

Advances in Myelin-Sensitive Quantitative MR Imaging for Alzheimer's Disease Applications

by

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ABSTRACT

Intact myelin is critical to healthy brain function. Many quantitative MRI (qMRI) techniques aim to quantify myelin content, including myelin water fraction (MWF) and magnetization transfer (MT) parameter mapping. This dissertation explores several open questions related to understanding and improving myelin characterization using qMRI, and is organized into three parts:

Myelin-sensitive qMRI parameter mapping is often conducted on formalin-fixed ex vivo tissue samples to enable validation with histology. Part 1 investigates the impact of tissue sample preparation (freezing vs. fixation) on these parameter estimates.

As accumulating evidence suggests that significant myelin degradation occurs in Alzheimer's disease and related dementias (ADRD), it is important to understand how ADRD histopathological features influence MR estimates of myelin content. Part 2 examines the specificity of existing qMRI parameters to ADRD pathology in ex vivo samples by comparing these parameters to histological markers of myelin content, axonal density, and oligodendrocyte count.

Beyond myelin content quantification, another tissue property of interest is myelin water exchange, which may be a complementary biomarker to MWF. Part 3 describes the development of a fast method for mapping MWF and exchange using steady-state MRI.

Together, these investigations contribute to advancing myelin-sensitive qMRI techniques, specifically in the context of ADRD.

CHAPTER 1

Introduction

Magnetic Resonance Imaging (MRI) is a non-invasive imaging modality that is useful for studying soft-tissue contrast in the body. Traditional MR images, such as in Figure 1.1, are qualitative, where images with different contrasts can be generated by using different MR sequences and scan parameters. It is a powerful tool for diagnosis; but the common clinical T_1 and T_2 weighted scans are not specific for diagnosis, for example, they cannot diagnose the cause of the characteristic T_2 lesions that indicate demyelination and brain white matter (WM) degradation [18]. In particular, white matter T_2 hyperintensities are clinically ambiguous and are not specific to a particular pathology, as illustrated in Figure 1.2. These lesions are seen in multiple sclerosis (MS), Alzheimer’s disease and related dementias (ADRD), and older adults undergoing healthy aging, so there is a strong need for better characterization.

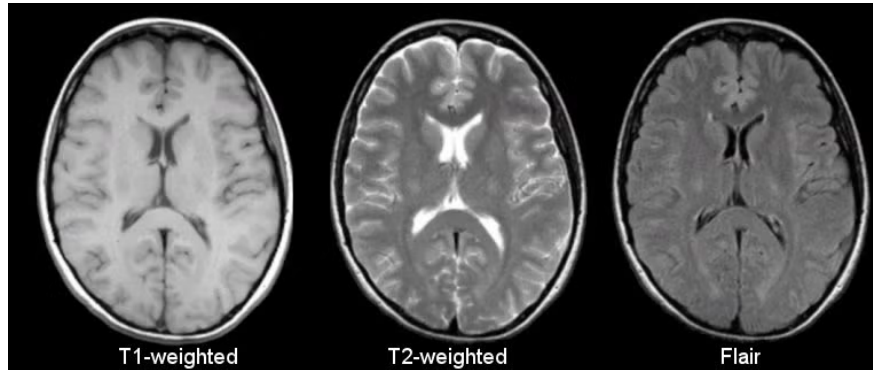


Figure 1.1: Sample T_1 -weighted, T_2 -weighted, and FLAIR qualitative MR images from [Case Western Reserve University MRI Basics](#).

Quantitative MRI (qMRI) aims to solve this limitation by acquiring images with different contrasts and conducting post-processing to create quantitative maps of tissue parameters, such as T_1 , T_2 , and M_0 in Figure 1.3. Quantitative MRI can also be helpful in obtaining information about tissue microstructure. MRI voxels (3D pixels) are usually on the order of 1mm^3 , but myelin is on a μm scale. This means that in one voxel there are often multiple tissue types with multiple T_1 and

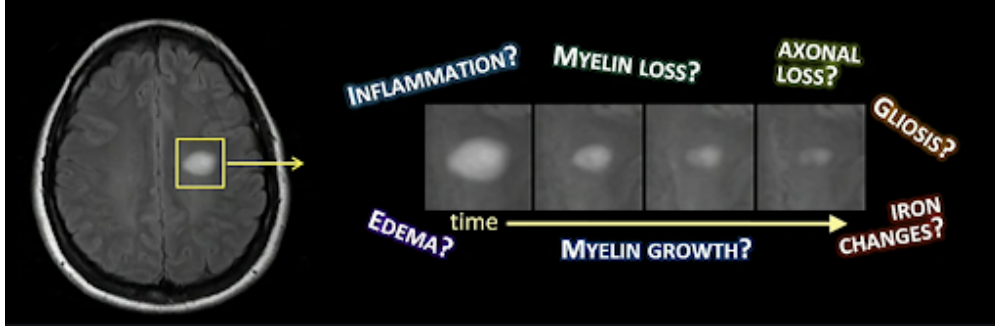


Figure 1.2: Adapted from Dr. Cornelia Laule, UBC, ISMRM 2024, Quantitative Neuroimaging in the Era of Precision Health & Personalized Medicine Weekend Course: Quantitative Imaging in Multiple Sclerosis. T_2 hyperintensities can be seen in contrast weighted images; however the scan is not specific to a particular pathology.

T_2 values. Specifically for brain imaging, a two-pool model assumes that there is a free water pool with longer T_1 and T_2 values and a myelin water pool with shorter T_1 and T_2 values. Quantitative MRI can be used to estimate the relaxation properties of the two pools and the fraction of the total MR signal originating from each pool; two common methods include myelin water imaging and magnetization transfer imaging.

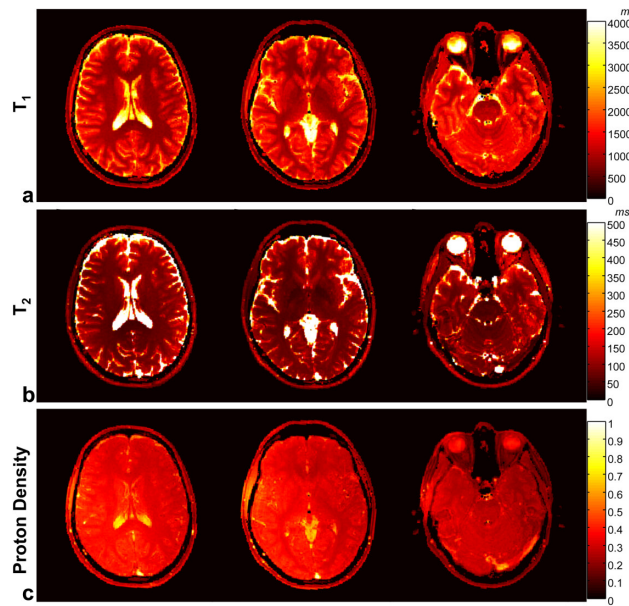


Figure 1.3: Image from [1]. T_1 , T_2 , and proton density quantitative MRI maps where the value at each voxel represents a specific quantity. They are created by conducting post-processing on a set of different contrast-weighted images; in particular, these maps were generated using MR fingerprinting [2].

Myelin water imaging (MWI) assumes that there are multiple tissue compartments and that the MRI signal is a superposition of water signals in different compartments, leading to multi-exponential decay [19]. The standard MWI experiment determines the relative signal contribution from each T_2 component. The fraction of the signal below a T_2 cutoff between the two peaks is defined as the myelin water fraction (MWF), which has been histologically validated and accepted as a measure of myelin content [8, 20–24]. Figure 1.4 shows an in vivo MWF map of a healthy subject from a prior study [3]; higher MWF values correspond to higher myelin content.

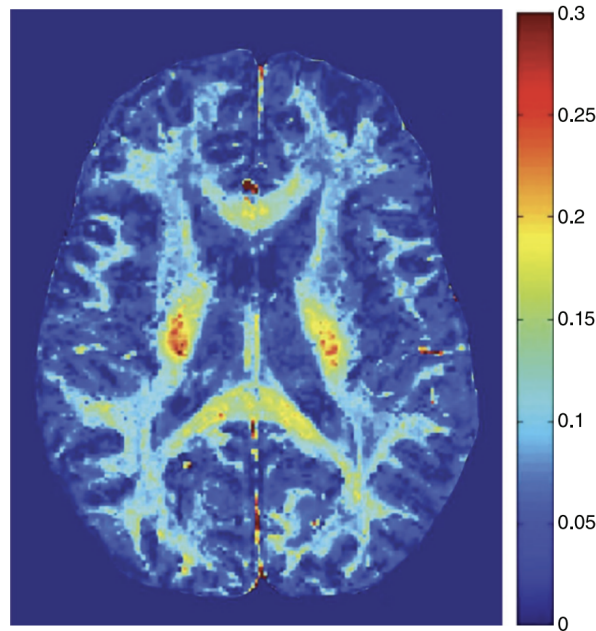


Figure 1.4: Image from [3]. Healthy in vivo MWF map.

Magnetization transfer (MT) imaging of brain white matter [10, 25, 26] depends on the fact that MT in myelin is dominated by exchange of water and macromolecular protons that have T_2 times of order $10\ \mu\text{s}$ and decay too fast to be observed using typical MRI sequences. Conventional MT imaging applies an MT preparation pulse far off-resonance from the water signal, directly saturating the macromolecular proton longitudinal magnetization. The semi-quantitative magnetization transfer ratio (MTR), the difference between the images obtained with and without the preparation pulse, normalized by the image without the preparation pulse [27], has been previously validated in tissue samples for its sensitivity to myelin content [28, 29]. Images acquired after applying preparation pulses at multiple RF power levels and off-resonance frequencies are fit to a quantitative MT (qMT) signal model to calculate the fractional size of the bound pool (F) [7, 17], which is another accepted measure of myelin content [30–32]. Inversion recovery MT experiments can also provide the macromolecular pool fraction [11, 33–35].

We are interested in calculating these qMRI measures for tissue samples with ADRD pathology. Specifically for ADRD, in addition to the hallmark β -amyloid and tau pathological changes [36], many other pathologies co-exist, including ones that affect WM tracts through ischemia, inflammation, or direct oligodendrocyte cytotoxicity. These WM lesions could be caused by gliosis, axonal loss, and demyelination, and there is accumulating evidence that myelin degradation is also an important pathological change in ADRD [37–39]. Due to the co-existence of underlying tissue pathologies, MR measures that are more specific to these pathologies would be valuable for diagnosing and monitoring ADRD. As mentioned above, qMRI has become a useful tool for making MR more specific, and using it to study ADRD tissue pathology [40, 41] may assist in understanding the effects of histopathological features in ADRD on MR measures of myelin.

In the literature, when qMRI is conducted on tissue samples, the tissue is usually fixed; however, the samples at the Michigan Alzheimer’s Disease Research Center (MADRC) brain bank are frozen at -80°C . We wanted to know whether the tissue preservation method (freezing vs. fixation) would significantly affect quantitative measures of myelin content, so we conducted a study where we scanned sheep brain tissue samples at three different tissue conditions: fresh, frozen, and subsequently thawed, and fixed. Then we scanned MADRC tissue samples to relate qMRI parameters in these ADRD samples to histological quantities of myelin content, axonal density, and oligodendrocyte count.

Despite their usefulness for myelin quantification and correlation to histology, traditional MWI and MT qMR imaging scans have extremely long scan times, making them impractical for clinical use. In addition, traditional MWI models the signals of multiple tissue compartments, but ignores exchange among these compartments, which has been theorized to bias MWF values [42]. Due to these limitations, we performed a preliminary investigation of estimating MWF and exchange parameters by fitting data acquired from faster balanced steady-state free precession (bSSFP) scans to an exchanging two-pool signal model. Since bSSFP signals are very sensitive to flip angle and off-resonance [43], we vary the flip angle and phase cycling factor (PCF) scan parameters to generate various contrast images to use in this model fitting process. A long-term goal of this work is to optimize the scan design (selection of the various flip angles and PCFs) for jointly estimating MWF and exchange parameters and quantify tissue myelin content in the ADRD samples.

The rest of the thesis (including my contributions, which are contained in Chapters 3-5 and in the provided citations) is organized as follows:

- Chapter 2 explains relevant background material.
- Chapter 3 explores the impact of different types of tissue sample preparation methods on myelin-sensitive quantitative MRI parameters. Using traditional myelin water imaging and magnetization transfer imaging methods, this chapter investigates how tissue freezing and

fixation affect qMRI estimates of white matter content. We found that both freezing and fixing tissue caused similar changes in qMRI metrics, so tissue freezing is reasonable alternative preservation method to fixation for use in qMRI analysis. Its content comes from published journal paper [44] that extends abstracts [6,45,46].

- Chapter 4 examines the relationships between these myelin-sensitive quantitative MRI parameters and histological markers in Alzheimer’s disease tissue samples. We found that myelin water fraction correlates with neurofilament in addition to myelin basic protein in white matter ROIs, and found that the incorporation of both histology metrics improved statistical modeling. This was not true for the MT parameters, indicating that MWF and the MT parameter provide distinct information about tissue white matter content in AD tissue. Its content extends conference abstract [47] that will be submitted as a journal paper [48].
- Chapter 5 describes an alternative balanced steady-state free precession method for estimating myelin water fraction and myelin water exchange. We test our method in simulations, a single-pool uniform phantom, a two-pool non-exchanging phantom, two-pool exchanging phantom, and human tissue sample. Our method results in precise estimates of the fast component fraction and mean residence time in a urea phantom. Fast fraction estimates in a human tissue sample were biased relative to MWF. Its content is based on research that will be submitted as a journal paper [49].
- Chapter 6 offers ideas for future investigations based on the work in this thesis.
- Appendices A and B provide additional information relevant to Chapters 3-4.

CHAPTER 2

Background

This chapter provides background information that is relevant to subsequent chapters. Section 2.1 provides an introduction to MR physics concepts that are important to the work in this thesis. Section 2.2 discusses current standard methods for imaging myelin with MRI. Section 2.3 reviews how quantitative MRI is currently used for studying ADRD. Section 2.4 gives background on balanced steady-state free precession MR imaging. Section 2.5 describes background information about exchange estimation with inversion-recovery nuclear magnetic resonance (IR-NMR).

2.1 MR Physics

This section briefly provides some background information about MR physics principles described in [4, 50] that are important to this thesis. Magnetic resonance imaging (MRI) is commonly based on the magnetization of hydrogen protons, as they are abundant in biological tissue and provide a strong MR signal. Hydrogen protons have a magnetic property called nuclear spin; these protons (which we will also call spins) rotate about their own axes, have a magnetic moment, and interact with external magnetic fields. In the absence of an external magnetic field, the spins are in random directions, so on a macroscopic scale there is no net magnetization. However, in the presence of an externally applied magnetic field $\mathbf{B}_0$, the spins form a net magnetization vector $\mathbf{M} = [M_x \ M_y \ M_z]^T$ that is aligned parallel to $\mathbf{B}_0$. The applied magnetic field is typically in the z-direction, and so when $\mathbf{B}_0$ is applied, the magnetization vector $\mathbf{M} = [M_x \ M_y \ M_0]^T$, where M_0 is the equilibrium magnetization. The spins precess around the B_0 field, which means that the transverse component $M_{xy} = M_x + iM_y$ rotates at a specific frequency, the frequency of precession which is called the Larmor frequency. This frequency is proportional to the strength of the magnetic field and is given by the Larmor equation:

$$\omega_0 = \gamma B_0. \tag{2.1}$$

Here, $B_0 = \|\mathbf{B}_0\|_2$ and γ is the gyromagnetic ratio. For hydrogen protons, the [NIST value](#) is $\gamma/2\pi = 42.5775 \text{ MHz/T}$. The precession frequency ω_0 is about 298 MHz for a 7T magnet. Precession causes time-varying magnetic flux, which, according to Faraday's law, induces a voltage in a receiver coil that has been tuned to the Larmor frequency.

If an external RF field is applied close to this frequency, the spins can absorb energy through resonance, allowing their net magnetization to be manipulated and subsequently detected. When this magnetization is perturbed, or excited, by an RF pulse in the transverse plane, it is disturbed from equilibrium and then relaxes back to its equilibrium state. The MR signal is the transverse component of the magnetization acquired as the magnetization goes back to equilibrium. This signal depends on the time the data is acquired, which is called the echo time TE. Different materials have different relaxation characteristics, which in conjunction with varying scan parameters such as the TE and the time between the RF pulses, repetition time TR, result in the different tissue contrast seen MR images. Section 2.1.1 further details free precession, longitudinal and transverse relaxation, and excitation. The reader is referred to [\[50\]](#) for more in-depth information about MR-related topics, such as using gradient fields for imaging, k-space, etc. Section 2.1.2 discusses an MR topic important to this thesis, the Bloch-McConnell Equations [\[51, 52\]](#).

2.1.1 Bloch Equations

The Bloch Equations are a set of differential equations that describe the behavior of the magnetization vector $\mathbf{M}$ in the presence of applied magnetic fields [\[53\]](#). They can be compactly written in the following vector form:

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma \mathbf{B} - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} - \frac{(M_z - M_0) \mathbf{k}}{T_1}. \quad (2.2)$$

Here $\mathbf{i}, \mathbf{j}$ and $\mathbf{k}$ are the unit vectors along the x, y and z directions. Equation (2.2) can also be written in matrix form:

$$\frac{d\mathbf{M}}{dt} = \begin{bmatrix} -\frac{1}{T_2} & \gamma B_z & -\gamma B_y \\ -\gamma B_z & -\frac{1}{T_2} & \gamma B_x \\ \gamma B_y & -\gamma B_x & -\frac{1}{T_1} \end{bmatrix} \mathbf{M} + \begin{bmatrix} 0 \\ 0 \\ \frac{M_0}{T_1} \end{bmatrix}. \quad (2.3)$$

We will break down this equation under the conditions of free precession, longitudinal relaxation, and transverse relaxation.

2.1.1.1 Free Precession

The precession of $\mathbf{M}$ around a $\mathbf{B}$ field at a frequency $\omega = \gamma B$ is described by [4, 50]:

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma \mathbf{B}, \quad (2.4)$$

Equation (2.4) can be written in matrix form:

$$\frac{d\mathbf{M}}{dt} = \begin{bmatrix} 0 & \gamma B_z & -\gamma B_y \\ -\gamma B_z & 0 & \gamma B_x \\ \gamma B_y & -\gamma B_x & 0 \end{bmatrix} \mathbf{M}. \quad (2.5)$$

This equation can be solved under various conditions; in the presence of only the main magnetic field $\mathbf{B} = [0 \ 0 \ B_0]^T$, where $\omega_0 = \gamma B_0$, the solution is [50]:

$$\mathbf{M}(t) = \begin{bmatrix} \cos \omega_0 t & \sin \omega_0 t & 0 \\ -\sin \omega_0 t & \cos \omega_0 t & 0 \\ 0 & 0 & 1 \end{bmatrix} \mathbf{M}(0), \quad (2.6)$$

where $\mathbf{M}(0)$ is the initial condition for $\mathbf{M}$.

2.1.1.2 Longitudinal Relaxation

After an RF pulse excites the spins, they eventually return to their equilibrium state along $\mathbf{B}_0$. Longitudinal relaxation, or spin-lattice relaxation, involves the return of M_z to equilibrium according to [4, 50]:

$$\frac{dM_z}{dt} = -\frac{M_z - M_0}{T_1}, \quad (2.7)$$

The spin-lattice time constant T_1 describes the recovery of the longitudinal magnetization back to equilibrium. [4, 50] also provides the solution to Equation (2.7):

$$M_z(t) = M_z(0) e^{-t/T_1} + M_0 (1 - e^{-t/T_1}), \quad (2.8)$$

where $M_z(0)$ is the initial condition for M_z .

2.1.1.3 Transverse Relaxation

In addition to relaxation of the longitudinal component after an RF pulse, the transverse component M_{xy} decays toward 0 according to [4,50]:

$$\frac{dM_{xy}}{dt} = -\frac{M_{xy}}{T_2}, \quad (2.9)$$

The spin-spin time constant T_2 describes the decay of the transverse magnetization. [50] also provides the solution to Equation (2.9):

$$M_{xy}(t) = M_{xy}(0) e^{-t/T_2}, \quad (2.10)$$

where $M_{xy}(0)$ is the initial condition for M_{xy} . Combining Equations (2.4), (2.7), and (2.9) results in Equation 2.2.

2.1.1.4 Excitation

The Bloch Equations in the previous section describe the behavior of the magnetization $\mathbf{M}$ after excitation by an RF pulse. MR scanners use RF coils that generate amplitude-modulated circularly polarized RF pulses in the transverse direction: [4,50]:

$$\mathbf{B}_1(t) = B_1(t) [\cos(\omega_{\text{rf}}t + \theta)\mathbf{i} - \sin(\omega_{\text{rf}}t + \theta)\mathbf{j}], \quad (2.11)$$

where $B_1(t)$ is an amplitude modulation function, ω_{rf} is the carrier frequency, and θ is the initial phase of the pulse. The total field is thus:

$$\mathbf{B}(t) = B_1(t) [\cos(\omega_{\text{rf}}t + \theta)\mathbf{i} - \sin(\omega_{\text{rf}}t + \theta)\mathbf{j}] + B_0\mathbf{k}, \quad (2.12)$$

and we plug $\mathbf{B}(t)$ into Equation (2.4) and solve:

$$\frac{d\mathbf{M}}{dt} = \mathbf{M} \times \gamma [B_1(t) [\cos(\omega_{\text{rf}}t + \theta)\mathbf{i} - \sin(\omega_{\text{rf}}t + \theta)\mathbf{j}] + B_0\mathbf{k}], \quad (2.13)$$

In matrix form, where $\omega_0 = \gamma B_0$ and $\omega_1(t) = \gamma B_1(t)$, this is:

$$\frac{d\mathbf{M}}{dt} = \begin{bmatrix} 0 & \omega_0 & \omega_1(t) \sin(\omega_{\text{rf}}t + \theta) \\ -\omega_0 & 0 & \omega_1(t) \cos(\omega_{\text{rf}}t + \theta) \\ -\omega_1(t) \sin(\omega_{\text{rf}}t + \theta) & -\omega_1(t) \cos(\omega_{\text{rf}}t + \theta) & 0 \end{bmatrix} \mathbf{M}. \quad (2.14)$$

For excitation, it is convenient to describe the solution to the Bloch Equations in a transformed coordinate system. The conventional coordinate system is called the laboratory frame; the transformed coordinate system is called the rotating frame. In the rotating frame, Equation (2.14) becomes [50]:

$$\frac{d\mathbf{M}_{\text{rot}}}{dt} = \begin{bmatrix} 0 & \omega_0 - \omega_{\text{rf}} & \omega_1(t) \sin \theta \\ -(\omega_0 - \omega_{\text{rf}}) & 0 & \omega_1(t) \cos \theta \\ -\omega_1(t) \sin \theta & -\omega_1(t) \cos \theta & 0 \end{bmatrix} \mathbf{M}_{\text{rot}}. \quad (2.15)$$

The excitation is on resonance when ω_{rf} is set to ω_0 :

$$\frac{d\mathbf{M}_{\text{rot}}}{dt} = \begin{bmatrix} 0 & 0 & \omega_1(t) \sin \theta \\ 0 & 0 & \omega_1(t) \cos \theta \\ -\omega_1(t) \sin \theta & -\omega_1(t) \cos \theta & 0 \end{bmatrix} \mathbf{M}_{\text{rot}}. \quad (2.16)$$

If θ is set to 0, (2.16) becomes [50]:

$$\frac{d\mathbf{M}_{\text{rot}}}{dt} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & \omega_1(t) \\ 0 & -\omega_1(t) & 0 \end{bmatrix} \mathbf{M}_{\text{rot}}. \quad (2.17)$$

The flip angle (also called tip angle/rotation angle) of this RF pulse is defined as:

$$\alpha(t) = \int_0^t \omega_1(\tau) d\tau, \quad (2.18)$$

when the RF pulse has duration t and no gradients are on during the pulse (see [50] for more information about selective excitation). Also, an RF pulse with flip angle α and phase θ relative to the x-axis uses the following matrix composition [54]:

$$\mathbf{R}_\theta(\alpha) = \mathbf{R}_z(-\theta) \mathbf{R}_x(-\alpha) \mathbf{R}_z(\theta), \quad (2.19)$$

where [54]:

$$\mathbf{R}_x(\alpha) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(\alpha) & -\sin(\alpha) \\ 0 & \sin(\alpha) & \cos(\alpha) \end{bmatrix}, \quad \mathbf{R}_z(\theta) = \begin{bmatrix} \cos(\theta) & -\sin(\theta) & 0 \\ \sin(\theta) & \cos(\theta) & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (2.20)$$

Using (2.18), (2.19), and (2.20), the solution to (2.16) for initial condition $\mathbf{M}_{\text{rot}}(0)$ is then [4, 50]:

$$\mathbf{M}_{\text{rot}}(t) = \mathbf{R}_z(-\theta)\mathbf{R}_x(-\alpha(t))\mathbf{R}_z(\theta)\mathbf{M}_{\text{rot}}(0), \quad (2.21)$$

where x and z are the axes in the rotating frame. Since RF pulses are usually very short ($\ll T_2$), signal models often ignore relaxation during each RF pulse.

2.1.2 Bloch-McConnell Equations

The Bloch equations described in [50] assume that the signal coming from each individual voxel comes from a material with a specific T_1 and T_2 value. In reality, voxels can be made up of multiple different materials, forming multiple compartments within a single voxel. The net magnetization in a voxel is then the sum of the magnetization from the individual compartments. The Bloch McConnell equations [51, 52] extend the Bloch equations in the case where there is more than one compartment within a voxel and these compartments exchange magnetization between them. The following equations, based on those in [4], assume there are two voxel compartments, but these equations can be extended to include three or more compartments.

In the two-pool case, where the two tissue compartments are denoted by a and b , each voxel includes spins in either compartment a or b , and the magnetization vector can be written as

$$\mathbf{M} = \begin{bmatrix} M_{x,a} & M_{y,a} & M_{z,a} & M_{x,b} & M_{y,b} & M_{z,b} \end{bmatrix}^T \in \mathbf{R}^6 \quad (2.22)$$

The Bloch McConnell Equation takes the following form in the rotating frame [51]

$$\frac{d\mathbf{M}}{dt} = \begin{bmatrix} \mathbf{L}_a - \mathbf{K}_a & \mathbf{K}_b \\ \mathbf{K}_a & \mathbf{L}_b - \mathbf{K}_b \end{bmatrix} \mathbf{M} + \mathbf{c}. \quad (2.23)$$

Here, $\mathbf{L}_a$ and $\mathbf{L}_b$ are defined as

$$\mathbf{L}_a \triangleq \begin{bmatrix} -\frac{1}{T_{2a}} & \Delta\omega_a & -\gamma B_y \\ -\Delta\omega_a & -\frac{1}{T_{2a}} & \gamma B_x \\ \gamma B_y & -\gamma B_x & -\frac{1}{T_{1a}} \end{bmatrix}, \quad \mathbf{L}_b \triangleq \begin{bmatrix} -\frac{1}{T_{2b}} & \Delta\omega_b & -\gamma B_y \\ -\Delta\omega_b & -\frac{1}{T_{2b}} & \gamma B_x \\ \gamma B_y & -\gamma B_x & -\frac{1}{T_{1b}} \end{bmatrix}.$$

T_{1a} and T_{1b} are the longitudinal magnetization relaxation constants for pools a and b , and T_{2a} and T_{2b} are the transverse magnetization relaxation constants for pools a and b . $\Delta\omega_a$ and $\Delta\omega_b$ are the off-resonance experienced by the spins in compartments a and b by frequency or chemical shift. The exchange terms appear in $\mathbf{K}_a$ and $\mathbf{K}_b$, which are defined as

$$\mathbf{K}_a \triangleq \begin{bmatrix} \frac{1}{\tau_{a \rightarrow b}} & 0 & 0 \\ 0 & \frac{1}{\tau_{a \rightarrow b}} & 0 \\ 0 & 0 & \frac{1}{\tau_{a \rightarrow b}} \end{bmatrix}, \quad \mathbf{K}_b \triangleq \begin{bmatrix} \frac{1}{\tau_{b \rightarrow a}} & 0 & 0 \\ 0 & \frac{1}{\tau_{b \rightarrow a}} & 0 \\ 0 & 0 & \frac{1}{\tau_{b \rightarrow a}} \end{bmatrix}, \quad (2.24)$$

where $\tau_{a \rightarrow b}$ is the mean residence time (MRT), or inverse of the exchange rate, from pool a to b and $\tau_{b \rightarrow a}$ is the MRT for exchange from pool b to a . $\mathbf{c}$ is defined as

$$\mathbf{c} = \begin{bmatrix} 0 & 0 & \frac{f_a M_0}{T_{1a}} & 0 & 0 & \frac{f_b M_0}{T_{1b}} \end{bmatrix}^T \quad (2.25)$$

where f_a and f_b are the fraction of spins in each voxel that correspond to compartments a and b , and M_0 is the equilibrium magnetization. $\mathbf{L}_a$ and $\mathbf{L}_b$ include the magnetization dynamics that are specific to pools a and b individually, and $\mathbf{K}_a$ and $\mathbf{K}_b$ include the magnetization dynamics between the two pools. In the absence of exchange (infinite MRT), $\mathbf{K}_a$ and $\mathbf{K}_b$ are all zeroes and then the Bloch equations can be used to separately analyze the magnetization dynamics of each compartment.

2.1.2.1 Excitation

In the case that the RF excitation pulse is of very short time duration, precession, relaxation, and exchange effects often can be neglected during the pulse. In this situation, (2.23) simplifies to the following equation:

$$\frac{d\mathbf{M}}{dt} = \begin{bmatrix} 0 & 0 & -\gamma B_{1,y} & 0 & 0 & 0 \\ 0 & 0 & \gamma B_{1,x} & 0 & 0 & 0 \\ \gamma B_{1,y} & -\gamma B_{1,x} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -\gamma B_{1,y} \\ 0 & 0 & 0 & 0 & 0 & \gamma B_{1,x} \\ 0 & 0 & 0 & \gamma B_{1,y} & -\gamma B_{1,x} & 0 \end{bmatrix} \mathbf{M}, \quad (2.26)$$

where (2.26) is solved separately for each pool. A general hard RF pulse with flip angle α and phase θ relative to the x-axis can use the following rotation matrices and matrix composition:

$$\mathbf{R}_x(\alpha) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(\alpha) & \sin(\alpha) \\ 0 & -\sin(\alpha) & \cos(\alpha) \end{bmatrix}, \quad \mathbf{R}_z(\theta) = \begin{bmatrix} \cos(\theta) & \sin(\theta) & 0 \\ -\sin(\theta) & \cos(\theta) & 0 \\ 0 & 0 & 1 \end{bmatrix}, \quad (2.27)$$

$$\mathbf{R}_\theta(\alpha) = \mathbf{R}_z(\theta) \mathbf{R}_x(\alpha) \mathbf{R}_z(-\theta), \quad (2.28)$$

leading to the solution of (2.26) in the rotating frame:

$$\mathbf{M}^+ = \begin{bmatrix} \mathbf{R}_z(\theta)\mathbf{R}_x(\alpha)\mathbf{R}_z(-\theta) & \mathbf{0}_{3 \times 3} \\ \mathbf{0}_{3 \times 3} & \mathbf{R}_z(\theta)\mathbf{R}_x(\alpha)\mathbf{R}_z(-\theta) \end{bmatrix} \mathbf{M}^-, \quad (2.29)$$

where $\mathbf{M}^- \in \mathbf{R}^6$ and $\mathbf{M}^+ \in \mathbf{R}^6$ represent the net magnetization before and after the RF excitation.

2.1.2.2 Free Precession, Relaxation, and Exchange

After the RF pulse, excitation effects can be neglected and free precession, relaxation, and exchange effects can be analyzed. In this situation, (2.23) simplifies to the following equation:

$$\frac{d\mathbf{M}}{dt} = \mathbf{A}\mathbf{M} + \mathbf{c}, \quad (2.30)$$

where

$$\mathbf{A} \triangleq \begin{bmatrix} \tilde{\mathbf{L}}_a - \mathbf{K}_a & \mathbf{K}_b \\ \mathbf{K}_a & \tilde{\mathbf{L}}_b - \mathbf{K}_b \end{bmatrix}. \quad (2.31)$$

$\mathbf{K}_a$, $\mathbf{K}_b$, and $\mathbf{c}$ are as defined above in (2.24) and (2.25) and $\tilde{\mathbf{L}}_{ba}$ and $\tilde{\mathbf{L}}_{bb}$ are defined as:

$$\tilde{\mathbf{L}}_a \triangleq \begin{bmatrix} -\frac{1}{T_{2a}} & \Delta\omega_a & 0 \\ -\Delta\omega_a & -\frac{1}{T_{2a}} & 0 \\ 0 & 0 & -\frac{1}{T_{1a}} \end{bmatrix}, \quad \tilde{\mathbf{L}}_b \triangleq \begin{bmatrix} -\frac{1}{T_{2b}} & \Delta\omega_b & 0 \\ -\Delta\omega_b & -\frac{1}{T_{2b}} & 0 \\ 0 & 0 & -\frac{1}{T_{1b}} \end{bmatrix},$$

similar to $\mathbf{L}_a$ and $\mathbf{L}_b$ without the RF excitation effects. The solution to (2.31) is given by:

$$\mathbf{M}(t) = e^{t\mathbf{A}} \mathbf{M}(0) + (e^{t\mathbf{A}} - \mathbf{I}_6) \mathbf{A}^{-1} \mathbf{c}. \quad (2.32)$$

Here we assume $\mathbf{A}$ is invertible, $\mathbf{M}(0)$ is the initial condition for the net magnetization, $\mathbf{I}_6$ is the 6×6 identity matrix, and $e^{\mathbf{X}} = \sum_{k=0}^{\infty} \frac{1}{k!} \mathbf{X}^k$ is the matrix exponential function. Assuming chemical equilibrium between the two pools, meaning that the exchange rates between the two pools are equal [25], $\frac{f_a}{\tau_{a \rightarrow b}} = \frac{f_b}{\tau_{b \rightarrow a}}$. This leads to $\mathbf{A}\mathbf{m}_0 = -\mathbf{c}$ where

$$\mathbf{m}_0 = \begin{bmatrix} 0 & 0 & f_a M_0 & 0 & 0 & f_b M_0 \end{bmatrix}^T. \quad (2.33)$$

In this case, (2.32) can be rewritten as:

$$\mathbf{M}(t) = e^{t\mathbf{A}} \mathbf{M}(0) + (\mathbf{I}_6 - e^{t\mathbf{A}}) \mathbf{m}_0. \quad (2.34)$$

2.2 Tissue White Matter Modeling

The Bloch-McConnell equations in the previous section describe the magnetization dynamics where there are two exchanging compartments within a single voxel. A practical application of this model is brain tissue white matter. Many models exist for modeling MR properties of white matter. Myelin water imaging and magnetization transfer imaging are two important and popular models. These are discussed in further detail in this section.

2.2.1 Traditional Myelin Water Imaging

The myelin water imaging method was developed by researchers at the University of British Columbia [3, 19, 55–58]. The model assumes that a voxel is made up of various components with a range of many different T_2 relaxation values.

Figure 2.1 shows an electron micrograph image of myelin. The water molecules trapped in the myelin sheath shown in blue are more tightly confined than the water molecules in the intracellular and extracellular space, also called free water, shown in purple. Due to being more tightly confined, the myelin water molecules have shorter T_2 values than the free water molecules. Myelin water imaging aims to estimate the amount of signal coming from the myelin water compared to the free water in a voxel. This is quantified by an MR biomarker called myelin water fraction (MWF), and has shown to correlate with myelin content [8, 20–22]. Myelin water imaging is typically done by acquiring multi-echo spin echo (MESE) data and then using non-negative least squares (NNLS) regression separately for each the signal decay curve of each voxel. The fitting procedure aims to determine the relative amplitudes of the signals originating from each of these T_2 components that combine to form a multi-exponential decay curve. Figure 2.2 visualizes an ideal resulting T_2 distribution from [3].

For this model, M is the total number of echoes in the MESE signal decay and N is the total number of T_2 components. Equation (2.35) below is solved for $\mathbf{x} \in \mathbb{R}^N$, where $\mathbf{y} \in \mathbb{R}^M$ is the magnitude of the MESE signal decay curve for one voxel and $\mathbf{A} \in \mathbb{R}^{M \times N}$ is a dictionary of N T_2 decay curves. Each column of $\mathbf{A}$ is an M -echo long MESE decay curve simulated with the n th T_2 value, where each element is the signal value at a specific TE. This can be done using a Bloch simulation or using the extended phase graph algorithm [59]. The vector $\mathbf{x}$ is thus the relative amplitude of each of the signal components corresponding to one of the N T_2 values. These

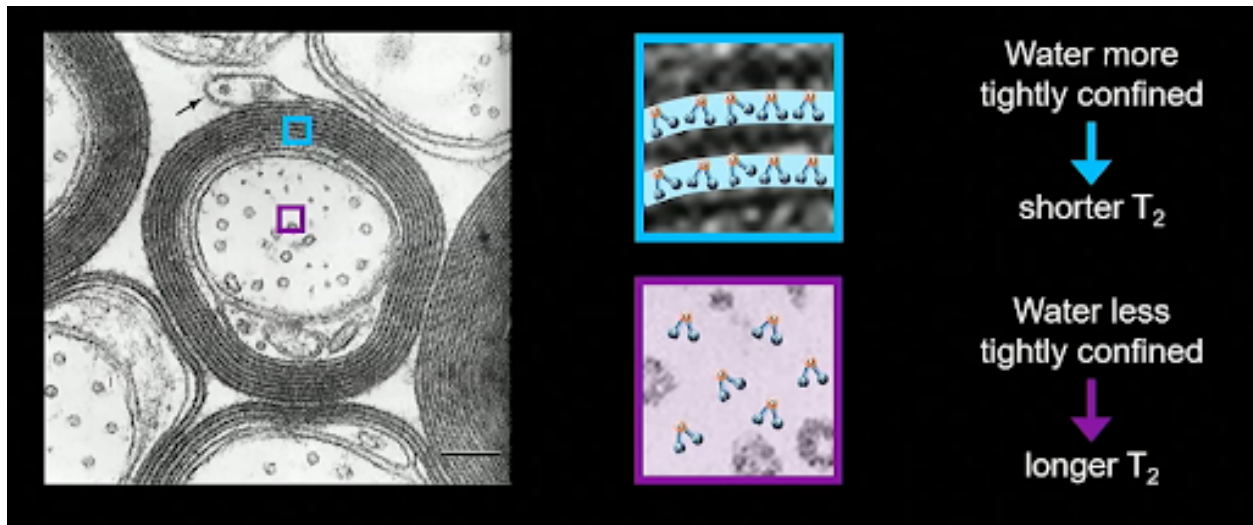


Figure 2.1: Adapted from Dr. Cornelia Laule, UBC, ISMRM 2023, Myelin Imaging: Strategies & Applications Weekend Course: Introduction to Myelin Water Imaging: Overview, Motivation, Historical Perspective & Classical Solutions. Water molecules trapped in the myelin bilayers (blue) are more tightly confined and have shorter T_2 values. Free water molecules in the intra/extracellular space (purple) are less tightly confined and have longer T_2 values.

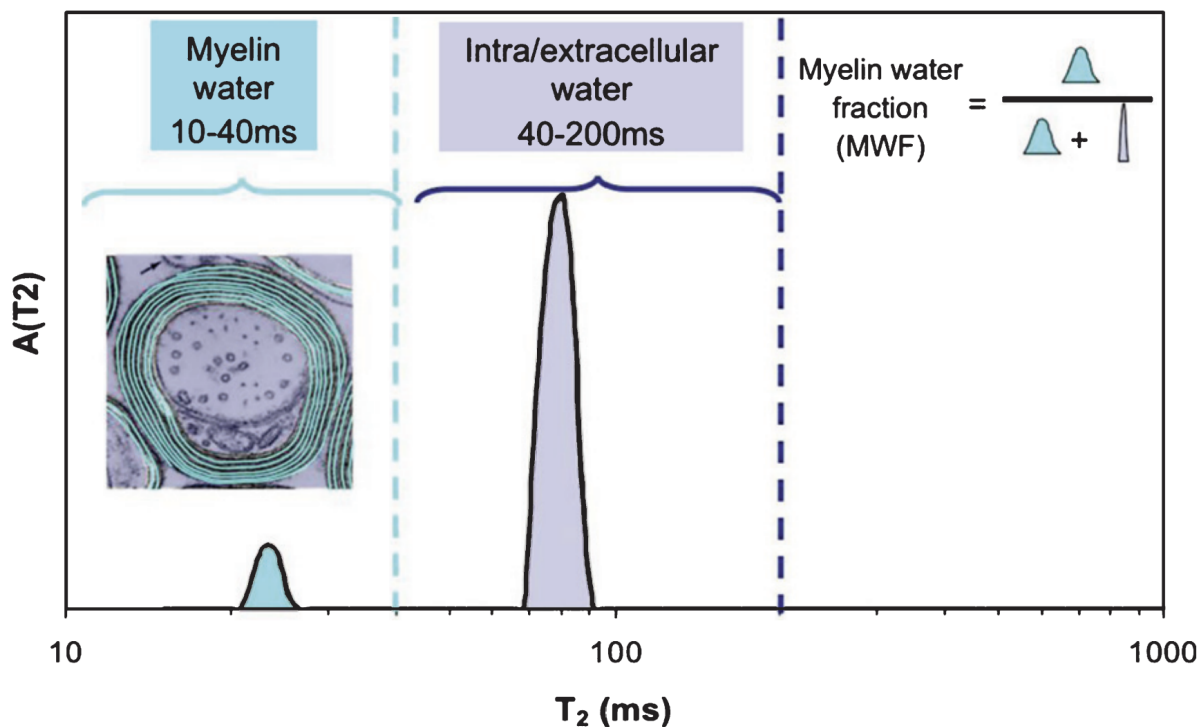


Figure 2.2: T_2 distribution from human white matter from [3]. The small peak corresponds to shorter T_2 values arising from myelin water (blue) and the tall peak corresponds to longer T_2 values arising from intra/extracellular water (purple).

amplitudes are constrained to be non-negative, and Tikhonov regularization is added to discourage the amplitudes from having excessively high values. This optimization problem is formulated as

$$\hat{\mathbf{x}} = \underset{\mathbf{x}}{\operatorname{argmin}} \|\mathbf{A}\mathbf{x} - \mathbf{y}\|_2^2 + \beta \|\mathbf{x}\|_2^2, \quad \mathbf{x} \geq 0. \quad (2.35)$$

The myelin water fraction (MWF) calculation is traditionally done by summing the amplitudes of the points in the T_2 spectrum up to a predetermined cutoff divided by the sum of the amplitudes of the entire spectrum [20, 57], according to the following equation:

$$\text{MWF} = \frac{\mathbf{1}'\hat{\mathbf{x}}(T_2\text{cutoff})}{\mathbf{1}'\hat{\mathbf{x}}}. \quad (2.36)$$

where $\hat{\mathbf{x}}(T_2\text{cutoff})$ is a vector of the first n elements of $\hat{\mathbf{x}}$ up to a predetermined cutoff. This cutoff divides the T_2 values that correspond to free water and myelin water, and should be between the two peaks in an ideal spectrum.

Expanding on this calculation, the T_2 spectrum that comes out of the NNLS calculation is a vector of length N where each index n corresponds to a T_2 value. It can also be thought of as a histogram where each index n corresponds to a bin for a range of T_2 values and the amplitude of the T_2 spectrum at that index n corresponds to the number of points in the bin. The number of elements in bin n divided by the total number of points in the distribution represents the probability of a point being in that bin. Then the probability mass function (PMF) for one point is the probability of a bin containing that point divided by the width of the bin. Thus the PMF of the distribution can be written as

$$\text{PMF}_{\text{MWF}} = \frac{\hat{\mathbf{x}}}{(\mathbf{1}'\hat{\mathbf{x}}) \text{diff}(T_2\text{vector})}, \quad (2.37)$$

where $\hat{\mathbf{x}}$ is the T_2 spectrum of length N returned from the NNLS fitting where each element is the number of elements in bin n , $\mathbf{1}'\hat{\mathbf{x}}$ is a scalar representing the total number of points in the histogram, and $\text{diff}(T_2\text{vector})$ is the bin width, or the difference between two consecutive T_2 values used in the fitting process. This is important as often the T_2 values are log-spaced for MWF-NNLS fitting.

The MWF is often described as the area under the T_2 spectrum up to the T_2 cutoff divided by the total area under the T_2 spectrum. This can be written with a Riemann sum vector $\mathbf{v}_{\text{RS}}$:

$$\mathbf{v}_{\text{RS}} = \text{PMF} \odot \text{diff}(T_2\text{vector}), \quad (2.38)$$

where $\odot$ denotes element-wise multiplication. The total area under the PMF is then the sum $\mathbf{1}'\mathbf{v}_{\text{RS}}$. Using this notation, the MWF can be written as:

$$\text{MWF} = \frac{\mathbf{1}'\mathbf{v}_{\text{RS}}(T_2\text{cutoff})}{\mathbf{1}'\mathbf{v}_{\text{RS}}}. \quad (2.39)$$

Setting $\text{diff}(T_2\text{vector}) = \mathbf{d}_{T_2}$:

$$\text{PMF} = \frac{\hat{\mathbf{x}}}{(\mathbf{1}'\hat{\mathbf{x}}) \mathbf{d}_{T_2}}, \quad \mathbf{v}_{\text{RS}} = (\text{PMF})(\mathbf{d}_{T_2}) = \frac{\hat{\mathbf{x}}}{\mathbf{1}'\hat{\mathbf{x}}}. \quad (2.40)$$

Plugging (2.40) into (2.39), and then canceling the $\mathbf{1}'\hat{\mathbf{x}}$ term:

$$\text{MWF} = \frac{\mathbf{1}'\mathbf{v}_{\text{RS}}(T_2\text{cutoff})}{\mathbf{1}'\mathbf{v}_{\text{RS}}} = \frac{\mathbf{1}'(\frac{\hat{\mathbf{x}}}{\mathbf{1}'\hat{\mathbf{x}}}(T_2\text{cutoff}))}{\mathbf{1}'(\frac{\hat{\mathbf{x}}}{\mathbf{1}'\hat{\mathbf{x}}})} = \frac{\mathbf{1}'(\hat{\mathbf{x}}(T_2\text{cutoff}))}{\mathbf{1}'\hat{\mathbf{x}}}, \quad (2.41)$$

which is the same as (2.36). Thus, these two definitions of MWF, (2.36) and (2.39), are equivalent. The MWF can be described as the area under the PMF of the T_2 histogram up to the T_2 cutoff divided by the total area under the PMF of the T_2 histogram, taking into account the log spacing with the $\text{diff}(T_2\text{vector})$ term, and as the sum of the amplitudes of the T_2 histogram up to the cutoff divided by the total sum of the amplitudes.

2.2.2 Myelin Water Imaging with Two-Pool Exchanging Model

The traditional myelin water imaging framework described in the previous section can be thought of as a multi-pool model, where each T_2 value represents a different pool. In this framework it is difficult to model exchange of water between the different pools as there can be as many pools as T_2 values used to create the dictionary for the NNLS fitting. A two-pool or two-compartment model is more practical when modeling exchange between the two pools. Figure 2.3 illustrates the parameters in this model. The non-myelin water is one compartment with its own T_1 and T_2 , called $T_{1,s}$ and $T_{2,s}$ where “s” stands for slow, because the non-myelin water magnetization relaxes slower than the myelin water magnetization. Thus the other compartment for myelin water has its own T_1 and T_2 , called $T_{1,f}$ and $T_{2,f}$ where “f” stands for fast. The fraction of the signal coming from the fast-relaxing compartment (myelin water compartment) is f_f . The myelin water also has its own orientation-dependent frequency shift $\Delta\omega_f$ on the order of 5-10 Hz, which is an additional off-resonance in addition to the bulk off-resonance $\Delta\omega$ (total $\Delta\omega + \Delta\omega_f$) that is only experienced by the myelin water pool [60]; the non-myelin water pool experiences the bulk off-resonance $\Delta\omega$. The mean residence time for exchange from myelin water to non-myelin water (inverse of the exchange rate) is $\tau_{f \rightarrow s}$. The myelin water and non-myelin water compartments are assumed to be

in chemical equilibrium, meaning that $f_f \tau_{s \rightarrow f} = (1 - f_f) \tau_{f \rightarrow s}$. Spin dynamics in the presence of exchange are governed by the Bloch-McConnell equations [51], as described in Section 2.1.2.

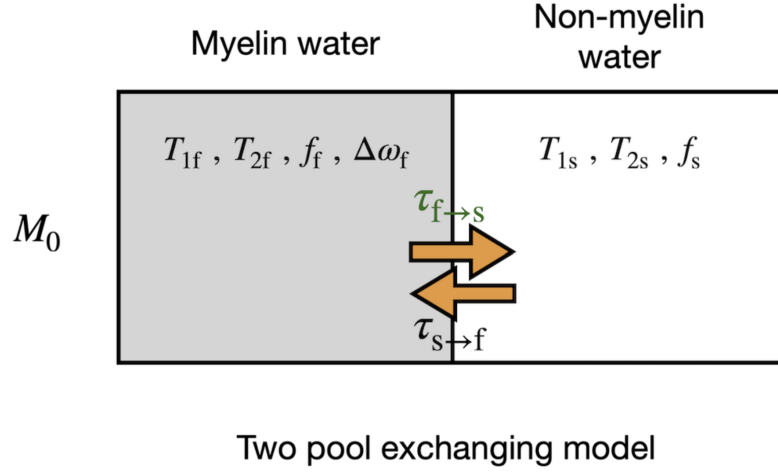


Figure 2.3: Two-compartment model for myelin water imaging; the two compartments are myelin water and non-myelin (free) water $T_{1,f}$ and $T_{2,f}$ are the fast-relaxing constants for the myelin water pool and $T_{1,s}$ and $T_{2,s}$ are the slow-relaxing constants for the non-myelin water pool. The fraction of the signal coming from the myelin water pool is f_f and the fraction of the signal coming from the free water pool is $f_s = 1 - f_f$. The mean residence time for exchange from myelin water to non-myelin water is $\tau_{f \rightarrow s}$ and the mean residence time for exchange from non-myelin water to myelin water is $\tau_{s \rightarrow f}$. $\Delta\omega_f$ is the additional off-resonance that is only experienced by the myelin water pool and M_0 is the equilibrium magnetization [4].

2.2.3 Magnetization Transfer Imaging

Magnetization transfer (MT), visualized in Figure 2.4, is another popular technique for imaging white matter. MT involves the interaction of protons in macromolecules, such as myelin, and protons in free water. The protons on the macromolecules have very short T_2 values ($< 1\text{ms}$), so their MR signal cannot directly collected fast enough. The free water protons have much longer T_2 values, which makes them MR visible. We can apply RF energy via an off-resonance MT preparation pulse exclusively to the macromolecular water protons, and then some of this energy is transferred to the free water pool via dipole-dipole interactions. Then we can image the free water pool and gain information about its interaction with the macromolecular pool. The MT effect also influences the observed T_1 value. The magnitude of the MT effect can be quantified by obtaining two images, one with the MT preparation pulse and one without it, and then subtracting them. The semi-quantitative magnetization transfer ratio (MTR), the difference between the images obtained with and without the preparation pulse, normalized by the image without the preparation

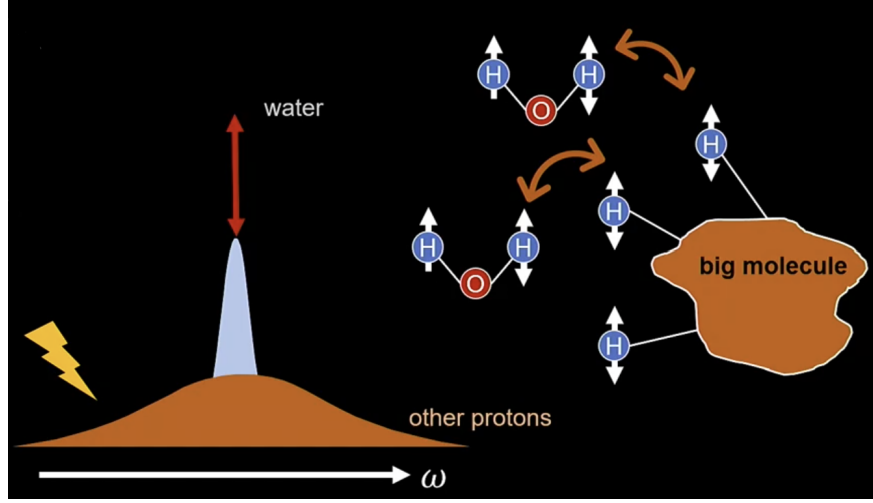


Figure 2.4: Adapted from Dr. Cornelia Laule, UBC, ISMRM 2024, Quantitative Neuroimaging in the Era of Precision Health & Personalized Medicine Weekend Course: Quantitative Imaging in Multiple Sclerosis. Visualization of magnetization transfer. Off-resonance MT preparation pulses provide RF energy exclusively to the macromolecular pool, and then this energy is transferred to the free water pool via dipole-dipole interactions. The free water pool can then be imaged.

pulse [27], is defined as $\frac{S_0 - S_+}{S_0}$, where S_+ is an image obtained with an MT preparation pulse and S_0 is the image obtained without it. The MTR value reflects the difference between these two measurements; for example, the MR signal from white matter is more suppressed than the MR signal from gray matter, causing white matter to have a higher MTR than gray matter. MTR is another accepted measure of myelin content [30–32].

Images acquired after applying preparation pulses at multiple RF power levels and off-resonance frequencies are fit to a quantitative MT (qMT) signal model to calculate the ratio of macromolecular to total free water protons $F_{\text{sat}} = \frac{M_m}{M_f}$, which is another accepted measure of myelin content [30–32].

Selective inversion recovery (SIR) methods can also be used to perform qMT analysis [11, 33, 34, 61, 62], where the observed signal recovery is driven by MT. An inversion pulse selectively inverts water magnetization but not macromolecular proton magnetization. After this pulse, magnetization quickly flows into the inverted water pool from the solid pool [35, 63]. Inversion recovery data is fit to a bi-exponential model to calculate the long and short T_1 components and their corresponding amplitudes [35, 64]. These parameters can be used to calculate the ratio of macromolecular to free water protons, $F_{\text{SIR}} = \frac{M_m}{M_f}$ as well; when calculated using SIR, this parameter is often referred to as the pool size ratio (PSR) [11, 33, 34], and can also be used as a measure of myelin content [64–67].

An extension of MT imaging is inhomogeneous magnetization transfer (ihMT) imaging; the ihMT ratio (ihMTR) can be calculated from ihMT data and has been shown to be more specific to myelin than MTR [68–70].

2.2.4 Three and Four Pool Models of White Matter

Sections 2.2.2 and 2.2.3 describe two different two-pool models for the different tissue compartments in a single white matter voxel. Figure 2.5 illustrates these two frameworks: myelin and non-myelin water exchanging pools and macromolecular and free water exchanging pools. Many



Figure 2.5: Two different two-pool models of white matter. A white matter voxel can be modeled as having a myelin water compartment and a non-myelin water compartment (left). It can also be modeled as having a macromolecular pool and a free water pool (right).

studies have combined these two models. Three-pool model frameworks such as in [14] assume three pools: a myelin water pool, a non-myelin water pool, and a macromolecular pool. The myelin water and non-myelin water pools are in exchange, and the myelin water and macromolecular pools are in exchange [25]. Figure 2.6 illustrates this model. Other studies utilize a four-pool model,

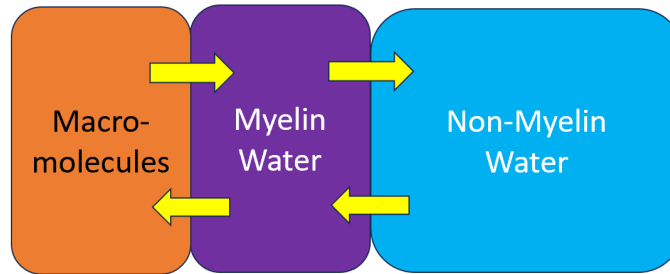


Figure 2.6: Three-pool model of white matter. A white matter voxel can be modeled as having a macromolecular compartment, a myelin water compartment, and a non-myelin water compartment. The macromolecules and myelin water exchange magnetization, and the myelin water and non-myelin water exchange magnetization.

which separates the macromolecular pool into myelin and non-myelin compartments [25, 71, 72];

the non-myelin compartment includes mostly lipids and proteins in glial cell membranes [71]. Figure 2.6 illustrates this model, where the myelin exchanges with myelin water and the non-myelin macromolecules exchange with non-myelin water. Selecting model complexity is an ongoing research area; for example, some works distinguish axonal water from extracellular water, rather than merging both into a single non-myelin water pool. Our studies focus on the two-pool models

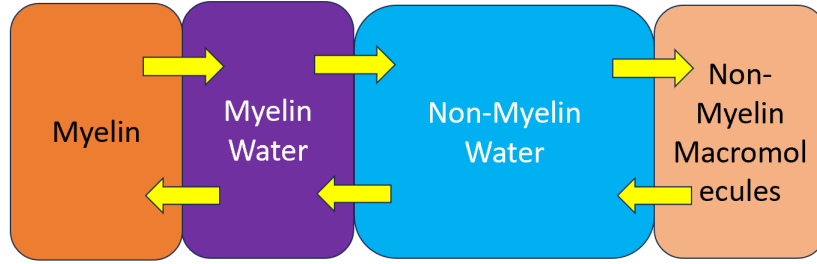


Figure 2.7: Four-pool model of white matter. A white matter voxel can be modeled as having a myelin compartment, a myelin water macromolecular compartment, a non-myelin water compartment, and a non-myelin macromolecular compartment. The macromolecules and myelin water exchange magnetization, the myelin water and non-myelin water exchange magnetization, and the non-myelin water and non-myelin macromolecules exchange magnetization.

in Figure 2.5.

2.2.5 G-Ratio

Another parameter of interest for studying white matter is the g-ratio that quantifies the comparative thickness of the myelin sheath to the axon, or the degree of myelination relative to the cross-sectional size of the axon [73–76]. It relies on the four-pool model of WM described in Section 2.2.4 [75], and is the ratio of the inner axon diameter (or radius) relative to that of the axon plus the myelin sheath. The g-ratio can be calculated for each voxel using myelin volume fraction (MVF) and axonal volume fraction (AVF) [73]. Previous studies [77, 78] defined the g-ratio as

$$g = \sqrt{\frac{1}{1 + (\text{MVF}/\text{AVF})}}. \quad (2.42)$$

The MVF value can be calculated using MWF according to this equation [75, 76]

$$\text{MVF} = \frac{\text{MWF} \times 0.859}{(\text{MWF} \times 0.384) + 0.475}. \quad (2.43)$$

The AVF value can be calculated according to this equation [76]

$$\text{AVF} = (1 - \text{MVf}) \times f_{\text{ia}} , \quad (2.44)$$

where f_{ia} , or the intra-axonal fraction, can be calculated from diffusion MRI data with multiple b-values and diffusion directions [79]. This parameter is of interest because it has been shown to correlate with axon diameter, which may vary along WM tracts depending on the amount of myelination in that part of the brain [73, 74].

2.3 Application of Quantitative MRI to Study ADRD

Accumulating evidence suggests that significant myelin degradation occurs in Alzheimer’s Disease and Related Dementias (ADRD) [37–39]. In addition to the hallmark amyloid beta and tau accumulation in the gray matter that occurs in Alzheimer’s disease (AD) [80], myelin and oligodendrocyte degeneration have been observed in the brains of AD patients, both in vivo and post-mortem. Myelin damage has been associated with the presence of amyloid beta and tau pathology in vivo [81, 82], and myelin and oligodendrocyte gene transcription is prominently dysregulated in AD [83, 84]. Conversely, white matter disease (WMD) pathological changes have also been shown to affect the progression of neurodegeneration and cognitive decline in patients with AD and affect AD pathology, as myelin degeneration has been shown to degrade microglia and impair amyloid beta clearance [82]. Additionally, impaired myelin formation was observed to precede amyloid beta and tau pathology in preclinical AD [85] and the APOE Apo-lipoprotein E gene, which is involved in lipid transport, homeostasis, and myelination, is a risk factor for AD.

Thus, many pathologies co-exist in AD, including ones that affect white matter (WM) tracts through demyelination, gliosis, axonal loss, ischemia, inflammation, or direct oligodendrocyte cytotoxicity. The various pathologies involving WM alter MR relaxation properties, resulting in T_2 WMH. The characteristic T_2 hyperintensities in brain MR images that indicate WM degradation are not specific to a particular pathology [18]. White matter hyperintensities (WMH) are a prominent feature specifically in vascular dementia, but are also seen in multiple sclerosis (MS) and older adults undergoing healthy aging.

Quantitative MRI (qMRI), which has been used to develop more specific markers of tissue properties than MRI contrast images, is often used to study myelin content of brain tissue [8, 9, 19–22, 42, 44], and has also been useful to study ADRD [86] and the relationship between ADRD and WMD [40]. Diffusion tensor imaging (DTI) has been used to look at WM damage in AD patients, and has shown that better WM integrity correlates to better cognitive ability [87]. DTI has also been used to study ex vivo brains from patients with AD, and found that WMH tissue

change was more severe in AD than control samples [41]. Magnetization transfer (MT) imaging has also been conducted in AD patients, and researchers found that the magnetization transfer ratio (MTR) in the temporal lobe decreased with AD duration, progressed, indicating that the amount of myelin present decreased as the disease progressed [88]. They also found a positive correlation between MTR and cognitive ability. Myelin water imaging studies have also been conducted on AD patients, and a negative correlation between myelin water fraction (MWF) and tau-PET scores in the cortical gray matter indicated that regions with more myelin corresponding to less tau build-up [81]. The authors also found that reduced myelination along fiber tracts corresponded to increased tau spreading along these tracts.

In summary, qMRI has been important to the study of white matter changes in ADRD. In addition to further studying the relationship between myelin-sensitive qMRI parameters and AD pathology, there is a need to lower scan duration and improve precision of parameter estimates to allow these methods to become more widespread in the future.

2.4 Balanced Steady-State Free Precession

2.4.1 Sequence and Signal Profile

The balanced steady-state free precession (bSSFP) MRI pulse sequence is an SNR-efficient gradient echo sequence where all gradients are balanced and rewound over one (usually small) TR, meaning that the net gradient area over a TR is zero. There is a single RF excitation per each TR with flip angle α and $TE = \frac{TR}{2}$. The bSSFP signal is dependent on and sensitive to many factors,

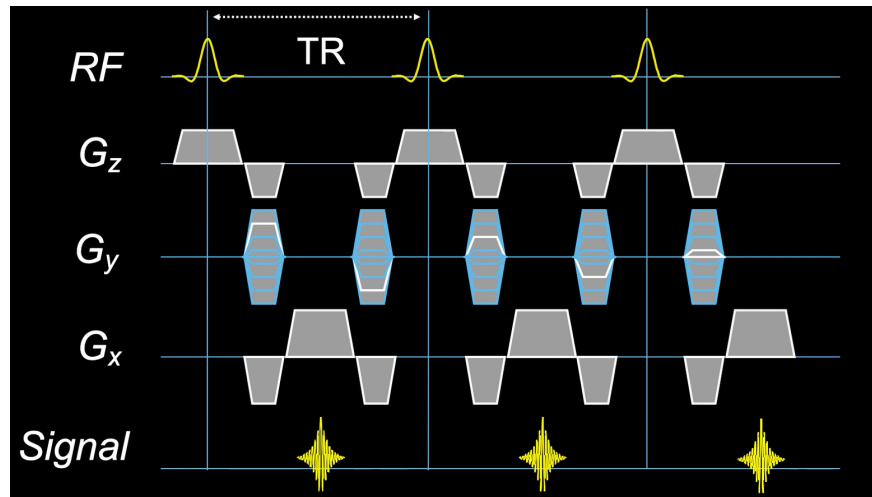


Figure 2.8: Typical bSSFP sequence diagram. All gradients are balanced and $TE = \frac{TR}{2}$. Image from [Stanford Rad229 lecture videos](#), Lecture-09A: Balanced-SSFP Dynamics, Daniel Ennis and Brian Hargreaves.

including T_1 and T_2 , the RF flip (α) and phase (θ) angles, TR and TE, and highly on resonant frequency. The signal magnitude of a bSSFP sequence is periodic with respect to resonant frequency with a period of $1/\text{TR}$. Figure 2.9 [43] shows the sensitivity of the bSSFP signal magnitude to resonant frequency and flip angle. Also, as seen in Figures 2.10-2.11, the signal decreases with increasing T_1 and increases with increasing T_2 as expected, as the signal magnitude is proportional to $\sqrt{T_2/T_1}$.

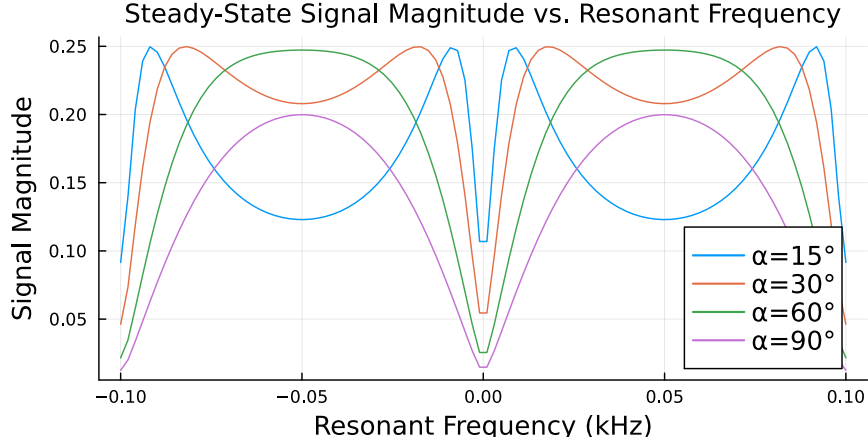


Figure 2.9: Reproduced from [5]. Signal magnitude vs. off-resonance frequency for a bSSFP sequence with $T_1 = 400$ ms, $T_2 = 100$ ms, $\text{TR} = 10$ ms, $\text{TE} = \frac{\text{TR}}{2}$ and four different flip angles. Signal nulls are spaced $\frac{1}{\text{TR}}$ apart. Data was simulated using a single pool model with [BlochSim.jl](#).

2.4.2 Steady-State Matrix Representation

As described in [5], for any steady-state sequence, including bSSFP, the magnetization change between periods can be described using a discrete-time system:

$$\mathbf{M}_{n+1} = \mathbf{A}\mathbf{M}_n + \mathbf{b}, \quad (2.45)$$

where $\mathbf{M}_n$ is a 3D magnetization vector at the n th period. For the classic single-pool model case, $\mathbf{A}$ is a 3×3 matrix and $\mathbf{b}$ is a 3D vector and are functions of T_1 , T_2 , and rotation and precession angles. For a steady-state sequence such as bSSFP, $\mathbf{M}_{n+1} = \mathbf{M}_n = \mathbf{M}_{\text{ss}}$, and

$$\mathbf{M}_{\text{ss}} = (\mathbf{I} - \mathbf{A})^{-1}\mathbf{b}, \quad (2.46)$$

where $\mathbf{M}_{\text{ss}}$ does not depend on n . $\mathbf{A}$ and $\mathbf{b}$ depend on the flip angle, TR, TE, T_1 , T_2 , and off-resonance frequency $\Delta\phi$, and are derived in [5, 89].

2.4.3 RF Phase Cycling

For bSSFP scans, the RF phase θ is often incremented each excitation by a phase cycling factor (PCF) $\Delta\theta$. Running the scan with alternating flip angles $(+\alpha, -\alpha, +\alpha, -\alpha\dots)$ is equivalent to running the scan with a constant flip angle with $\Delta\theta = \pi$ [90, 91]; this shifts the spectral profile so that the pass band occurs at the RF center. Another application is banding artifact removal by acquiring data with multiple phase cycling factors and then combining the data to get a composite image without the banding artifacts. Often 180° is used as the phase cycling factor [90, 92, 93], but arbitrary increments are possible [94–96]. The phase cycling factor has been previously shown to be able to be incorporated into the off-resonance term for a single pool bSSFP signal model [60, 97–99], which we write out here.

Using the terminology from Section 2.4, here we ignore relaxation during each RF pulse by treating it as instantaneous, modeling it as a rotation around the x-axis by the flip angle α . (See $\mathbf{R}_x$ in (2.47) below.) This approximation is reasonable when the RF pulse lengths are very short, such as $\approx 500\mu\text{s}$ for 2D imaging and even shorter in 3D imaging, so well below the relaxation times of even the fast relaxing pool. When the RF pulse is longer, such as on the order of 1 ms, relaxation effects must be taken into account during the RF pulse [100]. Using 3D imaging also allows us to ignore slice-selection effects (except for end slices that are discarded), whereas 2D imaging would require such modeling.

A general hard RF pulse with flip angle α and phase θ relative to the x-axis uses the following matrices and matrix composition:

$$\mathbf{R}_x(\alpha) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(\alpha) & \sin(\alpha) \\ 0 & -\sin(\alpha) & \cos(\alpha) \end{bmatrix}, \quad \mathbf{R}_z(\theta) = \begin{bmatrix} \cos(\theta) & \sin(\theta) & 0 \\ -\sin(\theta) & \cos(\theta) & 0 \\ 0 & 0 & 1 \end{bmatrix}, \quad (2.47)$$

$$\mathbf{R}_\theta(\alpha) = \mathbf{R}_z(\theta)\mathbf{R}_x(\alpha)\mathbf{R}_z(-\theta). \quad (2.48)$$

For a single-pool model, T_1 relaxation and T_2 exponential decay during a time interval of length t can be represented using the following matrix and vector:

$$\mathbf{E}(t) = \begin{bmatrix} e^{-t/T_2} & 0 & 0 \\ 0 & e^{-t/T_2} & 0 \\ 0 & 0 & e^{-t/T_1} \end{bmatrix}, \quad \mathbf{d}(t) = \begin{bmatrix} 0 \\ 0 \\ M_0(1 - e^{-t/T_1}) \end{bmatrix}, \quad (2.49)$$

and B_0 off-resonance can be represented as a rotation matrix around the z-axis (making the simplifying assumption here that off-resonance effects $\Delta\omega$ are constant during the interval):

$$\mathbf{R}_z(\Delta\omega t) = \begin{bmatrix} \cos(\Delta\omega t) & -\sin(\Delta\omega t) & 0 \\ \sin(\Delta\omega t) & \cos(\Delta\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix}. \quad (2.50)$$

Based on the solution to the Bloch equation for the free precession case [5], letting $\mathbf{M}_n$ denote the magnetization vector immediately before the n th RF pulse with phase θ_n , then the magnetization in the rotating frame after evolving for a TR can be represented by the following equation where we set $\mathbf{E} \triangleq \mathbf{E}(\text{TR})$, $\mathbf{d} \triangleq \mathbf{d}(\text{TR})$, and $\beta \triangleq \Delta\omega \text{TR}$:

$$\mathbf{M}_{n+1} = \mathbf{E} \mathbf{R}_z(\beta) \mathbf{R}_z(\theta_n) \mathbf{R}_x(\alpha) \mathbf{R}_z(-\theta_n) \mathbf{M}_n + \mathbf{d}. \quad (2.51)$$

It is impossible to directly compute a steady-state expression $\mathbf{M}_{\text{ss}}$ using this formula because $\mathbf{R}_z(\theta_n)$ depends on n , so we define an alternative coordinate system where we match the receiver phase to the RF excitation phase:

$$\begin{aligned} \tilde{\mathbf{M}}_n &\triangleq \mathbf{R}_z(-\theta_n) \mathbf{M}_n \\ \implies \mathbf{R}_z(\theta_n) \tilde{\mathbf{M}}_n &= \mathbf{M}_n. \end{aligned} \quad (2.52)$$

This coordinate system changes once per RF pulse. Assuming that the phase grows linearly (has a constant increment) with each RF pulse (each n):

$$\theta_{n+1} = \theta_n + \Delta\theta, \quad (2.53)$$

we rewrite the evolution of this magnetization vector as

$$\begin{aligned} \mathbf{M}_{n+1} &= \mathbf{R}_z(\theta_{n+1}) \tilde{\mathbf{M}}_{n+1} = \mathbf{E} \mathbf{R}_z(\beta) \mathbf{R}_z(\theta_n) \mathbf{R}_x(\alpha) \mathbf{R}_z(-\theta_n) \mathbf{R}_z(\theta_n) \tilde{\mathbf{M}}_n + \mathbf{d} \\ \implies \tilde{\mathbf{M}}_{n+1} &= \mathbf{R}_z(-\theta_{n+1}) \mathbf{R}_z(\beta) \mathbf{R}_z(\theta_n) \mathbf{E} \mathbf{R}_x(\alpha) \tilde{\mathbf{M}}_n + \mathbf{d} \\ &= \mathbf{R}_z(\beta + \theta_n - \theta_{n+1}) \mathbf{E} \mathbf{R}_x(\alpha) \tilde{\mathbf{M}}_n + \mathbf{d} \\ &= \mathbf{R}_z(\beta - \Delta\theta) \mathbf{E} \mathbf{R}_x(\alpha) \tilde{\mathbf{M}}_n + \mathbf{d}, \end{aligned}$$

because $\mathbf{R}_z$ and $\mathbf{E}$ commute and $\mathbf{R}_z \mathbf{d} = \mathbf{d}$. The steady-state magnetization vector [43] where $\tilde{\mathbf{M}}_n = \tilde{\mathbf{M}}_{n+1} = \tilde{\mathbf{M}}_{\text{ss}}$ immediately before the RF pulse and in the receiver coordinate system is thus

$$\tilde{\mathbf{M}}_{\text{ss}} = [\mathbf{I} - \mathbf{R}_z(\beta - \Delta\theta) \mathbf{E} \mathbf{R}_x(\alpha)]^{-1} \mathbf{d}. \quad (2.54)$$

Any time after the RF pulse $0 < t < \text{TR}$, the magnetization in the receiver coordinate system is defined as

$$\tilde{\mathbf{M}}(t) = \mathbf{R}_z(\beta_t) \mathbf{E}(t) \mathbf{R}_x(\alpha) \tilde{\mathbf{M}}_{\text{ss}} + \mathbf{d}(t), \quad (2.55)$$

where $\beta_t = \Delta\omega t$. There is no RF phase term in this equation because the effects of the phase-cycling factor in the RF excitation and the receiver phase were already considered in the steady-state derivation [101].

In summary, when calculating the steady-state magnetization of a bSSFP sequence, the RF phase-cycling term can be modeled by incorporating it into the off-resonance term by subtracting the phase cycling factor from off-resonance as seen in the $\beta - \Delta\theta$ term in (2.54), even for an arbitrary (non-180°) phase cycling factor. Also, there can only be a steady-state expression written in the receiver coordinate system and not the original coordinate system because the original system depends on θ_n that increments every n . However, to calculate the magnetization at a time between the RF pulse and the echo time, the non-shifted β value must be used as seen in (2.55).

Figures 2.10-2.11 show simulated bSSFP single-pool profiles ($\text{TR} = 5\text{ms}$ and $\text{TE} = 2.5\text{ms}$) vs. phase cycling factor for three different T_1 values while holding T_2 constant (Figure 2.10) and for three different T_2 values while holding T_1 constant (Figure 2.11). The signal profile varies with phase cycling factor as it does with off-resonance, similar to Figure 2.9.

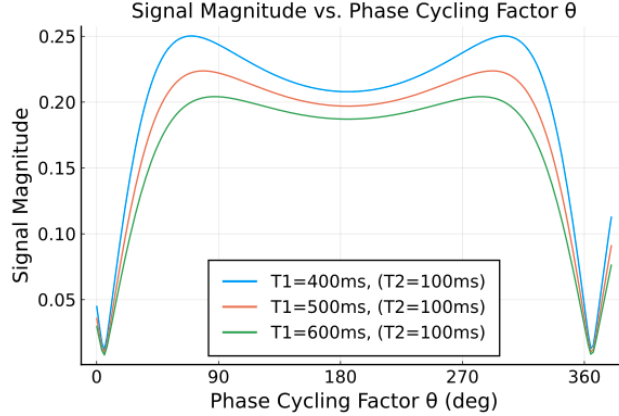


Figure 2.10: Simulated bSSFP signal magnitude vs. phase-cycling factor for a one-compartment voxel for three different T_1 values.

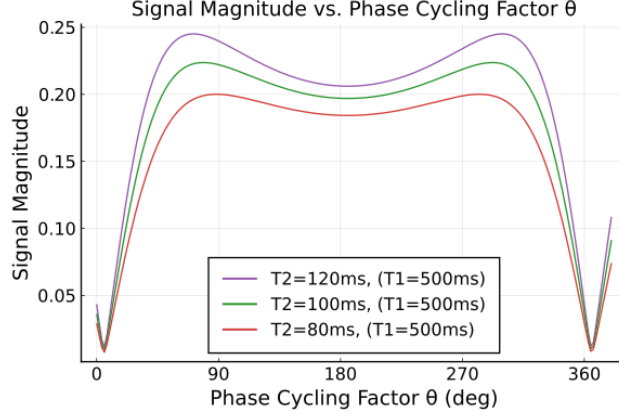


Figure 2.11: Simulated bSSFP signal magnitude vs. phase-cycling factor for a one-compartment voxel for three different T_2 values.

2.4.4 Elliptical Representation

When plotted on the complex plane, phase-cycled bSSFP signal profiles form an ellipse [93, 94, 102]. For a given phase cycling factor $\Delta\theta$, the signal equation takes the following form [94]:

$$S = M \frac{1 - ae^{-i(\phi - \Delta\theta)}}{1 - q \cos(-\phi + \Delta\theta)} e^{i(\phi(\frac{TE}{TR}) + \psi)} \quad (2.56)$$

where

$$M = M_0 \frac{(1 - E_1) \sin \alpha}{1 - E_1 \cos \alpha - E_2^2(E_1 - \cos \alpha)}, \quad (2.57)$$

$$a = E_2, \quad q = \frac{E_2(1 - E_1)(1 + \cos \alpha)}{1 - E_1 \cos \alpha - E_2^2(E_1 - \cos \alpha)}$$

In (2.56)-(2.57), ϕ is the phase due to off-resonance, $\Delta\theta$ is the phase cycling factor, and α is the RF flip angle as in Section 2.4.3. M , a , and q are functions of T_1 , T_2 , and M_0 , ψ is a constant accumulated phase term (from eddy-current induced and coil phase [94]), $E_1 = e^{-\frac{TR}{T_1}}$, and $E_2 = e^{-\frac{TR}{T_2}}$. When fitting a signal model using this elliptical representation, M , a , and q would be the fitted parameters (vs. fitting M_0 , T_1 , T_2 directly). This elliptical bFFSP signal model is equivalent to transverse component of the matrix representation [102].

2.5 Inversion Recovery NMR

Myelin water exchange is a difficult parameter to validate in vivo. Relaxation-exchange spectroscopy [103–105] has been used to estimate exchange, but is too slow for whole-brain scans.

An inversion recovery-based NMR experiment [4, 106] can be used to estimate a ground truth exchange rate for a two-pool system that includes a significant chemical shift, such as urea and water [107, 108]. The pulse sequence is shown in Figure 2.12. It is an NMR-based sequence so there is no imaging; data is collected for the whole sample, assumed to be homogeneous. The transmit offset frequency parameter is adjusted such that the pulse occurs at the resonant frequency of one of the two pools. Then the first two 90° pulses selectively invert that pool, which is done by setting T_S to $\frac{1}{2\Delta f_s}$, where Δf_s is the chemical shift between the two pools. The two pools exchange magnetization during the mixing time T_M , and then the free induction decay (FID) signal is measured at the echo time T_E . Data is collected for multiple values of T_M . Then the data is processed by integrating the area of each of the two peaks separately in the spectrum for each mixing time to generate a signal recovery curve vs. mixing time for each pool. A sample spectrum is shown in Figure 2.13. The integrated area vs. mixing time data for the two peaks are then fit to a bi-exponential recovery signal model [4, 35, 106] to extract the T_1 values corresponding to each pool, the exchange rates between the two pools, the overall exchange rate of the system, and the pool fractions. The following equations, based on those in [4], come from the Bloch-McConnell Equations and describe the longitudinal relaxation of two pools [35, 51], which we refer to as the urea and water pools

$$\begin{aligned}\frac{dM_w(t)}{dt} &= (M_{0,w} - M_w(t))R_w - k_w M_w(t) + k_u M_u(t) \\ \frac{dM_u(t)}{dt} &= (M_{0,u} - M_u(t))R_u - k_u M_u(t) + k_w M_w(t).\end{aligned}\quad (2.58)$$

Here, $M_w(t)$ and $M_u(t)$ denote the longitudinal relaxation in the water and urea pools at time t , and $M_{0,w}$ and $M_{0,u}$ denote the equilibrium magnetization of each pool. R_w and R_u represent the longitudinal relaxation rate of each pool, k_w is the exchange rate out of the water pool, and k_u is the exchange rate out of the urea pool. The magnetization can be rewritten in the form of reduced magnetization [35]:

$$S_w(t) = 1 - \frac{M_w(t)}{M_{0,w}}, \quad S_u(t) = 1 - \frac{M_u(t)}{M_{0,u}}, \quad (2.59)$$

which take the following form:

$$S_w(t) = c_{f,w}e^{-\lambda_f t} + c_{s,w}e^{-\lambda_s t}, \quad S_u(t) = c_{f,u}e^{-\lambda_f t} + c_{s,u}e^{-\lambda_s t}. \quad (2.60)$$

The parameters R_w , R_u , k_w , and k_u are fit with this bi-exponential signal model, as they are related to λ_f , λ_s , $c_{f,w}$, $c_{s,w}$, $c_{f,u}$, and $c_{s,u}$ by [4, 35]:

$$\lambda_{f,s} = \frac{(R_w + R_u + k_w + k_u) \pm \sqrt{(R_w - R_u + k_w - k_u)^2 + 4k_w k_u}}{2}. \quad (2.61)$$

$$\begin{aligned} c_{f,w} &= \frac{(R_w + k_w - \lambda_s)S_w(0) - k_w S_u(0)}{\lambda_f - \lambda_s} \\ c_{s,w} &= \frac{-(R_w + k_w - \lambda_f)S_w(0) + k_w S_u(0)}{\lambda_f - \lambda_s} \\ c_{f,u} &= \frac{(R_w + k_w - \lambda_f) \{ (R_w + k_w - \lambda_s)S_w(0) - k_w S_u(0) \}}{k_w(\lambda_f - \lambda_s)} \\ c_{s,u} &= \frac{-(R_w + k_w - \lambda_s) \{ (R_w + k_w - \lambda_f)S_w(0) - k_w S_u(0) \}}{k_w(\lambda_f - \lambda_s)}. \end{aligned} \quad (2.62)$$

Thus, the exchange rates k_w and k_u estimated using this method can serve as ground-truth exchange values to validate other exchange estimation methods.

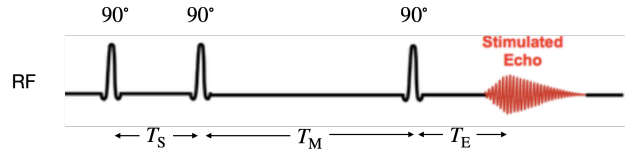


Figure 2.12: Image from [4]. Inversion recovery-based NMR pulse sequence that can provide exchange rate values. In this figure T_S is the selective inversion time, T_M is the mixing time, and T_E is the echo time.

This chapter has reviewed MR physics, tissue white matter modeling, qMRI in ADRD, and bSSFP and IR-NMR background; these details are applicable to the work in the following chapters.

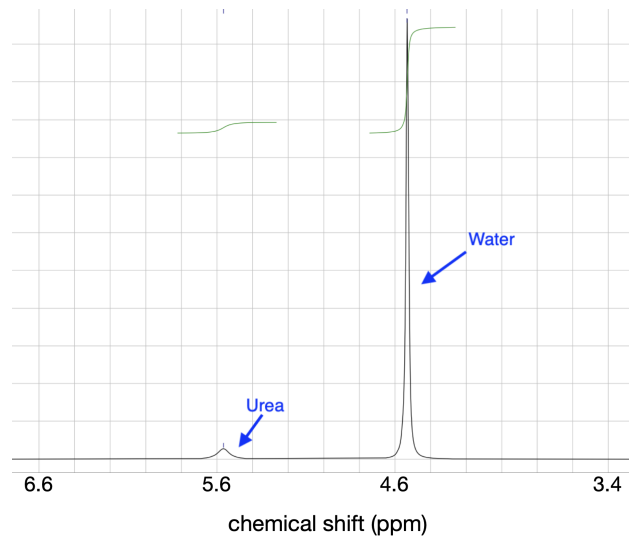


Figure 2.13: Image from [4]. NMR spectrum at one mixing time T_M for a urea-water phantom with a chemical shift of about 1 ppm. Area under the urea curve provides the urea curve signal at that T_M , and area under the water curve provides the water curve signal at that T_M .

CHAPTER 3

Impact of Tissue Sample Preparation Methods on Myelin-Sensitive Quantitative MR Imaging

3.1 Introduction

Quantitative MRI (qMRI) has been proposed as a more specific marker of disease pathology than MRI contrast images, and many qMRI methods have been developed to provide quantitative myelin-sensitive metrics [109]. Many prior qMRI studies have focused specifically on myelin-sensitive qMRI, as demyelination is the cause of many diseases, such as multiple sclerosis [19], and is involved in many others, such as Alzheimer’s disease [37]. Quantitative MRI parameters are often validated with histology in fixed tissue samples [8, 20–22, 28, 29]; however, fixation can alter MR tissue properties, including myelin-sensitive properties [9], and even immunohistochemistry and histological findings [110]. Freezing is another method for post-mortem storage that preserves nucleic acid components for genetic analysis on DNA, but a potential concern when using thawed tissue is the effect of the freeze-thaw cycle on cellular integrity, as ice crystals formed during freezing, cold storage, and thawing may destroy tissue components [111, 112]. This study compared the effects of both tissue preparation techniques, freezing and fixation, on myelin-sensitive qMRI parameters using three methods: myelin water imaging, off-resonance RF saturation magnetization transfer (MT) imaging, and selective inversion recovery MT imaging. These methods were chosen since they encompass fundamental contrast mechanisms that are widely accepted to be myelin-sensitive.

Myelin water imaging (MWI) is a common method for analyzing tissue myelin content and is based on the principle that water molecules within the myelin sheath interact more frequently with membrane molecules leading to shorter T_2 times than axonal or extracellular water. The MRI signal is a superposition of water signals in different compartments, leading to multi-exponential

This chapter is based largely on [44], that in turn extended [6, 45, 46].

decay [19]. The standard MWI experiment collects multiple spin echoes and fits the curve with non-negative least squares regression to determine the relative signal contribution from each T_2 component. T_2 spectra typically have two peaks corresponding to myelin water and free water [55, 57]. The fraction of the signal below a T_2 cutoff between the two peaks is defined as the myelin water fraction (MWF), which has been histologically validated and accepted as a measure of myelin content [8, 20–24]. However, it has been validated with fixed tissue, and fixation has been shown to decrease T_2 values [113–117] and increase MWF [9, 23, 118]. A study found that freezing and thawing tissue decreased water content, but did not significantly affect T_2 [119]. Another found that fresh and thawed samples exhibited similar signal behavior when imaged with ultrashort- T_2 techniques, but the thawed samples had consistently lower signal magnitudes than the fresh [120]. The effects of freezing and thawing tissue on MWF have not been widely studied.

Magnetization transfer (MT) is another common technique used in white matter (WM) imaging studies [10, 25, 26]. MT in myelin is dominated by exchange of water and macromolecular protons. The macromolecular protons have T_2 times of order 10 μ s and decay too fast to be observed using typical MRI sequences. Conventional MT imaging applies an MT preparation pulse far off-resonance from the water signal, directly saturating the macromolecular proton longitudinal magnetization. Proton exchange carries the depleted membrane magnetization to the water molecules, indirectly saturating the free (MR-visible) water magnetization. The semi-quantitative magnetization transfer ratio (MTR), the difference between the images obtained with and without the preparation pulse, normalized by the image without the preparation pulse [27], has been previously validated in tissue samples for its sensitivity to myelin content [28, 29]. Images acquired after applying preparation pulses at multiple RF power levels and off-resonance frequencies are fit to a quantitative MT (qMT) signal model to calculate the fractional size of the bound pool (F) [7, 17], which is another accepted measure of myelin content [30–32]. Fixation increases the effect of MT [9, 117, 121, 122], and freezing can increase MTR [119].

Quantitative T_1 mapping has also been used to study myelination [64–67] and perform qMT analysis [11, 33, 34, 61, 62], where the observed signal recovery is driven by MT. An inversion pulse selectively inverts water magnetization but not macromolecular proton magnetization. After this pulse, magnetization quickly flows into the inverted water pool from the solid pool. [35, 63]. Inversion recovery data is fit to a bi-exponential model to calculate the long and short T_1 components and their corresponding amplitudes. [35, 64] These parameters can be used to calculate the ratio of the sizes of the macromolecular and free water proton pools (F) as well [11, 33, 34].

Similar to T_2 relaxation, fixation decreases the T_1 value of water [113–117], and one study found that freezing tissue also decreases T_1 [119].

In this study, we extended preliminary work [6, 46] where we conducted MWI and qMT experiments and luxol fast blue (LFB) histological staining on fresh, thawed, and fixed sheep brain

tissue samples to analyze the effects of formalin fixation and freezing on qMRI measures of myelin content. We compared these qMRI parameter values across tissue conditions and correlated them with LFB values.

3.2 Methods

3.2.1 Samples and Equipment

Our process and procedures were compliant with and conducted with the approval of our university's Institutional Animal Care and Use Committee (IACUC). Seven unfixed, unfrozen ex vivo whole sheep brains were obtained from Nebraska Scientific (Omaha, NE, USA). Immediately after death, the tissue was transferred to a 4°C refrigerator. The time between death and brain extraction was seven days, and the whole brains were shipped to us five days after extraction, with a transit time of 12 hours on ice. We sectioned the specimens (see Figure 3.1 for an illustration) and placed them in the refrigerator at 4°C for up to one day. The total time between death and scanning was 12-13 days. The whole brain specimens were cut into ~13-mm thick coronal sections; sections at the level of caudate nucleus were placed into histology cassettes of size 40 × 26 × 13 mm for MR scanning (N=13). One specimen was cut as a parasagittal section of the same thickness resulting in 14 total samples. All samples allowed for examination of a variety of tissue types, including WM structures such as the corpus callosum, fornix, cortical white matter, and internal capsule, as well as cortical and subcortical gray matter (GM).

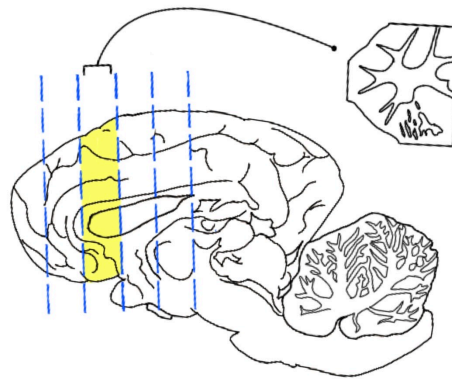


Figure 3.1: Illustration of the sheep brain dissection and sample selection. The sheep brains were cut into approximately 13 mm coronal sections. The 3rd most rostral section, which was at the level of caudate nucleus, was selected for MR and histological analysis.

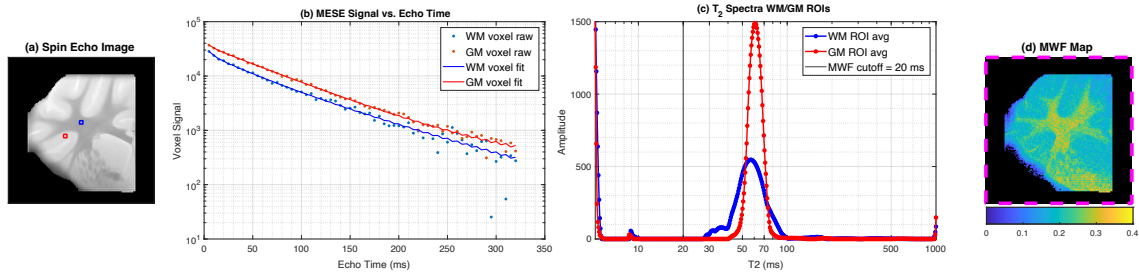


Figure 3.2: Myelin water imaging analysis for a representative fresh sample [6]. (a) Spin echo image ($TE = 5$ ms) marked with WM (blue) and GM ROIs (red). (b) Observed (dotted curve) and fitted (smooth curve) signal decay curves vs. echo time for one WM voxel and one GM voxel in the labeled ROI. (c) Average T_2 spectra for the WM and GM ROIs. (d) MWF map, calculated using a 20 ms T_2 cutoff (indicated in (c)). The MWF map is taken as a surrogate measure of myelin content.

Each cassette was submerged in Fluorinert FC-770 (3M, St. Paul, MN, USA) to minimize B_0 field inhomogeneity. All specimens were imaged fresh with our entire imaging protocol. Following fresh sample data collection, seven cassettes were placed in a -80°C freezer. After about three weeks the frozen samples were thawed for 48 hours at 4°C before their second round of imaging with the same protocol. The other seven cassettes were placed directly in 10% formalin for 36 hours after the fresh scan, washed in normal saline solution for 12 hours, and scanned again with the same protocol. 36 hours of fixation gives enough time for protein cross linking and tissue preservation without a significant effect on any subsequent immunohistological methods. This time frame was chosen after discussion with neuropathologists at the Unit for Laboratory Animal Medicine (ULAM) Pathology Core, and proved to be sufficient based on subsequent histological analysis. All samples were placed in 10% formalin and stored at room temperature before histological analysis.

For imaging, the samples were placed in a 40 mm Millipede quadrature coil and inserted into a 7.0 Tesla NMR/MRI scanner (Varian/Agilent, Walnut Creek, CA, USA) with 40 mT/m gradients with a 115-mm inner diameter. The studies were conducted below room temperature at $\sim 15\text{--}20^\circ\text{C}$ to ensure that the fresh and freshly thawed samples did not decay during the scanning process. Two rounds of B_0 shimming were performed voxel-wise across the sample using a 3D gradient echo shimming routine. Three of the 14 samples were scanned with a 40 mm saddle coil (Morris Inc., ON, CAN) due to equipment availability; statistical analyses in Section 3.2.5 accounted for this difference.

3.2.2 Myelin Water Imaging

Multi-echo spin echo (MESE) data were collected using a multi-echo multi-slice (MEMS) 2D slice-selective sequence with the following parameters: TR = 4000 ms, 64 echoes, echo spacing = 5 ms, matrix size = 128×128 , and Carr-Purcell-Meiboom-Gill (CPMG) phase cycling (custom or default Varian sequence) with two signal averages. The excitation pulse (90° flip angle) was a 5-lobe sinc pulse with a 1000 μs pulse duration and 5944 Hz bandwidth. The refocusing pulse (180° flip angle) was also an five-lobe sinc pulse with a 8000 μs width and 5877.5 Hz bandwidth. Data were acquired for five interleaved slices with thickness = 2 mm, slice gap = 0 mm, field of view = $35 \times 35 \text{ mm}^2$, and in-plane resolution = $273 \times 273 \mu\text{m}^2$. The total scan time per sample was 17 min.

The MESE data were analyzed voxel-wise with regularized non-negative least squares (NNLS) regression using the extended phase graph (EPG) formalism to estimate a T_2 spectrum for each voxel [55]. The fitting process used 50 T_2 values log spaced from 5-1000 ms and a regularization parameter of 0.001 to jointly estimate a B_1 field inhomogeneity scaling factor for each voxel using 16 values linearly spaced from 0.7-1.1; this B_1 map was then fixed and the T_2 spectrum was estimated with 500 T_2 values log spaced from 5-1000 ms and the same regularization parameter [59, 123]. The myelin water fraction (MWF) calculation used a cutoff value of 20 ms [8]. MWF was calculated for each voxel as the sum of the amplitudes of the points in the T_2 spectrum up to the cutoff divided by the sum of the amplitudes of the entire spectrum. These values were used to generate a MWF map. We conducted the NNLS fitting with the [NNLS package](#) in the [Julia programming language version 1.8.5](#). Figure 3.2 illustrates the steps in the MESE data processing pipeline for a representative fresh sample.

3.2.3 Quantitative Magnetization Transfer

Quantitative MT imaging was conducted using two different sequences and processing methods. The first was steady-state off-resonance RF saturation and MT parameter fitting according to the binary spin-bath model [7, 17], and the second was transient recovery of selectively inverted water proton magnetization and bi-exponential T_1 mapping [33].

3.2.3.1 Off-Resonance RF Saturation

Single-slice MT data were collected via a 2D gradient echo, slice-selective sequence with the following parameters: TR = 120 ms, TE = 3 ms, flip angle = 20° , and matrix size = 128×128 . The excitation pulse (90° flip angle) was a 5-lobe sinc pulse with a 1000 μs width. The MT preparation pulses consisted of a train of 20 Gaussian pulses of duration 1 ms, bandwidth 796 Hz, and duty cycle $\sim 16.7\%$ at 25 off-resonance frequencies from -60kHz to +60kHz at 5 kHz increments and

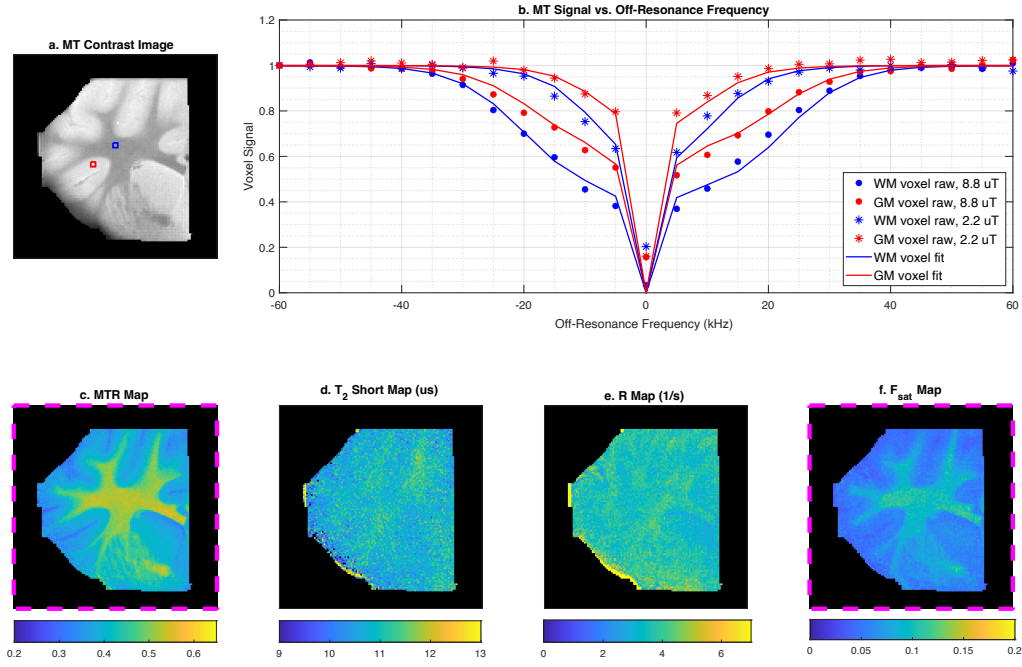


Figure 3.3: Saturation-based MT analysis for a representative fresh sample [6]. (a) MT contrast image at 10 kHz off-resonance and second highest power level (8.8 μ T) marked with WM (blue) and GM (red) ROIs. (b) Observed and fitted normalized signal curves vs. off-resonance frequencies from -60 kHz to 60 kHz for one WM voxel and one GM voxel in the ROI for two of the four power levels (8.8 and 2.2 μ T). (c) MTR map calculated with the average of the -10 kHz and 10 kHz images and 8.8 μ T power level. (d) Map of the estimated short T_2 value corresponding to the macromolecular pool in μ s for each voxel. (e) Map of the estimated rate constant R characterizing the exchange rate between the two pools in seconds for each voxel. (f) Map of the ratio of macromolecular to free water protons F_{sat} . Maps outlined in pink (c, f) are taken as surrogate measures of myelin content. The short T_2 and R maps have low WM/GM contrast compared to prior studies [7]; this may be due to temperature effects.

four RF B_1 RMS amplitudes at about 17.6, 8.8, 4.4 and 2.2 μT with flip angles of 8960°, 4480°, 2240°, and 1120°, respectively. The slice thickness = 2mm, field of view = $35 \times 35 \text{ mm}^2$, and in-plane resolution = $273 \times 273 \mu\text{m}^2$. The total scan time per sample was 26 min.

The magnetization transfer ratio (MTR) was calculated as $\frac{S_0 - S_+}{S_0}$, where S_+ is an image obtained with an MT preparation pulse at 10 kHz and S_0 is the image obtained at 60 kHz off-resonance. The reported MTR is the average of the MTR maps at +10kHz and -10kHz off-resonance and B_1 field of 8.8 μT .

A nonlinear parametric voxel-wise fit using the binary spin-bath MT model was performed on the MT data [7, 17, 124]. The two-pool model consists of a free water pool, M_f , with a Lorentzian lineshape and a macromolecular proton pool, M_m , with a super-Lorentzian lineshape [125–127]. For each voxel, three parameters were fixed. The long T_2 value of the free water pool, $T_{2,f}$, was assigned a value from a weighted average of the MWI T_2 spectrum data for all T_2 values beyond the 20 ms cutoff. $R_{1,f}$ was set to 0.33s^{-1} , the approximate R_1 of water at 7T in the absence of magnetization transfer, and $R_{1,m}$ was set to 1s^{-1} , as done in prior studies [7, 35, 128]. The remaining parameters were estimated: the T_2 value of the macromolecular pool $T_{2,m}$, the cross-relaxation rate of exchange between the pools R , the ratio of macromolecular to total free water protons $F_{\text{sat}} = \frac{M_m}{M_f}$, and the chemical shift of the macromolecular pool, $\Delta_{\text{cs},m}$; see Section A.1 in the appendix for further details about the signal model. Corrections for B_0 and B_1 inhomogeneities were also included in the model fitting using acquired maps. We conducted nonlinear fits with MATLAB R2023a (MathWorks, Natick, MA, USA). Figure 3.3 illustrates the steps in the MT data processing pipeline for a representative fresh sample.

3.2.3.2 Selective Inversion Recovery

Inversion recovery data were collected using a 2D slice selective inversion recovery (SIR) scan with a ramp-sampled spin-echo echo-planar imaging (EPI) readout at 21 inversion times (TI) logarithmically spaced from 10 ms to 5 s with the following parameters: TR = 8000 ms, TE = 36 ms, and matrix size = 64×64 . The excitation pulse (90° flip angle) was a 5-lobe sinc pulse with a 2000 μs width. The refocusing pulse (180° flip angle) was also a 5-lobe sinc pulse with a 1600 μs width. The inversion pulse (180° flip angle) was a hyperbolic secant (HS) adiabatic full passage (AFP) pulse [16] with a 4000 μs width and a power of 51 μT . A B_0 map, which was needed for EPI distortion correction, was acquired separately using a 2D gradient echo slice-selective sequence with multiple TE values at 4, 6, 8, and 10 ms, TR = 100 ms, flip angle = 20°, and matrix size = 128×128 . Data were acquired for five interleaved slices with thickness = 2 mm, slice gap = 0 mm, field of view = $35 \times 35 \text{ mm}^2$, and in-plane resolution = $547 \times 547 \mu\text{m}^2$. The total scan time per sample was 6 min for the EPI IR scan and 1 min for the B_0 mapping scan.

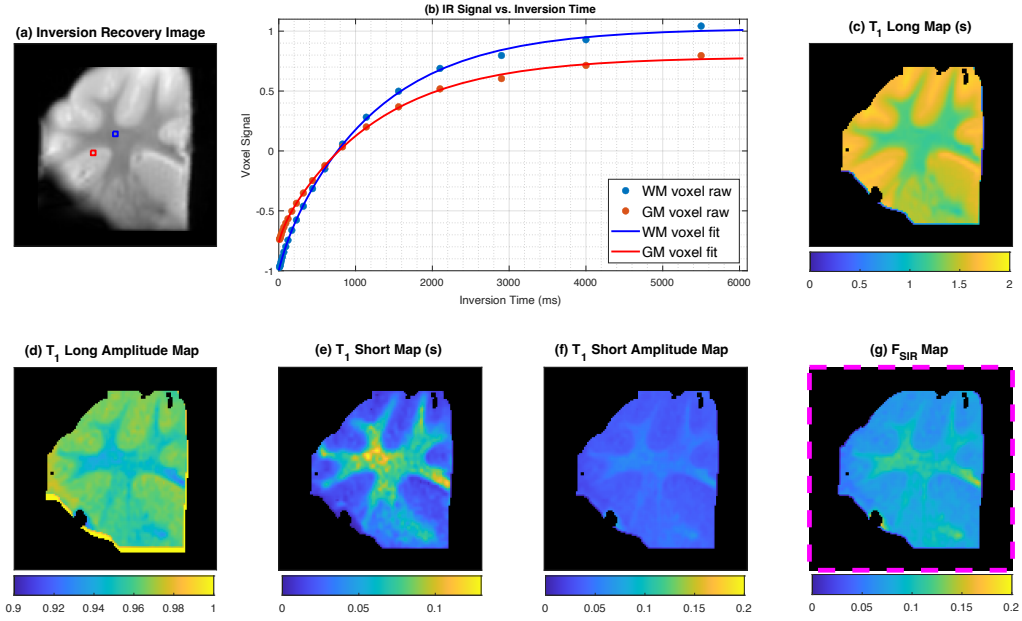


Figure 3.4: Selective inversion recovery bi-exponential analysis for a representative fresh sample [6]. (a) IR image (TI = 10 ms) marked with WM (blue) and GM (red) ROIs. (b) Observed and fitted signal recovery curves vs. inversion time for one WM voxel and one GM voxel in the ROI. (c) Map of the estimated long T_1 value for each voxel. (d) Normalized corresponding signal amplitude for the long T_1 component (a_{slow}) for each voxel. (e) Map of the estimated short T_1 value for each voxel. (f) Normalized corresponding signal amplitude for the short T_1 component (a_{fast}) for each voxel. (g) F_{SIR} map outlined in pink is taken as a surrogate measure of myelin content.

The IR EPI distorted images were unwarped, upsampled to a 128×128 matrix size, and registered to the MESE data [129]. Then a bi-exponential two-pool model fit was performed on the IR data using nonlinear least squares (NLLS) [33]. Five parameters were estimated voxel-wise: the short and long T_1 values corresponding to the fast and slow recovery rates, their corresponding amplitudes a_{fast} and a_{slow} , and a B_1 field inhomogeneity scaling factor. The ratio of macromolecular to free water protons, $F_{\text{SIR}} = \frac{M_m}{M_f}$ [33], was calculated as $2 \times$ the fast relaxing component amplitude a_{fast} [33] assuming full saturation of the macromolecular pool by the hyperbolic secant pulse; see Sections A.2-A.3 and Figure A.1 for further details. This quantity is the same as the definition of $F_{\text{sat}} = \frac{M_m}{M_f}$ in section 3.2.3.1 [130, 131], and is also called the pool size ratio (PSR) in other papers [33, 34, 130]. We conducted the nonlinear fit with MATLAB R2023a (MathWorks, Natick, MA, USA). Figure 3.4 illustrates the steps in the IR data processing pipeline for a representative fresh sample.

3.2.4 Histological Analysis

After imaging, all samples were immediately placed in 10% formalin to undergo fixation. After fixation, the tissue was bisected along the coronal plane with a razor to facilitate paraffin embedding. The top half, based on orientation in the magnet, was stored for further studies, and the other half underwent paraffin embedding followed by microtomy to obtain a $5 \mu\text{m}$ slice corresponding to the center of the original sample. The sample then underwent Luxol fast blue (LFB) staining for myelin visualization and Cresyl violet (CV) counterstaining for cell nuclei visualization; Figure 3.5 includes further details. Then they were scanned with a resolution of $0.5 \mu\text{m}/\text{pixel}$ using a Vectra Polaris brightfield whole-slide scanner (Akoya Biosciences, Marlborough, MA, USA). We used the blue channel minus the red channel as a measure of LFB-CV optical density (referred to as LFB in the following text).

3.2.5 ROI and Statistical Analysis

We used [Freeview](#) to label the histology and two corresponding MR center slice images for both tissue conditions (fresh/thawed and fresh/fixed). The images were labeled with WM and GM regions of interest (ROIs), which was done by manually looking for prominent anatomical WM/GM features in both the MR contrast and histology images. The ROIs were selected using a 4×4 region in the MR images, corresponding to an area of about 1 mm^2 . The histology ROIs were selected from an 8×8 region on a downsampled image, corresponding to approximately a $0.25\text{-}1 \text{ mm}^2$ range of tissue. Figure 3.6 shows a representative sample with corresponding MR and histology labeled ROIs.

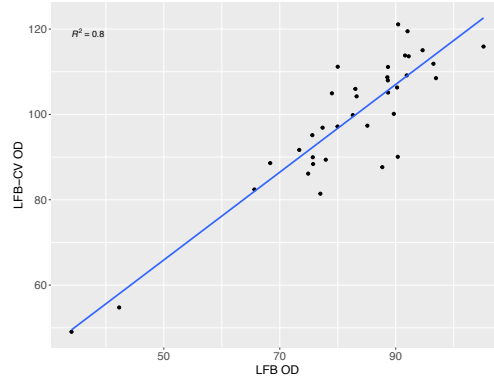


Figure 3.5: LFB + Cresyl Violet plotted against LFB only values (blue minus red channel) across WM ROIs (2 mm diameter circles) show a strong correlation between the two measures and a slope of nearly 1 from linear regression analysis. As expected, the intercept has a positive value (14), indicating a small residual stain for the nuclei in the absence of myelin staining. The plot shows that using a counterstain has little effect on the estimate of myelin content as compared to LFB alone, at least in ROIs on the order of 2 mm.

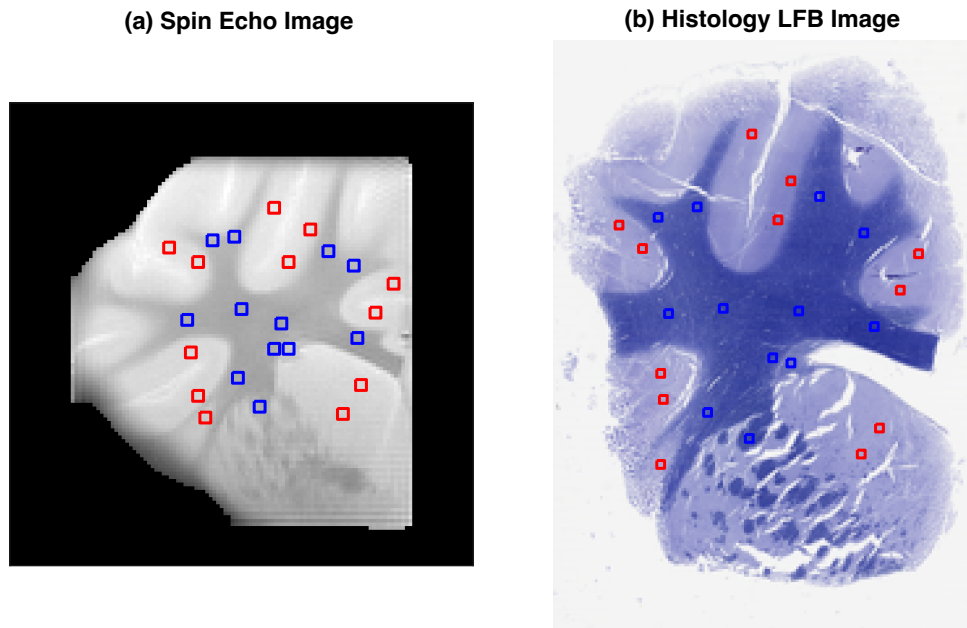


Figure 3.6: White matter (blue) and gray matter (red) ROIs labeled on a representative fresh sample spin echo image and corresponding histology LFB image.

All statistical analysis was done using [R version 4.4.1](#). LFB and qMRI values were averaged across the voxels within each ROI for each sample and were used as individual data points for the

subsequent statistical analyses. A mixed-effect linear model with a qMRI parameter as the dependent variable was used; the independent variables include: sample ID modeled as a random effect to account for variability across the samples, tissue condition (fresh/thawed/fixed) modeled as a fixed effect, LFB (or qMRI parameter) modeled as a fixed effect explanatory variable for regression analysis, and an interaction term between the tissue condition and the explanatory variable when comparing two or more regression terms. A significant interaction term would indicate a difference between the slopes of the regression lines, whereas a significant tissue condition term would indicate statistically different intercepts.

The plots were generated as mean-adjusted values generated from the mixed-effect models. The residuals after applying the random effect of sample ID were adjusted to the overall sample mean, which would account for the sample-to-sample variation including the variability in staining and use of different coils. Unpaired t-tests with a Bonferroni corrected p-value threshold of 0.00625 were used for post-hoc pair-wise comparison between the tissue conditions to determine whether the thawed or fixed tissues significantly differed from the fresh samples. Correlation analysis was conducted across ROIs across all samples and separately for each sample.

Power analysis: for a mixed-effect model, at least six samples per tissue preparation method and at least three ROIs per region (WM/GM) were needed to achieve a power of 90%, assuming an effect size of at least 10% of the control mean. These calculations were performed using the `simr` package (Version 1.07) function `powerSim` in R. We had 236 ROIs total (124 WM, 112 GM) across 14 samples (7 fixed, 7 thawed), with 5 to 24 ROIs per sample.

3.3 Results

Figure 3.7 shows qMRI parameter maps for two representative samples. Both samples were scanned fresh prior to tissue processing, followed by a second scan after a freeze-thaw cycle for Sample 1 and fixation for Sample 2. We observe similar contrasts in the maps across the different tissue conditions. As expected, a clear contrast was maintained between the WM and GM structures. Visually, freezing or fixation did not significantly affect MR maps or WM/GM contrast. Figure 3.7 also includes the corresponding histology images; the zoomed in images show myelinated axons and intact neurons, oligodendrocytes, and astrocytes for both tissue conditions. Freezing can cause morphological changes to the tissue, as demonstrated in the Sample 1 LFB image where portions of the sample have cracks. These cracks are also seen in fixed tissue, but based on visual inspection of the images, were more prevalent in samples that had been frozen.

Figure 3.8 pools average ROI data from all samples for the four qMRI parameters to show the effects of tissue preparation, separating data based on tissue condition and WM/GM data. The pooled data from the WM ROIs consistently showed a higher value than GM ROIs, supporting

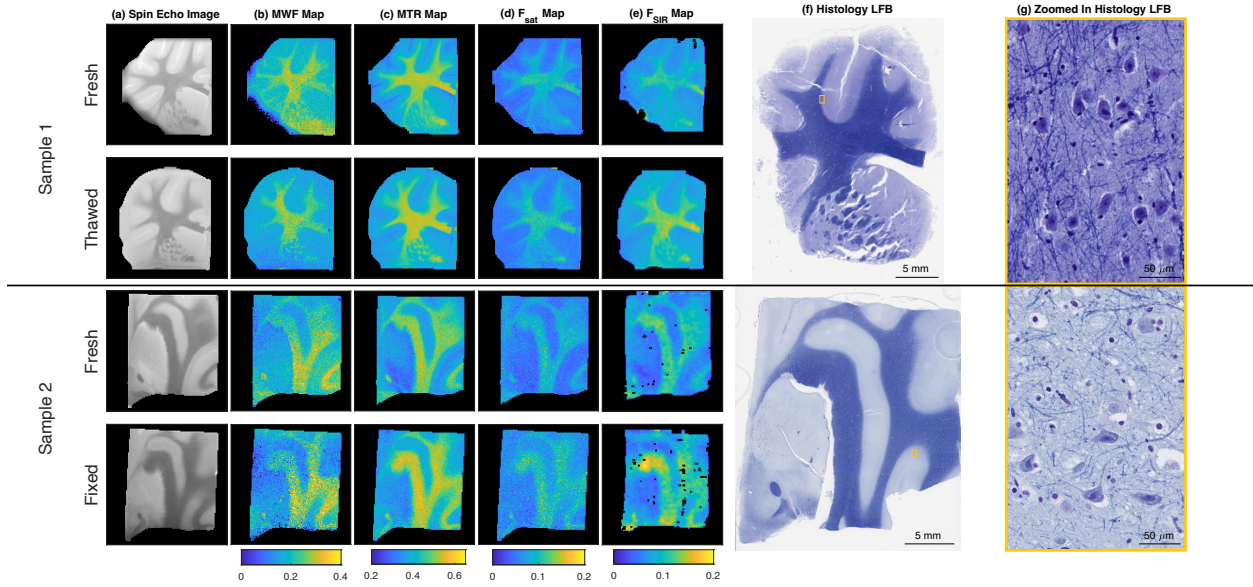


Figure 3.7: qMRI maps and histology stains for two representative sheep brain tissue samples. Sample 1 (rows 1-2, coronal slice) was scanned fresh and thawed, and Sample 2 (rows 3-4, sagittal slice) was scanned fresh and fixed. (a) Spin echo images at $TE = 5$ ms. (b) MWF maps calculated with a 20 ms cutoff; this value separated the peaks for most voxels, but black pixel outliers in maps are outlier voxels where the peaks were inconsistent with this cutoff. (c) MTR maps from MT contrast images at average of -10kHz and 10kHz off-resonance and $8.8 \mu T$ power level. (d) F_{sat} maps. (e) F_{SIR} maps; black pixel outliers in maps are voxels where the fitting routine produced NaN values. (f) LFB histology for Samples 1-2. (g) Zoomed in views showing cellular integrity.

the notion that these parameters are sensitive to myelin. Table 3.2 further quantifies the results in Figure 3.8 and includes the number of WM/GM ROIs and mean $\pm$ the standard deviation for each qMRI parameter for each tissue condition. The number of ROIs N represents the minimum number of ROIs pooled across samples because some samples are missing data from one or more parameters; see Table 3.1 for more details. Overall, all thawed and fixed average measures were similar (within ± 0.04) to those of the fresh samples. T-tests detected statistically higher MTR and F_{sat} in WM and GM and F_{SIR} in WM in both thawed and fixed tissue compared to fresh. The results were comparable across tissue condition for MWF, with the exception that GM MWF values were lower in fixed tissue compared to fresh, a finding that was not observed in the thawed samples.

Looking at average WM and GM values of qMRI parameters can mask the effects of tissue processing on estimation of myelin content. Figure 3.8 exhibits some WM outlier data points with values more in line with GM; for example, some MWF WM points have a very low MWF. We chose ROIs that include a wide range of myelin content, as seen in Figure 3.6, so the WM-labeled

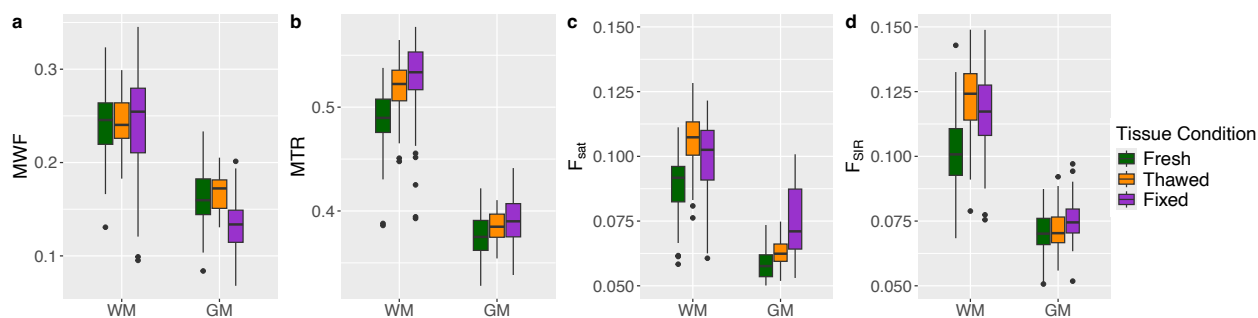


Figure 3.8: Box plots (interquartile range, whiskers: 1.5-times interquartile range, outliers as dots) of average mean-adjusted MR parameters (across individual samples) from ROIs from all tissue samples. Parameter values are consistent with literature values [8–11]. For MTR and F_{sat} , both thawed and fixed mean values were statistically different than fresh mean values for both WM and GM ROIs.

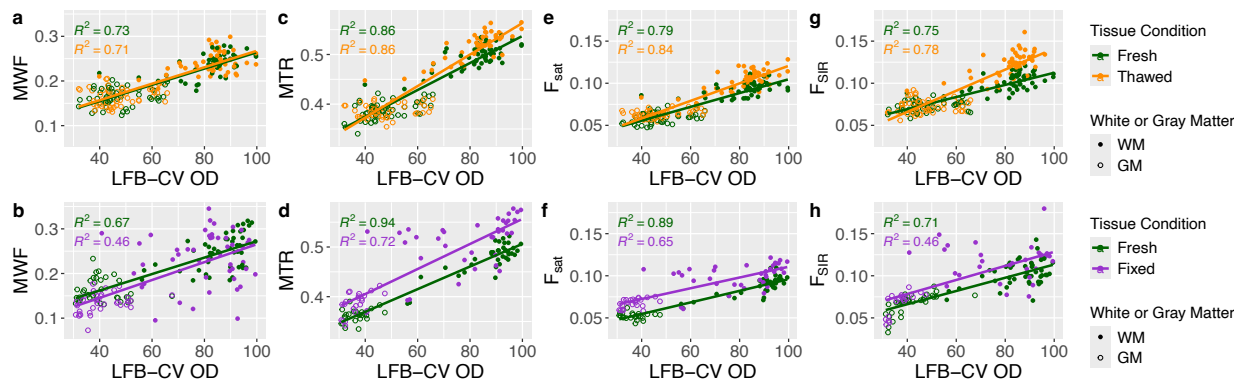


Figure 3.9: Correlation between qMRI parameter and histology Luxol fast blue stain/Cresyl violet counterstain (LFB-CV) mean-adjusted values across tissue samples for fresh and thawed (Row 1) and fresh and fixed (Row 2) samples. Fresh samples have been separated into two groups based on whether they went on to be frozen or fixed and were not pooled because each pair of scans for one sample corresponds to one LFB image. Fresh, thawed and fixed qMRI parameters showed a strong correlation with LFB-CV OD for all ROIs as indicated by the high R^2 values.

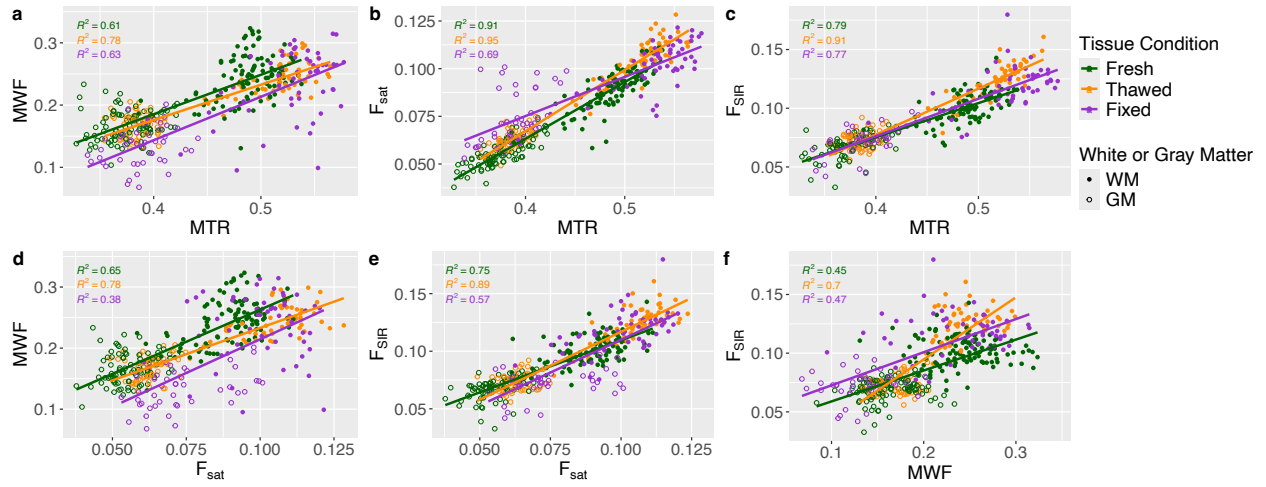


Figure 3.10: Correlation among each pair of qMRI parameter mean-adjusted values across tissue samples for fresh, thawed, and fixed samples. Fits are similar among tissue conditions for all pairs of parameters, further suggesting that thawed samples are a viable option for qMRI analysis. MTR and F_{sat} correlate well, likely because both are calculated from the off-resonance RF saturation MT scans. F_{sir} also correlates well with MTR and F_{sat}, which may result from it also being an MT parameter.

ROIs included some structures with less myelin, such as the fornix and juxtacortical WM. Additionally, intermediate myelin content in WM structures may be affected differently by different tissue preparations. Figure 3.9 addresses these limitations of the box plots; it shows qMRI vs. LFB values which demonstrates their correlation and displays intermediate LFB values between the distinct WM and GM clusters. These LFB values include a counterstain; the contribution of the nuclear staining to the overall tissue staining was found to be minimal and relatively uniform given our scale and ROI size as seen in Figure 3.5. The graph pools ROI data from samples scanned fresh/thawed and samples scanned fresh/fixed separately; this was done because each pair of scans corresponds to the same LFB data. All qMRI parameters correlated with LFB, and for some plots the qMRI parameters correlated with LFB within the WM ROI subgroups. Table 3.3 includes quantitative metrics corresponding to Figure 3.9. It shows the correlation R² values for each qMRI parameter for all ROIs pooled across all samples and for WM and GM ROIs separately in addition to the range of R² values from correlation analysis conducted on each sample separately. The R² values for all ROIs were high (≥ 0.45) and statistically significant across tissue conditions, as were many of the WM-only and GM-only ROI R² values, especially for MTR; this indicates that LFB accounts for a large variation in the MR signal. The MTR and F_{sir} fresh and thawed lines and MWF fresh and fixed fitted lines had statistically different slopes, and the MWF, MTR, and F_{sat} fresh and fixed fitted lines had statistically different intercepts.

Figure 3.10 shows the correlation between each pair of qMRI parameters across ROIs for all tissue conditions. Unlike histology images, where image co-registration is difficult, qMRI-qMRI correlation analysis uses the same ROI coordinates across images. Using similar mixed-model analysis as with the LFB plots, in subfigures 3.10c, e, and f the slopes and intercepts of the thawed sample data were significantly different from those of the fresh sample data. In subfigures 3.10d and e the slopes of the fixed sample data were significantly different from the fresh, and in subfigures 3.10a, d, e, and f, the intercepts of the fixed sample data were significantly different from the fresh.

3.4 Discussion

Our findings suggest that alterations in qMRI parameters due to freezing and thawing tissue are comparable to those due to fixation. Although our results are overall consistent with prior studies, some notable differences are discussed below. Our ranges of MWF, MTR, F_{sat} , and F_{SIR} values are consistent with those of prior studies [8, 10, 11, 20–22, 34, 62, 132]. The F_{sat} and F_{SIR} metrics had overall similar means, but the F_{SIR} means were $\sim 9 - 22\%$ higher, except for the fixed GM case, where the F_{SIR} was 3% lower; further comparing these estimates is an investigation for future studies.

A few groups have studied the effects of tissue fixation on qMRI parameters. In a study of MWF and F_{sat} values in fresh and fixed 20 mm human spinal cord samples [9], Seifert et al. reported an increase of around 40% in MWF for both WM and GM after 24 hours of fixation in 10% formalin compared to the pre-fixation measurement [9], which was similar to the observations by Chen et al. [118]. Our results, however, show that WM MWF values were not significantly affected by 36 hours of fixation in formalin, and GM values actually decreased by about 19%. Our study design differs from that of Seifert et al. in many aspects, as our studies involved different anatomical sites, and we used sheep brain tissue, whereas Seifert et al. used human spinal cord tissue. Our study was conducted at a lower temperature, and MWF has been shown to depend on temperature [68]. In addition, we submerged formalin-fixed tissue in buffered saline solution for 12 hours prior to scanning, whereas Seifert et al. washed the tissue for multiple 24-hour periods, which could have altered the tissue water content. Since WM and GM may have different susceptibilities to water fluctuations, differences in the duration of the saline wash could have affected GM MWF values more than WM.

Our MT related measures are consistent with previous studies. We saw a statistically significant increase in MTR and F_{sat} after fixation, although to a lesser degree than previously reported. For F_{sat} , Seifert et al. found approximately a 37% increase in WM and 12% increase in GM after one day of fixation [9]. We found an approximate 11% increase in WM and 32% increase in GM

after 36 hours of fixation. For MTR, we found an 8% increase in WM and 3% increase in GM, and for F_{SIR} a 20% increase in WM and 3% increase in GM. The overall smaller change in MT measures due to fixation in our studies could be due to similar factors as for MWF, or the choice of ROIs could contribute to some of the observed differences. We selected ROIs to include less myelinated regions of WM such as the fornix and juxtacortical myelin which may have affected the mean qMRI values. Our sequences and fitting methods also differed from Seifert et al., which may have affected the results.

A potential explanation for the observed changes in the qMT parameters after tissue fixation is that the fixation expands the spaces between layers of the myelin sheath, which may increase exchange between the macromolecular proton and water pools and increase F_{sat} [9]. Formalin cross-links protein lysine residues, which may increase semi-solid content, creating more pathways for MT and increasing MTR.

We also saw significant increases in thawed tissue MTR and F_{sat} in both WM (6% and 22%) and GM (3% and 13%) and in the F_{SIR} in WM (20%) on the same order of the increases from fixation. Evans et al. [119] also found that freezing muscle tissue increases MTR, which is consistent with our results, but given the different tissue types, any comparison must be viewed cautiously. Whether the increases in MTR, F_{sat} , and F_{SIR} in frozen tissue are due to similar mechanisms as the effect of fixation requires additional studies with closer inspection of morphological changes with electron microscopy.

Examining the relationship between qMRI and non-MR measures of myelin content across a wide range of myelin concentration affords additional insight into the effects of tissue preparation on qMRI measures as they relate to other measures such as LFB. Overall, all qMRI parameters correlated well with LFB as in previous studies [8, 20, 21, 28, 29], even when pooling ROIs from different samples and in WM ROIs alone [68, 69]. As mentioned in Section 3.3, the slopes of the qMRI vs. LFB plots are generally higher in thawed samples as compared to fresh, indicating that the freeze-thaw cycle increases the qMRI signal more for the WM than GM ROIs. This trend reached statistical significance only for MTR and F_{sat} . In the case of the fixed tissue, the intercepts of the fitted lines were affected for MWF, MTR, and F_{sat} . Tissue fixation appears to affect the WM to the same extent that it affects the GM, thereby increasing the intercepts of qMRI measures of myelin content without as strongly affecting the slopes. We saw a similar trend in the qMRI vs. qMRI plots, confirming that the observed trends hold without potential errors due to registration mismatch. The strong correlation in these plots was consistent with prior reports [9, 69]. We also observe a similar trend with MTsat, another qMRI parameter sensitive to myelin content [133–139]. Although this parameter has been excluded from the main text, we include MTsat maps and histological correlation analysis in Figures A.2-A.3.

The correlation analysis results indicate that freezing and fixation may affect qMRI tissue parameters differently; freezing could potentially alter the WM structures that contain thicker and more abundant myelin sheaths more robustly than thinner myelinated axons in the GM, whereas fixation could potentially affect the myelination in WM and GM uniformly. We hypothesize that formalin fixation, being a chemical process, may be less dependent on the morphological features of the tissue such as fiber diameter and the number of myelin layers, which may affect WM and GM equally. In contrast, freezing and thawing are thermo-mechanical processes that we conjecture may be affected by the microscopic morphology of the tissue and fiber diameter, thereby having a differential effect on WM and GM. MWF and MTR have been shown to depend on WM fiber orientation and thickness [42,69], but further studies are needed to elucidate the mechanisms behind our observations and to test these hypotheses.

Our study unveils many challenges and limitations in evaluating the effects of post-mortem tissue preparation on qMRI measures, including use of different coils for scanning, lack of strict temperature control, use of semi-quantitative measures of myelin (LFB-CV), variability in the time of death to scan across the samples, lack of stereotactic dissection of tissue and image registration, among others. We attempted to mitigate or account for some of these shortcomings as discussed below. Because we obtained our samples from a vendor, the post-mortem interval ranged from 12 to 13 days; however, care was taken to refrigerate or kept on ice to minimize tissue autolysis, and our histological examination confirmed tissue preservation throughout the process, as seen in Figure 3.7. Slight difference in postmortem interval may have affected qMRI results due to increased exchange from breakdown of the cellular membranes.

Additionally, freezing, thawing, and fixation deform the tissue and cause shrinkage and/or expansion, which makes histology-MR image registration challenging, leading us to choose to use manual matching for ROI analysis instead of registration. We selected regions that had clear anatomical demarcation to minimize the effects of slice selections between MR and histology images, but errors in ROI placement undoubtedly remain. These variations may lead to outliers in the data, for example impacting why the fixed GM MWF values were lower than their fresh counterparts and why the MWF plots had lower R^2 values in Figure 3.9. Figure 3.10 is immune to these ROI placement errors; however, imperfect unwarping of the EPI data and registration to the MESE data could also have led to outliers in the F_{SIR} values and lower R^2 values.

Our data indicate that the cells remained intact during the freezing/thawing process of around two weeks; however, the long-term effects of tissue freezing remain unknown. Further experiments such as those in Seifert et al [9] are needed to determine how freezing and fixation time affects each of the estimated parameters. Scanning tissue at lower temperature ($\sim 15-20^\circ\text{C}$) has the advantage of preserving tissue for longer, which is especially important when working with unfixed specimens; however, in a separate experiment (not shown) we conducted IR scans of a fixed sample at 19.8°C

and 16.3°C and found the F_{SIR} value to be $\sim 10 - 20\%$ higher at the lower temperature. Further experiments are needed to evaluate the effect of temperature on qMRI parameters for different tissue conditions. We did not monitor the temperature as the samples equilibrated from room temperature to the cooler ambient temperature of the magnet at $\sim 15^\circ\text{C}$. Therefore we cannot report the exact temperature, which is a limitation of our study.

Multi-slice MESE acquisitions are affected by MT [59, 140]. In a separate experiment (not shown) we compared parameter estimates from single and multi-slice MESE and IR acquisitions in a fixed sample. On average, the $\text{MWF}/F_{\text{SIR}}$ values estimated from the multi-slice acquisitions increased/decreased from the single-slice acquisition by $10 - 20\%$; thus, our use of multi-slice acquisitions is a limitation of our work. To estimate qMT parameters with the off-resonance RF saturation model we fixed $R_{1,f}$ to 0.33 s^{-1} [35] rather than estimating it using the IR data [7, 17] as this resulted in better qMT parameter fits. Fixing $R_{1,f}$ likely underestimated F_{sat} , made the F_{sat} values more sensitive to T_1 , and affected our WM/GM comparisons due to WM/GM differences in T_1 ; thus this is a limitation of this study.

Future studies could also examine the effects of freezing and fixation on MTsat further and on inhomogeneous magnetization transfer (ihMT) imaging, as the ihMT ratio (ihMTR) has been shown to be more specific to myelin than MTR [68–70]. Other modalities such as ultrashort TE (UTE) imaging, quantitative susceptibility mapping (QSM), and quantitative diffusion methods could also be explored.

3.5 Conclusion

Thawed and fixed tissue MR parameter values differed modestly compared to fresh values. Both tissue preparations had similar effects on myelin-sensitive qMRI parameter values, and the samples maintained cell integrity, allowing histology to be conducted. Thus, tissue freezing is a reasonable alternative tissue preservation method to fixation for use in qMRI analysis. However, it is important to consider that tissue preparation techniques may differentially affect regions with varying myelin content or morphology.

Table 3.1: Quantitative MRI Parameters and Histology Data Tally for All Samples

Samples		Parameter				
ID	Tissue Condition	MWF	MTR	F_{sat}	F_{SIR}	LFB-CV
S1	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S2	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S3	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S4	Fresh	✓	-	-	✓	-
	Thawed	✓	✓	✓	✓	-
S5	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S6	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S7	Fresh	✓	✓	✓	✓	✓
	Thawed	✓	✓	✓	✓	✓
S8	Fresh	✓	-	-	✓	✓
	Fixed	✓	-	-	-	✓
S9	Fresh	✓	✓	✓	✓	-
	Fixed	✓	✓	✓	✓	-
S10	Fresh	✓	✓	✓	✓	✓
	Fixed	✓	✓	✓	✓	✓
S11	Fresh	✓	✓	✓	✓	✓
	Fixed	✓	✓	✓	✓	✓
S12	Fresh	✓	✓	✓	✓	-
	Fixed	✓	✓	✓	✓	-
S13	Fresh	-	-	-	-	-
	Fixed	✓	✓	✓	✓	-
S14	Fresh	✓	✓	✓	✓	-
	Fixed	✓	✓	✓	✓	-

Quantitative MRI and histology metrics that we had for each sample. Some samples are missing certain scans or did not get sent for histological processing. For example, we do not have MTR or F_{sat} data for the fresh version of S4, nor do we have histology data for this sample.

Table 3.2: Quantitative summary of fresh, thawed, and fixed mean qMRI parameter values in Figure 3.8.

ROIs			Mean $\pm$ Standard Deviation P-Values			
Tissue Condition	Type	N	MWF	MTR	F _{sat}	F _{SIR}
Fresh	WM	102	0.24 $\pm$ 0.035	0.49 $\pm$ 0.026	0.09 $\pm$ 0.011	0.10 $\pm$ 0.012
	GM	90	0.16 $\pm$ 0.026	0.38 $\pm$ 0.022	0.056 $\pm$ 0.0071	0.070 $\pm$ 0.0096
Thawed	WM	51	0.24 $\pm$ 0.026 p=0.87	0.52 $\pm$ 0.027 p=4.1(10⁻¹⁰)	0.11 $\pm$ 0.011 p=1.4(10⁻¹⁴)	0.12 $\pm$ 0.016 p=1.9(10⁻¹³)
	GM	51	0.17 $\pm$ 0.020 p=0.17	0.39 $\pm$ 0.014 p=0.0011	0.063 $\pm$ 0.0053 p=8.2(10⁻¹⁰)	0.072 $\pm$ 0.0076 p=0.030
Fixed	WM	51	0.24 $\pm$ 0.054 p=0.91	0.53 $\pm$ 0.042 p=4.2(10⁻⁸)	0.10 $\pm$ 0.014 p=3.5(10⁻⁶)	0.12 $\pm$ 0.018 p=9.5(10⁻⁸)
	GM	39	0.13 $\pm$ 0.028 p=3.2(10⁻⁹)	0.39 $\pm$ 0.025 p=0.0026	0.074 $\pm$ 0.014 p=3.1(10⁻¹¹)	0.072 $\pm$ 0.013 p=0.16

Mean $\pm$ standard deviation of qMRI parameter values for WM and GM ROI data for the three tissue conditions. Bold values in the thawed/fixed rows indicate statistically significant differences in the mean qMRI values between the thawed or fixed samples compared to the fresh samples from t-tests with a Bonferroni corrected threshold ($p < 0.00625$). P-values are from these t-tests. All qMRI parameter values are unitless fractions. N is the minimum number of ROIs pooled across all samples (some qMRI measures have data from more than N ROIs), as for some samples, not every qMRI measure was collected; see Table 3.1.

Table 3.3: Quantitative summary of qMRI-LFB correlation in Figure 3.9.

ROIs			MWF	MTR	F _{sat}	F _{SIR}
Tissue Condition	Type	N	Overall R ² (Range)	Overall R ² (Range)	Overall R ² (Range)	Overall R ² (Range)
Fresh	WM	51	0.31	0.51	0.26	0.20
	GM	51	-	0.24	0.11	-
	All	102	0.73 (0.75-0.93)	0.86 (0.83-0.98)	0.79 (0.70-0.95)	0.75 (0.63-0.91)
Thawed	WM	51	0.16	0.67	0.48	0.46
	GM	51	-	-	0.14	-
	All	102	0.70 (0.64-0.95)	0.86 (0.74-0.98)	0.84 (0.80-0.94)	0.79 (0.70-0.96)
Fresh/Thawed Interaction	All	102	-	s (p=0.022)	s (p=0.028)	-
Fresh	WM	36	0.25	0.69	0.59	0.13
	GM	24	-	-	-	0.44
	All	60	0.66 (0.62-0.88)	0.94 (0.93-0.96)	0.89 (0.86-0.94)	0.75 (0.76-0.78)
Fixed	WM	36	-	0.15	0.11	-
	GM	24	0.097	0.29	0.19	0.48
	All	60	0.45 (0.42-0.86)	0.71 (0.52-0.98)	0.64 (0.46-0.92)	0.45 (0.32-0.93)
Fresh/Fixed Interaction	All	60	s (p=0.034) i (p=0.0076)	i (p=0.034)	i (p=8.3(10 ⁻⁶))	-

R² values for correlation between qMRI measures and LFB across WM ROIs only, GM ROIs only, and all ROIs (WM and GM). Values are shown as R² for mean-adjusted data pooled across all samples. Ranges of R² values for individual samples are shown in parenthesis for the “All” rows. With one exception (fresh MWF correlation), the overall R² values are within the range of the individual sample R² values. Dash marks represent R² values that were not statistically significant ($p < 0.05$). This table corresponds to the data in Figure 3.9. Yellow rows show results and corresponding p-values from the interaction term between tissue condition and LFB from the mixed-effect model analysis (“s:” statistically different slopes, “i:” statistically different intercepts); statistical significance indicates differential correlation based on tissue condition. N is the minimum number of ROIs pooled across all samples (some qMRI measures have data from more than N ROIs), as for some samples, not every measure (qMRI or LFB) was collected; see Table 3.1.

CHAPTER 4

Comparison of Myelin-Sensitive Quantitative MR and Histological Markers in Alzheimer’s Disease Tissue Samples

4.1 Introduction

Alzheimer’s disease was the seventh-leading cause of death in the US in 2021. Alzheimer’s disease and related dementias (ADRD) is an umbrella term that encompasses multiple disease processes that can result in a progressive decline in cognitive function, which in addition to Alzheimer’s disease, may include Lewy Body disease (caused by abnormal accumulation of alpha-synuclein), Frontotemporal dementia (due to abnormal accumulation of tau, TDP-43 or other proteins), as well as vascular dementia, among others. ADRD contributes a huge burden to healthcare costs in the United States, and early diagnosis could save \$7 trillion in medical and long-term care costs [36]. Since neuronal loss due to aggregation of β -amyloid and tau are hallmark pathological changes in Alzheimer’s disease, research has largely focused on their contribution to the disease [36]. However, multiple pathological processes co-exist in ADRD, including ones that affect white matter (WM) tracts. Ischemia, inflammation, or direct oligodendrocyte cytotoxicity can damage WM tracts, causing demyelination, axonal loss, and eventually replacement of these tracts by scar tissue (gliosis). Accumulating evidence suggests that significant myelin degradation occurs in ADRD [37–39, 141, 142]. MR measures that are more specific to myelin would be valuable for diagnosing and monitoring ADRD, quantifying the disease burden, and determining treatment effectiveness.

This chapter extends abstract [47] toward a journal submission.

A quantitative MR method that can accurately measure myelin content in ADRD would be valuable for determining the degree of myelin damage in these disorders, and can serve as a surrogate marker of efficacy of therapies that treat myelin loss in ADRD.

MRI is a powerful tool for diagnosis, but the common clinical T_1 and T_2 weighted scans are not specific for quantifying the degree of myelin loss or the degree of tissue damage [18]. For example, white matter hyperintensities (WMH) in multiple sclerosis (MS), which reflect profound myelin loss from inflammation, and those in ADRD due to a presumed vascular etiology, which reflect oligodendrocyte loss, and WMH in older adults undergoing healthy aging, which reflect benign enlargement of perivascular spaces, all have the same appearance on clinical imaging. Quantitative MRI (qMRI) can be more specific in determining the degree of myelin loss and potentially, other pathological features (e.g. axonal loss). Understanding the effects of histopathological features in ADRD on MR measures of myelin is necessary for developing myelin-specific MR measures that remain valid in the context of ADRD. ADRD affects myelin, and the qMRI measures of myelin content have not been validated in the context of ADRD. The broader impact of validating myelin-sensitive qMRI measures in the context of ADRD is that it can quantify the disease burden and has the potential to be a useful tool to determine treatment effectiveness.

This study focuses on three myelin-sensitive quantitative MRI (qMRI) parameters in ADRD tissue samples: MWF, MTR, and F_{sat} , and evaluates their accuracy in quantifying myelin as compared to histological quantities of myelin content. We also evaluate the specificity of myelin-sensitive qMRI parameters to myelin as compared to other histological measures of WM integrity such as axonal density and oligodendrocyte count. Myelin water imaging and magnetization transfer imaging have been previously discussed in Chapter 3; these imaging and data processing methods provide the three myelin-sensitive qMRI parameters listed above. Myelin basic protein (MBP) is present in the myelin sheath and is important to the myelination process of neurons, and MBP is a known biomarker of myelin content [68]. Neurofilament (NF) is a protein present in the cytoplasm of neurons that provides axonal support and correlates with axonal diameter [143]. OLIG2 is a protein present in the cell bodies of oligodendrocytes, which are glial (non-neuronal) cells that myelinate axons in the central nervous system. Loss of oligodendrocytes, or a lower oligodendrocyte count (OC), may be indicative of unhealthy tissue and may be related to the amount of myelin present. Oligodendrocytes have also been shown to play an important role in AD pathogenesis [144–151]. These three proteins can be detected with antibodies against them and quantified using immunofluorescence (IF) or immunohistochemistry (IHC) (specifically with the 3,3'-Diaminobenzidine (DAB) substrate to visualize the antibodies). We expect MBP to correlate with the myelin-sensitive qMRI measures based on prior studies [68]. We will assess the accuracy of myelin-sensitive qMRI measures using MBP and use other histological measures (NF and OLIG2) to identify potential non-myelin-related contributions to these qMRI measures.

Table 4.1: Human Tissue Sample Descriptions

Sample ID	Anatomical Location	Pathological Diagnosis
2194-S1	anterior periventricular WM	HL-AD, CAA
2194-S2	posterior periventricular WM	HL-AD, CAA
2468-S3	posterior/superior periventricular	HL-AD, CAA*
2468-S4	frontolateral periventricular WM	HL-AD, CAA*
1131-S3A	posterior parietal	IL-AD

FTD = frontotemporal dementia, HL-AD = high likelihood Alzheimer’s Disease, IL-AD = intermediate likelihood Alzheimer’s Disease, CAA = cerebral amyloid angiopathy. Samples with * also have: LBD/ND = lewy body dementia/neocortical diffuse, LPE = limbic-predominant age-related TDP-43 encephalopathy, ARTAG = aging related tau astroglipathy, Art = arteriolosclerosis, Ath = atherosclerosis

4.2 Methods

4.2.1 Samples and Equipment

Ex vivo frozen brain tissue samples were procured from the Michigan Alzheimer’s Disease Research Center (MADRC) brain bank. Each sample came from a donor patient who had been diagnosed with a high likelihood of AD based on postmortem neuropathology, see Table 4.1. These samples were defrosted for 48 hours from -80°C , cut into $\sim 13\text{-mm}$ thick coronal sections, and placed into histology cassettes of size $40 \times 26 \times 13\text{ mm}$ for MR scanning. While many ex vivo qMRI analysis studies are conducted with fixed tissue samples [8, 9, 20–22], it has been shown that the freeze-thaw process does not affect cellular integrity and allows for quantitative MRI analysis to be conducted with similar results to using fixed samples [44]. We chose to use freshly thawed tissue samples for this study because fixation has been shown to decrease T_2 values [113–117] and fixation of the tissue for a long period of time can potentially affect the immunohistochemistry and histological detection and findings [110]. Following the protocol outlined in [44], each cassette was submerged in Fluorinert FC-770 (3M, St. Paul, MN, USA) to minimize B_0 field inhomogeneity. For imaging, the samples were placed in a 40 mm Millipede quadrature coil [152] and inserted into a 7.0 Tesla NMR/MRI scanner (Varian/Agilent, Walnut Creek, CA, USA) with 40 mT/m gradients with a 115-mm inner diameter. The studies were conducted below room temperature at $\sim 15\text{-}20^{\circ}\text{C}$ to reduce the rate of autolysis in freshly thawed samples during the scanning process. Two rounds of B_0 shimming were performed using a 3D gradient echo shimming routine.

4.2.2 T_{2w} Imaging

T_{2w} images were collected first in our scanning protocol. The T_{2w} image was collected using a fast spin echo (FSE) sequence with the following parameters: TR = 4000 ms, $TE_{\text{effective}} = 40$ ms, echo spacing (ESP) = 10 ms, number of segments = 32, echo train length = 8, $k_{\text{zero}} = 4$, number of averages = 1, matrix size = 256×256 . Data were acquired for two slice plans: (1) five non-interleaved slices with thickness = 1 mm, slice gap = 1 mm and (2) 11 non-interleaved slices with thickness = 1 mm, slice gap = 0 mm, both with field of view = $40 \times 40 \text{ mm}^2$ and in-plane resolution = $156 \times 156 \mu\text{m}^2$. The total scan time per sample for each FSE scan was 2 min. These FSE T_{2w} images were used for ROI labeling and classification of tissue regions as normal appearing white matter (NAWM), deep white matter (DWM) lesions, periventricular (PV) lesions, and gray matter (GM).

4.2.3 Myelin Water Imaging

The myelin water imaging (MWI) experiments were similar to those conducted in [44] with some modifications. Multi-echo spin echo (MESE) data were collected using a multi-echo multi-slice (MEMS) 2D slice-selective sequence with the following parameters: TR = 4000 ms, 64 echoes, echo spacing = 5 ms, matrix size = 128×128 , and Carr-Purcell-Meiboom-Gill (CPMG) phase cycling with two signal averages. The excitation pulse (90° flip angle) was a 5-lobe sinc pulse with a 1000 μs pulse duration and 5944 Hz bandwidth. The refocusing pulse (180° flip angle) was also an five-lobe sinc pulse with a 8000 μs width and 5877.5 Hz bandwidth. Data were acquired for five non-interleaved slices with thickness = 1 mm, slice gap = 1 mm, field of view = $40 \times 40 \text{ mm}^2$, in-plane resolution = $313 \times 313 \mu\text{m}^2$. The total scan time per sample was 17 min.

The MESE data were analyzed voxel-wise with regularized non-negative least squares (NNLS) regression using the extended phase graph (EPG) formalism to estimate a T_2 spectrum for each voxel [55]. The fitting process used 50 T_2 values log spaced from 5-1000 ms and a regularization parameter of 0.001 to jointly estimate a B_1 field inhomogeneity scaling factor for each voxel using 16 values linearly spaced from 0.7-1.1; this B_1 map was then fixed and the T_2 spectrum was estimated with 500 T_2 values log spaced from 5-1000 ms and the same regularization parameter [59, 123]. The myelin water fraction (MWF) calculation used a cutoff value of 20 ms [8]. MWF was calculated for each voxel as the sum of the amplitudes of the points in the T_2 spectrum up to the cutoff divided by the sum of the amplitudes of the entire spectrum. These values were used to generate a MWF map. We conducted the NNLS fitting with the [NNLS package](#) in the [Julia programming language version 1.12.5](#).

4.2.4 Quantitative Magnetization Transfer

Single-slice MT data were collected via a 2D gradient echo, slice-selective sequence with the following parameters: TR = 120 ms, TE = 3 ms, flip angle = 20°, and matrix size = 128 × 128. The excitation pulse (90° flip angle) was a 5-lobe sinc pulse with a 1000 μ s width. The MT preparation pulses consisted of a train of 20 Gaussian pulses of duration 1 ms, bandwidth 796 Hz, and duty cycle $\sim$ 16.7% at 25 off-resonance frequencies from -60kHz to +60kHz at 5 kHz increments and four RF B_1 RMS amplitudes at about 17.6, 8.8, 4.4 and 2.2 μ T with flip angles of 8960°, 4480°, 2240°, and 1120°, respectively. The slice thickness = 2mm, field of view = 35 × 35 mm², and in-plane resolution = 273 × 273 μ m². The total scan time per sample was 26 min.

The magnetization transfer ratio (MTR) was calculated as $\frac{S_0 - S_+}{S_0}$, where S_+ is an image obtained with an MT preparation pulse at 10 kHz and S_0 is the image obtained at 60 kHz off-resonance. The reported MTR is the average of the MTR maps at +10kHz and -10kHz off-resonance and B_1 field of 8.8 μ T.

4.2.5 Histological Analysis

After imaging, all samples were immediately placed in 10% formalin to undergo fixation. After fixation, the tissue was bisected along the coronal plane with a razor to achieve a maximum thickness of 7 mm for paraffin embedding. The top half, based on orientation in the magnet, was stored for further studies, and the other half underwent paraffin embedding followed by microtomy to obtain 5 μ m slices corresponding to the center of each slice. The samples then underwent histological quantification of myelin and microglial population by the MADRC Neuropathology Core/Rogel Cancer Center Histology and Immunohistochemistry Cores and the Unit for Laboratory Animal Medicine (ULAM) Pathology Core, including luxol fast blue (LFB) staining for myelin visualization and Cresyl violet (CV) counterstaining for cell nuclei visualization, and myelin basic protein (MBP), neurofilament (NF), and OLIG2 (found in oligodendrocyte cell bodies) immunohistochemistry. Then they were scanned with a resolution of 0.5 μ m/pixel using a Vectra Polaris brightfield whole-slide scanner (Akoya Biosciences, Marlborough, MA, USA). The data was analyzed with QuPath [153]; we used the blue channel minus the red channel as a measure of LFB optical density (referred to as LFB in the following text), the mean intensity value for the DAB chromogen for MBP and NF, and the QuPath automated count of the number of oligodendrocytes detected per mm² for the oligodendrocyte count (OC). The accuracy of the automated count was verified by visual inspection.

4.2.6 ROI and Statistical Analysis

First the histology and MR FSE images were imported into Fiji (a distribution of ImageJ2) [154], and the [Fiji Warpy toolbox](#) was used to register the images to one another with landmark-based registration [155, 156]. The FSE and histology images and the resulting transformation function from the registration were then loaded into a QuPath project [153] for histological analysis. Then 0.25 - 1 mm² ROIs were selected in QuPath on one image and the transformation function was used to extract the corresponding ROIs from the other images. ROIs were extracted from histology in gray matter (GM), normal appearing WM (NAWM), and WM lesions (WML). Parameters from the maps were extracted and averaged over each ROI. Linear mixed-effects modeling was used for single and multivariable regression with each qMRI parameter as the dependent variable, MBP, NF, and OC as fixed-effect explanatory variables, and sample ID as a random effect to account for variability across samples.

4.3 Results

4.3.1 Initial Findings

Figure 4.1 shows qMRI parameter maps and histology images for two representative samples (2468-S3 and 2468-S4). The samples include labeled ROIs for gray matter, normal-appearing white matter (NAWM) tissue, and white matter lesions (WML). The locations of hyperintensities in the fast spin-echo images correspond to regions of lower myelin content in the quantitative parameter maps in (b-d). In contrast to experiments done with healthy brain tissue samples [44], there is not a clear contrast maintained between WM and GM structures due to these hyperintensities. Figure 4.2 provides high-power views of a NAWM ROI, WML ROI, and GM ROI in the MBP, NF, and OLIG2 histology images. The MBP images clearly show demyelination in the WML compared to the NAWM, and less MBP in the GM as expected. The NF images show less NF in the lesion compared to the NAWM. The GM NF image has an intermediate amount of NF, but it should be noted there is a large variability in the amount of NF in different GM ROIs. In the OLIG2 images, the red/brown cells are oligodendrocytes and the blue cells (counterstain) are other cell types; the OC is higher in the NAWM than GM as expected, and smaller in the WML as well. Figure 4.3 plots average MWF, MTR, and F_{sat} vs. average MBP, NF, and OC values in the ROIs labeled in Figure 4.1. Figure 4.3a shows that these three qMRI parameters have a strong correlation with myelin content across healthy GM and WM tissue, but Figure 4.3b shows that this correlation starts to break down across the WML that arise from varying and heterogeneous histopathological features. This example illustrates the importance of validating qMR techniques specifically in ADRD, and validating the correlation between qMR parameters and histological

measures of myelin content across the WM hyperintensities and normal-appearing white matter (NAWM) in ADRD.

4.3.2 Statistical Findings

We pooled data from ROIs from all samples except Sample 1131-S3A; this sample has additional pathology compared to the other samples and will be discussed in Appendix Section B.1. In Figure 4.4, the values for the qMR and histology measures show the expected patterns in GM and NAWM, and the values fall somewhere between the two for WMLs. The NAWM ROI values are similar to those in other studies, but some of the GM ROI values are higher than expected [8]. In addition, the MWF values are overall higher than the F_{sat} values; however MWF estimates the fractional myelin water content whereas F_{sat} estimates the fractional macromolecular content.

By looking at matched ROIs between the qMR and histology images, Figure 4.5 shows the relationship between qMR measures and the underlying histological profile across the ROIs without assumption about tissue type. Single variable regression analysis consisted of treating the qMRI parameters as dependent variables (y), the histology parameters as fixed-effect explanatory variables (x_1), where $y = \beta_0 + x_1\beta_1 + \epsilon$. The β terms are the fitted coefficients and ϵ is the error term. The Sample ID is also treated as a random effect to account for variability across samples. Results indicate a statistically significant correlation across the compared pairs of WM ROIs, except for F_{sat} vs. NF.

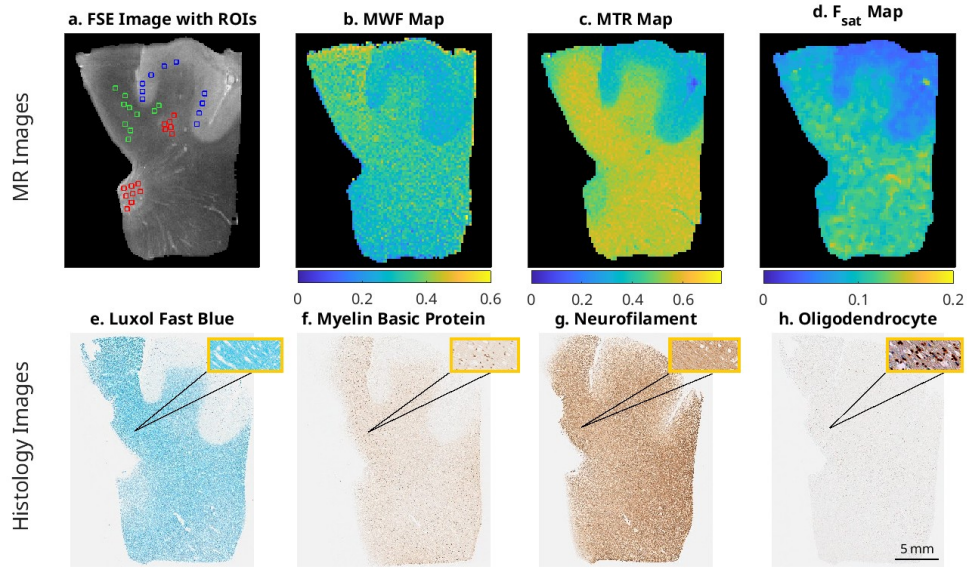
Since MR measures can potentially depend on fiber and cellular density, we used multivariable regression analysis to determine whether including other histological measures will better explain the variability in the qMRI parameters rather than only using MBP. In this case there were two predictors, x_1 and x_2 representing two histological measures (MBP and NF): $y = \beta_0 + x_1\beta_1 + x_2\beta_2 + \epsilon$. Multivariable regression models for MWF that included both MBP and NF as independent variables performed better than models using MBP alone, as indicated by significant differences in the maximum likelihood estimates using ANOVA, and lower Akaike information criterion (AIC) values for combined models (see Table 4.2). In contrast, MTR and F_{sat} were well modeled with MBP alone without NF information. OC contributed to improving MWF and F_{sat} multivariable regression models when combined with MBP. These findings are demonstrated in Figure 4.6, which shows regression plots for mean-adjusted residual qMR data in WM ROIs after regressing out MBP.

When doing the multivariable regression analysis, the explanatory variables NF and MBP should ideally provide independent information; however, this is not the case for MBP and NF, as where there is higher WM fiber density, there is likely more NF and MBP. Figure 4.7a shows that though MBP and NF are related, they do not always vary together in a fixed or proportional

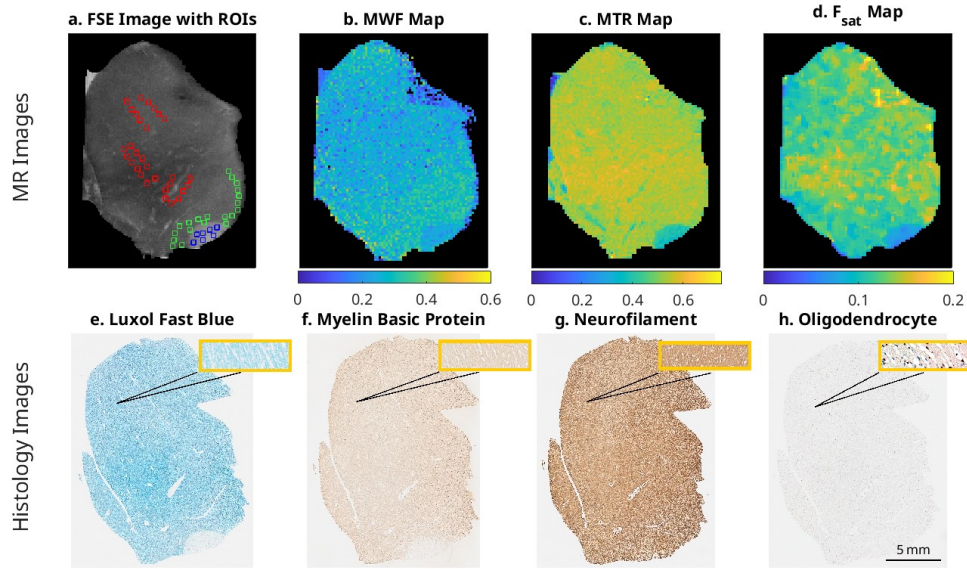
manner. This figure helps justify their inclusion as separate predictors in the multiple regression model. ROIs with low MBP are not always associated with low NF and vice versa, and the relation between MBP and NF is distinct in GM vs. WM. The high correlation between F_{sat} and MTR (Figure 4.7c), indicates that the deviation in MWF vs. MTR (Figure 4.7b) is not due to random effects (variability in ROI placement, etc.), and suggests that MWF and MTR provide distinct information about the ROIs.

qMRI Parameter	ΔAIC: MBP vs. MBP and NF	ΔAIC: MBP vs. MBP and OC
MWF	6.75	6.66
MTR	1.83	2.80
F_{sat}	0.12	5.36

Table 4.2: ΔAIC values between single and multivariable regression models analyzing qMRI and histology WM and WML ROI values. Lower AIC values are associated with models considered to have a better fit, and higher ΔAIC values are considered a more significant improvement. Adding NF information improved modeling for MWF to a more significant extent than for MTR or F_{sat} , and adding OC information improved modeling for MWF and F_{sat} to a more significant extent than for MTR.



(a) Sample 2468-S4



(b) Sample 2468-S3

Figure 4.1: qMRI maps and histology stains for two human brain tissue samples with AD pathology. (a) Fast spin echo image ($TR=4s$, $TE_{eff}=40ms$, $ESP=10ms$, segments/ETL/kzero = 32/8/4) with ROIs labeled; green = NAWM, red = WM lesion, black = GM. (b-d) qMRI parameter maps. (b) MWF map calculated with a 20 ms cutoff. (c) MTR maps from MT contrast images at average of -10kHz and 10kHz off-resonance and 8.8 μT power level. (d) F_{sat} map. (e-h) Corresponding histology images. (e) Luxol fast blue stain with a Cresyl violet counterstain. (f) Myelin basic protein immunofluorescence image. (g) Neurofilament immunofluorescence image. (h) Oligodendrocyte immunofluorescence image.

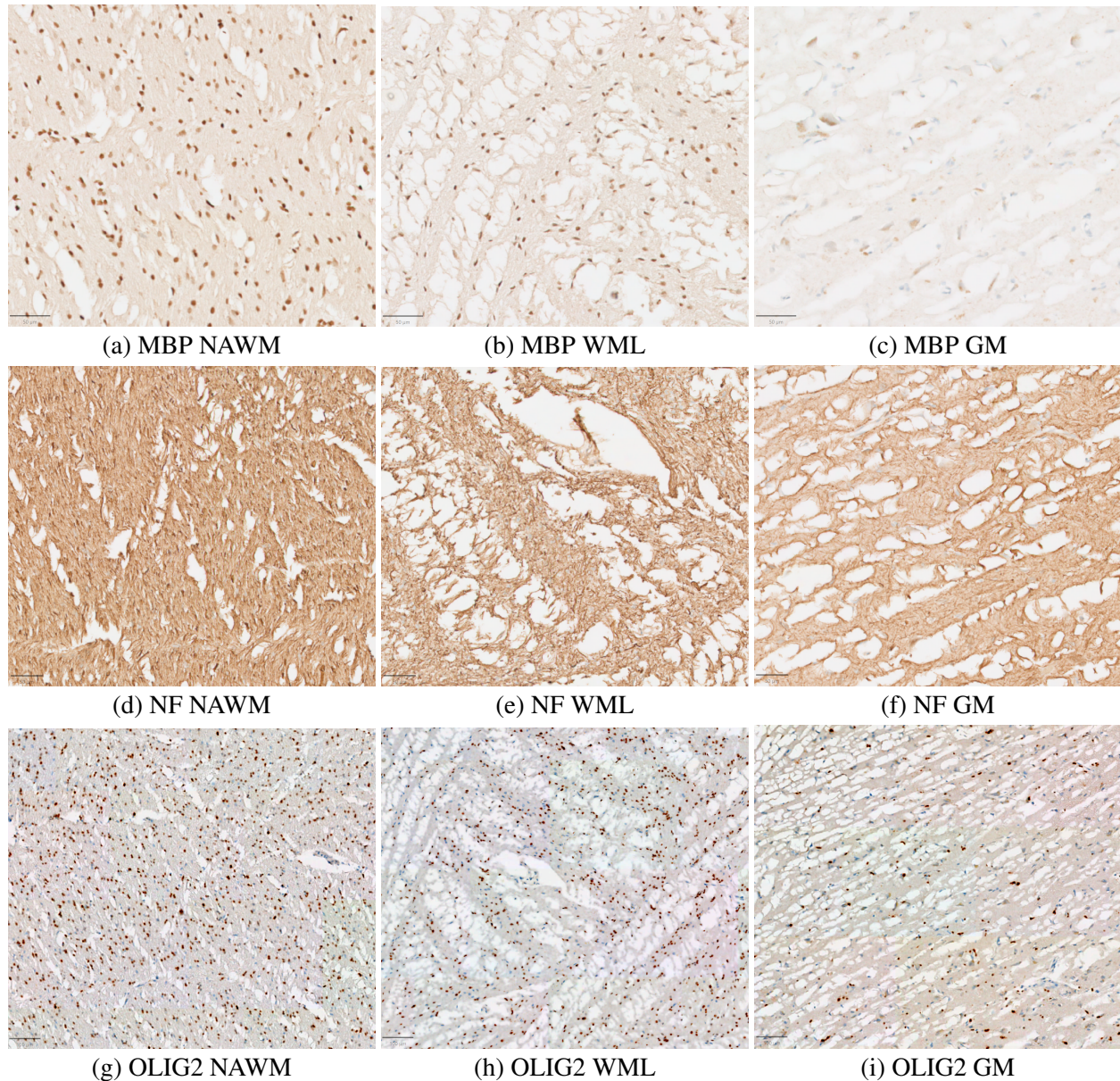


Figure 4.2: High-power views of MBP, NF, and OLIG2 images of a NAWM ROI, WML ROI, and GM ROI in Sample 2468-S4 in the MBP (scale bar 50 μm), NF (scale bar 50 μm), and OLIG2 (scale bar 100 μm) histology images. The MBP images clearly show demyelination in the WML compared to the NAWM, and less MBP in the GM as expected. The NF images show less NF in the lesion compared to the NAWM. The GM NF image has an intermediate amount of NF, but it should be noted there is a large variability in the amount of NF in different GM ROIs. In the OLIG2 images, the red/brown cells are oligodendrocytes and the blue cells (counterstain) are other cell types; the OC is higher in the NAWM than GM as expected, and smaller in the WML as well.

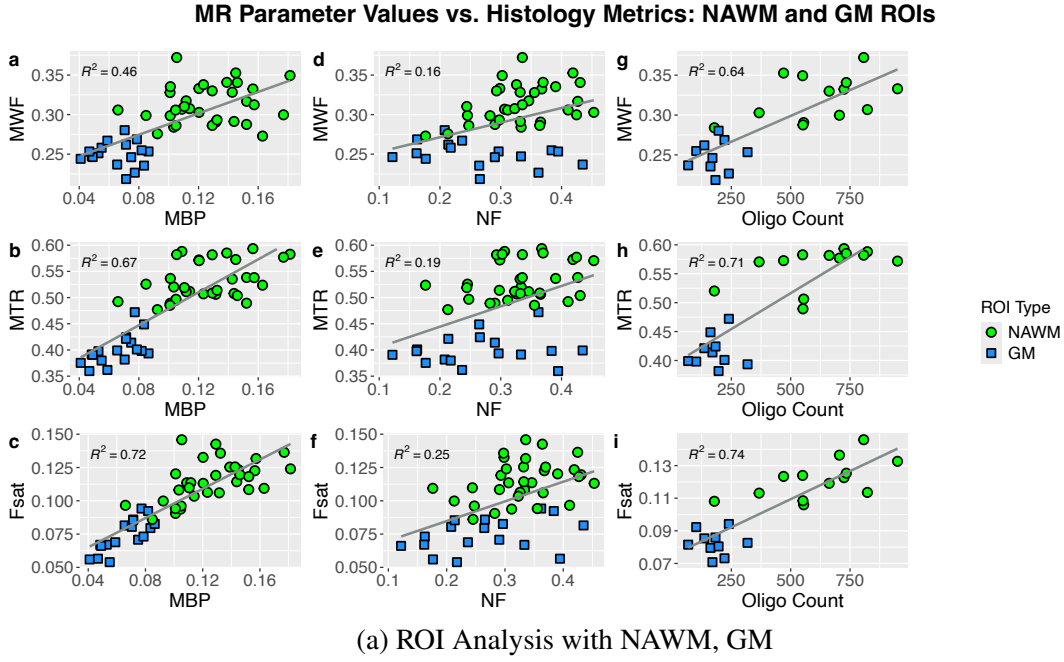
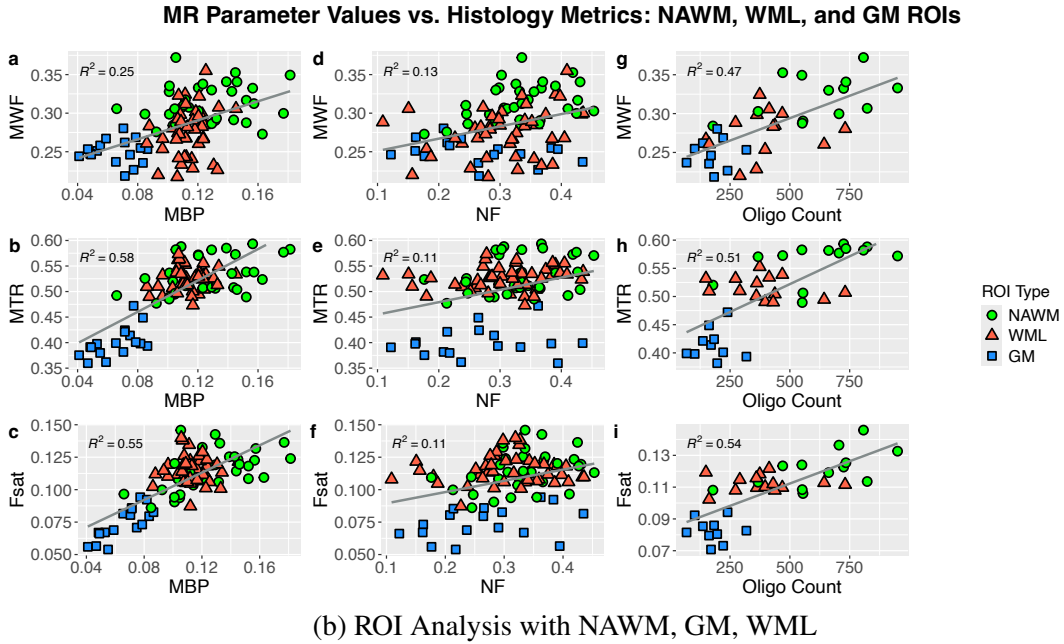


Figure 4.3: qMRI vs. histology averages in ROIs from the human tissue samples with AD pathology. All correlations are statistically significant ($p < 0.05$) for all plots. The high correlations of the qMRI parameters with MBP in subfigure (a) start to break down with the inclusion of lesional WM ROIs in subfigure (b) due to the heterogeneity of the parameter values in these regions. (a-c, g-i) MBP plots show a distinct separation between GM and NAWM ROIs. (d-f) MWF-NF plots have GM ROIs with NF content closer to WML values, whereas the MTR and F_{sat} plots have more of a separation.

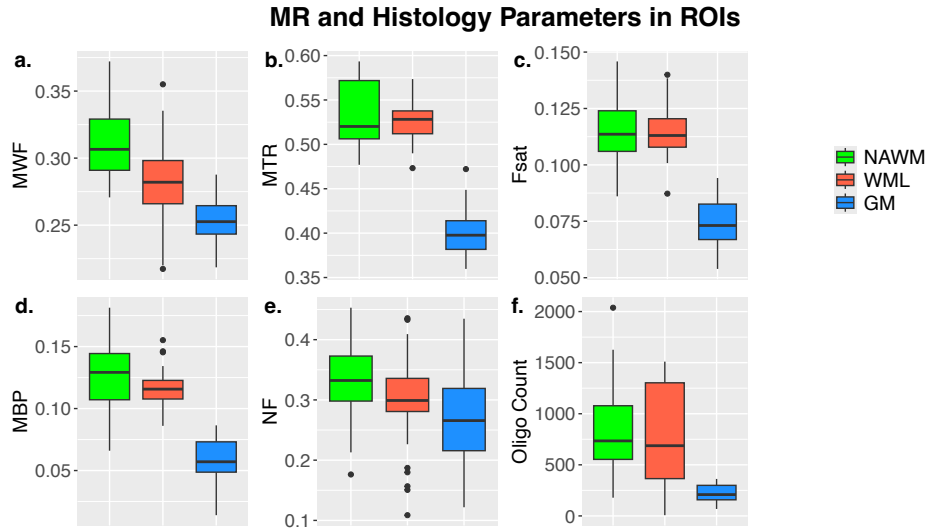


Figure 4.4: Box plots (interquartile range, whiskers: 1.5-times interquartile range, outliers as dots) of average mean-adjusted MR (a-c) and histology parameters (d-f) across tissue samples. (g) OC is not mean-adjusted. Parameter values are consistent with literature values [8, 10] and are consistently highest in NAWM and lowest in GM. The WML values for the two MT parameters and MBP are closer to the NAWM values, whereas for MWF and NF the values overlap with both WM and GM values.

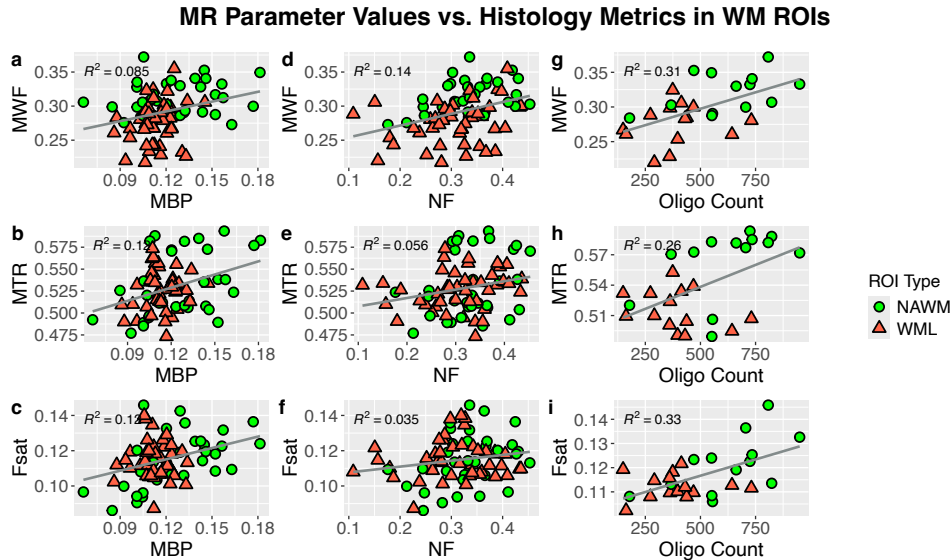


Figure 4.5: Single-variable regression analysis between qMRI and histology values across samples in WM ROIs. R^2 values are relatively low; however, the correlations are statistically significant ($p < 0.05$) for all plots except (f).

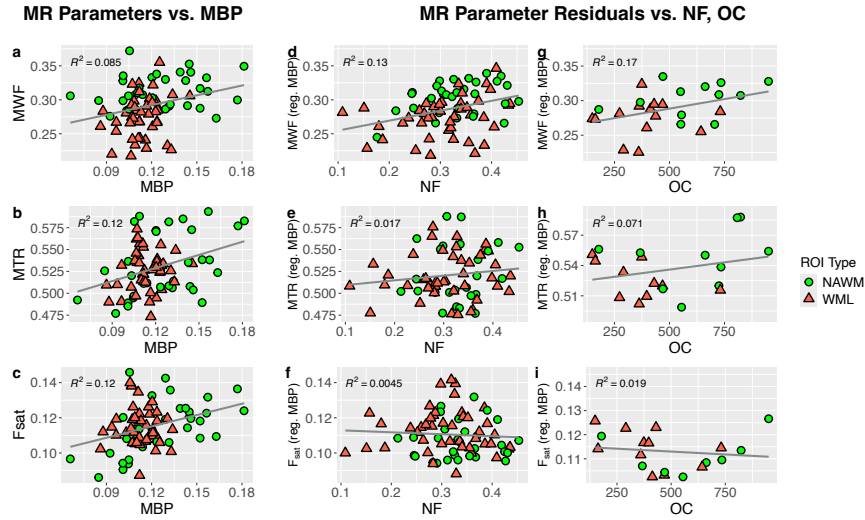


Figure 4.6: Pictorial representation of multivariable analysis in WM ROIs. (a-c) qMRI parameters vs. MBP. After regressing for MBP, the residual qMRI values were mean-adjusted and plotted (d-f) vs. NF and (g-i) vs. OC. After the correlation with MBP was removed from the data, MWF still correlated with NF with a statistically significant p-value ($p < 0.05$), but not OC after regressing out MBP. The correlations between MTR and F_{sat} with NF did not have statistically significant p-values; their correlations with OC did. Higher ΔAIC values indicated a better model with multivariable analysis (MBP and NF) as compared to MBP alone (See Table 4.2).

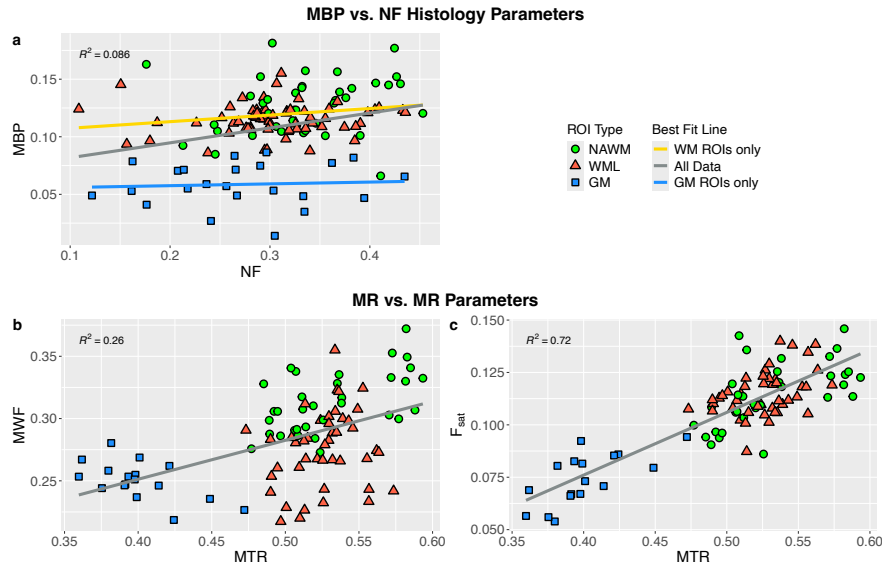


Figure 4.7: (a) MBP vs. NF; MBP variance is not well explained by the variation in NF indicating myelin and axonal loss can be independent. The difference in slopes of the fitted lines for GM vs. WM is consistent with more densely packed, thicker axons with more myelin in WM and areas of dense neuronal processes with less myelin in GM. (b) MWF vs MTR and (c) F_{sat} vs MTR; lesion ROIs deviate from the fitted line in (b) but not in (c), suggesting that MWF and MTR convey different information about WM lesions.

4.4 Discussion

The estimated qMRI parameters are overall consistent with literature values [8, 10]. There is statistically significant correlation between the qMRI and histological measures of myelin as expected based on prior studies [68]. The MWF and MT parameters show a strong correlation with myelin content (MBP), across WM and GM ROIs ($R^2=0.25, 0.58, 0.55$). This correlation indicates that a large majority of the variation seen in qMRI parameters can be explained by myelin content. However, within only WM regions, which includes both normal and lesioned regions, the correlation was weaker between qMRI measures and MBP ($R^2=0.085$, and $R^2=0.12$, and $R^2=0.13$ for MWF, MTR, and F_{sat} respectively). In other words, the variation in the qMRI measures seen across WM tracts could not be sufficiently explained by the variation in myelin content alone.

We expected a strong correlation between qMRI measures of myelin content and MBP based on prior studies [8, 9, 68]. Fewer studies have been conducted that compare NF with qMRI measures of myelin content, with conflicting results. The authors of [157] found a positive correlation between serum NF and MWF in patients with MS lesions, especially in patients with higher disability due to MS; however, the authors of [158] found a negative correlation between the two parameters in patients with MS lesions, and the authors of [159] found a negative correlation between NF and T_{1w}/T_{2w} values (another myelin-sensitive parameter) [135]. As described in [158], damaged axons release more NF, leading to higher MWF and lower NF associated with myelin damage. Another study that used mcDESPOT [160] to calculate MWF saw a positive trend between NF and MWF for a younger age group and a negative trend for an older age group [161]. A major difference between these four studies and ours is that NF was quantified in vivo as a blood biomarker in these studies and was compared for different subjects, whereas we conducted an ex vivo tissue sample study and performed histology on our tissue samples to quantify NF content.

Prior studies using ex vivo tissues have compared NF to MT measures of myelin content. The authors of [143] found that MTR negatively correlated with NfH-SMI34 (a hyperphosphorylated form of the neurofilament heavy protein (NfH)) in unfixed tissue samples from donor brains from patients with MS. This finding was consistent with the finding that there is more phosphorylation when injury occurs, which can also result in a lower MTR. Our study did not specifically look at NfH-SMI34. The authors of [162] found a significant increase in T_2 in mouse models where the mice could not produce MBP compared to the wild type; this result may be consistent with a decrease in myelin water content. In contrast, they found just a subtle increase in T_2 in mouse models where the mice had reduced NF but no change in myelin, indicating that there may have been a less subtle, but still present, decrease in myelin water content when there was decreased NF.

Also similarly to prior studies, we found that a large majority of the variation seen in these qMRI parameters can be explained by the presence of myelin [8]. A notable exception is that the

qMRI measures seem to be affected by not only MBP, but perhaps other histological measures such as NF. The findings presented by Figure 4.6 indicate that inclusion of NF and MBP in multivariable analysis resulted in a better model than MBP alone for MWF; thus NF and MBP may both contribute to the MWF variations observed across ROIs. This observation is consistent with previous studies that show MWF estimates of myelin biased by axonal diameter (g-ratio). [73,74] The histology correlation plot in Figure 4.7a indicates that although MBP and NF both reflect WM fiber density, they do not tightly correlate and contain distinct information that may help interpret WM-sensitive qMRI signals. This trend could be because in WM a decrease in axonal fibers results in a loss of NF density and myelin but in GM NF exists whether or not the axonal fibers are myelinated. However, future studies incorporating diffusion MRI measures such as g-ratio and intra-axonal fraction values (described in Section 2.2.5 could help disentangle the relative contributions of myelin, axonal density, and axonal content to MWF, MTR, and F_{sat} parameter values.

Myelin water exchange [12, 42], white matter fiber orientation [163], and iron accumulation [164, 165] may be able to explain why other histological parameters contribute to the variation in the MWF signal. In particular, [12] and [42] found that the apparent MWF increases with myelin thickness. Myelin thickness correlates with axonal diameter, which correlates with NF. This observed correlation is consistent with our finding that the MWF residuals (after removing all correlation with MBP (4.6) correlate with NF (since NF correlates with axonal diameter). The authors of [12] and [42] explain this correlation between MWF and myelin thickness by exchange; myelin water in thinner myelin has a shorter average lifetime in that compartment and faster exchange out of that compartment, leading to a smaller estimated MWF. Myelin water in thicker myelin has a longer average lifetime in the compartment and slower exchange out of that compartment, leading to a larger estimated MWF. Additional studies should be conducted to further investigate this connection between exchange, MWF, and myelin thickness. If myelin water exchange is involved in why MWF correlates with NF and why adding WML causes the breakdown of MBP-MWF correlation, quantification of this metric could provide another complementary, surrogate measure of disease severity.

A limitation of our study was that the histology slices were sampled between two MR slices, so we had to extract ROIs from two neighboring MR slices to ensure that we had the correct information in our ROI. Another limitation is partial voluming in the MR images. The MR image thickness is on the order of mm (1 mm), whereas the histology image thickness is on the order of μm (5 μm). There is also in-plane partial voluming in the MR images. The histology images for one MR slice are all from adjacent 5 μm slices (different histology stains were done on different slices). However, these slices combined still have a thickness less than one MR image, so MR partial voluming is more of an issue.

Our MWI protocol involves simultaneously collecting five slices, whereas the MT imaging protocol was a single-slice protocol. Magnetization transfer affects multi-slice MESE acquisitions [59, 140] and the resulting MWF values. Thus our use of multi-slice acquisitions for MESE is a limitation of this work. Future analysis comparing quantitative maps from single-slice MT and single-slice MESE experiments could be done to further validate our findings.

Future directions include examining data for a larger number of samples and ROIs, gaining MBP data for 1131-S3A, and incorporating measures such as inhomogeneous MTR that are more specific to myelin [166]. Other future directions include incorporating diffusion MRI metrics such as mean diffusivity (MD) and fractional anisotropy (FA), which are related to WM structural integrity, from diffusion tensor imaging (DTI) analysis. Intra-axonal fraction f_{ia} can also be calculated from diffusion MRI images [76], which can be combined with MWF to calculate the g-ratio. G-ratio may correlate with axon diameter, [73, 74], and would be interesting to compare to MBP and NF histology metrics. Using multiple histology metrics to explain variations in qMRI parameters is the “science” approach to the regression analysis; it would also be possible to take the more “engineering” approach and use multiple qMRI parameters to explain and predict histology values [167].

4.5 Conclusion

qMRI measures of WM content accurately measure ex vivo tissue myelin content in NAWM. However, the variability in qMRI measures is not sufficiently explained by the variation in myelin content in abnormal WM regions. Other, non-myelin related histological features such as axonal density appear to contribute to these qMRI measures. The correlation between MWF and LFB seems to be affected by axonal morphology and spatial distribution, as seen in ROIs with dysmorphic features, and MWI and MT parameters seem to provide complementary information about the tissue samples. Further examining the relationships between these parameters and knowing which histological parameters affect MR metrics in these samples may aid in understanding the impact of WM degradation on ADRD.

CHAPTER 5

Fast, Quantitative Mapping of Myelin Water Content and Exchange Using Steady-State MRI

5.1 Introduction

As discussed in previous chapters, myelin water imaging (MWI) is an important method for quantifying white matter content in the brain. Traditional MWI methodology involves fitting multi-echo spin echo (MESE) data to a T_2 distribution with non-negative least squares regression to estimate myelin water fraction (MWF) [19, 56]. This traditional fitting of MESE data to a multi-pool model with a range of T_2 values does not incorporate myelin water exchange between myelin and non-myelin tissue into the signal model. Previous studies have shown that the presence of exchange may cause traditionally estimated MWF to underestimate the amount of myelin [12, 42, 168, 169]. In addition, this rate of exchange between myelin and non-myelin water may be a complementary biomarker of interest to MWF for use in clinical applications [170, 171]. The exchange rate may be related to the thickness of the myelin sheath [104]; myelin thickness and axon diameter have been shown to affect the exchange rate between myelin and non-myelin water, and thicker myelin sheaths can also lead to higher MWF estimates [12, 42]. Rarefied myelin, which increases the extracellular space, and gliosis, the infiltration of myelinated axons by microglia cell processes, can also affect exchange [12, 42]. On a cellular level, the exchange rate across a cell membrane is related to the size of the cell, its membrane permeability [172], and its metabolic transport activity across the membrane [173]. Changes in the intercompartmental water exchange rate may be indicative of different diseases, such as pathological changes in myelin in multiple sclerosis [160] and pathophysiology changes in Alzheimer's disease [174]. Changes in exchange rate across cell membranes may also be indicative of benign vs. malignant breast and prostate cancer tissue [175–177] and carcinoma of head and neck [178].

5.1.1 Related Work

Prior studies have quantified myelin water fraction and exchange rates in white matter tissue using relaxation-exchange spectroscopy (REXS) [103–105, 179], and relaxation-exchange imaging (REXI) has extended this method for two-pool exchange quantification to imaging [180, 181]. Similar to the NNLS processing used on MESE data for MWI, REXS data analysis generates a two-dimensional T_2 spectrum and exchange is observed as off-diagonal components. Relaxation-exchange scan parameters have been optimized for parameter mapping in white matter [182]. However, these methods are currently too slow for whole-brain in vivo scans.

To address the long scan times of MESE and REXS, researchers have developed various alternative methods to estimate MWF and exchange. One popular method uses a combined gradient and spin echo sequence (GRASE) scan to produce rapid whole-brain MWF maps [58, 183, 184]. The ViSta method maps MWF based on T_1 and T_2^* [185, 186]. Multi-gradient echo (MGRE) methods have also been used to map MWF [187–189]; MGRE is useful since many signals can be acquired before the myelin signal decays due to the short TE, but is very sensitive to field inhomogeneity and may result in biased estimated long T_2 values [109]. Faster steady-state sequences have been used to estimate MWF; dual-echo steady-state (DESS) sequences have been used with a two-pool signal model [190–192]; however this signal model did not incorporate exchange [191]. Small-tip fast recovery (STFR) sequences have been successful in estimating MWF using two and three-pool modeling [14] and PERK for parameter estimation [193]. This method, which optimizes flip angle and phase cycling factor, considers the effects of exchange and exploits the differences in the effective magnetic field experienced by myelin water vs. non-myelin water [60]. However, it is optimized for MWF parameter mapping only.

Both magnetic resonance fingerprinting (MRF) and steady-state methods have been used to estimate exchange as well [2, 160, 170]. A popular method called mcDESPOT [91, 160, 194] estimates MWF and exchange using bSSFP [195] and spoiled gradient-recalled echo (SPGR) sequences collected at multiple flip angles and a two- or three-pool model with exchange [196]. Despite its popularity, mcDESPOT has some important limitations; analysis of this method has shown that it may overestimate MWF [168, 197], that it suffers from low precision (high coefficient of variation) of parameter estimates at feasible SNRs [198], that parameter estimates often hit the edges of the applied box constraints [168, 196], and that MT can bias the mcDESPOT signal [199]. Other studies have looked into using bSSFP to estimate MWF [200, 201], but these methods fix the exchange rate to some constant or assume there is no exchange. Thus, there exists a need to develop a fast method to quantify MWF and exchange with reasonable precision.

The authors of [198] investigated the mcDESPOT method with and without phase cycling with 0° and 180° increments, and found that including phase cycling improved the precision of the parameter estimates. They hypothesized that collecting bSSFP scans with arbitrary (non 0° and

180°) phase-cycling factors in addition to different flip angles may further improve the precision of parameter estimates. This has been further corroborated in [4, 13], where incorporating the phase cycling factor into the scan design, along with the flip angle of a bSSFP scan to estimate exchange, greatly improved the precision of the parameter estimates. Other works using the elliptical signal model bSSFP representation have included non 0° and 180° phase cycling factors into the signal model, [93, 94, 102]; however to our knowledge the work done by [4, 13] is the first to incorporate phase cycling into the two-pool signal model for myelin water exchange estimation. An arbitrary phase increment can be incorporated into the off-resonance term for a two-pool exchanging signal model. This had previously been shown analytically in the single-pool case [97, 98] and is described in Section 2.4.3, but to our knowledge has not been shown in the two-pool case. Thus, this work explores phase cycling as a novel contrast mechanism that may aid parameter estimation.

Additionally, it has been shown in [202] that modeling and estimating the additional off-resonance experienced by the myelin water pool compared to the free water pool can improve the precision of parameter estimates for estimating MWF with an STFR sequence and a two-pool model. The work done in [13, 14] takes this additional off-resonance into account as an estimated parameter.

5.1.2 Proposed Method

Our work expands upon prior methods [13, 14, 160] by analytically incorporating arbitrary (beyond 0° and 180°) phase increments and the additional myelin water off-resonance into an exchanging two-pool bSSFP signal model to aid the precision of our parameter estimates and better represent WM tissue. We tested our method using simulations, well-characterized phantoms, and brain tissue samples with Alzheimer’s Disease pathology. The simulated data is able to be fitted very well; however, model mismatch exists for the real data, especially the WM tissue ROI. To address model mismatch between our simulation and WM tissue data, we explored incorporating a third pool into our bSSFP signal model and slice profile effects.

5.1.3 Preliminary Experiments

Previous studies have shown that the presence of exchange affects conventional MWF estimates [12, 42, 168]. Conducting a similar simulation to one done in [12], we ran Bloch-McConnell simulations using the `BlochSim` package in the Julia programming language [203] to reinforce this importance. Fig. 5.1 shows MESE signal decay over time for three exchange rates. We generated this data by simulating multi-compartment spins with pre-determined $T_{1,f}$, $T_{1,s}$, $T_{2,f}$, and $T_{2,s}$ times for three different MRTs and passing an array of these spins through a MESE sequence with $TR = 4000$ ms, 64 echoes, echo spacing = 5 ms, CPMG phase cycling, and a 180° instantaneous RF

Table 5.1: True and MESE-based estimated parameters for multi-compartment spins used in simulation results depicted in Figure 5.1.

Parameter	True Values	No Exchange	MRT = 300 ms	MRT = 150 ms
$T_{1,f}$ (ms)	400	-	-	-
$T_{1,s}$ (ms)	1000	-	-	-
$T_{2,f}$ (ms)	20	19.9	19.2	17.7
$T_{2,s}$ (ms)	100	99.9	75.8	61.3
MWF	0.200	0.202	0.122	0.040

pulse. Then we conducted regularized NNLS fitting using the EPG formalism [55]. The fitting process used 500 T_2 values log spaced from 5-1000 ms and a regularization parameter of 0.00001 to estimate a T_2 spectrum. The myelin water fraction (MWF) calculation used a cutoff value of 25 ms [8]. The myelin frequency shift was set to 0 Hz for this simulation.

When the MRT is infinite, corresponding to no exchange between water pools, the NNLS fit results in a T_2 spectrum that estimates $T_{2,f}$, $T_{2,s}$, and MWF with good precision, as seen in Table 5.1. When the MRT is long (300 ms), corresponding to a slow exchange rate, the estimated $T_{2,f}$, $T_{2,s}$, and resulting MWF values all decrease. When the MRT is shorter (150 ms), which is closer for the true value in white matter, these estimates decrease even more. This simulation reinforces the experimental findings in [12, 42] that MWF estimated using conventional NNLS modeling is underestimated in an exchanging system.

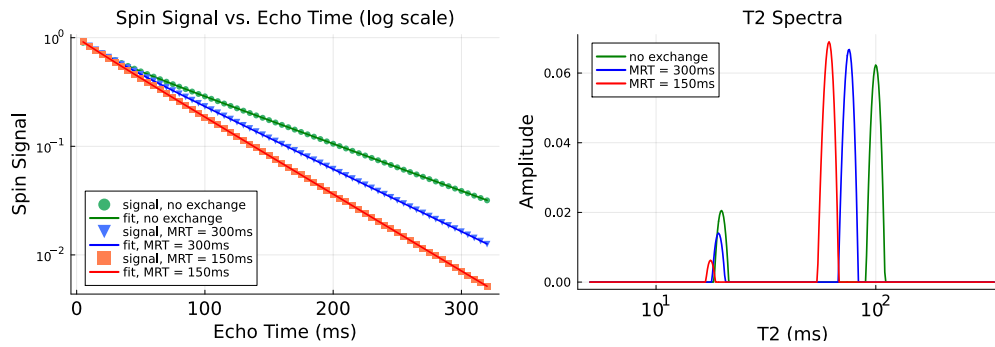


Figure 5.1: Simulation similar to one in [12]. Simulated MESE signal decay and NNLS fit for two exchange rates. Faster exchange rates lead to increased mismatch with the conventional model that ignores exchange, and under-estimation of MWF.

Balanced SSFP scans have been shown to be sensitive to myelin water exchange when optimizing over flip angle and phase cycling factor for exchange estimation, as shown in Figure 5.2 [13] and Figures 5.3- 5.5. In this work we expand upon these ideas to precisely estimate tissue parameters with bSSFP.

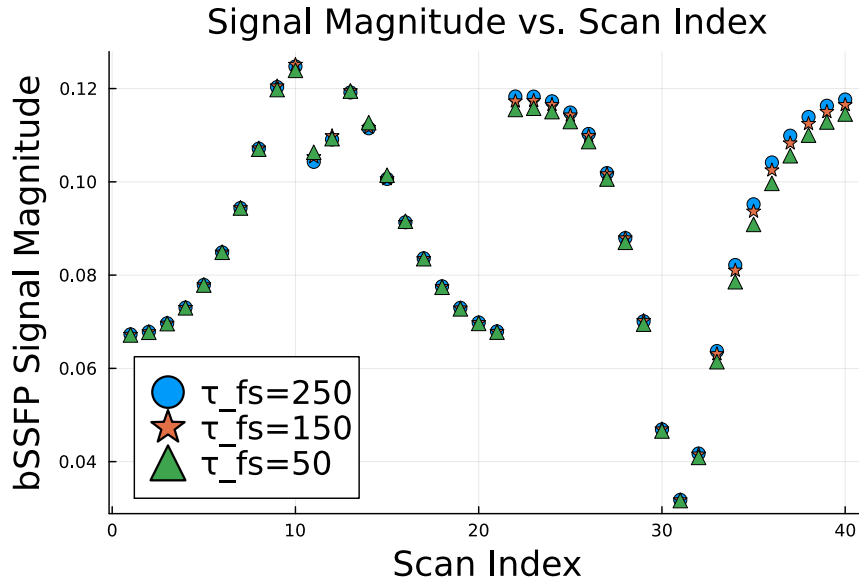


Figure 5.2: Reproduced from [13]. Plot of simulated bSSFP signal magnitude vs. scan index (flip angle/phase cycling factor pair) for an optimized scan design for three exchange rates for a typical WM voxel. With all other parameters kept constant, different exchange levels affect the signal.

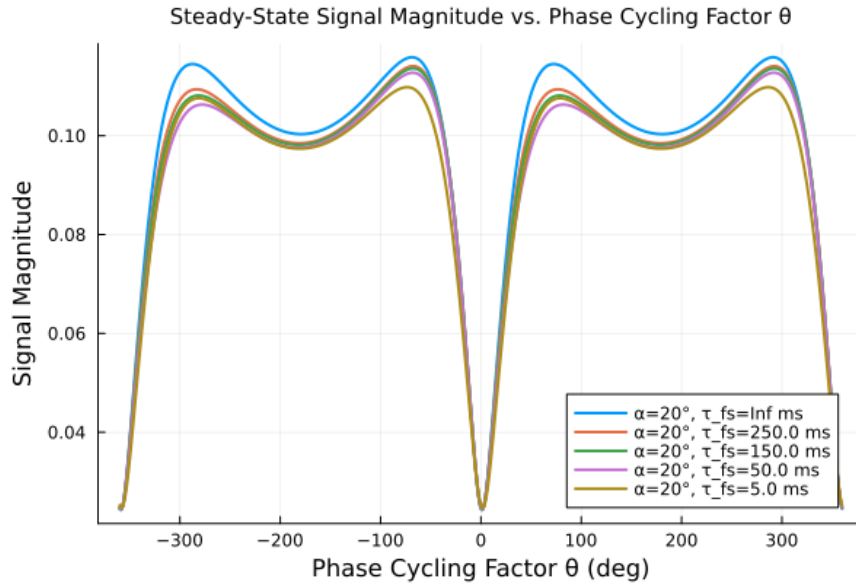


Figure 5.3: Plot of simulated bSSFP signal magnitude vs. phase cycling factor for a flip angle of 20° for two periods for various exchange rates using a two-pool signal model. The profile is asymmetric, and with all other parameters kept constant, different exchange levels affect the signal magnitude.

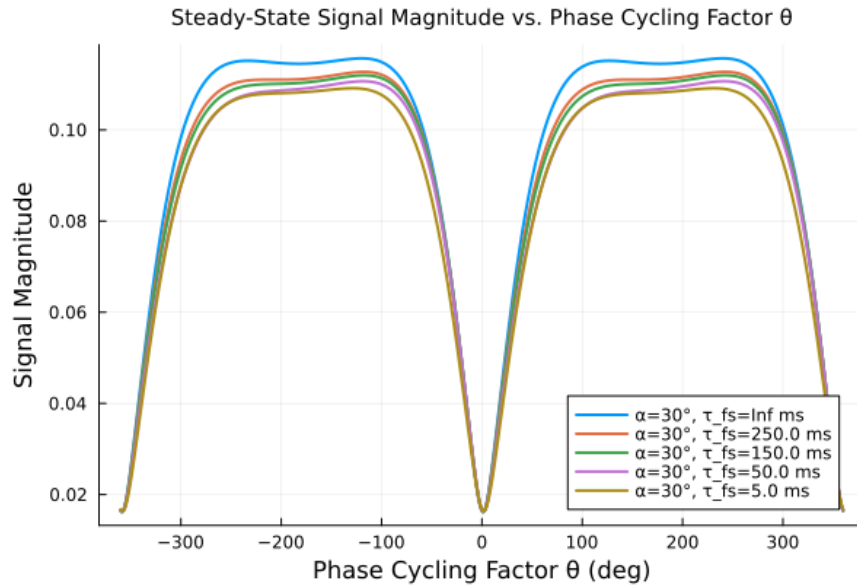


Figure 5.4: Plot of simulated bSSFP signal magnitude vs. phase cycling factor for a flip angle of 30° for two periods for various exchange rates using a two-pool signal model. The profile is asymmetric, and with all other parameters kept constant, different exchange levels affect the signal.

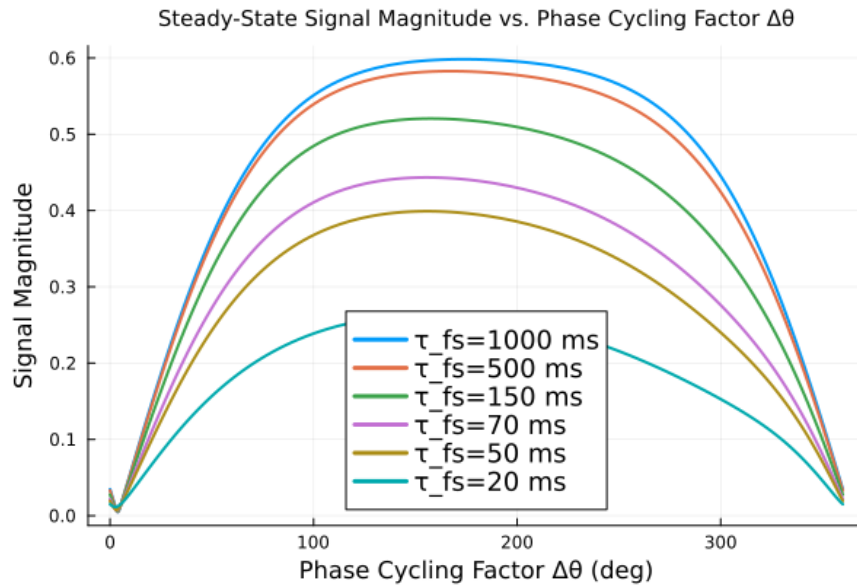


Figure 5.5: Plot of simulated bSSFP signal magnitude vs. phase cycling factor for a flip angle of 60° for one periods for various exchange rates using a two-pool signal model. The profile is asymmetric, and with all other parameters kept constant, different exchange levels affect the signal.

5.2 Theory

To estimate bSSFP parameters for a two-pool signal model, we must use the Bloch-McConnell equations to incorporate a two-compartment exchanging model into the bSSFP signal model. In addition, because we are optimizing the phase cycling factors, we incorporate an arbitrary phase cycling factor into our signal model. It has been shown that an arbitrary phase cycling factor can be incorporated into the bSSFP signal for a single pool model by altering the off-resonance term for phase cycle factors that are multiples of 180° [90,97–99,204]; Section 2.4.3 reviewed this property. These next sections introduce our notation and relevant parameters for a two-pool bSSFP signal model and generalize the matrix-based derivation of that fact for the single-pool case from Section 2.4.3 to the two-pool case.

5.2.1 Two-Pool Signal Model

Using the two-pool signal model described in Section 2.2.2, MR image data for a single voxel in a single scan are often modeled as [4, 13, 14, 205, 206]

$$y = s(\mathbf{x}; \mathbf{p}) + \epsilon, \quad (5.1)$$

where $s(\mathbf{x}; \mathbf{p}) \in \mathbb{C}$ is the MR signal that is a function of unknown tissue and inhomogeneity parameters ($\mathbf{x} \in \mathbb{C}^L$) and scan parameters ($\mathbf{p} \in \mathbb{R}^P$), and $\epsilon \sim \mathcal{N}(0, \sigma^2) \in \mathbb{C}$ is additive complex Gaussian noise; σ^2 is the noise variance of the complex signal. In a bSSFP sequence using a two-pool signal model, the tissue parameters often include a complex equilibrium magnetization M_0 , a fast and slow T_1 ($T_{1,s}$ and $T_{1,f}$), a fast and slow T_2 ($T_{2,s}$ and $T_{2,f}$), the fast-component fraction f_f , the mean residence time $\tau_{f \rightarrow s}$, and the myelin frequency shift $\Delta\omega_f$ [60]. The inhomogeneity parameters include B_0 and B_1 inhomogeneity: bulk off-resonance $\Delta\omega$ flip angle scaling factor κ . These can be considered as known parameters, or known parameters acquired through separate B_0 and B_1 mapping scans. Four important scan parameters of a bSSFP scan are TR, TE, the flip angle α , and the phase cycling factor θ .

When there are D scans, (leading to D contrast images), then the data for a single voxel across each scan is represented by the vector $\mathbf{y} \in \mathbb{C}^D$

$$\mathbf{y} = s(\mathbf{x}; \mathbf{P}) + \boldsymbol{\epsilon}, \quad s(\mathbf{x}; \mathbf{P}) = \begin{bmatrix} s_1(\mathbf{x}; \mathbf{p}_1) \\ \vdots \\ s_D(\mathbf{x}; \mathbf{p}_D) \end{bmatrix}. \quad (5.2)$$

Here, s_d is the signal given by the d th scan for $d = 1, 2, \dots, D$, $\mathbf{P} = [\mathbf{p}_1 \dots \mathbf{p}_D] \in \mathbb{R}^{P \times D}$ holds the scan parameters for the D scans, and $\epsilon \sim \mathcal{N}(\mathbf{0}, \Sigma) \in \mathbb{C}^D$, where $\Sigma = \sigma^2 \mathbf{I}_D$. We implement this signal model using [BlochSim.jl](#).

5.2.1.1 Cramér-Rao Lower Bound (CRLB) Analysis

The Cramér-Rao Lower Bound (CRLB) or Cramér-Rao Bound (CRB) has been previously used to analyze the precision of quantitative MRI parameter estimates [4, 13, 14, 205, 206]. The CRLB can be computed using the $L \times L$ Fisher information matrix of the signal model:

$$\mathbf{F}(\mathbf{x}; \mathbf{P}) = \frac{1}{\sigma^2} \underbrace{(\nabla_{\mathbf{x}} \mathbf{s}(\mathbf{x}; \mathbf{P}))}_{L \times D} \underbrace{(\nabla_{\mathbf{x}} \mathbf{s}(\mathbf{x}; \mathbf{P}))^T}_{D \times L}, \quad (5.3)$$

where $\nabla_{\mathbf{x}}$ is the gradient with respect to the L unknown parameters $\mathbf{x}$. For any unbiased estimator $\hat{\mathbf{x}}$, the CRLB is a lower bound on the variance of the parameter estimates $\hat{\mathbf{x}}$. This lower bound is given by the inverse Fisher information matrix $\mathbf{F}^{-1}(\mathbf{x}, \mathbf{P})$. Specifically, the variance of an unbiased estimator for the i th unknown parameter x_i has a lower bound given by the i th diagonal element of the inverse Fisher information matrix:

$$\text{var}(\hat{x}_i) \geq [\mathbf{F}^{-1}(\mathbf{x}; \mathbf{P})]_{i,i} \quad (5.4)$$

Thus $[\mathbf{F}^{-1}(\mathbf{x}; \mathbf{P})]_{i,i} = \text{CRLB}$ is a lower bound on the precision of the estimator $\hat{\mathbf{x}}$ for the i th unknown parameter x_i , and $\text{var}(\hat{x}_i) \geq \text{CRLB}$. Since the bSSFP signal model given by $\mathbf{s}$ in (5.2) involves complex data, when computing the CRLB, we use a stack of the real and imaginary parts of the signal to calculate $\mathbf{F}$:

$$\mathbf{s}_{\text{stacked}} = [\Re\{\mathbf{s}\}; \Im\{\mathbf{s}\}], \quad (5.5)$$

In this case, the σ^2 used in the calculation of $\mathbf{F}$ is half the complex noise variance $\sigma_{\text{stacked}}^2 = \frac{\sigma^2}{2}$, since the variance of a complex random variable Z is equal to the sum of the variances of its real and imaginary parts: $\text{Var}[Z] = \text{Var}[\Re(Z)] + \text{Var}[\Im(Z)]$. Thus we use:

$$\mathbf{F}(\mathbf{x}; \mathbf{P}) = \frac{1}{\sigma_{\text{stacked}}^2} \underbrace{(\nabla_{\mathbf{x}} \mathbf{s}_{\text{stacked}}(\mathbf{x}; \mathbf{P}))}_{L \times D} \underbrace{(\nabla_{\mathbf{x}} \mathbf{s}_{\text{stacked}}(\mathbf{x}; \mathbf{P}))^T}_{D \times L}. \quad (5.6)$$

We want to design $\mathbf{P}$ so that the CRLB is as small as possible. The CRLB can be used to analyze the precision of parameter estimates using the coefficient of variation (CV), which is defined as:

$$\text{coefficient of variation (CV)} = \frac{\text{std}}{\text{mean}} = \frac{\sqrt{\text{var}}}{\text{mean}}. \quad (5.7)$$

The CRLB can be used to calculate the CV as

$$\text{CV} = \frac{\sqrt{\text{CRLB}}}{\text{mean value of parameter}}. \quad (5.8)$$

It would be ideal for the empirical standard deviation from running multiple parameter estimation trials to approach the CLRB-based standard deviation; this would indicate that the estimation strategy has high precision.

5.2.2 RF Phase Cycling in a Two-Pool Model

The bSSFP signal model described in Section 5.2.1 involves a phase cycling factor scan parameter. We extend the steady-state derivation in (2.54) [97] to incorporate the phase cycling factor into the off-resonance term of the bSSFP signal model to the case of two pools in exchange. Other works have relied on the ability to incorporate the phase cycling factor into the off-resonance term for a two-pool model [99, 207, 208], but to our knowledge have not explicitly shown how this can be done. We show this below.

The solution to the Bloch-McConnell differential equation (see Section 2.1.2) for the magnetization at time t is given by

$$\mathbf{M}(t) = e^{t\mathbf{A}} \mathbf{M}(0) + (e^{t\mathbf{A}} - \mathbf{I}_6) \mathbf{A}^{-1} \mathbf{c}. \quad (5.9)$$

Here we assume $\mathbf{A}$ is invertible and constant over the interval $(0, t)$, the RF excitation pulse is of very short duration, allowing us to neglect the effects of free precession, relaxation, and exchange during excitation, and $\mathbf{M}(0)$ is the initial condition for the net magnetization. In the two-pool case, where the two tissue compartments are denoted by a and b ,

$$\mathbf{c} = \begin{bmatrix} 0 & 0 & \frac{f_a M_0}{T_{1a}} & 0 & 0 & \frac{f_b M_0}{T_{1b}} \end{bmatrix}^T \quad (5.10)$$

where f_a and f_b represent the fraction of spins in each voxel that correspond to compartments a and b , $e^{\mathbf{X}} = \sum_{k=0}^{\infty} \frac{1}{k!} \mathbf{X}^k$ is the matrix exponential function, and $\mathbf{A}$ is defined as the sum of four matrices: $\mathbf{L}_6$, $\mathbf{P}_6$, $\mathbf{P}_{06}$, and $\mathbf{K}_6$. Defining $R_{2a} = \frac{1}{T_{2a}}$, $R_{1a} = \frac{1}{T_{1a}}$, $R_{2b} = \frac{1}{T_{2b}}$, and $R_{1b} = \frac{1}{T_{1b}}$, the diagonal matrix $\mathbf{L}_6$ contains the relaxation rates for both pools:

$$\mathbf{L}_6 = \begin{bmatrix} -R_{2a} & 0 & 0 & 0 & 0 & 0 \\ 0 & -R_{2a} & 0 & 0 & 0 & 0 \\ 0 & 0 & -R_{1a} & 0 & 0 & 0 \\ 0 & 0 & 0 & -R_{2b} & 0 & 0 \\ 0 & 0 & 0 & 0 & -R_{2b} & 0 \\ 0 & 0 & 0 & 0 & 0 & -R_{1b} \end{bmatrix} = \begin{bmatrix} \mathbf{L}_a & \mathbf{0}_{3 \times 3} \\ \mathbf{0}_{3 \times 3} & \mathbf{L}_b \end{bmatrix}, \quad (5.11)$$

where

$$\mathbf{L}_a = \begin{bmatrix} -R_{2a} & 0 & 0 \\ 0 & -R_{2a} & 0 \\ 0 & 0 & -R_{1a} \end{bmatrix}, \quad \mathbf{L}_b = \begin{bmatrix} -R_{2b} & 0 & 0 \\ 0 & -R_{2b} & 0 \\ 0 & 0 & -R_{1b} \end{bmatrix}. \quad (5.12)$$

Please note that these definitions of $\mathbf{L}_a$ and $\mathbf{L}_b$ are slightly different than those in (2.1.2), which are based on [4]. The block skew-symmetric matrices $\mathbf{P}_6$ and $\mathbf{P}_{06}$ describe the off-resonance terms for pools a and b and the B_0 field map off-resonance terms, respectively:

$$\mathbf{P}_6 = \begin{bmatrix} 0 & \Delta\omega_a & 0 & 0 & 0 & 0 \\ -\Delta\omega_a & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \Delta\omega_b & 0 \\ 0 & 0 & 0 & -\Delta\omega_b & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} \mathbf{P}_a & \mathbf{0}_{3 \times 3} \\ \mathbf{0}_{3 \times 3} & \mathbf{P}_b \end{bmatrix}, \quad (5.13)$$

where

$$\mathbf{P}_a = \begin{bmatrix} 0 & \Delta\omega_a & 0 \\ -\Delta\omega_a & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}, \quad \mathbf{P}_b = \begin{bmatrix} 0 & \Delta\omega_b & 0 \\ -\Delta\omega_b & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}, \quad (5.14)$$

and

$$\mathbf{P}_{06} = \begin{bmatrix} 0 & \Delta\omega_0 & 0 & 0 & 0 & 0 \\ -\Delta\omega_0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \Delta\omega_0 & 0 \\ 0 & 0 & 0 & -\Delta\omega_0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} \mathbf{P}_{03} & \mathbf{0}_{3 \times 3} \\ \mathbf{0}_{3 \times 3} & \mathbf{P}_{03} \end{bmatrix}, \quad (5.15)$$

where

$$\mathbf{P}_{03} = \begin{bmatrix} 0 & \Delta\omega_0 & 0 \\ -\Delta\omega_0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}. \quad (5.16)$$

Defining $R_{ab} = \frac{1}{\tau_{a \rightarrow b}}$ and $R_{ba} = \frac{1}{\tau_{b \rightarrow a}}$, $\mathbf{K}_6$ is a block matrix with four diagonal blocks containing the exchange rates between pools a and b:

$$\mathbf{K}_6 = \begin{bmatrix} -R_{ab} & 0 & 0 & R_{ba} & 0 & 0 \\ 0 & -R_{ab} & 0 & 0 & R_{ba} & 0 \\ 0 & 0 & -R_{ab} & 0 & 0 & R_{ba} \\ R_{ab} & 0 & 0 & -R_{ba} & 0 & 0 \\ 0 & R_{ab} & 0 & 0 & -R_{ba} & 0 \\ 0 & 0 & R_{ab} & 0 & 0 & -R_{ba} \end{bmatrix} = \begin{bmatrix} -\mathbf{K}_a & \mathbf{K}_b \\ \mathbf{K}_a & -\mathbf{K}_b \end{bmatrix}, \quad (5.17)$$

where

$$\mathbf{K}_a = \begin{bmatrix} R_{ab} & 0 & 0 \\ 0 & R_{ab} & 0 \\ 0 & 0 & R_{ab} \end{bmatrix}, \quad \mathbf{K}_b = \begin{bmatrix} R_{ba} & 0 & 0 \\ 0 & R_{ba} & 0 \\ 0 & 0 & R_{ba} \end{bmatrix}. \quad (5.18)$$

The $\mathbf{A}$ in (5.9) is defined as: $\mathbf{A} = \mathbf{L}_6 + \mathbf{P}_6 + \mathbf{P}_{06} + \mathbf{K}_6$. Assuming chemical equilibrium between the two pools, meaning that the exchange rates between the two pools are equal [25], $R_{ba}f_b = R_{ab}f_a$.

This leads to the equality $\mathbf{A}\mathbf{m}_0 = -\mathbf{c}$ where

$$\mathbf{m}_0 = \begin{bmatrix} 0 & 0 & f_a M_0 & 0 & 0 & f_b M_0 \end{bmatrix}^T. \quad (5.19)$$

In this case, we rewrite (5.9) as:

$$\mathbf{M}(t) = e^{t\mathbf{A}} \mathbf{M}(0) + (\mathbf{I}_6 - e^{t\mathbf{A}}) \mathbf{m}_0. \quad (5.20)$$

Applying the bSSFP signal model in the two-pool case, we rewrite (2.51) for the two-pool model as

$$\mathbf{M}_{n+1} = e^{\text{TR} \cdot \mathbf{A}} \mathbf{R}_{z6}(\theta_n) \mathbf{R}_{x6}(\alpha) \mathbf{R}_{z6}(-\theta_n) \mathbf{M}_n + \mathbf{d}_6, \quad (5.21)$$

where

$$\mathbf{R}_{x6}(\alpha) = \mathbf{I}_2 \otimes \mathbf{R}_x(\alpha), \quad \mathbf{R}_{z6}(\theta) = \mathbf{I}_2 \otimes \mathbf{R}_z(\theta), \quad (5.22)$$

and

$$\mathbf{d}_6 = (\mathbf{I}_6 - e^{\text{TR} \cdot \mathbf{A}}) \mathbf{m}_0. \quad (5.23)$$

If we change coordinate systems as was done for the single pool case in Equation (2.52):

$$\begin{aligned} \tilde{\mathbf{M}}_n &\triangleq \mathbf{R}_{z6}(-\theta_n) \mathbf{M}_n \\ \mathbf{R}_{z6}(\theta_n) \tilde{\mathbf{M}}_n &\triangleq \mathbf{M}_n, \end{aligned} \quad (5.24)$$

then (5.21) becomes

$$\begin{aligned} \mathbf{M}_{n+1} &= \mathbf{R}_{z6}(\theta_{n+1}) \tilde{\mathbf{M}}_{n+1} = e^{\text{TR} \cdot \mathbf{A}} \mathbf{R}_{z6}(\theta_n) \mathbf{R}_{x6}(\alpha) \mathbf{R}_{z6}(-\theta_n) \mathbf{R}_{z6}(\theta_n) \tilde{\mathbf{M}}_n + \mathbf{d}_6 \\ \tilde{\mathbf{M}}_{n+1} &= \mathbf{R}_{z6}(-\theta_{n+1}) e^{\text{TR} \cdot \mathbf{A}} \mathbf{R}_{z6}(\theta_n) \mathbf{R}_{x6}(\alpha) \tilde{\mathbf{M}}_n + \mathbf{d}_6, \end{aligned} \quad (5.25)$$

where $\mathbf{R}_{z6} \mathbf{d}_6 = \mathbf{d}_6$. Next, we set $\mathbf{A}_6 = \mathbf{L}_6 + \mathbf{P}_6 + \mathbf{K}_6$ and write $e^{\text{TR} \cdot \mathbf{A}} = e^{\text{TR} \cdot (\mathbf{A}_6 + \mathbf{P}_{06})}$. We want to write $e^{\text{TR} \cdot (\mathbf{A}_6 + \mathbf{P}_{06})}$ as $(e^{\text{TR} \cdot \mathbf{A}_6})(e^{\text{TR} \cdot \mathbf{P}_{06}})$, which we can do if $\mathbf{A}_6$ and $\mathbf{P}_{06}$ commute ($\mathbf{A}_6 \mathbf{P}_{06} = \mathbf{P}_{06} \mathbf{A}_6$). This is because for matrices $\mathbf{X}$ and $\mathbf{Y}$, if $\mathbf{X}\mathbf{Y} = \mathbf{Y}\mathbf{X}$, then $e^{\mathbf{X}} e^{\mathbf{Y}} = e^{\mathbf{X} + \mathbf{Y}}$. In this case, $\mathbf{A}_6$ and $\mathbf{P}_{06}$ do commute:

$$\begin{aligned} \mathbf{A}_6 \mathbf{P}_{06} &= \begin{bmatrix} L_a + P_a - K_a & K_b \\ K_a & L_b + P_b - K_b \end{bmatrix} \begin{bmatrix} P_{03} & \mathbf{0}_{3 \times 3} \\ \mathbf{0}_{3 \times 3} & P_{03} \end{bmatrix} = \begin{bmatrix} (L_a + P_a - K_a) P_{03} & K_b P_{03} \\ K_a P_{03} & (L_b + P_b - K_b) P_{03} \end{bmatrix} \\ &= \begin{bmatrix} P_{03} (L_a + P_a - K_a) & P_{03} K_b \\ P_{03} K_a & P_{03} (L_b + P_b - K_b) \end{bmatrix} = \mathbf{P}_{06} \mathbf{A}_6, \end{aligned}$$

because the individual blocks commute. Specifically: P_{03} commutes with both K_a and K_b because P_{03} is skew-symmetric and K_a and K_b are both diagonal. Skew-symmetric matrices

commute with diagonal matrices if all the diagonal entries are equal, which is true for K_a and K_b . P_{03} also commutes with both $(L_a + P_a - K_a)$ and $(L_b + P_b - K_b)$. Thus we can write $(e^{\text{TR} \cdot A_6})(e^{\text{TR} \cdot P_{06}}) = e^{\text{TR} \cdot (A_6 + P_{06})} = e^{\text{TR} \cdot (P_{06} + A_6)} = (e^{\text{TR} \cdot P_{06}})(e^{\text{TR} \cdot A_6})$. We then write (5.25) as

$$\tilde{M}_{n+1} = R_{z6}(-\theta_{n+1})(e^{\text{TR} \cdot P_{06}})(e^{\text{TR} \cdot A_6})R_{z6}(\theta_n)R_{x6}(\alpha)\tilde{M}_n + d_6. \quad (5.26)$$

Furthermore, $e^{\text{TR} \cdot A_6}$ also commutes with $R_{z6}(\theta_n)$. We write $R_{z6}(\theta_n)$ as e^{Θ_6} where

$$\Theta_6 = \begin{bmatrix} 0 & \theta_n & 0 & 0 & 0 & 0 \\ -\theta_n & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \theta_n & 0 \\ 0 & 0 & 0 & -\theta_n & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} \Theta_3 & 0_{3 \times 3} \\ 0_{3 \times 3} & \Theta_3 \end{bmatrix}.$$

We showed that A_6 commutes with P_{06} , and P_{06} has the same block matrix format as Θ_6 ; thus, A_6 and Θ_6 also commute and so do their matrix exponentials. So we write (5.26) as:

$$\tilde{M}_{n+1} = R_{z6}(-\theta_{n+1})(e^{\text{TR} \cdot P_{06}})R_{z6}(\theta_n)(e^{\text{TR} \cdot A_6})R_{x6}(\alpha)\tilde{M}_n + d_6. \quad (5.27)$$

We write the rotation matrix $e^{\text{TR} \cdot P_{06}}$ as

$$e^{\text{TR} \cdot P_{06}} = \begin{bmatrix} \cos(\Delta\omega_0 \text{TR}) & \sin(\Delta\omega_0 \text{TR}) & 0 & 0 & 0 & 0 \\ -\sin(\Delta\omega_0 \text{TR}) & \cos(\Delta\omega_0 \text{TR}) & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & \cos(\Delta\omega_0 \text{TR}) & \sin(\Delta\omega_0 \text{TR}) & 0 \\ 0 & 0 & 0 & -\sin(\Delta\omega_0 \text{TR}) & \cos(\Delta\omega_0 \text{TR}) & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}.$$

Thus $e^{\text{TR} \cdot P_{06}} = R_{z6}(\Delta\omega_0 \text{TR})$. We set $R_{z6}(\beta) \triangleq R_{z6}(\Delta\omega_0 \text{TR})$ and assuming that the RF phase grows linearly as in Equation (2.53), we write:

$$\begin{aligned} \tilde{M}_{n+1} &= R_{z6}(-\theta_{n+1}) R_{z6}(\beta) R_{z6}(\theta_n) (e^{\text{TR} \cdot A_6}) R_{x6}(\alpha) \tilde{M}_n + d_6 \\ \tilde{M}_{n+1} &= R_{z6}(-\theta_{n+1} + \beta + \theta_n) (e^{\text{TR} \cdot A_6}) R_{x6}(\alpha) \tilde{M}_n + d_6 \\ \tilde{M}_{n+1} &= R_{z6}(\beta - \Delta\theta) (e^{\text{TR} \cdot A_6}) R_{x6}(\alpha) \tilde{M}_n + d_6. \end{aligned} \quad (5.28)$$

This means that, just as for a single pool model, for a two-pool model, an arbitrary phase-cycling factor can be incorporated into the bulk off-resonance term. This property readily generalizes to a multi-pool model with more than two pools. The steady-state value of this magnetization vector immediately before the RF pulse and in the receiver coordinate system is

$$\tilde{M}_{ss} = [I - R_{z6}(\beta - \Delta\theta)(e^{\text{TR} \cdot A_6})R_{x6}(\alpha)]^{-1}d_6, \quad (5.29)$$

and any time after the RF pulse $0 < t < \text{TR}$, the magnetization in the receiver coordinate system is defined as

$$\tilde{\mathbf{M}}(t) = \mathbf{R}_{z6}(\beta_t)(e^{t \cdot \mathbf{A}_6})\mathbf{R}_{x6}(\alpha)\tilde{\mathbf{M}}_{ss} + \mathbf{d}_6(t). \quad (5.30)$$

Thus, for a two-pool exchanging model, an arbitrary phase cycling factor can be incorporated into signal model by subtracting it from the off-resonance term just as for a single pool model. Figure 5.6 illustrates this, as changing the phase of the excitation pulse shifts the frequency profile along the resonant frequency axis for an arbitrary phase increment $\Delta\theta$.

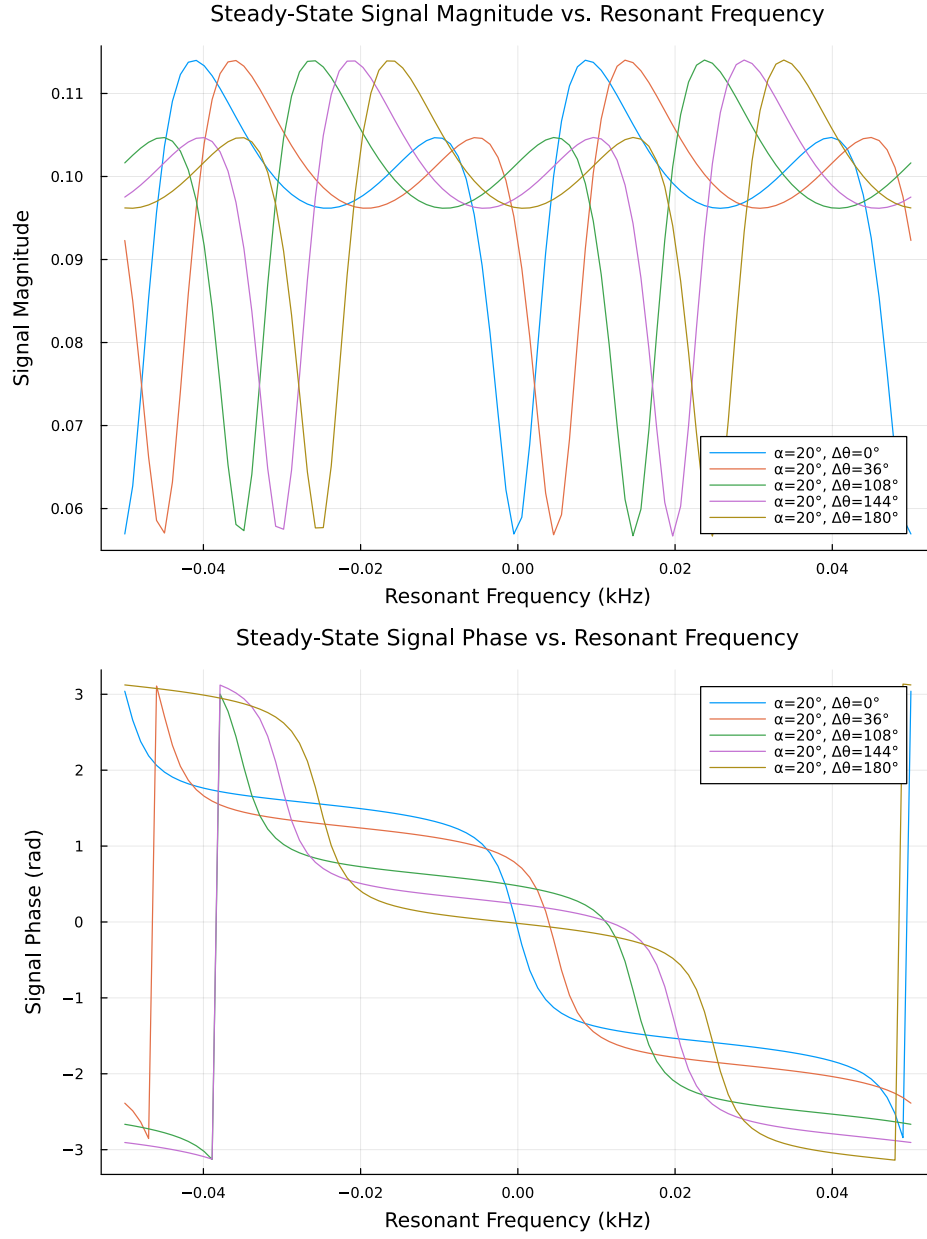


Figure 5.6: Signal magnitude vs. off-resonance frequency for a bSSFP sequence. Data was simulated using a two-pool model with [BlochSim.jl](#) with $TR = 8$ ms, $TE = 4$ ms, $T_{1,s} = 800$ ms, $T_{1,f} = 400$ ms, $T_{2,s} = 80$ ms, $T_{2,f} = 20$ ms, $M_0 = 0.77$, $f_f = 0.15$, mean residence time $\tau_{f \rightarrow s} = 50$ ms, and the additional off-resonance that is only experienced by the myelin water pool was set to 5 Hz. Plots for a flip angle of 20° and five different phase cycling factors are shown. The plotted resonance frequencies range from $-1/TR$ to $1/TR$; in the two-pool case, the signal magnitude is still periodic in frequency with a period of $1/TR$.

5.3 Methods

5.3.1 Simulations

First we tested our bSSFP fitting and parameter estimation method with simulated data. We generated bSSFP data using our phase-cycled bSSFP signal model implemented with [BlochSim.jl](#), and then generated a two-compartment voxel with the following parameters to mimic a WM voxel: $M_0 = 3.8$ (magnitude), $M_{0,\phi} = 1.7$ (phase), $T_{1,s} = 929$ ms, $T_{1,f} = 182$ ms, $T_{2,s} = 62$ ms, $T_{2,f} = 6$ ms, fast fraction $f_f = 0.15$, mean residence time $\tau_{f \rightarrow s} = 70$ ms [104], myelin frequency shift (additional off-resonance that is only experienced by the myelin water pool, $\Delta\omega_f = 5$ Hz [60], bulk off-resonance ($\Delta\omega$) = -27 Hz, flip angle scaling factor (κ) = 1.17. We used the following scan parameter to simulate the bSSFP data: TR = 8 ms, TE = 4 ms, six flip angles (10°, 15°, 20°, 30°, 40°, 50°, 60°) and 27 phase cycling factors linearly spaced from 0° to 520° (see Table 5.2). We added noise with

Table 5.2: Scan parameters used for all simulations and real data acquisitions.

Scan Parameter	Value
TR	8 ms
TE	4 ms
flip angle α	6 values linearly spaced 10°-60°
phase cycling factor $\Delta\theta$	27 values linearly spaced 0°-520°

a noise variance of $\sigma^2 = 2.23876(10^{-5})$ (SNR = 33.6 dB); this was done to mimic the SNR of an ROI in a bSSFP scan of one of the human tissue samples. Then we phase-corrected the data so that phase differences in the subsequent images were relative to the first image; we did this by taking the first complex bSSFP image as a baseline phase reference and then dividing the full complex image dataset by the baseline phase.

We then fitted the complex data of this simulated voxel through nonlinear least squares (NLLS) fitting with box constraints. We used the Variable Projection Method (VarPro) [209] to reduce the optimization search to the nine nonlinear parameters using the following simplified cost function (since the complex M_0 is a linear parameter).

$$\hat{\mathbf{x}} = \underset{\mathbf{x} \in \mathbb{R}^9}{\operatorname{argmin}} - \frac{|\mathbf{s}(\mathbf{x})' \mathbf{y}|^2}{\|\mathbf{s}(\mathbf{x})\|^2}. \quad (5.31)$$

Here $\mathbf{y}$ is the acquired data, $\mathbf{s}(\mathbf{x})$ is the signal model from (5.30), $\mathbf{x}$ are the parameters being estimated, $\hat{\mathbf{x}}$ are the final parameter estimates. We used automatic differentiation in Julia (ForwardDiff.jl) to calculate the gradient of the cost function. Our nonlinear least squares (NLLS)

parameter optimization used the BFGS algorithm [210–213] with a Hager-Zhang line search [214] with the Julia `Optim.jl` package [203].

These simulations were run for 20 random initializations distributed uniformly within the box constraints (same constraints as for the fitting) for 30 trials with different noise initializations. Then the estimates associated with the lowest cost of the 20 trials was chosen (since NLLS can fall into local minima, this step helps improve the odds that we find a global minimizer); many (about 15/20) of the initializations converge to the same minimum. We compared the empirical standard deviation of the 30 trials to the lower bound calculated with the CRLB.

We also investigated the three pool model described in Section 2.2.4 by simulating data with a three-pool bSSFP signal model and fitting with a two-pool model and compared the results to simulating with a three-pool model and fitting with a three-pool model. The three-pool model data was generated with noise with the same parameters as the two-pool model with an extra macromolecular pool with four extra parameters: the macromolecular pool fraction f_m , the T_1 and T_2 of the macromolecular pool $T_{1,m}$ and $T_{2,m}$, and the mean residence time of the myelin water pool exchanging into the macromolecular pool $\tau_{f \rightarrow m}$. These values were set to: $f_m = 0.1$, $T_{1,m} = 1$ sec, $T_{2,m} = 0.02$ ms, and $\tau_{f \rightarrow m} = 100$ ms based on [14]. The other three exchange times: $\tau_{s \rightarrow m}$, $\tau_{m \rightarrow f}$, and $\tau_{m \rightarrow s}$ were set to 0 per [202].

5.3.2 Single-Pool Phantom

We first tested our acquisition and parameter fitting methods in a single-pool uniform phantom made of water and nickel chloride in an agarose (T_2 shortening) gel. This gel was placed into a histology cassette to mimic the protocol outlined in [44]. Following this protocol, the cassette was submerged in Fluorinert FC-770 (3M, St. Paul, MN, USA) to minimize B_0 field inhomogeneity. For imaging, the samples were placed in a 40 mm Millipede quadrature coil and inserted into a 7.0 Tesla NMR/MRI scanner (Varian/Agilent, Walnut Creek, CA, USA) with 40 mT/m gradients with a 115-mm inner diameter. The studies were conducted below room temperature at ~ 15 - 20°C . Two rounds of B_0 shimming were performed using a 3D gradient echo shimming routine. Data for a B_0 map were acquired using a 2D gradient echo slice-selective sequence with multiple TE values at 4, 6, 8, and 10 ms, TR = 100 ms, flip angle = 20° , and matrix size = 128×128 . Data were acquired for five interleaved slices with thickness = 2 mm, slice gap = 0 mm, field of view = 35×35 mm², and in-plane resolution = 547×547 μm^2 . Data for a B_1 map were acquired using a 2D gradient echo single-shot EPI sequence with TR = 8 sec, TE = 9.55 ms, and matrix size = 32×32 at flip angles of 60° and 120° [215]. We conducted the default T_1 and T_2 mapping 1D NMR scans on the scanner and estimated the T_1 and T_2 values to be $T_1=252$ ms and $T_2=57$ ms. Balanced SSFP data were collected using a phase-cycled 2D bSSFP sequence with the same parameters as were

used in the simulations: $TR = 8$ ms, $TE = 4$ ms, matrix size = 128×128 , six flip angles (10° , 20° , 30° , 40° , 50° , 60°), and 27 phase cycling factors linearly spaced from 0° to 520° . The RF slice-selective excitation pulse was Varian's 3-lobe sinc pulse with a 1 ms duration, preceded and followed by prephasing and rephasing gradients. Data were collected for a single slice with a field of view (FOV) = 50×50 mm², in-plane resolution = 391×391 μm^2 and slice thickness of 2 mm. The total scan time was about 1.5 minutes per flip angle (about 11 minutes total).

5.3.3 Non-Exchanging Two-Pool Phantom

We then tested our acquisition and parameter fitting methods in a non-exchanging two-pool phantom made of water and dimethyl sulfoxide (DMSO) in an agarose (T_2 shortening) gel; thus the water was the fast-relaxing component and the DMSO was the slow-relaxing component. The chemical shift between the water and DMSO is 618 Hz at 7T, and the phantom was estimated to have a fractional component of water of 0.8344 and of DMSO of 0.1656 ($f_f = 0.8344$, $f_s = 0.1656$), calculated from the integrated areas under the two peaks from their NMR spectrum. This gel was placed into a histology cassette to mimic the protocol outlined in [44], and the same scanning setup procedure and B_0 and B_1 map collection was followed as described in Section 5.3.2. To characterize our phantom, an inversion recovery scan was conducted and the data fit to a bi-exponential signal model to generate short and long T_1 maps and a multi-echo spin echo scan was conducted and the data fit to a bi-exponential signal model to generate short and long T_2 maps [44]. The short and long T_1 values were estimated to be $T_{1,f} = 223$ ms and $T_{1,s} = 1050$ ms and the short and long T_2 values were estimated to be $T_{2,f} = 49$ ms and $T_{2,s} = 242$ ms. Then bSSFP data were collected; all parameters were the same as the single-pool phantom except with a field of view (FOV) = 60×60 mm² and in-plane resolution = 469×469 μm^2 .

5.3.4 Two-Pool Exchanging Phantom

We then tested our methods in an exchanging two-pool phantom composed of water and urea with 85 μM Mn and 200 μM Magnevist, a gadolinium-based contrast agent. The chemical shift between water and urea is about 300 Hz at 7T (about 1 ppm). The urea-water solution had a pH of about 8.2 and was designed to have a 10% urea fraction; it was based on phantoms from [4, 107]. The phantom was estimated to have a urea fraction of about 0.09 (about 9% urea protons and 91% water protons), calculated from the integrated areas under the two peaks from their NMR spectrum. The magnet was heated to about 21°C to aim for an exchange MRT between about 20-50 ms [108]. The urea-water solution was placed in a vial in a small syringe, surrounded by Fluorinert similarly to the DMSO phantom. The urea phantom was scanned in the same 7T scanner as the DMSO phantom in the same millipede coil and underwent the same shimming procedure as the DMSO phantom.

We collected B_0 , B_1 , and T_2 maps similarly to the DMSO phantom; from these scans we estimated $T_{2,f}$ to be 7 ms and $T_{2,s}$ to be 80 ms. We then collected one-dimensional inversion recovery data; a bi-exponential fit directly run on the scanner estimated both T_1 values to be 740 ms. We then collected an NMR inversion recovery spectrum according to the procedure in Section 2.5 where we selectively inverted the water pool to later estimate the pool fractions and exchange rates of the two pools. Then bSSFP data were collected; all parameters were the same as the DMSO phantom except the FOV was $20 \text{ mm} \times 20 \text{ mm}^2$, the matrix size was 64×64 , the in-plane resolution was $313 \times 313 \text{ } \mu\text{m}^2$, and the scan time was about 9.5 minutes. The bSSFP data were processed according to the method described in Section 5.3.1.

5.3.5 Ex Vivo Tissue Samples

Two ex vivo frozen brain tissue samples were procured from the Michigan Alzheimer’s Disease Research Center (MADRC) brain bank. These two samples came from a donor patient who had been diagnosed with a high likelihood of AD pre-mortem. The samples were defrosted for 48 hours from -80°C , cut into $\sim 13\text{-mm}$ thick coronal sections, and placed into histology cassettes of size $40 \times 26 \times 13 \text{ mm}$ for MR scanning [44]. The tissue sample underwent the same shimming procedure and B_0 , B_1 , T_1 , and T_2 map collection. The same 2D bSSFP sequence was used as for the DMSO phantom data collection except that the matrix size was 64×64 , FOV was $= 35 \times 35 \text{ mm}^2$, in-plane resolution in-plane resolution = $547 \times 547 \text{ } \mu\text{m}^2$ and slice thickness of 1 mm. The bSSFP data were processed according to the method described in Section 5.3.1.

5.4 Results

5.4.1 Noiseless Simulations

Figure 5.7 shows data from the simulated voxel described in Section 5.3.1 for the noiseless case where the parameters were initialized to their true values. Figure 5.7a shows the magnitude of the complex signal vs. the 27 phase cycling factors for the six flip angles. The periodic profile shows the typical off-resonance null for a bSSFP signal and is asymmetric due to the presence of the two pools. Figure 5.7b shows the linear phase of the signal, which starts at 0 due to the phase-correction pre-processing step. Figure 5.7c shows the elliptical signal profile of a bSSFP signal when the signal is plotted on the complex plane. The elliptical profiles are asymmetric due to the presence of the two pools, and each flip angle has its own profile. All parameters were initialized at their true values for the optimization method in this noiseless case (rather than initialized to

random values within the box constraints), and the estimates all came out to be identical (within numerical precision) to the correct values as seen in Figure 5.7d.

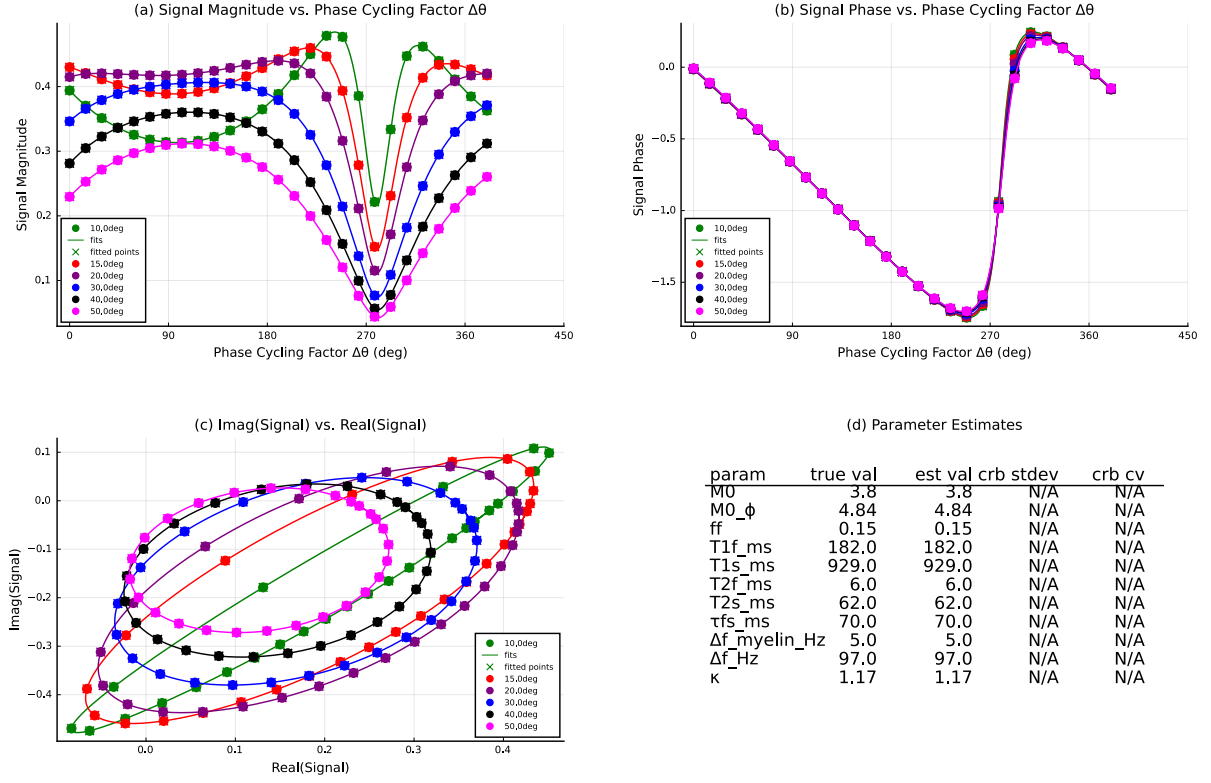


Figure 5.7: Simulated and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a two-compartment exchanging voxel with no noise. The dots correspond to the simulated data points, the lines correspond to the fits, and the Xs correspond to the fit at the exact phase cycling factor at which the data was acquired. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two exchanging pools. (d) Chart with true parameter values from the simulation and parameter estimates. There are no CRB values since the data is noiseless.

5.4.2 Two-Pool Model Noisy Simulations

Figure 5.8 show data and fits from a voxel with added noise described in Section 5.3.1. The parameters were initialized to a random value within the box constraints in Table 5.3, but the estimated converged near their true values, indicating the optimization works well when there is no model mismatch. The CRB and values in Figure 5.8 have been evaluated at the true values, as opposed to the estimated values, and the CVs have also been calculated with the true value as the mean. The table in Figure 5.9 shows the results from running 30 trials with different noise realizations. The empirical means approach the true values, and the empirical SD and CV values approach the CRB values. Figure 5.10 shows histograms with the parameter estimates from the 30 trials; most estimates are within $\pm 2\sigma_{\text{CRB}}$. These results indicate that the SNR is high enough that the asymptotic efficiency and unbiasedness of the maximum likelihood estimator can be applied to this study, and we can approach the CRB.

Table 5.3: Lower and upper parameter bounds used for NLLS fitting of noisy simulations.

Parameter	Lower Bound	Upper Bound
f_f	0.05	0.40
$T_{1,f}$ (ms)	100	700
$T_{1,s}$ (ms)	200	1200
$T_{2,f}$ (ms)	4	100
$T_{2,s}$ (ms)	20	500
$\tau_{f \rightarrow s}$ (ms)	20	10000
$\Delta\omega_f$ (myelin frequency shift) (Hz)	-10	10
$\Delta\omega$ (off-resonance)(Hz)	-63	63
κ	0.9	1.3

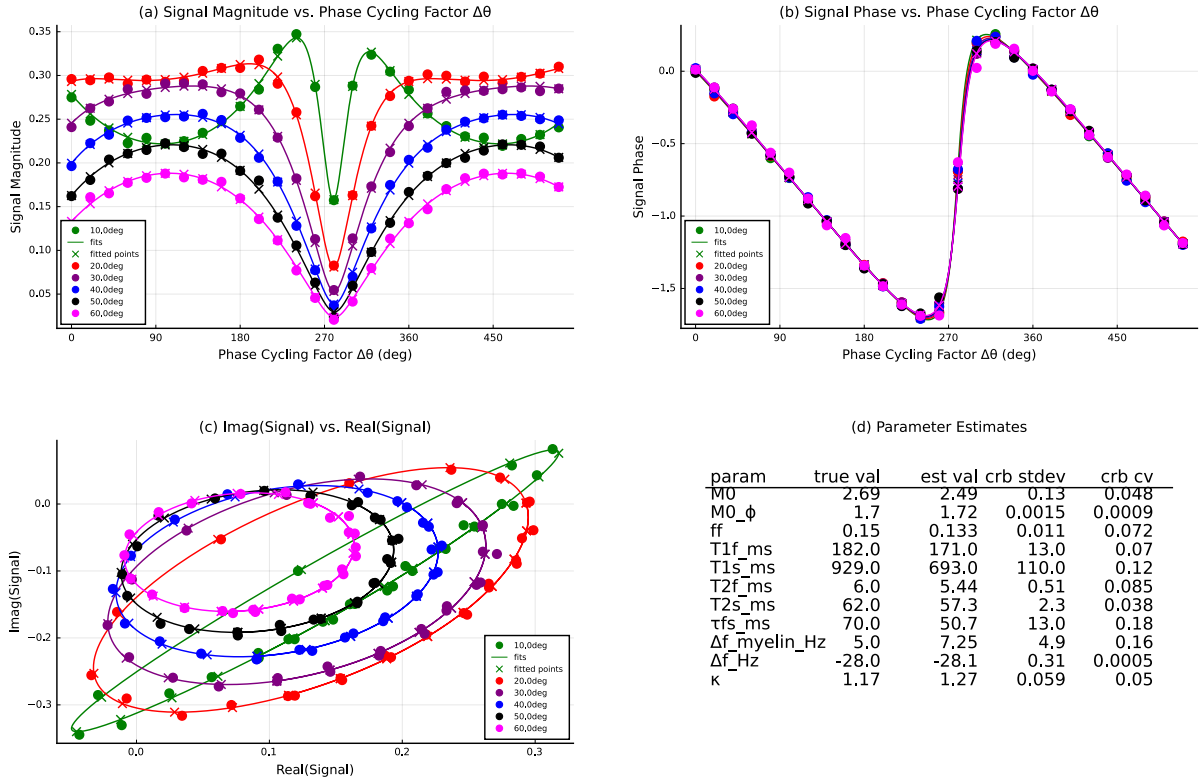


Figure 5.8: Simulated and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a two-compartment exchanging voxel with one realization of added noise corresponding to the noise of the WM ROI of the tissue sample. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two exchanging pools. (d) Table of true parameter values from the simulation and parameter estimates and the corresponding CRB values.

Param Name	True Param Value	Empirical Estimated Mean	Empirical SD	Empirical CV	CRB SD	CRB CV
M0	2.69	2.7	0.13	0.046	0.13	0.048
M0_φ	1.7	4.84	0.0092	0.0054	0.0015	0.00091
ff	0.15	0.15	0.009	0.061	0.011	0.072
T1f_ms	182	190	12	0.067	13	0.07
T1s_ms	929	960	120	0.13	110	0.12
T2f_ms	6	6	0.41	0.069	0.51	0.085
T2s_ms	62	62	2.1	0.034	2.3	0.038
τfs_ms	70	71	13	0.19	13	0.18
Δf_myelin_Hz	5	5.09	5.5	0.17	4.9	0.16
Δf_Hz	-28	-28.6	0.28	-0.0016	0.31	-0.00051
κ	1.17	1.2	0.059	0.051	0.059	0.05

Figure 5.9: Results from running 30 trials of our simulations with different noise realizations (2-pool voxel). The empirical means from the 30 trials approach the true values, and the empirical SDs and CVs approach the theoretical CRB values.

Noisy Simulation Histograms

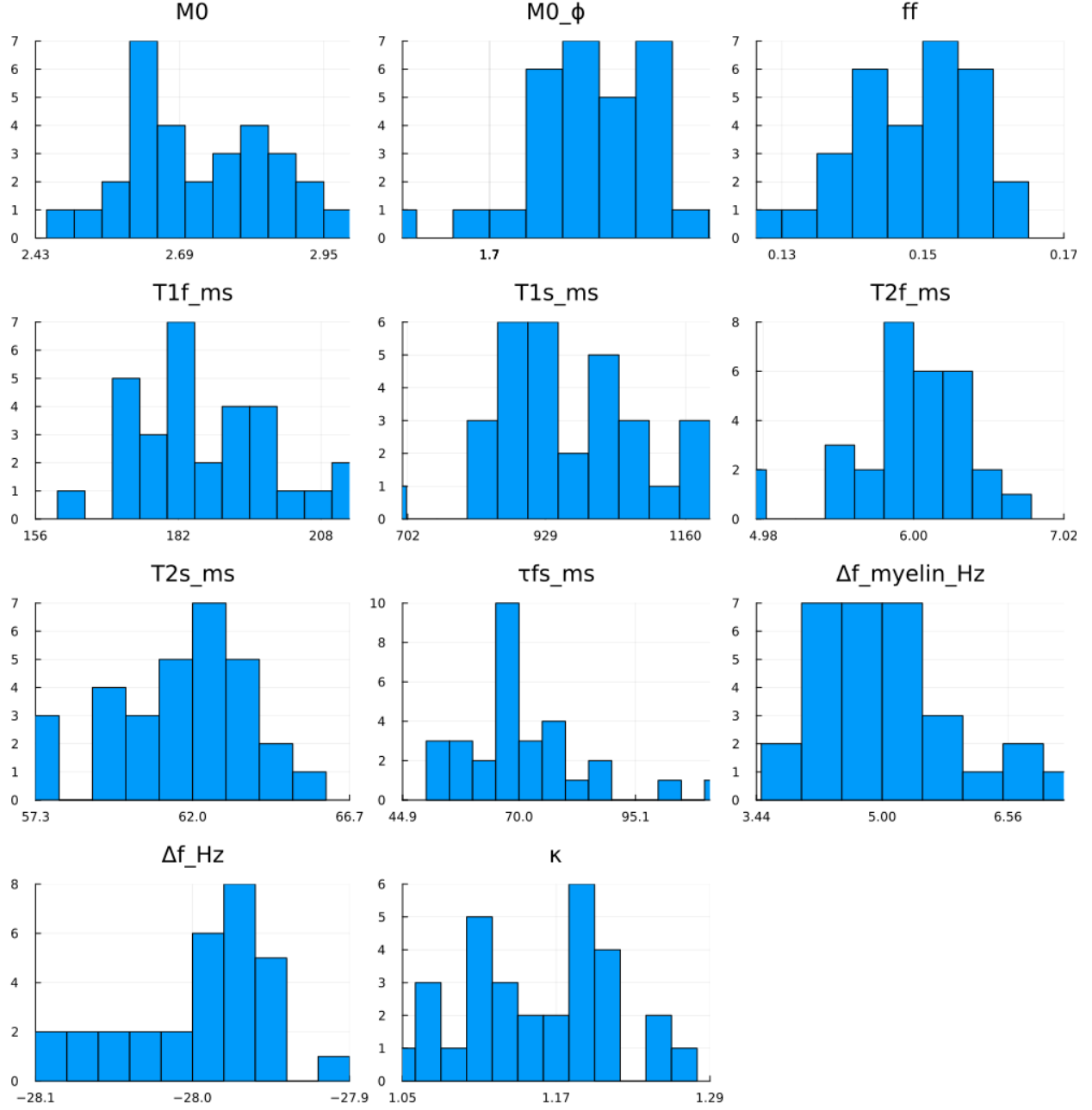


Figure 5.10: Histograms with results from running 30 trials with different noise realizations (2-pool voxel) where the parameter values were randomly initialized. Ticks are true parameter value (center tick) $\pm 2\sigma_{CRB}$.

5.4.3 Three-Pool Model Noisy Simulations

Figure 5.11 shows this simulated three-pool model data fit with a two-pool model. The f_f estimate is noticeably biased, and both T_1 values have reached the edge of their box constraints. In contrast, Figure 5.12 shows results from this same simulated three-pool voxel that was fit with the same three-pool model to avoid the model mismatch. The four extra parameters were kept fixed, so the same parameters were estimated. When there is no model mismatch, the estimated values are less biased, especially the f_f and both T_1 values.

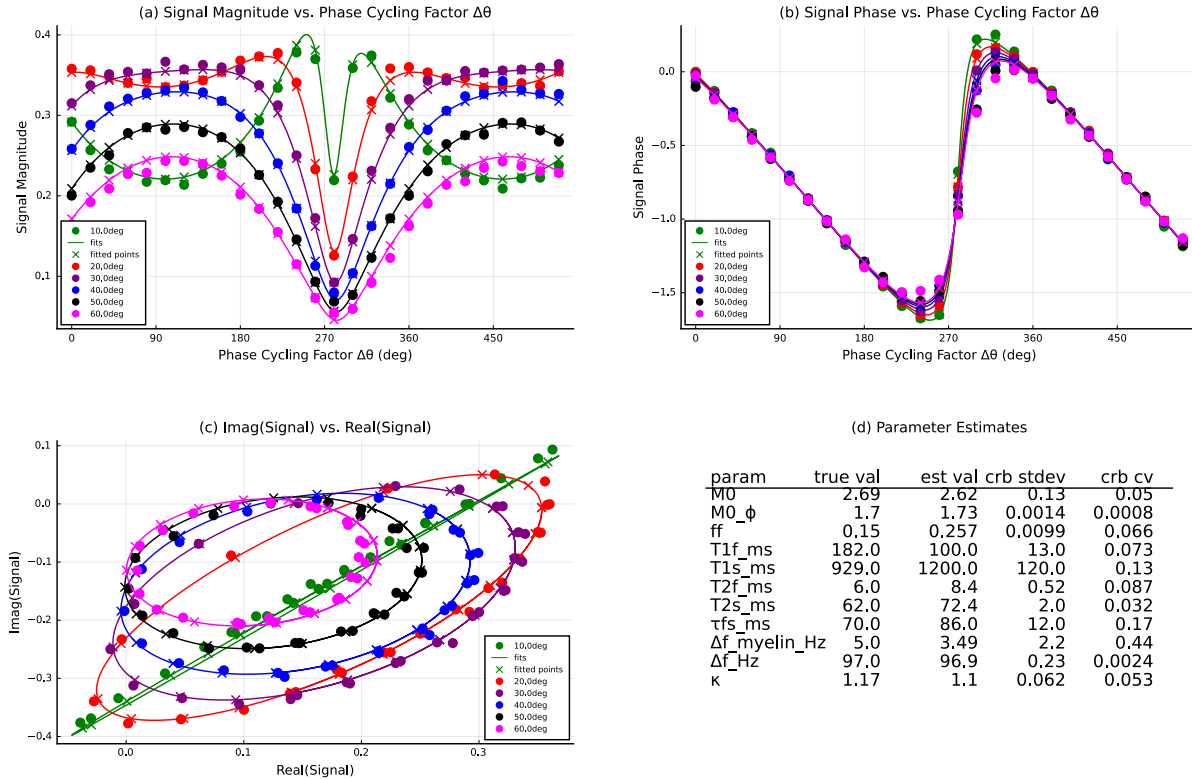


Figure 5.11: Simulated and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a three-compartment exchanging WM voxel. The data was generated for a three-pool exchanging voxel, but fit to a two-pool model. The parameters were initialized randomly within box constraints. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values.

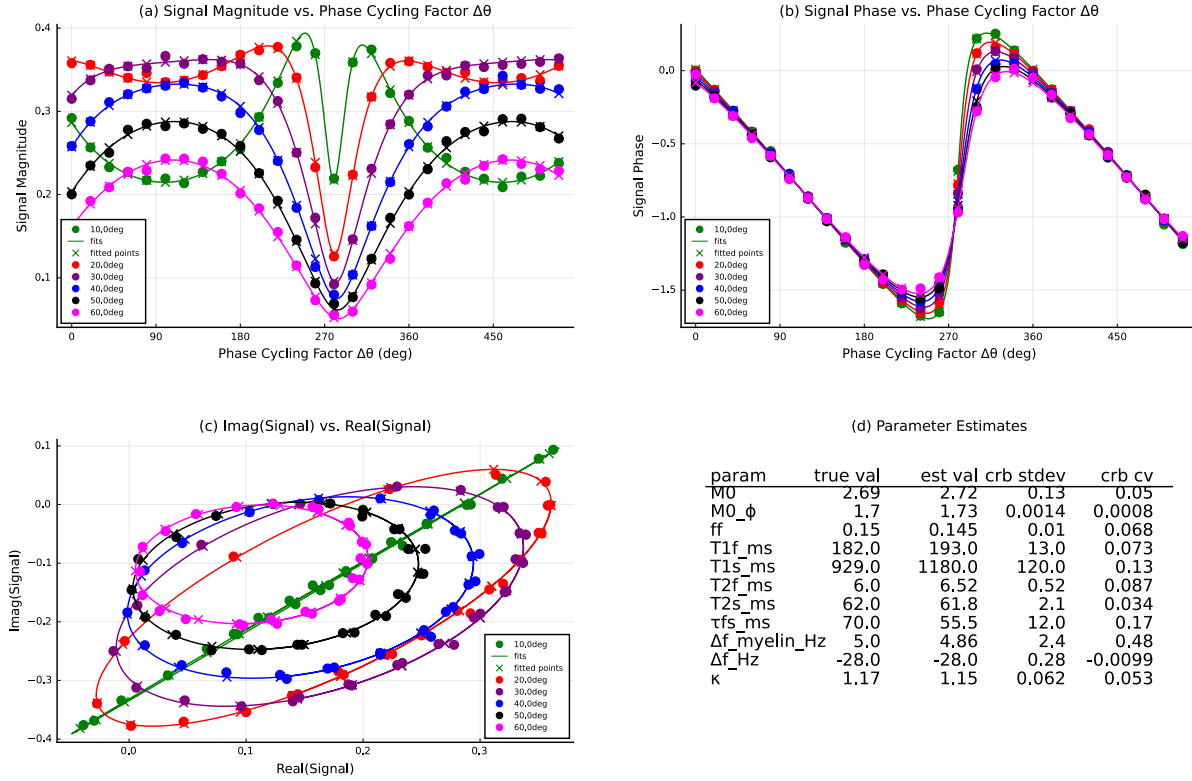


Figure 5.12: Simulated and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a three-compartment exchanging WM voxel. The data was generated for a three-pool exchanging voxel and fit with a three-pool model with the extra macromolecular pool parameters fixed to typical WM values [14]. The parameters were initialized randomly within box constraints. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values.

5.4.4 Single-Pool Phantom Experiments

Figure 5.13 shows bSSFP images of our preliminary experiments with a single-pool phantom acquired with the sequence described in Section 5.3.2 for 27 phase cycling factors linearly spaced from 0° - 520° and a flip angle 60° . Data for six flip angles linearly spaced from 10° - 60° were acquired for the 27 PCFs and processed according to the method described in Section 5.3.1, but with a single-pool bSSFP signal model. The estimated parameters were M_0 , $M_{0,\phi}$, T_1 , T_2 , B_0 offset $\Delta\omega$, and B_1 scaling factor κ . The parameter values were initialized to the estimated acquired from the other scans described in Section 5.3.2. The fitting procedure used the initial values and box constraints listed in Table 5.4. Figure 5.14 shows the the magnitude and phase plots of the acquired and fitted data from a center voxel in the single-pool phantom. The size parameters were estimated to be: $M_0 = 5.3$, $M_{0,\phi} = 2.5$, $T_1 = 273.5$ ms, $T_2 = 54.4$ ms, B_0 offset $\Delta\omega = -8.65$ Hz, and B_1 scaling factor $\kappa = 0.9$. The T_1 and T_2 values were approach their ground truth estimates of $T_1 = 252$ ms and $T_2 = 57$ ms. This preliminary study helped us establish confidence in our bSSFP protocol and NLLS fitting method, and next we moved on to two-pool phantoms.

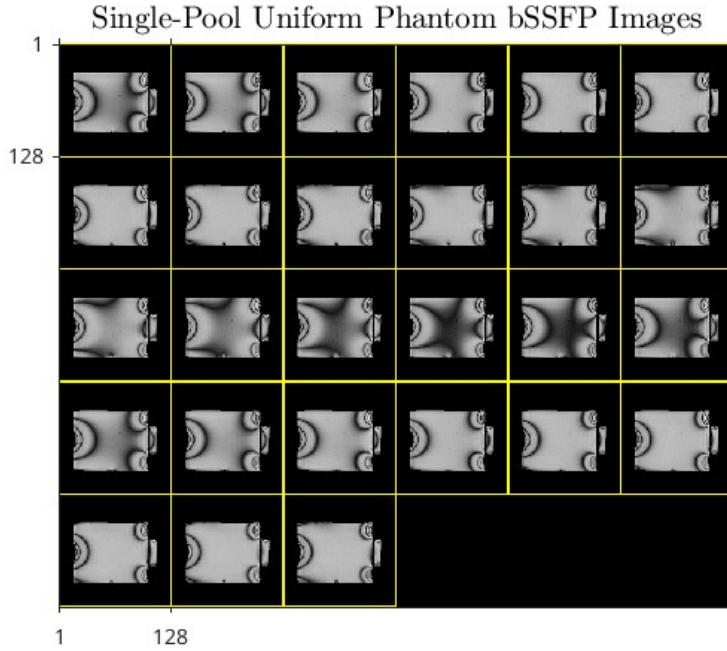


Figure 5.13: bSSFP images acquired with 27 phase cycling factors linearly spaced from 0° - 520° and flip angle of 60° . Shading due to off-resonance from the phase cycling are visible, providing different image contrasts.

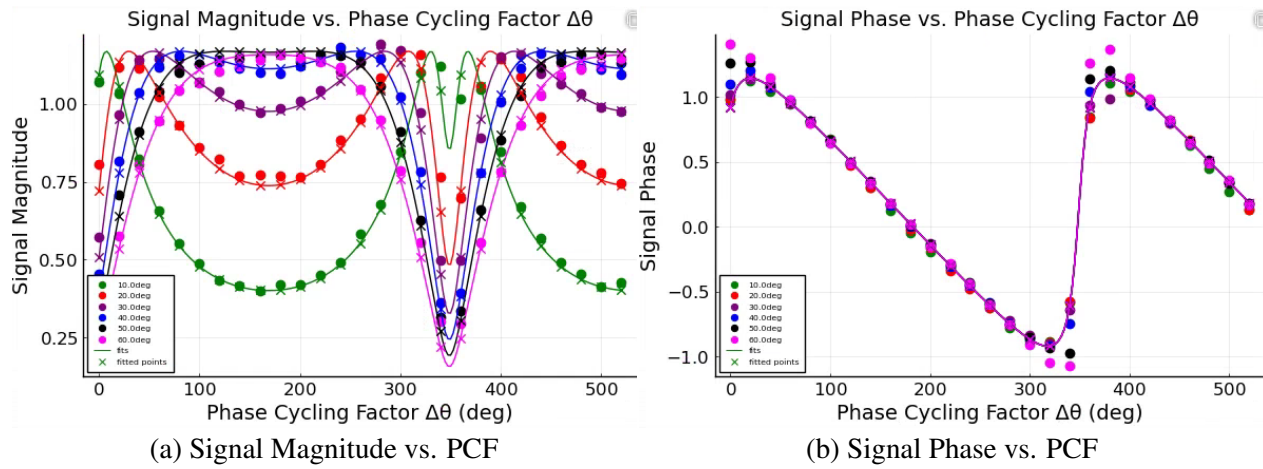


Figure 5.14: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a one compartment ROI in the center of uniform 1-pool phantom. Fits follow the data except for some mismatch in the signal nulls. Lower flip angles have better phase fits; a possible explanation could be slice profile effects.

5.4.5 Non-Exchanging Two-Pool Phantom Experiments

Figure 5.15 shows bSSFP images of the water+DMSO phantom acquired with the sequence described in Section 5.3.3 for 27 phase cycling factors linearly spaced from 0° - 520° and a flip angle 60° . Data for six flip angles linearly spaced from 10° - 60° were acquired for the 27 PCFs and processed according to the method described in Section 5.3.1. The parameter values were initialized to the estimated acquired from the other scans described in Section 5.3.3. f_f was initialized to 0.8344 as mentioned in Section 5.3.3, and $\tau_{f \rightarrow s}$ was initialized to 1000000 since this is a non-exchanging phantom. $\Delta\omega_f$ was initialized to 118 Hz since the chemical shift between water and DMSO is known to be about 618 Hz at 7T and bSSFP off-resonance is periodic with period $\frac{1}{TR}$. $\Delta\omega$ and κ were initialized to -18.26 Hz and 1.06; these values were obtained after a round of fitting was conducted where each parameter was fit individually. The fitting procedure used the initial values and box constraints listed in Table 5.4. Figure 5.16 shows acquired and fitted data from a center voxel in the water+DMSO phantom. The parameter estimates are shown in Figure 5.16d, and the CRBs and CVs were calculated using the estimated parameters and the noise variance, which was calculated from an ROI in the background of the bSSFP images. The f_f estimate deviates from the true value by 0.0186 and the $\tau_{f \rightarrow s}$ estimate is effectively Inf (no exchange). Data from a 25×25 ROI in the center of the phantom were processed for each voxel separately, and these results are summarized in the table in Figure 5.17. The estimate means show small deviations from the initialized values, and the standard deviations are largely consistent with the CRBs. Figure 5.18 shows histograms with the estimates from the voxels in this ROI; most parameters have a reasonable spread about the mean, although the chemical shift hit the edge of the box constraint. Figure 5.19 shows the acquired data and fit for one voxel in the center of the DMSO+water phantom with simulated data from a noiseless voxel with ground truth parameters acquired from MESE and IR scans. For this ground truth voxel, the B_0 and B_1 values estimated from the fit of all parameters to the acquired data for that voxel were used (future studies could use values from the acquired maps or fit the B_0 and B_1 using the other ground truth parameters). The MRT was fixed to 10,000 ms for this experiment. The ground truth data deviates from the acquired data more than the fits. Figure 5.20 shows scaled χ^2 statistics from 100 voxels in the center of the phantom with the same processing as for Figure 5.19. The red dot is the mean of the plotted values. The scaled chi-squared values are greater than one, meaning the observed data deviates from the model and that the fit is worse than expected from the noise variance. Some sources of model mismatch could be slice profile effects, finite RF effects, etc.

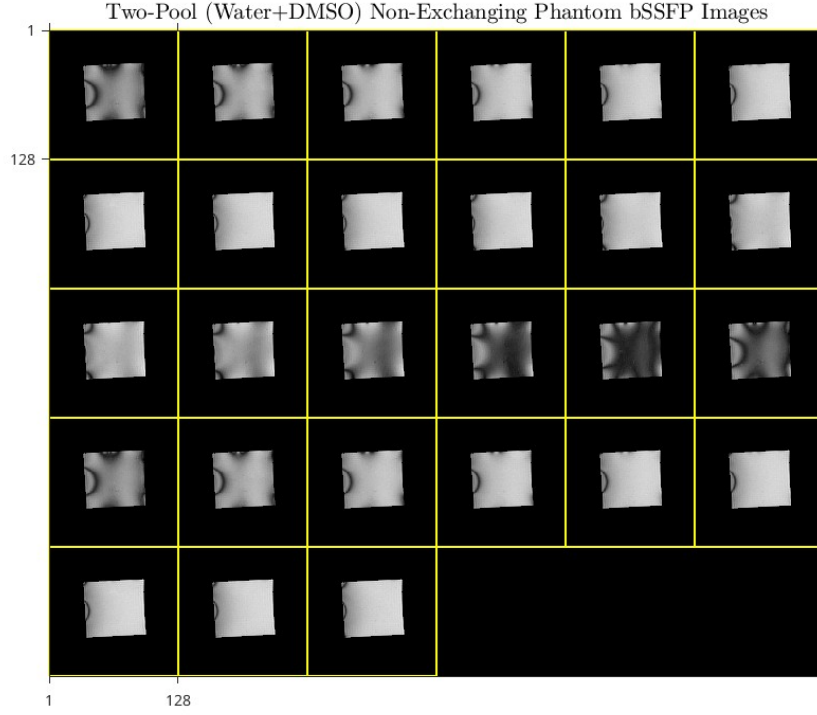


Figure 5.15: bSSFP images acquired with 27 phase cycling factors linearly spaced from 0° - 520° and flip angle of 60° . Shading due to off-resonance from the phase cycling are visible, providing different image contrasts.

Table 5.4: Initial values and lower and upper parameter bounds used for NLLS fitting of DMSO+water phantom data.

Parameter	Initialization	Lower Bound	Upper Bound
f_f	0.8344	0.75	0.95
$T_{1,f}$ (ms)	223	100	400
$T_{1,s}$ (ms)	1050	900	1200
$T_{2,f}$ (ms)	49	30	60
$T_{2,s}$ (ms)	242	230	260
$\tau_{f \rightarrow s}$ (ms)	10^6	1000	10^7
$\Delta\omega_f$ (chemical shift) (Hz)	118	-120	120
$\Delta\omega$ (off-resonance) (Hz)	-18.26	-20	20
κ	1.06	0.9	1.10

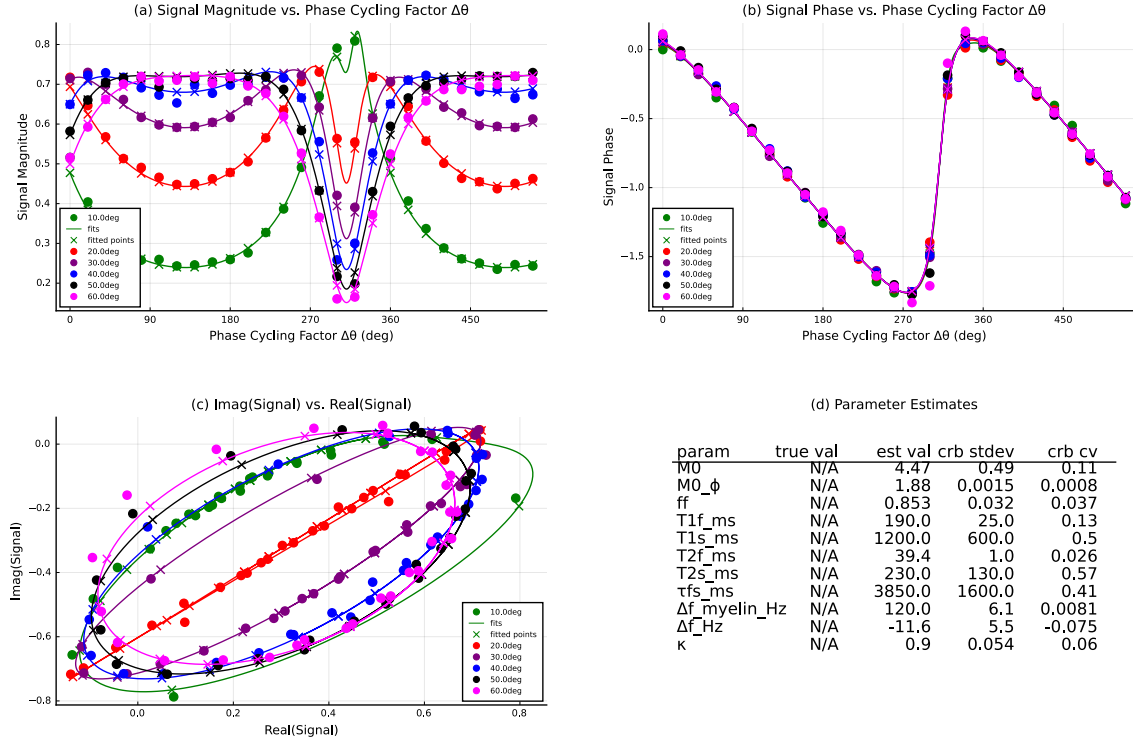


Figure 5.16: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a two-compartment non-exchanging voxel in the center of the DMSO+water phantom (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values. The Δf_{myelin} parameter here is the chemical shift of the phantom in Hz; the estimate is consistent with the true chemical shift of 618 Hz when the $1/TR$ periodicity is taken into account.

Param Name	Param Init Value	Empirical Estimated Mean	Empirical SD	Empirical CV	CRB SD	CRB CV
M_0	N/A	4.221	0.1667	0.03949	0.4037	0.09564
$M_{0,\phi}$	N/A	1.854	0.01474	0.007950	0.001236	0.0006667
ff	0.8344	0.8696	0.01270	0.01461	0.02506	0.02882
T1f_ms	223.0	183.9	7.688	0.04180	22.48	0.1222
T1s_ms	1050	1031	121.7	0.1180	1013	0.9830
T2f_ms	49.00	39.52	0.4088	0.01034	0.8089	0.02046
T2s_ms	242.0	248.5	12.26	0.04935	252.4	1.016
τ_{fs_ms}	1000000	246600	998700	4.049	1067000	4.328
chem_shift_Hz	118.0	120.0	0.006850	0.000009086	4.928	0.006536
Δf_Hz	-18.26	-11.56	0.3627	-0.004993	4.510	-0.06208
κ	1.060	0.9002	0.0005235	0.0005816	0.05047	0.05606

Figure 5.17: Results from processing 25 voxels from the DMSO+water phantom. The empirical means across the 25 trials show small deviations from the nominal parameter values, which were initialized based on the expected ground truth. For many parameters, the empirical SDs and CVs are consistent with the corresponding theoretical CRB values. Some empirical SDs are lower than the CRB SD, potentially from the parameter reaching the box constraint (such as for the chemical shift). The M_0 and $M_{0,\phi}$ inits are listed as N/A because they were calculated with VarPro.

DMSO+Water Phantom Histograms

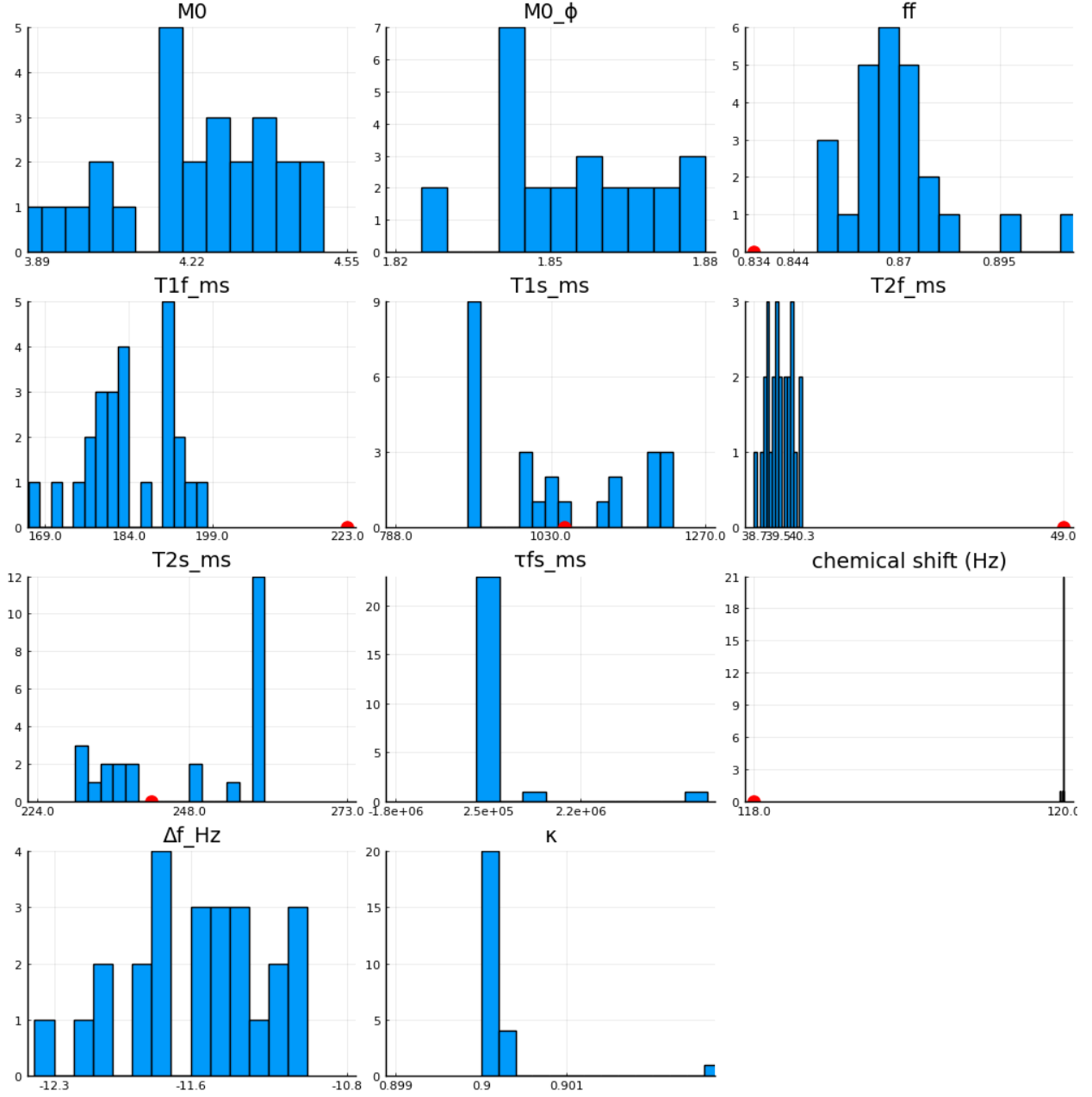


Figure 5.18: Histograms with results from processing 25 voxels in the center of the phantom where the parameter values were initialized to the values estimated from the other scans described in Section 5.3.3. The Δf_{myelin} parameter is the chemical shift in Hz for this phantom. Ticks are empirical mean estimated parameter value $\pm 2\sigma_{\text{empirical}}$.

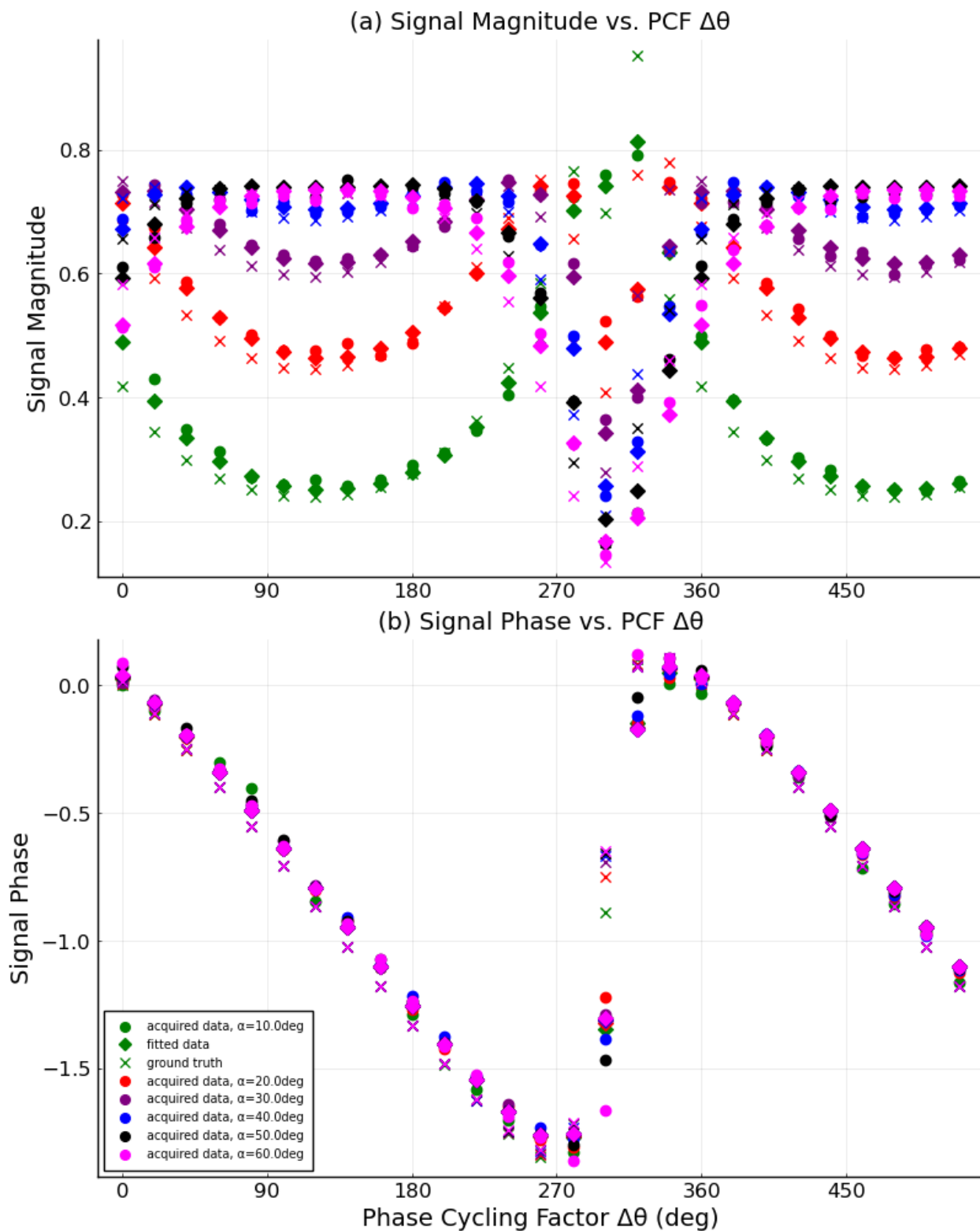


Figure 5.19: Center voxel in DMSO+water phantom acquired data and fit plotted with simulated signal using the ground truth parameters obtained from other scans (MESE, IR, etc). The ground truth deviates from the acquired data more than the fits.

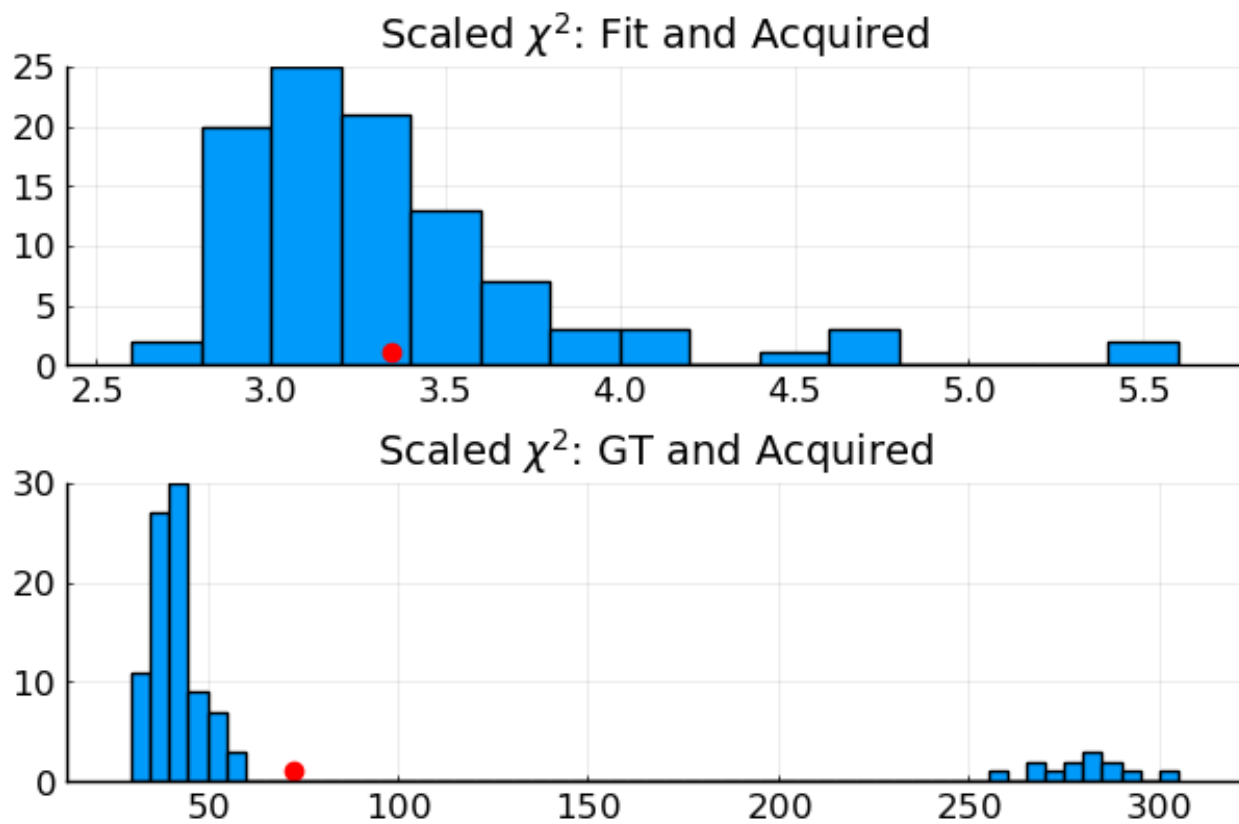


Figure 5.20: Histograms of scaled χ^2 statistics from 100 voxels in the center of the DMSO+water phantom. The scaled chi-squared values are greater than one, which means the observed data deviates from the model and that the fit is worse than expected from the assumed noise variance. The red dot is the mean of the plotted values.

5.4.6 Exchanging Two-Pool Phantom Experiments

Urea-water phantom data acquired from the IR-NMR spectrum was processed with NLLS and a bi-exponential fit according to the signal model and method described in Section 2.5. Figure 5.21 shows the acquired and fitted IR-NMR data. The T_1 values were constrained to be the same because the one-dimensional inversion recovery scan plus bi-exponential fit directly run on the scanner estimated both T_1 values to be 740 ms. The method from Section 2.5 estimated the T_1 values to be 766 ms and the urea mean residence time $\tau_{f \rightarrow s}$ to be 16 ms. This exchange time estimate and the 0.09 f_f value from Section 5.3.4 are taken as the ground truth values for these parameters. If the T_1 values were not constrained to be the same, $T_{1,s}$ was estimated to be 944 ms, $T_{1,f}$ was estimated to be 419 ms, and $\tau_{f \rightarrow s}$ was estimated to be 15 ms.

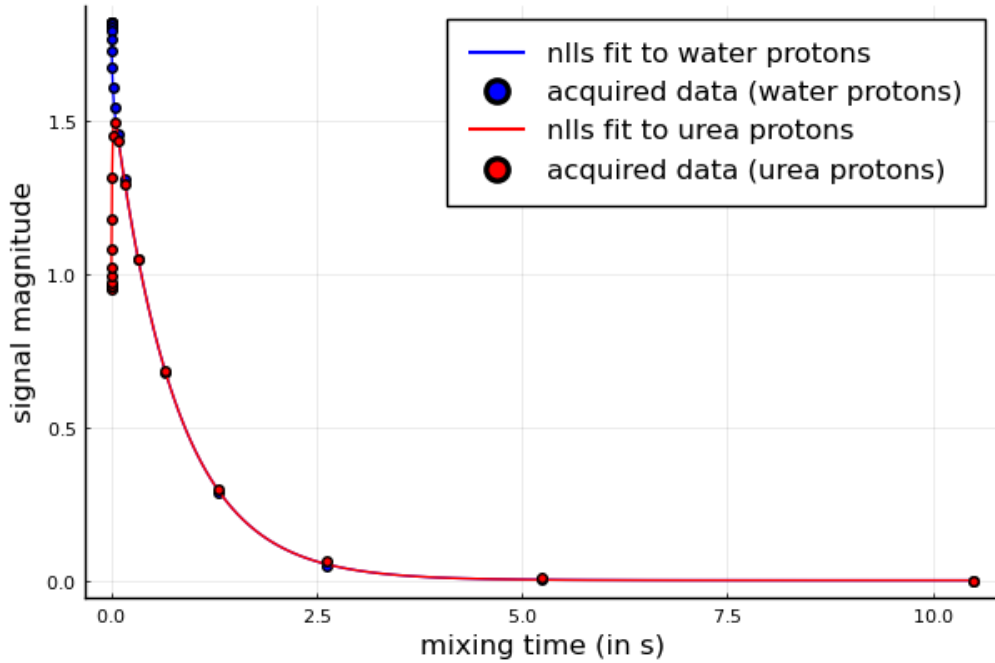


Figure 5.21: NLLS bi-exponential fit to the acquired IR-NMR data on the urea phantom to estimate the “ground truth” exchange rate. The water protons were selectively inverted here, and the T_1 values were constrained to be the same value.

Figure 5.22 shows bSSFP images of the water+urea phantom acquired with the sequence described in Section 5.4.6 for 27 phase cycling factors linearly spaced from 0° - 520° and a flip angle 60° . Data for six flip angles linearly spaced from 10° - 60° were acquired for the 27 PCFs. For this fit, the parameter values were randomly initialized within the box constraints in Table 5.5. Figure 5.23 shows acquired and fitted data from a 5×5 ROI in the water+urea phantom. The CRBs and CVs in Figure 5.23 were calculated using the estimated parameters. The table in Figure 5.24 shows the results from processing all voxels in the urea phantom with random initializations (with

the sliding window ROI). In this case, the CRB SD and CV values were calculated using the empirical mean values. The f_f mean of 0.16 is biased and is higher than the true urea fraction value of 0.09. However, its empirical standard deviation is relatively consistent with the CRB standard deviation, indicating that the estimator may be biased but relatively precise. The T_1 values were not constrained to be the same, and the IR-NMR estimate of 766 ms is between the two means, and both T_2 means are relatively consistent with their MESE+bi-exponential fit estimates of 7 ms and 80 ms; although the $T_{2,f}$ is approaches the box constraint. The MRT mean estimate of 22 ms is comparable to the IR-NMR estimate of 16 ms, differing by 6 ms. The estimated chemical shift of 59.54 Hz corresponds to 309.54 Hz under the $1/TR = 125$ Hz periodicity, differing from the true urea-water chemical shift of 300 Hz by 9.54 Hz. κ is also approaches the box constraint lower bound. The histograms in Figure 5.25 show the distribution of the estimates for the urea phantom map. The M_0 estimates were very consistent; however, the $M_{0,\phi}$ values converged to two estimates that fit the data well; This is likely related to why the off-resonance estimate also has two values that it converged to; the bSSFP signal is periodic with period $1/TR$, and the box constraints were wider than one period. The f_f , $T_{1,s}$, $T_{2,s}$, and $\tau_{f \rightarrow s}$ values have a reasonable spread about the mean, whereas $T_{1,f}$, $T_{2,f}$, the chemical shift, and κ are more clustered.

Thus, overall the estimation strategy did a good job at estimating the parameter values of this exchanging phantom with reasonable precision. A map of the entire phantom was generated for each parameter using a 5×5 sliding window ROI and random initialization of the parameter values for 10 trials (rather than 20). Figure 5.26 shows the f_f , $\tau_{f \rightarrow s}$, and Δf (chemical shift) parameter estimate maps for the whole urea phantom for two cases: when the T_1 and T_2 values were held constant at the values estimates from the MESE and IR-NMR experiments and bi-exponential fits described in Section 5.3.4 and when all parameters were estimated. The median values of these maps were 0.10 for f_f , 19.5 ms for $\tau_{f \rightarrow s}$, and 65 Hz for the chemical shift. Accounting for the $\frac{1}{TR}$ periodicity, the chemical shift corresponds to 315 Hz, or approximately 1.06 ppm. The median f_f and $\tau_{f \rightarrow s}$ estimates differ from the IR-NMR-derived ground truth values of 0.09 and 16 ms by 0.01 and 3.5 ms, respectively, and the estimated chemical shift differs from the expected value of approximately 1 ppm by about 0.06 ppm. These deviations are smaller than those obtained when T_1 and T_2 were not fixed, which yielded median f_f and $\tau_{f \rightarrow s}$ estimates of 0.16 and 22 ms. Figure 5.27 shows the maps for all parameters when all parameters were estimated (none held fixed).

Table 5.5: Lower and upper parameter bounds used for NLLS fitting of urea+water phantom data.

Parameter	Lower Bound	Upper Bound
f_f	0.05	0.4
$T_{1,f}$ (ms)	100	900
$T_{1,s}$ (ms)	100	900
$T_{2,f}$ (ms)	4	100
$T_{2,s}$ (ms)	20	500
$\tau_{f \rightarrow s}$ (ms)	2	100
$\Delta\omega_f$ (chemical shift) (Hz)	-120	120
$\Delta\omega$ (off-resonance) (Hz)	-120	120
κ	0.9	1.1

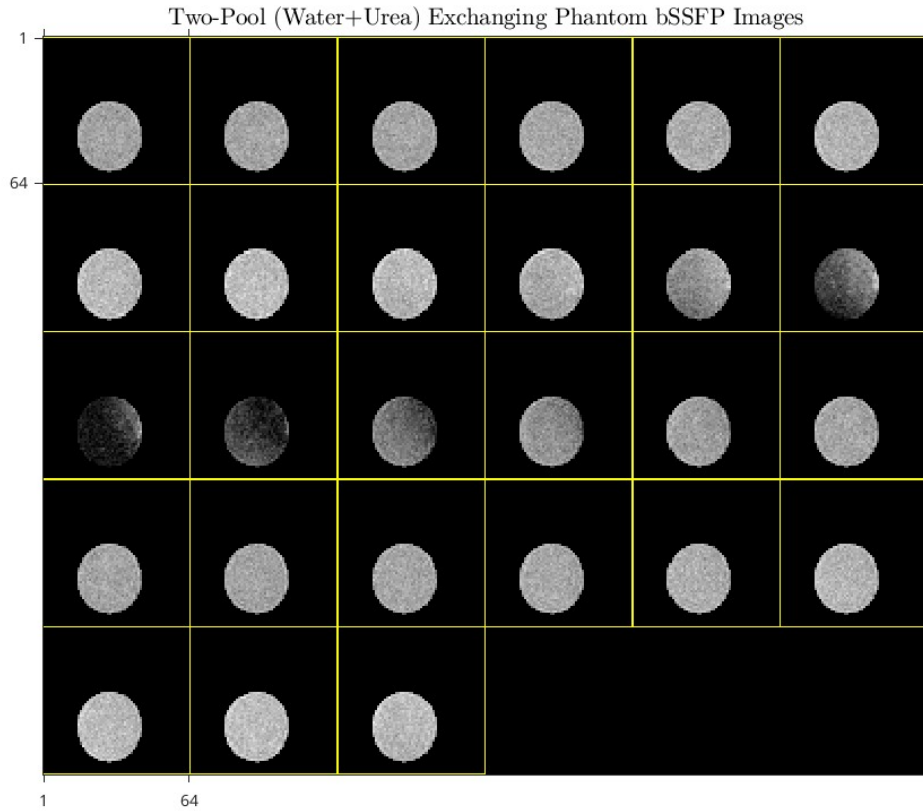


Figure 5.22: bSSFP images acquired with 27 phase cycling factors linearly spaced from 0° - 520° and flip angle of 60° . Shading due to off-resonance from the phase cycling are visible, providing different image contrasts.

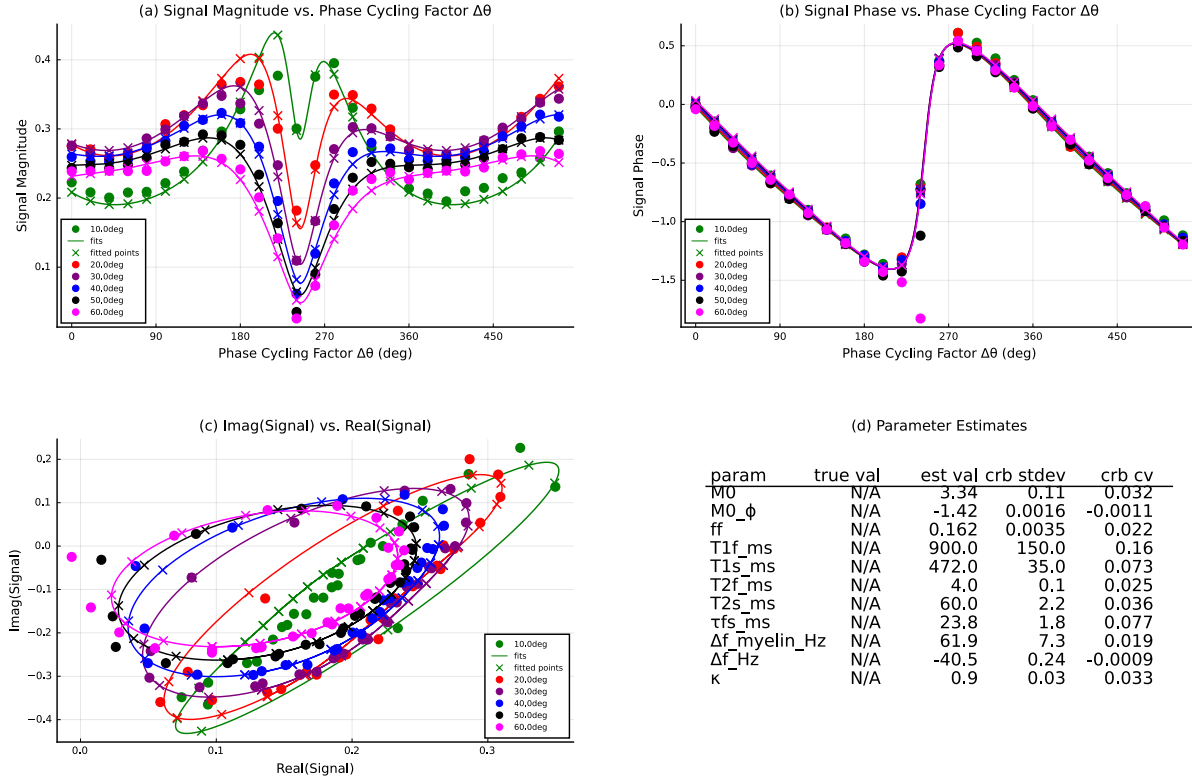


Figure 5.23: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a two-compartment exchanging ROI in the center of the urea+water phantom. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates for the ROI and the corresponding CRB values. The Δf_{myelin} parameter here is the chemical shift of the phantom in Hz. It is unexpected that $T_{1,f}$ is larger than $T_{1,s}$ here; the reason behind this remains to be explored.

Table 5.6: Median Values in Urea Phantom Parameter Maps

Parameter	Fix T_{1s} and T_{2s} Version	Estimate All Params Version
M_0	3.2	3.2
$M_{0,\phi}$	-1.4	-1.4
f_f	0.10	0.16
$T_{1,f}$ (ms)	[740]	900
$T_{1,s}$ (ms)	[740]	469
$T_{2,f}$ (ms)	[7]	4.0
$T_{2,s}$ (ms)	[80]	61.7
$\tau_{f \rightarrow s}$ (ms)	19.5	22.3
$\Delta\omega_f$ (chemical shift) (Hz)	65.4	61.2
$\Delta\omega$ (off-resonance)(Hz)	-34.4	-35.4
κ	0.9	0.9

Param Name	Param Init Value	Empirical Estimated Mean	Empirical SD	Empirical CV	CRB SD	CRB CV
M_0	N/A	3.007	0.5060	0.1683	0.09184	0.03055
$M_{0,\phi}$	N/A	-0.07113	1.559	-21.91	0.001587	-0.02231
ff	rand	0.1595	0.01320	0.08271	0.003316	0.02078
T_{1f_ms}	rand	878.4	74.14	0.08440	127.6	0.1453
T_{1s_ms}	rand	470.1	28.39	0.06039	32.59	0.06933
T_{2f_ms}	rand	4.131	0.6542	0.1584	0.1143	0.02767
T_{2s_ms}	rand	62.54	6.384	0.1021	2.255	0.03606
tfs_ms	rand	22.21	1.421	0.06396	1.580	0.07110
chem_shift_Hz	rand	59.54	14.77	0.03948	7.944	0.02124
Δf_Hz	rand	14.56	62.06	0.6786	0.2073	0.002266
κ	rand	0.9002	0.002993	0.003324	0.02843	0.03158

Figure 5.24: Results from processing urea+water phantom map. The empirical SDs and CVs approach the theoretical CRB values for many of the parameters. The M_0 and $M_{0,\phi}$ initializations are listed as N/A because they were calculated with VarPro; the rest were randomly initialized within box constraints. It is unexpected that T_{1f} is larger than T_{1s} here; the reason behind this remains to be explored.

Urea+Water Phantom Histograms

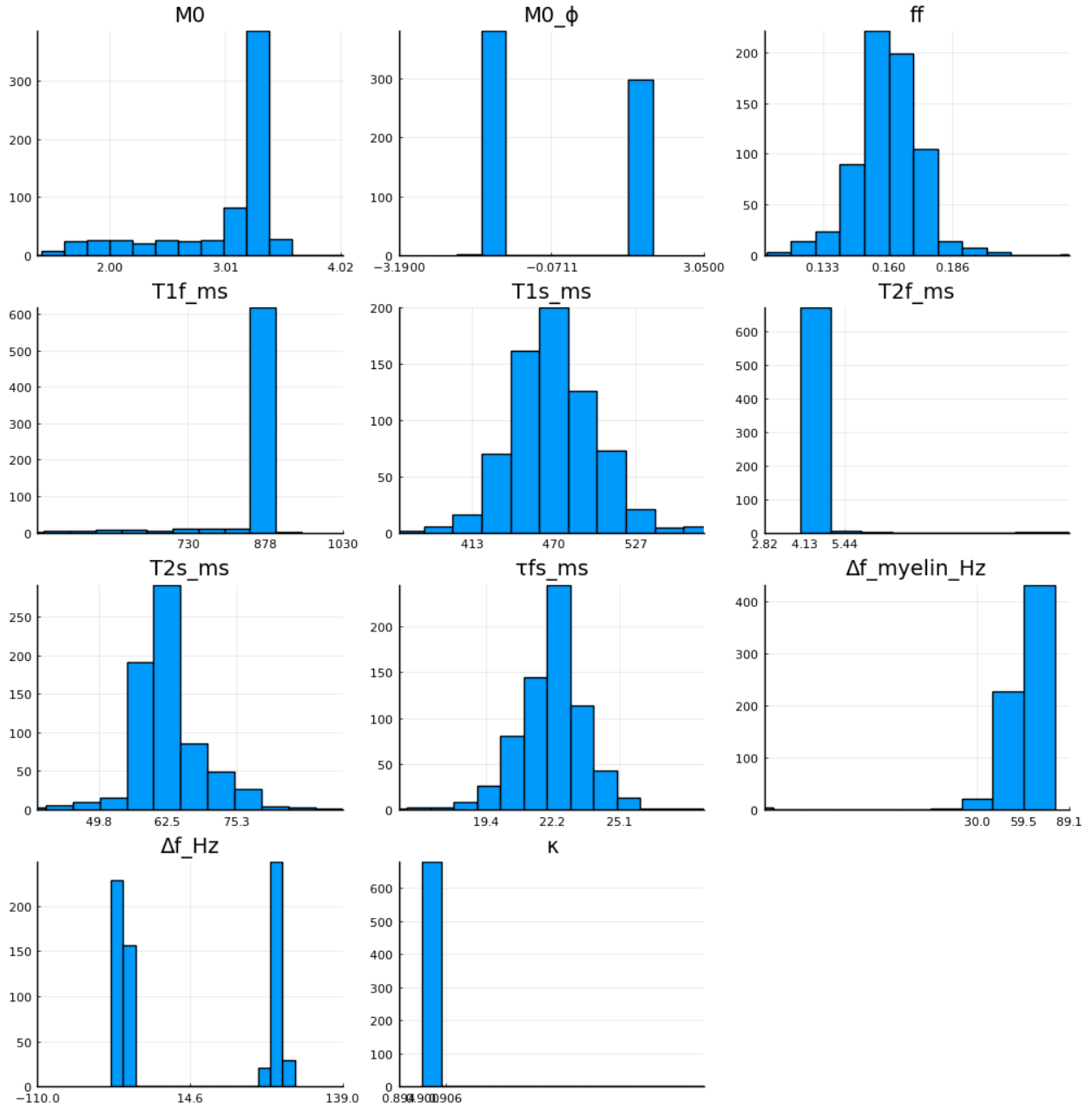
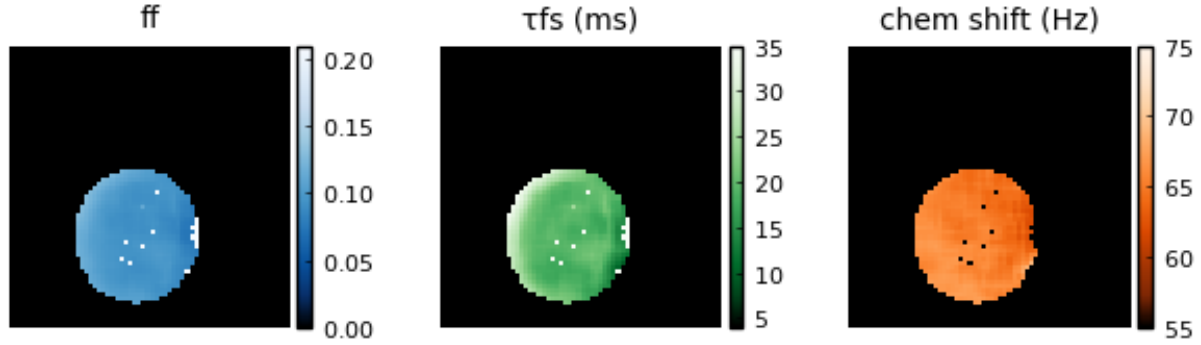
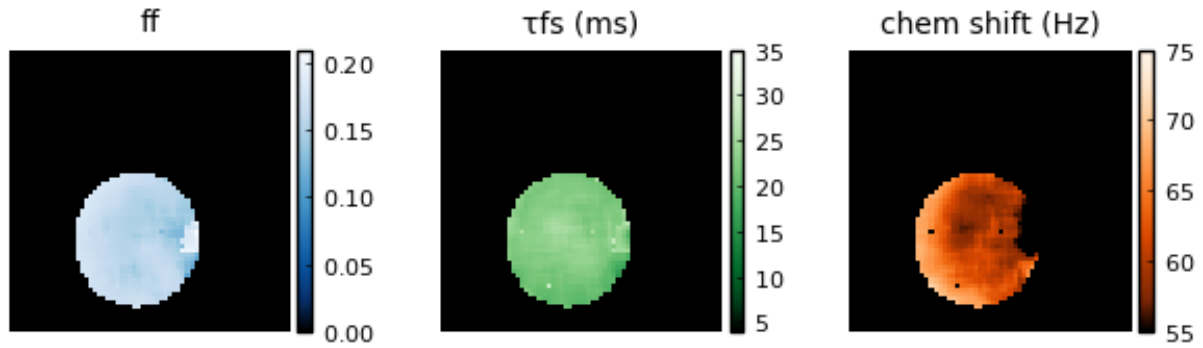


Figure 5.25: Histograms with results from processing the urea+water phantom map where the parameters were randomly initialized and all 11 parameters were estimated. The Δf_{myelin} parameter is the chemical shift in Hz for this phantom. Ticks are empirical mean estimated parameter value $\pm 2\sigma_{\text{empirical}}$. Two $M_{0,\phi}$ values fit this data well, likely due to the phase correction step, and two Δf values fit the data well due to the wide box constraints the the $1/\text{TR}$ periodicity of the off-resonance values. It is unexpected that T_{1f} is larger than T_{1s} here; the reason behind this remains to be explored.



(a) Urea Phantom Maps: $T_{1,f}$, $T_{1,s}$, $T_{2,f}$, $T_{2,s}$ Fixed



(b) Urea Phantom Maps: All Parameters Estimated

Figure 5.26: Parameter maps of urea+water phantom for f_f , $\tau_{f \rightarrow s}$, and chemical shift. These maps were (a) estimated when the T_1 and T_2 values were fixed to the estimates from the IR-NMR and MESE experiments described in Section 5.3.4 and (b) estimated when all parameters were estimated; these values correspond to Figures 5.23-5.25. The median values in these three maps are: (a) $f_f = 0.10$, $\tau_{f \rightarrow s} = 19.5\text{ms}$, and chemical shift = 65 Hz, corresponding to 315 Hz after accounting for the $1/\text{TR}$ periodicity, or approximately 1.06 ppm; and (b) $f_f = 0.16$, $\tau_{f \rightarrow s} = 22\text{ms}$, and chemical shift = 61 Hz, corresponding to 312 Hz after accounting for the $1/\text{TR}$ periodicity, or approximately 1.47 ppm. Relative to the ground truth values of $f_f = 0.09$ and $\tau_{f \rightarrow s} = 16\text{ms}$, the estimates in (a) differ by 0.01 and 3.5 ms, respectively, whereas the estimates in (b) differ by 0.07 and 6 ms.

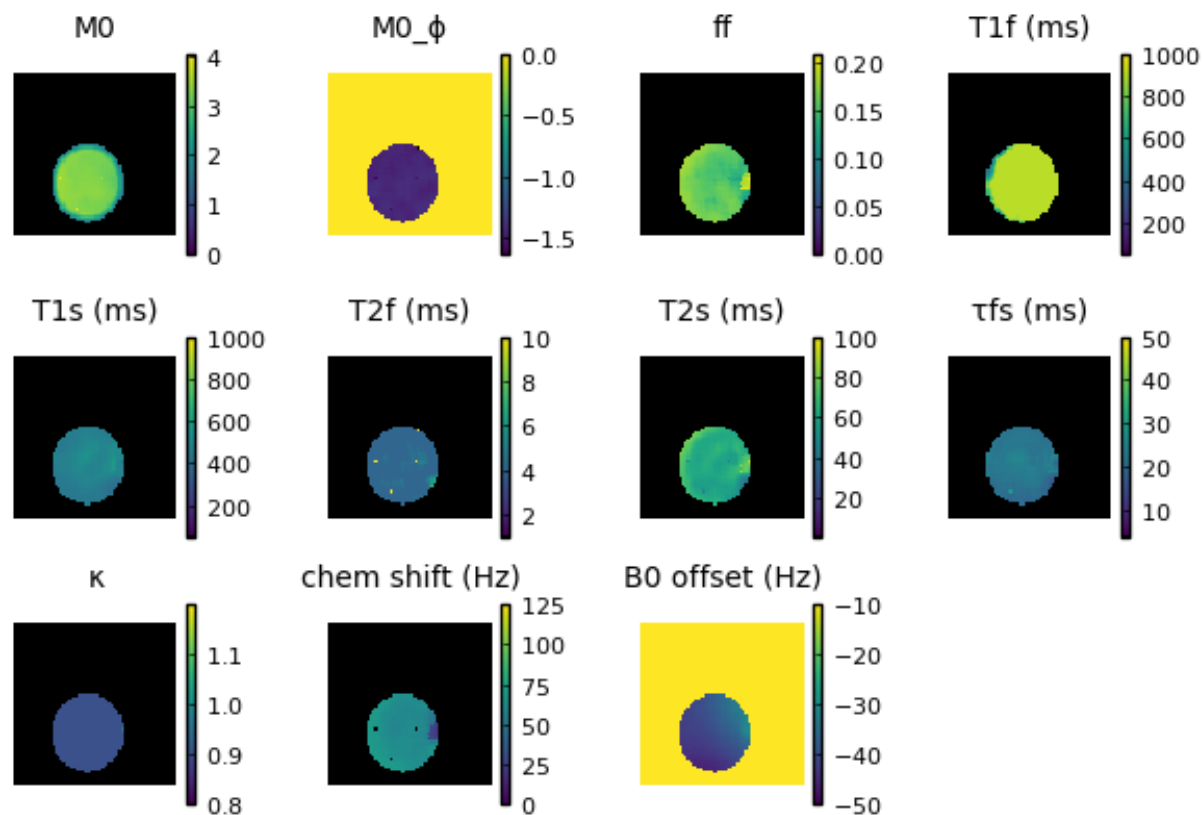


Figure 5.27: Maps of urea phantom for all 11 estimated parameters. The $M_{0,\phi}$ and B_0 offset maps were corrected based on the $\frac{1}{T_R}$ periodicity of the off-resonance. The $T_{1,f}$, $T_{2,f}$ and κ values hit the box constraints, but the $T_{1,s}$ and $T_{2,s}$ values are consistent with their ground truth values from the IR-NMR and MESE scans.

5.4.7 Ex Vivo Tissue Sample Two Pool Fit

Figure 5.28 shows bSSFP images of a MADRC tissue sample acquired with the sequence described in Section 5.3.5 for 27 phase cycling factors linearly spaced from 0° - 520° and a flip angle 60° . Data for six flip angles linearly spaced from 10° - 60° were acquired for the 27 PCFs and processed according to the method described in Section 5.3.1. The parameter values were initialized to the estimates acquired from the other scans described in Section 5.3.5 and also randomly within the box constraints in Table 5.7 (separate experiments). Figures 5.29-5.30 show acquired and fitted data from a 5×5 WM ROI in the tissue sample. The values in the two plots converge, showing that the estimates from random initialization (minimum cost of 20 trials) converge to the estimates from initializing with WM values (except for $M_{0,\phi}$ and the off-resonance which differ based on the $1/\text{TR}$ periodicity). The f_f value in this ROI is reasonable for tissue WM, but the fast-relaxing T_1 and T_2 and exchange values both hit box constraint edges. Figure 5.31 shows parameter maps where a sliding window 5×5 ROI was fit to the data with random initialization of all parameter values for 10 trials (rather than 20). The sliding window ROI was used to be able to have enough SNR to fit the data. The f_f map looks reasonable but the exchange map hits the lower edge of the box constraints, (in this case 50 ms), for most voxels, similar to the ROI in Figures 5.29-5.30. The $\Delta\omega_f$ myelin-specific off-resonance values are mostly around the expected 5 Hz for WM tissue [14]. The FSE image and MWF map corresponding to this sample generated from MESE data are shown in Figure 5.32. The f_f map in Figure 5.31 is smoother than the MWF map because each voxel was processed separately for the MWF calculation, rather than the sliding window 5×5 ROI used in the f_f calculation. The MWF in the WM ROI corresponding to Figures 5.29-5.30 is 0.26, so there is a bias between the f_f and MWF estimates.

Table 5.7: Lower and upper parameter bounds used for NLLS fitting of tissue sample WM ROI.

Parameter	Lower Bound	Upper Bound
f_f	0.05	0.40
$T_{1,f}$ (ms)	100	700
$T_{1,s}$ (ms)	200	1200
$T_{2,f}$ (ms)	4	100
$T_{2,s}$ (ms)	20	500
τ_{fs} (ms)	20	10000
$\Delta\omega_f$ (myelin frequency shift) (Hz)	-10	10
$\Delta\omega$ (off-resonance)(Hz)	-63	63
κ	0.9	1.3

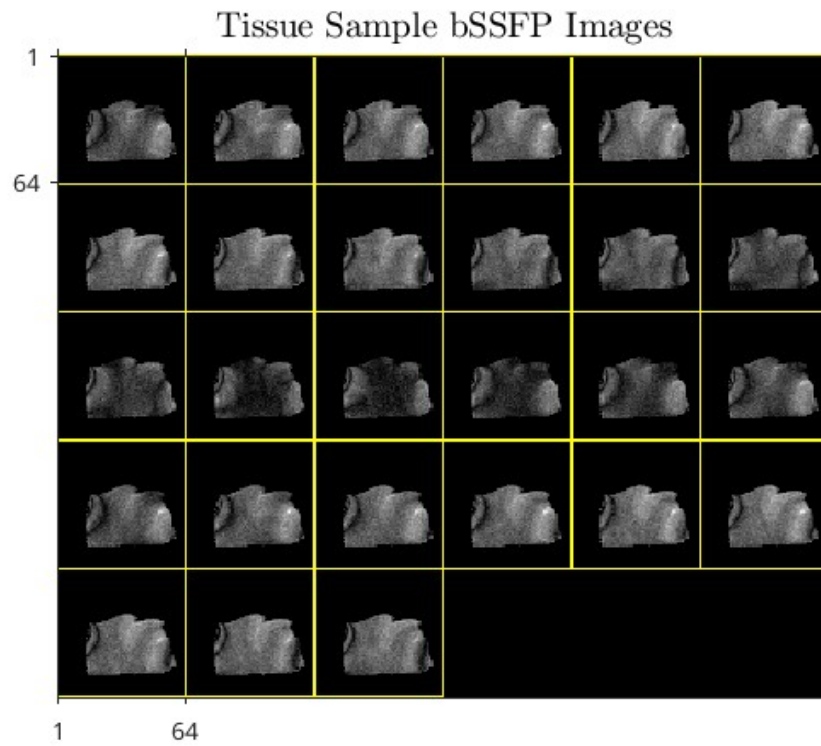


Figure 5.28: bSSFP images acquired with 27 phase cycling factors linearly spaced from 0° - 520° and flip angle of 60° . Shading due to off-resonance from the phase cycling are visible, providing different image contrasts.

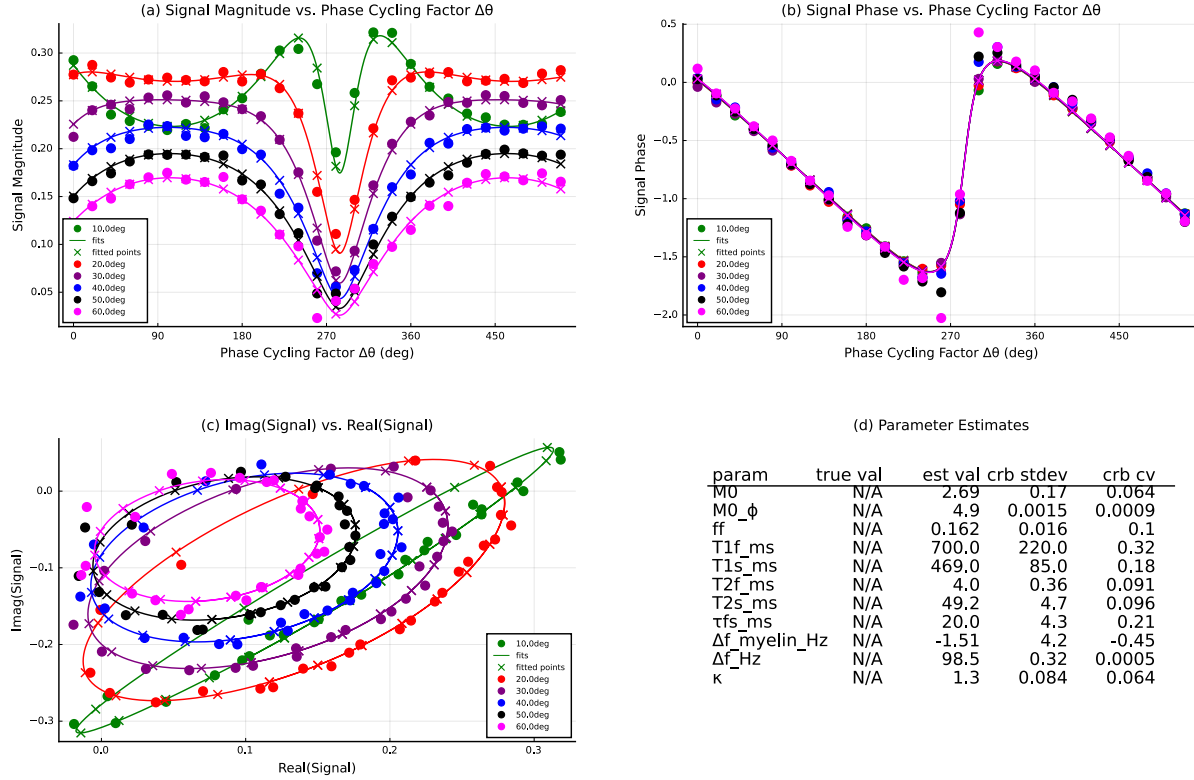


Figure 5.29: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a WM ROI in the tissue sample. The parameters were *initialized with WM values*. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values. The phase fit is better for lower flip angles; a possible explanation is slice profile effects.

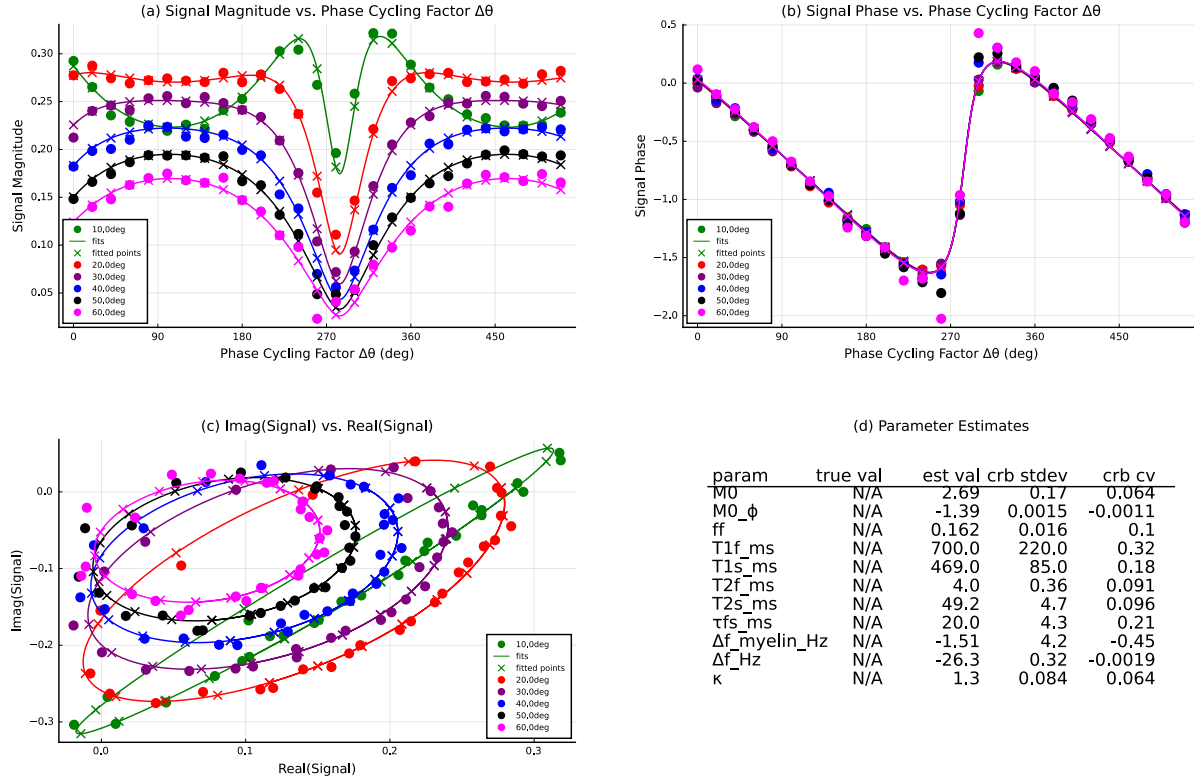


Figure 5.30: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for an exchanging WM ROI in the tissue sample. The parameters were *initialized randomly within box constraints*. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values. The estimates converged to the same values as when the parameters with initialized to typical WM values as seen in Figure 5.29.

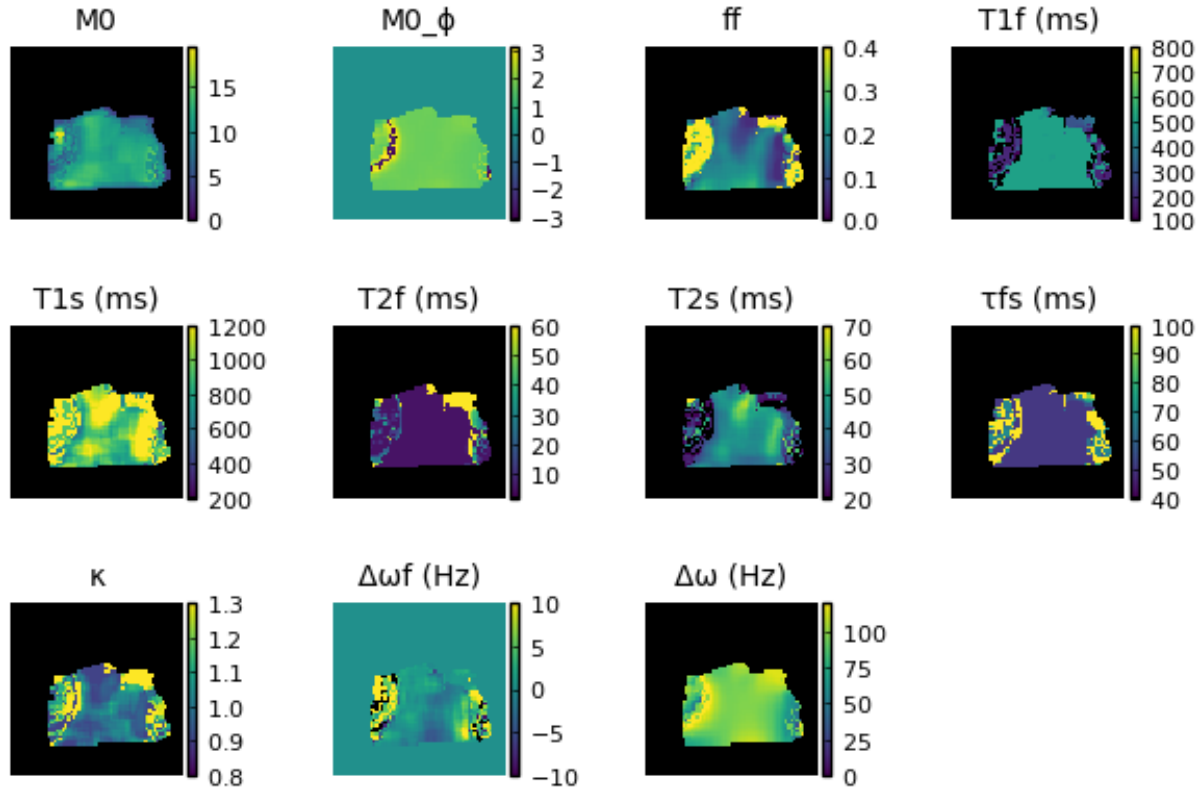


Figure 5.31: Parameter maps of tissue sample 1131-S3A. f_f , $T_{1,s}$, $T_{2,s}$ values are reasonable for an ex vivo tissue sample. $T_{1,f}$, $T_{2,f}$, and $\tau_{f \rightarrow s}$ values mostly hit their box constraints. $\Delta\omega_f$ values are reasonable for ex vivo tissue, around 5 Hz [15].

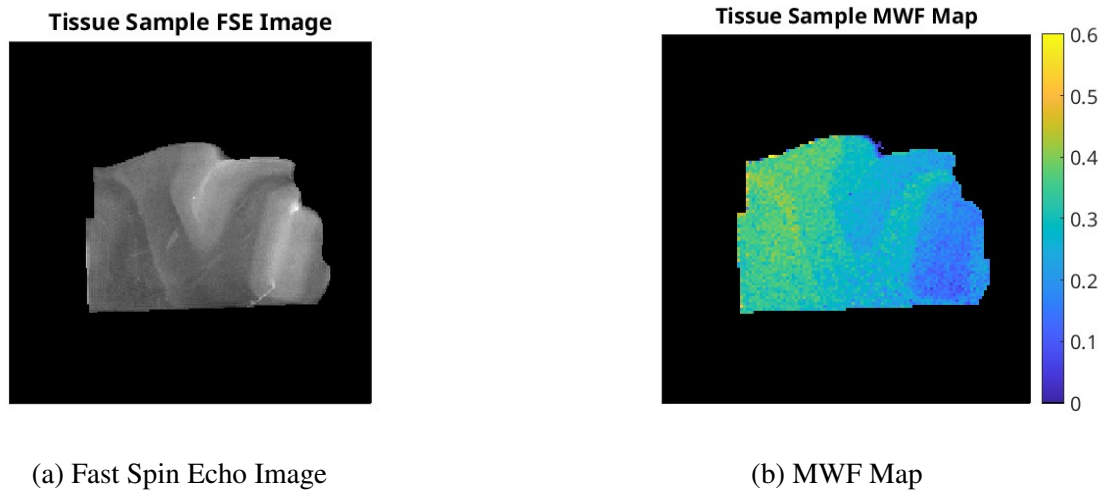


Figure 5.32: FSE image and MWF map of corresponding slice of 1131-S3A. The MWF in the WM ROI corresponding to Figures 5.29-5.30 is 0.26.

5.4.8 Ex Vivo Tissue Sample Three Pool Fit

Figure 5.33 shows data from a real WM ROI fitted with the three-pool model with the four extra parameters fixed. There is still significant model mismatch, and many parameter estimates have hit the edge of their box constraints, including f_f . However, it is of note that the exchange time $\tau_{f \rightarrow s}$ did not hit the box constraint edge. Future directions to explore this further would be to sweep and/or fit the extra four parameters, work with the four-pool model described in Section 2.2.4, and incorporate 3-4 pool modeling with the other sources of model mismatch described in this section.

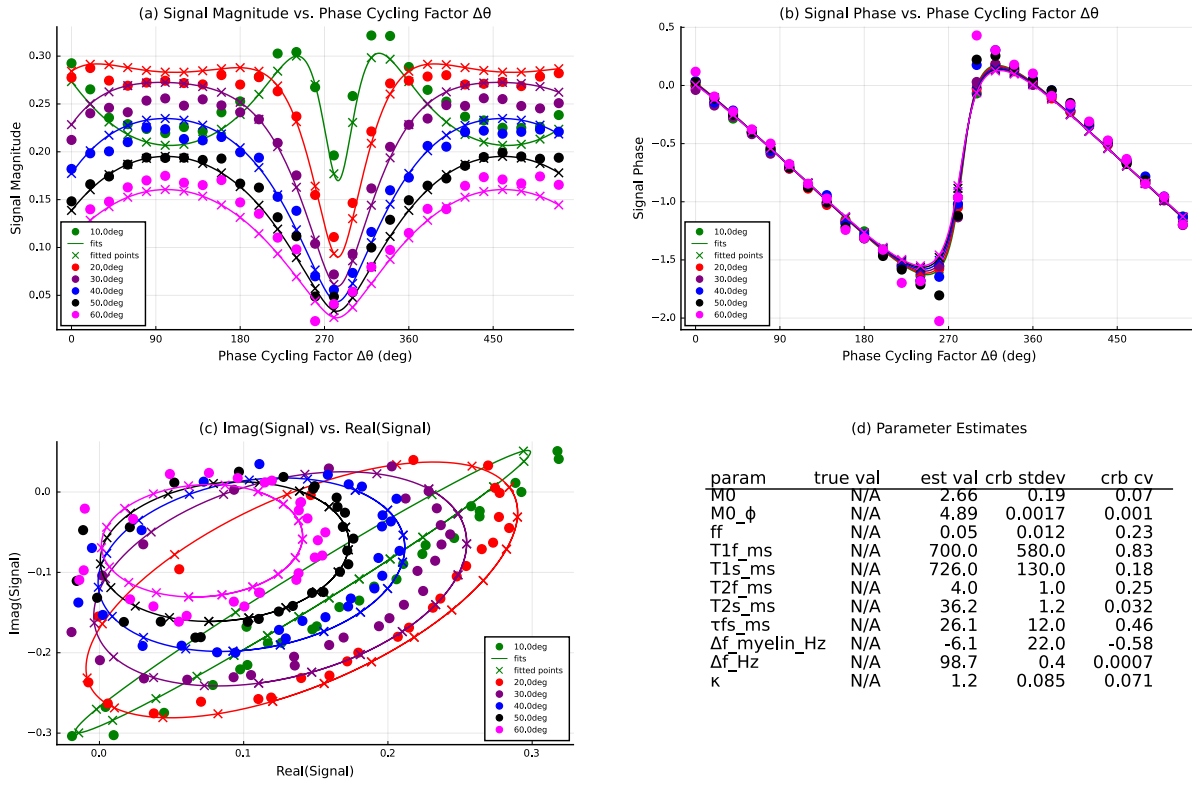


Figure 5.33: Acquired and fitted bSSFP signal vs. phase-cycling factor for six flip angles for an exchanging WM ROI in the tissue sample. The data was fit with a three-pool model with the extra macromolecular pool parameters fixed to typical WM values [14]. The parameters were initialized randomly within box constraints. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values.

5.5 Discussion

Our finding that the simulation empirical standard deviations approach the theoretical CRLB values indicates that 20 trials is likely enough for the NLLS fitting to find the global minimum (in the absence of model mismatch) and that the SNR is high enough and that we have enough scans to estimate the parameters with precision that is reasonably similar to the CRB predictions. The deviations from these results in the experimental findings (as opposed to the simulations), could be due to a number of factors that are described in the following sections.

Our method improves upon mcDESPOT [160] by incorporating a range of phase cycling factors in addition to flip angles into the scan design for the bSSFP scans for estimating the parameters for a two-pool exchanging model. The mcDESPOT method was able to generate reproducible parameter maps, including for exchange; however, its main limitation was that it was not able to generate maps with reasonable precision at feasible SNR values [198]. As indicated by [4] and suggested in [198], we have shown that adding the phase cycling factor as a scan parameter enabled the empirical precision of our estimates for simulated data to approach the CRLB. Despite reasonable precision, we often saw bias in our estimates, especially for f_f in the real WM ROI.

However, our phantom and ex vivo tissue sample experiments involved model mismatch. Other groups have examined the mismatch between parameter values generated by mcDESPOT and more traditional methods, specifically between GRASE-based MWF estimates and mcDESPOT-based f_f estimates [168]. This investigation found that when exchange was not included in the mcDESPOT signal model, the $T_{2,s}$ values were constrained to the lower limit of the box constraint. The study also considered a three-pool mcDESPOT model adding a macromolecular pool, and in this case the f_f estimates were constrained to the lower limit of the box constraint.

Our method differs with mcDESPOT and previous studies [160, 168, 196–199] in a few ways. We primarily focused on bSSFP, but mcDESPOT also includes spoiled gradient recalled echo (SPGR) scans, which is a fast scan primarily used for obtaining T_1 -weighted images. Unlike bSSFP, SPGR scans do not depend on the phase cycling factor parameter. Another difference is that our scan design incorporates many more bSSFP scans than mcDESPOT; we acquired data with a total of 162 acquisitions (18 unique phase cycling factors and 6 flip angles, 108 total unique scans), whereas mcDESPOT incorporated 18 total scans (9 bSSFP and 9 SPGR with different flip angles). Our very large number of scans results in a much lower CRLB than mcDESPOT; however, our incorporation of phase cycling factor into the signal model also results in lower CRLBs and more precise estimates [4]. We densely sampled the phase cycling factor for the purpose of improving the precision of the parameter estimates. Although this increase in the number of scans results in a much longer scan time, we are able to conduct this long scan since we have so far worked with ex vivo tissue samples. When we move to in vivo experiments we will need to lower the

number of scans to decrease the scan time. Optimizing the flip angles and phase cycling factors to maximize the precision of the included scans while limiting the number of scans has been previously investigated [4, 13], and is an important future direction of this work. A drawback of longer scan times is that B_0 drift will begin to come into play; this issue is further discussed in Section 5.4.3.

Another difference between our method and mcDESPOT is that we include the orientation-dependent myelin-specific frequency shift $\Delta\omega_f$ [60] in the signal model, in addition to B_0 off-resonance $\Delta\omega$ and B_1 inhomogeneity κ . mcDESPOT does incorporate $\Delta\omega$ and κ into the signal model, but acquired the data for these scans separately; we instead fit these as two additional parameters. We also acquired separate B_0 and B_1 maps; to compare to mcDESPOT in the future we could fix these parameters to the values estimated from the acquired maps. Our fitting method also differs from that of mcDESPOT. We conducted nonlinear least squares using the BFGS algorithm with a Hager-Zhang line search and automatic differentiation in Julia (`ForwardDiff.jl`) to calculate the gradient of the cost function. mcDESPOT also has a different fitting procedure, detailed in [160]. Likely the differences in these algorithms affect the results.

5.6 Conclusion

We have found that varying both flip angle and phase cycling factor allows for estimation of tissue parameters with reasonable accuracy and precision when there is no model mismatch in simulations. The method estimates parameters from a two-pool exchanging phantom with reasonable precision, but some bias. In WM tissue, the f_f estimate using this method is biased, and incorporating other factors including slice profile effects and the presence of a macromolecular pool will likely improve both f_f and exchange estimates.

CHAPTER 6

Summary and Future Work

This dissertation examined three aspects of using qMRI to study tissue myelin content: the impact of tissue sample preparation on qMRI parameter estimates, the relationship between qMRI and histological parameters in ADRD tissue, and the development of a method that also estimates exchange. The discussion and conclusion sections of Chapters 3-5 offer various future directions for this work. This chapter offers additional ideas for future directions related to this thesis, and some information about potential implementation details

6.1 Balanced SSFP Signal Model

As noted in the previous chapter, there is currently significant model mismatch between the acquired bSSFP data and signal model, even for the non-exchanging two-pool phantom studies. This section explores some of the potential sources of model mismatch that could be incorporated into the signal model in the future. These include slice profile effects, finite RF effects, T_2^* effects, two vs. three pool modeling, and B_0 drift effects.

6.1.1 Slice Profile Effects

An important difference between our acquired bSSFP data and signal model is slice profile effects. Our signal model does not take into account the distribution of flip angles across the slice, but our real data was collected with a 2D slice-selective sinc pulse, which has a slice profile due to an inhomogeneous flip angle across the slice. It has been previously shown that bSSFP data suffers from off-resonance-dependent slice profiles and signal intensity [216]. Using simulated data we investigated how the slice profile affects our model fits and estimated parameters. We simulated data with the parameters listed in Section 5.4.1, but instead of simulating one spin we simulated an array of 200 spins over a 0.5 cm FOV with different z positions. We calculated the effective

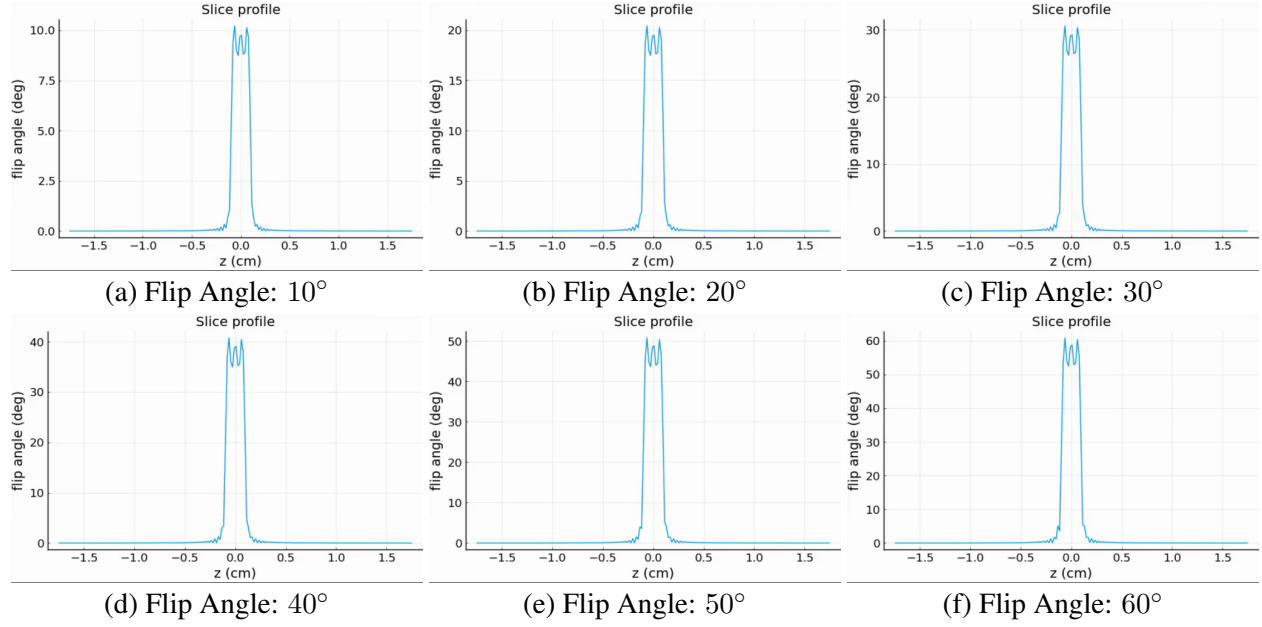


Figure 6.1: Simulated slice profiles used for Figure 6.2.

flip angle seen by each spin using [BlochSim.jl](#) to excite each spin with the RF pulse and slice-select gradient used in the real experiments (3-lobe, 1 ms sinc with $g_{ss} = 6.98$ G/cm, 2 mm slice thickness). Then after fitting the simulated data for each spin in the array, we summed the results to generate the total bSSFP signal over the slice profile. Figure 6.2 shows model mismatch between the simulated and fitted data when slice profile effects are incorporated into the simulated data but not the fitting routine, and the parameter estimates show f_f hitting its box constraint and a very biased $\tau_{f \rightarrow s}$. Figure 6.1 shows the slice profiles used in our simulation. The model mismatch observed in the higher-flip-angle phase plots is consistent with slice-profile effects, which are expected to be more significant at larger flip angles. For example, in Figures 5.29b and 5.30b, there is more model mismatch for the phase plots of the 50° and 60° flip angles. Slice profile effects are likely affecting our real data results and an important future direction would be to incorporate slice profile effects into the signal model. Another option would be to use a longer RF pulse helps to avoid slice profile effects; extra time for more lobes in the pulse's sinc function results in a better slice profile. However, using a longer RF pulse would result in finite RF effects, which are discussed in the following subsection. Another method to avoid slice profile effects is to conduct 3D imaging (a slab-selective pulse with phase-encoding in two dimensions); however, 3D imaging requires longer scan times. Slice profile effects are another direction for future investigation.

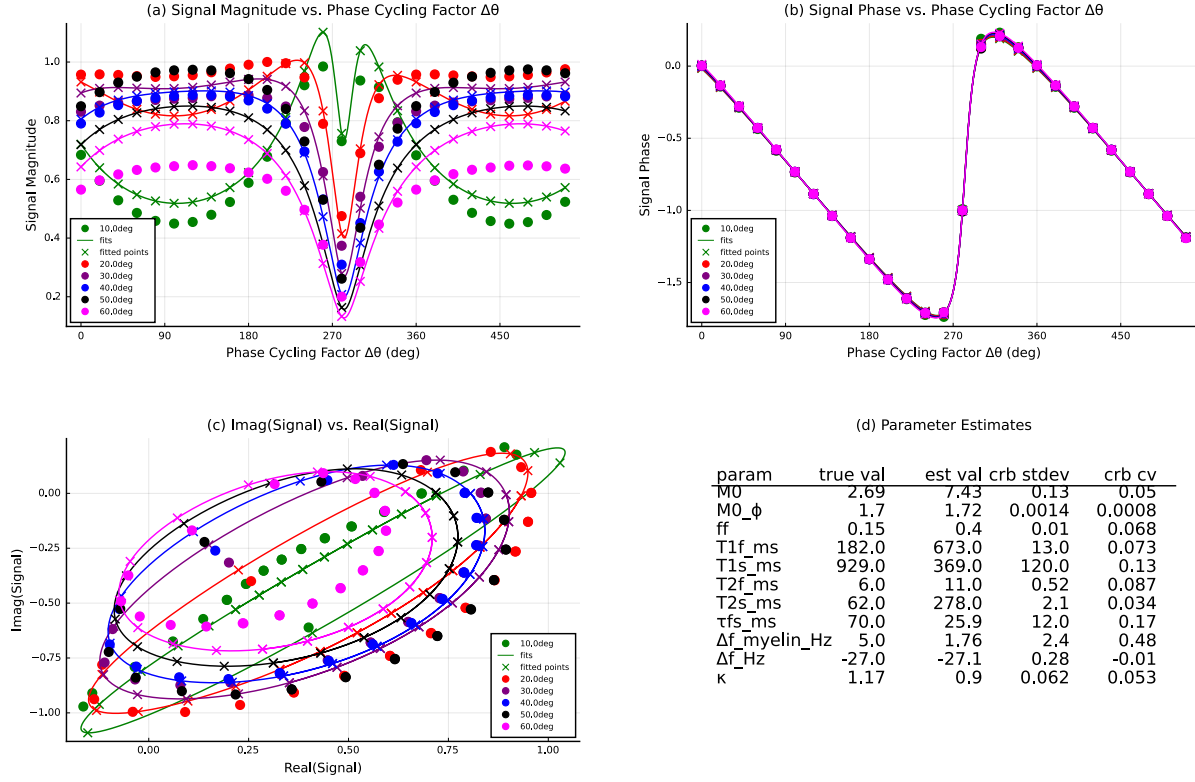


Figure 6.2: Simulated and fitted bSSFP signal vs. phase-cycling factor for six flip angles for a two-compartment exchanging WM voxel. The data was generated with slice profile effects included in the modeling, but fit to a model that does not include slice profile effects. The parameters were initialized randomly within box constraints. (a) Signal magnitude vs. phase-cycling factor. (b) Signal phase vs. phase-cycling factor. (c) Imaginary vs. real parts for the 27 phase-cycling factors. The elliptical shape is asymmetrical due to the two pools. (d) Chart with parameter estimates and the corresponding CRB values. There is severe model mismatch when slice profile effects are included in the simulated data but not the fit. Interestingly, the phase fits well for all flip angles.

6.1.2 Finite RF Effects

Another source of mismatch between our real bSSFP data and fitting model is that we collected WM tissue sample data with a 1 ms sinc RF pulse, but our signal model assumes an instantaneous RF pulse. Balanced SSFP data is known to be sensitive to the duration of the RF pulse [100, 217], and it has been shown that single-pool bSSFP data generated with a 1 ms rectangular RF pulse but fit with a signal model that assumes an instantaneous RF pulse can lead to biases in the estimated values of T_1 , M_0 , κ , and to a lesser extent T_2 ; see [this BlochSim.jl bSSFP RF duration example](#) for a 1-pool simulation-based investigation. Another prior study that ran mcDESPOT with different RF durations on in vivo white and gray matter found that both T_2 values were lower for experiments done using longer RF pulses. However, f_f estimates were not significantly different for the two RF

pulse durations. A different investigation incorporated a finite RF duration into the mcDESPOT signal model and found that the finite pulse model resulted in higher f_f values [218]. Increasing the length of the RF pulse results in less magnetization transfer effects in real MR experiments [199, 201, 219] and also less slice profile effects, but results in these finite RF effects that must be corrected for in the signal model or resulting signal [100]. Thus, the RF pulse duration can affect qMRI parameter estimates and incorporation into the bSSFP two-pool signal model is another avenue for future studies.

6.1.3 T_2^* Effects

Balanced SSFP data is known to have spin echo behavior at $TE = \frac{TR}{2}$ [102] when T_1 and T_2 are much longer than TR. It has been shown that when T_2 is short, the echo appears earlier; see [this BlochSim.jl bSSFP short echo time example](#). Thus, future studies could look at the T_2 dependence of the apparent echo time and choosing a TE that minimizes T_2^* effects or modeling T_2^* .

6.1.4 Two vs. Three Pool Modeling

Section 2.2.4 describes white matter models beyond the two-pool signal model. In addition to a myelin water pool and a free water pool, white matter tissue contains large macromolecules that lead to magnetization transfer effects. Three-pool models add a macromolecular pool with a long T_1 and a short T_2 that exchanges with the myelin water pool, and four-pool models add two macromolecular pools: a myelin pool that exchanges with the myelin water pool and a non-myelin macromolecular pool that exchanges with the non-myelin water pool. The presence of these large macromolecules in the WM tissue may account for model mismatch between the acquired data and signal model. This was explored in Section 5.4.3, where there was a bias in the f_f parameter when data was generated with three pools and fit with two pools. Incorporating 3-4 pool modeling when studying brain white matter is an important future direction.

6.1.5 B_0 Drift Effects

Another source of model mismatch between the simulated data and real data is B_0 drift, as bSSFP sequences are known to be extremely sensitive to B_0 drift [94, 160, 201]. Figure 6.3 shows B_0 maps of a uniform ball phantom generated from 2D gradient echo slice-selective sequence data from multiple TE values at 4, 6, 8, and 10 ms, TR = 100 ms, flip angle = 20°, and matrix size = 128×128 . These data were acquired at the beginning and end of a 30 min scanning session on the 7T scanner; the difference in these two maps in the third panel shows the B_0 field drifted on average by about 3.1 Hz. Figure 6.4 shows the bSSFP signal profile for one voxel in the uniform

ball phantom acquired at 10 different times, shown in the legend. The plot illustrates B_0 drift as the signal profile moved along the phase cycling factor axis with time. Future studies include updating the signal model to incorporate a time-varying B_0 field and implementing a method to correct for B_0 drift.

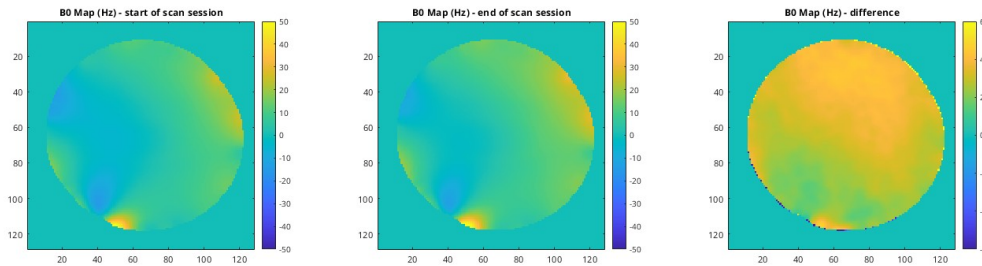


Figure 6.3: B_0 maps of a uniform ball phantom before and after scanning session, about 30 min apart, and difference image between the two maps. The B_0 field drifted on average by about 3.1 Hz.

6.1.6 Other Future Directions

Some other avenues for exploration for estimating myelin water exchange with bSSFP include varying TR to offer another avenue for tissue contrast. TR was previously held fixed since prior work found that optimizing it did not affect the precision of exchange measurements [4, 13]. Other future direction could be to explore multi-TR steady-state sequences such as OSSI [220] and multi-echo bSSFP methods [221] for exchange mapping.

We chose NLLS as our parameter estimation method; however, we could explore other method such as parameter estimation via regression with kernels (PERK) or neural network-based estimation. PERK [193, 205] is a fast, dictionary-free framework for MRI parameter estimation that learns nonlinear relationships between parameters of interest and training data obtained through Bloch-McConnell simulations with the bSSFP signal model. PERK lifts the inputs to a higher-dimensional space to capture the nonlinear dependence of the signal on the desired unknown parameters. Once trained, the resulting algorithm quickly estimates the underlying parameters from new data. PERK scales relatively well with an increasing number of estimated parameters, and thus is a good candidate for MWF mapping using a two-compartment model [14]. PERK could be beneficial over nonlinear least squares (NLLS) since NLLS requires iterative methods that can get stuck in local minima, which is why we chose the estimates corresponding to the trial with the lowest cost function value after multiple random initialization. Also, the NLLS optimization can be slow, so PERK's ability to train quickly may provide an advantage over NLLS.

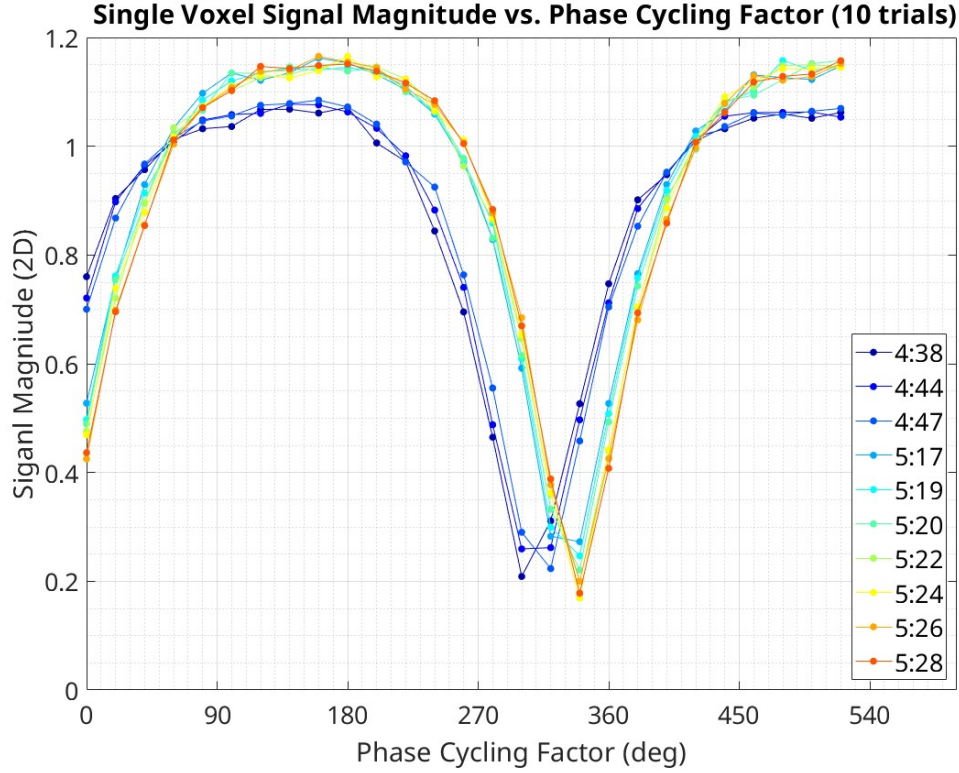


Figure 6.4: Signal Magnitude of one voxel in a 2D bSSFP sequence vs. phase cycling factor. With increasing time the signal profile moves along the phase cycling factor axis, illustrating scanner drift, as the phase cycling factor is incorporated into the off-resonance term in the signal model. The big jump between trials 1-3 and 4-10 is due to a longer time gap between scans (about 30 minutes).

Parameter estimation could also be conducted with a fully connected or convolutional neural network, drawing training data from Bloch-McConnell simulations with the bSSFP signal model similarly to PERK. We could also use a Bayesian neural network if we decide to quantify uncertainty in parameter estimates or an unrolled neural network, which mimics iterative optimization algorithms [222].

Another avenue for future studies is sampling k-space data during both the transient and steady-state periods and refining the optimization scheme to incorporate data collected during both regimes [43, 223]. We could also solely consider the transient response, instead of waiting for steady-state to be reached [224]. Exchange has been estimated during the transient regime of bSSFP previously [170, 182] and is an important direction to explore.

6.2 Cramér-Rao Lower Bound Scan Design Optimization

One future direction is to optimize the scan parameters α and $\Delta\theta$ instead of densely sampling them. The Cramér-Rao Lower Bound (CRLB) has been used to optimize MR sequence parameters for various pulse sequences and applications [206, 225–227]. MRI data for D scans is expressed as a function of known, unknown, and scan parameters: $\mathbf{y} = \mathbf{f}(\mathbf{x}, \boldsymbol{\nu}, \mathbf{P}) + \boldsymbol{\varepsilon}$, where $\boldsymbol{\nu}$ represents the known, $\mathbf{x}$ represents the unknown, $\mathbf{P}$ represents the scan parameters, $\boldsymbol{\varepsilon}$ is additive complex Gaussian noise. A future direction would be to minimize the expected weighted sum of the CRLB for each unknown parameter using a trace operator [14, 205]: $\hat{\mathbf{P}} = \operatorname{argmin}_{\mathbf{P} \in \mathcal{P}} \mathbb{E}_{\mathbf{x}, \boldsymbol{\nu}}[\operatorname{trace}(\mathbf{W}(\mathbf{I}(\mathbf{x}, \boldsymbol{\nu}, \mathbf{P}))^{-1})]$, where $\mathcal{P}$ denotes the scan parameter search space, $\mathbb{E}_{\mathbf{x}, \boldsymbol{\nu}}$ denotes an expectation over $\mathbf{x}$ and $\boldsymbol{\nu}$, and $\mathbf{W}$ is a diagonal weighting matrix used to indicate the relative importance of each unknown parameter. The expectation would be taken with respect to $\mathbf{x}$ and $\boldsymbol{\nu}$ because these parameters are represented by distributions with values that the voxels in the sample could have.

In our group’s prior work, the matrix $\mathbf{W}$ placed full weight on the CRLB for the MWF (fast fraction) parameter f_f to focus on estimating the MWF by setting the diagonal entries of $\mathbf{W}$ all to 0 except for 1 in the location corresponding to f_f [14]. Another study from our group placed full weighting on $\tau_{f \rightarrow s}$, the residence time for exchange from myelin water to non-myelin water [13]. We could generate a set of bSSFP [195] scans that optimize both the flip angles α (between 1° and 70°) and the RF phase cycling factors θ (between -180° and 180°). We could minimize the weighted sum of the CRLB for each parameter using the expected trace where we will set the elements of the weighting matrix $\mathbf{W}$ for f_f and for $\tau_{f \rightarrow s}$ to the reciprocals of their means. This weighting will ensure that the optimized flip angles and phase cycling factors will be sensitive to both MWF and exchange. If we use 40 scans as in [13], a TR of 5 ms (TE 2.5 ms), a matrix size of 128×128 , 5 fully sampled slices as in Aim 1, and add 1 sec for each scan to account for the transient time, the 2D bSSFP scan would be $128 \times 5 \times 40 \times 0.005 \text{ sec} + 40 \text{ sec} = 3 \text{ min}$, $5 \times$ faster than the MESE scan.

The scan design involves an expectation over unknown parameters $\mathbf{x}$ and known parameters $\boldsymbol{\nu}$, and the estimation step requires prior knowledge of possible ranges of these parameters. Prior works [13, 14] calculate this expectation over a range of tissue parameters consistent with literature values for WM [14, 25, 60, 194, 228]. We could work with pathological tissues with different parameter distributions than healthy tissue, so we would inform the choice of parameter distributions with the values estimated from scanning the MADRC samples in Chapter 4.

6.3 In Vivo Experiments

We want our proposed bSSFP method to be clinically translatable in the future. As a step toward this goal, a future direction would be to scan human subjects with our optimized bSSFP scans on a 3T human scanner to adapt our method toward a more clinically relevant setting. We would aim to make our MWF/exchange mapping method as fast as possible while maintaining acceptable precision and without sacrificing accuracy significantly compared to a fully sampled protocol.

6.3.1 Data Acquisition and Reconstruction

All subjects would be scanned on a 3T GE Discovery MR750. To optimize SNR and scan efficiency, this bSSFP method could be implemented as an undersampled 3D imaging protocol. The subjects could be scanned with the proposed optimized bSSFP sequence and a reference MESE scan using a Work in Progress (WIP) 3D GRASE pulse sequence developed by GE in MESE mode [58, 184]. For both whole-brain data would be acquired for 1.6 mm cubic voxels and a matrix size of $150 \times 150 \times 84$, incorporating undersampling along both k_y and k_z using our 32-ch Nova receive array. CAIPIRINHA undersampling [229] could be used and implemented with Pulseseq [230] with an acceleration factor of 6 ($R = 3 \times 2$), an echo planar (EPI) readout echo train length of 3 [184], and different random k -space undersampling patterns. For the reference GRASE scan if a TR of 1 sec is used and TE of 10 ms is used, then the total scan time would be approximately $(150 \times 84 \times 1 \text{ sec}) / (6 \times 3) = 12 \text{ min}$. For the bSSFP scan a TR of 5 ms, TE of 2.5 ms, and 40 scans with different flip angles/phase cycling factors could be used [13]. There could be an extra undersampling factor of 3 from jointly reconstructing the 40 images with a compressed sensing, low-rank regularization method that exploits the multiple contrasts from the 40 scans using subspace constraints. [231]. The total scan time for all 40 3D scans, including 1 sec for each scan to account for the transient time would be approximately $((150 \times 84 \times 0.005 \text{ sec} \times 40) / (6 \times 3)) + 40 \text{ sec} = 3 \text{ min}$, a $4 \times$ shorter scan than the 12 min GRASE scan. In addition to the undersampled scans, two repeated fully sampled 3D bSSFP scans could be conducted for each subject for a thin slab (5 slices, each 1.6 mm) that is $(150 \times 5 \times 0.005 \text{ sec} \times 40) + 40 \text{ sec} = 3 \text{ min}$. Altogether, repeating each scan twice, the scan time would be about 36 min. The fully sampled bSSFP data would define the ground truth for evaluating the potential bias of our undersampling and reconstruction strategies. Then we could ask the subject to leave/reenter the scanner and scan them again for a total 1 hour 12 min to evaluate test-retest reproducibility.

6.3.2 Evaluation and Statistical Analysis

To evaluate the method’s precision, we would calculate the intra-subject CV for both MWF and MRT across the repeated scans before and after the subject leaves the scanner. We would also compare the bSSFP fully sampled slab data to the undersampled version, starting with 40 scans and then varying the number of scans D . D may need to be increased to account for the lower SNR. Another step is to compare the MWF estimates to literature values for various WM ROIs [232], including variations along the corpus callosum; this would allow us to judge the measure sensitivity. We expect the MWF and MRT to vary with a similar trend to how the g-ratio, myelin thickness, and myelin volume fraction vary along WM tracts [73, 74]. This will be especially helpful for MRT since it currently cannot be validated in vivo.

6.3.3 Other Directions

Although we can conduct whole-brain GRASE scans on these subjects to estimate MWF, due to REXSY’s prohibitively long scan times, we would be unable to get in vivo REXSY-MWF or REXSY-MRT values. Therefore, we do not have a reference method for MRT calculation. Also, compared to 7T scanners, 3T scanners come with certain challenges (lower SNR) but also opportunities for further speedup (availability of 32-ch receive array). For further acceleration for the bSSFP scans, we may use segmented EPI k-space readout. A different direction would be to use PERK for parameter estimation, extrapolating the field strength-dependent parameters (e.g., T_1) based on known conversions [35]. We could also use an end-to-end optimization approach that includes a bias and variance term in the cost function as done in prior work [233]. Another option could be to use an unrolled neural network for the parameter estimation, which mimics iterative optimization algorithms [222]. Also, we expect fluctuations in B_0 inhomogeneity to be even more of a challenge in vivo due to breathing/motion, we could explore building in robustness to B_0 inhomogeneity fluctuations/drift into our signal model.

6.4 White Matter Lesion Classification

One possible extension of Chapter 4 could be to train a neural network to take qMRI ROI data as inputs and classify these data as normal appearing or lesional white matter using histology samples as the ground truth to label the images. This task is complicated by the fact that lots of paired data are needed and we have access to a limited amount of histology images. Methods exist to generate virtual histology images from MR images [167], which could be used to enhance the number of images in our dataset for this task.

APPENDIX A

Supporting Information for Chapter 3

This appendix provides information relevant to Chapter 3. Section A.1 offers background information about the off-resonance RF saturation MT signal model and Section A.2 provides details about the selective inversion recovery MT signal model. Section A.3 compares saturation inversion recovery results with a hyperbolic secant pulse vs. a Gaussian inversion pulse. Section A.4 shows MTsat maps, which is another myelin-sensitive parameter.

A.1 Off-Resonance RF Saturation MT Theory

Below outlines some extra information about the off-resonance RF saturation magnetization transfer (MT) signal model that was fit [7, 17, 124]. The water proton magnetization under steady-state RF saturation in a system with a free water proton pool M_f and macromolecular proton pool M_m is given by

$$\frac{M_{\text{sat},f}}{M_{0,f}} = \frac{R(R_{1,f} + FR_{1,m}) + R_{1,f}(R_{1,m} + R_{\text{RF},m})}{(R_{1,f} + R_{\text{RF},f})(R_{1,m} + R_{\text{RF},m}) + R(R_{1,f} + R_{\text{RF},f} + F(R_{1,m} + R_{\text{RF},m}))} \quad (\text{A.1})$$

where $\frac{M_{\text{sat},f}}{M_{0,f}}$ is the ratio of the free water pool magnetization with and without RF saturation, $R_{1,f}$ and $R_{1,m}$ are the rates of free water and macromolecular proton longitudinal relaxation, R is the cross-relaxation rate, F is the ratio of macromolecular protons to water protons, and $R_{\text{RF},f}$ and $R_{\text{RF},m}$ are the rates of RF saturation of the free and macromolecular proton pools given by:

$$R_{\text{RF},p}(\omega_1, \Delta) = \omega_1^2 g_p(\Delta) \quad (\text{A.2})$$

where $\omega_1 = \gamma B_1$ is the RMS amplitude of the applied saturation RF, Δ is the applied RF frequency in Hz, and $g_p(\Delta)$ is the normalized line shape for pool p ($p=f$ or $p=m$). The free water pool is given

by a Lorentzian function:

$$g_f(2\pi\Delta) = \frac{T_{2,f}}{1 + (2\pi\Delta T_{2,f})^2} \quad (\text{A.3})$$

and the macromolecular pool is given by a super-Lorentzian function [125–127]:

$$g_m(2\pi\Delta) = \pi \sqrt{\frac{2}{\pi}} T_{2,m} \int_0^1 \frac{1}{|3u^2 - 1|} \exp \left(-2 \left(\frac{2\pi(\Delta - \Delta_{cs,m}) T_{2,m}}{3u^2 - 1} \right)^2 \right) du \quad (\text{A.4})$$

where $T_{2,f}$ and $T_{2,m}$ are the water proton and macromolecular proton T_2 values and $\Delta_{cs,m}$ is the chemical shift in Hz between the water and macromolecular protons.

The parameters R , $T_{2,m}$, F (referred to as F_{sat} in the main text), and $\Delta_{cs,m}$ were fit on a voxel-by-voxel basis. $R_{1,f}$ was set to 0.33 s^{-1} [35], $R_{1,m}$ was set to 1 s^{-1} [7, 35, 128], and $T_{2,f}$ was set to a weighted average of the MWI T_2 spectrum data for all T_2 values beyond the 20 ms cutoff as discussed in the manuscript. Δ and ω_1 were adjusted by B_0 and B_1 mapping on a voxel-by-voxel basis.

A.2 Selective Inversion Recovery

We model the bi-exponential behavior of the inversion recovery T_1 mapping data as:

$$\frac{M_f(t)}{M_{f\infty}} = a_{\text{fast}}(1 - 2 \exp(-R_1^+ t)) + a_{\text{slow}}(1 - 2 \exp(-R_1^- t)) \quad (\text{A.5})$$

where $M_f(t)$ is the longitudinal magnetization of the mobile protons at time t , $M_{f\infty}$ is its equilibrium value, R_1^- is the slow recovery rate, R_1^+ is the fast recovery rate, and a_{slow} and a_{fast} are their corresponding amplitudes ($a_{\text{slow}} + a_{\text{fast}} = 1$). We calculate a short T_1 fraction map (short T_1 F) from the fitted amplitudes as: $\frac{a_{\text{fast}}}{a_{\text{fast}} + a_{\text{slow}}} = a_{\text{fast}}$ [64].

Setting $b_f^+ = -2 a_{\text{fast}}$ and $b_f^- = -2 a_{\text{slow}}$, (A.5) can be rewritten in the form of Equation 3 in Gochberg and Gore's paper [33]:

$$\frac{M_f(t)}{M_{f\infty}} = b_f^+ \exp(-R_1^+ t) + b_f^- \exp(-R_1^- t) + 1 \quad (\text{A.6})$$

The ratio of macromolecular to free water protons can be calculated using the formulation in Gochberg and Gore's paper [33]. We rewrite Equation 6 from Gochberg and Gore's paper using our paper's notation:

$$-2 a_{\text{fast}} = \left(\frac{M_f(0)}{M_{f\infty}} - \frac{M_m(0)}{M_{m\infty}} \right) \left(\frac{p_m}{p_f} \right) \quad (\text{A.7})$$

where $M_m(t)$ is the longitudinal magnetization of the macromolecular protons at time t , $M_{m\infty}$ is its equilibrium value, and p_m and p_f are the sizes of the macromolecular and free water proton pools. Solving for the ratio of macromolecular to free water protons $\frac{p_m}{p_f}$ (referred to as F_{SIR} in the main text and as the pool size ratio (PSR) by Gochberg and Gore), we get:

$$F_{\text{SIR}} = \frac{-2 a_{\text{fast}}}{\left(\frac{M_f(0)}{M_{f\infty}} - \frac{M_m(0)}{M_{m\infty}} \right)} \quad (\text{A.8})$$

It follows from (A.6) that the ratio $\frac{M_f(0)}{M_{f\infty}} = -1$. The hyperbolic secant pulse nearly saturates the macromolecular pool, which makes the ratio $\frac{M_m(0)}{M_{m\infty}} \approx 0$. Under these conditions/ approximations,

$$F_{\text{SIRsech}} \approx 2 a_{\text{fast}} \quad (\text{A.9})$$

Using a Gaussian lineshape and T_2 between 10-20 μs for the macromolecular pool would cause $\frac{M_m(0)}{M_{m\infty}}$ to be ≈ 0.88 according to [33,61]. Under these conditions/approximations,

$$F_{\text{SIRgauss}} \approx \frac{2 a_{\text{fast}}}{1.88} \approx 1.06 a_{\text{fast}} \quad (\text{A.10})$$

Gochberg and Gore also state that in the case where the macromolecular pool was saturated by the inversion pulse, the PSR values would increase by a factor of ~ 1.91 [33,61]. Thus,

$$F_{\text{SIRsech}} \approx 1.91 F_{\text{SIRgauss}} \approx 1.91(1.06 a_{\text{fast}}) \approx 2.02 a_{\text{fast}} , \quad (\text{A.11})$$

which is consistent with (A.9).

A.3 Comparison of Hyperbolic Secant vs. Gaussian Inversion Pulses

Our inversion recovery experiments utilized a high power (51 μT) hyperbolic secant pulse [16] that nearly saturated the macromolecular pool, reducing the fast relaxing component amplitude. Based on the calculations in Section A.2, in this case F_{SIR} would be approximately $2\times$ the fast relaxing component amplitude.

We scanned one thawed sample with two inversion recovery sequences, one with a 51 μT , 4000 μs hyperbolic secant pulse and another with a 7.3 μT , 4000 μs Gaussian pulse and show the results below. The T_1 long and T_1 short maps do not significantly change depending on the choice of pulse, but their corresponding amplitude maps do. The T_1 short amplitude map (a_{fast}) for the Gaussian pulse is about $2\times$ that of the hyperbolic secant pulse. Their corresponding F_{SIR} maps are consistent with one another and overall with literature values [11, 34, 62, 132], suggesting that the type of pulse used affects the F_{SIR} parameter less than it affects the short amplitude.

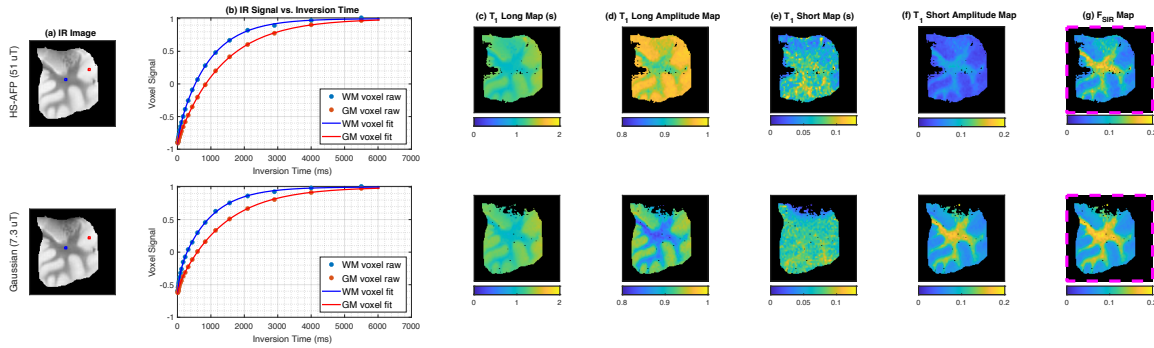


Figure A.1: Comparison of hyperbolic secant and Gaussian inversion pulses. (a) IR images (TI = 10 ms) for data collected with the the hyperbolic secant (HS) adiabatic full passage (AFP) inversion pulse [16] and Gaussian inversion pulse. (b) Observed and fitted signal recovery curves vs. inversion time for one WM voxel and one GM voxel in the ROI. (c) Map of the estimated long T_1 value for each voxel. (d) Normalized corresponding signal amplitude for the long T_1 component (a_{slow}) for each voxel. (e) Map of the estimated short T_1 value for each voxel. (f) Normalized corresponding signal amplitude for the short T_1 component (a_{fast}) for each voxel. (g) F_{SIR} map.

A.4 Additional Myelin-Sensitive Parameter: MTsat

MTsat is another parameter that is sensitive to myelin content with a reduced dependence on T_1 [133–139]. These data were added to the supporting information section rather than the main body because to generate these maps we had to generate T_1 weighted (T1w) images through simulation since we did not collect this data.

MTsat maps were calculated using Equations 7-8 from Helms et al [133]. The MT weighted (MTw) images acquired at 10 kHz off-resonance and 8.8 μ T were used, and the corresponding images acquired at 60 kHz off-resonance were used as the proton density weighted (PDw) images. Apparent R_1 (R_{1app}) maps were set to $\frac{1}{T_{1Long}}$ estimated in the bi-exponential T_1 fitting procedure, and A_{app} maps were calculated using Equation 7b from Helms et al [133]. Measured B_1 maps were incorporated according to Equation 5 in Rahman et al [134]. T1w images of each sample were simulated using TR = 10 ms, flip angle = 50°, our T_1 long map, and a proton density map simulated from the PDw image, its TR and flip angle, and our T_1 long map. Figure A.2 shows MTsat and MTR maps for comparison for two representative samples. Figure A.3 plots MTR and MTsat vs. LFB ROI values to examine their correlation.

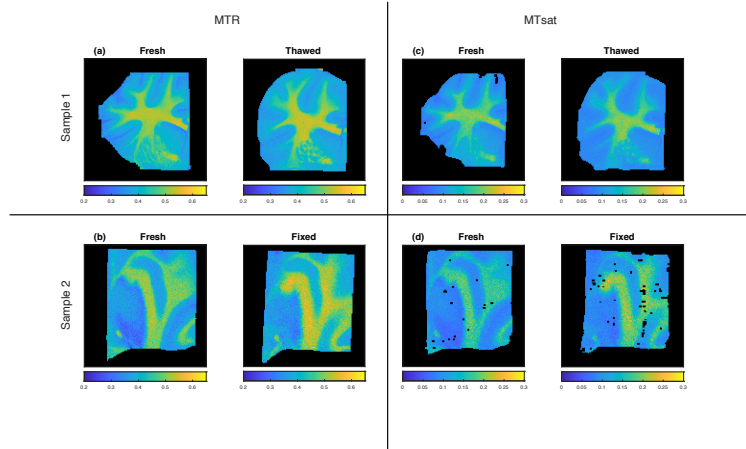


Figure A.2: MTR and MTsat maps for two representative samples including the fresh/thawed and fresh/fixed versions of each sample. (a) MTR maps from MT contrast images at average of -10kHz and 10kHz off-resonance and 8.8 μ T for fresh/thawed sample. (b) MTR maps for fresh/fixed sample. (c) MTsat maps generated as described above for fresh/thawed sample. (d) MTsat maps for fresh/fixed sample.

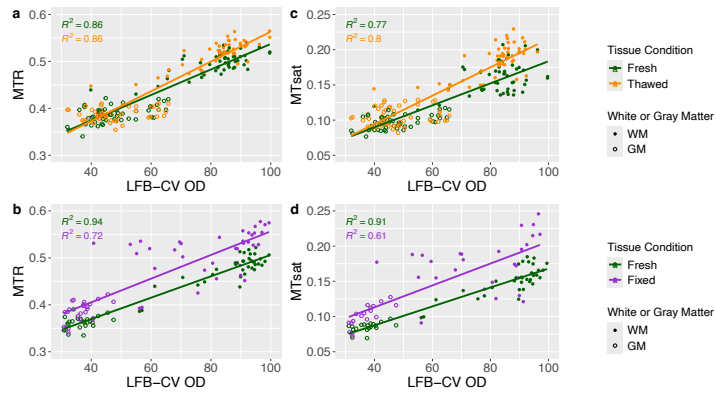


Figure A.3: Correlation between MTR and MTsat parameters and histology LFB-CV mean-adjusted values across tissue samples for fresh and thawed (Row 1) and fresh and fixed (Row 2) samples. Both MTR and MTsat show strong correlation with LFB-CV OD for all ROIs as indicated by the high R^2 values. MTsat exhibits a similar trend to the plots in Figure 3.9 in the main text based on the mixed-model analysis described in the methods section 3.2.5. (a-b) exhibit a statistically significant ($p < 0.05$) difference in slopes between the fresh/thawed fits (a: $p = 0.022$, b: $p = 0.0039$) and (c-d) exhibit a statistically significant ($p < 0.05$) difference in intercepts between the fresh/fixed fits (c: $p = 0.033$, d: $p = 0.028$).

APPENDIX B

Supporting Information for Chapter 4

This appendix provides additional information relevant to Chapter 4. It provides additional MR and histology images, ROI analysis, and correlation analysis results of a sample with an extra pathological feature that was not present in the previous sample: white matter lesions with dysmorphic myelin and axons.

B.1 Additional Results in Pathological Sample

Figure B.1 shows qMRI maps and histology images for one slice of human tissue sample 1131-S3A. The histology images include LFB-CV, NF, and OLIG2; we did not have MBP data for this sample so although less strictly quantitative than MBP data, LFB-CV is taken as the histological indicator of myelin content. This sample has different histopathological features from the previous samples, and its ROIs have been placed into four groups:

- Group A: Normal-Appearing White Matter (NAWM)
- Group B: White Matter Lesion with Pure Demyelination
- Group C: White Matter Lesion with Dysmorphic Myelin/Axons
- Gray Matter

Group A, located around the gray matter regions (juxtacortical), represents normal-appearing white matter, and shows robust staining with LFB, a high density of oligodendrocytes, and long fibers that stain with NF. Group B is the most similar to the white matter lesion groups of Figures 4.1-4.7, which shows a reduction in LFB staining, reduced OC, and NF staining, as compared to Group A. The morphology of fibers as seen in the NF and LFB, are similar to Group A, and has a lacy appearance in both groups. Group C, however, has a distinct histopathological feature that can be seen in images B.2-B.6. The LFB stain looks darker in the Group C areas of B.4 In Group C of the

LFB zoomed-in images in Figure B.4, the myelin looks darker. The zoomed-in view in Figure B.4 shows clumped LFB in these areas, as opposed to the more lacy appearance of Groups A-B. The neurofilament also appears dysmorphic in Group C in Figure B.5, where there are clumped fibers rather than long fibers in Group A. The term dysmorphic used here does not necessarily imply a pathological process, but simply refers to the different appearance of the fibers in this region as compared to Group A and B, which could represent either anatomical or pathological variations. Additionally, Group C has fewer oligodendrocytes per unit area in Figure B.6 compared to Groups A-B (more similar to the GM oligodendrocyte count).

For this sample, 80 ROIs (20 of each of the 4 categories) of size $0.5 \text{ mm} \times 0.5 \text{ mm}$ were labeled and registered between the FSE MR image and the three histology images. Box plots in Figure B.7, similar to those in Figure 4.4, show the values in the ROIs. The oligodendrocyte count plots show the expected trend, as there are the most in NAWM, fewer in WML with demyelination, fewer in WML with dystrophic myelin and axons, and then even fewer in the GM. The NF values are fairly consistent across the three groups of WM and the GM ROIs, with a greater range of NF values in the GM. The LFB-CV values show the expected trends in the NAWM, demyelinated lesions, and GM, with higher LFB in the healthier WM and lower LFB in the lesions and GM. However, an interesting result here is that the MWLs with dystrophic myelin and axons have higher LFB values than may be expected. Even though the axons are more sparse and have a globuloid appearance, as can be seen in the NF histology image, the myelin is still present, though with a similar appearance as in the NF figure. The MTR and F_{sat} plots mirror the LFB results. One interpretation is that the morphology of the myelin/axons allows for water to have more contact with the solid component of myelin, which increases the MT effect. This also explains the high MWF in Group C. However, a peculiar result is that in the MWF plot, the MWF values in the NAWM are consistently lower than in the typical Group B lesions, which have demyelination without complete destruction of the tissue. This does not follow the expected trend from the previous results.

These observations are further exemplified by the MR vs. histology plots in Figure B.8. As with the box plots, the MTR and F_{sat} vs. LFB plots follow the expected positive correlation. However, the MWF vs. LFB plot does not show the expected trend, as the Group B WMLs have higher MWF than expected. The MTR and F_{sat} plots follow the expected trend for Groups A and B with positive correlation between MR and histology; the MWF plot shows the opposite trend as expected. Interestingly, the NF plots have a significant positive correlation with all qMRI parameters, and the OC plots have a significant negative correlation due to low OC counts in Group C, but high qMRI parameters. Figure B.9 shows that the exclusion of Group C causes the NF plots to have much smaller correlations and flips the direction of correlation for the MTR and F_{sat} vs. OC plots. However, MWF vs. LFB and OC plots still have negative slopes, which contrasts with prior results that only have Groups A and B (such as for Samples 2468-S3 and 2468-S4).

The qMRI measures seems to be affected by morphological changes in the fibers such as orientation or shape (globular). Group C deviates from expected results, but also has different pathological features than previous studies [8]. The negative correlation between LFB and MWF excluding the Group C ROIs does deviate from literature and prior results [44, 47].

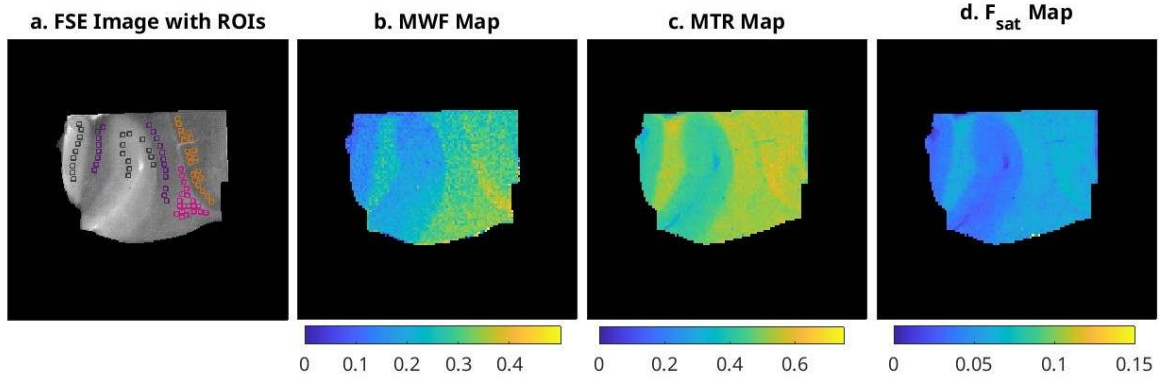
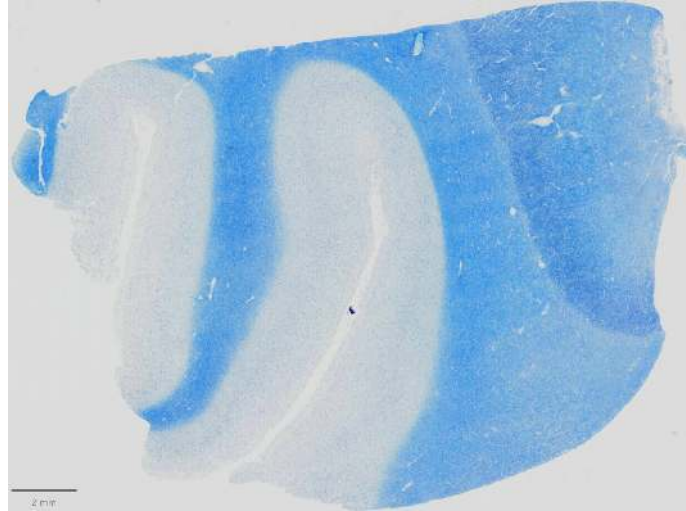


Figure B.1: qMRI maps for pathological human brain tissue sample 1131-S3A. (a) Fast spin echo image ($TR=4s$, $TE_{eff}=40ms$, $ESP=10ms$, segments/ETL/kzero = 32/8/4) with ROIs labeled; purple = NAWM, pink = WM lesion with demyelination, orange = WM lesion with axonal loss, gray = GM. (b-d) qMRI parameter maps. (b) MWF map calculated with a 20 ms cutoff. (c) MTR maps from MT contrast images at average of -10kHz and 10kHz off-resonance and $8.8 \mu T$ power level. For this sample, data was not acquired at exactly -10kHz and 10kHz off-resonance. The data acquired at multiple off-resonance frequencies and power levels was fit with the binary spin-bath model [7, 17] (for estimating F_{sat}), and this fit was used to extrapolate the MT signal at -10kHz and 10kHz off-resonance for the calculation of MTR. (d) F_{sat} map.



(a) LFB-CV image of Sample 1131-S3A



(b) NF image of Sample 1131-S3A



(c) OLIG2 image of Sample 1131-S3A

Figure B.2: LFB-CV images of four ROIs in Sample 1131-S3A (scale bar 2 mm).

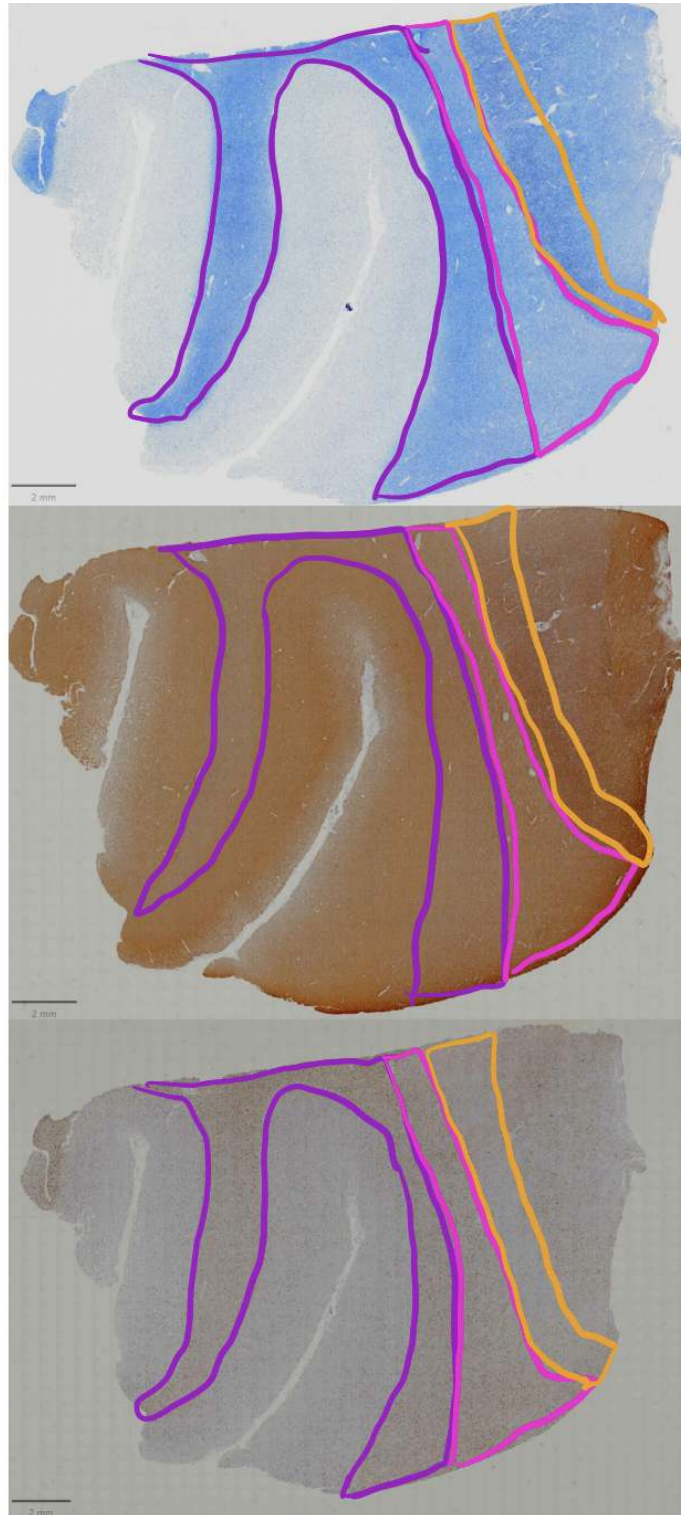
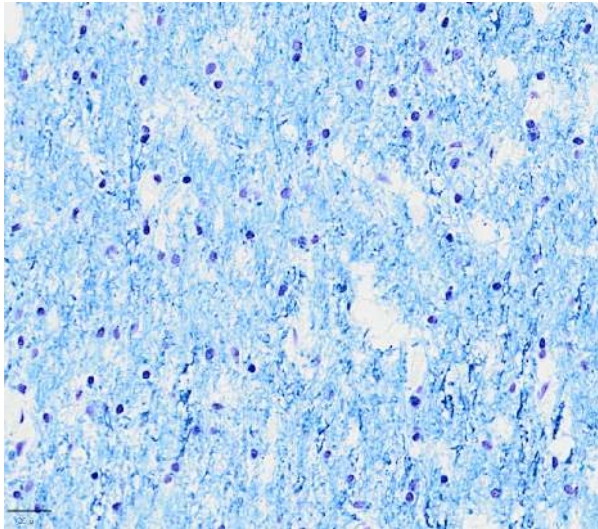
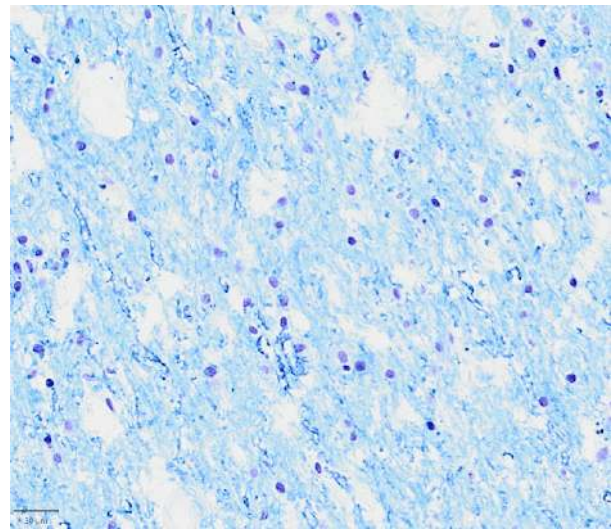


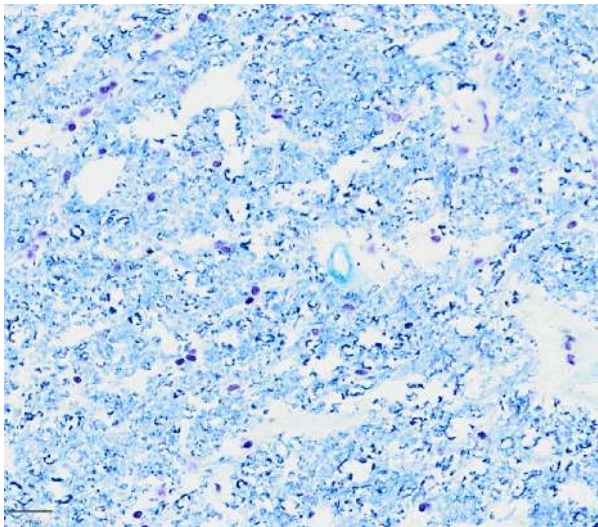
Figure B.3: LFB-CV, NF, and OLIG2 images of Sample 1131-S3A with Groups A-C ROIs labeled (Group A = purple, Group B = pink, Group C = orange) (scale bar 2 mm).



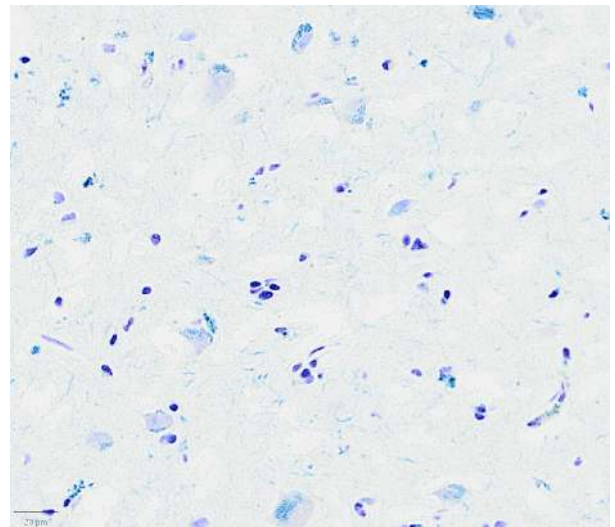
(a) Group A: Normal Appearing White Matter



(b) Group B: WML, Demyelination

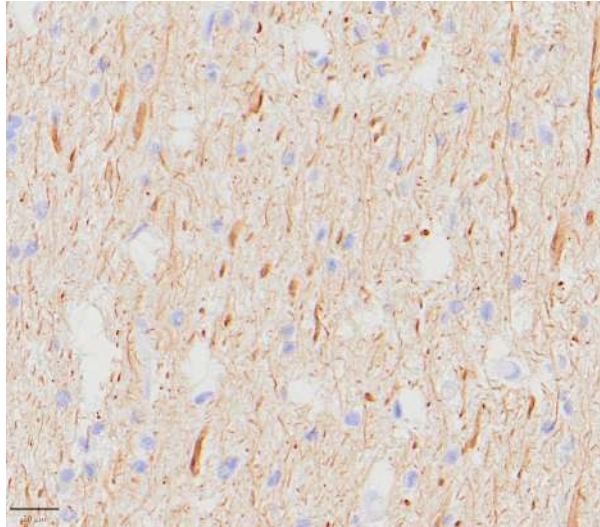


(c) Group C: WML, Dysmorphic Myelin/Axons

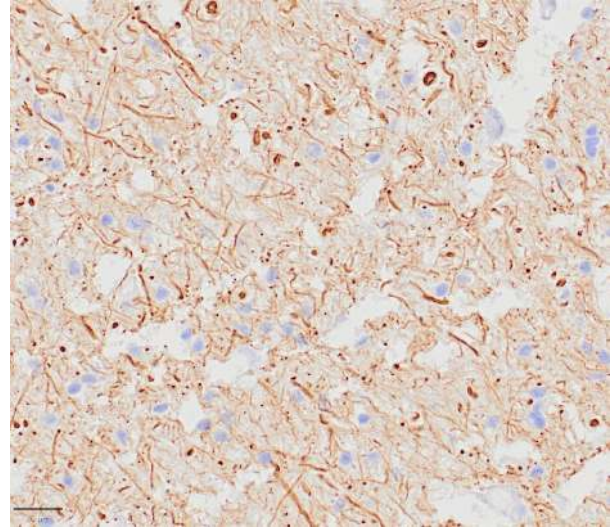


(d) Gray Matter

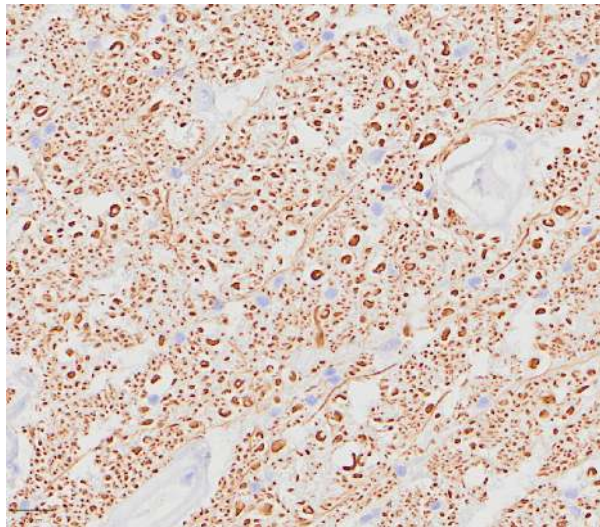
Figure B.4: LFB-CV images of four ROIs in Sample 1131-S3A (scale bar 20 μm). The NAWM in Group A is around the GM region (juxtacortical) and its LFB staining is robust with lacy appearance. The LFB staining for Group B is reduced compared to Group A. There is a similar lacy appearance of the fiber morphology. The Group C LFB stain is darker and more clumped.



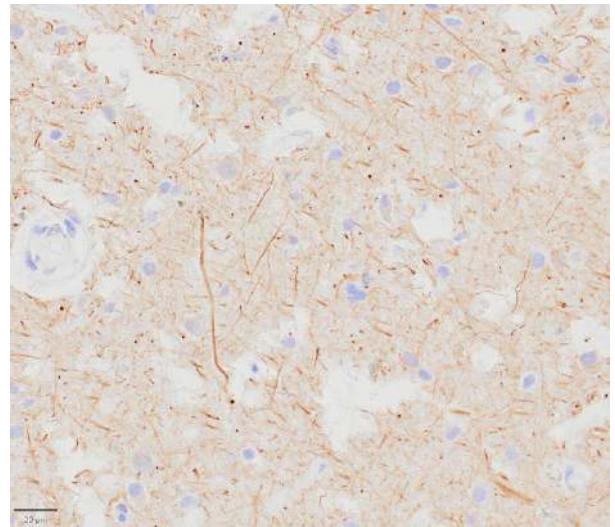
(a) Group A: Normal Appearing White Matter



(b) Group B: WML with Demyelination

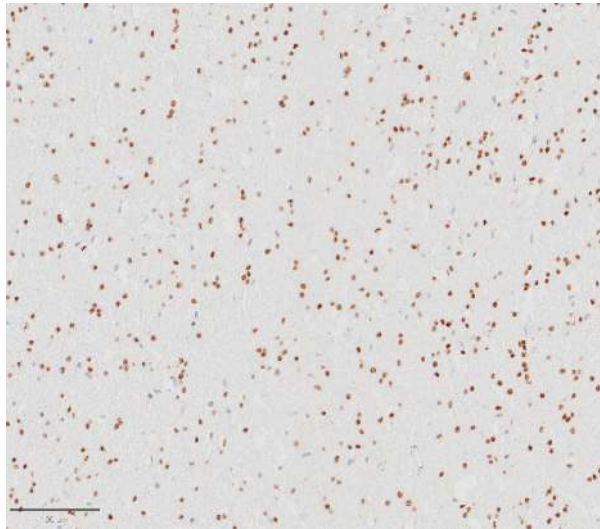


(c) Group C: WML with Axonal Destruction

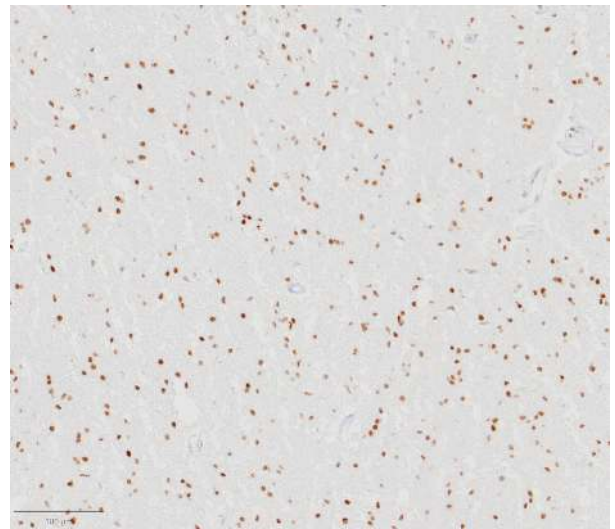


(d) Gray Matter

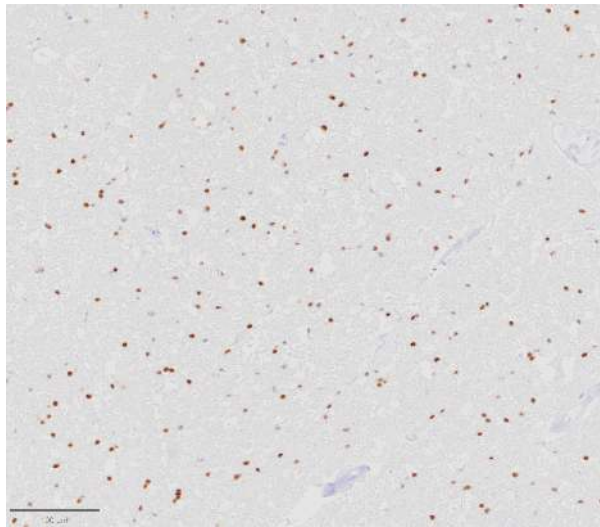
Figure B.5: NF images of four ROIs in Sample 1131-S3A (scale bar 20 μm). Group A has long fibers that stain with NF, whereas Group B has reduced NF staining. The NF appears dysmorphic compared to Groups A-B, where the fibers are clumped rather than long.



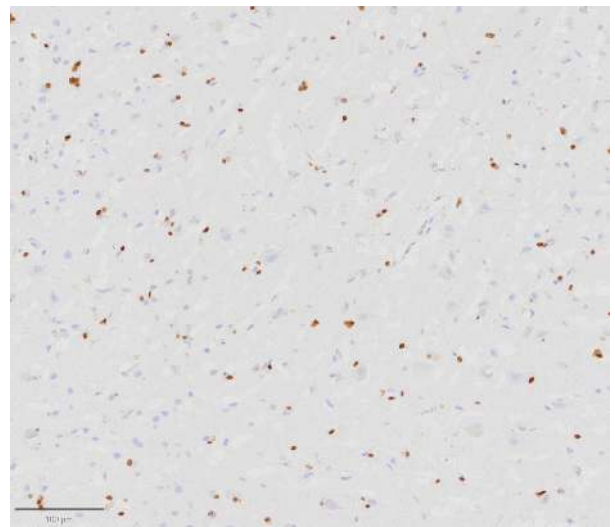
(a) Group A: Normal Appearing White Matter



(b) Group B: WML with Demyelination



(c) Group C: WML with Axonal Destruction



(d) Gray Matter

Figure B.6: OLIG2 images of four ROIs in Sample 1131-S3A (scale bar 100 μm). Group A has a high density of oligodendrocytes, and Group B has a reduced OC compared to Group A. Group C has even fewer oligodendrocytes per unit areas compared to Groups A-B; its count is more similar to that of the GM.

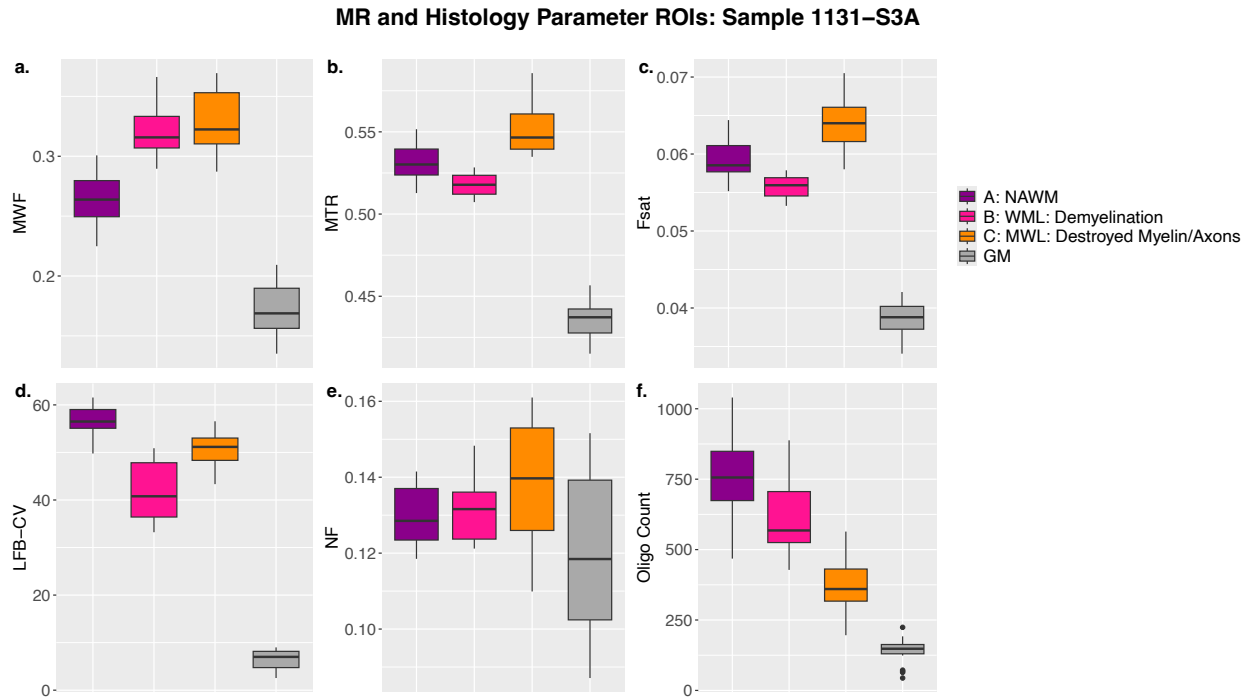


Figure B.7: Box plots (interquartile range, whiskers: 1.5-times interquartile range, outliers as dots) of MR (a-c) and histology parameters (d-f) for Sample 1131-S3A. The OC count plot follows the expected trend, and NF values are fairly consistent across ROI type. Group C has higher LFB values than may be expected. MTR and F_{sat} follow the expected trend from LFB; however the MWF Groups A and B ROIs do not follow this trend.

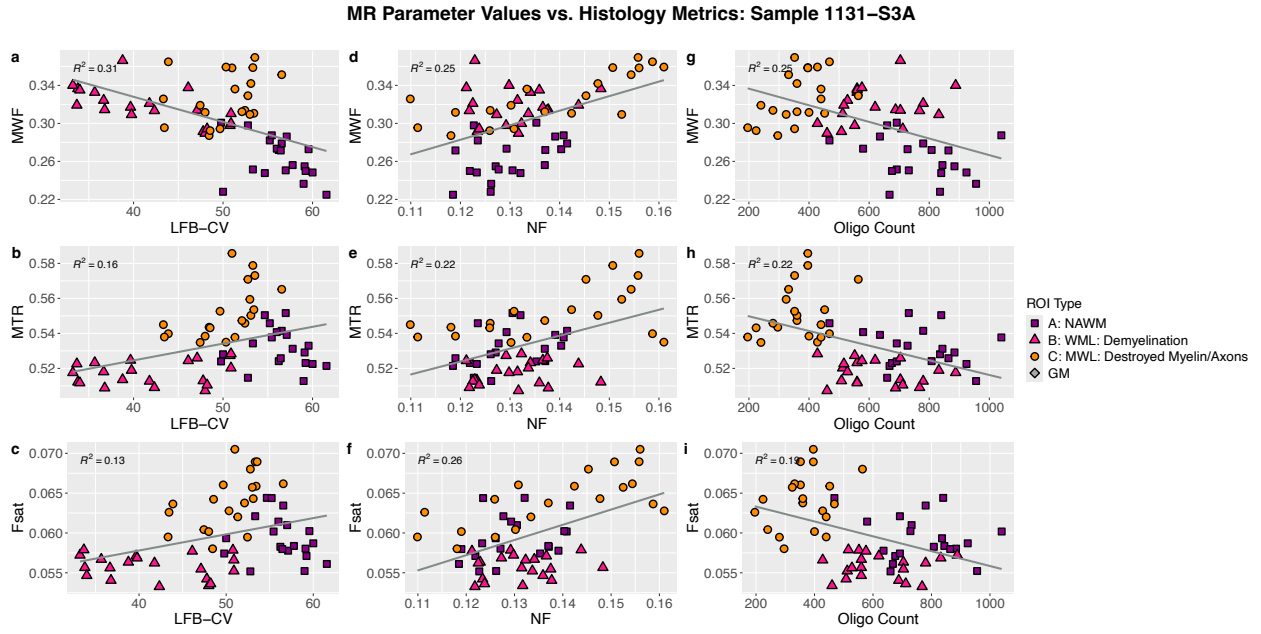


Figure B.8: Single-variable regression analysis between qMRI and histology values across for WM ROIs in Sample 1131-S3A. All correlations are statistically significant ($p < 0.05$). Plots b-f follow the expected trend based on Figure 4.5; plots a and g-i have the opposite of the expected trend.

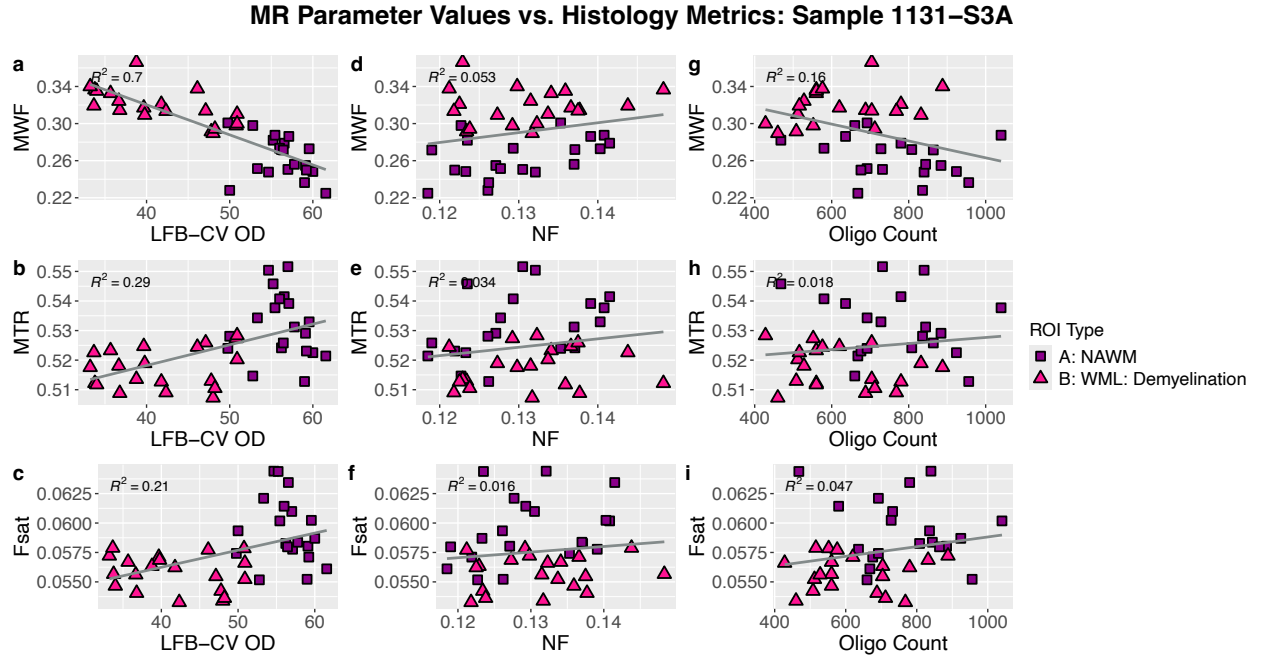


Figure B.9: Single-variable regression analysis between qMRI and histology values across for Groups A and B only for Sample 1131-S3A. NF correlation is significantly lower without Group C. Removing Group C switches the trends of h-i, but plots a and g still have the opposite trend as expected.

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