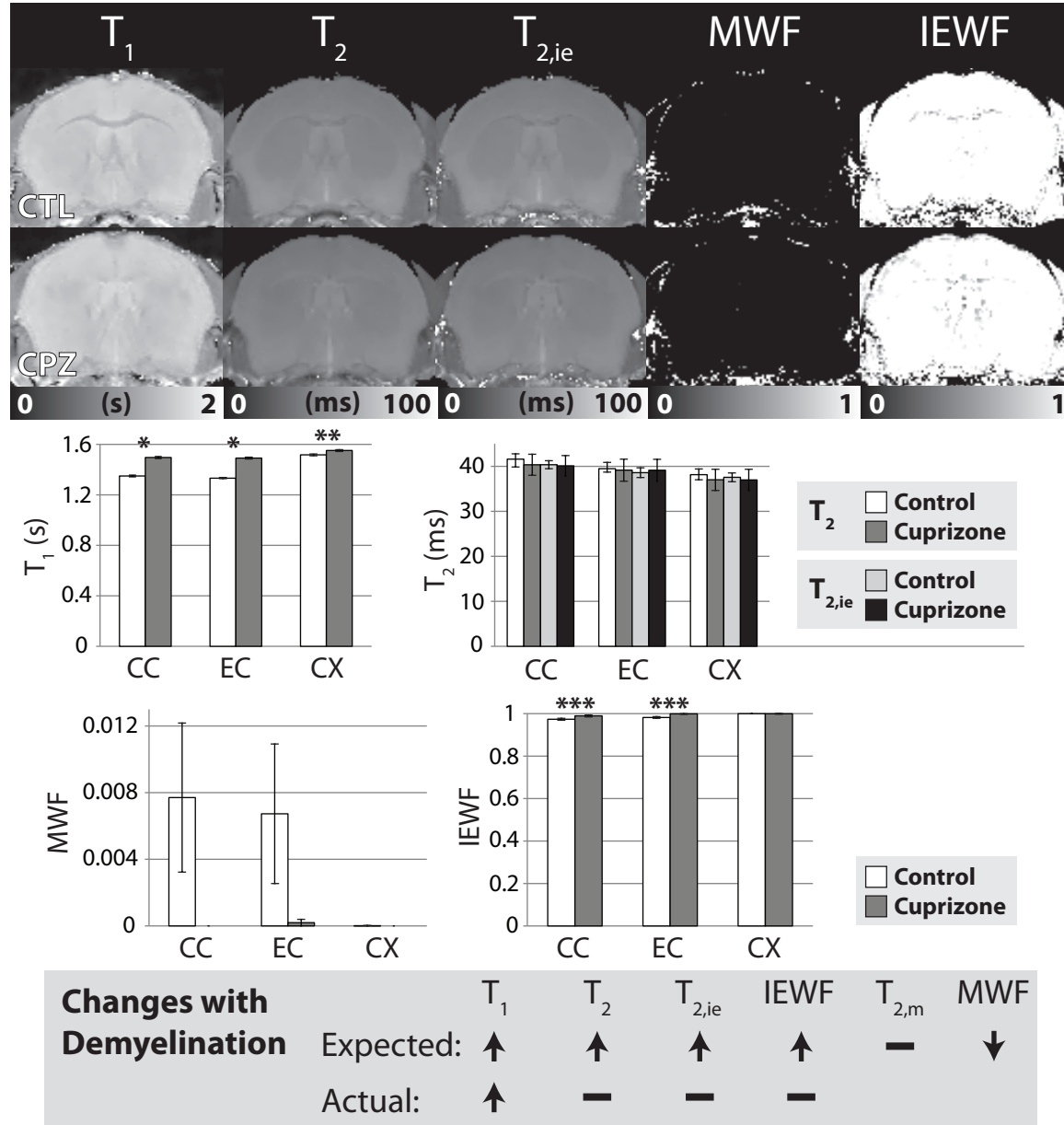


Figure 5.3: (a) Control (CTL) and (b) cuprizone-fed (CPZ) T_1 , T_2 , and water fraction maps. The scale from black to white is given below each map. Mean $\pm$ SEM values are presented for the corpus callosum (CC), external capsule (EC), and cerebral cortex (CX). T_1 increases with demyelination in white matter (as expected), however, T_2 and $T_{2,ie}$ were not significantly different and no significant myelin water component was detected. $*p < 0.001$, $**p < 0.01$, $***p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.

Representative T_1 , T_2 , $T_{2,ie}$, MWF, and IEWF maps for control and cuprizone-fed mice are shown in Figure 5.3) with numerical values in Table 5.2. T_1 was significantly larger in all three regions of the cuprizone-fed mice when compared to controls.

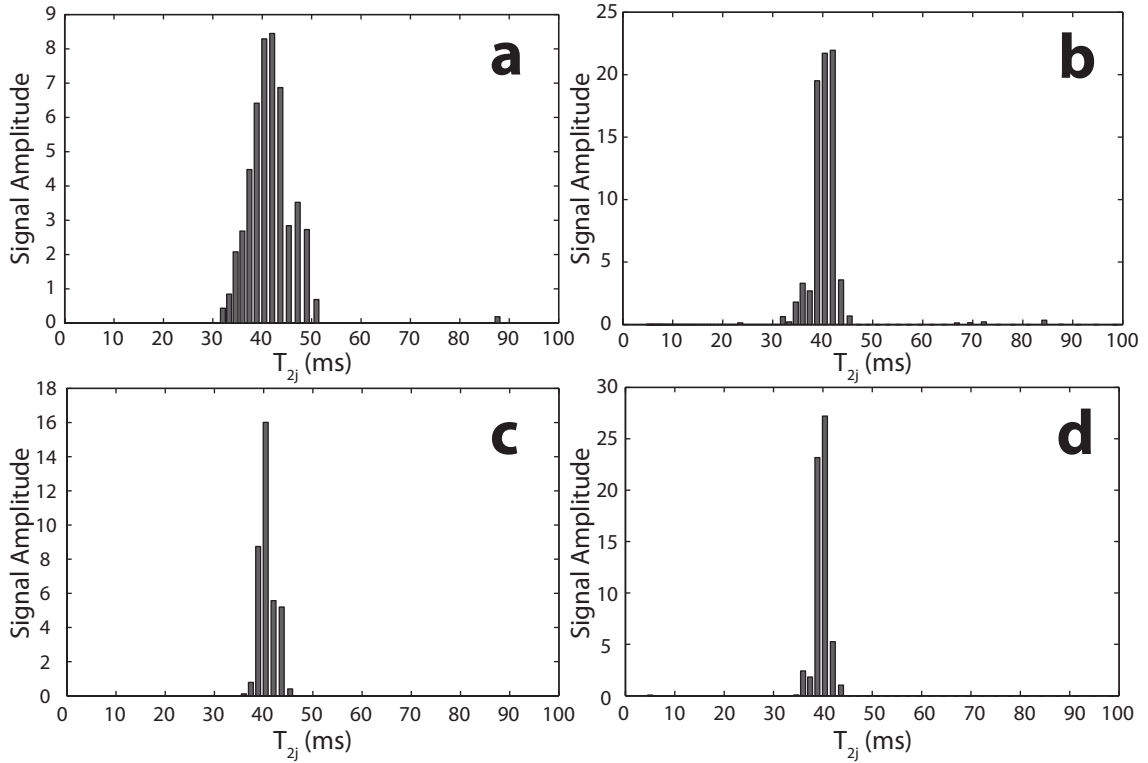


Figure 5.4: Distribution of multicomponent T_2 signal amplitudes. Representative control (a, b) and cuprizone-fed (c, d) signal amplitudes vs. the T_{2j} values in the corpus callosum (a, c) and external capsule (b, d).

Visual analysis of the distribution of signal amplitudes vs. T_{2j} values in the corpus callosum and external capsule revealed a single peak corresponding to the single component T_2 fit, with no significant values corresponding to a myelin water component (Figure 5.4). MWF was less than 0.01 in the control mice and essentially 0 in the cuprizone-fed mice. While the IEWF appears to be significantly larger in the cuprizone-fed corpus callosum and external capsule (equal variances not assumed), it is important to note that IEWF values were within 0.02 of 1 for the control mice and within 0.01 of 1 for the cuprizone-fed mice. Significance will be exaggerated by this ceiling, just as the MWF might appear significantly different because the majority of the cuprizone-fed mice had an MWF = 0. Because the MWF was essentially zero, only the geometric mean of the intra-/extracellular

T_2 distribution was used in any subsequent analysis. The T_2 values themselves had a large standard deviation, with mean values in the corpus callosum ranging from (37 ± 3) to (43 ± 4) ms in control mice and from (31 ± 1) to (45 ± 2) ms in cuprizone-fed mice. There were no significant differences between T_2 and $T_{2,ie}$ in any of the regions.

5.3.5 DTI

Representative $\langle D \rangle$, $\lambda_{\parallel}$, $\lambda_{\perp}$, FA, and RGB colour maps for control and cuprizone-fed mice are shown in [Figure 5.5](#) with numerical values in [Table 5.2](#). $\langle D \rangle$, $\lambda_{\parallel}$, and $\lambda_{\perp}$ were significantly higher in both the corpus callosum and external capsule of the cuprizone-fed corpus callosum when compared to controls. FA was significantly lower in both regions compared to controls. There was no significant difference in any of the DTI metrics in the cerebral cortex.

When comparing values between the corpus callosum and external capsule in the same treatment group, $\langle D \rangle$, $\lambda_{\perp}$, and FA were significantly different in the control group, with the latter two metrics also significantly different in the cuprizone-fed mice. This is indicative of sensitivity between white matter structures and not just treatment groups.

5.3.6 qMTI

Representative f , R_A , RM_0^A , T_2^A , and T_2^B maps for control and cuprizone-fed mice are shown in [Figure 5.6](#). f and R_A were significantly smaller in all three regions of the cuprizone-fed mice compared to controls. RM_0^A and T_2^A were significantly larger in the corpus callosum and external capsule. T_2^B was significantly smaller in just the external capsule.

When comparing values between the corpus callosum and external capsule in the same treatment group, RM_0^A and T_2^B were significantly different in both the control and cuprizone-fed groups. T_2^A was also significantly different in the cuprizone-fed group. As with the DTI metrics, this is indicative of sensitivity between white matter structures and not just treatment groups.

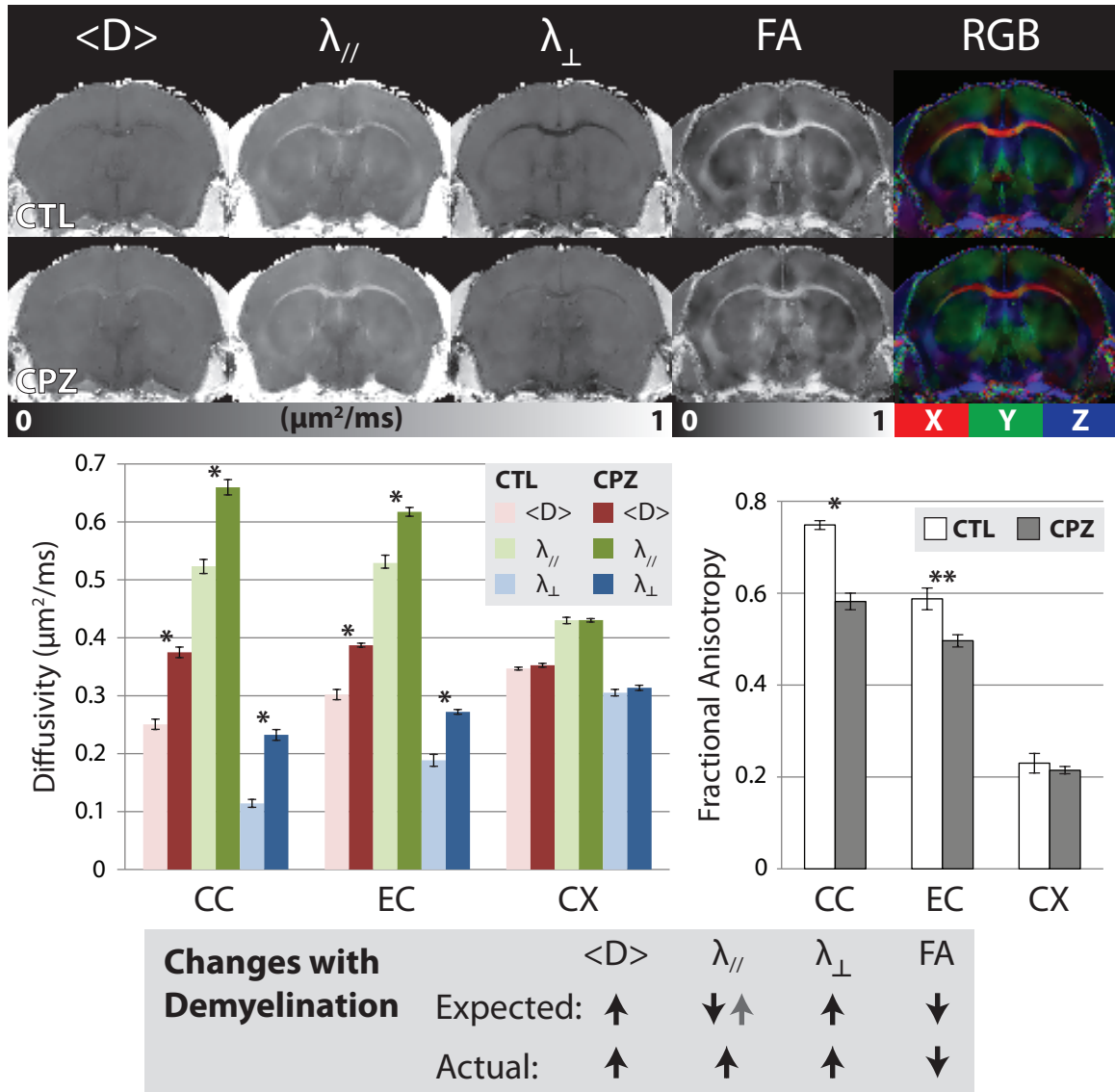


Figure 5.5: (a) Control (CTL) and (b) cuprizone-fed (CPZ) DTI maps. The scale from black to white is given below each map (except for the RGB colour map, which shows the colours corresponding to diffusion in the x, y, and z directions). Mean $\pm$ SEM values are presented for the corpus callosum (CC), external capsule (EC), and cerebral cortex (CX). $\langle D \rangle$, $\lambda_{\perp}$, and $\lambda_{||}$ were significantly larger and FA was significantly smaller with demyelination. * $p < 0.001$, ** $p < 0.01$ when comparing control and cuprizone-fed mice using an independent samples T-test.

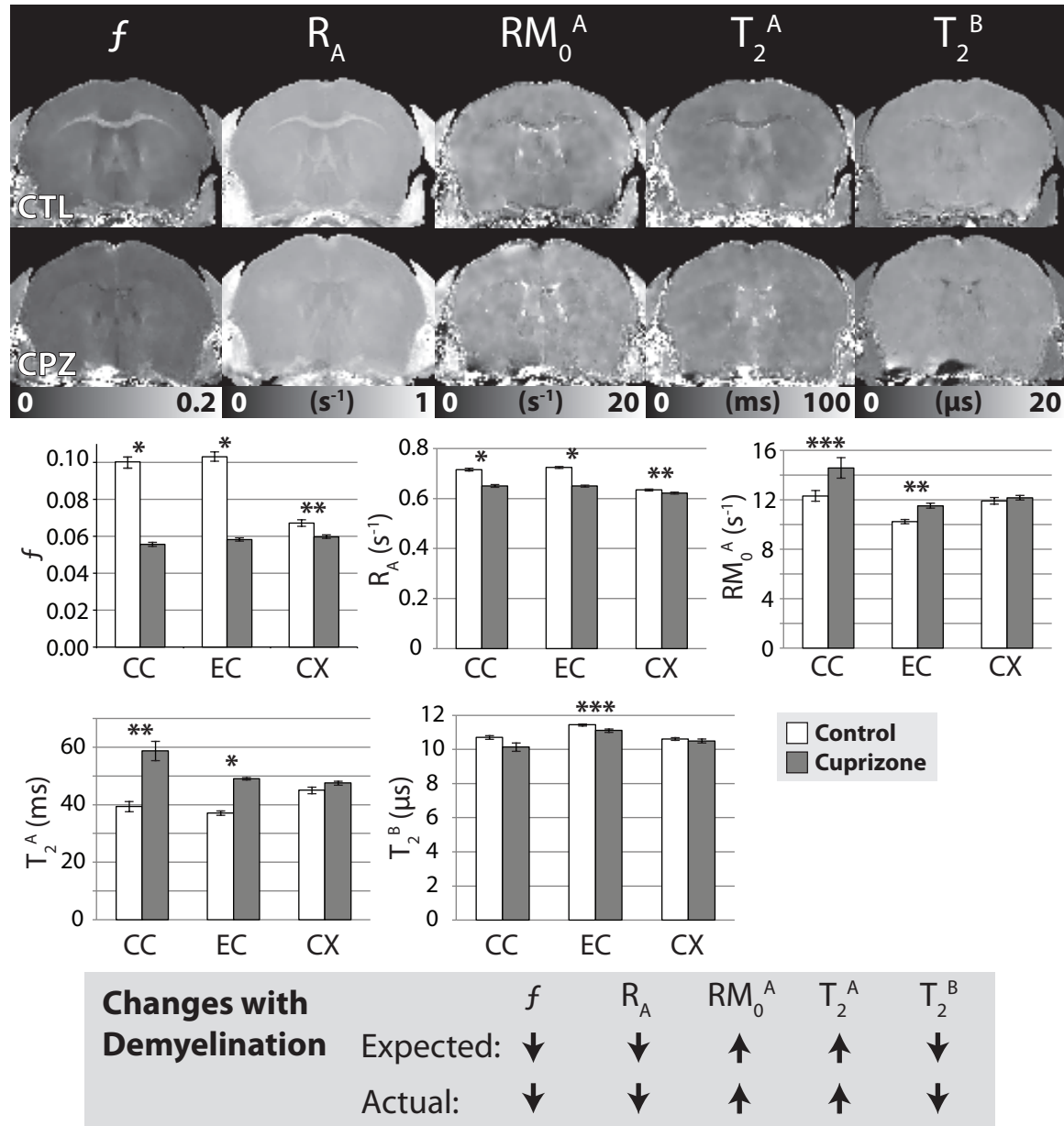


Figure 5.6: (a) Control and (b) cuprizone-fed quantitative MTI maps. The scale from black to white is given below each map. Mean $\pm$ SEM values are presented for the corpus callosum (CC), external capsule (EC), and cerebral cortex (CX). Significantly decreased f , R_A , and T_2^B and significantly increased RM_0^A and T_2^A reflect previous studies of demyelination in humans and animal models. * $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.

Table 5.2: Summary of *in vivo* and *ex vivo* MRI metrics. *In vivo* images were taken the same day of perfusion. Values reported as mean (standard error of the mean).

Method	Metric	Corpus Callosum		External Capsule		Cerebral Cortex	
		Control	Cuprizone	Control	Cuprizone	Control	Cuprizone
Relaxometry	T_1 (s)	1.350	1.496 [*] (0.007)	1.332	1.492 [*] (0.003)	1.517	1.552 ^{**} (0.006)
	T_2 (ms)	41.6	40.3 (2.3)	39.5	39.2 (2.4)	38.2	37.0 (2.4)
	$T_{2,te}$ (ms)	40.4	40.1 (2.3)	38.6	39.1 (2.4)	37.6	37.0 (2.4)
	MWF	0.008	0	0.007	0.002 (0.004)	0.000016	0
	IEWF	0.973	0.990 ^{***} (0.004)	0.982	0.999 ^{**†} (0.001)	0.99987	0.9995 (0.0003)
DTI	$\langle D \rangle$ ($\mu\text{m}^2/\text{ms}$)	0.251	0.375 [*] (0.009) ^{β}	0.302	0.387 ^{*†} (0.004) ^{β}	0.347	0.353 (0.003)
	$\lambda_{ }$ ($\mu\text{m}^2/\text{ms}$)	0.524	0.660 [*] (0.015)	0.529	0.617 ^{**} (0.014)	0.430	0.431 (0.002)
	$\lambda_{\perp}$ ($\mu\text{m}^2/\text{ms}$)	0.114	0.233 [*] (0.007) ^{α}	0.189	0.272 [*] (0.010) ^{α}	0.305	0.314 (0.005)
	FA	0.749	0.582 [*] (0.009) ^{α}	0.588	0.497 ^{**} (0.023) ^{α}	0.230	0.215 (0.008)
	f	0.100	0.056 [*] (0.003)	0.103	0.058 [*] (0.002)	0.067	0.060 ^{**} (0.001)
MTI	R_A (s^{-1})	0.715	0.650 [*] (0.004)	0.724	0.650 [*] (0.002)	0.635	0.622 ^{**} (0.003)
	RM_0^A (s^{-1})	12.31	14.57 ^{***} (0.42) ^{β†}	10.24	11.51 ^{**} (0.17) ^{β†}	11.91	12.16 (0.18)
	T_2^A (ms)	39.4	58.7 ^{**} (1.7)	37.1	49.0 [*] (3.4) ^{χ†}	45.1	47.5 (0.7)
	T_2^B (μs)	10.71	10.15 (0.10) ^{α}	11.45	11.11 ^{***} (0.24) ^{β}	10.62	10.51 (0.12)
	T_{2w}	0.279	0.421 [*] (0.011)	0.297	0.458 [*] (0.011)	0.398	0.395 (0.009)
<i>In Vivo</i>	MTR	0.514	0.394 [*] (0.009)	0.503	0.368 ^{*†} (0.007)	0.406	0.397 (0.003)

^{*} $p < 0.001$, ^{**} $p < 0.01$, and ^{***} $p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.

^{α} $p < 0.001$, ^{β} $p < 0.01$, and ^{χ} $p < 0.05$ when comparing values in the corpus callosum and external capsule using an independent samples T-test. Placed after (SEM) for clarity.

[†] equal variances not assumed

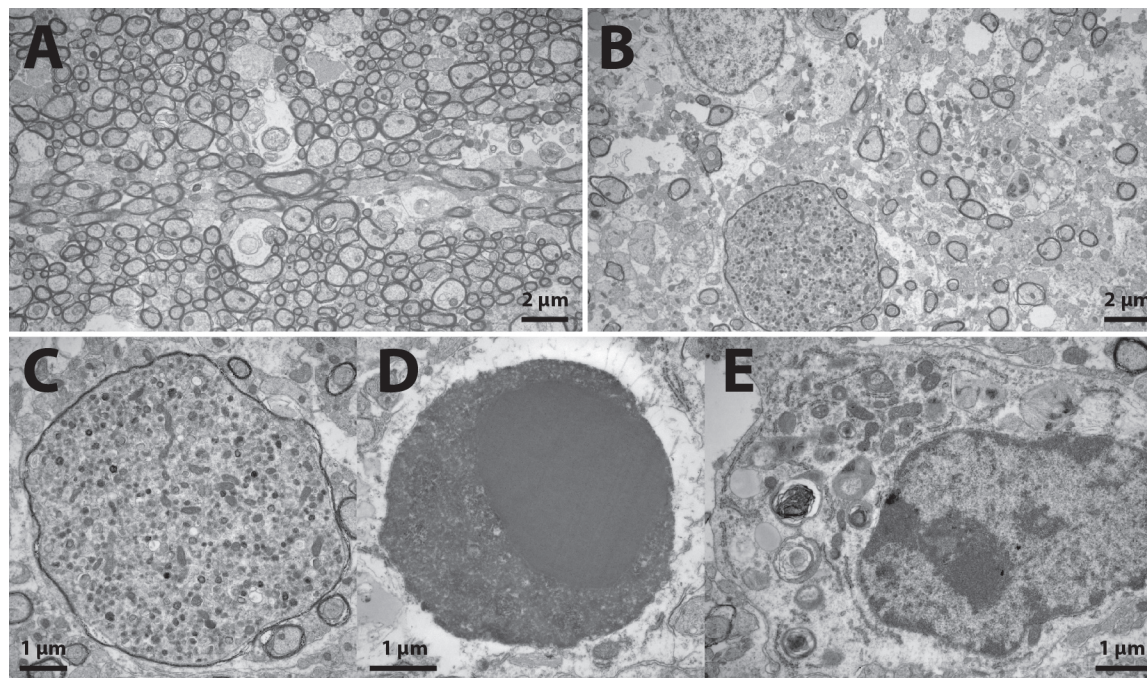


Figure 5.7: Myelinated axons in the control mouse (A) and demyelinated axons in the cuprizone-fed mice (B) with additional pathologies such as large, swollen axons with accumulation of organelles (C), apoptotic cells (D), and macrophage infiltration (E).

5.3.7 Electron Microscopy

After 6 weeks of cuprizone treatment, the corpus callosum was almost completely demyelinated. Along with demyelinated and thinly myelinated axons, there were large, swollen axons with accumulation of organelles. Corpora callosa in the cuprizone-fed mice also featured apoptotic glial cells (probably oligodendrocytes but could not be determined with certainty), reactive microglia with lipid debris, and macrophage infiltration (Figure 5.7).

All EM cellular distributions and measurements are summarized in Table 5.3. The distribution of cellular features in the 5000x EM images reflect the substantial changes seen in the cuprizone-fed mice (Figure 5.8). Myelinated axon fractions and myelin sheath fractions were both significantly smaller and non-myelinated cell fractions were significantly larger. Although extracellular space increased slightly in the cuprizone-fed mice, its relatively small size in both groups indicates its influence on MRI metrics should be minimal. Also it must be noted that tissue processing for EM tends to shrink tissue and contract the extracellular

Table 5.3: Summary of electron microscopy cellular distributions and measurements in the corpus callosum of control and cuprizone-fed mice. Values reported as mean (standard error of the mean).

	Metric	Control	Cuprizone
Distribution of Cells	Myelinated Axons (%)	29.8 (1.6)	6.4 ^{*,†} (0.7)
	Myelin Sheaths (%)	28.5 (2.1)	4.8 [*] (0.8)
	Non-Myelinated Cells (%)	41.2 (1.7)	85.1 [*] (2.0)
	Extracellular Space (%)	0.5 (0.2)	3.7 (1.4)
	Myelinated Axons/100 μm^2	116.1 (13.2)	19.2 ^{*,†} (2.2)
Myelinated Axon Diameter	Mean (μm)	0.43 (0.03)	0.59 (0.03)
	< 0.3 μm (%)	29.8 (5.3)	3.1 ^{**} (2.0)
	0.3 - 0.45 μm (%)	33.6 (2.1)	23.1 ^{***} (3.3)
	> 0.45 μm (%)	36.5 (7.3)	73.8 ^{**} (4.6)
Myelin Sheath Thickness	Mean (μm)	0.070 (0.003)	0.057 (0.002)
	< 0.06 μm (%)	32.4 (7.7)	54.8 (7.7)
	0.06 - 0.075 μm (%)	33.1 (2.9)	16.6 (6.8)
	> 0.075 μm (%)	34.5 (6.0)	28.6 (8.6)
Ratios	g-Ratio	0.734 (0.019)	0.830 ^{**} (0.014)
	M/A Ratio	0.190 (0.020)	0.106 ^{**} (0.010)

* $p < 0.001$, ** $p < 0.01$, and *** $p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.

[†] equal variances not assumed

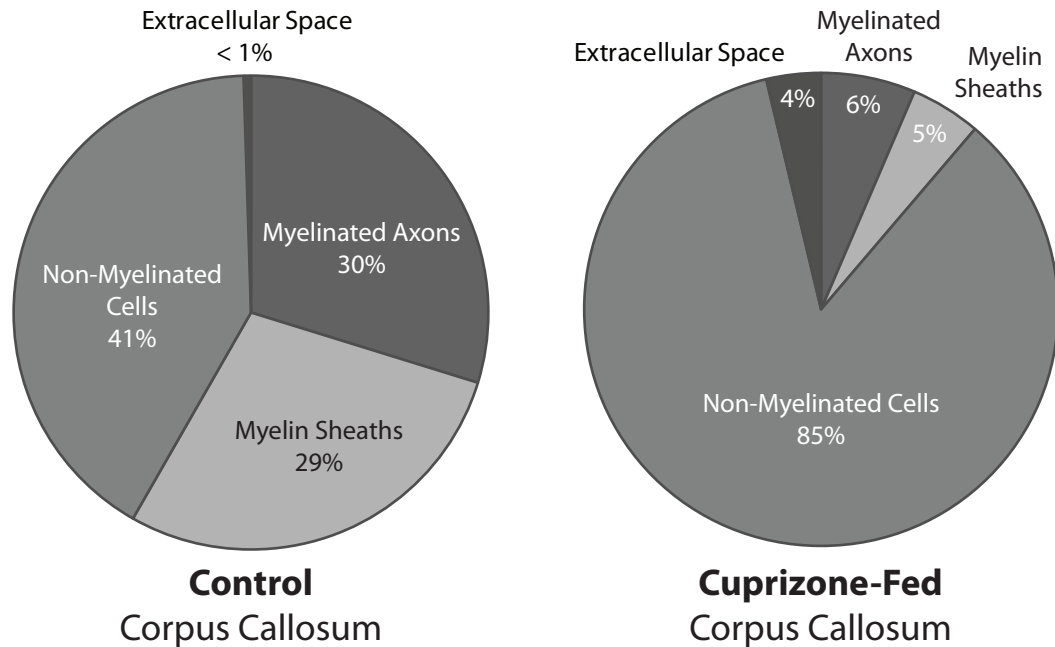


Figure 5.8: Distribution of features in control and cuprizone-fed mice.

compartment.

The mean myelinated axon diameter in the control mice ($0.43 \pm 0.03 \mu\text{m}$) was very

close to the value previously measured in the corpus callosum of 90-day old mice ($0.42 \pm 0.03 \mu\text{m}$) [235]. The number of myelinated axons per $100 \mu\text{m}^2$ was significantly smaller in the cuprizone-fed mice compared with controls (Table 5.3). The M/A ratio was also significantly smaller in myelinated axons of cuprizone-fed mice. In a histogram of the myelinated axon diameters, only larger axons in the cuprizone-fed mice tend to remain myelinated (Figure 5.9). The myelin sheath thickness histogram shows that, in the axons that remain myelinated, myelin sheath thickness tends to be thinner. Figure 5.9 and the M/A ratios in Table 5.3 show that cuprizone treatment causes significant demyelination of smaller myelinated axons while also thinning the myelin sheaths of larger myelinated axons. Some thin myelin sheaths might also be attributed to the start of remyelination.

5.3.8 MRI and EM Correlations

Spearman correlation coefficients of MRI metrics vs. EM cellular distributions and measurements in combined, control, and cuprizone-fed treatment groups are summarized in Table 5.4.

The strongest correlation with the myelinated axon fraction was shared by R_A and T_1 , with $\rho = 0.90$ and $\rho = -0.90$, respectively. Given that R_A is derived from T_1 , it is no surprise that R_A and T_1 have a voxel-based correlation coefficient of 1.00 in the corpus callosum. Correlations between T_1 and R_A with all EM measurements were the same in the combined group. Following R_A and T_1 , the next strongest correlations with the myelinated axon fraction were T_2^A ($\rho = -0.85$) and $\langle D \rangle$ ($\rho = -0.83$).

The strongest MRI correlate with the myelin sheath fraction was f ($\rho = 0.93$), followed by $\lambda_\perp$ ($\rho = 0.87$) and FA ($\rho = 0.84$). When myelinated axons and myelin sheaths are combined into a single fiber fraction, the strongest MRI correlates were f ($\rho = 0.89$), $\langle D \rangle$ ($\rho = -0.87$), and $\lambda_\parallel$ ($\rho = -0.85$). The non-myelinated cell fraction, which is closely tied to the fiber fraction, had the same top three correlations, albeit in a different order: $\lambda_\parallel$ ($\rho = 0.92$), $\langle D \rangle$ ($\rho = 0.90$), and f ($\rho = -0.90$).

The Pearson correlation coefficient, r is 0.98 ($p \ll 0.001$) when comparing f to the myelin sheath fraction. If we set the myelin sheath fraction as the dependent variable

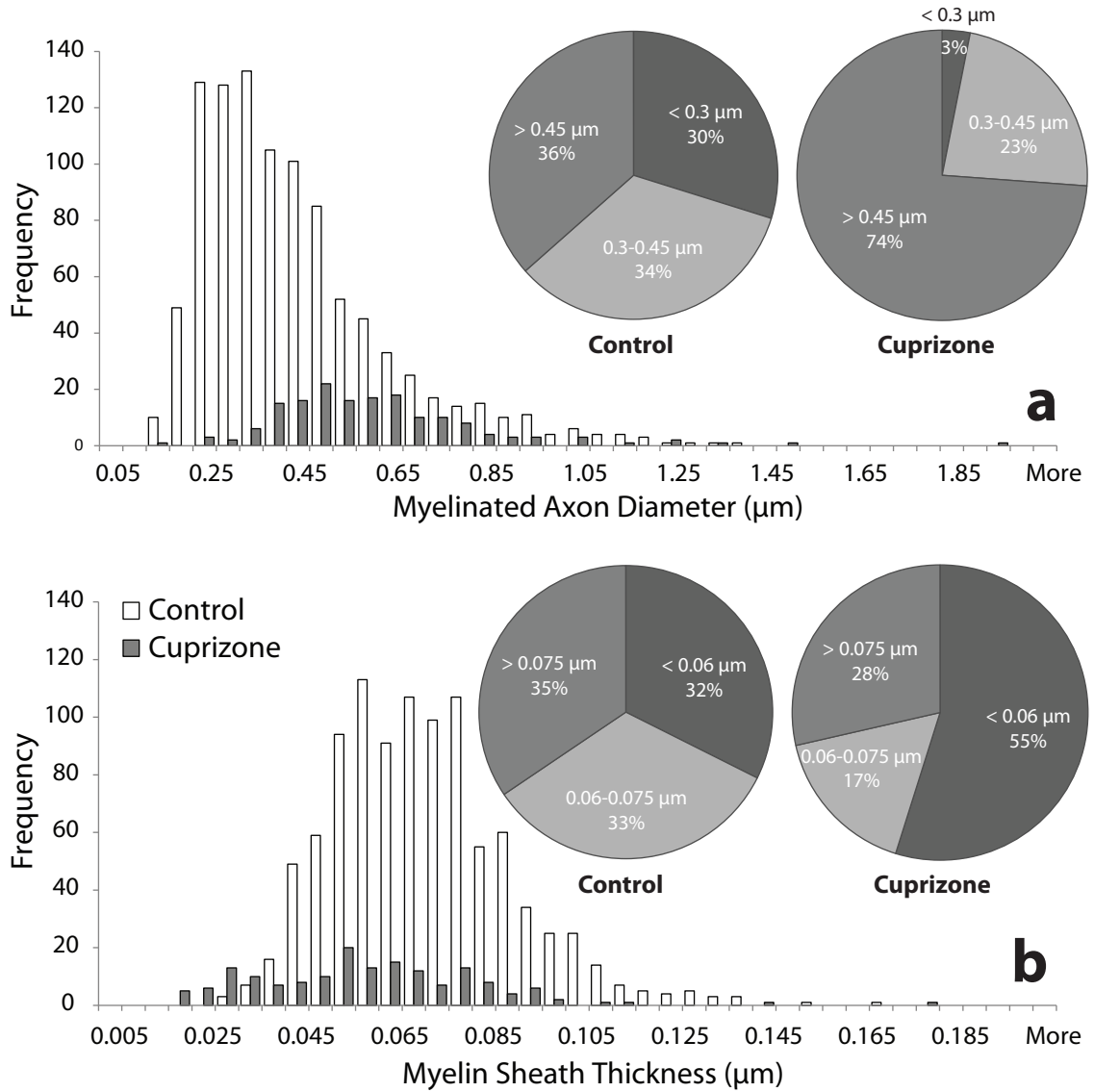


Figure 5.9: Myelinated axon diameter and myelin sheath thickness histograms for control and cuprizone-fed mice. Fraction of axon diameters and myelin thicknesses in control and cuprizone-fed mice (insets).

and f and $\lambda_{\perp}$ as the predictors, stepwise multiple regression also determines f is the best predictor of the myelin fraction. $\lambda_{\perp}$ has a tolerance of 0.061, suggesting the two predictors are colinear. The maximum Cook's Distance = 0.959 suggests there are no outliers that will dramatically affect the model, although there are outliers with intermediate affects on the coefficients in the model. Since we only have 10 dependent results (myelin sheath fractions), the minimum ratio of 1 predictor for every 5 dependent results limits us to 2 predictor variables when using multiple regression. More dependent results would allow

more predictor variables, possibly improving the linear regression by including more than one MRI metric when fitting to the myelin sheath fraction.

The strongest *in vivo* MRI correlation was between MTR and the myelinated axon fraction ($\rho = 0.81$). Given the relationship of MTR to T_1 and RM_0^A (which were both most strongly correlated to the myelinated axon fraction) as well as f , this is not a surprising result.

$\lambda_{\parallel}$ was most strongly correlated with the number of myelinated axons per $100 \mu\text{m}^2$ ($\rho = -0.89$) while f had the strongest correlation with the M/A ratio and fraction of axon diameters $< 0.3 \mu\text{m}$ ($\rho = 0.88$). The g-ratio is inversely proportional to the M/A ratio, and as such, gives negative correlation coefficients identical to those found with the M/A ratio (not shown). $\lambda_{\perp}$ had the strongest correlation with the fraction of axon diameters between 0.3 and $0.45 \mu\text{m}$ ($\rho = -0.78$) and axon diameters $> 0.45 \mu\text{m}$ ($\rho = 0.85$).

It is important to note that cellular distribution fractions are representative of all tissue in the sampled images, while EM measurements (starting with myelinated axons per $100 \mu\text{m}^2$) only pertain to the myelinated axons, which in the cuprizone-fed mice, are a minority of the cellular structures. While there were some strong correlations between these EM measurements and the MRI metrics, they may be reflective of underlying factors and should be interpreted with caution. This is particularly evident in the sudden shift in sign of correlations between f and diffusivity measurements with axon diameters $> 0.45 \mu\text{m}$, akin to the correlations with the non-myelinated cells. Similarly, correlations with smaller axon diameter fractions, the M/A ratio, and the number of myelinated axons / $100 \mu\text{m}^2$ appear to reflect correlations with the myelinated axon and myelin sheath fractions.

Correlations were much smaller and weaker in the separate control and cuprizone-fed mice (due in no small part to the small number of datapoints per group). Weaker correlations in the cuprizone-fed mice, such as f vs. the myelin sheath fraction ($\rho = 0.1$) are reflective of a narrow range of values along both axes (see [Figure 5.10](#)).

Scatter plots in [Figure 5.10](#) show the relationship between quantitative MRI metrics and various EM measurements in the corpus callosum. They are ordered by EM measurement from top to bottom and correlation strength from left to right.

Table 5.4: Spearman correlation coefficients (ρ) of mean qMRI values in the corpus callosum to electron microscopy measurements for all mice ($n = 10$), control mice ($n = 5$), and cuprizone-fed mice ($n = 5$). For the combined group, the three strongest correlation coefficients for each metric are highlighted with varying shades of grey. The three strongest *in vivo* MRI correlations are highlighted as well.

MRI														
	Metric	f	R_A	RM_0^A	T_2^A	T_2^B	$\langle D \rangle$	$\lambda_{\parallel}$	$\lambda_{\perp}$	FA	T_1	T_2	T_{2w}	MTR
ALL	Myelinated Axons	0.78**	0.90*	-0.70**	-0.85**	0.49	-0.83**	-0.79**	-0.75***	0.81**	-0.90**	0.12	-0.79	0.81
	Myelin Sheaths	0.93**	0.66***	-0.33	-0.60	0.39	-0.81**	-0.75**	-0.87*	0.84**	-0.66***	-0.26	-0.73	0.72
	Myelinated Axons + Myelin Sheaths	0.89**	0.76***	-0.53	-0.67***	0.58	-0.87*	-0.85**	-0.82*	0.82**	-0.76***	0.01	-0.68	0.76
	Non-Myelinated Cells	-0.90*	-0.81**	0.54	0.71***	-0.60	0.90*	0.92*	0.81**	-0.80**	0.81**	-0.05	0.70	-0.77
	Extracellular Space	-0.55	-0.47	0.27	0.44	-0.54	0.48	0.47	0.59	-0.59	0.47	0.44	0.64	-0.58
	Myelinated Axons/100 μm^2	0.81**	0.73***	-0.32	-0.71***	0.42	-0.81**	-0.89**	-0.77***	0.70***	-0.73***	-0.12	-0.72	0.73
	M/A Ratio	0.82**	0.65***	-0.20	-0.64	0.36	-0.73**	-0.75**	-0.79**	0.73***	-0.65***	-0.39	-0.73	0.73
	Axon Diameters < 0.3 μm	0.88*	0.71***	-0.28	-0.69***	0.26	-0.82**	-0.82**	-0.84**	0.78**	-0.71***	-0.20	-0.75	0.74
	Axon Diameters 0.3 - 0.45 μm	0.76***	0.53	-0.12	-0.54	-0.02	-0.75***	-0.67**	-0.78***	0.70***	-0.53	-0.16	-0.53	0.67
	Axon Diameters > 0.45 μm	-0.84**	-0.64***	0.20	0.64	-0.14	0.81**	0.77*	0.85**	-0.78**	0.64***	0.21	0.66	-0.76
CTL	Myelinated Axons	0.6	0.1	-0.1	0.6	0.9	0.1	0.1	0.1	0.1	-0.1	-0.4	-0.1	-0.3
	Myelin Sheaths	0.6	-0.4	0.4	0.9	0.6	-0.1	-0.4	-0.1	-0.1	0.4	-0.5	0.4	-0.3
	Myelinated Axons + Myelin Sheaths	0.6	-0.4	0.4	0.9	0.6	-0.1	-0.4	-0.1	-0.1	0.4	-0.5	0.4	-0.3
	Non-Myelinated Cells	-0.7	0.3	-0.3	-0.8	-0.7	-0.2	0.0	-0.2	0.2	-0.3	0.7	-0.3	0.6
	Extracellular Space	-0.10	0.67	-0.67	-0.97***	-0.56	-0.31	0.21	-0.31	0.56	-0.67	0.67	-0.67	0.31
	Myelinated Axons/100 μm^2	-0.1	-0.1	0.1	0.6	0.9	0.6	0.4	0.6	-0.4	0.1	-0.5	0.1	-0.2
	M/A Ratio	-0.2	-0.2	0.2	0.7	0.8	0.3	0.0	0.3	-0.3	0.2	-0.3	0.2	0.1
	Axon Diameters < 0.3 μm	0.15	0.15	-0.15	0.56	0.97***	0.15	0.10	0.15	0.05	-0.15	-0.21	-0.15	0.05
	Axon Diameters 0.3 - 0.45 μm	-0.2	0.3	-0.3	0.3	0.7	-0.2	-0.2	-0.2	0.3	-0.3	0.3	-0.3	0.6
	Axon Diameters > 0.45 μm	-0.1	-0.1	0.1	-0.6	-0.9	0.0	0.1	0.0	-0.1	0.1	0.1	0.1	-0.2
CPZ	Myelinated Axons	0.6	-0.6	0.6	0.8	-0.6	-0.1	0.5	-0.5	0.5	0.6	-0.3	0.3	0.2
	Myelin Sheaths	0.1	-0.9	0.5	0.7	-0.4	0.5	0.9	-0.1	0.1	0.9	-0.7	0.2	-0.3
	Myelinated Axons + Myelin Sheaths	0.1	-0.9	0.5	0.7	-0.4	0.5	0.9	-0.1	0.1	0.9	-0.7	0.2	-0.3
	Non-Myelinated Cells	-0.5	0.7	-0.8	-0.9	0.7	-0.2	-0.7	0.3	-0.3	-0.7	0.4	-0.1	0.1
	Extracellular Space	0.45	0.89	0.11	-0.22	-0.22	-0.67	-0.78	-0.22	0.22	-0.89	0.78	-0.45	0.45
	Myelinated Axons/100 μm^2	0.5	-0.7	0.8	0.9	-0.7	0.2	0.7	-0.3	0.3	0.7	-0.4	0.1	-0.1
	M/A Ratio	0.5	-0.7	0.8	0.9	-0.7	0.2	0.7	-0.3	0.3	0.7	-0.4	0.1	-0.1
	Axon Diameters < 0.3 μm	0.5	-0.7	0.8	0.9	-0.7	0.2	0.7	-0.3	0.3	0.7	-0.4	0.1	-0.1
	Axon Diameters 0.3 - 0.45 μm	0.6	-0.6	0.9	1.0***	-0.6	0.1	0.4	-0.1	0.1	0.6	0.0	0.3	-0.2
	Axon Diameters > 0.45 μm	-0.56	0.67	-0.87	-0.97***	0.67	-0.15	-0.56	0.21	-0.21	-0.67	0.21	-0.21	0.15

* $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$
 $|r| > 0.7$ in **bold**

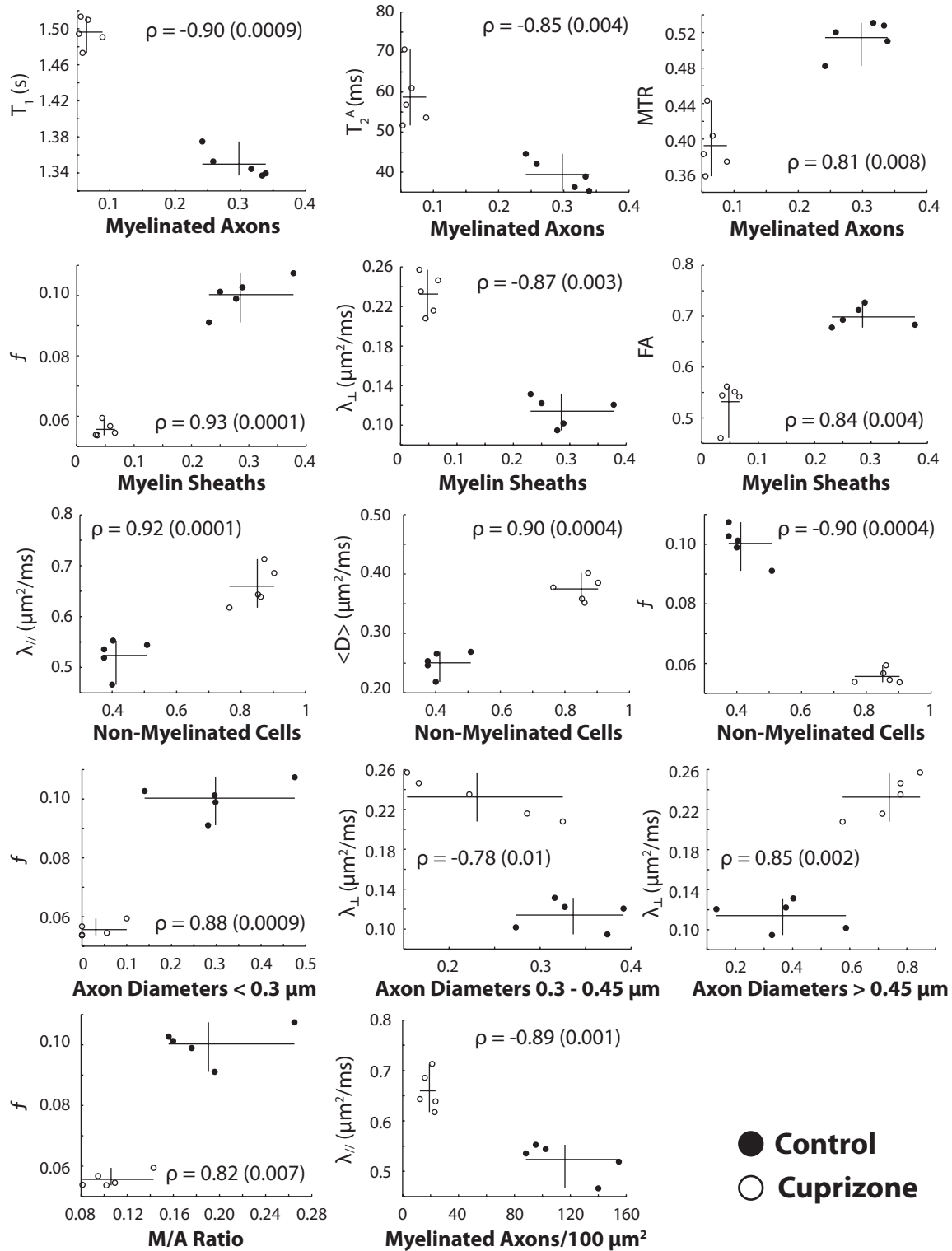


Figure 5.10: Scatter plots of quantitative MRI metrics vs. EM measurements in the corpus callosum, sorted by correlation strength from left to right. Horizontal line represents range of myelin sheath fractions and vertical line represents range of MRI metrics in control and cuprizone-fed mice. Spearman correlation coefficients are reported as ρ (p).

5.3.9 Quantitative MRI Correlations

Voxel-based Spearman correlations of quantitative MRI values in the corpus callosum and external capsule of the combined dataset are summarized in Table 5.5. Given the large number of values used for each correlation, all moderate and strong correlations have a $p \ll 0.001$. *In vivo* correlations for T_2 -weighted signal and MTR taken prior to perfusion are included for comparison.

In general, correlations do not vary significantly between the two white matter regions. Some notable exceptions are the correlations of FA vs. f and FA vs. R_A , which are both 0.71 in the corpus callosum and 0.49 and 0.50, respectively, in the external capsule. Likewise, correlations of FA vs. $\langle D \rangle$, FA vs. $\lambda_{\perp}$ and FA vs. T_1 are stronger in the corpus callosum, while FA vs. $\lambda_{\parallel}$ is weaker.

Given the relative homogeneity of correlations between the corpus callosum and external capsule, values were combined to represent changes in white matter when comparing correlations between control and cuprizone-fed mice and voxel-based and ROI-based correlations in all mice. Increasing the number of data points was also a consideration in improving the significance of the ROI-based correlations.

Table 5.5: Comparison of voxel-based Spearman correlation coefficients (r) in corpus callosum (upper right, *ex vivo* $n = 483$, *in vivo* $n = 410$) and external capsule (lower left, *ex vivo* $n = 736$, *in vivo* $n = 665$) of all mice. Correlations mentioned in the text are highlighted with grey.

Metric	f	RM_0^A	R_A	T_2^A	T_2^B	$\langle D \rangle$	$\lambda_{\parallel}$	$\lambda_{\perp}$	FA	T_1	T_2	T_{2w}	MTR
f	—	-0.64 [†]	0.79[†]	-0.78[†]	0.31 [†]	-0.64 [†]	-0.33 [†]	-0.74[†]	0.71[†]	-0.84[†]	-0.24 [†]	—	—
RM_0^A	-0.65 [†]	—	-0.29 [†]	0.62 [†]	-0.57 [†]	0.24 [†]	0.13 ^{**}	0.27 [†]	-0.25 [†]	0.35 [†]	0.19 [†]	—	—
R_A	0.83[†]	-0.34 [†]	—	-0.65 [†]	0.11 ^{***}	-0.44 [†]	-0.10 ^{***}	-0.62 [†]	0.71[†]	-1.00[†]	-0.38 [†]	—	—
T_2^A	-0.75[†]	0.56 [†]	-0.66	—	-0.23 [†]	0.60 [†]	0.37 [†]	0.64 [†]	-0.57 [†]	0.68 [†]	0.18 [†]	—	—
T_2^B	0.15 [†]	-0.18 [†]	0.07 ^{***}	-0.06	—	-0.08	-0.03	-0.12 ^{**}	0.12 ^{**}	-0.15 ^{**}	-0.08	—	—
$\langle D \rangle$	-0.72[†]	0.34 [†]	-0.61 [†]	0.65 [†]	-0.15 [†]	—	0.83[†]	0.89[†]	-0.62 [†]	0.48 [†]	-0.15 ^{**}	—	—
$\lambda_{\parallel}$	-0.31 [†]	0.16 [†]	-0.19 [†]	0.40 [†]	-0.14 [*]	0.71[†]	—	0.49 [†]	-0.12 ^{**}	0.14 ^{**}	-0.34 [†]	—	—
$\lambda_{\perp}$	-0.76[†]	0.37 [†]	-0.70 [†]	0.60 [†]	-0.12 ^{**}	0.79[†]	0.17 [†]	—	-0.89[†]	0.65 [†]	0.06	—	—
FA	0.49[†]	-0.25 [†]	0.50 [†]	-0.30 [†]	0.01	-0.31 [†]	0.38 [†]	-0.79[†]	—	-0.72[†]	-0.23 [†]	—	—
T_1	-0.87[†]	0.38 [†]	-1.00[†]	0.69 [†]	-0.09 ^{***}	0.64 [†]	0.22 [†]	0.72[†]	-0.50 [†]	—	0.37 [†]	—	—
T_2	-0.22 [†]	0.09 ^{***}	-0.30 [†]	0.14 [*]	-0.06	0.02	-0.11 ^{**}	0.13 [*]	-0.17 [†]	0.29 [†]	—	—	—
T_{2w}	—	—	—	—	—	—	—	—	—	—	—	—	-0.82[†]
MTR	—	—	—	—	—	—	—	—	—	—	—	-0.83[†]	—

[†] $p \ll 0.001$, $^*p < 0.001$, $^{**}p < 0.01$, $^{***}p < 0.05$

$|\rho| > 0.7$ in **bold**

Voxel-based and ROI-based correlations in the white matter of all mice are compared

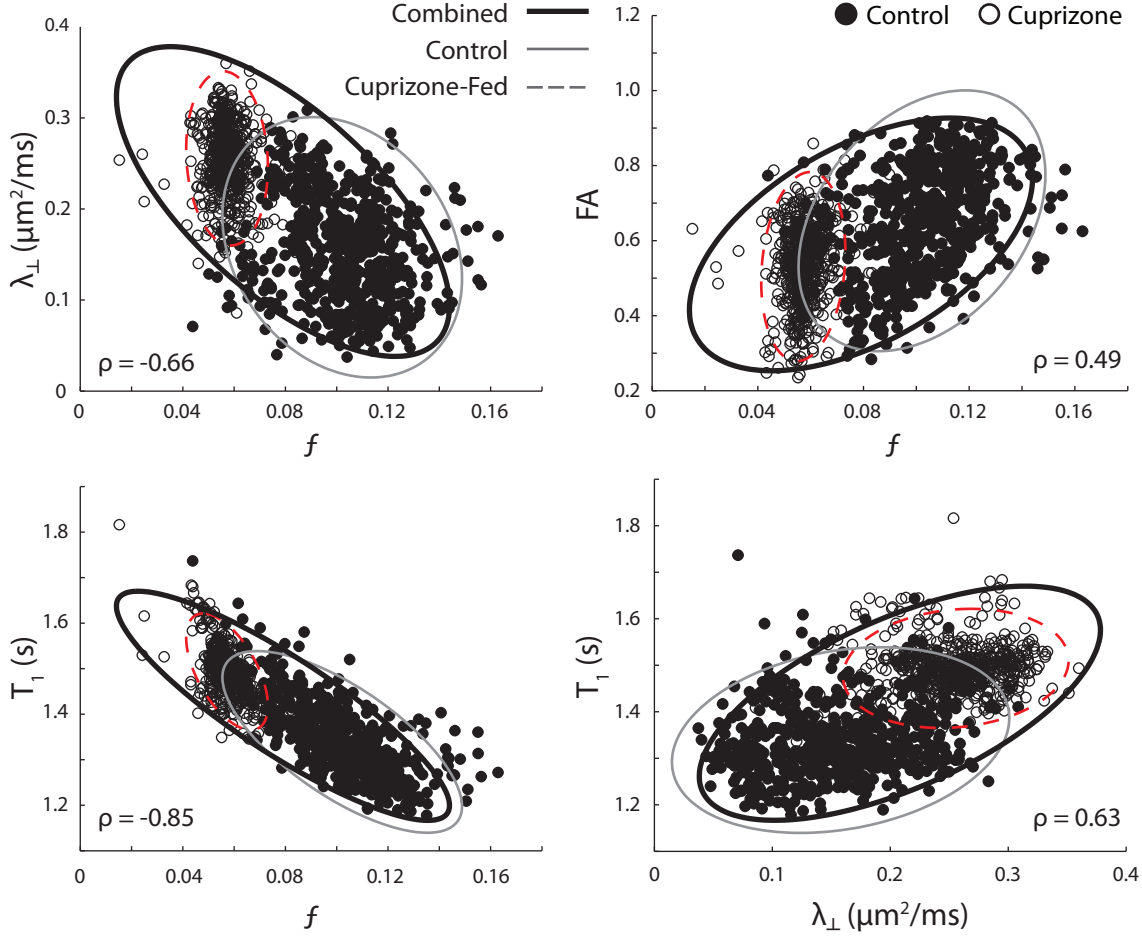


Figure 5.11: Selected scatter plots for quantitative MRI metrics with 95% confidence ellipses for combined (solid black line), control (solid grey line) and cuprizone-fed (dashed red line) mice. Spearman correlation coefficients are reported for the combined datasets as ρ with $p \ll 0.001$ for all four plots.

in Table 5.6. Selected voxel-based scatter plots are shown in Figure 5.11. Correlations were similar in both, although the ROI-based correlations tended to be larger, sometimes significantly, as in $\lambda_{\parallel}$ vs. f or T_2^B vs. RM_0^A . ROI-based correlations were used to correlate *in vivo* and EM measurements with *ex vivo* MRI results. T_{2w} and MTR were both most strongly correlated with $\lambda_{\perp}$. This was surprising for MTR in particular, which one might first assume would most strongly correlate with f .

Voxel-based Spearman correlations of quantitative MRI values in white matter of control and cuprizone-fed mice are summarized in Table 5.7. Correlations are more moderate when looking at the treatment groups separately, and, in general, smaller in the cuprizone-fed

Table 5.6: Comparison of voxel-based (upper right, *ex vivo* n = 1219, *in vivo* n = 1074) and ROI-based (lower left, n = 20) Spearman correlation coefficients (r) of MRI metrics in white matter (regions of interest encompassing corpus callosum and external capsule). Correlations mentioned in the text are highlighted with grey.

Metric	f	RM_0^A	R_A	T_2^A	T_2^B	$\langle D \rangle$	$\lambda_{\parallel}$	$\lambda_{\perp}$	FA	T_1	T_2	T_{2w}	MTR
f	—	-0.65 [†]	0.81[†]	-0.77[†]	0.23 [†]	-0.66 [†]	-0.33 [†]	-0.66 [†]	0.49 [†]	-0.85[†]	-0.23 [†]	—	—
RM_0^A	-0.55 ^{***}	—	-0.31 [†]	0.60 [†]	-0.39 [†]	0.24 [†]	0.17 [†]	0.20 [†]	-0.11 [*]	0.36 [†]	0.18 [†]	—	—
R_A	0.82[†]	-0.61 ^{**}	—	-0.65 [†]	0.09 ^{**}	-0.52 [†]	-0.15 [†]	-0.61 [†]	0.52 [†]	-1.00[†]	-0.33 [†]	—	—
T_2^A	-0.75[*]	0.65 ^{**}	-0.81[*]	—	-0.16 [†]	0.58 [†]	0.40 [†]	0.50 [†]	-0.30 [†]	0.68 [†]	0.17 [†]	—	—
T_2^B	0.46 ^{***}	-0.89[†]	0.47 ^{***}	-0.49 ^{***}	—	-0.04	-0.10 [*]	0.03	-0.10 [*]	-0.11 [*]	-0.12 [†]	—	—
$\langle D \rangle$	-0.74[*]	0.20	-0.70 [*]	0.73[*]	-0.06	—	0.72[†]	0.84[†]	-0.49 [†]	0.55 [†]	-0.08 ^{**}	—	—
$\lambda_{\parallel}$	-0.74[*]	0.45 ^{***}	-0.72[*]	0.87[†]	-0.41	0.85[†]	—	0.25 [†]	0.20 [†]	0.18 [†]	-0.20 [†]	—	—
$\lambda_{\perp}$	-0.72[*]	0.08	-0.65 ^{**}	0.60 ^{**}	0.09	0.92[†]	0.67 ^{**}	—	-0.86[†]	0.63 [†]	0.03	—	—
FA	0.52 ^{***}	0.16	0.41	-0.32	-0.35	-0.73[*]	-0.34	-0.90[†]	—	-0.52 [†]	-0.12 [†]	—	—
T_1	-0.84[†]	0.65 ^{**}	-0.99[†]	0.83[†]	-0.53 ^{***}	0.70 [*]	0.75[*]	0.62 ^{**}	-0.36	—	0.32 [†]	—	—
T_2	-0.28	0.29	-0.27	0.16	-0.42	-0.11	-0.03	-0.03	0.05	0.24	—	—	—
T_{2w}	-0.71[*]	0.23 ^{**}	-0.81^{***}	0.69	-0.06 [†]	0.88	0.72^{***}	0.89	-0.75	0.78^{***}	-0.02	—	-0.84[†]
MTR	0.70 [*]	-0.23	0.72[†]	-0.67 ^{**}	0.11	-0.88[†]	-0.77[*]	-0.90[†]	0.71[*]	-0.71[†]	0.10	-0.89[†]	—

[†] $p \leq 0.001$, $^*p < 0.001$, $^{**}p < 0.01$, $^{***}p < 0.05$
 $|\rho| > 0.7$ in **bold**

mice. FA is strongly correlated with $\lambda_{\perp}$ in both groups and also with $\lambda_{\parallel}$ in the cuprizone-fed mice. In control mice, FA is moderately correlated with $\langle D \rangle$ but is essentially zero in the cuprizone-fed mice. Correlations between f and various metrics are generally stronger in the control mice. As was previously observed in the *in vivo* correlations, MTR vs. T_{2w} was stronger in the control mice *in vivo* prior to perfusion.

Table 5.7: Comparison of voxel-based Spearman correlation coefficients (r) in white matter (region of interest encompassing corpus callosum and external capsule) between control (upper right, *ex vivo* n = 592, *in vivo* n = 515) and cuprizone-fed (lower left, *ex vivo* n = 627, *in vivo* n = 559) mice. Correlations mentioned in the text are highlighted with grey.

Metric	f	RM_0^A	R_A	T_2^A	T_2^B	$\langle D \rangle$	$\lambda_{\parallel}$	$\lambda_{\perp}$	FA	T_1	T_2	T_{2w}	MTR
f	—	-0.71[†]	0.63 [†]	-0.51 [†]	0.10 ^{***}	0.00	0.39 [†]	-0.27 [†]	0.38 [†]	-0.70 [†]	-0.30 [†]	—	—
RM_0^A	-0.62 [†]	—	-0.15 [†]	0.47 [†]	-0.25 [†]	-0.09 ^{***}	-0.11 ^{**}	-0.06	0.02	0.23 [†]	0.17 [†]	—	—
R_A	0.40 [†]	0.15 [*]	—	-0.36 [†]	-0.01	0.10 ^{***}	0.48 [†]	-0.22 [†]	0.36 [†]	-0.99[†]	-0.37 [†]	—	—
T_2^A	-0.39 [†]	0.53 [†]	-0.13 ^{**}	—	0.12 ^{**}	0.12 ^{**}	-0.06	0.15 [*]	-0.13 ^{**}	0.39 [†]	0.01	—	—
T_2^B	0.14 [*]	-0.45 [†]	-0.22 [†]	-0.17 [†]	—	0.24 [†]	0.01	0.27 [†]	-0.22 [†]	-0.01	-0.13 ^{**}	—	—
$\langle D \rangle$	0.08 ^{***}	-0.12 ^{**}	0.19 [†]	-0.06	0.14 [*]	—	0.52 [†]	0.78[†]	-0.51 [†]	-0.09 ^{***}	-0.41 [†]	—	—
$\lambda_{\parallel}$	0.14 [*]	0.07	0.31 [†]	0.11 ^{**}	-0.04	0.60 [†]	—	-0.07	0.41 [†]	-0.49 [†]	-0.43 [†]	—	—
$\lambda_{\perp}$	-0.05	-0.18 [†]	-0.12 ^{**}	-0.18 [†]	0.19 [†]	0.44 [†]	-0.40 [†]	—	-0.92[†]	0.24 [†]	-0.18 [†]	—	—
FA	0.15 [*]	0.14 [*]	0.23 [†]	0.15 [*]	-0.15 [*]	0.01	0.77[†]	-0.85[†]	—	-0.38 [†]	0.00	—	—
T_1	-0.47 [†]	-0.09 ^{***}	-0.99[†]	0.15 [*]	0.21 [†]	-0.19 [†]	-0.31 [†]	0.12 ^{**}	-0.24 [†]	—	0.38 [†]	—	—
T_2	-0.18 [†]	0.09 ^{***}	-0.31 [†]	0.21 [†]	-0.08 ^{***}	-0.29 [†]	-0.27 [†]	-0.02	-0.13 ^{**}	0.31 [†]	—	—	—
T_{2w}	—	—	—	—	—	—	—	—	—	—	—	—	-0.51 [†]
MTR	—	—	—	—	—	—	—	—	—	—	—	-0.24 [*]	—

[†] $p \leq 0.001$, $^*p < 0.001$, $^{**}p < 0.01$, $^{***}p < 0.05$
 $|\rho| > 0.7$ in **bold**

Given that some correlations appear to differentiate control and cuprizone-fed mice,

voxel-based correlation were calculated for individual control and cuprizone-fed mice and the mean values were compared with an independent samples T-test (see [Table 5.8](#)). As expected, results are very similar to the combined results found in [Table 5.7](#). This approach accounts for variations in individual mice and treats correlations between MRI measures as another metric that could be tested against healthy and diseased results. Mean correlation coefficients of FA vs. $\langle D \rangle$ and FA vs. $\lambda_{\parallel}$ were significantly different, as were several other mean correlations, such as T_1 vs. f and RM_0^A vs R_A .

Table 5.8: Comparison of voxel-based Spearman correlation coefficients (r) in white matter (region of interest encompassing corpus callosum and external capsule) between individual control (upper right, *ex vivo* $n = 108-130$, *in vivo* $n = 92-115$) and cuprizone-fed (lower left, *ex vivo* $n = 100-139$, *in vivo* $n = 98-118$) mice. Values reported as mean (standard error of the mean). Correlations mentioned in the text are highlighted with grey.

Metric	f	RM_0^A	R_A	T_2^A	T_2^B	$\langle D \rangle$	$\lambda_{ }$	$\lambda_{\perp}$	FA	T_1	T_2	T_{2w}	MTR
f	—	-0.68 (0.05)	0.64 (0.04)**	-0.49 (0.07)	0.08 (0.06)	0.02 (0.10)	0.34 (0.13)	-0.23 (0.04)	0.31 (0.07)	-0.70 (0.03)**	-0.32 (0.11)	—	—
RM_0^A	-0.65 (0.05)	—	-0.13 (0.05)*	0.46 (0.06)	-0.22 (0.05)	-0.11 (0.05)	-0.07 (0.11)	-0.11 (0.05)	0.07 (0.07)	0.20 (0.05)**	0.15 (0.06)***	—	—
R_A	0.36 (0.07)**	0.21 (0.04)*	—	-0.39 (0.05)**	-0.05 (0.07)***	0.08 (0.10)	0.46 (0.09)	-0.23 (0.11)	0.36 (0.11)	-0.995 (0.002)	-0.47 (0.11)	—	—
T_2^A	-0.43 (0.12)	0.52 (0.04)	-0.13 (0.05)**	—	0.14 (0.04)**	0.09 (0.04)	-0.06 (0.10)	0.10 (0.09)	-0.10 (0.12)	0.42 (0.04)**	0.09 (0.08)	—	—
T_2^B	0.13 (0.11)	-0.40 (0.09)	-0.25 (0.05)***	-0.17 (0.06)**	—	0.22 (0.06)	-0.04 (0.05)	0.26 (0.05)	-0.24 (0.03)	0.04 (0.07)***	-0.04 (0.08)	—	—
$\langle D \rangle$	0.06 (0.06)	-0.09 (0.05)	0.21 (0.09)	-0.02 (0.07)	0.15 (0.05)	—	0.52 (0.03)	0.75 (0.03)*	-0.47 (0.04)**	-0.08 (0.10)	-0.24 (0.06)	—	—
$\lambda_{ }$	0.04 (0.12)	0.13 (0.09)	0.25 (0.13)	0.16 (0.05)	-0.05 (0.05)	0.57 (0.06)	—	-0.10 (0.03)*	0.44 (0.03)*	-0.46 (0.09)	-0.35 (0.10)	—	—
$\lambda_{\perp}$	0.01 (0.11)	-0.19 (0.09)	-0.04 (0.15)	-0.17 (0.11)	0.21 (0.07)	0.44 (0.05)**	-0.42 (0.03)*	—	-0.91 (0.01)	0.24 (0.10)	-0.06 (0.11)	—	—
FA	0.04 (0.12)	0.18 (0.10)	0.13 (0.15)	0.17 (0.09)	-0.16 (0.07)	-0.03 (0.10)*	0.76 (0.03)*	-0.86 (0.02)	—	-0.36 (0.11)	-0.08 (0.13)	—	—
T_1	-0.44 (0.06)**	-0.14 (0.04)**	-0.994 (0.002)	0.16 (0.05)**	0.23 (0.05)***	-0.20 (0.09)	-0.24 (0.13)	0.03 (0.15)	-0.13 (0.15)	—	0.47 (0.11)	—	—
T_2	-0.31 (0.06)	-0.07 (0.07)***	-0.56 (0.03)	0.19 (0.06)	0.10 (0.15)	-0.19 (0.08)	-0.07 (0.09)***	-0.15 (0.10)	0.05 (0.11)	0.56 (0.03)	—	—	—
T_{2w}	—	—	—	—	—	—	—	—	—	—	—	-0.55 (0.09)	—
MTR	—	—	—	—	—	—	—	—	—	—	-0.41 (0.11)	—	—

* $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$ when comparing control and cuprizone-fed mice using an independent samples T-test.
 $|\rho| > 0.7$ in **bold**

5.4 Discussion

5.4.1 Effects of the Cuprizone Diet

Numerous studies have used a 0.2% cuprizone diet to model demyelination and remyelination in the mouse brain, with 0.4% cuprizone applied occasionally in older mice [30, 110]. Although this study used a 0.4% cuprizone diet, some of our measurements correspond to studies done at 0.2%.

Differences in mouse weight were similar to other studies that fed mice a 0.2% cuprizone diet (e.g. (19.3 ± 0.5) g vs. (23.2 ± 0.7) g in Ref. [43]). Some 0.2% cuprizone studies had significantly larger weight differences after 6 weeks ((16.1 ± 2.3) g vs. (28.3 ± 4.3) g in Ref. [141]).

Previous studies by our collaborators had focused on the rostral region of the corpus callosum when assessing the role quetiapine plays in reducing cuprizone-induced demyelination [278, 269, 272], thus our focus on the same region. The efficacy of cuprizone treatment, however, varies in different regions of the corpus callosum. In the rostral region of the corpus callosum, Xie *et al.* observed significantly lower myelinated fiber fractions in electron micrographs after 4 and 12 weeks of treatment, however, changes were more severe in the middle and caudal regions [270]. At 12 weeks of 0.2% cuprizone, a timepoint commonly associated with chronic demyelination, they reported a myelinated fiber percent of $\approx 25\%$, higher than our value of 13.2%. In two separate studies, the caudal region was more affected by 0.2% cuprizone treatment relative to rostral regions, with decreased $\lambda_{\parallel}$ and increased $\lambda_{\perp}$ [270, 267], a larger proportion of demyelinated fibers and reduced axonal diameters [270], prominent demyelination in LFB staining [267], and increased axonal injury and macrophage/microglial accumulation [270, 267]. Reduced susceptibility to the cuprizone treatment necessitated a larger cuprizone dose to achieve the same level of demyelination observed previously in the caudal region of mice fed 0.2% cuprizone. In our experiment, the effects of 0.4% cuprizone treatment on the rostral corpus callosum appear to have features associated with the caudal corpus callosum after 4–6 weeks of 0.2% treatment or the rostral corpus callosum after 12 weeks of 0.2% treatment (i.e. substantial demyelination, increased cellularity, axonal damage, and macrophage infiltration), but not the extensive

and irreversible tissue damage observed in the caudal corpus callosum after 12 weeks of 0.2% treatment [95, 270].

Besides regional differences, the reduced effects of our 0.4% cuprizone treatment might be related to the chow preparation. Our mouse chow was mixed with 0.4% and pressed into pellets. Pressing the pellets, however, can heat the cuprizone and reduce its effectiveness [218]. In Section 4.3, we demonstrated that two groups treated with the same concentration of cuprizone may have different responses. While cuprizone treatment can have variable effects on mice [109, 218], the effect of cuprizone was remarkably homogenous in mice from the same study (Figure B.2 and B.4). Subsequent ultrastructural examination enables us to assess how cell distributions and measurements co-vary with quantitative MRI metrics in control and cuprizone-fed mice.

5.4.2 Longitudinal MRI Measurements

Significant changes started in both the T_2 -weighted and MTR values of the corpus callosum at 3 weeks of treatment. This corresponds to the start of significant demyelination observed in LFB-PAS stained histology after 3 weeks of 0.2% cuprizone treatment [103, 202]. When assessed with electron microscopy, the fraction of myelinated fibers starts decreasing substantially at 3 weeks of treatment and by 4–5 weeks, almost all of the axons are demyelinated [161, 233, 218].

At 4 weeks, normalized T_2 -weighted signal peaked and was significantly larger in the corpus callosum when compared to the external capsule, but MTR values did not deviate between regions. In 0.2% cuprizone studies, this is the point of peak cellularity [103]. Significant changes in the T_2 -weighted image may also suggest vasogenic edema, previously associated with higher concentrations of cuprizone and prolonged exposure to 0.2% cuprizone treatment [121, 95]. In our results, inspection of toluidine blue stained sections showed thinning of the ependymal cell membranes in the cuprizone-fed mice, possibly allowing CSF from the lateral ventricles to infiltrate the extracellular spaces in the corpus callosum.

At 5 weeks, the T_2 -weighted signals were similar in the corpus callosum and external capsule and the MTR values were at their lowest in the corpus callosum. In 0.2% cuprizone studies, this is the point of peak demyelination [103, 161, 233]. At 6 weeks, decreasing

T_2 -weighted signal and increasing MTR in the corpus callosum may signify the start of remyelination in the cuprizone-fed mice. If cuprizone treatment is continued, remyelination only occurs briefly and at 12 weeks, the corpus callosum resembles a state of chronic demyelination [161].

Although somewhat speculative, it appears that the T_2 -weighted images co-vary more strongly with tissue cellularity and possible vasogenic edema, peaking at 4 weeks, and MTR maps co-vary more strongly with demyelination, hitting their lowest point at 5 weeks. Large decreases in MTR have been associated with demyelinated lesions, whereas slightly decreased [83] or slightly increased [59] MTR has been associated with lesions that exhibit edema but not demyelination. In a longitudinal study of cuprizone-fed mice, MTR was strongly correlated with myelin content ($r = 0.79$) [276], although in another animal study, MTR was also sensitive to inflammation [72]. Vavasour *et al.* showed a strong correlation between water content and MTR ($r = -0.65$), suggesting inflammation and edema might also alter MTR [260].

In a bi-weekly MRI study of the caudal corpus callosum, the T_2 relaxation time was significantly higher and MTR significantly lower after 4 weeks of 0.2% cuprizone treatment [277]. At 6 weeks, the T_2 relaxation time was even lower but the MTR appeared unchanged. It could be they missed the peak MTR value at 5 weeks. Similar studies found significantly increased T_2 -weighted signal [164] and decreased MTR [164, 276] after 6 weeks of treatment. Weekly MRI may be necessary to accurately monitor the progression of the cuprizone treatment. This is particularly relevant for experiments that require a specific timepoint, such as peak cellularity or demyelination, or that wish to track the progression of potential treatments.

Voxel-based correlations between the normalized T_2 -weighted and MTR values follow the same trends observed in the individual methods. In general, correlation will be stronger when there is a broader range of values in tissue. Greater breadth can be attributed to a broader range of tissue pathologies, such as varying degrees of myelination in white matter when correlating combined datasets of control and cuprizone-fed mice. In the weekly correlation measurements, this is illustrated by the sudden increase in $|\rho|$ starting at week 3 ($\rho = -0.58$) and peaking at weeks 4 and 5 in the corpus callosum and external capsule

($\rho = -0.83$ and -0.89), respectively. In correlations across all weeks, $|\rho|$ is larger in the cuprizone-fed ($\rho = -0.76$ and -0.83) and combined datasets ($\rho = -0.74$ and -0.79) because they encompass a wide range of tissue pathologies. The control group is relatively constant in both week-to-week correlations and all weeks combined ($\rho = -0.45$), suggesting there is moderate correlation between the two MRI methods.

At 6 weeks of 0.4% cuprizone treatment, complete demyelination has occurred, cellularity has started to decrease, and based on the T_2 -weighted images, vasogenic edema has started to clear. At this point, the range of tissue pathologies has decreased, and with that, the range of possible MRI measurements. Reflecting this narrowing of MRI measurements, the correlation coefficient decreased in both the corpus callosum ($\rho = -0.24$) and external capsule ($\rho = -0.15$) of the cuprizone-fed mouse. In moderately correlated MRI measurements such as T_2 -weighted images and MTR maps, this is indicative of severe and homogeneous tissue damage in white matter observed in the electron microscopy images. In the week-6 confidence ellipse (dashed blue line in [Figure 5.2](#)), the range of T_2 -weighted values decreases in both the corpus callosum and external capsule and the confidence ellipse becomes nearly circular in the external capsule. This sudden shift is not apparent in the individual methods, and may point to a means of determining the severity and breadth of tissue pathology at a single time-point.

5.4.3 Quantitative MRI Measurements

Relaxometry

T_1 times in the control corpus callosum fall within the expected range of values found previously in the *in vivo* mouse brain at 7 T ($T_1 = (1503 \pm 56)$ ms) [85] and in freshly excised bovine white matter at 3 T ($T_1 = 1084 \pm 45$ ms) [230]. Although no T_1 times have been reported for cuprizone-fed mice, T_1 increases with field strength [230] and in damaged or diseased white matter due to a variety of factors including demyelination, inflammation, gliosis, edema, and axonal or neuronal loss [173]. In a tellurium rat model of demyelination, Odrobina *et al.* found T_1 increased from (675 ± 22) ms in untreated sciatic nerves to (1036 ± 37) ms after 7 days of treatment (1.5 T at room temperature). Our results

increased by 11% in the corpus callosum, going from (1350 ± 7) ms in the control mice to (1496 ± 7) ms in the cuprizone-fed mice.

T_2 measurements were hampered by large variability between subjects and the inability to detect a myelin water component. Previously, 10 ms echo spacing was sufficient to detect myelin water components in human brain [156]. Animal studies that found a myelin water component have often used shorter echo spacing [263, 129, 162, 61] and spectroscopy instead of imaging [263, 229, 177]. Gareau *et al.* found a myelin water component in the corpora callosa of guinea pigs (MWF = $(28 \pm 6)\%$), but Does *et al.* only found a single T_2 component in the corpus callosum of rats [55]. Although myelin water components have been demonstrated in mouse spinal cords [162], to our knowledge, no study has shown myelin water in the mouse corpus callosum.

In a study of different white matter regions in rat spinal cord, Dula *et al.* found differing myelin water fractions despite similar myelin content in histology [61]. These discrepancies were attributed to smaller myelin water compartments and larger intercompartmental water exchange, both proportional to average fiber thickness. As intercompartmental water exchange increases, T_2 components shift to shorter T_2 values and measured myelin water fractions decrease. Thus, white matter with smaller axon diameters and thin myelin sheaths may lead to underestimation of the myelin content and T_2 times [61]. In a follow-up study, numerical simulations and *in vivo* measurements of the rat spinal cord demonstrated that regional differences in exchange, mediated by differences in axon radii and myelin sheath thicknesses, sufficiently accounted for variations in measured MWF [93]. In our EM measurements, the average axon diameter and myelin thickness in control mice ((0.43 ± 0.08) μm and (0.070 ± 0.006) μm) was significantly smaller than those observed in the rat spinal cord ($(1.1 - 4.5)$ μm and $(0.52 - 1.16)$ μm) [61]. It is reasonable to hypothesize that myelin water measurements in the mouse corpus callosum, with axon diameters and myelin thicknesses much smaller than those found in the rat spinal cord, will be even more strongly influenced by intercompartmental exchange. Our first echo had an $\text{SNR} = 128 \pm 19$, similar to McCreary *et al.*'s study of mouse spinal cord [162]; however, our echo spacing was twice as long (10 ms vs. 5 ms) and we used a less efficient slice-selective CPMG sequence. In order to see a myelin water component in the mouse corpus callosum, we may need to increase

the SNR, decrease the echo spacing, and use a single slice CPMG sequence [193].

The reported T_2 values, calculated from the geometric mean of the $T_{2,ie}$ distribution, had large variability, but were similar to previously reported *in vivo* values at 7 T ((37 ± 2) ms and (39 ± 2) ms in healthy corpus callosum and cortex) [85] and 9.4 T (≈ 39 ms and ≈ 43 ms in control and 6-week, 0.2% cuprizone-fed corpus callosum) [277].

Diffusion Tensor Imaging

Due to lower temperature, stoppage of cellular and axon transport, changes in membrane permeability, and fixation, *ex vivo* diffusivity measurements are lower than *in vivo* [239]. Despite this, DTI measurements in freshly excised mouse brains were still found to be sensitive to optic nerve damage, whereas fixation decreased their sensitivity [239]. In healthy fixed mouse brains, $\langle D \rangle = (0.2 - 0.4)\mu\text{s}/\text{mm}^2$, $\lambda_{\parallel} = (0.4 - 0.6)\mu\text{s}/\text{mm}^2$, and $\lambda_{\perp} = (0.1 - 0.3)\mu\text{s}/\text{mm}^2$ [180, 239, 277]. Freshly excised brains tend to have slightly larger diffusivity measurements ($\approx 0.1\mu\text{m}/\text{ms}^2$ larger in $\langle D \rangle$ and $\approx 0.05\mu\text{m}/\text{ms}^2$ larger in $\lambda_{\parallel}$ and $\lambda_{\perp}$) [239, 212]. Relative DTI measurements, such as FA, are relatively unchanged between *in vivo*, freshly excised, and fixed tissue measurements [238, 239, 212]. These results coincide with our own in Section 4.3.6.

Matching our own results, several DTI studies of 0.2% cuprizone-fed mice have found significantly lower FA and higher $\lambda_{\perp}$ in the *in vivo* and *ex vivo* corpus callosum [224, 240, 95, 270, 277, 261]. Unlike our results, significantly lower *in vivo* $\lambda_{\parallel}$ is often reported after 4 and 6 weeks of 0.2% cuprizone treatment, indicative of axonal injury early in the progression of the cuprizone diet [222, 270, 277, 261]. However, in studies of fixed *ex vivo* corpus callosum, $\lambda_{\parallel}$ was not significantly different [224] or slightly increased [277] after 6 weeks on a 0.2% cuprizone diet. In their comparison of *in vivo* and *ex vivo* measurements, Zhang *et al.* determined that $\lambda_{\perp}$ was a stronger indicator of demyelination in *ex vivo* tissue when compared to *in vivo* measurements and $\lambda_{\parallel}$ was a stronger indicator of axonal damage *in vivo* when compared to *ex vivo* measurements [277].

In our case, significantly larger $\langle D \rangle$ and $\lambda_{\parallel}$ might also reflect the lingering effects of vasogenic edema. Also, activated microglia, observed in the electron microscopy images, can phagocytose debris and stimulate oligodendrocyte precursor cells, but also can release

cytotoxins that further damage oligodendrocytes [202]. Cleared debris and increased extracellular space would allow for increased water movement. It should be noted that the fixative used for electron microscopy is hypertonic, reducing the size of the extracellular space in electron micrographs. Although extracellular space was slightly larger in the cuprizone-fed mice, it is difficult to assess its true extent based on the electron microscopy images alone.

Magnetization Transfer Imaging

Our results for quantitative MTI were as expected, with significantly lower f and R_A , and significantly higher RM_0^A and T_2^A in the cuprizone-fed corpus callosum and external capsule. Although no quantitative MTI studies have been published on the cuprizone mouse model, Ou *et al.* reported estimates of RM_0^A , R_1 and F in the corpus callosum and optic nerve of fixed control and shiverer (almost completely dysmyelinated) mouse brains [180, 181]. Using an inversion recovery method, they found values of $f = 0.090$ and $f = 0.071$ in the corpus callosum of control and shiverer mice, respectively [180]. Quantitative MT studies have consistently found decreased f in demyelinated rat sciatic nerve [177], shiverer mouse spine [194], glioma [273, 253], and multiple sclerosis [220, 198, 273, 248, 49, 211, 58]. Previously, fixation has been found to increase f by $\approx 50\%$ when compared to unfixed tissue [212], although correlation to myelin content remains strong [212, 61]. Our own results suggest a more modest increase of 13% when comparing f in the corpus callosum of fresh and fixed mouse brains (4.3.6).

Using an *in vivo* pulsed gradient method similar to our own, Underhill *et al.* found $f = 0.139$, $R_1 = 0.95 \text{ s}^{-1}$, $RM_0^A = 20.9 \text{ s}^{-1}$, $T_2^A = 33 \text{ ms}$, and $T_2^B = 11 \text{ }\mu\text{s}$ in healthy rat corpus callosum [253]. Although values for f , R_A , T_2^A , and T_2^B were consistent with previously reported results in healthy and demyelinated white matter, our RM_0^A value is lower than those reported for most pulsed gradient methods. However, it is surprisingly close to RM_0^A values determined with inversion recovery, which have been shown to be more accurate and less variable when determining the MT rate [79, 78]. Using the inversion recovery method, $RM_0^A = 11 \text{ s}^{-1}$ in human white matter [58] and $RM_0^A = 15 \text{ s}^{-1}$ in ferret brain [78].

Both RM_0^A and T_2^A have been shown to have large variability in tissue [194, 221, 198, 253]

while T_2^B shows little contrast between different tissues [220, 273, 248, 49]. Ramani *et al.*'s pulse approximation used here has also been shown to underestimate RM_0^A and overestimate T_2^A , but only by 12–15% [194]. The most consistent and relevant metric is f , which has been shown to correlate with myelin content in several studies [211, 61, 253].

5.4.4 Relationship Between Quantitative MRI Metrics and Electron Microscopy

Figure 5.12 shows the percent difference between cuprizone and control mice in the quantitative MRI and EM metrics reported in this study. After several EM metrics that describe the large decrease in myelinated axon and myelin sheath fractions, the largest change in MRI metrics are found in $\lambda_{\perp}$ and f , followed by the normalized T_2 -weighted signal (T_{2w}), $\langle D \rangle$, T_2^A , MTR, FA, and $\lambda_{\parallel}$. Judged purely on tissue contrast, the two largest metrics, $\lambda_{\perp}$ and f would be the best choice for revealing changes in the corpus callosum of the cuprizone model.

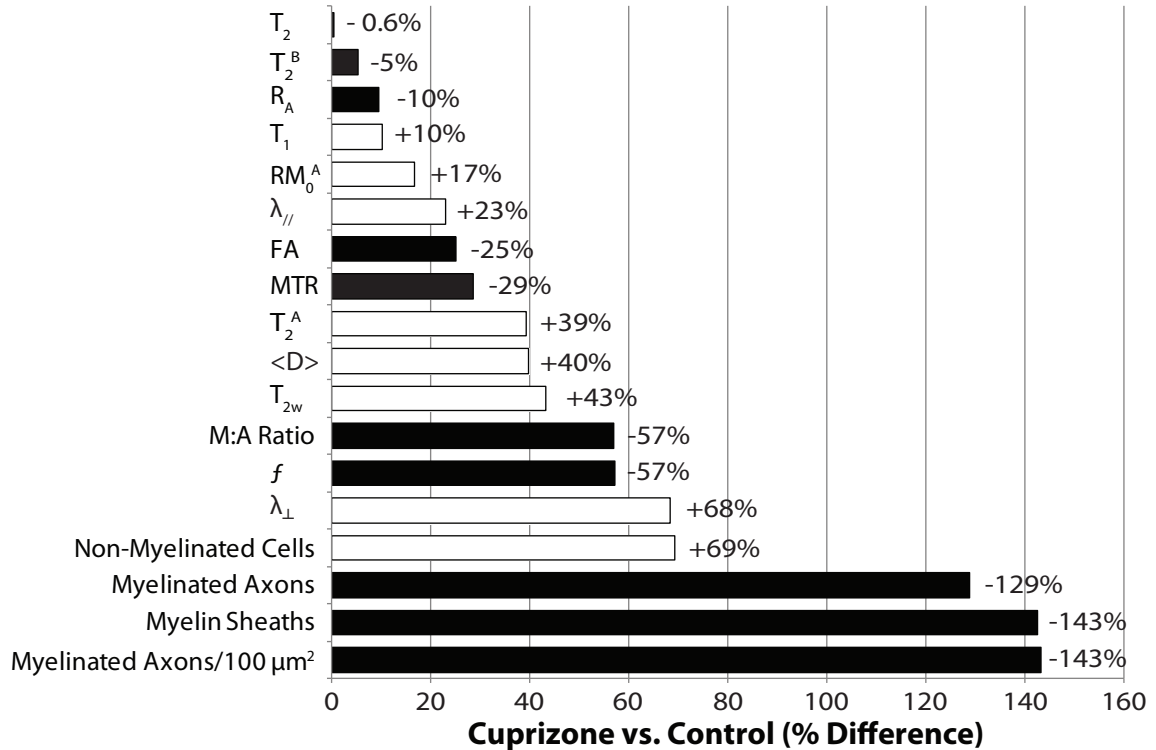


Figure 5.12: Percent difference for various qMRI and EM metrics in cuprizone vs. control mice. Black represent a negative difference and white a positive difference.

Correlations between MRI metrics and EM measurements confirmed several previous results.

In vivo measurements of the T_2 -weighted signal and MTR were strongly correlated with the fraction of myelinated axons, but only MTR was strongly correlated with the non-myelinated cells. This reflects previous studies showing MTR is more strongly influenced by tissue pathologies outside the myelinated axons, such as inflammation or edema [72, 260].

Although we were unable to detect a significant myelin water fraction in the corpus callosum, two studies of MS brain samples registered LFB-stained images to MWF maps to demonstrate strong correlation between optical density of the LFB stain (related to myelin content) and the MWF at 1.5 T ($r^2 = 0.67$) (0.45 – 0.92) [135] and 7 T ($r^2 = 0.78$) (0.56 – 0.95) [138]. In both studies, correlations included regions of grey matter, normal appearing white matter (NAWM), and lesions. If sufficient myelin water is present in the mouse corpus callosum, improving the accuracy and fidelity of the T_2 acquisition scheme would provide an additional metric strongly associated with myelin content and integrity.

R_A and T_1 were strongly correlated with the myelinated axon fraction. In soft tissue, water mobility and the surrounding lattice of macromolecules co-conspire to increase the longitudinal relaxation rate of the free pool (R_A) and shorten the T_1 relaxation time. In this context, we would expect longer T_1 times for tissue in which the lattice size has decreased and the water mobility has increased (e.g. demyelinated white matter, inflammation, or axonal loss). Strong correlation between $\langle D \rangle$ and the myelinated axon fraction suggest that water mobility is increasing as the myelinated axon fraction decreases (contributing to the lattice). Thus, the strong correlation with T_1 . In a recent study, detailed post-mortem T_1 -weighted imaging of the human brain at 7T had contrast in excellent agreement with MBP immunostaining [74].

$\lambda_{\perp}$ and FA were strongly correlated with the myelin fraction, although not as strongly as f . While this seems to confirm the notion that $\lambda_{\perp}$ is sensitive to myelin [223, 171, 224, 240, 122, 35, 277], other studies have found only marginal decreases in the anisotropy of demyelinated axonal fibres, suggesting factors such as axonal degeneration and other physiological changes might be contributing to changes in anisotropy [14, 171]. Demyelination is just one of the effects of the cuprizone treatment. Increased cellularity, axonal damage

and loss, and edema all alter the internal geometry of white matter tracts, thus altering the radial diffusivity by affecting the extracellular tortuosity and relative volumes of the different compartments.

Significant increases in $\lambda_{\parallel}$ indicate that axonal damage and increased cellularity are not a major influence, even though some damaged axons and nuclei were observed in the cuprizone-fed electron micrographs. Rather, strong correlation of $\lambda_{\parallel}$ and $\langle D \rangle$ with non-myelinated cells and myelinated axons per 100 μm^2 shows that increased water mobility along the direction of the axonal tracts is associated with the non-myelinated cell fraction and density of myelinated axons.

f was strongly correlated with myelin content and axonal density in studies of post-mortem MS brain ($r = 0.80$ and 0.72) [211]. Underhill *et al.* also found strong correlation between f and myelin content in an *in vivo* rat brain study ($r = 0.97$ in white matter), but correlations with axon content and cell count were insignificant in white matter [253]. When incorporating values across both white and grey matter, correlation were generally stronger, with significant correlations between f , T_2^A , and T_2^B vs. myelin content ($r = 0.99$, -0.90 , and 0.84) and vs. axon content ($r = 0.88$, -0.89 , and 0.84) [253]. Much like our *in vivo* observations, this reflects a broader range of tissue pathologies being sampled. Ou *et al.* found f to be specific to myelin content in the optic nerves of control and shiverer mice, but not to axonal degeneration.

Of the measured MRI parameters, f had the strongest correlation to the myelin sheath fraction. The linear equation describing the relationship between the myelin sheath fraction and f is

$$M = (5.37 \pm 0.38)f - (0.25 \pm 0.03) \quad (5.3)$$

with $p < 0.001$ for both coefficients. Figure 5.13 shows the linear fit and residuals for the myelin sheath fraction as a function of f . Underhill *et al.* found a similar linear relationship to the myelin density in white matter and grey matter of healthy rat brain, where myelin density was determined by measuring the mean intensity of the red channel in 5 μm thick sections stained with Luxol-Fast blue (LFB) [252]. Their linear regression fit,

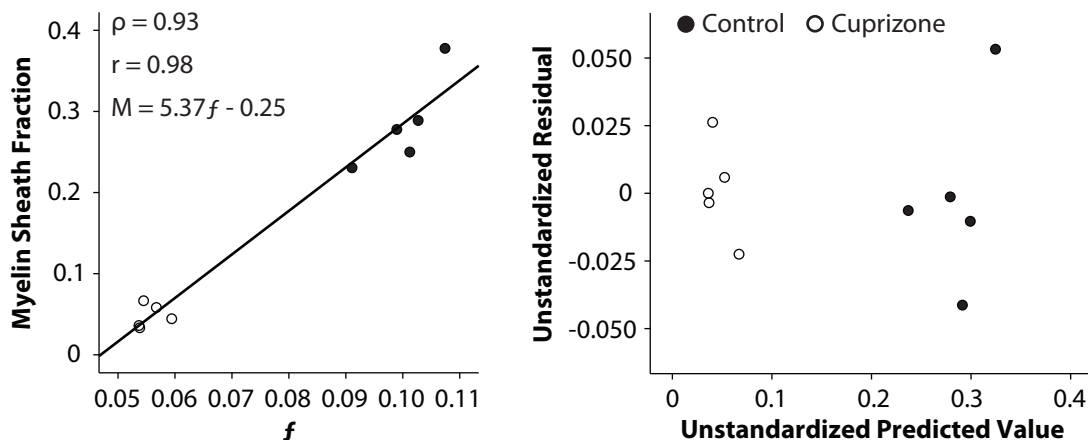


Figure 5.13: Linear fit and residuals for the myelin sheath fraction as a function of f . Spearman (ρ) and Pearson (r) correlation coefficients are both strong.

$f = 0.21M + 0.039$ and its inverse, $M = 4.76f - 0.186$, compare favorably to our equation. For $f = 0.1$, our linear fit yields $M = 0.28$ and theirs $M = 0.29$. LFB staining might overestimate the degree of organized myelination because lipid debris from degraded myelin still gives a positive signal [30]. In both results, $f \neq 0$ when $M = 0$, suggesting that features other than myelin contribute to the measured bound pool fraction. Of the evaluated MRI methods, f is the best predictor of myelin content.

Correlations to the distribution of myelinated axon diameters and M/A ratio are less informative, as they only take into account the myelinated axons in the cuprizone-fed mice, and are more reflective of changes in the underlying cellular distribution.

Correlation coefficients calculated in the control and cuprizone-fed mice were mostly small and insignificant. This is both a reflection of the limited sample size ($n = 5$) and limited breadth of tissue pathology in the individual groups. To improve the power of this analysis, future studies could incorporate several timepoints along the course of cuprizone treatment and several different regions of interest analyzed with both electron microscopy and MRI measurements.

5.4.5 Relationship Between Different Quantitative MRI Metrics

Correlating different MRI metrics may provide another way to assess changes in tissue pathology non-invasively. In particular, given its strong relationship to myelin content, the correlation of MRI metrics to f may reveal the influence of myelin integrity on measurements of DTI and relaxometry. While f is sensitive to the bound pool magnetization, of which myelin is a major constituent in healthy white matter, it is also sensitive to changes in the free pool magnetization. Increases in the size of the free pool, due to changes in the axonal density or edema, will also cause f to decrease. This confounding influence should be kept in mind, particularly when f is interpreted as varying only with myelin content.

In Table 5.5, voxel-based correlations between MRI metrics were compared in the corpus callosum and external capsule of all mice. Although most correlations were similar, correlations of FA vs. f and FA vs. R_A or T_1 were smaller in the external capsule. In Table 5.4, f is strongly correlated to the myelin sheath fraction and R_A/T_1 to the myelinated axon fraction. Decreases in correlation with FA suggest that anisotropy is more strongly influenced by the presence of myelinated axons in the corpus callosum. Although inherently related to each other, correlations of FA vs. $\langle D \rangle$, $\lambda_{\parallel}$, and $\lambda_{\perp}$ reveal the shift in influence of different DTI metrics on the overall diffusion anisotropy. FA vs. $\langle D \rangle$ and $\lambda_{\perp}$ were smaller in the external capsule, while FA vs. $\lambda_{\parallel}$ was larger. These show the shift in influence on anisotropy, from myelinated axons in the corpus callosum to non-myelinated cells in the external capsule.

In Table 5.6, voxel-based correlations were compared to ROI-based correlations in the white matter of all mice. ROI-based correlations allowed us to correlate *in vivo* and *ex vivo* MRI measurements as well as EM measurements in the corpus callosum. ROI-based correlations of *in vivo* T_2 -weighted signal and MTR were stronger with DTI metrics than with f , suggesting that water content, mobility and anisotropy have a stronger influence on T_{2w} and MTR than myelin content. Again, this reflects similar observations in the EM measurements, where correlations were stronger with myelinated axons than with myelin content itself.

Although ROI-based correlations tended to be larger, some obvious disadvantages of an

ROI-based approach are reduced number of samples (in this case, $n = 20$ vs. $n = 1000+$) and the inherent assumption that individual voxels can be represented by the average characteristics of the ROI, akin to the ecological fallacy [231]. Voxel-based analysis provides a more accurate picture of how MRI metrics co-vary due to varying tissue pathology within an ROI, albeit, a picture more strongly influenced by noisy measurements.

The interpretation of correlations reflecting breadth of tissue pathology seems to be supported by previous studies. Ou *et al.* found moderate correlation between average values of F and $\lambda_{\perp}$ in the combined values from the corpora callosa of control and shiverer mice ($r = -0.57$) but not in control and shiverer mice separately [180]. In a study of healthy human brains, Underhill *et al.* found that correlation between qMTI and DTI metrics was weak and insignificant in healthy white matter, but strong and significant when comparing metrics in combined values from both grey and white matter ($|r| = 0.48$ to 0.75) [252]. In a study of healthy human brains, Stikov *et al.* found voxel-based correlations between f and FA increased in white matter tracts with high directional coherence, such as the corpus callosum [234]. Based on a tissue model, Stikov *et al.* conclude that FA and f covary primarily due to variations in the fiber volume fraction. Differences arise due to variations in the directional coherence of fibers (affecting diffusion anisotropy but not f) and myelin density (affecting f more than it affects diffusion) [234].

In Table 5.7, voxel-based correlations were compared in the white matter of control and cuprizone-fed mice. Some of the regional differences observed in Table 5.5 are reflected here as well. Strong correlation between FA and $\lambda_{\parallel}$ in the cuprizone-fed mice reflect the reduced influence of myelinated axons on measurements of anisotropy. Correlations between f and various metrics are generally stronger in the control mice, where myelin is significantly more abundant and the free water pool is smaller. Stronger correlations of f with $\lambda_{\parallel}$, $\lambda_{\perp}$, and FA in the control mouse reflect the trend of stronger correlation in directionally coherent tracts [179, 234] and tracts with broader ranges of tissue pathology [181, 179, 252, 234].

To test the feasibility of using voxel-based correlations as an additional test of tissue pathology, correlations were calculated for individual control and cuprizone-fed mice (Table 5.8). Results are very similar to the combined results found in Table 5.7. Again, FA vs. $\lambda_{\perp}$ was significantly higher in the cuprizone-fed mice. Significantly lower values of f

vs. T_1 in the cuprizone-fed mice may reflect the varying influence of lattice size and water mobility on the bound pool fraction.

5.5 Conclusions

Several conclusions can be made regarding the application of longitudinal and quantitative MRI measurements to the cuprizone-mouse model:

1. The efficacy of the cuprizone treatment is dependent on preparation and region of interest. Using 0.4% cuprizone pressed into mouse chow pellets, changes in the rostral corpus callosum were similar to those observed previously in 0.2% cuprizone-fed mice after 6 weeks in the caudal corpus callosum or 12 weeks in the rostral corpus callosum.
2. In the corpus callosum, T_2 -weighted and MTR values peaked at 4 and 5 weeks, respectively. In order to effectively track the progression of demyelination and potential treatments, weekly MRI assessments are recommended.
3. To our knowledge, this study is the first to apply multicomponent T_2 analysis and quantitative MTI measurements to the cuprizone mouse model. In multicomponent T_2 analysis with 10 ms echo spacing and a first echo SNR ≈ 130 , there was no visible myelin water component associated with the short- T_2 value. Quantitative MTI metrics such as RM_0^A , T_2^A , and in particular, f showed significant differences in the corpus callosum and external capsule of the cuprizone-fed mice, similar to previous studies of multiple sclerosis in humans and animal models of demyelination.
4. FA was significantly lower and $\langle D \rangle$, $\lambda_{\parallel}$, and $\lambda_{\perp}$ were significantly higher in the cuprizone-fed mice. Besides $\lambda_{\parallel}$, DTI metrics were similar to previous measurements in the cuprizone mouse model. Significantly larger $\lambda_{\parallel}$ was attributed to the higher concentration cuprizone diet and possible vasogenic edema.

As a comparison of quantitative MRI metrics to ultrastructural measurements, the following conclusions can be made:

1. Cellular distributions measured in electron micrographs correlate strongly to several different quantitative MRI metrics. Ranked by correlation strength, the top two

correlations for each tissue type were: R_A/T_1 and T_2^A vs. the myelinated axon fraction ($\rho = 0.90/-0.90$ and -0.85), f and $\lambda_\perp$ vs. the myelin sheath fraction ($\rho = 0.93$ and -0.87), f and $\langle D \rangle$ vs. the myelinated axons + myelin sheath fraction ($\rho = 0.89$ and -0.87), $\lambda_\parallel$ and $f/\langle D \rangle$ vs. the non-myelinated cell fraction ($\rho = 0.92$ and $-0.90/0.90$). Correlations with measurements of myelinated axons reflected these trends.

2. Using Pearson's correlation coefficient, f was strongly correlated to the myelin sheath fraction ($r = 0.98$) with a linear equation predicting myelin content ($5.368f - 0.252$). Of the calculated MRI metrics, f was the strongest indicator of myelin content while longitudinal relaxation rates and diffusivity measurements were the strongest indicators of changes in tissue structure.
3. Correlations between different MRI metrics represent underlying tissue pathologies that influence the paired MRI metrics. In particular, variations in the correlations between f , T_1 and the DTI metrics revealed the varying influence of myelin content, axonal density, and water mobility in the white matter of control and cuprizone-fed mice.
4. Correlations also represent the breadth of tissue pathologies. The correlation coefficients of several quantitative MRI measurements were smaller after 6 weeks of cuprizone treatment. Correlation of *in vivo* T_2 -weighted signals and MTR values also reflected a shift in the range of tissue pathologies in the corpus callosum after 6 weeks of cuprizone treatment, where the variance of both metrics and their correlation coefficients were significantly decreased.

In conclusion, longitudinal and multiparametric MRI analysis gives new and complementary information about tissue pathology in the cuprizone mouse model of demyelination.

Chapter 6

Conclusions

6.1 Quantitative MRI Methods

In developing the quantitative MRI analysis methods, MATLAB scripts were written to calculate and compare T_1 and T_2 relaxation, diffusion tensors, and parameters from the two-pool model of magnetization transfer. To improve both the goodness-of-fit in individual methods and comparisons between multiple methods, a tool was built to manually and automatically register MR images. The anisotropic diffusion filter was used to reduce the influence of noise on quantitative fits. Quantitative magnetization transfer imaging was tested on agar phantoms and mouse brains. Diffusion tensor imaging was tested on a water phantom, fixed rat spinal cords, and mouse brains. Single component T_1 and T_2 analysis was tested on rat spinal cords and, along with multicomponent T_2 analysis, mouse brains.

6.1.1 Image Registration and the Anisotropic Diffusion Filter

The image registration tool automatically calculated an affine transformation matrix to match target images to a reference image. The matrix defines parameters for translation, scaling, shearing, and rotation in two-dimensions. Optimal parameters were searched for by minimizing functions defining the shared information between the two images. The three functions tested were: the absolute difference, 2-D correlation coefficient, and mutual information. Of these, the 2-D correlation coefficient gave the best compromise between accuracy and speed. Although an assessment of accuracy based on the percent difference works for identical MRI methods, variations in contrast between different methods makes

this less than ideal. Instead, each target image was compared visually to the reference image to ensure accuracy.

Improvements due to the application of the anisotropic diffusion filter were assessed for all quantitative MRI methods in freshly excised mouse brains. The filter improved both the goodness-of-fit and visual quality of all qMRI maps, but increased χ^2/ν values were observed at the transition between white matter and grey matter. Particularly noisy quantitative maps, such as T_2^A and RM_0^A , were markedly improved after filtration, with the corpus callosum more clearly defined against the surrounding grey matter. MRI metrics calculated with filtered and non-filtered images were similar, although non-filtered FA was higher in the corpus callosum and external capsule, reflecting previously observed increases due to noise [119, 64]. To our knowledge, the anisotropic diffusion filter has not been applied to quantitative MTI analysis previously, nor has there been an assessment of its efficacy across multiple quantitative MRI measurements.

6.1.2 Quantitative MTI

In various concentrations of agar, quantitative MTI results for f and R_A were similar to previous results. Differences in RM_0^A , T_2^A , and T_2^B led to a brief exploration of inaccurate estimations of MT pulse amplitude. When the MT saturation powers were scaled by a constant, the model fit was unchanged but certain parameters changed significantly (f , RM_0^A , and T_2^A) while others were relatively constant (R_A and T_2^B). This finding merits future studies looking at the influence of image noise and inaccuracies in offset frequency and saturation strength on the two-pool model of pulsed magnetization transfer.

Using quantitative MTI data from Chapter 5, the ideal offset frequency and MT pulse amplitude was determined for acquiring an MTR map of the corpus callosum in freshly excised mouse brain at 7 T. The three criteria used were: optimal contrast between white and grey matter, maximum correlation between MTR and f , and minimal direct saturation of the liquid pool. Based on 3 MT pulse amplitudes and 6 offset frequencies at each amplitude, an amplitude of 10 μ T and offset frequency of 6000 Hz was chosen for acquiring the M_{sat} image.

6.1.3 DTI, T_1 , and T_2 measurements in Fixed Rat Spinal Cord

Results from our study of fixed rat spinal cords suggest that the fractional anisotropy and radial diffusivity in grey matter is dependent on diffusion time at $\Delta < 20$ ms, but not in white matter. Corrections were needed to account for temperature drift in the water sample over the long imaging time. Differences in T_1 and T_2 before and after 48 hours of imaging were also attributed to this change in temperature as well as drift in the Larmor frequency of the sample. Results matched DTI, T_1 and T_2 measurements found previously in fixed rat spinal cord. For future studies, the shift in temperature could be corrected by allowing samples to equilibrate to the bore temperature for a longer period of time before imaging or by using a water bath with a feedback loop similar to *in vivo* imaging.

6.1.4 Comparison of Fresh and Fixed Mouse Brains

Comparison of quantitative MRI results in freshly excised and fixed mouse brains reflected previous studies [238, 212, 239]. Significant differences were observed in DTI metrics (except for fractional anisotropy), qMTI metrics, and T_1 . Given that fixation has varying influence on different quantitative MRI metrics, studies that compare different quantitative methods should use freshly excised or *in vivo* tissue.

6.2 Multiparametric MRI of the Cuprizone Mouse Model

6.2.1 Characterizing the Cuprizone Mouse Model

Based on the *in vivo* results for two separate studies of the cuprizone mouse model, caution is advised when combining MRI data, even when acquired with the same diet and imaging parameters. Subtleties in preparation and variability in the effects of the cuprizone-diet may lead to different degrees of demyelination in the same region of the corpus callosum. Due to different *in vivo* results suggesting different degrees of demyelination, only the second cuprizone study was used when analyzing the *in vivo* and *ex vivo* MRI results in Chapter 5. When assessing the sensitivity of different MRI methods to white matter integrity, pathohistology, as was done in these studies, is also advised.

In Chapter 5, several new results were found in the cuprizone model of demyelination. Using 0.4% cuprizone pressed into mouse chow pellets, changes in the rostral corpus callosum were similar to those observed previously in 0.2% cuprizone-fed mice after 6 weeks in the caudal corpus callosum or 12 weeks in the rostral corpus callosum. In the corpus callosum, T_2 -weighted and MTR values peaked at 4 and 5 weeks, respectively, suggesting that weekly MRI assessments might be necessary to effectively track the progression of demyelination and potential treatments.

To our knowledge, this study is the first to apply multicomponent T_2 analysis and quantitative MTI measurements to the cuprizone mouse model. In multicomponent T_2 analysis, there was no significant myelin water component associated with the short- T_2 value in either control or cuprizone-fed mice. Quantitative MTI metrics such as RM_0^A , T_2^A , and in particular, f showed significant differences in the corpus callosum and external capsule of the cuprizone-fed mice, similar to previous studies of multiple sclerosis in humans and animal models of demyelination. FA was significantly lower and $\langle D \rangle$, $\lambda_{\parallel}$, and $\lambda_{\perp}$ were significantly higher in the cuprizone-fed mice. Besides $\lambda_{\parallel}$, DTI metrics were similar to previous measurements in the cuprizone mouse model. Significantly larger $\lambda_{\parallel}$ was attributed to the higher concentration cuprizone diet and possible vasogenic edema.

6.2.2 Correlations Between Quantitative MRI and Electron Microscopy

Cellular distributions measured in electron microscopy images were strongly correlated to several different quantitative MRI metrics. f was the strongest indicator of myelin content while longitudinal relaxation measurements (T_1 and R_A) and diffusivity measurements ($\lambda_{\parallel}$, $\lambda_{\perp}$, and FA) were the strongest indicators of changes in tissue structure. Using Pearson's correlation coefficient, f was strongly correlated to the myelin sheath fraction ($r = 0.98$) with a linear equation predicting myelin content, $M = (5.368 \pm 0.379)f - (0.252 \pm 0.031)$.

Correlations between different MRI metrics might represent underlying tissue pathologies that influence the paired MRI metrics. In particular, variations in the correlations between f , T_1 and the DTI metrics revealed the varying influence of myelin content, axonal density, and water mobility in the white matter of control and cuprizone-fed mice. Correlations also represent the breadth of tissue pathologies. The correlation coefficients of

several quantitative MRI measurements were smaller after 6 weeks of cuprizone treatment. Correlation of *in vivo* T_2 -weighted signals and MTR values also reflected a shift in the range of tissue pathologies in the corpus callosum after 6 weeks of cuprizone treatment, where the variance of both metrics and their correlation coefficients were significantly decreased.

In this study, longitudinal and multiparametric MRI analysis gave new and complementary information about tissue pathology in the cuprizone mouse model of demyelination. The use of multiparametric rather than single parameter MR analysis may result in a better understanding of white matter pathology.

6.3 Future Studies

The imaging and analysis methods developed for this dissertation have a number of potential applications.

In vivo and longitudinal quantitative MRI in the cuprizone model are an obvious extension of Chapter 5. Quantitative MRI and ultrastructural examination at various timepoints spanning both demyelination and remyelination would improve the power of the correlations. The efficacy of anti-psychotic drugs in alleviating white matter pathology has been tested previously by our collaborators using the cuprizone mouse model [278, 269, 272]. *In vivo* multiparametric MRI could be a powerful tool for assessing white matter integrity in conjunction with potential treatments. Other models of multiple sclerosis, such as the dysmyelinated shiverer mouse model [215, 223, 180] or the immune-mediated experimental allergic encephalomyelitis model [59, 72, 199, 210, 33] can give additional insight into the effects different tissue pathology have on multiparametric MRI measurements.

There are numerous diseases which affect white matter in the brain, including but not limited to: Alzheimer’s disease, schizophrenia, epilepsy, Huntington’s disease, Parkinson’s disease, and various types of brain tumours. Preliminary *ex vivo* measurements were made in a mouse model of Alzheimer’s disease using the same quantitative MRI methods described here. *In vivo* DTI and MTI measurements have also been made in our lab using mouse models of Alzheimer’s disease.

Based on Ref. [241], a Monte Carlo simulation of diffusion in tissue has been developed

in our lab using a two compartment square lattice with a permeable barrier representing the myelin sheath. Measurements in fixed rat spinal cord are currently being compared to this simulation to test the effects varying gradient strength and diffusion time have on DTI measurements in tissue. This simulation could be extended by including magnetization transfer effects and ultrastructural measurements of cellular distributions. Using f to estimate the myelin volume fraction and measurements of diffusivity to estimate the density of myelinated axons might improve comparisons between simulation and experiment.

Appendix A

List of Acronyms

μ nuclear magnetic dipole moment

B_0 magnetic field vector

M net magnetization vector

M_0 net magnetization at equilibrium

ω_0 Larmor frequency

γ gyromagnetic ratio

MRI magnetic resonance imaging

FID free induction decay

RF radiofrequency

T_E echo time

T_R repetition time

T_I inversion time

FOV field of view

RARE Rapid Acquisition with Refocused Echoes

FLASH Fast Low Angle Shot

CPMG Carr Purcell Meiboom Gill

PGSE Pulsed Gradient Spin Echo

EPI Echo-Planar Imaging

T_1 longitudinal relaxation time

T_2 transverse relaxation time

T_{2w} T2-weighted image

mcT₂ multicomponent transverse relaxation time

MWI myelin water imaging

NNLS non-negative least squares

$T_{2,m}$ transverse relaxation time of the myelin water component

$T_{2,ie}$ transverse relaxation time of the intra-/extracellular water component

MWF myelin water fraction

IEWF intra-/extracellular water fraction

MT magnetization transfer

MTI magnetization transfer imaging

qMTI quantitative magnetization transfer imaging

MTC magnetization transfer contrast

MTR magnetization transfer ratio

Δf offset frequency

ω_1 angular frequency induced by RF pulse

ω_{1CWPE} continuous wave power equivalent angular frequency induced by RF pulse

f bound pool fraction

M_0^A equilibrium magnetization of the liquid pool

M_0^B equilibrium magnetization of the bound pool

R magnetization transfer rate between the bound and liquid pool

R_A longitudinal relaxation rate of the liquid pool

R_B longitudinal relaxation rate of the bound pool

R_{rfA} RF absorption rate of the liquid pool

R_{rfB} RF absorption rate of the bound pool

T_2^A transverse relaxation time of the liquid pool

T_2^B transverse relaxation time of the bound pool

DTI diffusion tensor imaging

D diffusion coefficient

ADC apparent diffusion coefficient

D diffusion tensor

b diffusion-weighting factor

$\lambda_{\parallel}$ axial diffusivity

$\lambda_{\perp}$ radial diffusivity

$\langle D \rangle$ mean diffusivity

FA fractional anisotropy

RGB red-green-blue

LS linear least-squares

WLS weighted linear least-squares

NLS non-linear least squares

SNR signal-to-noise ratio

A affine transformation matrix

CNS central nervous system

CSF cerebrospinal fluid

NAWM normal appearing white matter

MS multiple sclerosis

LFB Luxol fast blue

PAS periodic acid Schiff

MBP myelin basic protein

PBS phosphate-buffered saline

PFA paraformaldehyde

EM electron microscopy

A myelinated axons

M myelin sheaths

C non-myelinated cells

E extracellular space

M/A ratio myelin thickness/axon diameter ratio

CTL control mouse

CPZ cuprizone-fed mouse

CC corpus callosum

EC external capsule

CX cerebral cortex

ROI region of interest

RBA ROI-based analysis

VBA voxel-based analysis

ANOVA analysis of variance

ρ Spearman's correlation coefficient

r Pearson's correlation coefficient

Appendix B

Additional Figures

B.1 Longitudinal MRI Images

All *in vivo* T_2 -weighted images (Figures B.1 and B.2) and MTR maps (Figures B.3 and B.4) from Chapter 5 show consistent slice selection and a healthy corpus callosum in control mice from 0–6 weeks. Changes in the cuprizone-fed mice consistently start on the third week of treatment in the corpus callosum and radiate outwards to the external capsule in subsequent weeks. At 4 weeks, normalized T_2 -weighted signal is significantly larger in the corpus callosum compared to the external capsule, but MTR values don't deviate. By week-5, the T_2 -weighted signals are similar. At 6 weeks, decreasing T_2 -weighted signal and increasing MTR in the corpus callosum may signify remyelination in the cuprizone-fed mice.

B.2 Electron Microscopy Measurements

Example of electron microscopy cell distributions in a control (Figure B.5) cuprizone-fed corpus callosum (Figure B.6). Cell distributions were counted on two, 5000X magnification images per mouse with a $2 \times 2 \mu\text{m}$ grid, yielding 360 points per mouse. Labels include myelinated axons (A), myelin sheaths (M), non-myelinated cells (C), nuclei (N), damaged axons (DA), glial cell processes (G), and extracellular spaces (E). Ultimately, all cells that weren't myelinated axons were included in the non-myelinated cell category.

Myelinated axons were measured in two, 15000x magnification images from each control (Figure B.7) and cuprizone-fed mouse (Figure B.7). The narrow axon diameter and myelin sheath thickness of myelinated axons were measured using ImageJ 1.40 (National Institutes of Health). No measurements were made on non-myelinated cells, including non-myelinated axons, thus the number of measurements is much smaller in the cuprizone-fed mice.

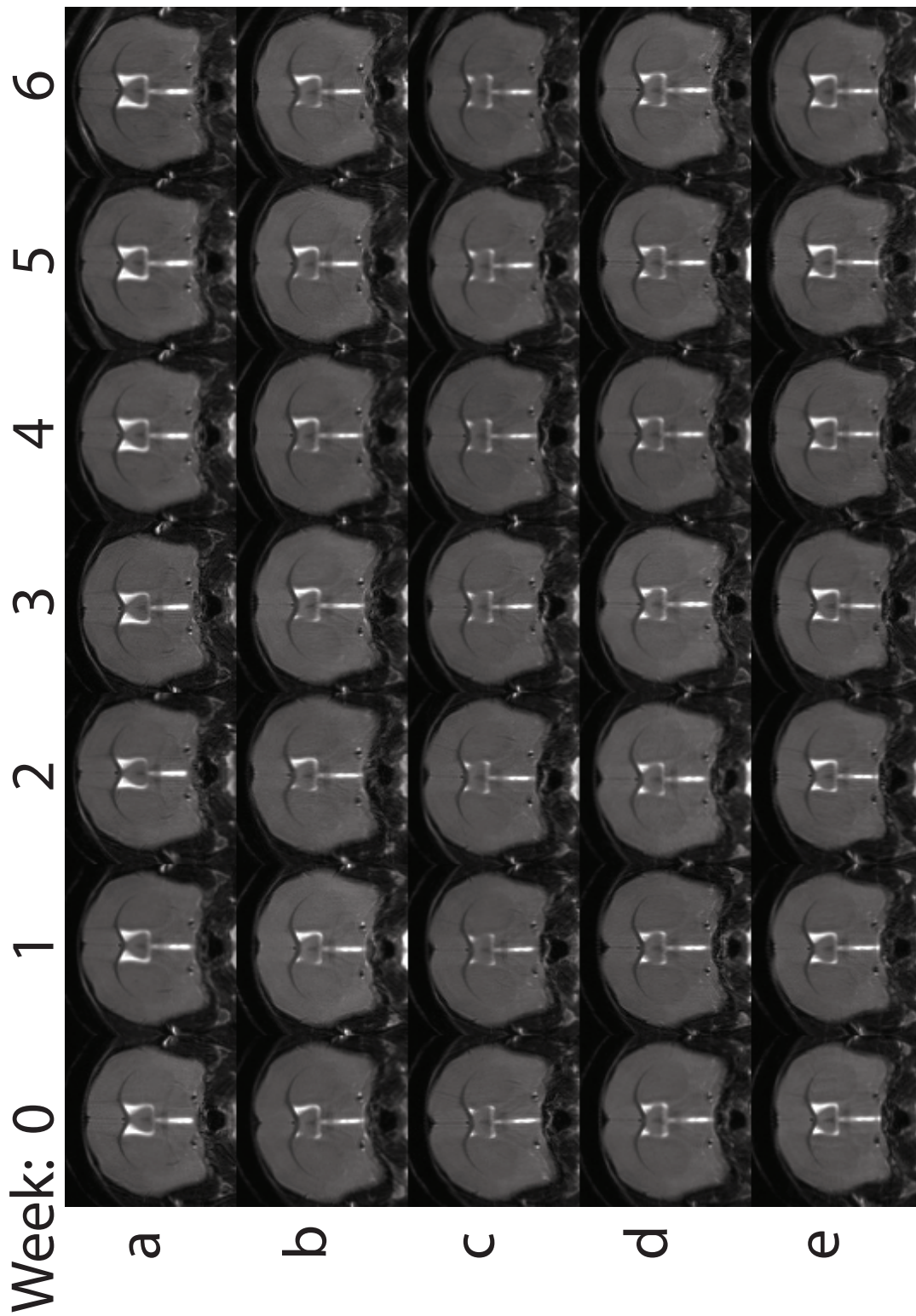


Figure B.1: Weekly T_2 -weighted images from all 5 control mice.

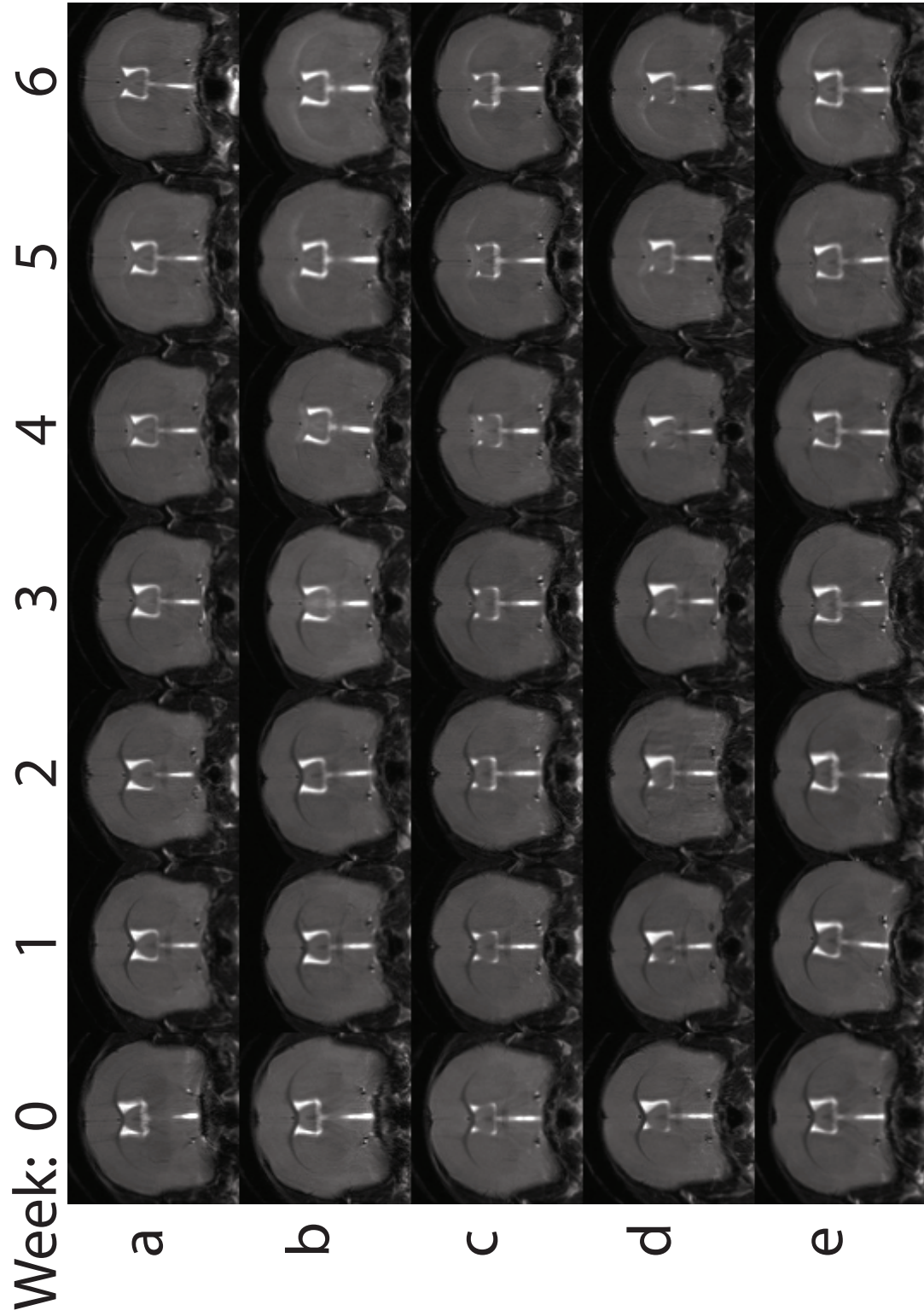


Figure B.2: Weekly T_2 -weighted images from all 5 cuprizone-fed mice.

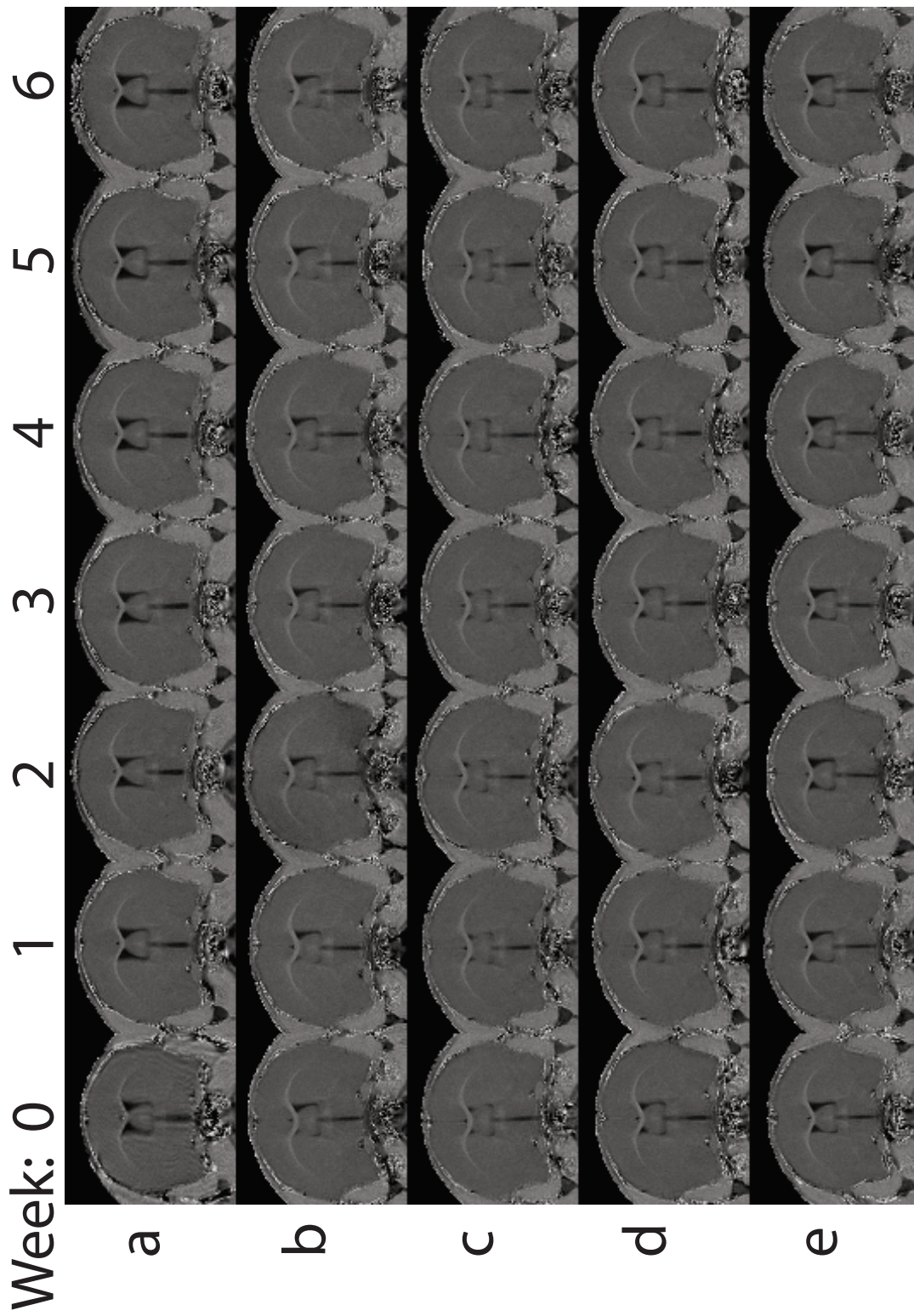


Figure B.3: Weekly MTR from all 5 control mice.

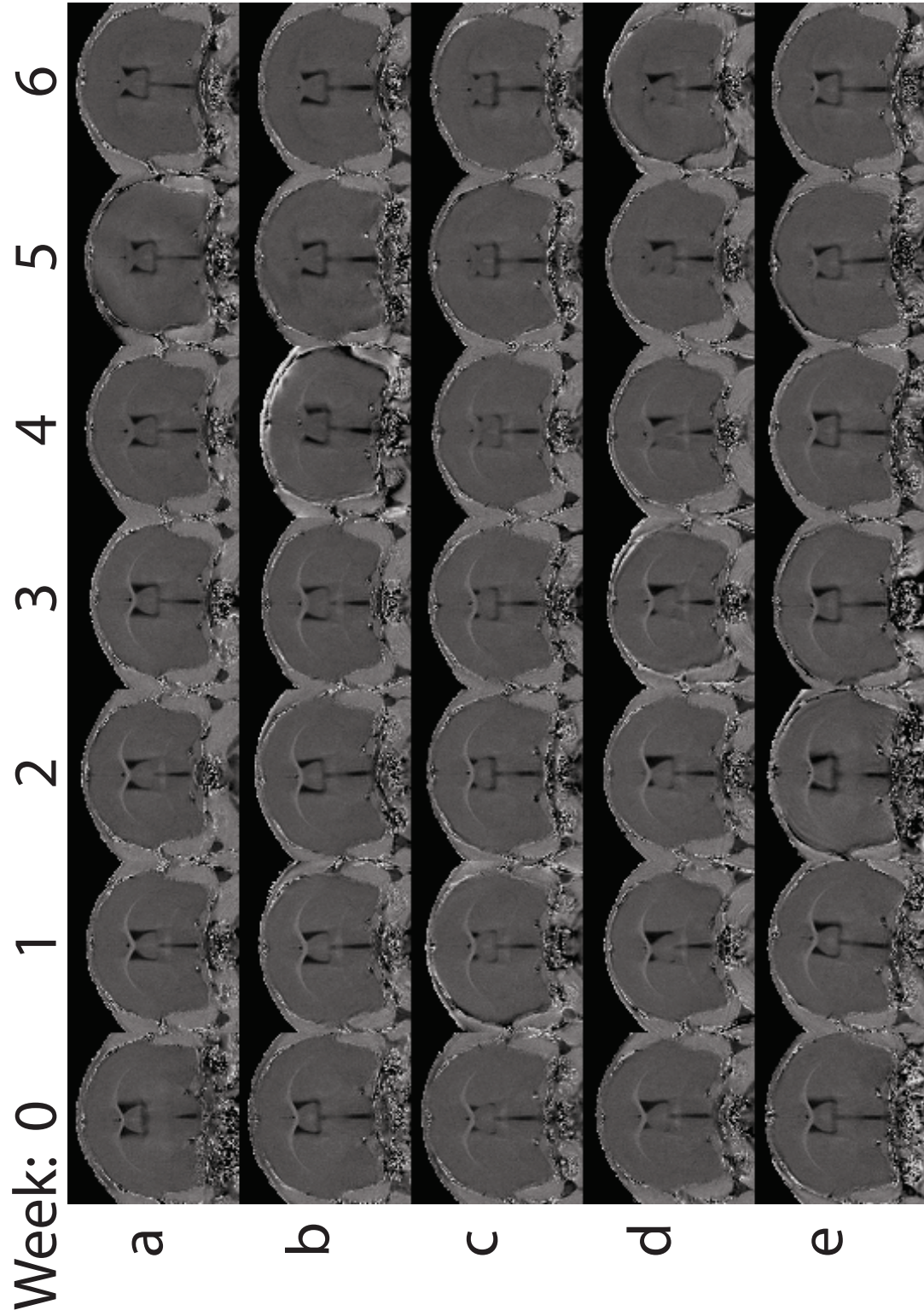


Figure B.4: Weekly MTR from all 5 cuprizone-fed mice.

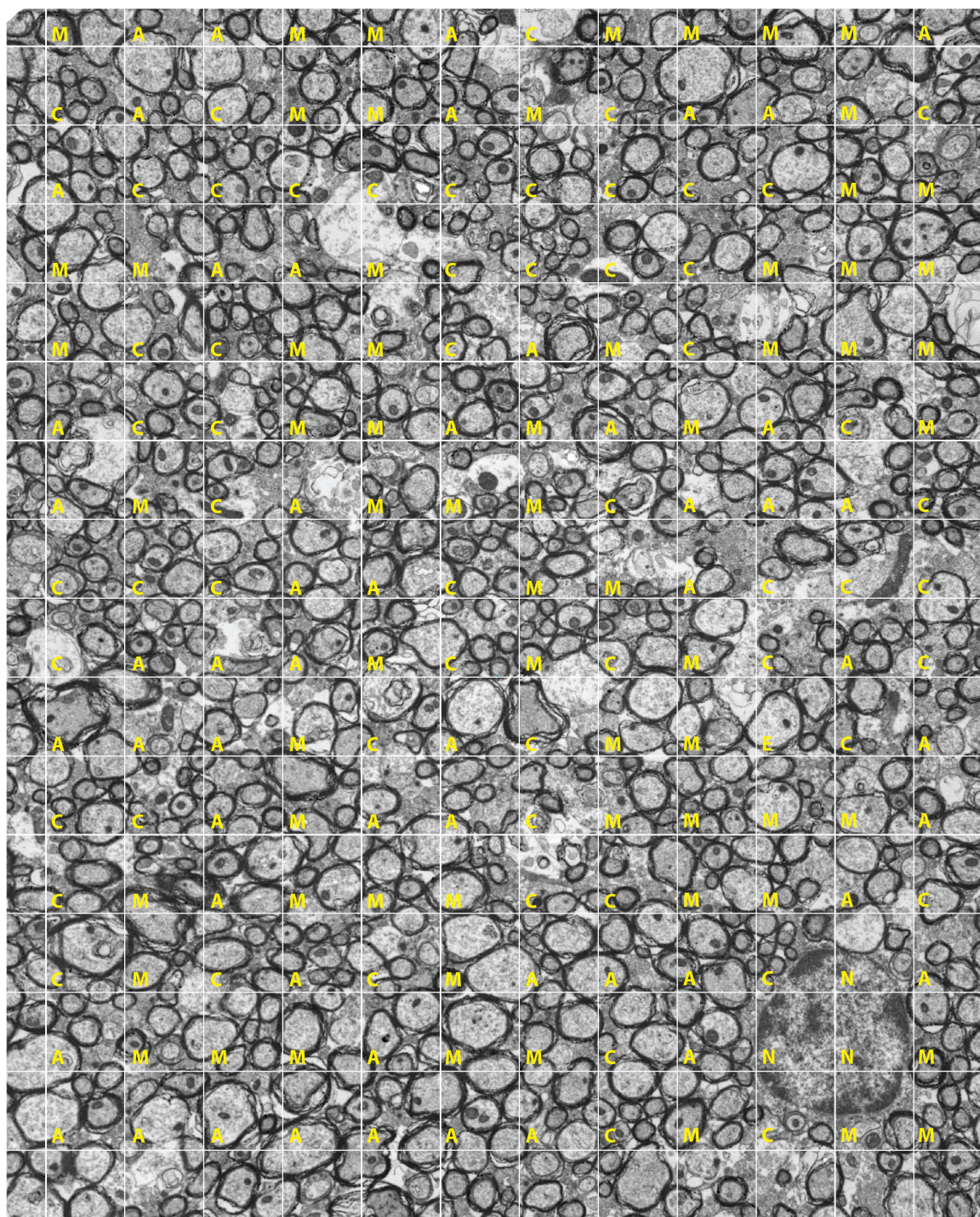


Figure B.5: Example of electron microscopy cell distribution in a control corpus callosum. Cell distributions were counted on two, 5000x magnification images per mouse with a $2 \times 2 \mu\text{m}$ grid, yielding 360 points per mouse. Labels include myelinated axons (A), myelin sheaths (M), non-myelinated cells (C), nuclei (N), and extracellular spaces (E). Ultimately, all cells that weren't myelinated axons were included in the non-myelinated cell category.

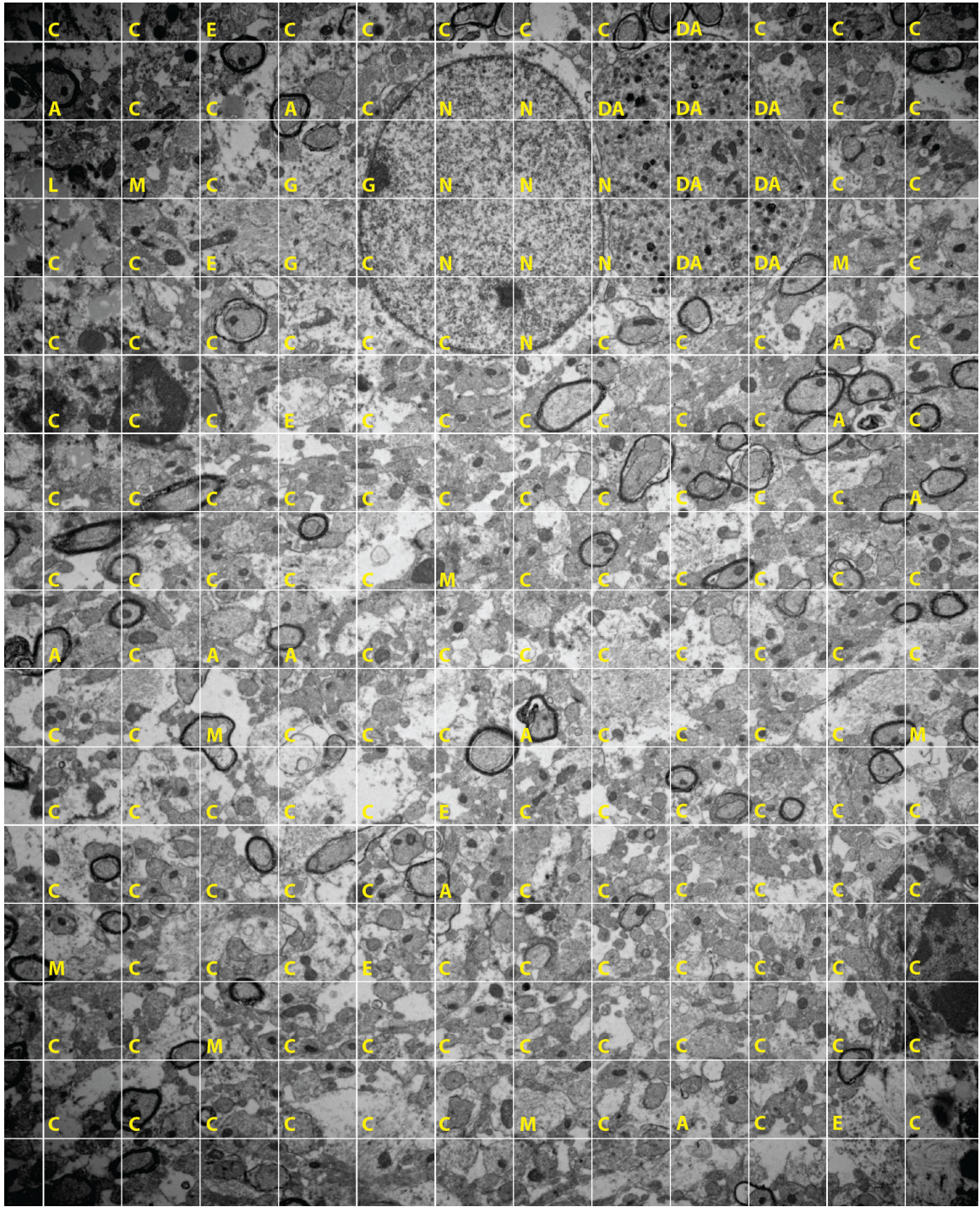


Figure B.6: Example of electron microscopy cell distribution in a cuprizone-fed corpus callosum. Cell distributions were counted on two, 5000X magnification images per mouse with a $2 \times 2 \mu\text{m}$ grid, yielding 360 points per mouse. Labels include myelinated axons (A), myelin sheaths (M), non-myelinated cells (C), nuclei (N), damaged axons (DA), glial cell processes (G), and extracellular spaces (E). Ultimately, all cells that weren't myelinated axons were included in the non-myelinated cell category.

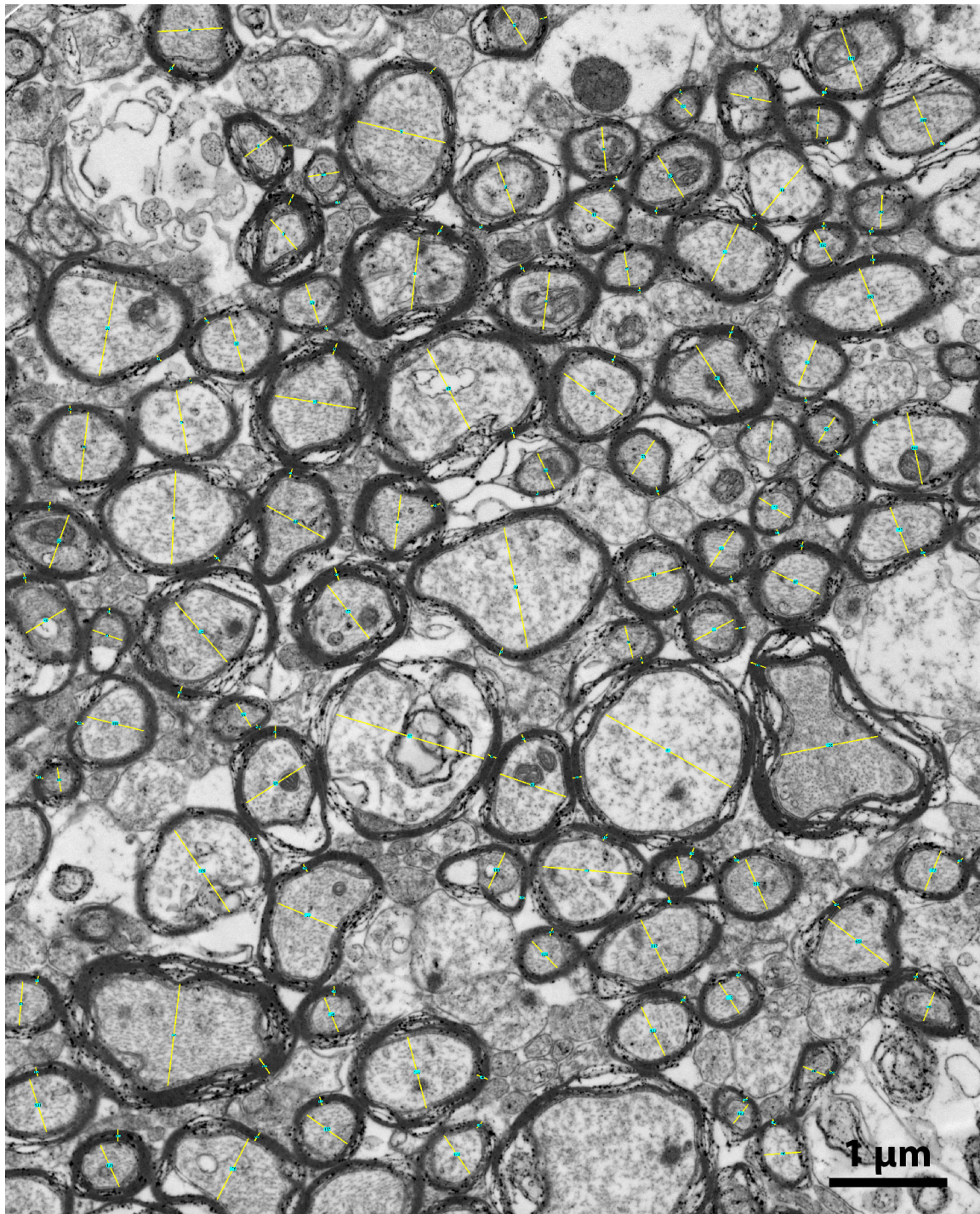


Figure B.7: Example of electron microscopy measurements of myelinated axons in a control corpus callosum. The narrow axon diameter and myelin sheath thickness of myelinated axons were measured on two, 15000X magnification images per mouse. Scale bar = 1 μm .

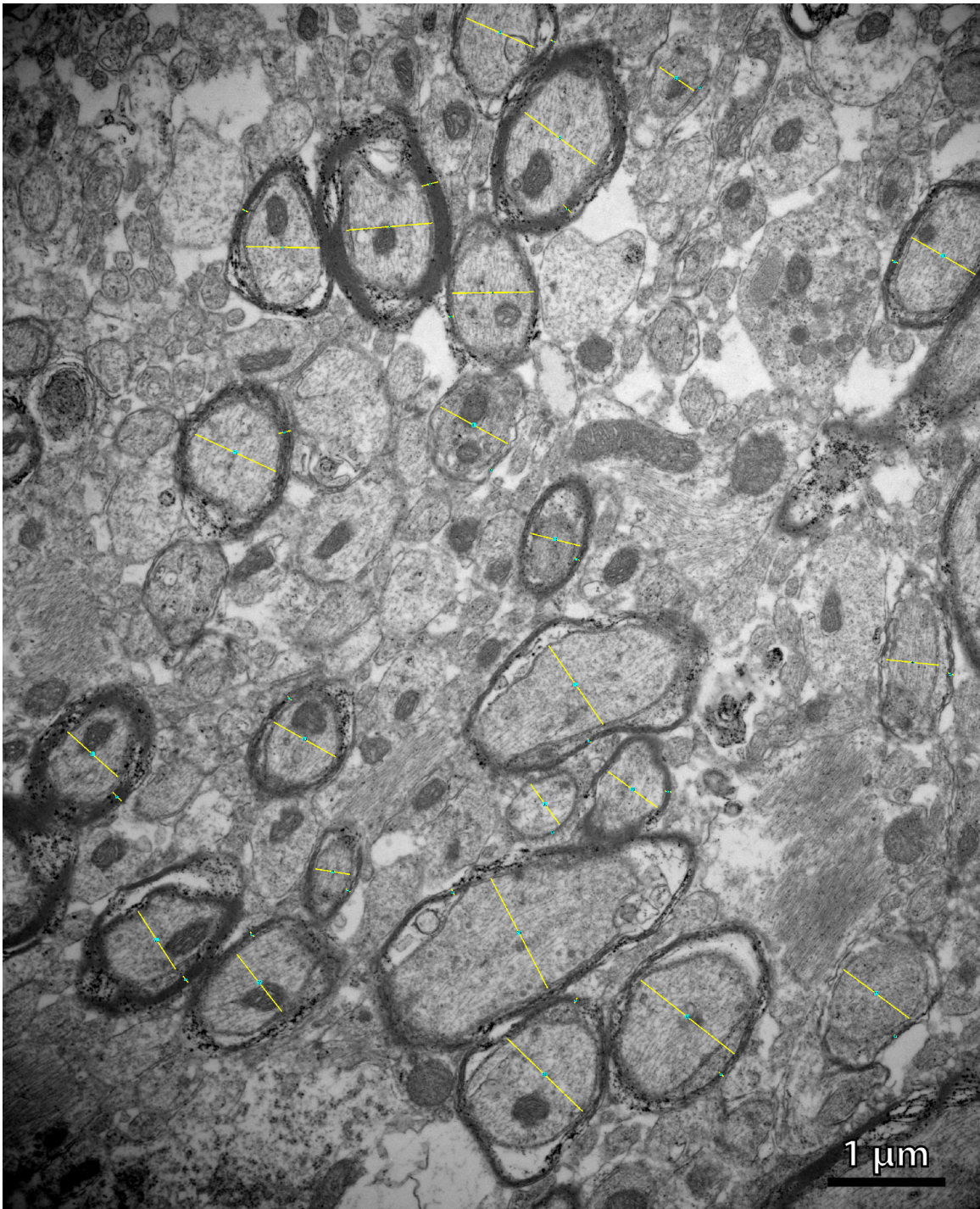


Figure B.8: Example of electron microscopy measurements of myelinated axons in a cuprizone-fed corpus callosum. The narrow axon diameter and myelin sheath thickness of myelinated axons were measured on two, 15000X magnification images per mouse. No measurements were made on non-myelinated cells, including non-myelinated axons, thus the number of measurements is much smaller in the cuprizone-fed mice. Scale bar = 1 μm .

Appendix C

MATLAB Programs

C.1 Image Preparation

C.1.1 Loading Images

After acquisition, Paravision reconstructs the MRI data and saves it as a scaled raw data file named 2dseq. `load_2dseq.m` relies on several scripts originally written by Mike Tyszka (Caltech, 2000) and later amended by Doug Story and Blair Cardigan Smith (University of Winnipeg, 2006). It ensures that the dimensionality, endianness (big or little), and data types (16-bit or 32-bit signed integers) are correct. Importantly, it removes any scaling and offsets applied to the images by Paravision. This is absolutely essential for creating quantitative maps from multiple acquisitions.

```
function [unscaled_image info] = load_2dseq(serdir, reconum)

% Load a Paravision 2dseq file
fname = 'load_2dseq';

% Loads the image information
info = load_info(serdir);

% Dimensions
if info.ndims == 2
    nz = info.nims;
    info.dim(3) = nz;
else
    nz = info.dim(3)*info.nims;
end

% RECO location
recofile = [serdir '\pdata\' in2str(reconum) '\reco'];

% Checks for existence of Reco file
if exist(recofile) ~= 2
    fprintf('RECO file does not exist')
end;

% Reads RECO file
recoinfo = textread(recofile,'%s','delimiter','\n','whitespace','');

% Check dimensions of reconstructed matrix
```

```

dim = extract_matrix(recoinfo, '$RECO_ft_size=');
nx = dim(1);
ny = dim(2);

% Read scaling info
slope = extract_matrix(recoinfo, '$RECO_map_slope=');
offset = extract_matrix(recoinfo, '$RECO_map_offset=');

% Check if slope and offset is the same for all slices
if (slope ~= slope(1)) || (offset ~= offset(1))
    errmsg = sprintf('Slices are individually scaled');
    waitfor(errordlg(errmsg));
end

% Open the file
filename = [sudir '\pdata\' int2str(reconum) '\2dseq'];
if strcmp(info.endian, 'be') == 1
    fd = fopen(filename, 'r', 'ieee-be'); % big endian
elseif strcmp(info.endian, 'le') == 1
    fd = fopen(filename, 'r', 'ieee-le'); % little endian
end
if (fd < 1)
    errmsg = sprintf('%s: Could not open %s to read\n', fname, filename);
    waitfor(errordlg(errmsg));
    return;
end

% Read the data
d = fread(fd, info.datatype); % info.datatype = 16bit or 32bit

% Close the file
fclose(fd);

% Check if data set is complete
if (nx*ny*nz ~= length(d))
    errmsg = sprintf('%s: 2dseq file is incomplete\n', fname);
    waitfor(errordlg(errmsg));
    return;
else
    % Unscale the data
    unscaled = round(d/slope(1) + offset(1));
    % Reshape the data
    unscaled_image = reshape(unscaled, nx, ny, nz);
end;

```

C.1.2 Image Registration

In order to register images for quantitative analysis, a GUI was written in MATLAB that allowed manual and automatic registration of 2-dimensional MR images (Figures C.1 and C.2). An affine transformation matrix is used to represent translation, scale, and shear along the x and y directions as well as rotation. Functionality includes:

- **Load Images:** Loads individual images from two separate Paravision datasets.
- **Affine Transformation Parameters:** Defines parameters for x/y translation, scale, and shear as well as rotation angle. Parameters are used as starting points for automatic registration. Automatic registration is also bounded by a user defined value for

each parameter. All values can be manually changed at any point with the option to redraw the images with the new parameters or reset to the default parameters.

- **Reference/Target Images:** Allows the user to switch between full or cropped views of both the reference and target images.
- **Percent Difference Image:** Shows the percent difference between the reference and target image, calculated as $(|reference - target|/reference) * 100\%$. In the case of a reference proton density image and a target magnetization transfer contrast image, this will be equivalent to the magnetizaion transfer ratio. The percent difference can be viewed at a specific point of interest.
- **Registration:** Registration can be done automatically using a bounded minimization search (`fminsearchbnd`). The `fminsearchbnd` function was written by John D’Erico and is available on the MATLAB File Exchange [54]. Bounds are applied to the variables by internally transforming them with $\sin(x)$ prior to using MATLAB’s included `fminsearch` algorithm. `fminsearch` itself is based on the Nelder-Mead simplex search method [133]. It is a direct search method for finding the minimum of an unconstrained multivariable function based on an initial guess for the variables. The difference between the reference and target images can be measured using mutual information, the 2D correlation coefficient, or the absolute difference.
- **File:** User can save or load previously registered images and parameters. Save/Load Images saves/loads all images and parameters, Save/Load Parameters saves/loads just the parameters, and Save/Load Aligned Image saves/loads just the aligned image.

imreg.m

An excerpt of the main registration program (`imreg.m`) is included below. This snippet is taken from the section that executes upon pressing the Mutual Information button in the Registration section of the program.

```
% imreg.m (EXCERPT)
% Executes upon Mutual Information button press in Registration section.
% -----

% Acquire bounds and parameters from GUI. Sets lower and upper parameter
% bounds.
bounds = handles.bounds;
initial_scale = handles.scale;
LB = initial_scale - bounds; % lower parameter bounds
UB = initial_scale + bounds; % upper parameter bounds

% Normalize reference and target images prior to image registration.
reference = handles.reference./max(max(handles.reference));
target = handles.target./max(max(handles.target));

% Sets optimization options for maximum number of function evaluations,
% iterations, and termination tolerance.
options = optimset('MaxFunEvals', 5000, 'MaxIter', 5000, 'TolX', 1e-12);
```

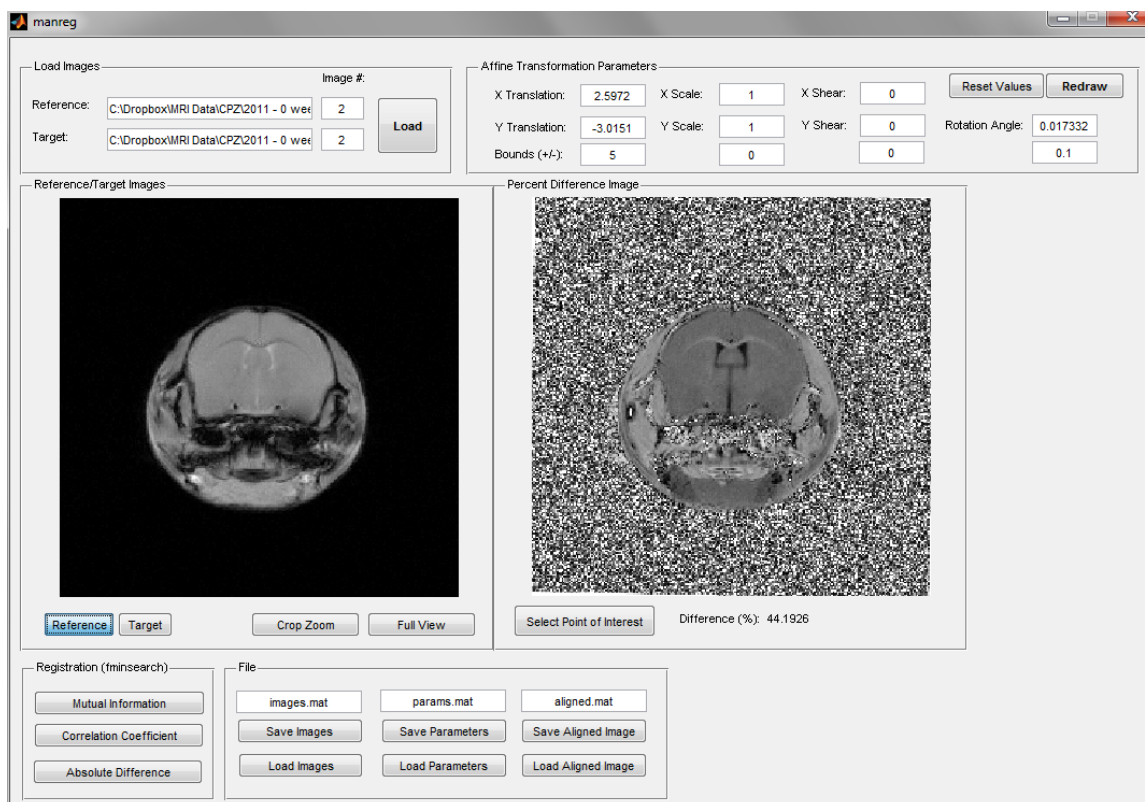



Figure C.1: Image registration tool. Functionality includes: (a) loading individual images from two separate raw Paravision datasets; (b) adjusting affine transformation parameters such as translation, scale, shear, and rotation for registering the target image; (c) viewing and cropping reference and target images; (d) viewing the percent difference between the two images; (e) automatic image registration based on a bounded minimization search of a function representing either the mutual information, correlation coefficient, or absolute difference of the two images; (f) saving and loading all or part of the images and parameters generated during image registration.



Figure C.2: Image registration with cropped view.

```
% Bound Nelder-Mead simplex search for error function rescale_mi().
% Searches for optimal affine transformation parameters (reg_scale) based
% on initial_scale and lower and upper bounds. Based on the button pressed,
% the error function that is minimized can be:
%   rescale_mi(): Mutual Information
%   rescale_corr(): 2-D Correlation Coefficient
%   rescale_abs(): Absolute Difference

reg_scale=fminsearchbnd(@(reg_scale) ...
    rescale_mi(reg_scale,reference,target),initial_scale,LB,UB,options);
handles.scale = reg_scale;

% Calculates aligned target image and percent difference image based on
% reg_scale.
[handles.reference, handles.target, handles.target_aligned, ...
    handles.difference] = calc_images(handles);

% Draws images in GUI
draw_images(handles);
```

rescale_mi.m

rescale_mi.m transforms the target image and calculates the error function for minimization based on the mutual information between the reference and target images.

rescale_corr.m does the same by calculating the 2-D correlation coefficient between the

two images. `rescale_diff.m` calculates the absolute difference between the two images. The mutual information function, `mi()`, is written by Jose Delpiano and is available on the MATLAB File Exchange [51].

```
function err = rescale_mi(scale, I, I_transform)

[M N]=size(I);

% Applies affine transformation based on the scaling parameters (scale)
% and calculates the mutual information between the reference image (I) and
% the aligned image (I_aligned).

tx=scale(1);
ty=scale(2);
sx=scale(3);
sy=scale(4);
shx=scale(5);
shy=scale(6);
u=scale(7);

% Creates affine transformation image from rotation, translation, scale,
% and shear parameters.
Trotation=[cos(u) sin(u) 0; -sin(u) cos(u) 0; 0 0 1;];
Ttranslation=[1 0 0; 0 1 0; tx ty 1;];
Tscale=[sx 0 0; 0 sy 0; 0 0 1;];
Tshear=[1 shy 0; shx 1 0; 0 0 1;];
T=Trotation*Ttranslation*Tscale*Tshear;
Tform=maketform('affine',T);

% Aligns target image using the affine transformation matrix.
I_aligned=imtransform(I_transform,Tform,'Xdata',[1 N],'Ydata',[1 M]);

% Find the mutual information between the reference and the aligned images.
% Mutual information (and 2-D correlation) will INCREASE as image becomes
% better aligned, thus, for minimization purposes, the negative value is
% returned in err.
err=-abs(mi(I_aligned,I));

% In rescale_corr(), the previous line is: err=-abs(corr2(I_aligned,I));
% In rescale_abs(), the previous line is: err=sum(sum(abs(I-I_aligned)));

end
```

calc_images.m

Calculates the aligned target image and percent difference image based on the affine transformation parameters.

```
function [reference target target_aligned difference] = calc_images(handles)
% Calculate images using affine transformation parameters and bicubic
% interpolation.

reference_num = str2double(get(handles.image_num_reference, 'String'));
target_num = str2double(get(handles.image_num_target, 'String'));
```

```

% Note that MATLAB loads raw image data differently than Paravision. Also,
% the original acquisition has the brain oriented upside down in order to
% reduce fat-shift into the brain. In order to display the image with the
% correct left/right and up/down orientation, it needs to be flipped and
% rotated first:
reference = fliplr(rot90(handles.reference_images(:,:,reference_num)));
target = fliplr(rot90(handles.target_images(:,:,target_num)));

[M N] = size(reference);

tx = str2double(get(handles.x_trans, 'String'));
ty = str2double(get(handles.y_trans, 'String'));
sx = str2double(get(handles.x_scale, 'String'));
sy = str2double(get(handles.y_scale, 'String'));
shx = str2double(get(handles.x_shear, 'String'));
shy = str2double(get(handles.y_shear, 'String'));
u = str2double(get(handles.rot_angle, 'String'));

% Construct affine transformation matrix
Trotation=[cos(u) sin(u) 0; -sin(u) cos(u) 0; 0 0 1;];
Ttranslation=[1 0 0; 0 1 0; tx ty 1;];
Tscale=[sx 0 0; 0 sy 0; 0 0 1;];
Tshear=[1 shy 0; shx 1 0; 0 0 1;];
T=Trotation*Ttranslation*Tscale*Tshear;
Tform=maketform('affine',T);

% Calculate transformed target image
target_aligned=imtransform(target,Tform,'bicubic','Xdata',[1 N],'Ydata',[1 M]);

% Calculate percent difference image with transformed image
for x=1:N
    for y=1:M
        if reference(y,x) == 0
            difference(y,x) = 0;
        else
            difference(y,x) = ...
                abs(reference(y,x)-target_aligned(y,x))/reference(y,x);

            if difference(y,x) > 1
                difference(y,x) = 1;
            end;
            if difference(y,x) < 0
                difference(y,x) = 0;
            end;
        end;
    end;
end;

```

C.1.3 Image Filter

After loading and registering images, a filter can be applied to smooth out noise. The anisotropic diffusion algorithm is a locally adaptive filter that improves SNR while still preserving features within the image itself [188, 73]. It has previously been demonstrated to improve quantitative analysis of multicomponent T_2 data [115, 107] and DTI data [183]. The anisotropic diffusion filter used in all of the analysis methods was written by Daniel Lopes and is available on the MATLAB File Exchange [154].

C.2 *In Vivo* Image Analysis

In vivo images would typically include a T_2 -weighted, proton density, and magnetization transfer contrast (MTC) image. These are loaded and registered to a common reference image using `imreg.m` (with the MTC image as the reference).

C.2.1 Defining Regions of Interest

In `define_Nroi.m`, noise regions in the registered images and MTR maps are removed using a binary mask and several regions of interest are defined in the corpus callosum, external capsule, cerebral cortex, ventricle and noise region for comparisons.

```
function roi = define_Nroi(mtr_file, t2_file, mouse, study, Nroi)
% Define in vivo regions of interest.

% Load aligned PD and MTC images
load(mtr_file);
PD = target_aligned;
MTC = reference;

% Create threshold mask
bthresh = 0.2;
bmin = 1000;
MTint = squeeze(mat2gray(MTC));
bw = im2bw(MTint, bthresh);
bw2 = imfill(bwareaopen(bw,bmin), 'holes');
[ylength, xlength] = size(PD);

% Calculate thresholded MTR (sets regions with low signal to 0)
MTR = ((PD - MTC)./PD).*bw2;

% Load aligned T2-weighted image
load(t2_file);
T2 = target_aligned;

% Define variables
T2mean = zeros(Nroi+4,1);
T2std = zeros(Nroi+4,1);
PDmean = zeros(Nroi+4,1);
PDstd = zeros(Nroi+4,1);
MTCmean = zeros(Nroi+4,1);
MTCstd = zeros(Nroi+4,1);
MTRmean = zeros(Nroi+4,1);
MTRstd = zeros(Nroi+4,1);
N = zeros(Nroi+4,1);

% Select ROI in MTC image
figure('Name', 'MTC');
imagesc(MTC); colormap(gray); colorbar; axis equal;

% Define Nroi regions of interest
for n=1:Nroi
    [roi(n).bw, roi(n).xi, roi(n).yi] = roipoly;
    line(roi(n).xi, roi(n).yi, 'Color', 'r', 'LineWidth', 1);
end;
```

```

roi(Nroi+1).bw = logical(roi(2).bw+roi(3).bw); % external capsule (ecl + ecr)
roi(Nroi+2).bw = logical(roi(1).bw+roi(2).bw+roi(3).bw); % cc + ecl + ecr
roi(Nroi+3).bw = logical(roi(4).bw+roi(5).bw); % cerebral cortex (cxl + cxr)

% Define noise region of interest
x = [5 55 55 5 5];
y = [5 5 55 55 5];
roi(Nroi+4).bw = poly2mask(x,y,xlength,ylength);

% Creates T2-weighted image normalized to CSF signal in the ventricle
bw_ventricle = logical(roi(6).bw);
T2norm = T2./mean(T2(bw_ventricle));

% Calculate mean values based on ROIs in each image and the MTR map.
for n=1:Nroi+4
    bw_temp = logical(roi(n).bw);

    N(n) = sum(sum(bw_temp));
    T2mean(n) = mean(T2norm(bw_temp));
    T2std(n) = std(T2norm(bw_temp));
    PDmean(n) = mean(PD(bw_temp));
    PDstd(n) = std(PD(bw_temp));
    MTCmean(n) = mean(MTC(bw_temp));
    MTCstd(n) = std(MTC(bw_temp));
    MTRmean(n) = mean(MTR(bw_temp));
    MTRstd(n) = std(MTR(bw_temp));
end;

savefile = [mouse '_' study '_roi.mat'];
save(savefile, 'T2', 'PD', 'MTC', 'MTR', 'N', 'T2norm', 'T2mean', 'T2std', ...
    'PDmean', 'PDstd', 'MTCmean', 'MTCstd', 'MTRmean', 'MTRstd', 'roi');

```

C.2.2 Correlating *In Vivo* Images

Spearman correlation coefficients were calculated for individual voxels in each ROI as well as for mean values of each ROI. Across all weeks and for each week individually. For all mice and for control and cuprizone-fed mice individually.

```

% corr_invivo.m (EXCERPT)
% Calculates Spearman correlation coefficients in control, cuprizone-fed,
% and all mice. Does so for every voxel in each ROI and for mean values in
% each ROI (_roi). Over all weeks (all/ctl/cpz) and for each week
% individually (allw/ctlw/cpzw).

correlation = 'Spearman';

for j=1:Nroi
    [all{j,3},all{j,4}] = corr(all{j,1}, all{j,2}, 'type', correlation);
    [ctl{j,3},ctl{j,4}] = corr(ctl{j,1}, ctl{j,2}, 'type', correlation);
    [cpz{j,3},cpz{j,4}] = corr(cpz{j,1}, cpz{j,2}, 'type', correlation);

    [all_roi{j,3},all_roi{j,4}] = corr(all_roi{j,1}, all_roi{j,2}, 'type', ...
        correlation);
    [ctl_roi{j,3},ctl_roi{j,4}] = corr(ctl_roi{j,1}, ctl_roi{j,2}, 'type', ...
        correlation);

```

```

[cpz_roi{j,3},cpz_roi{j,4}] = corr(cpz_roi{j,1}, cpz_roi{j,2}, 'type', ...
    correlation);
for k=1:Nweeks
    [allw{k,j,3}, allw{k,j,4}] = corr(allw{k,j,1}, allw{k,j,2}, 'type', ...
        correlation);
    [ctlw{k,j,3}, ctlw{k,j,4}] = corr(ctlw{k,j,1}, ctlw{k,j,2}, 'type', ...
        correlation);
    [cpzw{k,j,3}, cpzw{k,j,4}] = corr(cpzw{k,j,1}, cpzw{k,j,2}, 'type', ...
        correlation);

    [allw_roi{k,j,3}, allw_roi{k,j,4}] = corr(allw_roi{k,j,1}, ...
        allw_roi{k,j,2}, 'type', correlation);
    [ctlw_roi{k,j,3}, ctlw_roi{k,j,4}] = corr(ctlw_roi{k,j,1}, ...
        ctlw_roi{k,j,2}, 'type', correlation);
    [cpzw_roi{k,j,3}, cpzw_roi{k,j,4}] = corr(cpzw_roi{k,j,1}, ...
        cpzw_roi{k,j,2}, 'type', correlation);
end;
end;

```

C.3 Relaxometry Maps

Images were first registered using the image registration tool. For the purposes of multiparametric comparisons, all *ex vivo* images were registered to the b=0 image of the DTI dataset. For the RARE-T₁+T₂ acquisition, short-TE and varying-TR images (used for fitting the T₁ value to the saturation recovery curve) were labeled `mouse_t1_1` to `mouse_t1_N`, where N was the number of different TR values used. Varying-TE and long-TR (used for fitting T₂ value to the T₂ decay curve) were labeled `mouse_t2_1` to `mouse_t2_N`, where N was the number of different echo times used. Likewise, values from the MSME-T2 acquisition (used for multi-component T₂ fitting) were labeled `mouse_t2m_n`.

C.3.1 T1 Relaxation Maps

T₁ relaxation maps are generated by fitting the signal values in each voxel of each TR-weighted image to the saturation recovery curve,

$$S = S_0 \left(1 - e^{-T_R/T_1}\right) \quad (\text{C.1})$$

with two unknown variables: T₁ and S₀.

Calculate T1 Maps

`calc_T1map_filt.m` loads the registered T₁ data, filters it using the 2-D anisotropic diffusion filter, and calculates a T₁ map based on the registered and filtered data using the `T1fit()` function. For the sake of comparison, a non-filtered version of this function (`calc_T1map.m`) does the same thing on the registered T1 dataset without filtering the individual images (not shown here).

```

function [T1map,S0map,T1data,T1data_filt] = calc_T1map_filt(serdir,mouse,TR)

% Determine size and number of TR values based on first registered T1 image.
T1_file = [serdir '\' mouse '_t1_1.mat'];
load(T1_file);

```

```

[ylength, xlength] = size(target_aligned);
nTR = length(TR);

% Initialize variables
Tldata = zeros(ylength,xlength,nTR);
Tldata_filt = zeros(ylength,xlength,nTR);
Tlmap = zeros(ylength,xlength);
S0map = zeros(ylength,xlength);

% Load nTR T1 datasets filtering.
for m=1:nTR
    Tl_file = [sdir '\' mouse '_t1_' num2str(m) '.mat'];
    load(Tl_file);
    Tldata(:, :, m) = target_aligned./1e5;
end;

% Calculate standard deviation of square region outside of the
% brain (estimation of noise). kappa = 3*noise is used in the anisotropic
% diffusion filter.
Tltemp = Tldata(:, :, 1);
xn = [5 20 20 5 5];
yn = [5 5 20 20 5];
bw_noise = poly2mask(xn,yn,ylength,xlength);
noise = std(Tltemp(bw_noise))/sqrt(2-pi/2);
kappa = 3*noise;

% Calculate filtered T1 dataset.
for m=1:nTR
    Tltemp = Tldata(:, :, m);
    Tldata_filt(:, :, m) = anisodiff2D(Tltemp, 5, 1/7, kappa, 1);
end;

% Calculate T1 map by fitting T1 and S0 to each pixel in each image.
for m=1:ylength
    for n=1:xlength
        Tltemp = reshape(Tldata_filt(m,n,:), 1, nTR);
        [Tl_est, ~] = Tlfit(TR, Tltemp);
        Tlmap(m,n) = Tl_est(1);
        S0map(m,n) = Tl_est(2);
    end;
end;

% Save relevant data.
save([mouse '_Tlmap_filt.mat'], 'Tlmap','S0map','Tldata','Tldata_filt','TR');

```

Fitting T1 Curves

The T_1 fitting function, `Tlfit.m`, fits to signal by minimizing the sum of squares for error (SSE) between the known signal and the predicted signal using `fminsearchbnd` [54].

```

function [estimates, model] = Tlfit(TR, ydata)

% Starting point for T1 fitting: T1 ~ 1s, S0 = 25. Raw data is divided by
% 1e5 in calc_Tlmap.m to ensure the S0 value is < 100.
start_point = [1 25];
model = @M;
options = optimset('MaxFunEvals', 5000, 'MaxIter', 5000);

```

```
% Define lower and upper bounds for T1 and S0 respectively.
LB = [0 0];
UB = [100 100];

% Search for optimal estimates using bounded Nelder–Mead simplex search.
estimates = fminsearchbnd(model, start_point, LB, UB, options);

% Error function of saturation curve for minimization.
function [sse, FittedCurve] = M(p)
    T1 = p(1);
    S0 = p(2);

    FittedCurve = S0.*(ones(1,length(TR)) - exp(-TR./T1));
    ErrorVector = FittedCurve - ydata;
    sse = sum(ErrorVector.^ 2);
end
end
```

C.3.2 T₂ Relaxation Maps

T₂ relaxation maps are generated by fitting the signal values in each voxel of each TE-weighted image to the T₂ decay curve:

$$S = S_0 e^{-T_E/T_2} + C \quad (\text{C.2})$$

with three unknown variables: T₂, S₀, and C.

Calculate T₂ Maps

`calc_T2map_filt.m` loads the registered T₂ data, filters it using the 2-D anisotropic diffusion filter, and calculates a T₂ map based on the registered and filtered data using the `T2fit()` function. For the sake of comparison, a non-filtered version of this function (`calc_T2map.m`) does the same thing on the registered T₂ dataset without filtering the individual images (not shown here).

```
function [T2map,S0map,Cmap,T2data,T2data_filt]=calc_T2map_filt(serdir,mouse,TE)

% Determine size and number of TE values based on first registered T2 image.
T2_file = [serdir '\' mouse '_t2m_1.mat'];
load(T2_file);
[ylength, xlength] = size(target_aligned);
nTE = length(TE);

% Initialize variables
T2data = zeros(ylength,xlength,nTE);
T2data_filt = zeros(ylength,xlength,nTE);
T2map = zeros(ylength,xlength);
S0map = zeros(ylength,xlength);
Cmap = zeros(ylength,xlength);

% Load nTE T2 datasets filtering.
for m=1:nTE
    T2_file = [serdir '\' mouse '_t2m_' num2str(m) '.mat'];
    load(T2_file);
```



```

    T2data(:,:,m) = target_aligned./1e5;
end;

% Calculate standard deviation of square region outside of the
% brain (estimation of noise). kappa = 3*noise is used in the anisotropic
% diffusion filter.
T2temp = T2data(:,:,1);
xn = [5 20 20 5 5];
yn = [5 5 20 20 5];
bw_noise = poly2mask(xn,yn,ylength,xlength);
noise = std(T2temp(bw_noise))/sqrt(2-pi/2);
kappa = 3*noise;

% To improve fitting speed, a binary mask is made (bw2) based on a signal
% threshold (bthresh) with holes filled in based on a minimum pixel size
% (bmin).
bthresh = .3;
bmin = 500;
T2_int = squeeze(mat2gray(T2temp));
bw = im2bw(T2_int, bthresh);
bw2 = imfill(bwareaopen(bw,bmin), 'holes');

% Calculate filtered T2 dataset.
for m=1:nTE
    T2temp = T2data(:,:,m);
    T2data_filt(:,:,m) = anisodiff2D(T2temp, 5, 1/7, kappa, 1);
end;

% Calculate T2 map by fitting T2, S0 and C to each pixel in each image
% (only where binary mask, bw2 = 1).
for y=1:ylength
    for x=1:xlength
        if bw2(y,x)==1
            T2temp = reshape(T2data_filt(y,x,:), 1, nTE);
            [T2_est, ~] = T2fit(TE, T2temp);
            T2map(y,x) = T2_est(1);
            S0map(y,x) = T2_est(2);
            Cmap(y,x) = T2_est(3);
        end;
    end;
end;

% Save relevant data.
save([mouse ...
    '_T2m_map_filt.mat'], 'T2map', 'S0map', 'Cmap', 'T2data', 'T2data_filt', 'TE');

```

Fitting T2 Curves

The T_2 fitting function, `T2fit.m`, fits to signal by minimizing the sum of squares for error (SSE) between the known signal and the predicted signal using `fminsearchbnd` [54]. `T2fit_w.m` introduces a weighting vector, w , based on the square of the pixel signal divided by the variance of the noise region in the corresponding T_2 -weighted image:

$$w_i = S_i^2 / \sigma_i^2 \quad (\text{C.3})$$

Results from `T2fit.m` and `T2fit_w.m` were subsequently compared to evaluate the efficacy of weighting the fits.

```
function [estimates, model] = T2fit(TE, ydata)

% Starting point for T2 fitting: T2 ~ 40 ms, S0 = 100, C = 0. Raw data is
% divided by 1e5 in calc_T2map.m to ensure the S0 value is < 1000.
start_point = [40 100 0];
model = @M;
options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000);

% Define lower and upper bounds for T2, S0, and C respectively.
LB = [0 0 0];
UB = [100 1000 0];

% Search for optimal estimates using bounded Nelder-Mead simplex search.
estimates = fminsearchbnd(model, start_point, LB, UB, options);

% Error function of T2 decay curve for minimization.
function [sse, FittedCurve] = M(p)
    T2 = p(1);
    S0 = p(2);
    C = p(3);

    FittedCurve = S0.*exp(-TE./T2) + C;
    ErrorVector = FittedCurve - ydata;
    sse = sum(ErrorVector.^ 2);
end
end

function [estimates, model] = T2fit_w(TE, ydata, noise)

% Starting point for T2 fitting: T2 ~ 40 ms, S0 = 100, C = 0. Raw data is
% divided by 1e5 in calc_T2map.m to ensure the S0 value is < 1000.
start_point = [40 100 0];
model = @M;
options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000);

% Define lower and upper bounds for T2, S0, and C respectively.
LB = [0 0 0];
UB = [100 1000 0];

% Calculate a weight for weighting the error function based on relative
% noise in each image.
w = ydata.^2./noise.^2;

% Search for optimal estimates using bounded Nelder-Mead simplex search.
estimates = fminsearchbnd(model, start_point, LB, UB, options); % fminsearch ...
    with bounds

% Weighted error function of T2 decay curve for minimization.
function [sse, FittedCurve] = M(p)
    T2 = p(1);
    S0 = p(2);
    C = p(3);

    FittedCurve = S0.*exp(-TE./T2) + C;
    ErrorVector = FittedCurve - ydata;
    sse = sum(w.*ErrorVector.^ 2);
end
end
```

end

C.3.3 Multicomponent T₂ Relaxation

Multicomponent T₂ relaxation describes the T₂-decay curve using M T₂ time coefficients (T_{2j}) with corresponding relative amplitudes (s_j). Given N spin echo measurements at echo times, T_{Ei}, the decay curve, y_i can be expressed as:

$$y_i = \sum_{j=1}^M s_j e^{-T_{Ei}/T_{2j}} \quad (\text{C.4})$$

Nonnegative Least Squares Solution

A nonnegative least squares algorithm (NNLS) can be used to solve for the s_j values in (C.4). This creates a fixed number of discrete T₂ amplitudes corresponding to a minimization of the χ^2_{min} -value of the estimated decay curve and experimental signal,

$$\chi^2_{min} = (y_i - y_{experimental}) / \sigma^2. \quad (\text{C.5})$$

This method is sufficient for the analysis of a region of interest, where T₂ components are first fitted to individual voxels and then analyzed as a distribution of voxels [29]. The NNLS solution is demonstrated in `calc_mcT2_maps_filt.m`. For the sake of comparison, a non-filtered version was also made (`calc_mcT2_maps.m`).

NNLS and regularized NNLS solutions were originally based on a series of tutorials posted on Dr. Craig K. Jones' website [114].

```
function [chi2_map, yfit_map, s_map, mwf, iewf, T2data_filt] = ...
    calc_mcT2_maps_filt(serdir, mouse, TE, Nbins)

T2_file = [serdir '\' mouse '_t2m_1.mat'];
load(T2_file);
[ylength, xlength] = size(target_aligned);
nTE = length(TE);

%% Initialize variables
T2data = zeros(ylength,xlength,nTE);
T2data_filt = zeros(ylength,xlength,nTE);

mwf = zeros(ylength,xlength);
iewf = zeros(ylength,xlength);
mw_gmT2 = zeros(ylength,xlength);
iew_gmT2 = zeros(ylength,xlength);

for m=1:nTE
    T2_file = [serdir '\' mouse '_t2m_' num2str(m) '.mat'];
    load(T2_file);
    T2data(:, :, m) = target_aligned./1e5;
end;

T2temp = T2data(:, :, 1);
xn = [5 20 20 5 5];
yn = [5 5 20 20 5];
bw_noise = poly2mask(xn,yn,ylength,xlength);
```

```

noise = std(T2temp(bw_noise))/sqrt(2-pi/2);
kappa = 3*noise;

bthresh = .3;
bmin = 500;
T2_int = squeeze(mat2gray(T2temp));
bw = im2bw(T2_int, bthresh);
bw2 = imfill(bwareaopen(bw,bmin), 'holes');

for m=1:nTE
    T2temp = T2data(:, :,m);
    T2data_filt(:, :,m) = anisodiff2D(T2temp, 5, 1/7, kappa, 1);
end;

y = zeros(1,nTE);

yfit_map = zeros(ylength,xlength,nTE);
chi2_map = zeros(ylength,xlength);
s_map = zeros(ylength,xlength,Nbins);

%% Create the TE and T2 vectors
t2 = logspace(log10(5), log10(500), Nbins); % t2(45) = 19.89 ms t2(83) = ...
101.89 ms
for n=1:Nbins
    if t2(n) < 20
        Nmwf = n;
    end;
    if t2(n) < 100
        Niewf = n;
    end;
end;

%% Create the A matrix
te = TE';
A = exp(-kron(te, 1./t2));
A = [A ones(size(A,1), 1)];

options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000, 'TolX', 1e-12);

for k=1:ylength
    for l=1:xlength
        if bw2(k,l)==1
            for m=1:nTE
                y(m) = T2data_filt(k,l,m);
            end;

            s = lsqnonneg(A, y', options);
            y_fit = A*s;
            s = s./sum(s);
            s = s(1:end-1);
            chi2 = sum((y_fit - y_e).^2./noise.^2);

            chi2_map(k,l) = chi2;
            yfit_map(k,l,:) = y_fit;
            s_map(k,l,:) = s;

            mwf(k,l) = sum(s(1:Nmwf))/sum(s);
            iewf(k,l) = sum(s(Nmwf+1:Niewf))/sum(s);
        end
    end
end

```

```

mw_gmT2(k,l) = exp( sum(s(1:Nmwf)'.*log(t2(1:Nmwf))) / ...
    sum(s(1:Nmwf)) );
iew_gmT2(k,l) = exp( ...
    sum(s(Nmwf+1:Niewf)'.*log(t2(Nmwf+1:Niewf))) / ...
    sum(s(Nmwf+1:Niewf)) );
end;
end;
end;

save([mouse '_mcT2_maps_filt.mat']);

```

Regularized Nonnegative Least Squares Solution

When calculating the decay curve based on the mean signal from a region of interest, it is desirable to create a continuous T_2 distribution as opposed to a few discrete T_2 components. To do this, the NNLS algorithm is regularized to create a smooth distribution. A regularization parameter, $\mu > 0$ is introduced to smooth the T_2 distribution according to a misfit of the non-regularized χ_{min}^2 value. A value for μ is typically found for $1.02\chi_{min}^2 \leq \chi^2 \leq 1.025\chi_{min}^2$ by minimizing the function,

$$\Xi = \chi^2 + \mu \sum_{j=1}^M s_j^2 \quad (C.6)$$

For our purposes, this is accomplished using the `nnls_reg.m` function after first calculating the non-regularized NNLS χ_{min}^2 value.

```

function [y_calculated,chi2_misfit,mu,s_reg] = ...
    nnls_reg(y,A,t2,noise_std,chi2_min,misfit)

% Determines regularized NNLS solution for the T2 spectrum.
% Searches for regularizer, mu, using fminsearch that satisfies a chi2
% misfit from the chi2_min.

% Define H matrix and initial value for mu.
H = eye(size(A,2)-1, size(A,2)-1);
H = [H zeros(size(H,1), 1)];
start_point = 0;

% Search for mu that that minimizes the difference between misfit*chi2_min
% and chi2.
model = @M;
options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000);
estimates = fminsearch(model, start_point, options); % fminsearch with bounds

mu = estimates(1);
AH = [A; mu*H];

% Regularized T2 amplitudes (s_reg), calculated signal (y_calculated), and
% chi2_misfit based on mu.
s_reg = lsqnonneg(AH, [y; zeros(length(t2), 1)]);
y_calculated = A*s_reg;
chi2_misfit = sum((y_calculated - y).^2./noise_std.^2);

% Normalizes T2 amplitudes
s_reg = s_reg./sum(s_reg);

```

```
% Error function for minimizing chi2 and misfit*chi2_min
function [sse, chi2] = M(p)
    mu = p(1);

    AH = [A; mu*H];
    s = lsqnonneg(AH, [y; zeros(length(t2), 1)]);
    y_calculated = A*s;
    chi2 = sum((y_calculated - y).^2 ./ noise_std.^2);

    ErrorVector = chi2 - misfit*chi2_min;
    sse = sum(ErrorVector.^2);
end
end
```

C.4 Magnetization Transfer Imaging

C.4.1 Calculating Quantitative Magnetization Transfer Images

`calc_qmti_filt.m` calculates qMTI parameters on a series of MT contrast images and a T1 map. Filtered and non-filtered versions were made for the sake of comparison (non-filtered version not shown).

```
function [qmti, MTCmap, MTCmap_filt] = calc_qmti_filt(mti_dir, mouse, nMTI)
% Calculate filtered and non-filtered parameter maps for qMTI

% Load proton density image and initialize variables
load([mti_dir '\' mouse '_qmti_pd.mat']);
MT_0 = target_aligned;

[ylength, xlength] = size(MT_0);
MTCmap = zeros(ylength, xlength, nMTI);
MTCmap_filt = zeros(ylength, xlength, nMTI);

% Filtered qMTI parameters
qmti.gM0a = zeros(ylength, xlength);
qmti.RM0a = zeros(ylength, xlength);
qmti.Rb = zeros(ylength, xlength);
qmti.fRaf = zeros(ylength, xlength); % f/Ra(1-f)
qmti.RaT2a = zeros(ylength, xlength); % 1/RaT2a
qmti.T2a = zeros(ylength, xlength);
qmti.T2b = zeros(ylength, xlength);
qmti.RA = zeros(ylength, xlength);
qmti.f = zeros(ylength, xlength);
qmti.chi2 = zeros(ylength, xlength);
qmti.Sf = zeros(ylength, xlength, nMTI);

% Calculate filtered T1 maps
TR = [4895.5 2895.5 1395.5 695.5 295.5 95.5]./1000;
[T1map, ~, ~, ~] = calc_T1map_filt(mti_dir, mouse, TR);
R1map = 1./T1map;

% Calculate standard deviation of the noise region and filtered MT0 image
xn = [5 20 20 5 5];
yn = [5 5 20 20 5];
bw_noise = poly2mask(xn, yn, ylength, xlength);
```

```

noise = std(MT_0(bw_noise))/sqrt(2-pi/2);
kappa = 3*noise;
MT0filt = anisodiff2D(target_aligned, 5, 1/7, kappa, 1);

% Load MT contrast images and normalize to the MT0 image
for n=1:nMTI
    load([mti_dir '\' mouse '_qmti_' num2str(n) '.mat']);
    MTCmap(:, :, n) = target_aligned./MT_0;
    MTIfilt = anisodiff2D(target_aligned, 5, 1/7, kappa, 1);
    MTCmap_filt(:, :, n) = MTIfilt./MT0filt;
end;

% MT saturation pulse offsets and continuous wave equivalent saturation
% flip angles for the Gaussian RF saturation pulses (omegae), calculated in
% pulse_integral.m.
offset = [1000 2000 4000 6000 10000 30000];
omega = [279.7452336621382 559.4904673242763 1118.980934648553];
n_omega = length(omega);
n_offset = length(offset);
Rrfb_mn = zeros(n_offset, n_omega);
omega_mn = zeros(n_offset, n_omega);
offset_mn = zeros(n_offset, n_omega);

% Create binary mask for removing noise regions in image.
bthresh = .3;
bmin = 500;
MT0_int = squeeze(mat2gray(MT_0));
bw = im2bw(MT0_int, bthresh);
bw2 = imfill(bwareaopen(bw, bmin), 'holes');

% Define starting point for qMTIfit() function.
start_point = [1 25 1 0.3 50 0.00001]; % superL white matter
LB = [0 0 1 0 5 0];
UB = [1e8 100 1 1 500 0.00005];
X = 0:pi/1000:pi/2;
Rrfb = zeros(1, 18);

fprintf('Computing qMTI fit in row: \n');
for y=1:ylength
    fprintf('    %d\n', y);
    for x=1:xlength
        if bw2(y, x) == 1
            % Calculate qMTI parameters based for MTCpoint using qMTIfit()
            MTCpoint = reshape(MTCmap_filt(y, x, :), 1, 18);
            [est, ~] = qMTIfit(offset, MTCpoint, omega, start_point, LB, UB, ...
                               'sl');
            qmti.gM0a(y, x) = est(1);
            qmti.RM0a(y, x) = est(2);
            qmti.Rb(y, x) = est(3);
            qmti.fRaf(y, x) = est(4); % f/Ra(1-f)
            qmti.RaT2a(y, x) = est(5); % 1/RaT2a
            qmti.T2b(y, x) = est(6);
            qmti.RA(y, x) = Rlmap(y, x)/(1 + ...
                (qmti.RM0a(y, x)*qmti.fRaf(y, x)*(qmti.Rb(y, x) - ...
                Rlmap(y, x)))/(qmti.Rb(y, x) - Rlmap(y, x) + qmti.RM0a(y, x)));
            qmti.f(y, x) = qmti.fRaf(y, x)*qmti.RA(y, x)/(qmti.fRaf(y, x) + 1);
            qmti.T2a(y, x) = 1/(qmti.RA(y, x)*qmti.RaT2a(y, x));

            % Calculate Rrfb using super-Lorentzian lineshape.

```

```
for m=1:n_omega
    for n=1:n_offset
        Rrfb_mn(n,m) = omega(m).^2.*pi .* trapz( ...
            X, sin(X)*sqrt(2/pi)*qmti.T2b(y,x) .* ...
            exp(-2*(2*pi.*offset(n).*qmti.T2b(y,x) ./ ...
            abs(3*cos(X).^2-1)).^2 ) ./ abs(3*cos(X).^2-1) ); % ...
            super-Lorentzian
        omega_mn(n,m) = omega(m);
        offset_mn(n,m) = offset(n);
    end;
end;

Rrfb = reshape(Rrfb_mn, 1, n_omega*n_offset);
omega_fit = reshape(omega_mn, 1, n_omega*n_offset);
offset_fit = reshape(offset_mn, 1, n_omega*n_offset);

% Estimated signal and chi2 values.
qmti.Sf(y,x,:) = qmti.gM0a(y,x) .* ((qmti.Rb(y,x) .* ...
    qmti.RM0a(y,x) .* qmti.fRaf(y,x) + Rrfb + qmti.Rb(y,x) + ...
    qmti.RM0a(y,x)) ./ (qmti.RM0a(y,x) .* qmti.fRaf(y,x) .* ...
    (qmti.Rb(y,x) + Rrfb) + (1 + (omega_fit ./ ...
    (2.*pi.*offset_fit)).^2 .* qmti.RaT2a(y,x)) .* (qmti.Rb(y,x) ...
    + Rrfb + qmti.RM0a(y,x))));
qmti.chi2(y,x) = sum((squeeze(MTCmap_filt(y,x,:)) - ...
    squeeze(qmti.Sf(y,x,:))).^2 ./ noise^2);
end;
end;
end;

save([mouse '_qmti_all.mat'], 'MTCmap', 'MTCmap_filt', 'qmti', 'Tlmap');
```

C.4.2 Fitting Two-Pool Model with Gaussian and Super-Lorentzian Lineshapes

Previous studies demonstrated heterogenous white matter fits better to a Super-Lorentzian shaped bound pool, while more homogenous phantoms, such as agar, fit better to a Gaussian-shaped bound pool. `qMTIfit.m` lets the user select the initial values, range of expected values, and suitable lineshape.

```
function [estimates, model] = qMTIfit(offset, ydata, omega, start_point, LB, ...
    UB, lineshape)
% Calculates parameters for the two-pool model of quantitative MTI.

model = @M;
options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000);

% Define variables
n_omega = length(omega);
n_offset = length(offset);
Rrfb_mn = zeros(n_offset,n_omega);
omega_mn = zeros(n_offset,n_omega);
offset_mn = zeros(n_offset,n_omega);
X = 0:pi/1000:pi/2;

% Search for optimal estimates using bounded Nelder-Mead simplex search.
estimates = fminsearchbnd(model, start_point, LB, UB, options);
```



```

% Error function of two-pool qMTI signal for minimization.
function [sse, FittedCurve] = M(params)
    gM0a = params(1);
    RM0a = params(2);
    Rb = params(3);
    fRaf = params(4); % f/Ra(1-f)
    RaT2a = params(5); % 1/RaT2a
    T2b = params(6);

    % Calculate Rrfb using super-Lorentzian lineshape.
    if strcmp(lineshape, 'sl')
        for m=1:n_omega
            for n=1:n_offset
                Rrfb_mn(n,m) = omega(m).^2.*pi .* trapz( ...
                    X, sin(X)*sqrt(2/pi)*T2b.*exp( ...
                        -2*(2*pi.*offset(n).*T2b./abs(3*cos(X).^2-1)).^2 ) ...
                    ./ abs(3*cos(X).^2-1));
                omega_mn(n,m) = omega(m);
                offset_mn(n,m) = offset(n);
            end;
        end;
    % Calculate Rrfb using Gaussian lineshape.
    elseif strcmp(lineshape, 'g')
        for m=1:n_omega
            for n=1:n_offset
                Rrfb_mn(n,m) = omega(m)^2*sqrt(pi/2)*T2b * ...
                    exp(-(2*pi*offset(n)*T2b)^2/2);
                omega_mn(n,m) = omega(m);
                offset_mn(n,m) = offset(n);
            end;
        end;
    end;

    Rrfb_fit = reshape(Rrfb_mn, 1, n_omega*n_offset);
    omega_fit = reshape(omega_mn, 1, n_omega*n_offset);
    offset_fit = reshape(offset_mn, 1, n_omega*n_offset);

    FittedCurve = gM0a.*((Rb.*RM0a.*fRaf + Rrfb_fit + Rb + ...
        RM0a)./(RM0a.*fRaf.*(Rb + Rrfb_fit) + (1 + ...
        (omega_fit./(2.*pi.*offset_fit)).^2.*RaT2a).*(Rb + Rrfb_fit + ...
        RM0a)));
    ErrorVector = FittedCurve - ydata;
    sse = sum(ErrorVector.^2);
end
end

```

C.4.3 Calculation of the Pulse Integral

The Gaussian pulse integral is described and implemented by Bruker as a series of discrete pulses. The pulse shape described by Paravision in a 256-element array (pY) was used to calculate the continuous wave equivalent power pulse for the 10.25 ms Gaussian pulse used in all of the MT contrast images (Figure 3.18).

```

X = -5.125:10.25/255:5.125; % 256 time steps for 10.25ms pulse.

```

```
% pY is a 256-element array describing the discrete amplitudes of the
% Gaussian RF pulse used by Paravision (for brevity, it isn't shown here).
% pY is normalized to 100. Y is normalized to 1.
Y = pY./100;

% Calculate ratio of integral and integral squared.
integral_ratio = sum(Y.*10.25/256)/10.25;
integral_squared_ratio = sum(Y.*Y.*10.25./256)/10.25;

% Calculate continuous wave equivalent flip angles for the Gaussian pulse.
flip_angle_5 = (267.513*5*.01025*integral_ratio);
omega_5 = flip_angle_5/0.01025;
omega_cwpe_5 = omega_5*sqrt(integral_squared_ratio*10.25/70)/integral_ratio;

flip_angle_10 = (267.513*10*.01025*integral_ratio);
omega_10 = flip_angle_10/0.01025;
omega_cwpe_10 = omega_10*sqrt(integral_squared_ratio*10.25/70)/integral_ratio;

flip_angle_20 = (267.513*20*.01025*integral_ratio);
omega_20 = flip_angle_20/0.01025;
omega_cwpe_20 = omega_20*sqrt(integral_squared_ratio*10.25/70)/integral_ratio;
```

C.4.4 Noise Propagation

Simulated Noise

Simulated qMTI and T1 curves for both agar and mouse brain tissue with different SNR levels were fitted using the two-pool qMTI model. Quadratic noise was added to the simulated signal to emulate the Rician noise seen in MR magnitude images.

```
function [T1, qmti] = qmti_noise(T1val, TR, T1_SNR, offsets, omegae, N, ...
    MTC_SNR, lineshape, params)

% Initialize variables
N_omega = length(omegae);
N_offset = length(offsets);
T1.N = length(TR);
qmti.N = N_omega*N_offset;
start_point = params;
LB = [0 0 1 0 5 0];
UB = [5 10000 1 100 10000 0.001];

MTCtemp = zeros(1,qmti.N);
T1temp = zeros(1,T1.N);
T1.vals = zeros(1,N);
T1.S = zeros(1,N);
qmti.gM0a = zeros(1,N);
qmti.RM0a = zeros(1,N);
qmti.Rb = zeros(1,N);
qmti.fRaf = zeros(1,N);
qmti.RaT2a = zeros(1,N);
qmti.T2b = zeros(1,N);
qmti.Robs = zeros(1,N);
qmti.RA = zeros(1,N);
qmti.f = zeros(1,N);

% Calculate simulated T1 curves.
```

```

% T1.data is calculated using TR (experimental values)
% T1.curve is based on T1.TRfit (smooth curve)
T1.TRfit = 0:0.01:ceil(max(TR));
T1.data = ones(1,length(TR)) - exp(-TR./T1val);
T1.curve = ones(1,length(T1.TRfit)) - exp(-T1.TRfit./T1val);
T1.std = T1.data./T1_SNR;

% Calculate simulated MT curves.
% qmti.data is calculated using offsets (experimental values)
% qmti.curve is based on qmti.offsets_model (smooth curve)
qmti.offsets_model = 10:10:max(offsets);
qmti.data = MTcurve(offsets, omegae, params, lineshape);
qmti.curve = MTcurve(qmti.offsets_model, omegae, params, lineshape);
qmti.std = qmti.data./MTC_SNR;

% Calculate error using Monte Carlo simulation and N normal distributions
for n=1:N
    % Add quadratic noise to simulated qMTI and T1 curves.
    for m=1:qmti.N
        MTCtemp(m) = sqrt((qmti.data(m) + qmti.std(m)*randn(1))^2 + ...
            (qmti.std(m)*randn(1))^2);
    end;
    for l = 1:T1.N
        T1temp(l) = sqrt((T1.data(l) + T1.std(l)*randn(1))^2 + ...
            (T1.std(l)*randn(1))^2);
    end;

    % T1 fit to noisy simulated data.
    [T1test, ~] = T1fit(TR, T1temp);
    T1.vals(n) = T1test(1);
    T1.S(n) = T1test(2);

    % qMTI fit to noisy simulated data.
    [MTest, ~] = qMTIfit(offsets, MTCtemp, omegae, start_point, LB, UB, ...
        lineshape);
    qmti.gM0a(n) = MTest(1);
    qmti.RM0a(n) = MTest(2);
    qmti.Rb(n) = MTest(3);
    qmti.fRaf(n) = MTest(4);
    qmti.RaT2a(n) = MTest(5);
    qmti.T2b(n) = MTest(6);
    qmti.Robs(n) = 1/T1.vals(n);
    qmti.RA(n) = qmti.Robs(n)/(1 + (qmti.RM0a(n)*qmti.fRaf(n)*(qmti.Rb(n) - ...
        qmti.Robs(n)))/(qmti.Rb(n) - qmti.Robs(n) + qmti.RM0a(n)));
    qmti.f(n) = qmti.fRaf(n)*qmti.RA(n)/(qmti.fRaf(n) + 1);
end;

% Calculate mean T1 parameter values.
T1.vals_mean = mean(T1.vals);
T1.S_mean = mean(T1.S);
T1.vals_gm = geomean(T1.vals);
T1.S_gm = geomean(T1.S);

% Calculate mean qMTI parameter values.
[qmti.gM0a_fit, qmti.gM0a_std] = normfit(qmti.gM0a);
[qmti.RM0a_fit, qmti.RM0a_std] = normfit(qmti.RM0a);
[qmti.fRaf_fit, qmti.fRaf_std] = normfit(qmti.fRaf);
[qmti.RaT2a_fit, qmti.RaT2a_std] = normfit(qmti.RaT2a);
[qmti.T2b_fit, qmti.T2b_std] = normfit(qmti.T2b);

```

```
[qmti.RA_fit, qmti.RA_std] = normfit(qmti.RA);
[qmti.Robs_fit, qmti.Robs_std] = normfit(qmti.Robs);
[qmti.f_fit, qmti.f_std] = normfit(qmti.f);

function FittedCurve = MTcurve(offset, omega, params, lineshape)
% Calculates simulated magnetization transfer curve for the two-pool model
% for the given offset frequencies, saturation powers, qMTI parameters and
% lineshape.

% Define variables.
gM0a = params(1);
RM0a = params(2);
Rb = params(3);
fRaf = params(4); % f/Ra(1-f)
RaT2a = params(5); % 1/RaT2a
T2b = params(6);

n_omega = length(omega);
n_offset = length(offset);
Rrfb_mn = zeros(n_offset, n_omega);
omega_mn = zeros(n_offset, n_omega);
offset_mn = zeros(n_offset, n_omega);
X = 0:pi/1000:pi/2;

% Calculate Rrfb using super-Lorentzian lineshape.
if strcmp(lineshape, 'sl')
    for m=1:n_omega
        for n=1:n_offset
            Rrfb_mn(n,m) = omega(m).^2.*pi .* trapz(X, sin(X) * ...
                sqrt(2/pi)*T2b .* exp(-2*(2*pi .* offset(n) .* T2b ./ ...
                    abs(3*cos(X).^2-1)).^2) ./ abs(3*cos(X).^2-1)); % ...
            % super-Lorentzian
            omega_mn(n,m) = omega(m);
            offset_mn(n,m) = offset(n);
        end;
    end;

% Calculate Rrfb using Gaussian lineshape.
elseif strcmp(lineshape, 'g')
    for m=1:n_omega
        for n=1:n_offset
            Rrfb_mn(n,m) = ...
                omega(m)^2*sqrt(pi/2)*T2b*exp(-(2*pi*offset(n)*T2b)^2/2); ...
            % Gaussian
            omega_mn(n,m) = omega(m);
            offset_mn(n,m) = offset(n);
        end;
    end;
end;

Rrfb_fit = reshape(Rrfb_mn, n_omega*n_offset, 1);
omega_fit = reshape(omega_mn, n_omega*n_offset, 1);
offset_fit = reshape(offset_mn, n_omega*n_offset, 1);

% Simulated MT curve.
FittedCurve = gM0a.*((Rb.*RM0a.*fRaf + Rrfb_fit + Rb + ...
    RM0a)./(RM0a.*fRaf.*(Rb + Rrfb_fit) + (1 + ...
```

```
(omega_fit./(2.*pi.*offset_fit)).^2.*RaT2a).*(Rb + Rrfb_fit + RM0a)));
```

Calculating Experimental Agar Values

An excerpt of `calc_qmti_agar.m` is given below for measuring the values experimentally. The sole differences from `calc_qmti_filt.m` is the creation of circular ROI masks over the 2%, 4% and 8% agar samples, thresholding to remove bubbles when fitting, and use of a Gaussian lineshape as opposed to the super-Lorentzian lineshape used for fitting tissue.

```
% Excerpt from calc_qmti_agar.m
% Creation of circular ROIs and removing air bubbles based on a threshold
% value.
figure('Name', 'ROI');
h_im = imagesc(MTCmap(:, :, 6), [0 2]); colormap(gray); axis off; axis equal;
e2 = imellipse(gca, [58 145 75 75]);
qmti.BW2 = createMask(e2, h_im);
e4 = imellipse(gca, [142 82 75 75]);
qmti.BW4 = createMask(e4, h_im);
e8 = imellipse(gca, [47 38 75 75]);
qmti.BW8 = createMask(e8, h_im);

%% Remove air bubbles
load([mti_dir, '\agar32_qmti_1.mat'], 'reference');
MT_mean2 = mean(mean(reference(qmti.BW2)));
MT_mean4 = mean(mean(reference(qmti.BW4)));
MT_mean8 = mean(mean(reference(qmti.BW8)));

for m=1:256
    for n=1:256
        if qmti.BW2(m,n) == 1
            if reference(m,n) < 0.93*MT_mean2
                qmti.BW2(m,n) = 0;
            end;
        elseif qmti.BW4(m,n) == 1
            if reference(m,n) < 0.93*MT_mean4
                qmti.BW4(m,n) = 0;
            end;
        elseif qmti.BW8(m,n) == 1
            if reference(m,n) < 0.93*MT_mean8
                qmti.BW8(m,n) = 0;
            end;
        end;
    end;
end;
```

Comparison of Experimental and Simulated Agar Values

Comparisons between experimental and simulated agar signal was conducted. Given the relatively homogeneous nature of agar gels, it is expected that variations in the distribution of fitted parameters will be primarily due to the contribution of noise in the fitted images.

```
% Comparing calculations from agar experiment to noise simulations.
close all; clear all;
```

```
% Define variables
TR = [4895.5 2895.5 1395.5 695.5 295.5 95.5]./1000;
offsets = [1000 2000 4000 6000 10000 30000];
omegae = [279.7452336621382 559.4904673242763 1118.980934648553];
lineshape = 'g'; % g = Gaussian, sl = superLorentzian
nMTI = 18;
nTR = length(TR);
MTC_SNR = zeros(nMTI,3);
T1_SNR = zeros(3,nTR);
T1val = zeros(3,1);
params = zeros(3,6);

% Load experimental data for agar experiment with 32 averages.
load('C:\Dropbox\Matlab\qMTI\agar32_qmti_all.mat');

% Calculate estimated values for T1 and qMTI parameters based on high SNR
% agar experiment (32 averages).
T1val(1) = mean(T1map_nf(qmti.BW2));
T1val(2) = mean(T1map_nf(qmti.BW4));
T1val(3) = mean(T1map_nf(qmti.BW8));
N(1) = sum(sum(qmti.BW2));
N(2) = sum(sum(qmti.BW4));
N(3) = sum(sum(qmti.BW8));

params(1,1) = mean(qmti.gM0a(qmti.BW2));
params(1,2) = mean(qmti.RM0a(qmti.BW2));
params(1,3) = mean(qmti.Rb(qmti.BW2));
params(1,4) = mean(qmti.fRaf(qmti.BW2));
params(1,5) = mean(qmti.RaT2a(qmti.BW2));
params(1,6) = mean(qmti.T2b(qmti.BW2));

params(2,1) = mean(qmti.gM0a(qmti.BW4));
params(2,2) = mean(qmti.RM0a(qmti.BW4));
params(2,3) = mean(qmti.Rb(qmti.BW4));
params(2,4) = mean(qmti.fRaf(qmti.BW4));
params(2,5) = mean(qmti.RaT2a(qmti.BW4));
params(2,6) = mean(qmti.T2b(qmti.BW4));

params(3,1) = mean(qmti.gM0a(qmti.BW8));
params(3,2) = mean(qmti.RM0a(qmti.BW8));
params(3,3) = mean(qmti.Rb(qmti.BW8));
params(3,4) = mean(qmti.fRaf(qmti.BW8));
params(3,5) = mean(qmti.RaT2a(qmti.BW8));
params(3,6) = mean(qmti.T2b(qmti.BW8));

% Load experimental data for agar experiment with 2 averages.
load('C:\Dropbox\Matlab\qMTI\agar2_qmti_all.mat');
for m=1:6
    T1temp = T1data(:, :, m);
    T1_SNR(1,m) = 0.655*mean(T1temp(qmti.BW2))/qmti.noise_t1(m);
    T1_SNR(2,m) = 0.655*mean(T1temp(qmti.BW4))/qmti.noise_t1(m);
    T1_SNR(3,m) = 0.655*mean(T1temp(qmti.BW8))/qmti.noise_t1(m);
end;

for n=1:nMTI
    MTCtemp = MTCmap(:, :, n).*MT_0;
    MTC_SNR(n,1) = 0.655*mean(MTCtemp(qmti.BW2))/qmti.noise_mtc(n);
    MTC_SNR(n,2) = 0.655*mean(MTCtemp(qmti.BW4))/qmti.noise_mtc(n);
    MTC_SNR(n,3) = 0.655*mean(MTCtemp(qmti.BW8))/qmti.noise_mtc(n);
```

```

end;

% Calculate simulated values for T1 and qMTI based on measured parameters
% in agar and number of fitted voxels (N).
for m=1:3
    [t1M(m), qmtiM(m)] = qmti_noise(T1val(m), TR, squeeze(T1_SNR(m,:)), ...
        offsets, omegae, N(m), squeeze(MTC_SNR(:,m)), lineshape, ...
        squeeze(params(m,:)));
end;

% Create histograms for comparing experimental and noisy simulated values.
N_bins = 100;
gM0a_bins = 0.8:0.4/N_bins:1.2;
RM0a_bins = 0:100/N_bins:100;
fRaf_bins = 0:0.05/N_bins:0.05;
RaT2a_bins = 5:55/N_bins:60;
T2b_bins = 0:40e-6/N_bins:40e-6;
Robb_bins = 0.2:0.4/N_bins:0.6;
RA_bins = 0.2:0.4/N_bins:0.6;
f_bins = 0:0.03/N_bins:0.03;
T1_bins = 1:3/N_bins:4;

% Simulated histograms.
gM0a_hist2 = hist(qmtiM(1).gM0a, gM0a_bins);
RM0a_hist2 = hist(qmtiM(1).RM0a, RM0a_bins);
fRaf_hist2 = hist(qmtiM(1).fRaf, fRaf_bins);
RaT2a_hist2 = hist(qmtiM(1).RaT2a, RaT2a_bins);
T2b_hist2 = hist(qmtiM(1).T2b, T2b_bins);
RA_hist2 = hist(qmtiM(1).RA, RA_bins);
f_hist2 = hist(qmtiM(1).f, f_bins);
T1_hist2 = hist(t1M(1).vals, T1_bins);

% Experimental histograms.
gM0a_hist2b = hist(qmti.gM0a(qmti.BW2), gM0a_bins);
RM0a_hist2b = hist(qmti.RM0a(qmti.BW2), RM0a_bins);
fRaf_hist2b = hist(qmti.fRaf(qmti.BW2), fRaf_bins);
RaT2a_hist2b = hist(qmti.RaT2a(qmti.BW2), RaT2a_bins);
T2b_hist2b = hist(qmti.T2b(qmti.BW2), T2b_bins);
RA_hist2b = hist(qmti.RA(qmti.BW2), RA_bins);
f_hist2b = hist(qmti.f(qmti.BW2), f_bins);
T1_hist2b = hist(T1map_nf(qmti.BW2), T1_bins);

```

C.5 Diffusion Tensor Imaging

The DTI analysis code automates the extraction of information about the DTI method used by Paravision (`load_dti_info.m`), loads and reshapes the diffusion-weighted images (`load_dwi.m`), and generates DTI metrics after applying appropriate filters and automatic image registration (`calc_dti.m`). These methods are wrapped up in the function, `analyze_dti.m` shown below.

```

function [info, dwi, dti] = analyze_dti(serdir, mouse)
% Analyzes DTI data for mouse found in directory serdir.

if exist(serdir, 'dir') == 7
    info = load_dti_info(serdir);

```

```
dwi = load_dwi(serdir, info);
dti = calc_dti(dwi, info);
else
    fprintf('Directory does not exist.')
end;

save([mouse '_dti.mat'])
```

C.5.1 Load DTI Information

`load_dti_info.m` loads information generated by Paravision relevant to generating DTI parameters.

```
function info = load_dti_info(serdir)
% Loads DTI info from the method and visu_pars files.

% Load method file
methodfile = [serdir '\method'];
visufile = [serdir '\pdata\2\visu_pars'];

if exist(methodfile, 'file') == 2
    % Loads the method file information into the matrix recoinfo
    methodinfo = textread(methodfile, '%s', 'delimiter', '\n', 'whitespace', '');

    % Extract general image parameters
    info.fov = extract_matrix1(methodinfo, '##$PVM_Fov=');
    info.dim = extract_matrix1(methodinfo, '##$PVM_Matrix=');
    info.method = extract_string(methodinfo, '##$Method=');
    info.dimension = extract_string(methodinfo, '##$PVM_SpatDimEnum=');
    info.slices = extract_matrix1(methodinfo, '##$PVM_SPackArrNSlices=');
    info.ndims = length(info.dim);

    % Diffusion-weighting gradient duration, separation, direction and
    % amplitude.
    info.graddur = extract_matrix1(methodinfo, '##$PVM_DwGradDur=');
    info.gradsep = extract_matrix1(methodinfo, '##$PVM_DwGradSep=');
    info.graddir = extract_matrix2(methodinfo, '##$PVM_DwDir=');
    info.gradamp = extract_matrix1(methodinfo, '##$PVM_DwGradAmp=');
    % Number of diffusion-weighted directions (Ndir), diffusion-weighted
    % images per experiment (Ndwi), number of experiments (Nexp), and b=0
    % images (NA0).
    info.Ndir = extract_double(methodinfo, '##$PVM_DwNDiffDir=');
    info.Ndwi = extract_double(methodinfo, '##$PVM_DwNDiffExpEach=');
    info.Nexp = extract_double(methodinfo, '##$PVM_DwNDiffExp=');
    info.NA0 = extract_double(methodinfo, '##$PVM_DwAoImages=');
    % B-matrix derived from diffusion-weighting and gradient directions for
    % each diffusion-weighted image. Beff is the effective B-matrix after
    % considering the effect of imaging gradients.
    info.B = extract_matrix1(methodinfo, '##$PVM_DwBvalEach=');
    info.Bmat = extract_matrix3(methodinfo, '##$PVM_DwBMat=');
    info.Beff = extract_matrix1(methodinfo, '##$PVM_DwEffBval=');
    % Description of diffusion-weighting gradients along the read, phase,
    % and slice directions.
    info.gradvec = extract_matrix2(methodinfo, '##$PVM_DwGradVec=');
    info.gradread = extract_matrix1(methodinfo, '##$PVM_DwGradRead=');
    info.gradphase = extract_matrix1(methodinfo, '##$PVM_DwGradPhase=');
    info.gradslice = extract_matrix1(methodinfo, '##$PVM_DwGradSlice=');
```



```

else
    fprintf('Method file does not exist.\n');
end;

if exist(visufile, 'file') == 2
    % Loads the visu_pars file information into the matrix recoinfo
    visu_pars = textread(visufile, '%s', 'delimiter', '\n', 'whitespace', '');

    % Extract image slope and number of images.
    info.slope = extract_matrix1(visu_pars, '##$VisuCoreDataSlope=');
    info.Ndti = extract_double(visu_pars, '##$VisuCoreFrameCount=');
else
    fprintf('visu_pars file does not exist.\n');
end;

```

C.5.2 Load Diffusion-Weighted Images

`load_dwi.m` loads diffusion-weighted images according to the number of experiments and slices.

```

function dwi = load_dwi(serdir, info)
% Loads diffusion-weighted images based on info and reshapes by experiment
% and slice.

dwi_raw = load_2dseq(serdir);

dwi = zeros(info.dim(1), info.dim(2), info.Nexp, info.slices);

for n=1:info.Nexp
    for m=1:info.slices
        % Reshapes dwi matrix.
        % fliplr accounts for differences in how Paravision and MATLAB load
        % images. rot90 accounts for prone orientation used to acquired
        % images.
        dwi(:, :, n, m) = fliplr(rot90(dwi_raw(:, :, m + (n-1)*info.slices)));
    end;
end;

```

C.5.3 Calculating Diffusion Tensor Images

`calc_dti.m` calculates diffusion tensor maps using the previously loaded information and diffusion-weighted images. Non-filtered and multislices methods were also created (not shown here). Functions called inside of `calc_dti.m` are detailed in the following sections.

```

function dti = calc_dti(dwi, info, dt_fit)
% Calculate DTI parameters based on diffusion-weighted images and method.
% dwi and info should be generated previously using load_dti_info.m and
% load_dwi.m respectively.
%
% dt_fit is a string defining the type of fit to be used for determining
% the diffusion tensor: least squares 'ls', weighted least squares 'wls',
% non-linear least squares 'nls', along with constrained versions of each:
% 'cls', 'cwls', and 'cnls'.

```

```
% Initialize Variables
dti.lambda = zeros(info.dim(1), info.dim(2), 3);
dti.ev = zeros(info.dim(1), info.dim(2), 3, 3);
dti.Davg = zeros(info.dim(1), info.dim(2));
dti.Drad = zeros(info.dim(1), info.dim(2));
dti.Daxi = zeros(info.dim(1), info.dim(2));
dti.FA = zeros(info.dim(1), info.dim(2));
dti.RGB = zeros(info.dim(1), info.dim(2), 3);
dti.posdef = zeros(info.dim(1), info.dim(2));
dti.D = zeros(info.dim(1), info.dim(2), 3, 3);
dti.chi2 = zeros(info.dim(1), info.dim(2));

% Calculate B
B = calc_B(info.Bmat, info.Nexp);

% Filter diffusion-weighted images using anisotropic diffusion filter, then
% register.
[dti.dwi_filt, dti.dwi_noise, dti.dwi_filt_noise] = calc_dwi_filt(dwi, info, ...
    15, 5);
dwi_base = dti.dwi_filt(:, :, 1, :);
[dti.dwi_reg, dti.B_reg, dti.B_mat, dti.reg] = calc_dwi_reg(dwi_base, ...
    dti.dwi_filt, info, B);

% Calculate thresholded binary mask.
bthresh = 0.1;
bmin = 100;
A0_int = squeeze(mat2gray(dwi(:, :, 1)));
bw = im2bw(A0_int, bthresh);
bw2 = imfill(bwareaopen(bw, bmin), 'holes');

%% Calculate DTI parameters
for y=1:info.dim(1)
    for x=1:info.dim(2)
        if bw2(y,x)==1
            S = squeeze(dti.dwi_reg(y,x,:));

            % Calculate diffusion tensor based on dt_fit.
            if strcmp(dt_fit, 'ls')
                [dti.D(y,x,:), dti.S(y,x,:)] = calc_dt_ls(S, dti.B_reg);
            elseif strcmp(dt_fit, 'cls')
                [dti.D(y,x,:), dti.S(y,x,:)] = calc_dt_cls(S, dti.B_reg);
            elseif strcmp(dt_fit, 'wls')
                [dti.D(y,x,:), dti.S(y,x,:)] = ...
                    calc_dt_wls(S, dti.B_reg, dti.dwi_noise);
            elseif strcmp(dt_fit, 'cwls')
                [dti.D(y,x,:), dti.S(y,x,:)] = ...
                    calc_dt_cwls(S, dti.B_reg, dti.dwi_noise);
            elseif strcmp(dt_fit, 'nls')
                [dti.D(y,x,:), dti.S(y,x,:)] = ...
                    calc_dt_nls(S, dti.B_reg, dti.dwi_noise);
            elseif strcmp(dt_fit, 'cnls')
                [dti.D(y,x,:), dti.S(y,x,:)] = ...
                    calc_dt_cnls(S, dti.B_reg, dti.dwi_noise);
            else
                error('ERROR: dt_fit must be one of the following strings: ...
                    ls, cls, wls, cwls, nls, or cnls.')
            end;

            % Calculate chi2 for experimental signal, S and fitted signal,
```

```

% dti.S
dti.chi2(y,x) = sum( (S - squeeze(dti.S(y,x,:))).^2 ./ ...
    dti.dwi_noise'.^2 );

% Calculate eigenvalues, dti.lambda and sorted eigen vectors,
% dti.ev
[dti.lambda(y,x,:), idx] = sort(eig(squeeze(dti.D(y,x,:,:))), ...
    'descend');
[ev, ~] = eig(squeeze(dti.D(y,x,:,:)));
for m=1:3
    dti.ev(y,x,:,m) = ev(:,idx(m));
end;

% Calculate DTI parameters: mean diffusivity (Davg), axial
% diffusivity (Drad), radial diffusivity (Drad), fractional
% anisotropy (FA), and FA-weighted RGB color map (RGB).
dti.Davg(y,x) = mean(dti.lambda(y,x,:));
dti.Drad(y,x) = (dti.lambda(y,x,2) + dti.lambda(y,x,3))/2;
dti.Daxi(y,x) = dti.lambda(y,x,1);
dti.FA(y,x) = calc_FA(dti.lambda(y,x,1), dti.lambda(y,x,2), ...
    dti.lambda(y,x,3), dti.Davg(y,x));
dti.RGB(y,x,1) = abs(dti.ev(y,x,1,1))*dti.FA(y,x);
dti.RGB(y,x,2) = abs(dti.ev(y,x,2,1))*dti.FA(y,x);
dti.RGB(y,x,3) = abs(dti.ev(y,x,3,1))*dti.FA(y,x);
end;
end;
end;

function B = calc_B(Bmat, Nexp)
% Calculate B.

B = zeros(Nexp,7);

for m=1:Nexp
    B(m,1) = -Bmat(1,1,m);
    B(m,2) = -Bmat(2,2,m);
    B(m,3) = -Bmat(3,3,m);
    B(m,4) = -2*Bmat(1,2,m);
    B(m,5) = -2*Bmat(1,3,m);
    B(m,6) = -2*Bmat(2,3,m);
    B(m,7) = 1;
end;

function FA = calc_FA(e1, e2, e3, Davg)

% Calculate fractional anisotropy and its error.

A = sqrt((e1-Davg)^2 + (e2-Davg)^2 + (e3-Davg)^2);
B = sqrt(e1^2 + e2^2 + e3^2);

FA = abs(sqrt(3/2)*A/B);

```

C.5.4 DWI Filtering

`calc_dwi_filt.m` filters diffusion-weighted images using an anisotropic diffusion filter.

```
function [dwi_filt, dwi_noise, dwi_filt_noise] = calc_dwi_filt(dwi, info, ...
    length, iterations)
% Filters diffusion-weighted images based on region of noise and
% anisotropic diffusion filter.
% dwi = dim(1) x dim(2) x Nexp x Nslices
% info = DTI info structure loaded by load_dti_info()
% length = length of noise region
% iterations = # of iterations performed by filter

% Initialize variables
x = [5 5+length 5+length 5 5];
y = [5 5 5+length 5+length 5];
bw = poly2mask(x,y,info.dim(1),info.dim(2));
dwi_filt = zeros(size(dwi));
dwi_noise = zeros(1,info.Nexp);
dwi_filt_noise = zeros(1,info.Nexp);

% Calculate filtered diffusion-weighted images using an anisotropic
% diffusion filter.
for n=1:info.slices
    for m=1:info.Nexp
        dwi_temp = dwi(:, :, m, n);
        dwi_noise(m) = std(dwi_temp(bw))/sqrt(2-pi/2);
        kappa = 3*dwi_noise(m);
        dwi_filt(:, :, m, n) = anisodiff2D(dwi_temp, iterations, 1/7, kappa, 1);
        dwi_filt_temp = dwi_filt(:, :, m, n);
        dwi_filt_noise(m) = std(dwi_filt_temp(bw));
    end;
end;

end
```

C.5.5 DWI Registration

`calc_dwi_reg.m` automatically registers diffusion-weighted images to a $b=0$ image using a Nelder-Mead simplex search with mutual information or the 2D correlation coefficient as the error function.

```
function [dwi_reg, B_reg, B_mat, reg] = calc_dwi_reg(dwi_base, dwi, info, B)
% Registers diffusion-weighted images to a base image using 2D correlation
% coefficient.
% dwi = dim(1) x dim(2) x Nexp x Nslices
% info = DTI info structure loaded by load_dti_info()
% length = length of noise region
% iterations = # of iterations performed by filter

% Initialize variables
reg = zeros(3,info.Nexp, info.slices);
dwi_reg = zeros(size(dwi));
B_reg = zeros(info.Nexp,7,info.slices);
B_mat = zeros(3, 3, info.Nexp,info.slices);

R = @rescale2;
init_scale = [0 0 0];
LB = init_scale + [-1 -5 -5];
UB = init_scale + [1 5 5];
```

```

for n=1:info.slices
    B_reg(:,:,n) = B;
    for m=1:info.Nexp
        I = dwi_base(:,:,n);
        I_transform = dwi(:,:,m,n);
        R = @rescale2;

        % Bound Nelder-Mead simplex search for error function rescale_mi().
        % Searches for optimal affine transformation parameters (reg_scale) ...
        % based
        % on initial_scale and lower and upper bounds.
        reg_scale=fminsearchbnd(R,init_scale,LB,UB);

        % Calculate transformed image.
        dwi_reg(:,:,m,n) = calc_image(I_transform, reg_scale);
        reg(:,m,n) = reg_scale;

        % Rotate B matrix for each registered image.
        u=(reg_scale(1)/360)*2*pi;
        R = [cos(u) -sin(u) 0; sin(u) cos(u) 0; 0 0 1];
        B_mat(:,:,m,n) = R*info.Bmat(:,:,m)*transpose(R);
        B_reg(m,1,n) = -B_mat(1,1,m,n);
        B_reg(m,2,n) = -B_mat(2,2,m,n);
        B_reg(m,3,n) = -B_mat(3,3,m,n);
        B_reg(m,4,n) = -2*B_mat(1,2,m,n);
        B_reg(m,5,n) = -2*B_mat(1,3,m,n);
        B_reg(m,6,n) = -2*B_mat(2,3,m,n);
        B_reg(m,7,n) = 1;
    end;
end;

% Error function for registration.
function [err]=rescale2(scale)
    [M N]=size(I);

    % Apply transformation with various scale parameters until the correlation
    % between the base and the aligned image is acceptable
    u=scale(1);
    dx=scale(2);
    dy=scale(3);

    % Creates affine transformation image from rotation and translation
    % parameters.
    Trotation=[cos(u) sin(u) 0; -sin(u) cos(u) 0; 0 0 1];
    Ttranslation=[1 0 0; 0 1 0; dx dy 1];
    T=Trotation*Ttranslation;
    Tform=maketform('affine',T);

    % Aligns target image using the affine transformation matrix.
    I_aligned=imtransform(I_transform,Tform,'Xdata',[1 N],'Ydata',[1 M]);

    % Find the 2D correlation coefficient between the base and the aligned
    % images (substitute mi for Mutual Information value).
    err=-abs(corr2(I_aligned,I));
    %err=-abs(mi(I_aligned,I));
end

end

```

```
function I_aligned = calc_image(I_transform, scale)
% Calculate images using affine transformation parameters and bicubic
% interpolation.

% Initialize variables
[M N]=size(I_transform);
u=(scale(1)/360)*2*pi;
dx=scale(2);
dy=scale(3);

% Construct affine transformation matrix for rotation and translation
Trotation=[cos(u) sin(u) 0; -sin(u) cos(u) 0; 0 0 1];
Ttranslation=[1 0 0; 0 1 0; dx dy 1];
T=Trotation*Ttranslation;

Tform=maketform('affine',T);

I_aligned=imtransform(I_transform,Tform,'bicubic','Xdata',[1 N],'Ydata',[1 M]);
```

C.5.6 Diffusion Tensor Fitting: Least Squares, Weighted Least Squares, and Non-Linear Least Squares Methods

Solutions for the diffusion tensor can be found using least-squares, weighted least-squares, or non-linear least squares fitting methods. In the presence of noise or high anisotropy, sometimes the generated diffusion tensor will yield negative eigenvalues or fractional anisotropy greater than 1, both of which are physically impossible. To address this, diffusion tensors that are not positive definite are modified using the Cholesky decomposition to ensure physical values. For comparison, non-constrained methods were also developed (`calc_dt_ls.m`, `calc_dt_ls.m`, and `calc_dt_ls.m`), but are not shown here. The modified Cholesky decomposition function (`mchol.m`) was written by Brian Borchers and is available on the MATLAB File Exchange [32].

```
function [D, Scls, posdef] = calc_dt_cls(S, B)
% Calculate diffusion tensor using least-squares fitting with constraints
% on diffusion tensor to ensure it is symmetric and positive definite
% (using Cholesky parameterization of D).

% Initialize variables.
[M,N] = size(B);
Scls = zeros(1,M);

% Calculate inverse if B is symmetric or Moore-Penrose inverse if B is
% asymmetric.
if M == N
    X = inv(B);
else
    X = pinv(B);
end;

% Least-squares solution for diffusion tensor.
lnS = log(S);
Dv = X*lnS;
C = [Dv(1) Dv(4) Dv(5); Dv(4) Dv(2) Dv(6); Dv(5) Dv(6) Dv(3)];

% Derive modified cholesky decomposition than reconstruct diffusion tensor
```

```

% Check if D is positive-definite
if (Dv(1) > 0) && (Dv(1)*Dv(2) - Dv(4)^2 > 0) && (det(C) > 0)
    D = C;
    posdef = 1;
else
    [L,D,E,pneg] = mchol(C);
    D = C + E;
    posdef = 0;
end;

for m=1:M
    Scwls(m) = exp(Dv(7))*exp(squeeze(B(m,1:6))*Dv(1:6));
end;

function [D, Scwls, posdef] = calc_dt_cwls(S, B, dwi_noise)
% Calculate diffusion tensor using weighted least-squares fitting
% with constraints on diffusion tensor to ensure it is symmetric and positive
% definite (using Cholesky parameterization of D).

% Initialize variables.
[M,N] = size(B);
Scwls = zeros(1,M);

% Calculate weighted least-squares solution for diffusion tensor.
lnS = log(S);
w = S.^2./dwi_noise'.^2;
W = diag(w);

Dv = (B'*W*B)\(B'*W)*lnS;

C = [Dv(1) Dv(4) Dv(5); Dv(4) Dv(2) Dv(6); Dv(5) Dv(6) Dv(3)];

% Derive modified cholesky decomposition than reconstruct diffusion tensor
if (Dv(1) > 0) && (Dv(1)*Dv(2) - Dv(4)^2 > 0) && (det(C) > 0) % Check if D ...
    is positive-definite
    D = C;
    posdef = 1;
else
    [L,D,E,pneg] = mchol(C);
    D = C + E;
    posdef = 0;
end;

for m=1:M
    Scwls(m) = exp(Dv(7))*exp(squeeze(B(m,1:6))*Dv(1:6));
end;

function [D, Scnls] = calc_dt_cnls(S, B, dwi_noise)
% Calculate diffusion tensor using non-linear least-squares fitting
% with constraints on diffusion tensor to ensure it is symmetric and
% positive definite (using Cholesky parameterization of D).

% Initialize variables.
[M,N] = size(B);
Scnls = zeros(1,M);

```

```
% Calculate weighted least-squares solution for initial value, Dstart.
lnS = log(S);
w = S.^2./dwi_noise'.^2;
W = diag(w);
Dstart = (B'*W*B)\(B'*W)*lnS;

% Search for optimal diffusion tensor estimates using Nelder-Mead simplex ...
search.
model = @Sd;
options = optimset('MaxFunEvals', 50000, 'MaxIter', 50000);
Dvals = fminsearch(model, Dstart, options);

C = [Dvals(1) Dvals(4) Dvals(5); Dvals(4) Dvals(2) Dvals(6); Dvals(5) ...
     Dvals(6) Dvals(3)];

% Derive modified cholesky decomposition than reconstruct diffusion tensor
% Check if D is positive-definite
if (Dvals(1) > 0) && (Dvals(1)*Dvals(2) - Dvals(4)^2 > 0) && (det(C) > 0)
    D = C;
    posdef = 1;
else
    [L,D,E,pneg] = mchol(C);
    D = C + E;
    posdef = 0;
end;

% Calculate fitted signal
for m=1:M
    Scnls(m) = exp(Dvals(7))*exp(squeeze(B(m,1:6))*Dvals(1:6));
end;

% Error function for minimization search of diffusion tensor values.
function [sse, FittedCurve] = Sd(Dv)
    S0 = exp(Dv(7));

    for n=1:M
        FittedCurve(n) = S0*exp(squeeze(B(n,1:6))*Dv(1:6));
    end;

    ErrorVector = FittedCurve' - S;
    sse = sum(ErrorVector.^2);
end
end
```

C.6 *Ex Vivo* Image Analysis

Ex vivo images would typically include DTI, qMTI, T1 and T2 relaxometry datasets. These are loaded and registered to a common reference image using `imreg.m` (with the `b=0` diffusion-weighted image as a reference). Image registration across different quantitative MRI methods allows for the application of common regions of interest and voxel-based correlations within those regions of interest.

C.6.1 Defining Regions of Interest

In `define_roi_dti.m`, regions of interest are defined using the RGB color map in the corpus callosum, external capsule, cerebral cortex, and noise region for comparisons. These regions of interest were subsequently applied to the registered qMTI and relaxometry maps.

```
function roi = define_roi_dti(dti_dir, mouse, Nroi)
% Define regions of interest in DTI dataset.

% Load aligned images
dti_file = [dti_dir '\ ' mouse '_dti.mat'];
load(dti_file);

% Define variables
FA_mean = zeros(Nroi+3,1);
FA_std = zeros(Nroi+3,1);
Davg_mean = zeros(Nroi+3,1);
Davg_std = zeros(Nroi+3,1);
Daxi_mean = zeros(Nroi+3,1);
Daxi_std = zeros(Nroi+3,1);
Drad_mean = zeros(Nroi+3,1);
Drad_std = zeros(Nroi+3,1);
N = zeros(Nroi+3,1);

% Select ROIs in RGB color map.
figure('Name', 'RGB'); imagesc(RGB); axis equal;

for n=1:Nroi
    [roi(n).bw,roi(n).xi,roi(n).yi] = roipoly;
    line(roi(n).xi,roi(n).yi,'Color','r','LineWidth',1);
end;

roi(Nroi+1).bw = logical(roi(2).bw+roi(3).bw); % ec
roi(Nroi+2).bw = logical(roi(1).bw+roi(2).bw+roi(3).bw); % cc + ec
roi(Nroi+3).bw = logical(roi(4).bw+roi(5).bw); % cerebral cortex

% Calculate mean DTI values in each ROI.
for n=1:Nroi+3
    bw_temp = logical(roi(n).bw);

    N(n) = sum(sum(bw_temp));
    FA_mean(n) = mean(dti.FA(bw_temp));
    FA_std(n) = std(dti.FA(bw_temp));
    Davg_mean(n) = mean(dti.Davg(bw_temp));
    Davg_std(n) = std(dti.Davg(bw_temp));
    Daxi_mean(n) = mean(dti.Daxi(bw_temp));
    Daxi_std(n) = std(dti.Daxi(bw_temp));
    Drad_mean(n) = mean(dti.Drad(bw_temp));
    Drad_std(n) = std(dti.Drad(bw_temp));
end;

save([mouse '_roi.mat']);
```

C.6.2 Correlating *Ex Vivo* Images

Spearman correlation coefficients were calculated for individual voxels in each ROI as well as for mean values of each ROI, for all mice and for control and cuprizone-fed mice individually. ROI-based correlations included *in vivo* T_2 -weighted and MTR measurements in all regions of interest, and electron microscopy measurements in the corpus callosum.

```
% corr_exvivo.m (EXCERPT)
% Calculates Spearman correlation coefficients in control, cuprizone-fed,
% and all mice. Does so for every voxel in each ROI and for mean values in
% each ROI (_roi). Filtered and non-filtered results were also compared
% (not shown).
% Nparams = number of MRI parameters (in vivo and ex vivo)
% Nem = number of electron microscope measurements

correlation = 'Spearman';

% Calculate correlation coefficients of mean MRI values in the corpus
% callosum along with corresponding electron microscopy measurements.
for k=1:Nparams+Nem
    for l=1:Nparams+Nem
        [all_roi_r{k,l,1},all_roi_r{k,l,2}] = corr(all_roi{1,k}, ...
            all_roi{1,l}, 'type', correlation);
        [ctl_roi_r{k,l,1},ctl_roi_r{k,l,2}] = corr(ctl_roi{1,k}, ...
            ctl_roi{1,l}, 'type', correlation);
        [cpz_roi_r{k,l,1},cpz_roi_r{k,l,2}] = corr(cpz_roi{1,k}, ...
            cpz_roi{1,l}, 'type', correlation);
    end;
end;

for j=1:10
    for k=1:Nparams
        for l=1:Nparams
            % Calculate voxel-based correlation coefficients.
            [all_r{j,k,l,1},all_r{j,k,l,2}] = corr(all{j,k}, all{j,l}, ...
                'type', correlation);
            [ctl_r{j,k,l,1},ctl_r{j,k,l,2}] = corr(ctl{j,k}, ctl{j,l}, ...
                'type', correlation);
            [cpz_r{j,k,l,1},cpz_r{j,k,l,2}] = corr(cpz{j,k}, cpz{j,l}, ...
                'type', correlation);
        end;
    end;
    for k=1:Nparams+2
        for l=1:Nparams+2
            % Calculate ROI-based correlation coefficients (includes two
            % more parameters for in vivo T2w and MTR measurements).
            [all_roi_r{j,k,l,1},all_roi_r{j,k,l,2}] = corr(all_roi{j,k}, ...
                all_roi{j,l}, 'type', correlation);
            [ctl_roi_r{j,k,l,1},ctl_roi_r{j,k,l,2}] = corr(ctl_roi{j,k}, ...
                ctl_roi{j,l}, 'type', correlation);
            [cpz_roi_r{j,k,l,1},cpz_roi_r{j,k,l,2}] = corr(cpz_roi{j,k}, ...
                cpz_roi{j,l}, 'type', correlation);
        end;
    end;
end;
end;
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References

- [1] F. Aboitiz, A. B. Scheibel, R. S. Fisher, and E. Zaidel. Fiber composition of the human corpus callosum. *Brain Research*, 598(1-2):143–53, Dec. 1992. ISSN 0006-8993. URL <http://www.ncbi.nlm.nih.gov/pubmed/1486478>.
- [2] M. Aggarwal, M. V. Jones, P. a. Calabresi, S. Mori, and J. Zhang. Probing mouse brain microstructure using oscillating gradient diffusion MRI. *Magnetic Resonance in Medicine*, 000, May 2011. ISSN 1522-2594. doi: 10.1002/mrm.22981. URL <http://www.ncbi.nlm.nih.gov/pubmed/21590726>.
- [3] A. L. Alexander, J. E. Lee, M. Lazar, and A. S. Field. Diffusion Tensor Imaging of the Brain. *Neurotherapeutics*, 4(July):316–329, 2007.
- [4] D. C. Alexander and G. J. Barker. Optimal imaging parameters for fiber-orientation estimation in diffusion MRI. *NeuroImage*, 27(2):357–67, Aug. 2005. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2005.04.008. URL <http://www.ncbi.nlm.nih.gov/pubmed/15921931>.
- [5] D. C. Alexander, P. L. Hubbard, M. G. Hall, E. a. Moore, M. Ptito, G. J. M. Parker, and T. B. Dyrby. Orientationally invariant indices of axon diameter and density from diffusion MRI. *NeuroImage*, 52(4):1374–89, Oct. 2010. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2010.05.043. URL <http://www.ncbi.nlm.nih.gov/pubmed/20580932>.
- [6] I. V. Allen, S. Mcquaid, M. Mirakhur, and G. Nevin. Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. *Journal of Neurological Sciences*, 22:141–144, 2001.
- [7] T. J. Andrews, M. T. Osborne, and M. D. Does. Diffusion of myelin water. *Magnetic Resonance in Medicine*, 56(2):381–5, Aug. 2006. ISSN 0740-3194. doi: 10.1002/mrm.20945. URL <http://www.ncbi.nlm.nih.gov/pubmed/16767712>.
- [8] Y. Assaf, R. Z. Freidlin, G. K. Rohde, and P. J. Basser. New modeling and experimental framework to characterize hindered and restricted water diffusion in brain white matter. *Magnetic Resonance in Medicine*, 52(5):965–78, Nov. 2004. ISSN 0740-3194. doi: 10.1002/mrm.20274.
- [9] A. V. Avram, A. Guidon, and A. W. Song. Myelin water weighted diffusion tensor imaging. *NeuroImage*, 53(1):132–8, Oct. 2010. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2010.06.019. URL <http://www.ncbi.nlm.nih.gov/pubmed/20587369>.
- [10] M. M. Bahn. Invariant and Orthonormal Scalar Measures Derived from Magnetic Resonance Diffusion Tensor Imaging. *Journal of Magnetic Resonance*, 41:68–77, 1999.

- [11] M. M. Bahn. Comparison of Scalar Measures Used in Magnetic Resonance Diffusion Tensor Imaging. *Journal of Magnetic Resonance*, 139:1–7, 1999.
- [12] R. Bakshi, A. J. Thompson, M. a. Rocca, D. Pelletier, V. Dousset, F. Barkhof, M. Inglese, C. R. G. Guttmann, M. a. Horsfield, and M. Filippi. MRI in multiple sclerosis: current status and future prospects. *Lancet Neurology*, 7(7):615–25, July 2008. ISSN 1474-4422. doi: 10.1016/S1474-4422(08)70137-6. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2586926&tool=pmcentrez&rendertype=abstract>.
- [13] R. Bammer, B. Acar, and M. E. Moseley. In vivo MR tractography using diffusion imaging. *European Journal of Radiology*, 45:223–234, 2003.
- [14] P. J. Basser and D. K. Jones. Diffusion-tensor MRI: theory, experimental design and data analysis Ás a technical review. *NMR in Biomedicine*, 15:456–467, 2002.
- [15] P. J. Basser, J. Mattiello, and D. Le Bihan. Estimation of the Effective Self-Diffusion from the NMR Spin Echo. *Journal of Magnetic Resonance, Series B*, 103:247–254, 1994.
- [16] P. J. Basser, S. Pajevic, C. Peierpaoli, D. J, and A. Aldroubi. In vivo fiber tractography using DT-MRI data. *Magnetic Resonance in Medicine*, 44(4):625–632, 2000.
- [17] P. G. Batchelor, D. Atkinson, D. L. G. Hill, F. Calamante, and A. Connelly. Anisotropic noise propagation in diffusion tensor MRI sampling schemes. *Magnetic Resonance in Medicine*, 49(6):1143–51, June 2003. ISSN 0740-3194. doi: 10.1002/mrm.10491.
- [18] C. Beaulieu. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR in Biomedicine*, 15(7-8):435–55, 2002. ISSN 0952-3480. doi: 10.1002/nbm.782.
- [19] C. Beaulieu and P. S. Allen. Water diffusion in the giant axon of the squid: Implications for diffusion-weighted MRI of the nervous system. *Magnetic Resonance in Medicine*, 32(5):579–583, Nov. 1994. ISSN 07403194. doi: 10.1002/mrm.1910320506. URL <http://doi.wiley.com/10.1002/mrm.1910320506>.
- [20] C. Beaulieu, F. R. Fenrich, and P. S. Allen. Multicomponent Water Proton Transverse Relaxation and T2-Discriminated Water Diffusion in Myelinated and Nonmyelinated Nerve. *Magnetic Resonance Imaging*, 16(10):1201–1210, 1998.
- [21] P. Belton, B. Hills, and E. Raimbaud. The effects of morphology and exchange on proton N.M.R. relaxation in agarose gels. *Molecular Physics*, 63(5):825–842, Apr. 1988. ISSN 0026-8976. doi: 10.1080/00268978800100591. URL <http://www.tandfonline.com.proxy2.lib.umanitoba.ca/doi/abs/10.1080/00268978800100591>.
- [22] J. A. Benjamins. Molecular Structure of the Myelin Membrane. In R. M. Herndon, editor, *Multiple Sclerosis: Immunology, Pathology and Pathophysiology*, pages 31–50. Demos, New York, 2003.
- [23] D. Berg and M. B. H. Youdim. Role of iron in neurodegenerative disorders. *Neurodegeneration*, 17(1):5–17, Feb. 2006. ISSN 0899-3459. doi: 10.1097/01.rmr.0000245461.90406.ad. URL <http://www.ncbi.nlm.nih.gov/pubmed/17179893>.

-
- [24] M. A. Bernstein, K. F. King, and X. J. Zhou. *Handbook of MRI Pulse Sequences*. Academic Press, 2004. ISBN 0120928612.
 - [25] M. Bilgen, B. Al-hafez, T. M. Malone, and I. V. Smirnova. Ex vivo magnetic resonance imaging of rat spinal cord at 9.4 T. *Magnetic Resonance Imaging*, 23:601 – 605, 2005. doi: 10.1016/j.mri.2005.02.003.
 - [26] R. Bitar, G. Leung, R. Perng, S. Tadros, A. R. Moody, J. Sarrazin, C. McGregor, M. Christakis, S. Symons, A. Nelson, and T. P. Roberts. MR pulse sequences: what every radiologist wants to know but is afraid to ask. *Radiographics*, 26(2):513–37, 2006. ISSN 1527-1323. doi: 10.1148/rg.262055063. URL <http://www.ncbi.nlm.nih.gov/pubmed/16549614>.
 - [27] T. A. Bjarnason. *Introduction to Quantitative T2 with emphasis on Medical Imaging*. 2010. ISBN 9780557674039.
 - [28] T. a. Bjarnason, I. M. Vavasour, C. L. L. Chia, and a. L. MacKay. Characterization of the NMR behavior of white matter in bovine brain. *Magnetic Resonance in Medicine*, 54(5):1072–81, Nov. 2005. ISSN 0740-3194. doi: 10.1002/mrm.20680. URL <http://www.ncbi.nlm.nih.gov/pubmed/16200557>.
 - [29] T. A. Bjarnason, C. R. McCreary, J. F. Dunn, and J. R. Mitchell. Quantitative T2 Analysis : The Effects of Noise , Regularization , and Multivoxel Approaches. *Magnetic Resonance in Medicine*, 217(October 2009):212–217, 2010. doi: 10.1002/mrm.22173.
 - [30] W. F. Blakemore and R. J. M. Franklin. Remyelination in Experimental Models of Toxin-Induced Demyelination. *Current Topics in Microbiology and Immunology*, 318: 193–212, 2008.
 - [31] F. Bloch. Nuclear Induction. *Physical Review*, 70(7):460–474, 1946.
 - [32] B. Borchers. Cholesky Factorizations, 2002. URL <http://www.mathworks.com/matlabcentral/fileexchange/47-ldlt>.
 - [33] S. Boretius, A. Escher, T. Dallenga, C. Wrzos, R. Tammer, W. Brück, S. Nessler, J. Frahm, and C. Stadelmann. Assessment of lesion pathology in a new animal model of MS by multiparametric MRI and DTI. *NeuroImage*, 59(3):2678–88, Feb. 2012. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2011.08.051. URL <http://www.ncbi.nlm.nih.gov/pubmed/21914485>.
 - [34] M. A. Brown and R. C. Semelka. MR Imaging Abbreviations , Definitions , and Descriptions: A Review. *Radiology*, 213:647–662, 1999.
 - [35] M. D. Budde, J. H. Kim, H.-F. Liang, R. E. Schmidt, J. H. Russell, A. H. Cross, and S.-K. Song. Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. *Magnetic Resonance in Medicine*, 57(4):688–95, 2007. ISSN 0740-3194. doi: 10.1002/mrm.21200. URL <http://www.ncbi.nlm.nih.gov/pubmed/17390365>.
 - [36] M. D. Budde, M. Xie, A. H. Cross, and S.-K. Song. Axial diffusivity is the primary correlate of axonal injury in the experimental autoimmune encephalomyelitis spinal cord: a quantitative pixelwise analysis. *The Journal of Neuroscience*,

- 29(9):2805–13, Mar. 2009. ISSN 1529-2401. doi: 10.1523/JNEUROSCI.4605-08.2009. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2673458&tool=pmcentrez&rendertype=abstract>.
- [37] G. H. Caines, T. Schleich, and J. M. Rydzewski. Incorporation of magnetization transfer into the formalism for rotating-frame spin-lattice proton NMR relaxation in the presence of an off-resonance-irradiation field. *Journal of Magnetic Resonance*, 95(3):558–566, Dec. 1991. ISSN 00222364. doi: 10.1016/0022-2364(91)90169-T. URL <http://linkinghub.elsevier.com/retrieve/pii/002223649190169T>.
- [38] P. Callaghan. *Principles of Nuclear Magnetic Resonance Microscopy*. Oxford University Press, USA, 1994. ISBN 0198539975.
- [39] H. Y. Carr and E. M. Purcell. Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Physical Review*, 94(3):630–638, 1954.
- [40] M. Cercignani and D. C. Alexander. Optimal acquisition schemes for in vivo quantitative magnetization transfer MRI. *Magnetic Resonance in Medicine*, 56(4):803–10, Oct. 2006. ISSN 0740-3194. doi: 10.1002/mrm.21003.
- [41] M. Cercignani and G. J. Barker. A comparison between equations describing in vivo MT: the effects of noise and sequence parameters. *Journal of Magnetic Resonance*, 191(2):171–183, Apr. 2008. ISSN 1090-7807. doi: 10.1016/j.jmr.2007.12.012. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2323944&tool=pmcentrez&rendertype=abstract>.
- [42] M. Cercignani, G. Iannucci, M. A. Rocca, G. Comi, M. A. Horsfield, and M. Filippi. Pathologic damage in MS assessed by diffusion-weighted and magnetization transfer MRI., 2000. URL http://ovidsp.tx.ovid.com.proxy1.lib.umanitoba.ca/sp-3.5.1a/ovidweb.cgi?&S=PNOLFPHPPJDDKEDANCALFEJCGGADAA00&Link+Set=S.sh.15.16.20.24|27|sl_10.
- [43] P. Chandran, J. Upadhyay, S. Markosyan, A. Lisowski, W. Buck, C.-L. Chin, G. Fox, F. Luo, and M. Day. Magnetic resonance imaging and histological evidence for the blockade of cuprizone-induced demyelination in C57BL/6 mice. *Neuroscience*, 202:446–53, Jan. 2012. ISSN 1873-7544. doi: 10.1016/j.neuroscience.2011.10.051. URL <http://www.ncbi.nlm.nih.gov/pubmed/22119061>.
- [44] J. T. Chen, D. L. Collins, H. L. Atkins, M. S. Freedman, and D. L. Arnold. Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. *Annals of Neurology*, 63(2):254–62, Feb. 2008. ISSN 1531-8249. doi: 10.1002/ana.21302.
- [45] C.-L. Chin, F. W. Wehrli, S. N. Hwang, M. Takahashi, and D. B. Hackney. Biexponential diffusion attenuation in the rat spinal cord: computer simulations based on anatomic images of axonal architecture. *Magnetic Resonance in Medicine*, 47(3):455–60, Mar. 2002. ISSN 0740-3194. doi: 10.1002/mrm.10078.
- [46] K. A. Christensen, D. M. Grant, E. M. Schulman, and C. Walling. Optimal Determination of Relaxation Times of Fourier Transform Nuclear Magnetic Resonance. Determination of Spin-Lattice Relaxation Times in Chemically Polarized Species. *The Journal of Physical Chemistry*, 7(79), 1974.

-
- [47] J. W. Cooley and J. W. Tukey. An Algorithm for the Machine Calculation Complex Fourier Series. *Mathematics of Computation*, 19(90):297–301, 1965.
 - [48] A. P. Crawley and R. M. Henkelman. A comparison of one-shot and recovery methods in T1 imaging. *Magnetic Resonance in Medicine*, 7(1):23–34, May 1988. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/3386519>.
 - [49] G. Davies, D. Tozer, M. Cercignani, A. Ramani, C. Dalton, A. Thompson, G. Barker, P. Tofts, and D. Miller. Estimation of the macromolecular proton fraction and bound pool T2 in multiple sclerosis. *Multiple Sclerosis*, 10(6):607–613, Nov. 2004. ISSN 13524585. doi: 10.1191/1352458504ms1105oa. URL <http://msj.sagepub.com/cgi/doi/10.1191/1352458504ms1105oa>.
 - [50] C. A. DeBoy, J. Zhang, S. Dike, I. Shats, M. Jones, D. S. Reich, S. Mori, T. Nguyen, B. Rothstein, R. H. Miller, J. T. Griffin, D. A. Kerr, and P. A. Calabresi. High resolution diffusion tensor imaging of axonal damage in focal inflammatory and demyelinating lesions in rat spinal cord. *Brain*, 130(8):2199–210, 2007. ISSN 1460-2156. doi: 10.1093/brain/awm122.
 - [51] J. Delpiano. Fast mutual information of two images or signals, 2011. URL <http://www.mathworks.com/matlabcentral/fileexchange/13289-fast-mutual-information-of-two-images-or-signals>.
 - [52] S. C. L. Deoni, B. K. Rutt, and D. K. Jones. Investigating the Effect of Exchange and Multicomponent T1 Relaxation on the Short Repetition Time Spoiled Steady-State Signal and the DESPOT1 T1 Quantification Method. *Journal of Magnetic Resonance Imaging*, 25:570–578, 2007. doi: 10.1002/jmri.20836.
 - [53] S. C. L. Deoni, B. K. Rutt, T. Arun, C. Pierpaoli, and D. K. Jones. Gleaning multicomponent T1 and T2 information from steady-state imaging data. *Magnetic Resonance in Medicine*, 60(6):1372–87, Dec. 2008. ISSN 1522-2594. doi: 10.1002/mrm.21704.
 - [54] J. D’Errico. fminsearchbnd, fminsearchcon, 2012. URL <http://www.mathworks.com/matlabcentral/fileexchange/8277-fminsearchbnd-fminsearchcon>.
 - [55] M. D. Does and J. C. Gore. Compartmental Study of T1 and T2 in Rat Brain and Trigeminal Nerve In Vivo. *Magnetic Resonance in Medicine*, 47:274–283, 2002. doi: 10.1002/mrm.10060.
 - [56] M. D. Does and R. E. Snyder. Multiexponential T2 Relaxation in Degenerating Peripheral Nerve. *Magnetic Resonance in Medicine*, 35:207–213, 1996.
 - [57] R. D. Dortch, T. E. Yankeelov, Z. Yue, C. C. Quarles, J. C. Gore, and M. D. Does. Evidence of multiexponential T2 in rat glioblastoma. *NMR in Biomedicine*, 22(6): 609–18, July 2009. ISSN 1099-1492. doi: 10.1002/nbm.1374. URL <http://www.ncbi.nlm.nih.gov/pubmed/19267385>.
 - [58] R. D. Dortch, K. Li, D. F. Gochberg, E. B. Welch, A. N. Dula, A. a. Tamhane, J. C. Gore, and S. a. Smith. Quantitative magnetization transfer imaging in human brain at 3 T via selective inversion recovery. *Magnetic Resonance in Medicine*, 000:1–7, May 2011. ISSN 1522-2594. doi: 10.1002/mrm.22928.

- [59] V. Dousset, R. I. Grossman, K. N. Ramer, M. D. Schnall, L. H. Young, F. Gonzalez-Scarano, E. Lavi, and J. A. Cohen. Experimental Allergic Encephalomyelitis and Multiple Sclerosis: Lesion Characterization with Magnetization Transfer Imaging. *Radiology*, 182:483–491, 1992.
- [60] A. N. Dula, D. F. Gochberg, and M. D. Does. Optimal echo spacing for multi-echo imaging measurements of bi-exponential T2 relaxation. *Journal of Magnetic Resonance*, 196(2):149–56, Feb. 2009. ISSN 1557-8968. doi: 10.1016/j.jmr.2008.11.002.
- [61] A. N. Dula, D. F. Gochberg, H. L. Valentine, W. M. Valentine, and M. D. Does. Multiexponential T2 , Magnetization Transfer , and Quantitative Histology in White Matter Tracts of Rat Spinal Cord. *Magnetic Resonance in Medicine*, 63:902–909, 2010. doi: 10.1002/mrm.22267.
- [62] A. Einstein. *Investigations on the Theory of the Brownian Movement*. Dover Publications, Inc., 1956.
- [63] B. M. Ellingson, S. N. Kurpad, S.-J. Li, and B. D. Schmit. In vivo diffusion tensor imaging of the rat spinal cord at 9.4T. *Journal of Magnetic Resonance Imaging*, 27(3):634–42, Mar. 2008. ISSN 1053-1807. doi: 10.1002/jmri.21249. URL <http://www.ncbi.nlm.nih.gov/pubmed/18224673>.
- [64] J. a. D. Farrell, B. a. Landman, C. K. Jones, S. a. Smith, J. L. Prince, P. C. M. van Zijl, and S. Mori. Effects of signal-to-noise ratio on the accuracy and reproducibility of diffusion tensor imaging-derived fractional anisotropy, mean diffusivity, and principal eigenvector measurements at 1.5 T. *Journal of Magnetic Resonance Imaging*, 26(3):756–67, Sept. 2007. ISSN 1053-1807. doi: 10.1002/jmri.21053. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2862967&tool=pmcentrez&rendertype=abstract>.
- [65] K. T. M. Fernando, D. J. Tozer, K. A. Miskiel, R. M. Gordon, J. K. Swanton, C. M. Dalton, G. J. Barker, G. T. Plant, A. J. Thompson, and D. H. Miller. Magnetization transfer histograms in clinically isolated syndromes suggestive of multiple sclerosis. *Brain*, 128:2911–2925, 2005. doi: 10.1093/brain/awh654.
- [66] M. Filippi and F. Agosta. Imaging biomarkers in multiple sclerosis. *Journal of Magnetic Resonance Imaging*, 31(4):770–88, Apr. 2010. ISSN 1522-2586. doi: 10.1002/jmri.22102. URL <http://www.ncbi.nlm.nih.gov/pubmed/20373420>.
- [67] D. A. Finelli, G. C. Hurst, P. Amantia, R. P. Gullapali, and A. Apicella. Cerebral White Matter: Technical Development and Clinical Applicatiosn of Effective Magnetization Transfer (MT) Power Concepts for High-Power, Thin-Section, Quantitative MT Examinations. *Radiology*, 199:219–226, 1996.
- [68] J. C. Ford and D. B. Hackney. Numerical Model for Calculation of Apparent Diffusion Coefficients (ADC) in Permeable Cylinders - Comparison with Measured ADC in Spinal Cord White Matter. *Magnetic Resonance in Medicine*, 37:387–394, 1997.
- [69] F. Franconi, L. Lemaire, L. Marescaux, P. Jallet, L. Jeune, J.j, and J. J. L. Jeune. In vivo quantitative microimaging of rat spinal cord at 7T. *Magnetic Resonance in Medicine*, 44(July):893– 898, 2000.

- [70] R. A. Y. Freeman and M. H. Levitt. Compensation for pulse imperfections in NMR spin-echo experiments. *Journal of Magnetic Resonance*, 80(1):65–80, Apr. 1981. ISSN 00222364. doi: 10.1016/0022-2364(81)90082-2. URL <http://linkinghub.elsevier.com/retrieve/pii/0022236481900822>.
- [71] M. Garcia, M. Gloor, S. G. Wetzel, E.-W. Radue, K. Scheffler, and O. Bieri. Characterization of normal appearing brain structures using high-resolution quantitative magnetization transfer steady-state free precession imaging. *NeuroImage*, 52(2): 532–7, Aug. 2010. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2010.04.242. URL <http://www.ncbi.nlm.nih.gov/pubmed/20430101>.
- [72] P. J. Gareau, B. K. Rutt, S. J. Karlik, and J. R. Mitchell. Magnetization Transfer and Multicomponent T2 Relaxation Measurements With Histopathologic Correlation in an Experimental Model of MS. *Journal of Magnetic Resonance Imaging*, 11:586–595, 2000.
- [73] G. Gerig, O. Kubler, and F. A. Jolesz. Nonlinear Anisotropic Filtering of MRI Data. *IEEE Transactions on Medical Imaging*, 11(2):221–232, 1992.
- [74] S. Geyer, M. Weiss, K. Reimann, G. Lohmann, and R. Turner. Microstructural Parcellation of the Human Cerebral Cortex - From Brodmann’s Post-Mortem Map to in vivo Mapping with High-Field Magnetic Resonance Imaging. *Frontiers in Human Neuroscience*, 5(19):1–7, 2011. ISSN 1662-5161. doi: 10.3389/fnhum.2011.00019. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3044325&tool=pmcentrez&rendertype=abstract>.
- [75] P. E. Gill and W. Murray. Newton-Type Methods for Unconstrained and Linearly Constrained Optimization. *Mathematical Programming*, 7:311–350, 1974.
- [76] M. Gloor, K. Scheffler, and O. Bieri. Quantitative magnetization transfer imaging using balanced SSFP. *Magnetic Resonance in Medicine*, 60(3):691–700, Sept. 2008. ISSN 1522-2594. doi: 10.1002/mrm.21705. URL <http://www.ncbi.nlm.nih.gov/pubmed/18727085>.
- [77] M. Gloor, K. Scheffler, and O. Bieri. Nonbalanced SSFP-based quantitative magnetization transfer imaging. *Magnetic Resonance in Medicine*, 64(1):149–56, July 2010. ISSN 1522-2594. doi: 10.1002/mrm.22331. URL <http://www.ncbi.nlm.nih.gov/pubmed/20572130>.
- [78] D. F. Gochberg and J. C. Gore. Quantitative magnetization transfer imaging via selective inversion recovery with short repetition times. *Magnetic Resonance in Medicine*, 57(2):437–41, Feb. 2007. ISSN 0740-3194. doi: 10.1002/mrm.21143.
- [79] D. F. Gochberg, R. P. Kennan, M. D. Robson, and J. C. Gore. Quantitative imaging of magnetization transfer using multiple selective pulses. *Magnetic Resonance in Medicine*, 41(5):1065–1072, May 1999. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/10332891>.
- [80] S. J. Graham and R. M. Henkelman. Understanding Pulsed Magnetization Transfer. *Journal of Magnetic Resonance Imaging*, 7:903–912, 1997.

- [81] S. J. Graham and R. M. Henkelman. Pulsed Magnetization Transfer Imaging : Evaluation of Technique. *Radiology*, 212:903–910, 1999.
- [82] S. J. Graham, P. L. Stanchev, M. J. Bronskill, and P. D. Criteria for analysis of multicomponent tissue T2 relaxation data. *Magnetic Resonance in Medicine*, 35(3): 370–378, Mar. 1996. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/8699949>.
- [83] R. I. Grossman. Magnetization Transfer in Multiple Sclerosis. *Annals of Neurology*, 36:S97–S99, 1994.
- [84] H. Gudbjartsson and S. Patz. The Rician Distribution of Noisy MRI Data. *Magnetic Resonance in Medicine*, 34(6):910–914, 1995.
- [85] D. N. Guilfoyle, V. V. Dyakin, J. O. Shea, G. S. Pell, and J. A. Helpert. Quantitative Measurements of Proton Spin-Lattice (T1) and Spin–Spin (T2) Relaxation Times in the Mouse Brain at 7.0 T. *Magnetic Resonance in Medicine*, 49:576 –580, 2003. doi: 10.1002/mrm.10371.
- [86] A. C. Guo, V. L. Jewells, and J. M. Provenzale. Analysis of Normal-Appearing White Matter in Multiple Sclerosis: Comparison of Diffusion Tensor MR Imaging and Magnetization Transfer Imaging. *American Journal of Neuroradiology*, 22(December): 1893–1900, 2001.
- [87] E. M. Haacke, R. W. Brown, M. R. Thompson, and R. Venkatesan. *Magnetic Resonance Imaging: Physical Principles and Sequence Design*. John Wiley & Sons, New York, 1999.
- [88] A. Haase. Snapshot FLASH MRI. Applications to T1, T2, and Chemical-Shift Imaging. *Magnetic Resonance in Medicine*, 13:77–89, 1990.
- [89] E. L. Hahn. Nuclear Induction Due to Free Larmor Precession. *Physical Review*, 77 (3):297–298, 1950.
- [90] E. L. Hahn. Spin Echoes. *Physical Review*, 80(4):580–594, 1950.
- [91] C. D. Hansen and C. R. Johnson. *Visualization Handbook*. Academic Press, 2004. ISBN 012387582X.
- [92] L. G. Hanson. Is Quantum Mechanics Necessary for Understanding Magnetic Resonance? *Concepts In Magnetic Resonance*, 32A(5):329–340, 2008. doi: 10.1002/cmr.a.
- [93] K. D. Harkins, A. N. Dula, and M. D. Does. Effect of intercompartmental water exchange on the apparent myelin water fraction in multiexponential T2 measurements of rat spinal cord. *Magnetic Resonance in Medicine*, 67(3):783–800, June 2012. ISSN 1522-2594. doi: 10.1002/mrm.23053. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3188335&tool=pmcentrez&rendertype=abstract>.
- [94] R. Harrison, M. J. Bronskill, and R. M. Henkelman. Magnetization transfer and T2 relaxation components in tissue. *Magnetic Resonance in Medicine*, 33(4):490–6, Apr. 1995. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/7776879>.

-
- [95] L.-A. Harsan, J. Steibel, A. Zaremba, A. Agin, R. Sapin, P. Poulet, B. Guignard, N. Parizel, D. Grucker, N. Boehm, R. H. Miller, and M. S. Ghandour. Recovery from chronic demyelination by thyroid hormone therapy: myelinogenesis induction and assessment by diffusion tensor magnetic resonance imaging. *The Journal of Neuroscience*, 28(52):14189–201, Dec. 2008. ISSN 1529-2401. doi: 10.1523/JNEUROSCI.4453-08.2008. URL <http://www.ncbi.nlm.nih.gov/pubmed/19109501>.
 - [96] L.-A. Harsan, D. Paul, S. Schnell, B. W. Kreher, J. Hennig, J. F. Staiger, and D. von Elverfeldt. In vivo diffusion tensor magnetic resonance imaging and fiber tracking of the mouse brain. *NMR in Biomedicine*, 23(7):884–96, Aug. 2010. ISSN 1099-1492. doi: 10.1002/nbm.1496. URL <http://www.ncbi.nlm.nih.gov/pubmed/20213629>.
 - [97] K. M. Hasan, D. L. Parker, and A. L. Alexander. Comparison of Gradient Encoding Schemes for Diffusion-Tensor MRI. *Journal of Magnetic Resonance Imaging*, 13:769–780, 2001.
 - [98] K. M. Hasan, R. K. Gupta, R. M. Santos, J. S. Wolinsky, and P. A. Narayana. Diffusion tensor fractional anisotropy of the normal-appearing seven segments of the corpus callosum in healthy adults and relapsing-remitting multiple sclerosis patients. *Journal of Magnetic Resonance Imaging*, 21(6):735–43, June 2005. ISSN 1053-1807. doi: 10.1002/jmri.20296.
 - [99] R. M. Henkelman, X. Huang, Q.-s. Xiang, G. J. Stanisz, S. D. Swanson, and M. J. Bronskill. Quantitative Interpretation of Magnetization Transfer. *Magnetic Resonance in Medicine*, 29:759–766, 1993.
 - [100] R. M. Henkelman, G. J. Stanisz, and S. J. Graham. Magnetization transfer in MRI: a review. *NMR in Biomedicine*, 14(2):57–64, Apr. 2001. ISSN 0952-3480. doi: 10.1002/nbm.683.
 - [101] J. Hennig, A. Nauerth, and H. Friedburg. RARE Imaging: A Fast Imaging Method for Clinical MR. *Magnetic Resonance in Medicine*, 3:823–833, 1986.
 - [102] C. Hildebrand and S. Mohens. The Structure of Myelinated Axons in the CNS. In S. Waxman, editor, *Multiple Sclerosis as a Neuronal Disease*, chapter 1, pages 1–28. Elsevier Academic Press, London, 2005.
 - [103] M. M. Hiremath, Y. Saito, G. W. Knapp, J. P.-Y. Ting, K. Suzuki, and G. K. Matsushima. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *Journal of Neuroimmunology*, 92:38–49, 1998.
 - [104] K. Hoffmann, M. Lindner, I. Grötcke, M. Stangel, and W. Löscher. Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in C57BL/6 mice. *Experimental Neurology*, 210(2):308–21, Apr. 2008. ISSN 0014-4886. doi: 10.1016/j.expneurol.2007.11.005. URL <http://www.ncbi.nlm.nih.gov/pubmed/18096162>.
 - [105] M. Holz, S. R. Heila, and A. Saccob. Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate 1H NMR PFG measurements. *Physical Chemistry Chemical Physics*, 2:4740–4742, 2000.
 - [106] M. A. Horsfield and D. K. Jones. Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review. *NMR in Biomedicine*, 15(7-8):570–7, 2002. ISSN 0952-3480. doi: 10.1002/nbm.787.

- [107] D. Hwang, H. Chung, Y. Nam, Y. P. Du, and U. Jang. Robust mapping of the myelin water fraction in the presence of noise: synergic combination of anisotropic diffusion filter and spatially regularized nonnegative least squares algorithm. *Journal of Magnetic Resonance Imaging*, 34(1):189–95, July 2011. ISSN 1522-2586. doi: 10.1002/jmri.22586. URL <http://www.ncbi.nlm.nih.gov/pubmed/21618330>.
- [108] G. Iannucci, M. Rovaris, L. Giacomotti, G. Comi, and M. Filippi. Correlation of Multiple Sclerosis Measures Derived from T2-Weighted, T1-Weighted, Magnetization Transfer, and Diffusion Tensor MR Imaging. *American Journal of Neuroradiology*, 22 (September):1462–1467, 2001.
- [109] K. A. Irvine and W. F. Blakemore. Age increases axon loss associated with primary demyelination in cuprizone-induced demyelination in C57BL / 6 mice. *Journal of Neuroimmunology*, 175:69–76, 2006. doi: 10.1016/j.jneuroim.2006.03.002.
- [110] K. A. Irvine and W. F. Blakemore. Remyelination protects axons from demyelination-associated axon degeneration. *Brain*, 131(6):1464–77, June 2008. ISSN 1460-2156. doi: 10.1093/brain/awn080. URL <http://www.ncbi.nlm.nih.gov/pubmed/18490361>.
- [111] J. H. Jensen, J. a. Helpert, A. Ramani, H. Lu, and K. Kaczynski. Diffusional Kurtosis Imaging : The Quantification of Non- Gaussian Water Diffusion by Means of Magnetic Resonance Imaging. *Magnetic Resonance in Medicine*, 1440(6):1432–1440, June 2005. ISSN 0740-3194. doi: 10.1002/mrm.20508. URL <http://www.ncbi.nlm.nih.gov/pubmed/15906300>.
- [112] B. Jian, B. C. Vemuri, E. Ozarslan, P. R. Carney, and T. H. Mareci. A novel tensor distribution model for the diffusion-weighted MR signal. *NeuroImage*, 37(1):164–76, 2007. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2007.03.074.
- [113] C. K. Jones. *T2 Decay Curve Acquisition and Analysis in MRI: Noise Considerations, Short T2, and B1 Field Encoding*. PhD thesis, 2003.
- [114] C. K. Jones. T2 Curve Fitting, 2012. URL <http://mri.brechmos.org>.
- [115] C. K. Jones, K. P. Whittall, and A. L. MacKay. Robust myelin water quantification: averaging vs. spatial filtering. *Magnetic Resonance in Medicine*, 50(1):206–9, July 2003. ISSN 0740-3194. doi: 10.1002/mrm.10492.
- [116] D. K. Jones. The effect of gradient sampling schemes on measures derived from diffusion tensor MRI: a Monte Carlo study. *Magnetic Resonance in Medicine*, 51(4): 807–15, 2004. ISSN 0740-3194. doi: 10.1002/mrm.20033. URL <http://www.ncbi.nlm.nih.gov/pubmed/15065255>.
- [117] D. K. Jones and C. Pierpaoli. Confidence mapping in diffusion tensor magnetic resonance imaging tractography using a bootstrap approach. *Magnetic Resonance in Medicine*, 53(5):1143–1149, May 2005. ISSN 0740-3194. doi: 10.1002/mrm.20466. URL <http://www.ncbi.nlm.nih.gov/pubmed/15844149>.
- [118] D. K. Jones, M. A. Horsfield, and A. Simmons. Optimal Strategies for Measuring Diffusion in Anisotropic Systems by Magnetic Resonance Imaging. *Magnetic Resonance in Medicine*, 42:515–525, 1999.

-
- [119] R. A. Jones, S. Palasis, and J. D. Grattan-smith. MRI of the Neonatal Brain: Optimization of Spin Echo Parameters. *American Journal of Roentgenology*, 182 (February):367–372, 2004.
- [120] S. Kalantari, C. Laule, T. a. Bjarnason, I. M. Vavasour, and A. L. Mackay. Insight into in vivo magnetization exchange in human white matter regions. *Magnetic Resonance in Medicine*, 66(4):1142–51, Oct. 2011. ISSN 1522-2594. doi: 10.1002/mrm.22873. URL <http://www.ncbi.nlm.nih.gov/pubmed/21381107>.
- [121] J. W. Kesterson and W. W. Carlton. Histopathologic and Enzyme Histochemical Observations of the Cuprizone-Induced Brain Edema. *Experimental and Molecular Pathology*, 15:82–96, 1971.
- [122] J. H. Kim, M. D. Budde, H.-F. Liang, R. S. Klein, J. H. Russell, A. H. Cross, and S.-K. Song. Detecting axon damage in spinal cord from a mouse model of multiple sclerosis. *Neurobiology of Disease*, 21(3):626–32, Mar. 2006. ISSN 0969-9961. doi: 10.1016/j.nbd.2005.09.009.
- [123] P. B. Kingsley. Introduction to Diffusion Tensor Imaging Mathematics: Part III. Tensor Calculation, Noise, Simulations, and Optimization. *Concepts In Magnetic Resonance*, 28A:155–179, 2006. doi: 10.1002/cmr.a.
- [124] C. G. Koay, J. D. Carew, A. L. Alexander, P. J. Basser, and M. E. Meyerand. Investigation of anomalous estimates of tensor-derived quantities in diffusion tensor imaging. *Magnetic Resonance in Medicine*, 55(4):930–6, Apr. 2006. ISSN 0740-3194. doi: 10.1002/mrm.20832. URL <http://www.ncbi.nlm.nih.gov/pubmed/16526013>.
- [125] C. G. Koay, L.-C. Chang, J. D. Carew, C. Pierpaoli, and P. J. Basser. A unifying theoretical and algorithmic framework for least squares methods of estimation in diffusion tensor imaging. *Journal of Magnetic Resonance*, 182(1):115–25, Sept. 2006. ISSN 1090-7807. doi: 10.1016/j.jmr.2006.06.020. URL <http://www.ncbi.nlm.nih.gov/pubmed/16828568>.
- [126] S. H. Koenig. Cholesterol of Myelin Is the Determinant of Gray-White Contrast in MRI of Brain. *Magnetic Resonance in Medicine*, 20(2):285–291, 1991.
- [127] S. H. Kolind, C. Laule, I. M. Vavasour, D. K. B. Li, A. L. Traboulsee, G. R. W. Moore, and A. L. Mackay. Complementary information from multi-exponential T2 relaxation and diffusion tensor imaging reveals differences between multiple sclerosis lesions. *Magnetic Resonance Imaging*, 40:77 – 85, 2008. doi: 10.1016/j.neuroimage.2007.11.033.
- [128] P. Kozlowski, C. Lam, J. Liu, D. Raj, W. Tetzlaff, and A. C. Yung. Characterizing white matter damage in rat spinal cord with quantitative MRI and histology. *Journal of Neurotrauma*, 25(6):653–676, 2008.
- [129] P. Kozlowski, J. Liu, A. C. Yung, and W. Tetzlaff. High-Resolution Myelin Water Measurements in Rat Spinal Cord. *Magnetic Resonance in Medicine*, 59:796 – 802, 2008. doi: 10.1002/mrm.21527.
- [130] K. S. Krane. *Introductory Nuclear Physics*. Wiley, 1987. ISBN 047180553X.

- [131] K. Kuroda, T. Iwabuchi, M. Obara, M. Honda, K. Saito, and Y. Imai. Temperature dependence of relaxation times in proton components of fatty acids. *Magnetic Resonance in Medicine*, 10(3):177–83, Jan. 2011. ISSN 1880-2206. URL <http://www.ncbi.nlm.nih.gov/pubmed/21960000>.
- [132] C. Labadie, J.-H. Lee, G. Vetek, and C. S. Springer. Relaxographic Imaging. *Journal of Magnetic Resonance, Series B*, 105:99–112, 1994.
- [133] J. C. Lagarias, J. A. Reeds, M. H. Wright, and P. E. Wright. Convergence Properties of the Nelder-Mead Simplex Method in Low Dimensions. *SIAM Journal of Optimization*, 9(1):112–147, 1998.
- [134] C. Laule, I. M. Vavasour, G. R. W. Moore, J. Oger, D. K. B. Li, D. W. Paty, and A. L. MacKay. Water content and myelin water fraction in multiple sclerosis. A T2 relaxation study. *Journal of Neurology*, 251(3):284–93, Mar. 2004. ISSN 0340-5354. doi: 10.1007/s00415-004-0306-6.
- [135] C. Laule, E. Leung, D. K. B. Li, a. L. Traboulsee, D. W. Paty, a. L. MacKay, and G. R. W. Moore. Myelin water imaging in multiple sclerosis: quantitative correlations with histopathology. *Multiple Sclerosis*, 12(6):747–53, Dec. 2006. ISSN 1352-4585. URL <http://www.ncbi.nlm.nih.gov/pubmed/17263002>.
- [136] C. Laule, I. M. Vavasour, S. H. Kolind, D. K. B. Li, T. L. Traboulsee, G. R. W. Moore, and A. L. Mackay. Magnetic Resonance Imaging of Myelin. *Neurotherapeutics*, 4(July):460 – 484, 2007.
- [137] C. Laule, I. M. Vavasour, B. Madler, S. H. Kolind, S. M. Sirrs, E. E. Brief, A. L. Traboulsee, G. R. Moore, D. K. Li, and A. L. Mackay. MR evidence of long T2 water in pathological white matter. *Journal of Magnetic Resonance Imaging*, 26:1117–1121, 2007.
- [138] C. Laule, P. Kozlowski, E. Leung, D. K. B. Li, A. L. Mackay, and G. R. W. Moore. Myelin water imaging of multiple sclerosis at 7 T: Correlations with histopathology. *NeuroImage*, 40:1575 – 1580, 2008. doi: 10.1016/j.neuroimage.2007.12.008.
- [139] P. Lauterbur. Image Formation by Induced Local Interactions: Examples Employing Nuclear Magnetic Resonance. *Nature*, 242:190–191, 1973.
- [140] D. Le Bihan, E. Breton, D. Lallemand, P. Grenier, E. Cabanis, and M. Laval-Jeantet. MR Imaging of Intravoxel Incoherent Motions: Application to Diffusion and Perfusion in Neurologic Disorders. *Radiology*, 161:401–407, 1986.
- [141] J. Lee, K. Shmueli, B.-T. Kang, B. Yao, M. Fukunaga, P. van Gelderen, S. Palumbo, F. Bosetti, A. C. Silva, and J. H. Duyn. The contribution of myelin to magnetic susceptibility-weighted contrasts in high-field MRI of the brain. *NeuroImage*, 59(4):3967–75, Feb. 2012. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2011.10.076. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3288572&tool=pmcentrez&rendertype=abstract>.
- [142] J.-H. Lee and C. S. Springer. Effects of equilibrium exchange on diffusion-weighted NMR signals: the diffusigraphic "shutter-speed". *Magnetic Resonance in Medicine*, 49(3):450–8, Mar. 2003. ISSN 0740-3194. doi: 10.1002/mrm.10402. URL <http://www.ncbi.nlm.nih.gov/pubmed/12594747>.

-
- [143] A. Leemans. *Modeling and Processing of Diffusion Tensor Magnetic Resonance Images for Improved Analysis of Brain Connectivity*. PhD thesis, University of Antwerp, 2006.
 - [144] A. Leemans and D. K. Jones. The B-matrix must be rotated when correcting for subject motion in DTI data. *Magnetic Resonance in Medicine*, 61(6):1336–49, June 2009. ISSN 1522-2594. doi: 10.1002/mrm.21890. URL <http://www.ncbi.nlm.nih.gov/pubmed/19319973>.
 - [145] I. R. Levesque and G. B. Pike. Characterizing healthy and diseased white matter using quantitative magnetization transfer and multicomponent T(2) relaxometry: A unified view via a four-pool model. *Magnetic Resonance in Medicine*, 62(6):1487–96, 2009. ISSN 1522-2594. doi: 10.1002/mrm.22131. URL <http://www.ncbi.nlm.nih.gov/pubmed/19859946>.
 - [146] I. R. Levesque, P. S. Giacomini, S. Narayanan, L. T. Ribeiro, J. G. Sled, D. L. Arnold, and G. B. Pike. Quantitative magnetization transfer and myelin water imaging of the evolution of acute multiple sclerosis lesions. *Magnetic Resonance in Medicine*, 63(3):633–40, Mar. 2010. ISSN 1522-2594. doi: 10.1002/mrm.22244. URL <http://www.ncbi.nlm.nih.gov/pubmed/20146232>.
 - [147] I. R. Levesque, J. G. Sled, and G. B. Pike. Iterative optimization method for design of quantitative magnetization transfer imaging experiments. *Magnetic Resonance in Medicine*, 66(3):635–43, July 2011. ISSN 1522-2594. doi: 10.1002/mrm.23071. URL <http://www.ncbi.nlm.nih.gov/pubmed/21748796>.
 - [148] M. H. Levitt. *Spin Dynamics: Basics of Nuclear Magnetic Resonance*. Wiley, West Sussex, second edition, 2008.
 - [149] J. G. Li, S. J. Graham, and R. M. Henkelman. A Flexible Magnetization Transfer Line Shape Derived from Tissue Experimental Data. *Magnetic Resonance in Medicine*, 37: 866–871, 1997.
 - [150] Z.-P. Liang and P. C. Lauterbur. *Principles of Magnetic Resonance Imaging: A Signal Processing Perspective*. Wiley-IEEE Press, 1999. ISBN 0780347234.
 - [151] H. G. Lipinski. Monte Carlo simulation of extracellular diffusion in brain tissues. *Physics in Medicine and Biology*, 35(3):441–7, Mar. 1990. ISSN 0031-9155. URL <http://www.ncbi.nlm.nih.gov/pubmed/2320671>.
 - [152] F. Liu, L. Vidarsson, J. D. Winter, H. Tran, and A. Kassner. Sex differences in the human corpus callosum microstructure: a combined T2 myelin-water and diffusion tensor magnetic resonance imaging study. *Brain Research*, 1343:37–45, July 2010. ISSN 1872-6240. doi: 10.1016/j.brainres.2010.04.064. URL <http://www.ncbi.nlm.nih.gov/pubmed/20435024>.
 - [153] D. C. Look and D. R. Locker. Time Saving in Measurement of NMR and EPR Relaxation Times. *Review of Scientific Instruments*, 41(2):250–51, 1970. ISSN 00346748. doi: 10.1063/1.1684482. URL <http://link.aip.org/link/?RSI/41/250/1&Agg=doi>.

- [154] D. Lopes. Anisotropic Diffusion (Perona & Malik), 2006. URL <http://www.mathworks.com/matlabcentral/fileexchange/14995-anisotropic-diffusion-perona-malik>.
- [155] K.-O. Lövgren, N. Anzalone, A. Dörfler, M. Essig, B. Hurwitz, L. Kappos, S.-K. Lee, and M. Filippi. MR imaging in multiple sclerosis: review and recommendations for current practice. *American journal of neuroradiology*, 31(6):983–9, June 2010. ISSN 1936-959X. doi: 10.3174/ajnr.A1906. URL <http://www.ncbi.nlm.nih.gov/pubmed/20019103>.
- [156] A. MacKay, C. Laule, I. Vavasour, T. Bjarnason, S. Kolind, and B. Mädler. Insights into brain microstructure from the T2 distribution. *Magnetic Resonance Imaging*, 24(4):515–25, May 2006. ISSN 0730-725X. doi: 10.1016/j.mri.2005.12.037. URL <http://www.ncbi.nlm.nih.gov/pubmed/16677958>.
- [157] A. L. Mackay, K. P. Whittall, J. Adler, D. K. B. Li, D. W. Paty, and D. Graeb. In Vivo Visualization of Myelin Water in Brain by Magnetic Resonance. *Magnetic Resonance in Medicine*, 31:673–677, 1994.
- [158] S. Madi, K. M. Hasan, and P. A. Narayana. Diffusion Tensor Imaging of In Vivo and Excised Rat Spinal Cord at 7T with an Icosahedral Encoding Scheme. *Magnetic Resonance in Medicine*, 125:118–125, 2005. doi: 10.1002/mrm.20304.
- [159] B. Mädler, S. a. Drabycz, S. H. Kolind, K. P. Whittall, and A. L. MacKay. Is diffusion anisotropy an accurate monitor of myelination? Correlation of multicomponent T2 relaxation and diffusion tensor anisotropy in human brain. *Magnetic Resonance Imaging*, 26(7):874–888, Sept. 2008. ISSN 0730-725X. doi: 10.1016/j.mri.2008.01.047. URL <http://www.ncbi.nlm.nih.gov/pubmed/18524521>.
- [160] R. a. Marrie, N. Yu, J. Blanchard, S. Leung, and L. Elliott. The rising prevalence and changing age distribution of multiple sclerosis in Manitoba. *Neurology*, 74(6): 465–71, Feb. 2010. ISSN 1526-632X. doi: 10.1212/WNL.0b013e3181cf6ec0. URL <http://www.ncbi.nlm.nih.gov/pubmed/20071664>.
- [161] G. K. Matsushima and P. Morell. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathology*, 11: 107–116, 2001.
- [162] C. R. McCreary, T. A. Bjarnason, V. Skihar, J. R. Mitchell, V. W. Yong, and J. F. Dunn. Multiexponential T2 and magnetization transfer MRI of demyelination and remyelination in murine spinal cord. *NeuroImage*, 45(4):1173–1182, 2009. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2008.12.071. URL <http://dx.doi.org/10.1016/j.neuroimage.2008.12.071>.
- [163] S. Meiboom and D. Gill. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Review of Scientific Instruments*, 29(8):688, 1958. ISSN 00346748. doi: 10.1063/1.1716296. URL <http://link.aip.org/link/RSINAK/v29/i8/p688/s1&Agg=doi>.
- [164] D. Merkler, S. Boretius, C. Stadelmann, T. Ernsting, T. Michaelis, J. Frahm, W. Brück, and W. Brück. Multicontrast MRI of remyelination in the central ner-

- vous system. *NMR in Biomedicine*, 18(6):395–403, Oct. 2005. ISSN 0952-3480. doi: 10.1002/nbm.972.
- [165] E. P. Minty, T. A. Bjarnason, C. Laule, and A. L. Mackay. Myelin Water Measurement in the Spinal Cord. *Magnetic Resonance in Medicine*, 61(February):883–892, 2009. doi: 10.1002/mrm.21936.
- [166] G. R. Moore, E. Leung, a. L. MacKay, I. M. Vavasour, K. P. Whittall, K. S. Cover, D. K. Li, S. a. Hashimoto, J. Oger, T. J. Sprinkle, and D. W. Paty. A pathology-MRI study of the short-T2 component in formalin-fixed multiple sclerosis brain. *Neurology*, 55(10):1506–10, Nov. 2000. ISSN 0028-3878. URL <http://www.ncbi.nlm.nih.gov/pubmed/11094105>.
- [167] S. Mori. *Introduction to Diffusion Tensor Imaging*. Elsevier, Amsterdam, 2007.
- [168] S. Mori, P.c.m, and P. C. M. van Zijl. Fiber tracking: principles and strategies – a technical review. *NMR in Biomedicine*, 15(7-8):468–480, 2002. ISSN 0952-3480. doi: 10.1002/nbm.781.
- [169] C. Morrison and R. M. Henkelman. A Model for Magnetization Transfer in Tissues. *Magnetic Resonance in Medicine*, 33:475–482, 1995.
- [170] M. E. Moseley, Y. Cohen, J. Mintorovitch, L. Chileuitt, H. Shimizu, J. Kucharczyk, M. F. Wendland, and P. R. Weinstein. Early detection of regional cerebral ischemia in cats: Comparison of diffusion- and T2-weighted MRI and spectroscopy. *Magnetic Resonance in Medicine*, 14(2):330–346, May 1990. ISSN 07403194. doi: 10.1002/mrm.1910140218. URL <http://doi.wiley.com/10.1002/mrm.1910140218>.
- [171] G. Nair, Y. Tanahashi, H. P. Low, S. Billings-Gagliardi, W. J. Schwartz, and T. Q. Duong. Myelination and long diffusion times alter diffusion-tensor-imaging contrast in myelin-deficient shiverer mice. *NeuroImage*, 28(1):165–74, Oct. 2005. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2005.05.049.
- [172] P. Narayana, D. Fenyes, and N. Zacharopoulos. In Vivo Relaxation Times of Gray Matter and White Matter In Spinal Cord. *Magnetic Resonance Imaging*, 17(4):623–626, 1999.
- [173] M. Neema, J. Stankiewicz, A. Arora, V. S. R. Dandamudi, C. E. Batt, Z. D. Guss, A. Al-Sabbagh, and R. Bakshi. T1- and T2-based MRI measures of diffuse gray matter and white matter damage in patients with multiple sclerosis. *Journal of Neuroimaging*, 17 Suppl 1:16S–21S, Apr. 2007. ISSN 1051-2284. doi: 10.1111/j.1552-6569.2007.00131.x.
- [174] T. R. Nelson and S. M. Tung. Temperature Dependence of Proton Relaxation Times In Vitro. *Magnetic Resonance Imaging*, 5:189–199, 1987.
- [175] T. Niendorf, R. M. Dijkhuizen, D. G. Norris, M. van Lookeren Campagne, and K. Nicolay. Biexponential diffusion attenuation in various states of brain tissue: implications for diffusion-weighted imaging. *Magnetic Resonance in Medicine*, 36(6):847–57, Dec. 1996. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/8946350>.

- [176] D. G. Norris. The effects of microscopic tissue parameters on the diffusion weighted magnetic resonance imaging experiment. *NMR in Biomedicine*, 14(2):77–93, Apr. 2001. ISSN 0952-3480. doi: 10.1002/nbm.682.
- [177] E. E. Odrobina, T. Y. J. Lam, T. Pun, R. Midha, and G. J. Stanisz. MR properties of excised neural tissue following experimentally induced demyelination. *NMR in Biomedicine*, 18(5):277–284, Aug. 2005. ISSN 0952-3480. doi: 10.1002/nbm.951. URL <http://www.ncbi.nlm.nih.gov/pubmed/15948233>.
- [178] J. Oh, E. T. Han, M. C. Lee, S. J. Nelson, and D. Pelletier. Multislice Brain Myelin Water Fractions at 3T in Multiple Sclerosis. *Journal of Neuroimaging*, 17(2):156–163, 2007. doi: 10.1111/j.1552-6569.2007.00098.x.
- [179] M. C. Otaduy, D. Callegaro, L. A. Bacheschi, and C. C. Leite. Correlation of magnetization transfer and diffusion magnetic resonance imaging in multiple sclerosis. *Multiple Sclerosis*, 12(6):754–759, Nov. 2006. ISSN 1352-4585. doi: 10.1177/1352458506070824.
- [180] X. Ou, S.-w. Sun, H.-f. Liang, S.-k. Song, and D. F. Gochberg. The MT pool size ratio and the DTI radial diffusivity may reflect the myelination in shiverer and control mice. *NMR in Biomedicine*, (November), 2008. doi: 10.1002/nbm.1358.
- [181] X. Ou, S.-w. Sun, H.-f. Liang, S.-k. Song, and D. F. Gochberg. Quantitative Magnetization Transfer Measured Pool-Size Ratio Reflects Optic Nerve Myelin Content in Ex Vivo Mice. *Magnetic Resonance in Medicine*, 61:364–371, 2009. doi: 10.1002/mrm.21850.
- [182] E. Ozarslan and T. H. Mareci. Generalized diffusion tensor imaging and analytical relationships between diffusion tensor imaging and high angular resolution diffusion imaging. *Magnetic Resonance in Medicine*, 50(5):955–65, Nov. 2003. ISSN 0740-3194. doi: 10.1002/mrm.10596. URL <http://www.ncbi.nlm.nih.gov/pubmed/14587006>.
- [183] G. J. Parker, J. a. Schnabel, M. R. Symms, D. J. Werring, and G. J. Barker. Nonlinear smoothing for reduction of systematic and random errors in diffusion tensor imaging. *Journal of Magnetic Resonance Imaging*, 11(6):702–10, June 2000. ISSN 1053-1807. URL <http://www.ncbi.nlm.nih.gov/pubmed/10862071>.
- [184] A. Parry, S. Clare, M. Jenkinson, S. Smith, and J. Palace. MRI Brain T1 Relaxation Time Changes in MS Patients Increase Over Time in Both the White Matter and the Cortex. *Journal of Neuroimaging*, 13(3):234–239, 2003. doi: 10.1177/1051228403254648.
- [185] E. Parsons. Modified oscillating gradient pulses for direct sampling of the diffusion spectrum suitable for imaging sequences. *Magnetic Resonance Imaging*, 21(3-4):279–285, May 2003. ISSN 0730725X. doi: 10.1016/S0730-725X(03)00155-3. URL <http://linkinghub.elsevier.com/retrieve/pii/S0730725X03001553>.
- [186] G. Paxinos and K. B. J. Franklin. *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego, 2nd edition, 2001.
- [187] S. Peled, D. G. Cory, S. a. Raymond, D. a. Kirschner, and F. a. Jolesz. Water diffusion, T(2), and compartmentation in frog sciatic nerve. *Magnetic Resonance in Medicine*, 42(5):911–8, Nov. 1999. ISSN 0740-3194. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2826629&tool=pmcentrez&rendertype=abstract>.

- [188] P. Perona and J. Malik. Scale-Space and Edge Detection Using Anisotropic Diffusion. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 12(7):629–639, 1990.
- [189] J. R. Petrella, R. I. Grossman, J. C. McGowan, G. Campbell, and J. A. Cohen. Multiple sclerosis lesions: relationship between MR enhancement pattern and magnetization transfer effect. *American Journal of Neuroradiology*, 17(6):1041–1049, 1996. ISSN 0195-6108. URL <http://www.ncbi.nlm.nih.gov/pubmed/8791914>.
- [190] J. Pfeuffer, U. Flo, and D. Leibfritz. Monitoring of cell volume and water exchange time in perfused cells by diffusion-weighted ^1H NMR spectroscopy. *NMR in Biomedicine*, 11:11–18, 1998.
- [191] C. Pierpaoli and P. J. Basser. Toward a quantitative assessment of diffusion anisotropy. *Magnetic Resonance in Medicine*, 36(6):893–906, Dec. 1996. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/8946355>.
- [192] J. P. W. Pluim, J. B. A. Maintz, and M. A. Viergever. Mutual information based registration of medical images: a survey. *IEEE Transactions on Medical Imaging*, 22(8):986–1004, 2003.
- [193] C. S. Poon and R. M. Henkelman. Practical T2 Quantitation for Clinical Applications. *Journal of Magnetic Resonance Imaging*, 2:541–553, 1992.
- [194] S. Portnoy and G. J. Stanisz. Modeling pulsed magnetization transfer. *Magnetic Resonance in Medicine*, 58(1):144–55, July 2007. ISSN 0740-3194. doi: 10.1002/mrm.21244.
- [195] J. G. Powles, M. J. D. Mallett, G. Rickayzen, and W. A. B. Evans. Exact analytic solutions for diffusion impeded by an infinite array of partially permeable barriers. *Proceedings: Mathematical and Physical Sciences*, 436(1897):391–403, 1992.
- [196] E. M. Purcell, H. C. Torrey, and R. V. Pound. Resonance absorption by nuclear magnetic moments in a solid. *Physical Review*, pages 37–38, 1946.
- [197] D. Purves, G. J. Augustine, D. Fitzpatrick, L. C. Katz, A.-S. LaMantia, J. O. McNamara, and S. M. Williams, editors. *Neuroscience*. Sunderland, 2nd edition, 2001.
- [198] A. Ramani, C. Dalton, D. H. Miller, P. S. Tofts, and G. J. Barker. Precise estimate of fundamental in-vivo MT parameters in human brain in clinically feasible times. *Magnetic Resonance Imaging*, 20(10):721–31, Dec. 2002. ISSN 0730-725X. URL <http://www.ncbi.nlm.nih.gov/pubmed/12591568>.
- [199] M. Rausch, P. Hiestand, D. Baumann, C. Cannet, and M. Rudin. MRI-based monitoring of inflammation and tissue damage in acute and chronic relapsing EAE. *Magnetic Resonance in Medicine*, 50(2):309–14, Aug. 2003. ISSN 0740-3194. doi: 10.1002/mrm.10541.
- [200] M. Rausch, P. Tofts, P. Lervik, A. Walmsley, A. Mir, A. Schubart, and T. Seabrook. Characterization of white matter damage in animal models of multiple sclerosis by magnetization transfer ratio and quantitative mapping of the apparent bound proton fraction f . *Multiple Sclerosis*, 15(1):16–27, Jan. 2009. ISSN 1352-4585. doi: 10.1177/1352458508096006. URL <http://www.ncbi.nlm.nih.gov/pubmed/18971220>.

- [201] D. S. Reich, S. a. Smith, K. M. Zackowski, E. M. Gordon-Lipkin, C. K. Jones, J. a. D. Farrell, S. Mori, P. C. M. van Zijl, and P. a. Calabresi. Multiparametric magnetic resonance imaging analysis of the corticospinal tract in multiple sclerosis. *NeuroImage*, 38(2):271–279, Nov. 2007. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2007.07.049. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2082136&tool=pmcentrez&rendertype=abstract>.
- [202] L. T. Remington, A. a. Babcock, S. P. Zehntner, and T. Owens. Microglial recruitment, activation, and proliferation in response to primary demyelination. *The American Journal of Pathology*, 170(5):1713–1724, May 2007. ISSN 0002-9440. doi: 10.2353/ajpath.2007.060783. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1854965&tool=pmcentrez&rendertype=abstract>.
- [203] B. H. Ridha, D. J. Tozer, M. R. Symms, K. C. Stockton, E. B. Lewis, D. G. Macmanus, M. N. Rossor, N. C. Fox, and P. S. Tofts. Quantitative Magnetization Transfer Imaging in Alzheimer Disease. *Radiology*, 244(3):832–837, 2007.
- [204] I. Ronen, S. Moeller, K. Ugurbil, and D.-S. Kim. Investigation of multicomponent diffusion in cat brain using a combined MTC-DWI approach. *Magnetic Resonance Imaging*, 24(4):425–31, May 2006. ISSN 0730-725X. doi: 10.1016/j.mri.2005.12.022. URL <http://www.ncbi.nlm.nih.gov/pubmed/16677949>.
- [205] W. D. Rooney, G. Johnson, X. Li, E. R. Cohen, S.-G. Kim, K. Ugurbil, and C. S. Springer. Magnetic field and tissue dependencies of human brain longitudinal $^1\text{H}_2\text{O}$ relaxation in vivo. *Magnetic Resonance in Medicine*, 57(2):308–18, Feb. 2007. ISSN 0740-3194. doi: 10.1002/mrm.21122. URL <http://www.ncbi.nlm.nih.gov/pubmed/17260370>.
- [206] M. Rovaris, F. Agosta, M. P. Sormani, M. Inglese, V. Martinelli, G. Comi, and M. Filippi. Conventional and magnetization transfer MRI predictors of clinical multiple sclerosis evolution: a medium-term follow-up study. *Brain*, 126(10):2323–32, Oct. 2003. ISSN 0006-8950. doi: 10.1093/brain/awg232.
- [207] M. A. Sahraian and A. Eshaghi. Role of MRI in diagnosis and treatment of multiple sclerosis. *Clinical Neurology and Neurosurgery*, 112(7):609–615, Apr. 2010. ISSN 1872-6968. doi: 10.1016/j.clineuro.2010.03.022. URL <http://www.ncbi.nlm.nih.gov/pubmed/20417027>.
- [208] M. Schachter. *Development of Methods to Assess Water Compartmentation and Restricted Diffusion Using Oscillating Gradient Nuclear Magnetic Resonance*. Phd, Yale, 2000.
- [209] M. Schachter, M. D. Does, a. W. Anderson, and J. C. Gore. Measurements of restricted diffusion using an oscillating gradient spin-echo sequence. *Journal of Magnetic Resonance*, 147(2):232–7, Dec. 2000. ISSN 1090-7807. doi: 10.1006/jmre.2000.2203. URL <http://www.ncbi.nlm.nih.gov/pubmed/11097814>.
- [210] A. E. Schellenberg, R. Buist, V. W. Yong, and M. R. D. Bigio. Magnetic Resonance Imaging of Blood–Spinal Cord Barrier Disruption in Mice With Experimental Autoimmune Encephalomyelitis. *Magnetic Resonance in Medicine*, 58:298–305, 2007. doi: 10.1002/mrm.21289.

- [211] K. Schmierer, D. J. Tozer, F. Scaravilli, D. R. Altmann, G. J. Barker, P. S. Tofts, D. H. Miller, and D. R. Quantitative Magnetization Transfer Imaging in Postmortem Multiple Sclerosis Brain. *Journal of Magnetic Resonance Imaging*, 26(1):41–51, 2007.
- [212] K. Schmierer, C. a. M. Wheeler-Kingshott, D. J. Tozer, P. a. Boulby, H. G. Parkes, T. a. Yousry, F. Scaravilli, G. J. Barker, P. S. Tofts, and D. H. Miller. Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. *Magnetic Resonance in Medicine*, 59(2):268–77, Feb. 2008. ISSN 0740-3194. doi: 10.1002/mrm.21487. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2241759&tool=pmcentrez&rendertype=abstract>.
- [213] P. N. Sen and P. J. Basser. Modeling diffusion in white matter in the brain: a composite porous medium. *Magnetic Resonance Imaging*, 23(2):215–20, 2005. ISSN 0730-725X. doi: 10.1016/j.mri.2004.11.014. URL <http://www.ncbi.nlm.nih.gov/pubmed/15833615>.
- [214] T. M. Shepherd, P. E. Thelwall, G. J. Stanisz, and S. J. Blackband. Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*, 62(1):26–34, July 2009. ISSN 1522-2594. doi: 10.1002/mrm.21977. URL <http://www.ncbi.nlm.nih.gov/pubmed/19353660>.
- [215] R. L. Sidman and M. K. Wolf. Myelin Deficient (shimld) Mutant Allele: Morphological Comparison with Shiverer (shi) Allele on a B6C3 Mouse Stock. *Brain Research*, 360: 235–247, 1985.
- [216] S. Skare, M. Hedehus, M. E. Moseley, and T. Q. Li. Condition number as a measure of noise performance of diffusion tensor data acquisition schemes with MRI. *Journal of Magnetic Resonance*, 147(2):340–52, Dec. 2000. ISSN 1090-7807. doi: 10.1006/jmre.2000.2209.
- [217] R. Skoff. Morphology of Oligodendrocytes and Myelin. In R. M. Herndon, editor, *Multiple Sclerosis: Immunology, Pathology and Pathophysiology*, pages 7–23. Demos, New York, 2003.
- [218] T. Skripuletz, V. Gudi, D. Hackstette, and M. Stangel. De- and remyelination in the CNS white and grey matter induced by cuprizone: The old, the new, and the unexpected. *Histology and Histopathology*, 26:1585–1597, 2011.
- [219] J. G. Sled and G. B. Pike. Quantitative interpretation of magnetization transfer in spoiled gradient echo MRI sequences. *Journal of Magnetic Resonance*, 145(1):24–36, July 2000. ISSN 1090-7807. doi: 10.1006/jmre.2000.2059. URL <http://www.ncbi.nlm.nih.gov/pubmed/10873494>.
- [220] J. G. Sled and G. B. Pike. Quantitative imaging of magnetization transfer exchange and relaxation properties in vivo using MRI. *Magnetic Resonance in Medicine*, 46: 923–931, 2001.
- [221] J. G. Sled, I. Levesque, a. C. Santos, S. J. Francis, S. Narayanan, S. D. Brass, D. L. Arnold, and G. B. Pike. Regional variations in normal brain shown by quantitative magnetization transfer imaging. *Magnetic Resonance in Medicine*, 51(2):299–303, Feb. 2004. ISSN 0740-3194. doi: 10.1002/mrm.10701. URL <http://www.ncbi.nlm.nih.gov/pubmed/14755655>.

- [222] G. Song, W. Xiaoying, and B. Shanglian. Effects of diffusion time and diffusion gradient strength on the mean diffusivity of water molecules in healthy human brain. *Progress in Natural Science*, 16(7):706–711, 2006.
- [223] S. Song, S. Sun, M. Ramsbottom, C. Chang, J. Russell, and a. Cross. Demyelination Revealed through MRI as Increased Radial (but Unchanged Axial) Diffusion of Water. *NeuroImage*, 17(3):1429–1436, Nov. 2002. ISSN 10538119. doi: 10.1006/nimg.2002.1267. URL <http://linkinghub.elsevier.com/retrieve/pii/S105381190291267X>.
- [224] S.-K. Song, J. Yoshino, T. Q. Le, S.-J. Lin, S.-W. Sun, A. H. Cross, and R. C. Armstrong. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *NeuroImage*, 26(1):132–40, 2005. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2005.01.028.
- [225] B. Spanò, M. Cercignani, B. Basile, S. Romano, R. Mannu, D. Centonze, C. Caltagirone, P. Bramanti, U. Nocentini, and M. Bozzali. Multiparametric MR investigation of the motor pyramidal system in patients with 'truly benign' multiple sclerosis. *Multiple Sclerosis*, 16(2):178–88, Mar. 2010. ISSN 1477-0970. doi: 10.1177/1352458509356010. URL <http://www.ncbi.nlm.nih.gov/pubmed/20007429>.
- [226] G. J. Stanisz and R. M. Henkelman. Diffusional Anisotropy of T2 Components in Bovine Optic Nerve. *Magnetic Resonance in Medicine*, 40:405–410, 1998.
- [227] G. J. Stanisz, A. Szafer, G. A. Wright, and R. M. Henkelman. An Analytical Model of Restricted Diffusion in Bovine Optic Nerve. *Magnetic Resonance in Medicine*, 37: 103–111, 1997.
- [228] G. J. Stanisz, A. Kecojevic, M. J. Bronskill, and R. M. Henkelman. Characterizing white matter with magnetization transfer and T2. *Magnetic Resonance in Medicine*, 42(August):1128–1136, 1999.
- [229] G. J. Stanisz, S. Webb, C. a. Munro, T. Pun, and R. Midha. MR properties of excised neural tissue following experimentally induced inflammation. *Magnetic Resonance in Medicine*, 51(3):473–479, Mar. 2004. ISSN 0740-3194. doi: 10.1002/mrm.20008. URL <http://www.ncbi.nlm.nih.gov/pubmed/15004787>.
- [230] G. J. Stanisz, E. E. Odrobina, J. Pun, M. Escaravage, S. J. Graham, M. J. Bronskill, and R. M. Henkelman. T1, T2 relaxation and magnetization transfer in tissue at 3T. *Magnetic Resonance in Medicine*, 54(3):507–12, Sept. 2005. ISSN 0740-3194. doi: 10.1002/mrm.20605.
- [231] D. G. Steel and D. Holt. Analysing and Adjusting Aggregation Effects: The Ecological Fallacy Revisited. *International Statistical Review*, 64(1):39–60, 1996.
- [232] E. O. Stejskal and J. E. Tanner. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient. *The Journal of Chemical Physics*, 42 (1):288–292, 1965.
- [233] M. F. Stidworthy, S. Genoud, U. Suter, N. Mantei, and R. J. M. Franklin. Quantifying the early stages of remyelination following cuprizone-induced demyelination. *Brain Pathology*, 13(3):329–39, July 2003. ISSN 1015-6305. URL <http://www.ncbi.nlm.nih.gov/pubmed/12946022>.

- [234] N. Stikov, L. M. Perry, A. Mezer, E. Rykhlevskaia, B. a. Wandell, J. M. Pauly, and R. F. Dougherty. Bound pool fractions complement diffusion measures to describe white matter micro and macrostructure. *NeuroImage*, 54(2):1112–1121, Sept. 2011. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2010.08.068. URL <http://www.ncbi.nlm.nih.gov/pubmed/20828622>.
- [235] R. R. Sturrock. Myelination of the Mouse Corpus Callosum. *Neuropathology and Applied Neurobiology*, 6:415–420, 1980.
- [236] A. L. Sukstanskii, D. A. Yablonskiy, and J. J. H. Ackerman. Effects of permeable boundaries on the diffusion-attenuated MR signal: insights from a one-dimensional model. *Journal of Magnetic Resonance*, 170(1):56–66, Sept. 2004. ISSN 1090-7807. doi: 10.1016/j.jmr.2004.05.020.
- [237] E. V. Sullivan and A. Pfefferbaum. Diffusion tensor imaging in normal aging and neuropsychiatric disorders. *European Journal of Radiology*, 45:244–255, 2003.
- [238] S.-W. Sun, J. J. Neil, and S.-K. Song. Relative indices of water diffusion anisotropy are equivalent in live and formalin-fixed mouse brains. *Magnetic Resonance in Medicine*, 50(4):743–8, Oct. 2003. ISSN 0740-3194. doi: 10.1002/mrm.10605. URL <http://www.ncbi.nlm.nih.gov/pubmed/14523960>.
- [239] S.-W. Sun, H.-F. Liang, M. Xie, U. Oyoyo, and A. Lee. Fixation, not death, reduces sensitivity of DTI in detecting optic nerve damage. *NeuroImage*, 44(3):611–9, Feb. 2009. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2008.10.032. URL <http://www.ncbi.nlm.nih.gov/pubmed/19027864>.
- [240] S.-W. W. Sun, H.-F. F. Liang, K. Trinkaus, A. H. Cross, R. C. Armstrong, and S.-K. K. Song. Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. *Magnetic Resonance in Medicine*, 55(2):302–308, Feb. 2006. ISSN 0740-3194. doi: 10.1002/mrm.20774.
- [241] A. Szafer, J. Zhong, and J. C. Gore. Theoretical Model for Water Diffusion in Tissues. *Magnetic Resonance in Medicine*, 33:697–712, 1995.
- [242] S. Takahashi, H. Yonezawa, J. Takahashi, M. Kudo, T. Inoue, and H. Tohgi. Selective reduction of diffusion anisotropy in white matter of Alzheimer disease brains measured by 3.0 Tesla magnetic resonance imaging. *Neuroscience Letters*, 332:45–48, 2002.
- [243] P. E. Thelwall, T. M. Shepherd, G. J. Stanisz, and S. J. Blackband. Effects of temperature and aldehyde fixation on tissue water diffusion properties, studied in an erythrocyte ghost tissue model. *Magnetic Resonance in Medicine*, 56(2):282–9, Aug. 2006. ISSN 0740-3194. doi: 10.1002/mrm.20962. URL <http://www.ncbi.nlm.nih.gov/pubmed/16841346>.
- [244] D. L. Thomas, M. F. Lythgoe, G. S. Pell, F. Calamante, and R. J. Ordidge. The measurement of diffusion and perfusion in biological systems using magnetic resonance imaging. *Physics in Medicine and Biology*, 45:97–138, 2000.
- [245] O. Torkildsen, L. A. Brunborg, K.-M. Myhr, and L. Bo. The cuprizone model for demyelination. *Acta Neurologica Scandinavica*, 117(4):72–76, 2008. doi: 10.1111/j.1600-0404.2008.01036.x.

- [246] O. i. Torkildsen, L. Anne, F. Thorsen, S. Jarl, M. Stangel, K.-m. Myhr, and L. Bø. Effects of dietary intervention on MRI activity , de- and remyelination in the cuprizone model for demyelination. *Experimental Neurology*, 215(1):160–166, 2009. ISSN 0014-4886. doi: 10.1016/j.expneurol.2008.09.026. URL <http://dx.doi.org/10.1016/j.expneurol.2008.09.026>.
- [247] H. C. Torrey. Bloch Equations with Diffusion Terms. *Physical Review*, 104(3):563–565, 1956.
- [248] D. J. Tozer, A. Ramani, G. J. Barker, G. R. Davies, D. H. Miller, and P. S. Tofts. Quantitative magnetization transfer mapping of bound protons in multiple sclerosis. *Magnetic Resonance in Medicine*, 50(1):83–91, July 2003. ISSN 0740-3194. doi: 10.1002/mrm.10514.
- [249] D. J. Tozer, G. R. Davies, D. R. Altmann, D. H. Miller, and P. S. Tofts. Correlation of apparent myelin measures obtained in multiple sclerosis patients and controls from magnetization transfer and multicompartamental T2 analysis. *Magnetic Resonance in Medicine*, 53(6):1415–1422, July 2005. ISSN 0740-3194. doi: 10.1002/mrm.20479. URL <http://www.ncbi.nlm.nih.gov/pubmed/15906291>.
- [250] D. J. Tozer, P. S. Tofts, M. Cercignani, M. R. Symms, G. R. Davies, A. Ramani, and G. J. Barker. Correction: Quantitative Magnetization Transfer Mapping of Bound Protons in Multiple Sclerosis. *Magnetic Resonance in Medicine*, 53(2):492–493, Feb. 2005. ISSN 0740-3194. doi: 10.1002/mrm.20370.
- [251] D. S. Tuch. *Diffusion MRI of Complex Tissue Structure*. PhD thesis, MIT, 2002.
- [252] H. R. Underhill, C. Yuan, and V. L. Yarnykh. Direct quantitative comparison between cross-relaxation imaging and diffusion tensor imaging of the human brain at 3.0 T. *NeuroImage*, 47(4):1568–78, Oct. 2009. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2009.05.075. URL <http://www.ncbi.nlm.nih.gov/pubmed/19500678>.
- [253] H. R. Underhill, R. C. Rostomily, A. M. Mikheev, C. Yuan, and V. L. Yarnykh. Fast bound pool fraction imaging of the in vivo rat brain: association with myelin content and validation in the C6 glioma model. *NeuroImage*, 54(3):2052–2065, Feb. 2011. ISSN 1095-9572. doi: 10.1016/j.neuroimage.2010.10.065. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3008309&tool=pmcentrez&rendertype=abstract>.
- [254] M. A. van Buchem, J. C. McGowan, D. L. Kolson, M. Polansky, and R. I. Grossman. Quantitative Volumetric Magnetization Transfer Analysis in Multiple Sclerosis : Estimation of Macroscopic and Microscopic Disease Burden. *Magnetic Resonance in Medicine*, 36:632–636, 1996.
- [255] L. van Der Weerd, S. M. Melnikov, F. J. Vergeldt, E. G. Novikov, and H. Van As. Modelling of Self-diffusion and Relaxation Time NMR in Multicompartment Systems with Cylindrical Geometry. *Journal of Magnetic Resonance*, 156(2):213–221, June 2002. ISSN 10907807. doi: 10.1006/jmre.2002.2550.
- [256] D. van Dusschoten. Unraveling Diffusion Constants in Biological Tissue by Combining Carr-Purcell-Meiboom-Gill Imaging and Pulsed Field Gradient NMR. *Magnetic Resonance in Medicine*, 36:907–913, 1996.

-
- [257] I. M. Vavasour, K. P. Whittall, a. L. MacKay, D. K. Li, G. Vorobeychik, and D. W. Paty. A comparison between magnetization transfer ratios and myelin water percentages in normals and multiple sclerosis patients. *Magnetic Resonance in Medicine*, 40(5):763–8, Nov. 1998. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/9797161>.
 - [258] I. M. Vavasour, K. P. Whittall, D. K. Li, and a. L. MacKay. Different magnetization transfer effects exhibited by the short and long T(2) components in human brain. *Magnetic Resonance in Medicine*, 44(6):860–6, Dec. 2000. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/11108622>.
 - [259] I. M. Vavasour, C. M. Clark, D. K. B. Li, and A. L. Mackay. Reproducibility and reliability of MR measurements in white matter: clinical implications. *NeuroImage*, 32(2):637–42, Aug. 2006. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2006.03.036. URL <http://www.ncbi.nlm.nih.gov/pubmed/16677833>.
 - [260] I. M. Vavasour, C. Laule, D. K. B. Li, A. L. Traboulsee, and A. L. MacKay. Is the magnetization transfer ratio a marker for myelin in multiple sclerosis? *Journal of Magnetic Resonance Imaging*, 33(3):713–8, Mar. 2011. ISSN 1522-2586. doi: 10.1002/jmri.22441. URL <http://www.ncbi.nlm.nih.gov/pubmed/21563257>.
 - [261] Y. Wang, Q. Wang, J. P. Halder, F.-C. Yeh, M. Xie, P. Sun, T.-W. Tu, K. Trinkaus, R. S. Klein, A. H. Cross, and S.-K. Song. Quantification of increased cellular-ity during inflammatory demyelination. *Brain*, 134(12):3590–601, Dec. 2011. ISSN 1460-2156. doi: 10.1093/brain/awr307. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3235568&tool=pmcentrez&rendertype=abstract>.
 - [262] S. Warach, J. Gaa, B. Siewert, P. Wielopolski, and R. R. Edelman. Acute human stroke studied by whole brain echo planar diffusion-weighted magnetic resonance imaging. *Annals of Neurology*, 37:231–241, 1995.
 - [263] S. Webb, C. a. Munro, R. Midha, and G. J. Stanis. Is multicomponent T2 a good measure of myelin content in peripheral nerve? *Magnetic Resonance in Medicine*, 49(4):638–645, Apr. 2003. ISSN 0740-3194. doi: 10.1002/mrm.10411. URL <http://www.ncbi.nlm.nih.gov/pubmed/12652534>.
 - [264] C.-F. Westin, S. E. Maier, H. Mamata, A. Nabavi, F. a. Jolesz, and R. Kikinis. Processing and visualization for diffusion tensor MRI. *Medical Image Analysis*, 6(2): 93–108, June 2002. ISSN 1361-8415. URL <http://www.ncbi.nlm.nih.gov/pubmed/12044998>.
 - [265] K. Whittall and A. Mackay. Quantitative interpretation of NMR relaxation data. *Journal of Magnetic Resonance*, 84(1):134–152, Aug. 1989. ISSN 00222364. doi: 10.1016/0022-2364(89)90011-5. URL <http://linkinghub.elsevier.com/retrieve/pii/0022236489900115>.
 - [266] K. P. Whittall, a. L. MacKay, D. a. Graeb, R. a. Nugent, D. K. Li, and D. W. Paty. In vivo measurement of T2 distributions and water contents in normal human brain. *Magnetic Resonance in Medicine*, 37(1):34–43, Jan. 1997. ISSN 0740-3194. URL <http://www.ncbi.nlm.nih.gov/pubmed/8978630>.

- [267] Q.-z. Wu, Q. Yang, H. S. Cate, D. Kemper, M. Binder, H.-x. Wang, K. Fang, M. J. Quick, M. Marriott, T. J. Kilpatrick, and G. F. Egan. MRI Identification of the Rostral-Caudal Pattern of Pathology Within the Corpus Callosum in the Cuprizone Mouse Model. *Journal of Magnetic Resonance Imaging*, 27:446 – 453, 2008. doi: 10.1002/jmri.21111.
- [268] X. Wu. Lineshape of magnetization transfer via cross relaxation. *Journal of Magnetic Resonance*, 94(1):186–190, Aug. 1991. ISSN 00222364. doi: 10.1016/0022-2364(91)90308-G. URL <http://linkinghub.elsevier.com/retrieve/pii/002223649190308G>.
- [269] L. Xiao, H. Xu, Y. Zhang, Z. Wei, J. He, W. Jiang, X. Li, L. E. Dyck, R. M. Devon, Y. Deng, and X. M. Li. Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. *Molecular Psychiatry*, 13(7):697–708, July 2008. ISSN 1476-5578. doi: 10.1038/sj.mp.4002064. URL <http://www.ncbi.nlm.nih.gov/pubmed/17684494>.
- [270] M. Xie, J. E. Tobin, M. D. Budde, C.-I. Chen, K. Trinkaus, A. H. Cross, D. P. McDaniel, S.-K. Song, and R. C. Armstrong. Rostrocaudal Analysis of Corpus Callosum Demyelination and Axon Damage Across Disease Stages Refines Diffusion Tensor Imaging Correlations With Pathological Features. *Journal of Neuropathology and Experimental Neurology*, 69(7):704–716, July 2010. ISSN 0022-3069. doi: 10.1097/NEN.0b013e3181e3de90. URL <http://www.ncbi.nlm.nih.gov/pubmed/20535036>.
- [271] J. Xu, M. D. Does, and J. C. Gore. Quantitative characterization of tissue microstructure with temporal diffusion spectroscopy. *Journal of Magnetic Resonance*, 200(2):189–97, Oct. 2009. ISSN 1096-0856. doi: 10.1016/j.jmr.2009.06.022. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2758625&tool=pmcentrez&rendertype=abstract>.
- [272] H.-J. Yang, H. Wang, Y. Zhang, L. Xiao, R. W. Clough, R. Browning, X.-m. Li, and H. Xu. Region-specific susceptibilities to cuprizone-induced lesions in the mouse forebrain : Implications for the pathophysiology of schizophrenia. *Brain Research*, 1270:121–130, 2009. ISSN 0006-8993. doi: 10.1016/j.brainres.2009.03.011. URL <http://dx.doi.org/10.1016/j.brainres.2009.03.011>.
- [273] V. L. Yarnykh. Pulsed Z-Spectroscopic Imaging of Cross-Relaxation Parameters in Tissues for Human MRI : Theory and Clinical Applications. *Magnetic Resonance in Medicine*, 47(5):929 – 939, May 2002. ISSN 0740-3194. doi: 10.1002/mrm.10120. URL <http://www.ncbi.nlm.nih.gov/pubmed/11979572>.
- [274] V. L. Yarnykh and C. Yuan. Cross-relaxation imaging reveals detailed anatomy of white matter fiber tracts in the human brain. *NeuroImage*, 23(1):409–24, Sept. 2004. ISSN 1053-8119. doi: 10.1016/j.neuroimage.2004.04.029. URL <http://www.ncbi.nlm.nih.gov/pubmed/15325389>.
- [275] O. Yu, J. Steibel, Y. Mauss, B. Guignard, B. Eclancher, J. Chambron, and D. Grucker. Remyelination assessment by MRI texture analysis in a cuprizone mouse model. *Magnetic Resonance Imaging*, 22:1139 – 1144, 2004. doi: 10.1016/j.mri.2004.08.017.

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- [276] W. Zaaraoui, M. Deloire, M. Merle, C. Girard, G. Raffard, M. Biran, M. Inglese, K. G. Petry, O. Gonen, B. Brochet, and J.-m. F. Vincent. Monitoring demyelination and remyelination by magnetization transfer imaging in the mouse brain at 9.4 T. *Magnetic Resonance Materials In Physics, Biology, And Medicine*, 21:357–362, 2008. doi: 10.1007/s10334-008-0141-3.
- [277] J. Zhang, M. V. Jones, M. T. McMahon, S. Mori, and P. a. Calabresi. In vivo and ex vivo diffusion tensor imaging of cuprizone-induced demyelination in the mouse corpus callosum. *Magnetic Resonance in Medicine*, 67(3):750–9, June 2012. ISSN 1522-2594. doi: 10.1002/mrm.23032. URL <http://www.ncbi.nlm.nih.gov/pubmed/21656567>.
- [278] Y. Zhang, H. Xu, W. Jiang, L. Xiao, B. Yan, J. He, Y. Wang, X. Bi, X. Li, J. Kong, and X.-M. Li. Quetiapine alleviates the cuprizone-induced white matter pathology in the brain of C57BL/6 mouse. *Schizophrenia Research*, 106(2-3):182–91, Dec. 2008. ISSN 0920-9964. doi: 10.1016/j.schres.2008.09.013. URL <http://www.ncbi.nlm.nih.gov/pubmed/18938062>.