Quantitative magnetic resonance imaging of magnetization transfer and $T_2$ relaxation in human white matter pathology

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<th>Notation</th>
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<tbody>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
<td></td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
<td></td>
</tr>
<tr>
<td>FoV</td>
<td>field of view</td>
<td></td>
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<tr>
<td>GM</td>
<td>grey matter</td>
<td></td>
</tr>
<tr>
<td>GRE</td>
<td>gradient echo</td>
<td></td>
</tr>
<tr>
<td>IE</td>
<td>intra/extracellular</td>
<td></td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
<td></td>
</tr>
<tr>
<td>MR/MRI</td>
<td>magnetic resonance / magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>magnetization transfer</td>
<td></td>
</tr>
<tr>
<td>MTR</td>
<td>magnetization transfer ratio</td>
<td></td>
</tr>
<tr>
<td>MW</td>
<td>myelin water</td>
<td></td>
</tr>
<tr>
<td>MWF</td>
<td>myelin water fraction</td>
<td></td>
</tr>
<tr>
<td>NAGM</td>
<td>normal appearing grey matter</td>
<td></td>
</tr>
<tr>
<td>NAWM</td>
<td>normal appearing white matter</td>
<td></td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
<td></td>
</tr>
<tr>
<td>NNLS</td>
<td>non-negative least squares</td>
<td></td>
</tr>
<tr>
<td>QMT(I)</td>
<td>quantitative magnetization transfer (imaging)</td>
<td></td>
</tr>
<tr>
<td>QT2</td>
<td>quantitative multi-component $T_2$</td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>radio-frequency</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>spin echo</td>
<td></td>
</tr>
<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
<td></td>
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<tr>
<td>SPGR</td>
<td>spoiled gradient echo</td>
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</tbody>
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Abbreviations & Notation

TE  echo time
TI  inversion time
TR  repetition time
WM  white matter

$B_0$  main magnetic field
$B_1$  RF magnetic field
$F$  ratio of semi-solid to liquid pool sizes
$^1H$  hydrogen nucleus
$k_f$  forward two-pool (MT) exchange rate constant
$k_r$  reverse two-pool (MT) exchange rate constant
$M_0$  equilibrium magnetization
$R$  non-directional two-pool (MT) exchange rate constant
$R_{1f}$  spin-lattice relaxation rate constant of the free pool ($= 1/T_{1f}$)
$R_{D}$  non-directional inter-compartmental water exchange rate constant
$S(T_2)$  $T_2$ distribution
$T$  Tesla
$T_1$  spin-lattice relaxation time constant ($= 1/R_1$)
$T_{1obs}$  observed spin-lattice relaxation time constant
$T_2$  spin-spin relaxation time constant ($= 1/R_2$)
$T_{2obs}$  spin-spin relaxation time constant ($= 1/R_2$)
$T_2^*$  transverse relaxation time constant ($= 1/R_2^*$)
$\langle T_2 \rangle$  geometric mean of the $T_2$ distribution
$T_{2f}$  spin-spin relaxation time constant of the free pool
$T_{2r}$  spin-spin relaxation time constant of the restricted pool
$T_{2ie}$  spin-spin relaxation time constant of the intra/extracellular water pool
$T_{2mw}$  spin-spin relaxation time constant of the myelin water pool
$T_{cr}^D$  exchange time for diffusional cross-relaxation
$\omega$  angular frequency
I am first and foremost grateful to my doctoral advisor, mentor, and friend, Professor Bruce Pike. His patience and trust allowed me to explore various aspects of MR imaging and research. His guidance ensured that I remained focused and completed the task at hand. His generosity and support made my graduate studies an experience that I will cherish for a very long time.

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I also wish to thank my colleagues in the Shark Tank, past and present, who contributed each in their own way to a friendly and stimulating working environment: Michael Ferreira, Dr. Jennifer Campbell, Dr. Bojana Stefanovic, Dr. Jean Chen, Ilana Leppert, Charmaine Chia, Vivian Woo, Dr. Jan Warnking, Christine Tardif, Clarisse Mark, Eric Stinson, and Claire Cohalan. In particular, Ms. Chia and I collaborated closely during her time in our group; she made very important contributions to our common understanding of multi-component $T_2$ imaging, and to some of the work presented herein. The laughter and good humour of these lab-mates was excellent compensation for the lack of natural light in our
office. I am very fortunate to have shared our oddly-shaped space with such fantastic people, and am proud to call them friends.

I was once told to “take care of the little things, and the big things will take care of themselves”. I am very grateful to the people who took care of the million little things: Jennifer Chew, André Comier, Ron Lopez, David Costa, Louise Marcotte, Stacey Peixoto, and Hélène Day. Thanks to them the MRI unit runs “comme sur des roulettes”. Jean-François Malouin, Dale Einarson, and Sylvain Milot solved computer issues with nary a sys-admin grumble.

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I wish to thank my family, who provided me with the tools to get this far, and were always incredibly supportive and proud of me throughout my graduate work.

Finally, I am extremely grateful to my fiancee Janice. She endured countless conversations about “spin-this, magnet-that”, my extended evenings at the computer, and the random rant about results or reviewers. She kindly proofread the first manuscript of the thesis. Most importantly, Janice provided me with the energy and motivation I needed to see this project through to the end. This thesis is as much hers as it is mine.

This thesis is dedicated to my grandmother, Jeanne (Robert) Fafard, who had always wanted me to become “un docteur”.

Ives Levesque
Montreal, June 2009
Preface

The core of this thesis consists of four manuscripts:


At the time of this writing, all of these manuscripts are at various stages of review. They appear in full and in sequence in this thesis.
Contributions of authors

As the first author of all four manuscripts, I designed, implemented and validated all imaging methodology, conducted the bulk of the experiments, performed the data analysis, and drafted the papers. The contributions of the various co-authors to the four manuscripts, including specific contributions of co-authors to manuscripts 2, 3, and 4, were as follows.

**G. Bruce Pike, PhD:** As the candidate’s supervisor, Professor Pike provided essential guidance and mentorship throughout the project, and reviewed all manuscripts extensively.

**Douglas L. Arnold, MD:** For manuscripts 2 and 4 (Chapters 4 and 6, respectively), provided guidance and revision of the manuscripts, particularly with respect to the clinical aspects.

**Paul S. Giacomini, MD and Luciana T. Ribeiro, MD:** For manuscripts 2 and 4, recruited the study subjects (healthy and MS), contributed to the data acquisition and to the revision of the manuscripts.

**Sridar Narayanan, PhD and John G. Sled, PhD:** For manuscripts 2 and 4, provided specific guidance with regards to the experimental design, data acquisition and analysis, and contributed to the revision of the manuscripts.

**Charmaine L. L. Chia, MSc:** In the specific case of manuscript 3 (Chapter 5), Ms. Chia, as a Masters student under the supervision of Professor Pike, developed the multi-component $T_2$ analysis software, acquired the experimental data for the first part of that study (protocol comparison), drafted a portion of the text, and provided two of the Figures.
Other Publications

The following are additional peer-reviewed journal and conference publications that were produced over the course of this project, which have not been included in this thesis:


v. Paul S. Giacomini, Ives R. Levesque, Sridar Narayanan, G. Bruce Pike, and Douglas L. Arnold, “In vivo measurement of remyelination and edema resolution in the core and peripheral zone of acute multiple sclerosis lesions”. The 43rd Annual Congress of


The primary aim of this thesis is the reconciliation of two seemingly disparate quantitative magnetic resonance imaging (MRI) techniques proposed to characterize human brain white matter (WM) in health and disease. Quantitative magnetization transfer imaging (QMTI) and multi-component analysis of T\textsubscript{2} relaxation (QT2) both attempt to quantify myelin content \textit{in vivo}, but are based on fundamentally different models of WM. QMTI probes the macromolecular component of tissue using a two-pool model of magnetization transfer, while QT2 isolates the water signal from distinct micro-anatomical compartments. The specific objectives were to determine the interrelationship between measurements made with both techniques in the context of potential pathological changes associated with multiple sclerosis (MS), and to apply both to track WM changes in the acute phase of MS lesions. First, simulations were used to evaluate the theoretical sensitivity of each technique to the characteristics of a model of WM that incorporates four pools of magnetization, based on published \textit{in vitro} measurements. Next, the experimental reproducibility of each technique was investigated, and the impact of certain basic variations in the data acquisition and analysis procedures was evaluated. In the final stage, both methods were applied longitudinally \textit{in vivo} to assess the dynamic changes that occur in acute, contrast-enhancing lesions of MS. The theoretical results illustrate the sensitivity and limitations of QMTI and QT2 to specific pathology-inspired modifications of WM, and shed new light on the potential specificity of often-neglected QMTI parameters. The reproducibility of both techniques is acceptable for use in repeated clinical measurements, and QMTI has lower variability overall. The importance of corrections for magnetic field inhomogeneity in QMTI is demonstrated, and a simple optimization of the QMTI data acquisition is introduced. Both techniques were sensitive to active disease pathology in the longitudinal study.
of MS patients. Overall, this thesis demonstrates the complementary nature and usefulness of QMTI and QT2 in the characterization of the natural disease course of a degenerative disease of the human central nervous system. With further refinement, these techniques could play an important role in the study of other diseases, and have the potential to serve as outcome measures in clinical trials.
L’objectif principal de cette thèse est la réconciliation de deux techniques quantitatives d’imagerie par résonance magnétique, en apparence différentes, utilisées pour la caractérisation de la substance blanche du cerveau humain en santé ou affectée par la maladie. Les techniques d’imagerie quantitative par transfert de magnétisation (QTM) et d’analyse de la relaxation $T_2$ par de multiples composantes (QT2) proposent toutes deux des mesures *in vivo* de la quantité de myéline, mais à l’aide de modèles fondamentalement différents. D’un côté, l’imagerie QTM sonde la composante macro-moléculaire des tissus à l’aide d’un modèle à deux réservoirs pour le transfert de magnétisation. De l’autre, l’imagerie QT2 sépare les signaux acqueux provenant de compartiments micro-anatomiques distincts. Plus spécifiquement, cet ouvrage cherche à mieux comprendre l’interdépendance des mesures de ces deux techniques dans le contexte pathologique de la sclérose en plaques (SEP), pour ensuite les appliquer à l’étude de lésions aigues de SEP. En premier lieu, des simulations ont été effectuées pour évaluer la sensibilité de chaque technique aux caractéristiques d’un modèle plus complet de la substance blanche, qui découle de résultats *in vitro* publiés et incorpore quatre réservoirs de magnétisation. Ensuite, la reproductibilité de chacune des techniques a été évaluée; de plus, quelques variations élémentaires des méthodes d’acquisition et d’analyse des données ont été examinées. En dernier lieu, les deux techniques ont été utilisées *in vivo* afin de mesurer les changements dynamiques des lésions aigues de sclérose en plaques, présentant un hyper-signal rehaussé par un agent de contraste. Les résultats des simulations démontrent d’un point de vue théorique la sensibilité et les limites de chacune de ces technique aux changements dans la substance blanche. Ces résultats apportent également de nouvelles connaissances sur le rôle potentiel que peuvent jouer certains paramètres souvent négligés de l’imagerie QTM. La reproductibilité acceptable de
ces techniques ouvre la voie à leur utilisation répétée en recherche clinique, en sachant que l’imagerie QTM est généralement la moins variable. L’importance des corrections des inhomogénéités de champs magnétiques pour l’imagerie QTM a été démontrée, et une technique simple d’optimisation d’acquisition des données d’imagerie QTM a été présentée. Cette thèse démontre la complémentarité et l’utilité des méthodes de d’imagerie QTM et QT2 dans la caractérisation de l’évolution d’une maladie dégénérative du système nerveux central humain. Suivant leur amélioration, ces techniques joueront des rôles importants dans l’étude d’autres maladies du système nerveux central, et pourraient servir d’outil de mesure lors d’essais cliniques.
Original Contributions

The original scientific contributions made in the course of this Ph.D. project are:

1. Establishing the sensitivity and limitations of the quantitative magnetization transfer (QMTI) and quantitative multi-component $T_2$ (QT2) MRI techniques to an underlying, more complete, model of white matter with four pools of magnetization;

2. Evaluation of the variability of the QMTI and QT2 MRI techniques over repeated measurements in healthy subjects;

3. Assessment of the importance of corrections for static and radio-frequency field inhomogeneities in QMTI for achieving more uniform parameter maps and significantly better longitudinal reproducibility;

4. Development of an optimized data acquisition scheme for QMTI, using a simple data reduction technique with error analysis, that achieves high parameter map quality and longitudinal reproducibility;

5. Demonstration that basic parameters in the QT2 data acquisition protocol, such as the image geometry and repetition time, can have an important impact on the output of the multi-component $T_2$ analysis;

6. Evaluation of the impact of the myelin water $T_2$ range on estimates of the myelin water fraction, and on their variability across healthy subjects;

7. Measurement of the dynamic changes of myelin content and inflammation in acute gadolinium-enhancing lesions of multiple sclerosis with QMTI and QT2, characterizing the recovery after the resolution of enhancement.
“Essentially, all models are wrong. Some are useful.”

– George E. P. Box

“Insanity is doing the same thing over and over again and expecting different results.”

– attributed to Rita Mae Brown, Benjamin Franklin, and even Albert Einstein
Magnetic resonance imaging (MRI) has become a powerful and flexible tool for biophysical and medical research, in addition to its important role in diagnostic radiology. The sensitivity of MRI to subtle variations in soft tissue microstructure, molecular composition, and physiological processes \textit{in vivo} is unparalleled. When coupled with models that describe the behaviour of the image signal, MRI can be used as an instrument for mapping fundamental physical parameters that relate, directly or indirectly, to tissue microstructure or function.

The motivation, and primary application, of this work is the characterization of cerebral tissue damage in multiple sclerosis (MS), a debilitating autoimmune disease of the human central nervous system (CNS). MRI is a powerful tool in the study of MS [1–3], mainly due to its high sensitivity to the lesions of MS, which appear hyperintense on clinical $T_2$-weighted scans. However, conventional MRI techniques such as $T_2$ or $T_1$-weighted imaging offer poor pathological specificity, due to the confounding effects of edema, demyelination, axonal loss, and gliosis [4]. Furthermore correlation of MRI findings with clinical status remains elusive.

Many quantitative MRI techniques have been proposed to measure tissue composition and organization in the CNS. More specifically, certain techniques purport to measure myelin content and changes resulting from disease or aging, in brain white matter (WM). These techniques have the potential to expand our understanding of the natural course of neurodegenerative diseases such as MS, and to serve as outcome measures in therapeutic trials. To achieve this standing, an MRI method must be able to robustly and reproducibly
measure quantities that can be directly related to one (or more) aspects of disease, such as tissue destruction, inflammation, edema, and tissue repair. Such techniques must also be practical, fast, and reliable.

This thesis focuses on two quantitative MRI methods that have been proposed to measure myelin content in WM. The first, quantitative magnetization transfer imaging (QMTI), exploits the inherent physical and chemical interaction between water and the macromolecular components of biological membranes to measure the non-water content of tissue, and to probe the interaction process. The other method, quantitative multi-component $T_2$ relaxometry (QT2), measures the relative content and $T_2$ relaxation properties of separate water populations in micro-compartment. Each offers a useful, yet limited, window into WM structure and pathology, and has been used and validated in vivo. A brief review of certain essential principles of magnetic resonance imaging and an overview of the basic characteristics of MS are presented in Chapter 2. A more comprehensive review of NMR relaxation and MT in WM is also presented in Chapter 2, leading to a detailed description of the QMTI and QT2 methods.

The first objective of this work was to reconcile these two quantitative imaging techniques and to shed light on their combined observations in vivo. At the core of this work was a set of simulations to investigate the sensitivity and limitations of QT2 and QMTI methods, based on a more complete model of human WM constructed from in vitro observations in bovine WM. This is presented in Chapter 3. The second aim was to evaluate the experimental reproducibility of each technique in healthy individuals; moreover, these investigations were used to explore the impact of certain experimental parameters on measurements and reproducibility. The results of these reproducibility studies are reported in Chapters 4 and 5. The final aim was to apply both techniques in vivo to track disease progression during an active phase of MS. A longitudinal study of acute gadolinium-enhancing lesions in MS patients, which believed to be the initial stage of plaque formation. The observations in acute MS lesions are presented in Chapter 6. The thesis concludes with summary discussion of the major findings in Chapter 7.
Chapter 2

Background

MRI is rooted in nuclear magnetic resonance (NMR), the phenomenon by which nuclei with net nuclear magnetic moment (or magnetic spin) immersed in a magnetic field can be probed by electromagnetic induction using radio-frequency (RF) magnetic fields [5, 6]. The behaviour of the macroscopic magnetization (or polarization) vector can be described by the classical Bloch equations, which can be derived from principles of quantum and statistical mechanics [7]. These equations combine terms for the Larmor precession, relaxation, and RF irradiation, and are a powerful tool in the development and analysis of MRI techniques. The fundamentals of nuclear magnetism and NMR are well documented [7–9].

In MRI, the NMR signal is spatially encoded by modulation of the local resonance frequency, encoding the spatial distribution of spins in the phase and frequency of the signal [10]. Image reconstruction is achieved by means of a Fourier transform. Image contrast is determined by the combination of the NMR properties of the tissue and data acquisition parameters; in addition, other manipulations can be performed to sensitize the signal to tissue properties such as water diffusion, magnetization transfer, or blood flow. The result is a flexible imaging modality with excellent soft tissue contrast, volumetric capability, and good spatial resolution, which has become an essential tool in diagnostic radiology. Further information about MRI is presented elsewhere [11, 12].
2.1 Fundamentals of NMR relaxation

A group of $N$ nuclear magnetic spins $I = 1/2$ immersed in a magnetic field $B_0$ will align either parallel or anti-parallel to the applied field, defining low and high energy states. The net excess of parallel spins at a temperature $T$, determined by Boltzmann statistics, results in a net magnetization $M_0$:

$$M_0 = \frac{N\gamma^2\hbar^2}{4kT}B_0,$$

where $\gamma$ is the gyromagnetic ratio of the spins. Relaxation is the phenomenon by which the spins, and by extension the macroscopic magnetization of a group of spins, achieve equilibrium, through the exchange of energy with their environment. It consists of two “distinct” processes: recovery of equilibrium longitudinal magnetization and decay of transverse magnetization. The Bloembergen-Purcell-Pound (BPP) theory of relaxation was derived from statistical mechanics by considering a spin system immersed in a thermal bath, or lattice, with a very large number of degrees of freedom. This theory describes relaxation mechanisms based on the correlation of random fluctuations in the magnetic field experienced by spins due to their neighbours [7, 13]. Further discussion of NMR relaxation will be mostly limited to the classical description [11], which is appropriate in this context.

The recovery of longitudinal magnetization $M_z$ – the component of magnetization oriented along the main static field – is the result of thermal interaction between the system of nuclear spins and their broad atomic and molecular environment, and is termed spin-lattice relaxation. The system returns to its minimum-energy equilibrium state with a characteristic time-constant $T_1$. For a system of like spins, the rate of recovery of longitudinal magnetization is proportional to the difference between the instantaneous magnetization $M_z(t)$ and the equilibrium magnetization:

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

which, taking $M_z(t_0)$ as the initial magnetization, has the solution:

$$M_z(t > t_0) = M_0 - [M_0 - M_z(t_0)]e^{-\frac{t-t_0}{T_1}}.$$
2.1 Fundamentals of NMR relaxation

Spin-lattice relaxation times are generally longer in pure liquids and in solids than in biological tissue, for different reasons. In liquids, the rapid motion, rotation, and vibration of molecules result in short correlation times and thus longer relaxation times. In tissue, other pathways for relaxation are present, resulting in shorter $T_1$s.

Interaction within the spin system also contributes to relaxation, but the effect is limited to the component of magnetization perpendicular to the static magnetic field, $M_{xy}$. Spin-spin interaction has a dramatic effect on signal decay by destroying the coherence of transverse magnetization. This decay is faster than spin-lattice relaxation, with characteristic time $T_2$. The decay of coherent transverse magnetization in liquids is proportional to the remaining $xy$ magnetization,

$$\frac{dM_{xy}}{dt} = -\frac{M_{xy}(t)}{T_2}, \quad (2.4)$$

which, assuming an initial state $M_{xy}(t_0)$, results in the exponential solution:

$$M_{xy}(t) = M_{xy}(t_0)e^{-\frac{t-t_0}{T_2}}. \quad (2.5)$$

This form of decay is equivalent to having an RF absorption spectrum that is Lorentzian, of width proportional to $1/T_2$. Spin-spin interaction is much greater for spins with restricted motion (in solids or gels) than for spins that are relatively free (in liquids). The extremely short decay of $M_{xy}$ in solids (on the order of microseconds) generally precludes direct observation by MRI. In liquids, the rapid tumbling and free motion of the molecules result in short correlation times and longer $T_2$s (tens of milliseconds or more), maintaining coherent signal long enough for imaging.

Inhomogeneity in the static applied magnetic field $B_0$ introduces additional dephasing of the spins. This additional phase dispersion results in a further reduction of the transverse relaxation time, described by the term $T_2'$, which results in an overall decay constant $T_2^*$ ($T_2$-“star”, Eq. 2.6).

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \quad (2.6)$$
The contribution of $T'_2$ can be reduced by making the $B_0$ field more spatially uniform. The remaining $T'_2$ decay is recoverable by making use of RF pulses to reverse the phase dispersion and to “refocus” $M_{xy}$, producing a “spin echo” that depends only $T_2$.

### 2.2 White Matter and WM Pathology

#### 2.2.1 Biology of the central nervous system and white matter

The CNS comprises the brain and spinal chord, and is composed of four major types of cell [14]. Neurons are the principal active cells of the nervous system, and have four important sections: the dendrites, which receive input from other neurons; the neuronal body, or soma, where the nucleus resides and which is the primary centre of neuronal metabolism; the axon, along which neuronal impulses travel, and axon terminals (or synaptic endings), which relay the information to other neurons. The other three major cell types in the CNS are support, or glial cells: i) astrocytes, which provide structural and metabolic support, ii) oligodendrocytes, whose projections form the myelin sheath that envelops the axons of neurons, and iii) microglia, which play an important role in response to tissue injury. A myelinated neuron is illustrated in Figure 2.1.

Cells in the central nervous system (CNS) are spatially organized to form white matter (WM) and grey matter (GM). The neuronal somata reside in GM, either in the cortex, or deep within the brain. WM is composed mainly of myelinated axons and glial cells, and constitutes a large portion of the brain tissue. Axons in WM are generally organized in bundles that connect the various areas of GM and enable communication.

Oligodendrocytes have projections which extend to form the myelin sheath. Myelin wraps the axons in a spiral fashion, as shown in the electron micrograph of Figure 2.2. By dry weight, WM consists of about 50% myelin; the rest is composed of axons and other glial cells [15]. The spiral arrangement of myelin results in a concentric lamellar structure of phospholipid bilayers, separated by alternating bands of oligodendrocyte cytoplasm and extra-cellular fluid. Each section of myelinated axon is separated by a very
Figure 2.1: Illustration of a myelinated neuron, showing its major parts. The oligodendrocyte bodies, which produce the myelin sheath, are not shown.

A short section of bare axon called a node of Ranvier, which plays a very important role in conduction of neuronal impulses. Each oligodendrocyte can wrap up to 60 different axons. The myelin sheath consists of lipids and proteins in a ratio of about 70%-30% [16], a lipid content that is greater than is generally observed in other cellular membranes. The high lipid content confers myelin its insulating properties, and gives WM its characteristic pale color. The major lipids of myelin are phospholipids (46% of lipids), galactolipids (29%), and cholesterol (25%) [17]. Four specific proteins play a role in myelin structure: the proteolipid and myelin basic proteins, and the myelin oligodendrocyte and myelin-associated glycoproteins.

Myelin plays a very important role of electrical insulation of axons. It also enables faster conduction of electrical impulses, or *action potentials*, along the axon [14]. In the resting state, the inside membranes of axons are negatively polarized (around -65 mV). Action potentials travel resistively along unmyelinated axons, much like current down a network of resistors and capacitors. The electric impulse is sustained by a continuous wave...
of depolarization mediated by ionic channels distributed along the axon, and conduction velocity is limited by this depolarization. Myelin forces the axons to concentrate these ionic channels at the nodes of Ranvier, and lowers the capacitance of the combined axon-myelin membrane [17]. Conduction of the action potential happens more efficiently between the nodes, in a process called saltatory conduction, at a velocity 10-50 times greater than in unmyelinated axons. This phenomenon has enabled vertebrates to develop more compact brains while retaining fast impulse conduction.

WM and myelin can be affected by injury, toxins, neurodegenerative diseases (such as Alzheimer’s disease), and autoimmune diseases such as multiple sclerosis (MS) and acute disseminated encephalomyelitis. Damage to the myelin translates to a reduction of the conduction speed and loss of trophic support to the axons. MS is the primary application of the MRI techniques in this thesis.
2.2 White Matter and WM Pathology

2.2.2 Multiple sclerosis and MRI of MS

Multiple sclerosis, an acquired autoimmune disorder of the CNS in humans affecting the brain and the spine, is characterized by inflammation, demyelination, and neurodegeneration [17]. The disease is most prevalent in northern Europe, North America, and southern Australia, affecting between 1 and 3 million worldwide. The age of onset of MS is most often in early adulthood, and affected women outnumber affected men roughly two-to-one. MS is considered as the most common cause of neurological disability in young adults [18, 19]. Individuals with MS usually suffer from physical disability, visual impairment, as well as cognitive and sensory impairment. Clinically, MS most commonly presents as a cycle of short-term disability separated by periods of recovery (the relapsing-remitting form). With time, MS often results in lasting, progressive impairment (the secondary progressive form). In fewer cases, MS can be progressive from the onset (the primary progressive form).

MS is characterized by focal damage to WM, referred to as lesions or plaques. The damage consists mainly of demyelination accompanied by edema and gliosis, with some measure of axonal loss [20]. Demyelination is a critical component of the disease, and is the focus of much research. There is also evidence for diffuse involvement of “lesion-free” areas of WM (normal-appearing WM, or NAWM), where myelin breakdown has also been observed [21]. GM is also involved in MS, through the formation of GM lesions [22].

The formation of WM lesions is due to the engagement of activated immune system cells, penetrating from the blood stream into the CNS, and attacking the myelin sheath. Breakdown of the blood-brain barrier (BBB) allows monocytes and lymphocytes to reach and attack the myelin sheath, causing acute inflammation and loss of myelin. Demyelination is often accompanied by axonal injury, and eventually leads to the formation of chronic sclerotic plaques as the disease progresses [18]. Neurodegeneration is considered to be the cause of lasting clinical disability, and may result from the loss of myelin trophic support [17] or from direct inflammatory processes, independently of demyelination [23]. Remyelination is believed to occur in some cases, more commonly at the edge of
lesions [17]: this partial recovery may play a role in the cyclic nature of some MS symptoms [19]. Remyelination usually results in thinner, less compact myelin, with shorter inter-node distances.

MRI has provided very useful insight into MS, and constitutes a powerful tool for studying the disease in-vivo. MRI is used in diagnosis [24], in patient monitoring, as an outcome measure for clinical trials of new therapies, and as a tool for basic research on the genesis and progression of MS. Basic MRI methods can be used to characterize MS. $T_2$-weighted ($T_2$w) and proton-density-weighted (PDw) images are very sensitive to the presence of lesions, which appear as hyper-intense regions. These likely reflect the increased presence of water at various stages of lesion evolution, i.e., edema in the initial stages, and later, replacement of lipids by water [4, 17]. PD weighting has the particular characteristic of enabling the distinction between lesions and cerebro-spinal fluid, useful for peri-ventricular lesions. Fluid-attenuated inversion recovery (FLAIR), a variation of $T_2$w imaging, is very sensitive to lesions of the cerebrum (the superior portion of the brain).

$T_1$w imaging reveals fewer lesions that $T_2$w imaging: this has been suggested to reflect acute edema in young lesions, and advanced tissue destruction in chronic lesions [4, 25]. $T_1$w can be used in conjunction with an injected contrast agent containing paramagnetic gadolinium, which enhances $T_1$ relaxation. This contrast agent does not normally cross the BBB into the CNS, and thus post-injection $T_1$w images are sensitive to the opening of the BBB which occurs during the initial stage of lesion formation.

While conventional MRI images are very sensitive to the presence of WM lesions, they lack the necessary pathological specificity to separate the effects of demyelination, inflammation, and neurodegeneration in MS. As a results, other MRI techniques have been explored for potential specificity, many of which exploit the direct measurement of specific NMR or other biophysical properties of tissue. This work focuses on two such techniques used in the study of MS: quantitative magnetization transfer (MT) imaging and multi-component analysis of $T_2$ relaxation (QT2). These are described in greater detail in the following section.
2.3 Relaxation and magnetization transfer in WM

2.3.1 Overview

MRI is sensitive to the water hydrogen spins, the image contrast is in large part determined by their NMR properties. The complex, heterogeneous structure of biological tissue provides many factors that influence the relaxation of these spins, and conventional MRI techniques present contrast that reflects a combination of the physical properties (proton density, relaxation, magnetization transfer, diffusion, etc.). In turn, MRI contrast can be exploited to extract meaningful quantitative information about the water micro-environment.

The physical basis of relaxation processes in liquids and solutions can be viewed in terms of rotational motion of the molecules [7–9], but this is insufficient for complex heterogeneous systems such as biological tissue [26]. In fact, the former interpretation incorrectly assumes independence of water and macromolecular components. Interactions with the lipids and proteins of biological membranes contribute significantly to water hydrogen spin relaxation [27]. Hydrogen in distinct molecules can notably experience mutual spin flips by magnetic dipolar interaction, or might undergo chemical exchange. The sum interaction plays a very important role in determining tissue $T_1$ s, as originally demonstrated in hydrated collagen [26].

Studies of spin-lattice relaxation times of protons and deuterons in muscle and brain tissue samples demonstrated that intermolecular dipolar interaction between hydrogen spins is a major $T_1$ relaxation mechanism in tissue [28]. This also demonstrated using NMR dispersion measurements in protein solutions with various levels of deuterization [29]. Other experiments highlighted the impact of lipids on $T_1$-weighted signal in WM [30–33]. It is straightforward to show that cross-relaxation in a two-phase model results in bi-exponential $T_1$ relaxation; generally, the slow component is observed. This spawned the study and use of magnetization transfer (MT) MRI, discussed in Section 2.3.2.

$T_2$ decay is also influenced by the presence of other species in the environment. The presence of other spins provides for additional avenues for interaction, which results in
additional dephasing of the observable transverse magnetization. While pure dipolar interaction between water and other macromolecules does not contribute to the dephasing component of $T_2$ decay [7], variations in the local magnetic field due to other spins may contribute to $T_2$ decay. Chemical exchange of hydrogen atoms, especially with short-$T_2$ macromolecular species such as lipids and proteins, can also enhance $T_2$ decay. As a result, $T_2$ decay is shorter in gels, protein solutions, and tissue [27].

In addition to atomic and molecular interactions, the compartmental nature of tissue micro-anatomy, due to the presence of semi-permeable cell membranes, introduces another level of complexity in tissue NMR relaxation. In WM, this effect is most easily observed with $T_2$ relaxation, as we will see in Section 2.3.3, but impacts all relaxation mechanisms, to be discussed in Section 2.3.4.

Mapping of the relaxation properties of tissue can be achieved via in a number of ways, by extending NMR relaxometry techniques to imaging applications. Relaxometry describes the quantitative measurement methods by maps of relaxation time constants are obtained, in contrast to the qualitative weighting of conventional MRI methods. Although techniques for $T_1$ and $T_2$ relaxometry are different, the general concept is the same for both. The evolution of the components of magnetization must be sampled, transiently or in the steady-state, to observe the relaxation process and enable robust determination of the constants using the phenomenological equations.

As we will see in the following sections, the quantitative analysis of MT and water proton $T_2$ relaxation can be exploited to map more specific information about tissue composition and structure. Detailed information about the mechanisms of $T_2$ and MT in WM will be presented, along with a survey of the most popular methods to quantify these effects in MRI (QT2 and QMTI). As $T_1$ relaxation is an integral part of the global picture, but is not specifically investigated in this work, a short review of methods of *in vivo* $T_1$ mapping is presented first.
Mapping $T_1$ relaxation in WM

Inversion recovery (IR) methods are the gold standard for $T_1$ relaxometry. The recovery of longitudinal magnetization is sampled with repeated experiments, modulating the recovery (or inversion) time; however, long acquisition times are a major obstacle to IR techniques. Two-point inversion recovery may be acceptable in certain circumstances [34], i.e. when fast determination of the observed $T_1$ is desired, but it is unacceptable for accurate determination of tissue characteristics. Fast, single-shot, and steady-state techniques have also been developed. These faster techniques include, but are not limited to [35, 36]: IR with fast readout such as EPI [37], fast spin-echo, or FLASH [38], Look-Locker multi-shot sampling of the IR [39–46]; steady-state variable-nutation methods based on steady-state GRE sequences [47–51]; and composite-echo methods, where stimulated echoes are exploited to yield $T_1$ estimates [35, 52, 53].

Spin-lattice relaxation in cerebral tissue is most often regarded as mono-exponential and characterized by a single observed $T_1$, which is regionally dependent and reflects the average behaviour of the underlying $T_1$ contributions. Multi-component $T_1$ in tissue can result from MT, discussed in Section 2.3.2, or the variation of $T_1$ between micro-anatomical water compartments, as discussed for $T_2$ in Section 2.3.3. A two-component water exchange model for $T_1$ was recently investigated for steady-state variable-nutation methods [54], and for $T_2$ in steady-state free precession [55], revealing that water exchange between micro-anatomical compartments (see Sections 2.3.3 and 2.3.4) is not negligible under certain experimental conditions. This effect has very recently been exploited to separate signal components based on relaxation [56]. MT has also shown to play a role in determining the signal of such steady-state sequences [57], and may significantly impact $T_1$ measurements depending on the acquisition parameters, or result in multi-component behaviour. In short, the experimental observation of multiple $T_1$ components is difficult and depends on the acquisition method. Sensitivity is also affected by the difference between the $T_1$ values and interaction between the components, all of which happen on a similar time scale as will be discussed in Section 2.3.4. Multi-component $T_1$ is most reliably observed with
multi-dimensional experiments, to be discussed in Section 2.3.4 as well.

2.3.2 Magnetization Transfer in WM

Magnetization transfer (MT) refers broadly to the general mechanism of interaction between nuclear spins in different chemical environments. In the context of this work, MT more specifically refers to interaction between the hydrogen spins of water molecules and of larger molecules in biological membranes, through a combination of dipolar interaction (also known as the nuclear Overhauser effect, or NOE), and chemical exchange. MT can be exploited to produce MRI contrast distinct from the conventional forms ($T_1w$, $T_2w$, PDw), and allows the indirect observation of the macromolecular content of tissue. Comprehensive reviews of MT and MT imaging have been offered previously [36,58–60]. This section reviews the mechanisms of MT, and the methods for quantitative MT imaging (QMTI).

Fundamental concepts of MT imaging

The majority of hydrogen protons in biological tissue are found in water molecules, which experience rapid rotational and translational motion that average out mutual magnetic dipole interactions. These relatively free spins are characterized by a narrow Larmor spectrum (peak width $\sim 20$ Hz) and longer $T_2$ values ($> 10$ ms) [32]. MRI signal originates from this tissue water, as its $T_2$ is long enough for imaging gradients to be employed. Hydrogen protons are also found in “semi-solid” macromolecules, in smaller proportion. In contrast to the free protons, these restricted spins experience relatively slow rotational and translational motion. Thus, they present a broader absorption spectrum (peak width $\sim$ tens of kHz). Macromolecular proton signal decays too quickly ($T_2 \sim$ few $\mu$s, $\ll$ TE) to permit direct imaging with conventional methods [26, 61, 62]. There have been efforts to image the ultra-short $T_2$ component of tissue directly [63], but these are beyond the scope of this work. Two general techniques have been developed to investigate the MT effect between free and restricted spins: the first derives from selective inversion of the water pool, and the second, used throughout this work, is based on selective saturation of the semi-solid pool.
Both techniques are discussed below, and subsequent developments and applications of the techniques will be discussed.

Selective inversion was proposed by Edzes and Samulski, who also proposed a description of MT mechanisms [26, 64]. The method, originally coined selective hydration inversion, quickly and selectively inverts the water signal to study the effect of MT by careful observation of the ensuing bi-exponential recovery. Based on this technique, MT has been studied in model systems to demonstrate its importance in relaxation [65]. The Goldman-Shen and Edzes-Samulski selective inversion techniques were used to study cross-relaxation between proton phases in model systems (agar and BSA) [66]. Selective inversion was applied to imaging using on-resonance binomial pulses to study the approach to steady-state [67]. This has also inspired a host of development of imaging techniques [68, 69], culminating in a recent application of the technique for quantitative mapping of MT in vivo, using fast-spin-echo sequences [70]. A related technique using stimulated echoes has also been demonstrated [71].

Saturation transfer consists of studying a system of two or more freely-exchanging NMR species by selective saturation of one species and observation of the effect on the total system. The technique was initially proposed to observe the NOE between proton species with different resonant frequencies [72], and was also used in the study of chemical exchange rates [73,74]. In biological tissue, where both spin species have (nearly) identical resonance frequencies, the technique exploits the very different widths of the absorption spectra of the water and macromolecular spin pools. The semi-solid pool can be selectively saturated with off-resonance or on-resonance techniques, and transfer of this saturation to the liquid pool results in an observable signal decrease. Selective semi-solid saturation can be performed prior to image acquisition, or interleaved within the pulse sequence. Signal attenuation occurs in areas of the image with significant MT, producing MT contrast (MTC) [75].

Saturation transfer was originally performed using continuous wave (CW) irradiation [75]. Long periods of narrow-bandwidth, off-resonance RF irradiation are used to prepare the
magnetization prior to data acquisition. Semi-solid pool selectivity is ensured by the frequency offset from the water resonance (5-10 kHz). The effective coupling between restricted pool spins ensures the spread of saturation throughout the pool. This method has been employed in combination with a number of NMR [76, 77] and MRI techniques [75, 78–86]. Signal behaviour was characterized as a function of the frequency and power of the CW saturation (the “Z-spectrum”) in an in vitro model of MT in tissue [76]. A method for quantitative MT using low power CW saturation was demonstrated [87], and it is still used for in vitro and NMR studies of WM [88, 89]. It is straightforward to describe CW saturation analytically. However, this technique is impractical for clinical MRI because it is not time-efficient, it is not compatible with standard MRI hardware (which is not generally configured for CW generation), and it leads to excessive power deposition.

The alternative method of pulsed RF irradiation is more widely used in MRI applications. It is compatible with MRI equipment, and pulse sequence interleaving makes it more efficient than CW saturation. The on-resonance variant of pulsed saturation [90, 91] consists of brief, binomial pulses (e.g. $\hat{1}\bar{1}$, $\hat{2}\bar{1}$, $\hat{3}\bar{3}$). Free pool spins are returned to their original state by these self-compensating (total angle of 0°) pulses, while restricted protons are saturated due to much faster dephasing and $T_2$ decay. Pulsed saturation can also be applied off-resonance [92–94] as brief, shaped, off-resonance RF pulses. Like CW saturation, restricted pool selectivity is ensured by the frequency offset. Off-resonance pulses are widely used for QMTI as they enable more complete characterization of the MT effect. The effects of CW, pulsed on-resonance, and pulsed off-resonance saturation methods are illustrated in Figure 2.3. Saturation pulses have been added to a variety of standard acquisition sequences [83, 95–98], which usually include gradient or RF spoiling, to eliminate any transverse magnetization (signal) produced directly by the MT pulse. The MT effect is most widely reported as the percent difference between images with and without saturation, known as the MT ratio (MTR). The MTR combines all of the MT effect into a single semi-quantitative index. Applications of the MTR are reviewed elsewhere [36].
Biophysical aspects of MT

The mechanism of MT was first described by a 3-stage process involving mobile bulk water, water loosely bound to macromolecules (the hydration layer), and semi-solid macromolecules [26]. It was postulated that magnetization is quickly transferred between the bulk and hydration water, then between the hydration water and macromolecular protons by nuclear dipole coupling, and then rapidly distributed among macromolecular protons via spin diffusion [64].

The predominance of dipolar coupling over chemical exchange as the major mode of MT in biological systems was first revealed by \textit{in vitro} experiments in solutions of cross-linked bovine serum albumin (BSA), a model of MT in tissue [76], by using a solvent with no exchangeable protons. Experiments on the effect of isotopic composition of the spin population (hydrogen vs. tritium) in model systems of egg phosphatidylycholine (EPC) confirmed the predominance of dipolar coupling in MT [100]. This was also supported by a study of the influence of pH in various \textit{in vitro} models of MT using brain sphingomyelin [101]. pH and temperature had little effect over the range studied, supporting the predominance of dipolar coupling in that model system. The effect of temperature was
further investigated in experiments on cooked egg white [102] between 10 and 60°C. The MT effect was relatively independent of temperature between 25-45°C, suggesting that MT is not limited by diffusion at biological temperatures. Slow diffusion exchange between the bulk and hydration layer water populations became important below 25°C.

Cholesterol in the myelin membrane was first proposed as the dominant component of MT in WM and $T_1$ [103], validated in saturation transfer experiments on solutions of EPC/cholesterol mix [104]. MT was only observed in the presence of both components, and increased non-linearly with cholesterol content. A later study of an *in vitro* model system demonstrated the importance of galactocerebrosides and other lipids in WM relaxation and MT [105]. A substantial effect of pH was also observed in that study, suggesting that chemical exchange could not be dismissed in this model and, by extension, in human WM.

**Quantification of MT**

The MT effect is generally described using models with two (or more) pools, each with a set of Bloch equations [76, 106], including exchange terms based on the mathematical formalism for chemical exchange [107]. Initial models took into account only the longitudinal magnetization, and MT was viewed as a $T_1$-specific process [64, 83, 106]. The transverse magnetization of the liquid protons was eventually included in the model to account for the direct effect of saturation [108], leading to a more accurate estimation of the MT effect.

The exchange terms in the two-pool model equations can be derived from first principles [74]; the formalism applies to dipolar coupling as well, but only to the longitudinal magnetization [7]. Exchange of transverse magnetization can occur by chemical exchange, and has been explored [62, 109]. While transverse exchange is realistic and occurs in systems with significant chemical exchange, it does not need to be explicitly considered for MT in biological systems. Any transverse magnetization in the semi-solid pool is dissipated in a matter of microseconds, and the effect on the liquid pool is indistinguishable from pure $T_2$ relaxation.

While the majority of MT experiments are modeled using two spin baths, other models
have been proposed. Certain models sought to improve the description of semi-solid pool behaviour, by incorporating two separate macromolecular pools, in addition to a liquid pool [110]. Such models were applied to the study of ocular lens [111] and cartilage [112]. A comparison of two- and three-pool models in CW-saturated NMR experiments in a variety of model systems [113] showed that the MT effect depended more strongly on one of the macromolecular pools, and that two semi-solid pools are not necessary. Alternatively, the importance of the hydration layer was studied using a different three-pool model (bulk water, hydration-layer water, and immobile macromolecules) [114]. This model was shown to reduce easily to a two-pool system in cases where the hydration layer population is small, and that the effective exchange constant includes dipolar and chemical exchange, as is the case in tissue. In short, exchange between the hydration and bulk water pools is commonly accepted to be much faster than the interaction between water and macromolecules [115], so MT is usually modeled using one water pool (free, $^1$H$_f$) proton pool and one semi-solid (or restricted, $^1$H$_r$) pool [60]. From the point of view of MRI, mobile and restricted protons may exchange magnetization by dipole interactions or rapid chemical exchange, and the two processes are indiscernible by *in vivo* imaging methods.

Early experiments involving the two-pool model assumed constant and complete saturation of the semi-solid pool, which greatly simplified the signal equations and allowed for quantification of the MT effect with as little as 2 measurements (MT and $T_1$) to produce *in vivo* maps of the MT rate constant [83]. Under this assumption, however, the MT model parameters are power- and time-dependent. Experiments in the ocular lens showed that complete saturation could not be assumed [111], and that neglecting any remaining semi-solid pool magnetization resulted in a saturation-dependent exchange constant, a contradiction of the physical premise of the model.

While the majority of MT imaging is performed in the steady-state, solutions exist to describe the transient approach. Investigations of MT effects incorporating off-resonance saturation transfer into spin-lattice relaxation yielded a method general enough to allow study of transient effects [116]. A simplified version of that model, under realistic ex-
perimental conditions, revealed that separate terms for chemical exchange could be safely neglected, but that the direct effect had to be incorporated [117]. In order to observe an MT effect, it was concluded that the exchange rate must be at least on the order of the liquid proton relaxivity. A solution by projection operators was used to describe the transient effects observed in the approach to steady-state [118, 119], applied to both continuous (CW) and pulsed saturation. This model was extended to treat the semi-solid pool with the Redfield-Provotorov theory [120], lifting the constraint of a Lorentzian absorption lineshape for the semi-solid pool to provide more realistic description of the signal from cross-linked BSA [121]. Measurements based on the approach to steady-state have received limited attention mainly due to data acquisition considerations. One implementation using fast echo-planar-imaging (EPI) readouts has been reported [122], and others have investigated the transient behaviour seeking optimized MT contrast [123, 124].

The two-pool model of MT for MRI, illustrated in Figure 2.4, was formalized and validated by Henkelman and collaborators [125]. Using the notation of Sled and Pike [126], the fully-relaxed absolute sizes of the free and restricted pools are denoted respectively $M_{0f}$ and $M_{0r}$. The time-dependent magnetization components for the respective pools are denoted by $M_{(x,y,z),(f,r)}(t)$. The pools are characterized by their respective relaxation constants, $R_{1f,r} (= 1/T_{1f,r})$ and $T_{2f,r}$. The spins freely exchange longitudinal magnetization, and this is modeled by adding exchange terms to the Bloch equations of each pool. Assuming chemical equilibrium, exchange is represented by a single rate constant $R$, which is scaled by the pool sizes to yield the forward and reverse transfer rates, $k_f (= RM_{0r})$ and $k_r (= RM_{0f})$. Exchange of transverse magnetization is not explicitly modelled, due to the extremely short $T_{2r}$ of the semi-solid pool. For a binary system subjected to off-resonance irradiation of amplitude $B_1(t)$ (nutation frequency $|\hat{\omega}_1| = \gamma B_1$) and offset $\Delta$, the model can be described by the set of Equations 2.7 to 2.10, formulated in a reference frame rotating at the offset frequency $\Delta$ from resonance.
2.3 Relaxation and magnetization transfer in WM

\[
\begin{align*}
\frac{dM_{zf}}{dt} &= -\frac{M_{zf}}{T_{2f}} - \Delta M_{zf} - \text{Im}(\hat{\omega}_1) M_{zf} \\
\frac{dM_{zf}}{dt} &= -\frac{M_{zf}}{T_{2f}} + \Delta M_{zf} + \text{Re}(\hat{\omega}_1) M_{zf} \\
\frac{dM_{zf}}{dt} &= R_{1f}(M_{0f} - M_{zf}) - k_f M_{zf} + k_r M_{zr} + \text{Im}(\hat{\omega}_1) M_{zf} - \text{Re}(\hat{\omega}_1) M_{zf} \\
\frac{dM_{zr}}{dt} &= R_{1r}(M_{0r} - M_{zr}) - k_r M_{zr} + k_f M_{zf} - W M_{zr}
\end{align*}
\]

where \( \hat{\omega}_1(t) \) is the complex, time-dependent pulse envelope of the saturating field.

Equation 2.10 contains a term \( W \) for the off-resonance RF saturation, which for CW off-resonance saturation is the product of the square of amplitude of the applied RF \( (\omega_1 = \gamma B_1) \), and the absorption lineshape of the NMR species:

\[
W = \pi \omega_1^2 G(\Delta).
\]

This treatment is accurate for CW off-resonance saturation, as the off-resonance frequency and CW amplitude are constant, and the bandwidth of the irradiation is very narrow.

Figure 2.4: Two-pool model of magnetization transfer. \(^1\text{H}_f\) represents the “free” pool associated with bulk and hydration water molecules. \(^1\text{H}_r\) denotes the motionally-“restricted” macromolecular proton pool.
For a steady-state CW saturation experiment, Equations 2.7-2.10 can be decoupled yielding a closed-form solution for the observable magnetization of the liquid pool, $M_{zf}$. To validate this solution, CW off-resonance saturation measurements were performed in agarose gels and a water phantom doped with paramagnetic manganese ($\text{Mn}^{2+}$) ions [125], varying the MT saturation parameters $\omega_1$ and $\Delta$ to map the Z-spectrum. The signal equation was fitted to this data to yield two-pool model parameters. Isolation of the fundamental model parameters required a separate IR measurement of $T_{1\text{obs}}$, which can be expressed as a function of the MT parameters.

Strict Bloch formalism results in a Lorentzian lineshape for $G(\Delta)$ [76, 83, 106]; alternatively, the absorption lineshape of the semi-solid pool can be adjusted to suit the needs of the experiment. The restricted pool for agar was modelled by a Gaussian lineshape [127], an empirical choice inspired from NMR absorption lineshapes observed in solids, which improved fit quality over the Lorentzian lineshape not only in agar, but in BSA and cartilage as well. Semi-solids in WM are better described by using a super-Lorentzian (SL) lineshape [128] which arises in partially ordered materials such as lamellar liquid crystals [129] and lipids [130], and is defined in Equation 2.12.

$$G(\Delta) = T_{2r} \int_0^1 \frac{1}{|3u^2 - 1|} \exp \left[ -2 \left( \frac{2\pi \Delta T_{2r}}{3u^2 - 1} \right)^2 \right] du . \tag{2.12}$$

The interpretation of the SL lineshape in biological tissue can be understood by considering the various Hamiltonians that determine the NMR spectrum [131], where the restricted motion of membrane phospholipids and proteins results in angular-dependent interactions, captured by the dipolar factor included in the SL equation. The SL lineshape has resulted in better fits of the two-pool model to data from in vitro solutions of EPC and cholesterol, bovine WM [132], and from various tissues (such as WM, GM, optic nerve, liver, muscle, blood, and CSF) [131]. Alternatives exist to the SL lineshape [133–135], but these are more complex and are described by more than a single parameter, making their computation and interpretation too complex for routine applications.

Pulsed saturation has become the preferred technique for in vivo MT imaging [90–94].
Pulsed saturation was investigated and compared to CW saturation in a set of simulations and experiments on agarose gels [136]. The general treatment of arbitrary pulses was formalised, and the average saturation rate of a shaped pulse with narrow-bandwidth can be computed from the instantaneous pulse power as per Equation 2.13, where $\Delta$ is taken as the central frequency of the pulse, and $T$ is its duration.

\[ \langle W \rangle = \pi \gamma^2 \frac{1}{T} \int_0^T B_1^2(t)G(\Delta)dt \]  

(2.13)

By inspection of Equation 2.13, the instantaneous saturation rate of the pulse can be written as:

\[ W(t) = \pi \omega_1^2(t)G(\Delta) = \pi [\gamma B_1(t)]^2 G(\Delta) \]  

(2.14)

*In vivo* QMTI was developed independently by three groups. The following sections review the techniques for mapping of MT parameters with pulsed off-resonance saturation, the signal equations used to analyse the data, and a brief survey of *in vivo* observations. Details of on-resonance and selective inversion techniques [67–71] are beyond the scope of this review.

**Mapping of MT – Techniques and Sequences**

The spoiled gradient echo (SPGR) imaging sequence with pulsed off-resonance saturation is the most widely used sequence for *in vivo* mapping of MT model parameters. It takes advantage of long restricted pool $T_1$ recovery by using short MT pulse repetition times (duty cycles of 30 to 50 %). The MT-weighted SPGR sequence is illustrated in Figure 2.5. It uses a non-selective shaped off-resonance MT pulse (Gaussian [137–140] or Hanning-windowed Gaussian [141]) for saturation of the restricted pool, followed by a small-angle excitation pulse and a gradient echo readout. Protocol parameters are often selected to produce a PD-weighted baseline (without MT saturation). Single-slice [141], 2D multi-slice with long repetition time (TR) [137], and slab-selective 3D versions [139, 140, 142] have all been used. Partial $k$-space acquisition has also been used to accelerate the acquisition [137]. In most cases, a single protocol is used to acquire all MT-weighted images, with
one exception where two TRs were used while maintaining average saturation power [141]. A series of images are acquired while modulating the saturation parameters, in a manner similar to [125]: acquisitions range from 4 to 60 MT-weighted images. Saturation parameters are selected for the data acquisition to suit the signal equation, which is then used to produce maps of the tissue parameters. In some cases, offset frequencies and flip angles must be selected to accommodate limitations of the model [139], or by making reasonable assumptions about model parameters [140]. The separate measurement of the apparent longitudinal recovery rate $R_{1obs}$, required to constrain all the model parameters, is usually performed either with the Look-Locker technique [141], or with the variable nutation method [48, 137, 139].

Optimal data acquisition of QMTI data, in particular the sampling of the Z-spectrum, has received limited consideration. Limited benefit is obtained from using more than two pulse powers, and using two TRs may result in more precise estimates of $k_f$ [141]. In the lone exception, optimization of quantitative MT measurements was achieved using the theory of Cramer-Rao lower bounds (CRLB) [143], to search for optimal combinations of saturation pulse flip angles and offsets, using the CWPE model for pulsed saturation
showed a large improvement in parameter standard deviation, and visibly improved quality of the parameter maps from \textit{in vivo} experiments.

**Signal equations for pulsed MT imaging**

The use of pulsed saturation in imaging requires an accurate description of signal behaviour, which is slightly more complex to model than that of CW off-resonance saturation. Signal description should consider the pulse design, the saturation power, the frequency of irradiation, and duration (or duty cycle) of irradiation. The various approaches to QMTI with pulsed off-resonance saturation are based on the two-pool model for MT, but differ in their treatment of the pulsed nature of imaging sequences, the semi-solid pool saturation, and the consideration of direct saturation of the liquid pool. While equations 2.7 through 2.10 can be solved directly by numerical methods for the pulsed MT sequences, applying Equation 2.14, their complexity makes this time-consuming, especially for parameter estimation.

As a first approximation, the solution for steady-state CW saturation \cite{125} can be used, approximating pulsed saturation by CW saturation of equivalent power \cite{144}, \textit{i.e.} setting $\omega_1^2$ in Equation 2.11 equal to the pulse power $\bar{P}_{RF}$ averaged over the repetition time. The constant-wave-power-equivalence method (CWPE) is a refinement on that approximation that takes into account the pulse shape \cite{145}. The factor $\omega_1$ in Equation 2.11 is replaced with $\omega_{CWPE}$:

$$\omega_{CWPE} = \gamma B_{1,CWPE} = \gamma \sqrt{P_{sat}} = \sqrt{\frac{p_2 \theta^2}{p_1^2 \text{TR}' \gamma \tau_{sat}}}$$

(2.15)

where $\tau_{sat}$ and TR' are the duration and repetition time of the saturation pulse, respectively; $p_1$ and $p_2$ are parameters related to the pulse shape; and $\theta$ is the resulting angle if the pulse is applied on-resonance. $P_{sat}$ is the mean squared saturation field of the pulse, adjusted for the pulse shape, and the CWPE $B_1$ field ($B_{1,CWPE}$) is the root mean square of the average saturating field. The CWPE approach was used by Ramani \textit{et al.} to derive a solution for pulsed MT experiments \cite{146}. The signal equation was also modified by the introduction of the bound pool fraction, denoted here by $f_b (= M_{0r}/(M_{0f} + M_{0r})$. While a
Gaussian lineshape was originally selected to describe the semi-solid pool behaviour, this has since been replaced by a super-Lorentzian [147].

More detailed signal equations have been derived for spin echo [97] and spoiled gradient echo sequences [98] with pulsed saturation, resulting in accurate signal description in gel phantoms and human brain tissue. In the work of Sled and Pike [126], the free pool magnetization was normalized to 1, and $F$, the ratio of semi-solid to liquid pool sizes, was defined as in Equation 2.16.

$$F \equiv \frac{M_{0e}}{M_{0f}} = \frac{f_b}{1 - f_b}$$ (2.16)

The restricted pool was modeled by an equation for the inverse spin temperature, from the Redfield-Provotorov theory [120], as proposed by [121]. Solutions to special cases of the Bloch equations were developed, allowing for rapid estimation from closed-form solutions. The SPGR sequence was decomposed into periods of free-precession of both pools, instantaneous saturation of the free pool, and off-resonance CW irradiation of the semi-solid pool. In these cases, Equations 2.7 to 2.10 are reduced to first-order equations with constant coefficients which have closed-form solutions under (periodic) steady-state conditions. Solutions were derived for a modified CW approximation, and a rectangular pulse (RP) approximation. In the former, pulsed saturation of the semi-solid pool was averaged over the entire TR. In the RP approximation, illustrated in Figure 2.6, the shaped pulse was replaced by a rectangular pulse with equivalent power and offset frequency, and a duration equal to the full-width-at-half-maximum of the shaped pulse. In both cases, the effects of off-resonance pulses on the free pool were reduced to instantaneous fractional saturation of the longitudinal magnetization, and on-resonance pulses were assumed to have no effect on the restricted pool. Off-resonance saturation of the restricted pool was computed with Equation 2.11, where $\omega_1$ was the amplitude of the equivalent rectangular pulse, and $\Delta$ was the frequency offset from resonance. Corrections were applied to the results to compensate for static and transmit RF field non-uniformities [141, 148]. Experiments on agar gels agreed with previously published data [125] and confirmed that the dipolar term in the theory could be safely neglected. This methods was validated with in vitro experi-
ments on uncooked beef – selected for its homogeneity and super-Lorentzian lineshape – and extended to in vivo imaging.

Figure 2.6: Breakdown of MT spoiled-GRE sequence into periods of free precession, on-resonance free pool saturation, and off-resonance restricted pool saturation (from [141]).

In the work of Yarnykh [139], the signal was described using a modified version of the model proposed by Graham et al. [136], and the method of Listerud [149]. The direct effect on the free pool was ignored, and thus $T_{2f}$ could not be determined. The effect saturation on the solid pool was also modeled with a rectangular pulse approximation, but with the same duration as the shaped pulse (as opposed to the FWHM). Simulations were performed to demonstrate the validity of the model for data acquired far off-resonance. The solid pool $T_2$ did not vary greatly, and fixing its value seemed to have little impact on the results. This model was later extended to include direct saturation effects [140], and with additional assumptions regarding model parameters $T_{2f}$ and $T_{2r}$, resulted in whole-brain mapping of the other parameters in a time acceptable for clinical imaging.

Portnoy and Stanisz [89] performed a review and comparison of three signal equations for pulsed MT experiments [137, 139, 141]. The minimal approximations MT (MAMT)
model was introduced to describe saturation pulses: it partitions the shaped pulse into very small (50 $\mu$s) increments, allowing for more accurate signal computation. All three models were fit to simulated data generated with the MAMT model, and experimental data acquired in wild-type and *shiverer* mice. MT estimates from CW saturation experiments were used as the gold standard. All three models agreed with the MAMT model above roughly 2-3kHz off-resonance, and approximations made by each model were found to be robust over the prescribed range of data for each. The Sled-Pike RP model was the most accurate, down to roughly 0.1 kHz, while the Ramani model had a tendency to underestimate signal below 1 kHz. Estimates of $M_0r(F)$ and $T_2r$ were consistent, while $R$ and $T_2f$ showed some small discrepancies between the three models.

Another comparison of three pulsed MT signal equations (Sled and Pike’s CW and RP solutions, and Ramani’s solution) was performed with simulations and *in vivo* imaging [150], focused on the effect of noise and sequence parameters (duty cycle, $T_1$-weighting) on model parameter estimates. The Sled-Pike CW model was most robust and accurate over a range of duty cycles and $T_1$-weighting. Ramani’s model fared better at low SNR but was more affected by duty cycle. The differences between models were below the between-voxel standard deviation observed in ROIs.

**In vivo MT parameters**

Initial demonstrations of QMTI methods were performed in healthy control WM, MS, and cancer [137, 139, 141], and included validation of the technique, either in model systems or postmortem tissue. In healthy controls, initial estimates of $F$ were approximately 0.16 in WM, and around 0.06 in GM [141]. $k_f$ values were about 4.5 s$^{-1}$ in WM, and about 2.3 s$^{-1}$ in GM. $T_{2f}$ values were longer in GM (55 ms) than in WM (about 34 ms). $T_{2r}$ values were relatively constant across tissues ($\sim$ 12 $\mu$s). Others reported slightly lower values of $F$ in WM (0.10 - 0.13), yet similar values in GM [139]; the same study reported larger exchange rates in all tissues (5.5 to 7.5 s$^{-1}$ in WM, 3.6 - 4.8 s$^{-1}$ in GM), and smaller estimates of $T_{2r}$. In general, MS lesions in fixed postmortem human brain had dramatic
2.3 Relaxation and magnetization transfer in WM

decreases in $F$ (0.02 in lesions vs. 0.16 in WM), increases of $T_{2r}$ (20 $\mu$s in lesions vs. 13 $\mu$s in WM), and generally reduced free pool relaxation times due to formalin fixing [137]. In vivo, MS lesions had decreased semi-solid pool size ratio ($F = 0.09 - 0.11$, vs. 0.16 in NAWM and 0.19 in WM [137]) and $T_{2r}$. Evidence of a relation to lesion age was also reported [141]. Glioma pathology had a lower exchange rate compared to MS lesions, but similar semi-solid pool ratio. Example maps of QMTI parameters $F$, $k_f$, $R_{1f}$, $T_{2f}$, and $T_{2r}$ from a healthy control are presented in Figure 2.7, along with the associated map of $T_{1obs}$.

Normative regional values for the MT pool sizes, exchange, and relaxation parameters were reported in seven healthy subjects at 1.5 T [151]. $F$ ranged from 10.6 to 14.2 % in WM, and 6.2 - 7.0 % in GM; $k_f$ ranged from 3.7 to 4.8 s$^{-1}$ in WM ROIs, and 1.7 to 2.5 s$^{-1}$ in GM. $T_{2r}$ did not differ according to tissue type, ranging from 9.9 to 11.8 $\mu$s. $R_{1f}$ was much greater in WM (1.6 - 1.9 s$^{-1}$) than in GM (1.0 - 1.2 s$^{-1}$). Significant variations were found among WM and GM regions, and symmetry was observed between left/right homologous ROIs. A methodology optimized for fast acquisition reported consistent parameter estimates and highlighted WM fibre tracts in three healthy volunteers [140]. Initial studies using the Ramani protocol reported consistently lower values of $F$ ($\sim 9$-11 %), but similar values $T_{2r}$ of 11.4 - 13.4 $\mu$s, compared to other studies [137, 142]. These studies also reported variable success at constraining the exchange rate.

Quantitative MT measurements have been performed with the CW method in a variety of tissues in a spectrometer at 3 T et al. [88]: $F$, $k_f$, $R$, and $T_{2r}$ were very similar to values reported at 1.5 T. An pulsed MT imaging study reported composite parameters at 3 T (that is, without a measurement of $T_{1obs}$), with a view on optimizing MT contrast in MTR acquisitions. These observations were also consisten with measurements reported at 1.5 T.

Whole brain mapping of QMT in 7 controls and 20 MS patients [152] showed reduced $F$ and $T_{2r}$ in lesions ($0.09 \pm 0.02$ and $15.9 \pm 1.0 \mu$s, respectively) versus control WM ($0.19 \pm 0.01$ and $18.4 \pm 0.5 \mu$s, respectively). A larger study (60 MS patients and 27 control) reported even lower average values in lesions ($F = 0.05$) [138], but significant increases of $T_{2r}$ in lesions ($10.6 \mu$s vs. 11.5 $\mu$s). In general, no differences have been observed between
Figure 2.7: Example maps of QMTI parameters $F$, $k_f$ (s$^{-1}$), $R_{1f}$ (s$^{-1}$), $T_{2f}$ (ms), and $T_{2r}$ ($\mu$s), acquired from a healthy control at 1.5 T. The associated map of $T_{1obs}$ (s) is also presented. As can be seen here, $F$ and $k_f$ are highest in WM where the MT effect is strongest, and near essentially zero in CSF, located in the ventricles. GM presents lower $F$ and $k_f$, reflecting the lower myelin content. The small patch of signal in the upper left corner is a vial of water doped with paramagnetic ions, where $F$ is correctly estimated as 0. The data acquisition time for these maps was just under 45 minutes.
parameter values in lesions from different MS patient subgroups. It has been suggested that changes in the parameters \( f_b, T_{2r}, \) and \( (R_{1,f}T_{2f})^{-1} \) are potentially specific to pathology in lesions. A cross-sectional study of MS patients investigated the nature of the chronic \( T_1 \) hypointensities observed on clinical scans of MS patients [153], thought to be indicative of greater tissue destruction. \( F \) values in lesions ranged from 0.03 to 0.12 in all lesions, but only down to 0.06 in \( T_1 \) w isointense lesions.

NAWM has slightly smaller \( F \) versus control WM [152], and is thus potentially sensitive to alterations in NAWM. This has been reproduced in two studies [138, 154], where the mean value of \( F \) was significantly reduced by approximately 10% in NAWM relative to controls. Mean \( T_{2r} \) estimates are generally the same in control WM and MS NAWM. As is the case for lesions, MS subgroups are not differentiated on the basis of MT parameters in NAWM. Axonal injury in NAWM, evaluated with MR spectroscopic imaging of axonal metabolites (N-acetylaspartate, NAA), was shown to correlate with tissue destruction in lesions evaluated with \( F \), perhaps reflecting lesion-induced Wallerian degeneration in the NAWM [154]. On the other hand, QMTI has not revealed any significant differences between the GM of MS patients and healthy controls [152].

QMTI of postmortem fresh MS brain showed \( f_b \) (and thus, \( F \)) to be strongly correlated with Luxol Fast Blue (LFB) histological staining [155]; furthermore, \( f_b \) was sensitive to differences between NAMW, demyelination and remyelination. The semi-solid pool \( T_{2r} \) was not indicative of any histopathological measures. The effect of formalin fixation on some of these measures was also investigated [156], revealing increased \( F \) after formalin fixation. QMT measurements have also been performed in murine brain, with pulsed saturation, and the results were used to optimize MTR acquisitions [157]. The semi-solid pool ratio has also been shown to be sensitive to demyelination in the cervical cord of individuals affected by adrenomyeloneuropathy [158].

In summary, the MT effect in WM can be exploited to obtain information about biological macromolecular content indirectly. The important role of myelin in determining WM MT has been established. In this work, the interpretation of MT from a more complete
model of WM is explored in Section 2.3.4 and Chapter 3. An evaluation of the longitudinal variability of the QMTI method will be presented in Chapter 4, and results of its application in vivo to study a dynamic phase of MS lesions are presented in Chapter 6.

2.3.3 $T_2$ relaxation in WM

$T_2$ relaxometry

The spin echo (SE) experiment produces $T_2$-weighted MRI signal that can be modulated to permit estimation of the $T_2$ characterizing a region-of-interest in the imaging volume, or on a voxel-wise basis. Simple estimation of $T_2$ can be achieved by using a dual-echo SE sequence to sample the decay curve at two time points, and computing $T_2$ from Equation 2.5. Accurate $T_2$ mapping is usually performed using SE sequences with at least 32 echoes. Acquisitions are based on the Carr-Purcell-Meiboom-Gill (CPMG) sequence [159, 160]. In this type of experiment, originally devised by Carr and Purcell [159] based on Hahn echoes [161], a $90^\circ$ is followed by a set of $180^\circ$ pulses, creating a series of spin echoes. The scheme was modified by Meiboom and Gill [160] to use $180^\circ$ pulses, reducing the cumulative errors from non-ideal $180^\circ$ pulses. Increasing the number of echoes allows for more extensive sampling of the decay curve, and more reliable fitting. However, increasing the number of echoes is more demanding on sequence design, as the impact of the measurement on the relaxation is greater. Spectrometer experiments, not constrained by imaging gradients, can incorporate thousands of tightly-spaced echoes.

As a first approximation, relaxation in biological tissue can be described with mono-
2.3 Relaxation and magnetization transfer in WM

Figure 2.8: Illustration of the multi-echo spin-echo MRI sequence, with Poon-Henkelman gradient scheme. The refocusing pulses in this example follow the CPMG convention; these are often replaced by $90^\circ_x-180^\circ_y-90^\circ_x$ combination, for reduced sensitivity to transmit and static field inhomogeneities.

exponential decay (Equation 2.5) using a single “observed” $T_2$ ($T_{2obs}$), which has been measured in various tissues [36]. Disease pathology such as tumors, inflammation, or tissue degeneration yield noticeably different $T_{2obs}$ values compared to healthy tissue, which reflect the different molecular and/or cellular composition of these pathologies. However, the mono-exponential model is too limited to accurately describe tissues such as WM and muscle.

$T_2$ decay of certain tissues exhibits multiple observable components, dependent on the complexity of the local spin environment. Multi-exponential spin-spin decay was predicted very early [5, 13]. Although initial attempts failed to isolate more than one component in tissue [165, 166], multi-component $T_2$ measurements have been proposed [167, 168], demonstrated in in vitro models and ex vivo animal systems [169, 170], and used to map tissue characteristics and [171–173]. The remainder of this section surveys multi-component analysis techniques, and closes with a discussion of the major findings. Further details on these methods, their application to WM imaging, and findings with respect to WM microstructure, are also available in recent reviews [173, 174].
Analysing multiple $T_2$ components

In structurally complex biological tissue, MRI signal arises from almost liquid-like free water existing in multiple distinct states, most likely in exchange with each other: this compartmentalization results in multi-exponential signal decay. A sufficient number of echoes must be collected (e.g. $N = 32$) with enough SNR to allow differentiation of the components. The sampled NMR signal at time $t$ from a given distribution of $T_2$ components, $S(T_2)$, can be represented via integration of $S(T_2)$ over the range of observable $T_2$ values:

$$y(t) = \int_{T_2,\text{min}}^{T_2,\text{max}} S(T_2)e^{-t/T_2}dT_2 + \beta \quad (2.17)$$

where $\beta$ is a baseline signal value, which can be arbitrarily set to zero.

Analysis of transverse relaxation data using $T_2$ distributions is generally done using least-squares methods. Due to noise in the data, the basic least-squares solution (in essence, an inverse Laplace transform) is likely to contain spurious fine detail. A general method was first proposed to select the most appropriate solution by statistical means [175]. Smooth solutions were obtained by adding a “regularization” term to the minimization function, and the fits were performed using the CONTIN method [176, 177]. Simulations and experiments on water phantoms doped with paramagnetic agents indicated that with an SNR of 150, resolution of $T_1/T_2$ peaks required these to be separated by a factor of at least 4. Three methods for the multi-component analysis of NMR relaxation data were later explored [178]: non-negative least-square (NNLS), least-distance programming (LDP), and linear programming (LP). These were applied to simulations and decay data from an experimental model (red cedar), using a logarithmically-spaced $T_2$ distribution. The outcome favoured the NNLS [179] with regularization, which is explained as follows. The distribution $S(T_2)$ from Equation 2.17 can be represented discretely over a range of $M$ possible $T_{2,j}$ values using delta functions modulated by the amplitudes $S_j$:

$$S(T_2) = \sum_{j=1}^{M} S_j \delta(T_2 - T_{2,j}) \, , \quad (2.18)$$
which in turn fully discretizes the signal equation for fitting to the acquired data samples \(y(t_i)\):

\[
y(t_i) = y_i = \sum_{j=1}^{M} S_j e^{-t_i/T_{2,j}}. \tag{2.19}
\]

The NNLS method minimizes the \(\chi^2\) value of the fit by isolating the best-fit model, with a positive-signal \((S_j > 0)\) constraint:

\[
\min \left\{ \sum_{i=1}^{N} \left| \sum_{j=1}^{M} A_{ij} S_j - y_i \right|^2 \right\}. \tag{2.20}
\]

In this case, the \(A_{ij}\) are kernels for exponential decay, \(\exp(-t_i/T_{2,j})\).

In situations of high SNR, the presence of noise can be accounted for by allowing the misfit to increase and reach the statistical “balance” point \((\chi^2 \approx N)\) between an overly structured solution \((\chi^2 \ll N)\), and an overly smooth solution which may lack information \((\chi^2 \gg N)\). An additional constraint, the regularization term, is added to the NNLS method to force a smoother distribution, as described by the second term in Equation 2.21. This constraint can take the form of the energy of the distribution, its derivative, or its curvature. The parameter \(\mu\) governs the level of smoothing applied to the solution. Iterative, controlled increase of the total misfit has been deemed the most suitable technique for determining \(\mu\) in low SNR studies [180]

\[
\min \left\{ \sum_{i=1}^{N} \left| \sum_{j=1}^{M} A_{ij} S_j - y_i \right|^2 + \mu \sum_{k=1}^{K} \left| \sum_{j=1}^{M} H_{kj} S_j - f_k \right|^2 \right\}. \tag{2.21}
\]

An illustration of a distribution resulting directly from a standard NNLS fit, and a smooth distribution (regularized using an energy term) is presented in Figure 2.9.

There are a few metrics derived from the \(T_2\) distribution that yield important information about the tissue micro-anatomy. The portion of signal at short \(T_2\) values is usually assigned to myelin water: this is quantified as the myelin water fraction (MWF), the fraction of signal in the myelin water \(T_2\) range divided by the total distribution signal, as expressed in Equation 2.22.
Figure 2.9: *Top:* SE decay data, with mono-exponential fit (green), and multi-exponential fit with NNLS (blue), and regularized NNLS (red). The data are from a WM ROI (major forceps) acquired in a healthy subject at 1.5 T. The direct and constrained NNLS fits are indistinguishable. *Bottom:* Plots of example and constrained $T_2$ distributions. The NNLS fit is plotted in blue, and the red line is NNLS regularized with the energy of the distribution, resulting in a 2-2.5% increase of the $\chi^2$ value. Both distributions have been normalized so that the maximum value is 1.
2.3 Relaxation and magnetization transfer in WM

\[
MWF = \sum_{T_{MW,\text{max}}}^{T_{MW,\text{min}}} S(T_{2,j}) \frac{\sum S(T_{2,j})}{\sum_{j=1}^{N_{MW}} S(T_{2,j})} \left(\sum_{j=1}^{M} S(T_{2,j})\right) (2.22)
\]

There is no set definition for the range of myelin water \(T_2\)s. Ideally, only the short \(T_2\) peak signal should be retained; however, because of the vagaries of the regularized NNLS analysis, and also due to biological variability, this peak is not necessarily easy to identify. The widely adopted solution is to simply define the \(T_2\) range with hard limits, most often 10-50 ms.

The geometric mean is the natural choice for a distribution of values defined on the logarithmic scale; \(\langle T_2 \rangle\) (Equation 2.23) yields information about the centroid of the distribution. The \(T_2\) values of the peaks in the distribution can be similarly defined as the geometric mean of the distribution over a limited range of \(T_2\) values.

\[
\langle T_2 \rangle = \exp \left[ \frac{\sum_{j=1}^{M} S(T_{2,j}) \log(T_{2,j})}{\sum_{j=1}^{M} S(T_{2,j})} \right] (2.23)
\]

Observations of multiple \(T_2\) components

Multi-component \(T_2\) distributions have been extracted from spin-echo data acquired in \textit{in vitro} cat brain [170], collected in a spectrometer using the CPMG method with 16,384 echos with 0.4-ms spacing. Four components were observed in the WM: a short \(T_2\) component (12.7 ms, representing a 6.8% fraction of the total signal), assigned to hydration water within myelin in slow exchange with cellular water; a major component (89 ms, 86% of the signal), attributed to water in fast exchange between the bulk cellular water and hydration sites on both the soluble proteins and cellular cytoskeleton; and finally, two very small components, one with ultrashort \(T_2\) (\(\approx 1\) ms, \(\approx 4\)-5% of the signal) assigned to phospholipid hydrogen, and one with long \(T_2\) (\(\approx 340\) ms, \(\approx 2\)% of the signal), with an assignment left open to speculation. It was noted that an SNR greater than \(10^3\) was required to separate the different \(T_2\) components, and that signal acquisition with many echos extending well into the noise was required to robustly establish the long-lived component.
In vivo SE MRI has revealed three components in human brain [171]: a short $T_2$ component located in the 10-55 ms window, accounting for about 16% of the signal in WM, 4% in GM and 4-6% in MS lesions, associated with water compartmentalized within the myelin bilayer (the MWF); an dominant $T_2$ component located in the 70-95 ms range, attributed to water in cytoplasmic and extracellular spaces; and a small-amplitude long $T_2$ component (1 s or more), assigned to the presence of CSF. The short $T_2$ component was assigned to myelin water based on in vitro observations, and its spatial distribution was observed to scale qualitatively with the distribution and density of myelin. The ultrashort component previously observed was not observed directly in MRI, reflecting the relatively long first echo time ($TE_1 = 10$ ms), in agreement with observations of water $T_2$s in the human brain [32].

Normative observations of QT2 in 12 healthy controls [172] reported MWFs between 8.4% and 15% in WM, averaging 11.3%. Geometric mean $T_2$, $\langle T_2 \rangle$ (Eq. 2.23), was 77.4 ms in WM [range 70-83 ms], and 80 ms in GM [range 74-87 ms]. The width of the main water peak was more than double in WM (33 ms) compared to GM (14 ms). Total water content (in g/ml) can also be estimated by integrating the $T_2$ distribution, correcting for incomplete $T_1$ relaxation due to the finite repetition time of the imaging sequence, and scaling by the signal in an external water standard doped with paramagnetic ions. In the same study [172], total water content in WM was 0.71 g/ml, with a range of 0.70 to 0.72 g/ml, and 0.83 [0.79-0.87] gm/l in GM. Example maps of the MWF and $\langle T_2 \rangle$ from a healthy individual are shown in Figure 2.10.

Advancements in QT2 methodology have yielded moderate improvements in the observations. Filtering has been used to improve the quality of myelin water maps, by decreasing the noise in MW maps acquired with the necessary minimum SNR [181]. The method has also been used with a variable-TR scheme to enable the acquisition of 48 echoes [182]. Direct imaging of the short $T_2$ component has been proposed as an alternative to full $T_2$ quantification, either by linear combination filtering [183, 184], or by selective excitation of the short $T_2$ component [185, 186], but these methods have not found significant ap-
Figure 2.10: Example maps of the MWF and $\langle T_2 \rangle$ (ms), acquired from a healthy control at 1.5 T. As can be seen on the left, the MWF is highest in WM regions, very low in GM regions, and essentially zero in the CSF, in accordance with myelin content. Note the relatively noisy appearance of the map, reflecting the variability of MWF estimates. The $\langle T_2 \rangle$ is shortest in WM, closely followed by GM, and much greater in the CSF. The data acquisition time is approximately 25 minutes.
plication and are beyond the scope of this review. Alternatively, QT2 imaging has also been achieved with a magnetization-preparation scheme [187]. Recent QT2 measurements performed in a variety of tissues in a spectrometer at 3 T et al. [88] showed good correspondence with measurements reported at 1.5 T. $T_2$ values were consistent with those at 1.5 T, as expected since $T_2$ should not be affected by field strength. QT2 imaging at 3 T has also recently been reported [188], resulting in improved MWF map SNR, but also significantly larger estimates of the MWF in vivo when compared to 1.5T.

Significant decreases in myelin water content and increases of $T_2$ in MS lesions [171] supported the hypothesis that demyelination could be indirectly measured in vivo using QT2, not only in lesions but in WM in general. Significant $\langle T_2 \rangle$ increases in NAWM ROIs of MS patients (range 72 to 81 ms) versus WM ROIs in controls (range 67 to 80 ms) were attributed to diffuse pathology [189]. A study of 18 controls and 33 patients, showed that this increased $\langle T_2 \rangle$ in NAMW was related to significantly increased total water content and decreased MWF [190]. The MWF in that study ranged from 7.3 % to 15.6 % in WM regions of controls, and 1% to 4.2 % in GM regions. Lesions had even greater decreases in the MWF. This paper also presented a model of the effects of edema, demyelination, and cellular infiltration on the $T_2$ spectrum. In more recent work using the 48-echo technique, QT2 analysis has revealed an additional signal component with a $T_2$ longer than the intra/extracellular peak in patients affected by MS and phenylketonuria [191, 192]. While chronic $T_1$ hypointensity is proposed as a marker of more severe tissue damage, myelin water measurements revealed no differences between chronic $T_1$-hypointense and $T_1$-isotense lesions [193].

Imaging and histopathology of formalin-fixed postmortem brains of MS patients have demonstrated the correlation between the MWF and histological tissue staining by Luxol Fast Blue (LFB), a marker for lipids (dominated by myelin lipids in WM) [194, 195].

Studies of peripheral nerve samples and animal models of injury and disease have provided further support to the interpretation of multi-component $T_2$ relaxation in biological tissue. QT2 measurements in peripheral nerve were initially proposed as a means to over-
come contrast limitations due to the muscle signal [196]. Three stable components were observed in peripheral nerve: a first at 16-19 ms (16-26% of signal), a second at 63-78 ms (29-48% of signal), and a third at 247-317 ms (36-45% of signal). These were assigned to water (i) trapped inside the myelin sheath, (ii) in axonal space, and (iii) in extracellular space. A study of Wallerian degeneration in peripheral nerve caused by crush injury [197] resulted in the general merger of the peaks in the distribution, supporting the assignment of components.

QT2 was combined with histomorphometric analysis in a study of experimental models of tissue injury in rat sciatic nerve [198]. Demyelination, inflammation, and Wallerian degeneration caused by either transection or crushing of the nerve resulted in an obvious change in the $T_2$ distribution; moreover, the short $T_2$ component was observed to reflect the total (as opposed to healthy) myelin content determined by histological staining. Myelin regeneration, which follows crush injury, was identified post hoc by the position of the intermediate and long $T_2$ components. QT2 was also performed at 1.5 and 4 T in guinea pig brain [199], to study experimental autoimmune encephalomyelitis (EAE), a model of MS in animals. Histopathological correlations in that study supported the link between MWF and myelin content [200].

In summary, the multi-component nature of $T_2$ relaxation in WM has been reproducibly observed, and shown to reflect the compartmental nature of tissue microstructure. Despite its expanding use in the study of MS, questions regarding the reproducibility and interpretation of the measurements in vivo remain. The interaction of multi-component $T_2$ and MT are addressed next and in Chapter 3. Questions regarding the variability of the QT2 method will be addressed in Chaper 5. Finally, results of its application in vivo to study a dynamic phase of MS are presented in Chapter 6.

### 2.3.4 A unified view of relaxation and MT in WM

Relaxation and MT in biological tissues are best described by compartmental models which represent hydrogen spin populations associated with water in microanatomical compart-
ments or macromolecules in tissue membranes. These assignments are generally supported by the results of NMR studies of in vitro tissue and tissue models, histopathology, and in vivo studies of healthy and diseased WM. The most basic compartmental model which encompasses all of the relaxation and MT features of WM has four pools [201]: two water compartments (myelin water and intra/extracellular water) and 2 semi-solid pools, each associated with one of the water compartments (myelin and non-myelin). The model incorporates exchange of water between the compartments, and MT between each water/semi-solid pair. This section reviews the handful of experiments, mainly in vitro, that have combined relaxation and/or MT measurements to study WM, and the properties of the four-pool model of WM.

**Combined and multi-dimensional studies of ex vivo systems**

MT-weighted $T_2$ distributions were measured in freshly excised rat muscle, porcine WM, and bovine WM using 6,000-echo CPMG experiments (with 0.2-ms echo spacing) prepared with off-resonance CW saturation, in a spectrometer at 1.5 T [201]. Combined relaxation measurement of $T_1$ and $T_2$ values were also performed using an inversion-prepared CPMG sequence. $T_2$ distributions were estimated from the CPMG data for the reference and MT-prepared decay curves. WM exhibited a small short-$T_2$ component (8.5 % of the signal with $T_1/T_2 = 15/540$ ms) and a much larger long-$T_2$ component (90 % of the signal with $T_1/T_2 = 108/740$ ms), while the distribution was reversed in muscle. The fractional signal of each $T_2$ component was computed for each MT preparation. After isolation of the MT effect from the direct effect of saturation, the observed MT effect was equal for both $T_2$ components in WM, while in muscle the short $T_2$ component exhibited greater MT effects than the long $T_2$ component. While the proposed four-pool model was not quantitatively applied to the data, it was used to illustrate the compartmental nature of relaxation and MT in tissue. No combination of parameters could explain the identical MT effect for the different mobile pools, and this was treated as evidence that the mobile pools must to be connected. On the timescale of multi-echo $T_2$ experiments, water molecules diffuse...
greater distances than cellular dimensions (about 50 µm in 200 ms); the observation of multi-component relaxation suggests that water diffusion is restricted on that timescale.

Spectrometer studies of MT, $T_1$, and $T_2$ in peripheral nerve explored the effect of multiple communicating water compartments on MT in WM [202], using saturation-recovery (SR)-prepared CPMG measurements. $T_2$ distributions showed three distinct $T_2$ components (myelin, axons, and extra-axonal spaces). While simultaneous fits did not isolate the $T_1$-$T_2$ components, the $T_1$ distribution was obtained from the saturation recovery curve of each peak in the $T_2$ distributions. Each $T_2$ component had its own identifiable $T_1$ value. The short $T_2$ signal exhibited biexponential $T_1$ recovery, while the other two exhibited mono-exponential recovery. MT-prepared CPMG measurements were acquired in the transient and steady states. The transient data for each of the $T_2$ peaks were analysed with a model of MT to yield their MT exchange constants. The short and intermediate $T_2$ components had similar steady-state MT (but not the long $T_2$ component), but their respective transient MT behaviour was different.

Near-complete characterization of the four-pool model of WM was obtained from investigations of multi-component relaxation and MT in bovine optic nerve at 1.5 T using CW-prepared CPMG experiments (with 1,000 1-ms echoes) [203]. The MWF and $F$ reported in bovine optic nerve were about double that seen in human WM. Model behaviour was described by coupled equations for each compartment, with terms for MT and compartmental water exchange. The time evolution of the signal was described in two stages, for saturation preparation and CPMG read-out, and fit to the data simultaneously. Transient MT data were essential to ensure convergence and precision of the parameter estimates. The fit results suggested that MT occurs more efficiently between the water and semi-solid pools of myelin. The similarities between the MT behaviour of the short and long $T_2$ components were a direct result of the presence of water exchange, in contradiction of some [202], but in agreement with others [204]. The rate of water exchange reported in this work ($6.2 \text{s}^{-1}$ from myelin water to intra/extracellular water) was considerably slower than the rate previously proposed from theoretical arguments [31].
Combined mapping of $T_1$ and $T_2$ was performed using saturation-prepared CPMG in rat brain and peripheral nerve [205]. The RATE technique [163] and variable TRs were used to avoid long scan times. $T_1$-$T_2$ data were processed with a method which uses an iterative procedure to define a sparsely-sampled spectral ($T_1$-$T_2$) domain [206]. The fit results were clustered into three components for peripheral nerve, but not so for brain ROIs. Observations in brain GM ROIs were mainly mono-exponential, consistent with previous work by the same group [207], while observations in the corpus callosum (WM) surprisingly did not identify multiple $T_2$ components. These measurements demonstrated that distinct $T_2$ components may have different $T_1$s; furthermore, resolution of these $T_1$s implies that exchange rates between compartments may not be fast on a $T_1$ scale. If exchange was slow on a $T_1$ scale, then one would identify each $T_1$-$T_2$ component clearly. On the other hand, if exchange is comparable to $1/T_1$, one might expect to observe multiple $T_1$s for each $T_2$; simulations demonstrated that this is not the case, and that a single best fit $T_1$ would more likely be observed.

Of primary interest to this work, an extensive set of measurements in a spectrometer at 2.1 T was used to study the compartmental nature of relaxation and MT in bovine WM [208]. The mobile and restricted proton signals were isolated using a modified FID sequence and analysis by the method of moments [8]. Spin-spin relaxation was measured with a CPMG sequence, and spin-lattice relaxation was measured using a modified positive IR technique. A combined $T_1$/$T_2$ measurement was performed with IR-prepared CPMG. All three relaxation experiments were analysed using appropriate distributions of relaxation [178, 206]. MT between restricted and mobile protons was probed using two variations of a Goldman-Shen pulse sequence, the first with an FID read-out and the second using a CPMG read-out, to separate MT effects of the different mobile populations. Measurements were performed at 24$^\circ$C and 37$^\circ$C.

The motionally-restricted proton FID signal, evaluated via orientational disorder, yielded a value for the second moment, $M_2^{mr}$, intermediate to liquid crystalline phospholipids and proteins. IR-measured $T_1$ components were separable at 24$^\circ$C, but not at 37$^\circ$C. The $T_1$-
2.3 Relaxation and magnetization transfer in WM

$T_2$ dependence results showed that at 24°C, exchange between the water compartments is slow enough to allow isolated $T_1$-$T_2$ components to be observed, while at 37°C, $T_1$ became mono-exponential. This suggests that significant exchange occurs between the intra/extracellular components and the myelin water at body temperature. However, multiple $T_2$ components were observed, suggesting that the exchange time at both temperatures is greater than the measured $T_2$ and thus slow mixing on a $T_2$ timescale. MT data showed peak signal from the restricted protons at 150 ms in the IR signal, for either temperature.

A four-pool model of WM was used to describe the results. Model parameters were not fitted directly, but were determined by simulations of the Bloch equations, adjusting the parameters until the predicted signal of the set of experiments matched the measured data. Simulations yielded $T_{cr}^D = 1021$ ms at 37°C, and $T_{cr}^D = 2064$ ms at 24°C, more than an order of magnitude slower than early theoretical predictions [31], and much slower than measured in bovine optic nerve ($T_{cr}^D \approx 500$ ms at 20°C) [203]. MT rates were also significantly different from those measured in bovine optic nerve: the MT rate between myelin liquids and solids was similar, but the MT between intra/extracellular water and the non-myelin solids was considerably more important in bovine WM than in bovine optic nerve, prompting the conclusion that MT is not necessarily dominated by myelin. This set of exchange and MT rates implies that the MT of the water compartments reaches equilibrium in a estimated time of 142 ms, very close to the 200 ms predicted by Vavasour et al. [209]. Measured $T_1$ values were also different, but were of the same magnitude, and myelin water had a shorter $T_1$ than the intra/extracellular component at 24°C. Discrepancies in the results were attributed to differences in the methods, the temperature, and the differences between bovine WM and optic nerve.

**MT and $T_2$ in animal models of pathology**

Important insight about relaxation and MT has come from a set of spectrometer relaxometry studies of peripheral nerve injury and regeneration, initially in a model of trauma-induced Wallerian degeneration in rat sciatic nerve [198, 210]. QT2 and QMT techniques
were combined to study an experimental model of inflammation in the sciatic nerves of rats induced with tumor necrosis factor α (TNF-α) [211]. Myelin content, axonal integrity and extra-cellular volume fraction (EM) were evaluated by histomorphometry, while immunohistochemistry was used to evaluate the inflammatory process. Inversion recovery data were analysed for mono-exponential $T_1$ recovery. CPMG data were analysed using a model for the $T_2$ distribution [198, 212]. CW saturation data were also collected, and processed using the two-pool model for WM. Myelin content was observed to decrease in the injected animals (31 ± 4% to 23 ± 7%) and this was reflected in the MWF (32 ± 5% to 22 ± 6%). The EM fraction was observed to increase (42 ± 2% to 52 ± 6%) in treated animals. Treated animals also had lower $F$ (0.05 ± 0.02 vs. 0.10 ± 0.02 in controls) and increased $T_{1obs}$ (819 ± 80 ms up from 631 ± 30 ms). Most MT measures, the average $T_2$ and the $T_2$ of the intermediate $T_2$ peak correlated with the EM fraction, reflecting the impact of inflammation. The $T_2$ distribution changed dramatically, especially the intermediate $T_2$ peak (up 83%), suggesting that it might serve as the best means to distinguish between myelin loss and inflammation.

QMT and $Q_T^2$ were also combined in a study of tellurium-induced demyelination in rat sciatic nerve [213]. Histomorphometry revealed myelin content reduced to 8 ± 4% in affected rats, versus 28 ± 3% in controls. In the affected rats, the $T_2$ distribution showed smaller MWF (14 ± 1%, down from 28 ± 4%) with almost normal myelin water $T_2$ (19 ± 1 ms, versus 23 ± 3 ms), and slight increases in the major (intra/extracellular water) component $T_2$ and amplitude (108 ± 3 ms up from 75 ± 4 ms, and 83 ± 1% up from 74 ± 4%, respectively). $T_{1obs}$ increased in treated nerves (from 675 ± 22 ms to 1036 ± 37 ms). $F$ was less sensitive to the demyelination ($F = 3.8 ± 0.2\%$ in treated rats, versus $6.3 ± 0.4\%$ in controls). The MT rate was insensitive to demyelination. The degree of tissue damage was variable across the animals, and so comparisons with controls were not computed. Quantitative MR parameters correlated with myelin content to varying degrees: very high ($r < 0.8$) for $T_1$, mean and intermediate $T_2$, high ($0.7 < r < 8.8$) for the MWF and $F$, and moderate for MTR ($r = 0.56$). Interpretation of the previously observed changes in the $T_2$
distribution was supported in this work.

**Combined in vivo studies**

Investigations of human WM that combine quantitative MT, $T_1$, and $T_2$ have been limited. The sheer amount of data required currently precludes combined measurements and full quantification of the four-pool model. Studies have generally measured the MT and $T_2$ effects separately in the same patients, and compared the outcome. In a study involving 10 healthy subjects and 9 MS patients [204], measurements from pulsed-saturation MTR and multi-component $T_2$ analysis of 32-echo spin-echo data were compared in WM, GM (normal-appearing WM and GM in patients), and MS lesions. The MWF and the MTR gave largely independent information in MS.

In [209], pulsed-saturation-prepared multi-echo SE measurements in healthy human WM were reported, to analyse the differential MT effects of the multiple water components. The CPMG data were analysed by fitting the amplitudes of four discrete $T_2$ components ($T_2 = 20, 80, 120, \text{and} 2,000 \text{ ms}$). The MT effect of each peak was computed, and a greater effect was observed for the short $T_2$ component. The observation of MT effects after just an 18-ms delay also suggested fast MT. The differential MT effect disappeared when the delay between the pulsed preparation and the multi-echo read-out exceeded roughly 200 ms, suggesting equilibration of the magnetization through water exchange. Water exchange was deemed to be intermediate, slow on a $T_2$ timescale ($>100 \text{ ms}$), but fast on a $T_1$ timescale (<700 ms), in agreement with [201, 203], but contrasting with other measurements [208].

A comparison of the QT2 relaxometry and QMTI techniques was performed in 9 MS patients and 19 healthy controls [214], in what is essentially the only study combining the two techniques *in vivo*. The semi-solid pool ratio $f_b$ and MWF were both significantly decreased in MS lesions, compared to controls. The WM of patients also had significantly low $f_b$. Of greater interest in that study was the relationship between the two putative markers of myelin content: the $f_b$ and MWF were only weakly correlated in MS lesions ($r \approx 0.2$), in the WM of patients ($r \approx 0.6$), and in GM ($r \approx -0.3$). It was concluded that
the two measures provide complementary information.

Having reviewed experimental findings of relaxation and MT in WM, and the develop-
ment of the compartmental models of WM, the next chapter addresses the implications of
this more complete model with respect to in vivo imaging, and explores the sensitivity and
limitations of current QMTI and QT2 methods.
Chapter 3

A unified view of QMTI and $T_2$ relaxometry in WM

3.1 Preface

As discussed in Chapter 2, MT and multi-component $T_2$ have demonstrated potential to provide more specific information about WM and WM disease. However, in vitro and ex vivo experiments have demonstrated the validity of a more complete description of WM with four distinct, coupled compartments that capture all of the observable features of $T_2$ relaxation and MT [201, 203, 208]. While the four-pool model of WM is too complex for current in vivo human applications, it can be used to gain insight into the observations from the techniques of QMTI and QT2. In this chapter, the four-pool model is used to investigate the sensitivity and limitations of the QMTI and QT2 techniques to plausible variations in the underlying model. The results of an extensive set of simulations covering a series of modifications, constructed to mimic WM changes due to pathology such as demyelination and edema, are presented.

These results indicate that the phenomenon of MT in WM is adequately described with a two-pool model, in agreement with observations in bovine optic nerve [203] and rodent WM [89], and that the existence of separate water and semi-solid compartments does not impede the robust estimation of the semi-solid-to-liquid pool ratio $F$. We observe that other MT model parameters ($k_f$ and $T_{2f}$) may be useful in discriminating between subtle changes to the four-pool model. Furthermore, we show that compartmental water exchange is fast on an MT time-scale and in fact enables the observation of MT from all compart-
ments. Our investigations demonstrate that QT2 measures of the signal amplitude and \( T_2 \) of the different water compartments of the four-pool model are generally reliable; however, the potential increase of compartmental exchange of water due to pathology can mimic decreases in the MWF.

The observations presented here derive from simulations in a model with very specific parameter values, and the modifications introduced are over a limited range. For instance, the parameter values from bovine optic nerve reported in [203] could equally have been used. Simulations were in fact performed with those model parameters as well. Identical trends were observed in the quantities obtained from QMTI and QT2, for all simulations. In some cases, such as the increase in the inter-compartmental water exchange rate, the effect of the modification was more pronounced for the bovine optic nerve model than for the bovine WM model. For these reasons, and because the bovine WM model more closely resembles human WM, only the results from bovine WM simulations are reported.
Characterizing healthy and diseased white matter using quantitative magnetization transfer and multi-component $T_2$ relaxometry: a unified view via a four-pool model

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3.2 Abstract

NMR relaxation and magnetization transfer (MT) in cerebral white matter (WM) can be described using a four-pool model: two for water protons (in separate myelin and intra/extracellular compartments) and two for protons associated with the lipids and proteins of biological membranes (of myelin and non-myelin semi-solids). This model was used to gain insight into the observations from multi-component quantitative $T_2$ relaxometry (QT2) and quantitative magnetization transfer imaging (QMTI), both based on simplified WM models and experimentally feasible in vivo. Using a set of coupled Bloch equations describing the behaviour of the magnetization in a four-pool model of WM, simulations of the QT2 and QMTI techniques were performed. Pathology-inspired modifications were made to the four-pool model to gauge their impact on QT2 and QMTI observations. Our results show that changes in the rate of water movement between micro-anatomical compartments may impact otherwise stable QT2 observations; that the measure of the QMTI-based semi-solid pool population is robust, despite the presence of two distinct semi-solid components; and that QMTI compartment size estimates are not influenced by changes in the $T_2$ of the intra/extracellular water pool. The four-pool model, while impractical for in vivo characterization, yields important insight into the interpretation of changes observed with these quantitative MRI methods based on simplified models of WM.
3.3 Introduction

The sensitivity of magnetic resonance imaging to white matter (WM) damage in diseases such as multiple sclerosis (MS) has made it indispensable in diagnosis, but its lack of pathological specificity remains problematic. In response, investigators have turned to quantitative MRI techniques to identify putative indicators of specific pathological features, such as myelin loss. Quantitative analysis of spin-spin relaxation data using $T_2$ distributions (QT2), also known as myelin water imaging, can notably distinguish two major $T_2$ components in human WM [171, 173]. In particular, QT2 yields an estimate of the myelin water fraction (MWF), the fraction of water contained between the layers of myelin in WM. Magnetization transfer (MT) imaging [60, 75], most often measured as the MT ratio (MTR), is a widely available technique that is sensitive to the semi-solid constituents of tissue (e.g. lipids, proteins), and in WM the MT effect is believed to be dominated by myelin [105]. The MTR is a semi-quantitative index that presents a limited view of the MT effect. More detailed analysis of pulsed, off-resonance MT data using the two-pool model of tissue [125, 131] has been extended to in vivo imaging, in particular in human WM [137, 139, 141]. The resulting method, termed quantitative MT imaging (QMTI), allows mapping of the parameters of the two-pool (liquid and semi-solid) model of tissue: the ratio of semi-solid to liquid protons $F$, the first order forward and reverse exchange rate constants $k_f$ and $k_r$ (where $k_r = k_f / F$), the spin-lattice relaxation rate $R_{1f}$ of the free pool ($R_{1f} = T_{1f}^{-1}$), the spin-spin relaxation time constant of the free pool $T_{2f}$, and the “$T_2$” of the restricted pool, $T_{2r}$ (inversely related to the width of the restricted pool resonance). Separating the fundamental parameters of the two-pool model requires an additional measurement of the “long” observed $T_1$ ($T_{1obs}$), from which the free pool $R_{1f}$ can then be computed [125]. The MWF, MTR, and certain QMTI parameters have respectively been shown to correlate with myelin content and demyelination [155, 194, 215].

QT2 and QMTI techniques each present a different limited view of WM characteristics. QT2 imaging relies on the fundamental assumptions that water exists in isolated compartments, and that variations in the estimated MWF are equal to variations in myelin. On
the other hand, the two-pool model for MT neglects the existence of multiple water pools. A more comprehensive model for WM that includes four communicating proton pools (myelin solids, myelin water, intra/extracellular water, and non-myelin solids), comprising most of the basic features of the multi-compartment $T_2$ water model and the two-pool MT model, has been proposed [201, 203, 216]. The intra/extracellular (IE) water pool refers to the intra/extracellular water component reported in multi-component $T_2$ experiments. Originally characterized in bovine optic nerve [203], the properties of the four-pool model have recently been estimated in fresh ex vivo bovine WM [208]. While it is not feasible to fully characterize a four-pool model with in vivo imaging, this model can provide valuable insight into assumptions and observations made when applying and interpreting the established simpler QMTI and QT2 methods. Furthermore, it can be used to reveal potential limitations of each technique and to assess validity of parameter estimates obtained from both.

In this paper, we present simulations of pulsed MT and multi-echo spin echo experiments conducted using a four-pool model of WM to study the potential of QMTI and QT2 to characterize normal and diseased WM. We first considered how well the two techniques are able to reflect the properties of the four-pool model of healthy WM (baseline model). Following this, modifications were made to certain key parameters of the model to explore what impact these might have on quantities measurable by both imaging techniques. The presence of two distinct yet communicating water compartments was explored, in particular by modifying the rate of diffusion-mediated exchange between compartments. The influence of the separate semi-solid pools was studied by modifying the size of each pool separately. The effects of simultaneous reductions in the size of the myelin water and semi-solid pool sizes were explored, as were the impact of increases in the size and $T_2$ of the IE water compartment. Finally, we discuss our observations by relating them to typical MS pathology such as demyelination and edema.
3.4 Methods

3.4.1 Four-pool model of white matter

A modified four-pool model of WM, illustrated in Figure 3.1, was constructed from published bovine WM data [208]. The total equilibrium magnetization (water plus semi-solid) was normalized to 1. The total water and the total semi-solid pool sizes were set to 0.813 and 0.187, which results in a ratio of total semi-solids to total water equal to 0.23. The MWF was 0.13. The individual semi-solid pools were defined to be of equal size ($M_{0m} = M_{0nm} = 0.0935, m = myelin, nm = non-myelin$), based on histological observations [15]. Relaxation times reported in bovine WM at 37°C were selected (all $T_1$ values for semi-solids and water = 830 ms, $T_{2mw} = 13$ ms, $T_{2ie} = 90$ ms, $mw = myelin$ water, $ie =$ intra/extracellular water). The behaviour of the semi-solid pool was based on observations from MT experiments [131]; more specifically, both semi-solid compartments were assumed to behave like the single semi-solid pool observed in MT experiments, characterized by a super-Lorentzian absorption lineshape with $T_{2m} = T_{2nm} = 10$ µs. The coupling between compartments was modeled by first-order, non-directional exchange rate constants $R_D$, $R_m$, and $R_{nm}$. This is based on the mathematical formalism for chemical exchange at equilibrium, which extends naturally to cross-relaxation for two-pool MT [125]. $R_D$ was the compartmental water exchange rate for diffusion between water compartments. $R_m$ and $R_{nm}$ were the rates for MT between the water and semi-solid compartments of myelin and non-myelin, respectively. Directional exchange constants were computed by scaling with the appropriate pool size (i.e. $k_{ij} = R_{ij}M_{0j}$), as listed in the Appendix (3.9). The exchange time for diffusional cross-relaxation $T_{cr}^{D}$, defined in [208], was 1.09 s$^{-1}$ in this model.

3.4.2 Simulations

This model was used in simulations of QMTI and QT2 imaging experiments. In both cases, a set of coupled Bloch equations was used to describe the behavior of the magnetization vector of all four compartments of the model. Exchange of magnetization was included
Figure 3.1: Illustration of the four-pool model of WM with relevant physical parameters, based on observations reported in bovine WM [208] (IE = intra/extracellular).

between all compartments for the \( z \) component of magnetization, but only between water compartments for the \( x \) and \( y \) components. In general, the set of equations can be summarized by Equation 3.1, expanded from previous work [89, 141, 150]. \( A(t) \) and \( R_1 \) are both \( 12 \times 12 \) (4 compartments \( \times \) 3 components) matrices: \( A(t) \) contains terms for longitudinal and transverse relaxation, first-order exchange, RF \( B_1 \) fields, and off-resonance effects, while \( R_1 \) represents recovery of equilibrium magnetization. Both are written out in full in the Appendix (3.9).

\[
\frac{dM(t)}{dt} = A(t)M(t) + R_1M_0 \tag{3.1}
\]

In cases where these matrices are constant, as in the case of free precession or constant RF irradiation (on- or off-resonance), the differential equation can be solved analytically,
3.4 Methods

as in Equation 3.2, where the exponential terms are matrix exponentials [89, 150, 217]. By sequentially combining time blocks of constant conditions, pulse sequences were simulated in the four-pool model. All simulations and analyses were performed using MATLAB software (The MathWorks, Natick, MA, USA).

\[
\mathbf{M}(t_0 + t) = e^{-\mathbf{A}(t)}\mathbf{M}(t_0) + \left(\mathbf{I} - e^{-\mathbf{A}(t)}\right)\mathbf{A}^{-1}\mathbf{R}_1\mathbf{M}_0
\]  

(3.2)

The 32-echo CPMG sequence was simulated with 10-ms echo spacing, a repetition time (TR) of 3 s, and composite \((90^\circ_x-180^\circ_y-90^\circ_x)\) refocusing pulses, identical to that used in the overwhelming majority of QT2 imaging studies published to date. The saturation effects of on-resonance, composite CPMG refocusing pulses on the semi-solid pools were computed using a model for pulsed saturation [136], using the general formula for continuous wave irradiation while neglecting relaxation and exchange.

MT experiments were simulated as gradient echo experiments with pulsed off-resonance saturation to match the imaging protocol of [141], using 2 combinations of TR and excitation flip angle, each with 2 saturation pulse flip angles, and 20 and 10 offsets, respectively. The shaped off-resonance pulses were approximated by the minimal approximations MT (MAMT) model [89], in which the pulse is broken up into 50-\(\mu\)s increments of continuous wave saturation. The saturation rate for each increment is computed as suggested for narrow-bandwidth pulses [136].

Simulated measurements of the \(T_{1\text{obs}}\) were also performed for each modification to the model, because they are of independent interest and they are required to compute the fundamental MT parameters. Inversion recovery (IR) experiments were simulated with inversion times (TI) of 0.2, 0.5, 0.75, 1, 2, 4, and 10 seconds, and infinite TR. Conventional IR was selected – despite long scan times that limit its use \textit{in vivo} – to yield the most reliable and least-biased observations of \(T_{1\text{obs}}\). The simulated IR signal by combining cases of Equation 3.2 for free-precession and constant \(B_1\) irradiation, assuming had pulses for inversion and excitation. Semi-solid saturation was also incorporated into the IR simulations, in the same manner as the refocusing pulses of the spin echo simulations.
3.4.3 Analysis methods

Simulated decay curves were analyzed using a regularized, non-negative least-squares (NNLS) fit to estimate the $T_2$ distribution [178]: we used 120 logarithmically-spaced bins between 10 and 4,000 ms [181]. Regularization of the $T_2$ distribution was performed by including a term for the total energy of the distribution in the function to be minimized. The weight of the regularization term in the minimization was determined iteratively for each fit, allowing the chi-squared value to increase by 2-2.5% above the non-regularized fit value. The parameters for this analysis were reproduced from published in vivo imaging work [181]. Prior to the analysis, noise was added to the simulated signal to achieve an SNR value of 200 in the first echo. This SNR value was chosen as it is the value we obtain using the standard QT2 imaging protocol [171, 173] on the 1.5 T Siemens scanner at our institute; moreover, it is double the value recommended for imaging experiments in the literature [173]. Results from 10,000 noise iterations were averaged.

The pulsed saturation data were analyzed using the two-pool model of WM [131], with the rectangular pulse model of [126] for MT-weighted spoiled gradient echo sequences. This analysis yields five model parameters ($F$, $k_f$, $R_{1f}$, $T_{2f}$, and $T_{2r}$). The restricted pool spin-lattice relaxation constant $R_{1r}$, to which the fit of the two-pool model is relatively insensitive, was fixed to a value of 1 s$^{-1}$. This is identical to the analysis we perform on in vivo imaging data [141, 151, 153, 154]. In the MT experiments, Gaussian noise was added to the simulated data to produce an SNR of 75 in the data without MT saturation, to match roughly what we obtain in routine QMTI experiments at 1.5 T. Again, results from 10,000 noise iterations were averaged.

$T_{1obs}$ was estimated from the simulated IR signal with the classic 3-parameter solution to the $z$ component of the Bloch equations, as per Equation 3.3, where $S_0$ is the total available signal (for TI = ∞), and $k$ represents the inversion fraction.

$$S(TI) = S_0 \left( 1 - 2k e^{-TI/T_{1obs}} \right)$$ (3.3)
3.4 Methods

3.4.4 Modification of the model

We performed three main modifications to the basic WM model: first, increase of the water exchange rate constant; second, reductions of certain semi-solid and water compartment sizes; and third, increases of the IE water compartment size and $T_{2ie}$.

The exchange of water between the micro-anatomical compartments of myelin and the intra- and extra-cellular space is not accounted for in QMTI and QT2 imaging, and may lead to biases in parameter estimates in both techniques as reported in a model of bovine optic nerve [203]. This rate of exchange was recently estimated in fresh bovine WM [208], and was observed to be temperature-dependent, suggesting that water exchange between micro-anatomical compartments is largely mediated by diffusion. Pathology such as MS can modify water diffusion in WM, as reflected by increases in mean diffusivity and isotropy. The breakdown of myelin may thus increase movement of water between myelin and IE compartments. We investigated the impact of variations in the intercompartmental water exchange rate on multi-component $T_2$, and on two-pool parameter estimates from pulsed MT. Since diffusivity increases of up to 50% are reported in the MS literature [218–220], the effect of increased exchange from potential pathological changes was simulated by increases in the water exchange rate ($R_D$) of 25, 50, and 100%. Simulations were also performed at the fast- and slow-exchange limits ($R_D \rightarrow 0$ and $R_D = 10^4$ s$^{-1}$, respectively).

In their analysis, Bjarnason et al. assumed that restricted pool protons were equally divided between myelin and non-myelin components [208], based on published histological information [15]. To better understand the influence that each restricted proton pool can wield, simulations were performed with reductions in the size of either of the semi-solid proton pools, separately, with relative decreases of 25, 50, 75 and 100%.

Because a loss of myelin semi-solids inevitably entails a loss of myelin water, we also investigated the impact of a more realistic model of myelin loss by reducing the size of both the water and semi-solid pools of myelin. Specifically, we performed simulations reducing both compartments by the same relative amount of 25 to 100%. Finally, edema simulations were also performed with increases of up to 25% in the IE water compartment size, in
5% steps, increases larger than reported in MS [190], but selected to cover of the potential range of experimentally induced inflammation [211]. In addition, these simulations were performed with and without an increase of $T_{2ie}$ of up to 100%, linearly scaled with the increase of IE water content.

### 3.5 Results

We evaluated the impact of WM model modifications (increased water exchange, reduction of semi-solids, reduction of myelin water and semi-solids, increase of IE water) on $T_2$-distribution metrics, such as the MWF (fraction of observed signal with $T_2 < 50$ ms), the geometric mean $T_2$ of the entire distribution $\langle T_2 \rangle$ and the geometric mean $T_2$ of the IE water peak, above 50 ms, $\langle T_{2ie} \rangle$. The geometric mean is a natural choice for the logarithmic scale of $T_2$ [172]. Changes in the two-pool QMTI parameter estimates, described earlier, were also evaluated.

#### 3.5.1 General observations from CPMG and MT simulations

Multi-component analysis (with unregularized NNLS) of noise-free simulated $T_2$ decay in the base model yielded accurate estimates of the model parameters: a $\langle T_2 \rangle$ of 68.8 ms, a MWF of 10.6% (input 13%), and a $\langle T_{2ie} \rangle$ of 84.6 ms (input 90 ms). The total water signal observed by multi-component $T_2$ analysis was lower than the total input in all simulations, mainly due to $T_1$ relaxation over the finite TR of 3 seconds: semi-solid pool saturation from the refocusing pulses and MT only accounted for an additional signal decrease of 1%. Experiments performed with shorter TRs demonstrated that the impact of MT increased as the TR was reduced. Other $T_2$ distribution metrics were smaller than the model input values due to the presence of water exchange in the baseline model.

Residuals from the two-pool MT model fit to noise-free simulated off-resonance saturation data from the baseline four-pool model were less than 1%. Two-pool parameter estimates for this MT simulation in the baseline model of WM were: $F = 0.216$ (input
3.5 Results

0.23), \( k_f = 5.7 \text{ s}^{-1} \) \((R = F / k_f = 26.4 \text{ s}^{-1})\), \( R_{1f} = 1.2 \text{ s}^{-1} \) \((T_{1f} = 830 \text{ ms})\), \( T_{2f} = 71.3 \text{ ms} \), \( T_{2r} = 10 \mu\text{s} \).

3.5.2 The observed \( T_1 \)

The \( T_{1\text{obs}} \) obtained in the IR simulation was generally shorter than the nominal value used for the model (= 830 ms). In the base model, a \( T_{1\text{obs}} \) of 823.8 ms was measured. When the simulated values of \( T_{1\text{obs}} \) were used in fitting the simulated QMTI data, instead of the nominal value, no significant impact was observed on the two-pool MT parameters. This exercise was repeated for all of the various modifications imposed on the model in these experiments. The QMTI fit results presented below were obtained using the simulated value of \( T_{1\text{obs}} \) for each model variation, keeping in mind that no modifications of the input \( T_1 \) values were introduced in any of the simulations. Notably, the variations of \( T_{1\text{obs}} \) over all modifications to the model were small, ranging from 821 to 830 ms: the shortest \( T_{1\text{obs}} \) occurred when all non-myelin solids were removed \((M_{0nm} = 0)\), and the longest \( T_{1\text{obs}} \) resulted from the complete removal of myelin solids \((M_{0nm} = 0)\).

3.5.3 The impact of increased compartmental water exchange

The impact of increased water movement between the myelin and IE water compartments on the observed MWF and \( \langle T_2 \rangle \) is presented in Figure 3.2 (top). In the case of fast exchange, the MWF is not plotted because the \( T_2 \) distribution merges into a single peak. Initial computation of the MWF using the pre-defined threshold of 50 ms in the fast exchange case resulted in a surprising average value of 25.8 ± 8.8%. A closer look at the \( T_2 \) distributions revealed that all signal merges into a single broad peak in the fast exchange limit, which overlaps the myelin water \( T_2 \) upper limit of 50 ms and results in the artifactual MWF estimate. Results of fitting with the two-pool model of MT are plotted in Figure 3.2 (bottom). MT model parameters \( F \), \( k_f \), and \( T_{2f} \) were most different in the slow exchange limit. \( T_{2r} \) and \( R_{1f} \) were consistently fitted to 10 \( \mu\text{s} \) and 1.2 \( \text{s}^{-1} \), and are not plotted in
the Figure. When comparing the impact of increased water exchange on $F$ and MWF, the former is relatively stable while the latter shows a linear variation with the exchange rate.

### 3.5.4 Reduced semi-solid pool sizes

In all simulations, saturation effects of the CPMG refocusing pulses on the semi-solid pools were negligible, reflecting the relative insensitivity of CPMG MR images to MT effects. As a result, reducing the size of either of the restricted pools had no effect on multi-component $T_2$ observations.

Reductions of the semi-solid pool sizes had a pronounced effect on the QMTI observations. Parameter estimates for the two-pool model are plotted in Figure 3.3. The $T_{2r}$ and $R_{1f}$ were both consistently recovered by the fit (10 $\mu$s and 1.2 s$^{-1}$, respectively), and are excluded from Figure 3.3.

### 3.5.5 Reduced myelin water and semi-solid pool sizes

Simulations were performed with simultaneous reductions of the myelin water and semi-solid pool sizes, from 0 to 100% in 25% steps. The changes in MWF, $\langle T_{2ie} \rangle$, and $\langle T_2 \rangle$ resulting from these myelin modifications are plotted in Figure 3.4 (top), and the MT model parameters from the same modifications are plotted in Figure 3.4 (bottom). The average MWF estimates track the input value well, and the estimate of $F$ is accurate. Note that the effect of water exchange (held constant here) decreases with the MWF. The loss of the short $T_2$ component results in a slight increase of the $\langle T_2 \rangle$, and a larger non-linear increase of $T_{2f}$. The two-pool MT exchange constant $k_f$ also increases in these simulations, but only for large reductions of the myelin water and semi-solid content. The grey line in Figure 3.4 (bottom) represents the true variation of $F$ with the simultaneous reduction of myelin water and semi-solids. Note that for a complete elimination of myelin ($M_{0\text{mw}} = M_{0m} = 0$), the true $F$ is equal to 0.132 ($= M_{0\text{nm}}/M_{0ie} = 0.0935/0.707$), which is 57% of the baseline model’s $F$ ($= 0.23$). The same reasoning applies to all levels of this modification.
3.5 Results

Figure 3.2: Plots of the variation of $T_2$ distribution (QT2) metrics MWF, $\langle T_2 \rangle$, and $\langle T_{2ie} \rangle$ (top), and two-pool MT model parameter estimates $F$, $k_f$, and $T_{2f}$ (bottom), as a function of increasing water exchange in a model of WM. Results are averaged from 10,000 trials, with SNR = 200 for QT2 and SNR = 75 for MT. $F$, MWF, and $\langle T_{2ie} \rangle$ are normalized to their model input values (see Methods), while $\langle T_2 \rangle$, $T_{2f}$, and $k_f$ are normalized to their respective fit values for the baseline model ($R_D = 10 \text{ s}^{-1}$), as these are not defined for the input model.
Figure 3.3: Variations of two-pool MT model parameter estimates $F$ (top), $k_f$ (middle), and $T_{2f}$ (bottom), as a function of the reduction of the solid pool sizes of non-myelin (circles) and myelin (triangles). Parameter values are normalized to their respective fit values for the baseline model. The solid grey line in the plot of $F$ indicates the true value of $F$ in the simulation input.
Figure 3.4: (Top) Variations of $T_2$ metrics MWF, $\langle T_2 \rangle$, and $\langle T_{2ie} \rangle$ as a function of simultaneous decreases in the water and semi-solid pools of myelin (0-100%, in 25% steps). (Bottom) Variations of two-pool MT parameter estimates as a function of the myelin water and semi-solid compartment reductions. The solid grey line each plot represents the true value of the MWF (top), and $F$ (bottom), respectively.
3.5.6 Increase of IE water pool size and $T_2$

Increases in the IE water compartment size of 0 to 25% had noticeable effects on observations from QT2 and QMTI. In particular, the MWF and $F$ changed linearly with the model input, as expected. Additional changes to the $T_{2ie}$ also had a marked effect on the observations from both techniques. The impact of variations in the $T_{2ie}$ on the observed $\langle T_{2ie} \rangle$ and the $T_{2f}$ were essentially decoupled from the MWF and $F$, respectively, as seen in Figure 3.5.

3.6 Discussion

The four-pool model is the simplest model that captures all of the characteristics of WM observable by current in vivo quantitative $T_2$ relaxometry and MT techniques: compartmental structure, multi-component relaxation, and cross-relaxation/exchange. It has been employed here to provide insight into the interpretation of quantitative MRI observations, from a forward-problem perspective.

Prior to discussing the observations, a few facts bear mentioning. In a real-world situation, each water compartment is believed to have a $T_2$ distribution with finite width, as opposed to the delta function peaks simulated here. In our simulations, the regularization of the NNLS-based multi-component analysis of $T_2$ decay data deals purely with the noise added to the simulation results and with the underdetermined nature of the problem (more parameters are being estimated than observations are being made). These simulations also do not include real sources of error such as subject motion and image artifacts and inhomogeneities, which are fundamentally different from the additive Gaussian noise included here. Despite these limitations, we believe that our results provide a critical perspective to observations from emerging quantitative MRI techniques sensitive to WM structure and integrity. Thus, we have made judicious modifications to a basic four-pool model of WM, and observed their effect on quantities measurable with current in vivo MRI techniques.

Increased exchange of water between micro-anatomical compartments has very dif-
3.6 Discussion

Figure 3.5: Effect of increases in IE water compartment size, with and without increase of $T_{2ie}$, on QT2 metrics MWF and $\langle T_{2ie} \rangle$ (top), and QMTI parameters $F$ and $T_{2f}$ (bottom). The solid grey line each plot represents the true value of the MWF (top), and $F$ (bottom), respectively.
different effects on the QT2 and QMTI techniques. The MWF and the $\langle T_{2\text{ie}} \rangle$ are generally underestimated in simulations with non-zero exchange, and increased exchange enhances this effect. MWF estimates decrease linearly with increasing exchange, and are between 10 and 25% for the range of plausible exchange rates considered here. As expected, the mean $T_2$ value of each peak decreases with increasing exchange. Exchange affects $\langle T_2 \rangle$ only slightly over the range considered (< 2%, Figure 3.2). Water exchange had a marked effect on MWF estimates in the model of bovine optic nerve, as reported by Stanisz et al. [203]. The observations in this model of WM – which more closely resembles human WM – are slightly less sensitive to the variations in the exchange rate than in the model of optic nerve, likely due to generally slower exchange and a smaller myelin water compartment.

A model considering the layered structure of myelin has been proposed to estimate the rate of water exchange between myelin and IE compartments [31], which can be extended to a population of myelinated axons. Assuming a membrane permeability of 10 $\mu$m/s [31, 221], the reported water exchange rate in bovine WM [208] corresponds to a median number of 50 myelin wrappings, which falls within the range of counts reported in histology [222]. In this model, the non-directional exchange rate $R_D$ is proportional to the membrane permeability, and inversely proportional to the number of and spacing of the myelin lamellae. Changes in any combination of these factors with pathology could result in important increases in the exchange rate. Pathology affecting water exchange between the myelin and IE compartments, perhaps via the effects listed above, can result in variations in the MWF and could therefore be incorrectly interpreted as changes in myelin content. Furthermore, the decrease of apparent $T_2$ values of the IE water peak with increased exchange may affect the choice of $T_2$ threshold used to separate the water compartments. In particular, as the observed $T_2$ value of the IE water peak becomes smaller, the lower tail of the peak could extend below the upper threshold set for the myelin water $T_2$ range, resulting in an artifactual increase of the MWF.

With regards to MT simulations, our results also agree qualitatively with the observations of Stanisz et al. in bovine optic nerve [203]; however, water exchange has a less dra-
matic impact on the parameters of the two-pool MT model than on the MT of the individual water components. Increased water exchange has a modest impact on two-pool QMTI parameters in this model of WM, over the range of plausible water exchange rates considered. Estimates of $F$ are independent of the water exchange rate $R_D$ (variation < 1%) over the range of finite values considered. The greatest effect of increased water exchange over the same range was seen for $T_{2f}$ (up to a 8% decrease, similar to that seen for $\langle T_{2ie} \rangle$), while the impact on $k_f$ was smaller (up to an 5% increase). The outright absence of water exchange (slow limit) had the greatest impact on two-pool MT observations, in agreement with the notion that this exchange is fast on the time-scale of an MT experiment [89]. In fact, water exchange enables the probing of both semi-solid proton populations and justifies considering them as a single semi-solid component for QMTI analysis. These observations suggest that increased inter-compartmental water exchange has a negligible impact on parameter estimates from in vivo two-pool MT measurements in WM, in contrast to QT2 imaging.

As expected, our simulations of multi-component $T_2$ imaging were not directly sensitive to the solid components of WM when the TR was long enough to allow sufficient recovery of longitudinal magnetization. However, this may be an issue to be considered in the case of multi-slice CPMG measurements recently proposed [164], which employ slice-selective refocusing pulses that fill the entire TR, potentially resulting in significant MT effects.

QMTI measures are very sensitive to changes in the semi-solid components of the four-pool model of WM, as expected. The observed $F$ reflects the input value quite well; reduction of the size of either semi-solid pool results in robust decreases in the measured $F$, and any difference between the two semi-solid pools would be difficult to discern experimentally. On the other hand, the impact on estimates of the forward exchange rate $k_f$ is drastically different depending on which pool is affected. In the case of a reduction in myelin solids, $k_f$ is barely affected, while in the case of a loss of non-myelin solids, $k_f$ is greatly reduced. If we compute the non-directional exchange rate $R$ (= $k_f / F$), which should be independent of the sizes of the water and solid pools, we see that the behaviour of $k_f$ translates to an increase in $R$ in the case of a reduction of myelin solids, and to a
decrease in $R$ in the case of a reduction of non-myelin solids. It is quite interesting to note that a change in estimated $R$ can result simply from a change in the pool sizes, and not in the actual exchange rates themselves. This is quite straightforward to understand from the model, but it is important to consider when interpreting observations in MS pathology, in which lesions have significantly reduced $k_f$ and only slightly reduced $R$. The $T_{2f}$ is slightly affected by a reduction in the myelin semi-solid pool size, decreasing with the loss of myelin solids. In a more realistic model of pathology, the lost solids would presumably be replaced by water, thereby increasing the $T_2$ of the water compartments (primarily the IE water component) and potentially overshadowing this $T_{2f}$ decrease.

Two-pool MT estimates were also sensitive to demyelination, simulated as simultaneous reductions in the size of the myelin water and myelin semi-solid compartments. We observed a robust linear decrease in $F$ that reflected the input value, accompanied by a relatively large increase in $T_{2f}$, and a comparatively smaller increase in $k_f$ for simulated myelin loss greater than 50%. Our simulations of QT2 were also robustly sensitive to the simulated demyelination. The MWF decreased linearly with demyelination, and the underestimation of absolute MWF due to exchange was reduced for low myelin content. The $\langle T_2 \rangle$ increased slightly as the signal becomes more weighted towards IE water, while the observed $\langle T_{2ie} \rangle$ remained relatively stable.

Increases in the IE water pool size were correctly reflected by the estimates of both MWF and $F$. The 25% increase in IE water, which is relatively large, resulted in decreases of the MWF and $F$ (25% and 17%, respectively) that are modest when compared to the effect of severe demyelination (up to 100% decrease of the MWF, and close to 50% for $F$). Interestingly, the $T_{2ie}$ increase introduced in these simulations directly affected the $\langle T_{2ie} \rangle$ and $T_{2f}$ (of QT2 and QMTI, respectively), but did not influence the estimates of the MWF and $F$. 
3.6 Discussion

3.6.1 Clinical implications: pathology models

To illustrate the implications of our findings, we now frame them in terms of pathological observations. Modifications of the four-pool model were designed to simulate very simple representations of pure demyelination and pure edema. These observations highlight changes observed in the multi-component $T_2$ distribution and MT under these basic, but very specific, WM pathology models.

Myelin reductions and IE water increases both result in reduced MWF estimates, and a subtle increase in the $\langle T_2 \rangle$, the former due to the partial loss of short $T_2$ contributions, and the latter due to the increased contribution of IE water $T_2$ species. The position of the IE water $T_2$ was not affected in either of those cases. Large increases in IE water content were required to produce large decreases in MWF in our simulations: this supports the argument that edema alone cannot explain the important MWF decreases in MS [190]. Real $T_2$ increases likely to be present in the case of true edema clearly affected the observed $\langle T_2 \rangle$ and $\langle T_{2ie} \rangle$; moreover, we observed that the position of the $\langle T_{2ie} \rangle$ peak in the distribution does not strictly follow the model value of $T_{2ie}$, but is underestimated by the QT2 analysis. The different effects of demyelination and edema on the $T_2$ distribution may allow the distinction of the two effects using in vivo imaging in human WM, as previously suggested by Stanisz and colleagues [211, 213]. A linear relationship was assumed between increases in the IE water content and $T_{2ie}$ in these simulations as the functional form of this relationship is not distinguishable from published data [211]: better knowledge regarding this relationship would help determine the potential of QT2 to distinguish between edema and demyelination.

These simple models of pure demyelination and pure edema were also reflected in obvious changes in the two-pool MT model parameters. Estimates of the semi-solid to liquid pool ratio, $F$, were robust in both cases; moreover, our results show that pure edema can only result in a small change in $F$. In the case of pure edema (with $T_2$ increase), it was interesting to see that changes to $F$ were unaffected by the increase in $T_{2ie}$. This suggests that $T_{2f}$ may have some potential to evaluate the edema, especially in situations of partial
demyelination. The observed increase in $T_{2f}$ was relatively greater than the increase in $T_{2ie}$, an effect likely compounded by the increased contribution of the larger IE water pool.

To isolate the change in semi-solid content, the observable semi-solid pool magnetization ($PD_r$) was computed by multiplying $F$ by the observable water magnetization ($PD_f$, which is obtained by correcting the total observed signal from QT2 for incomplete $T_1$ relaxation), that is, $PD_r = F \times PD_f$. $PD_r$ tracked the input solid content well (not shown), and while it does not have zero intercept with total demyelination, due to the presence of non-myelin semi-solids, it is very tightly linked to myelin content changes.

### 3.7 Conclusion

We have investigated the implications of using simplified tissue models to study the complex behaviour of WM using two emerging quantitative MRI techniques that may provide improved pathological specificity. QT2 simulations confirmed the sensitivity of the technique to changes in water pool sizes and its insensitivity to semi-solid tissue constituents. However, inter-compartment water exchange had a marked effect on myelin water estimates, mimicking real myelin content changes. Our results show that a two-pool (liquid and semi-solid) model is sufficient to describe off-resonance MT behavior in WM. Water exchange between myelin and IE water sub-compartments, which is fast on the MT time scale, has a negligible impact on the QMTI estimate of the semi-solid to liquid ratio ($F$). $F$ is also robustly estimated, despite the existence of multiple semi-solid pools; furthermore, other QMTI parameters, such as $k_f$ and $T_{2f}$, might provide additional insight into changes in the more complete four-pool model based on limited two-pool observations. Simulated demyelination produces tightly correlated changes in both the semi-solid pool ratio $F$ and the myelin water fraction MWF.
3.8 Acknowledgments

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3.9 Appendix

Directional exchange constants are defined in Equations 3.4-3.9.

\[ k_{m,mw} = R_m M_{0mw} \quad (3.4) \]
\[ k_{mw,m} = R_m M_0 \quad (3.5) \]
\[ k_{mw,ie} = R_D M_{0ie} \quad (3.6) \]
\[ k_{ie,mw} = R_D M_{0mw} \quad (3.7) \]
\[ k_{ie,nm} = R_{nm} M_{0nm} \quad (3.8) \]
\[ k_{nm,ie} = R_{nm} M_{0ie} \quad (3.9) \]

If chemical equilibrium is assumed, the time constant for diffusional cross-relaxation, \( T_{cr}^D \), is related to the compartmental water exchange constant by Equation 3.10.

\[ T_{cr}^D = \frac{1}{k_{mw,ie}} + \frac{1}{k_{ie,mw}} = \frac{1}{R_D} \left( \frac{M_{0mw} + M_{0ie}}{M_{0mw}M_{0ie}} \right) \quad (3.10) \]

The total magnetization vector \( M(t) \) groups the magnetization of all compartments, as per Equations 3.11 and 3.12. \( M_0 \) is the fully relaxed magnetization vector (Equation 3.13). The superscript T denotes the transpose operation.

\[ M(t) = \begin{bmatrix} M_m(t) & M_{mw}(t) & M_{ie}(t) & M_{nm}(t) \end{bmatrix}^T \quad (3.11) \]

\[ M_i(t) = \begin{bmatrix} M_{i,x}(t) & M_{i,y}(t) & M_{i,z}(t) \end{bmatrix}, \quad i = \{m, mw, ie, nm\} \quad (3.12) \]

\[ M_0 = \begin{bmatrix} 0 & 0 & M_0m & 0 & 0 & M_{0mw} & 0 & 0 & M_{0ie} & 0 & 0 & M_{0nm} \end{bmatrix}^T \quad (3.13) \]
The matrix $A(t)$ contains terms for relaxation, exchange, $B_1$ fields, and off-resonance effects, expressed separately in Equation 3.14, in a frame of reference rotating at a frequency offset $\delta$ from resonance ($\delta = |\omega - \omega_0|$).

$$A(t) = R_1 + R_2 + K + \Delta + \Omega_1(t) \quad (3.14)$$

Relaxation terms are contained in diagonal matrices $R_1$ and $R_2$ (Equations 3.15 and 3.16).

$$R_1 = -diag \begin{bmatrix} 0 & 0 & T_{1m}^{-1} & 0 & 0 & T_{1mw}^{-1} & 0 & 0 & T_{1ie}^{-1} & 0 & 0 & T_{1nm}^{-1} \end{bmatrix} \quad (3.15)$$

$$R_2 = -diag \begin{bmatrix} T_{2m}^{-1} & T_{2m}^{-1} & 0 & T_{2mw}^{-1} & T_{2mw}^{-1} & 0 & T_{2ie}^{-1} & T_{2ie}^{-1} & 0 & T_{2nm}^{-1} & T_{2nm}^{-1} \end{bmatrix} \quad (3.16)$$

Exchange terms occupy diagonal and off-diagonal positions in $K$ (Equation 3.17). Off-resonance and $B_1$ field terms are expressed in a single matrix (Equation 3.18). In the case of on-resonance pulses, $\delta = 0$. $\omega_1(t)$ is the complex pulse envelope of the $B_1$ magnetic field pulse expressed in the rotating frame, which vanishes in the case of free precession. $W_m(t)$ and $W_{nm}(t)$ represent the instantaneous saturation rates of the myelin and non-myelin semi-solid compartments, computed using $\omega_1(t)$ and the absorption lineshape of the semi-solid pool.
\[ \mathbf{K} = \begin{bmatrix}
0 & 0 & \ldots & \ldots & 0 \\
0 & -k_{m,mw} & 0 & \cdots & k_{mw,m} \\
0 & 0 & -k_{mw,ie} & \cdots & k_{ie,mw} \\
0 & 0 & 0 & \cdots & -k_{ie,mw} \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
k_{m,mw} & 0 & 0 & \cdots & -(k_{m,m} + k_{m,ie}) \\
k_{mw,ie} & 0 & 0 & \cdots & -k_{ie,mw} \\
k_{mw,ie} & 0 & 0 & \cdots & -k_{ie,nm} \\
k_{mw,ie} & 0 & 0 & \cdots & 0 \\
0 & 0 & 0 & \cdots & k_{ie,nm} \\
k_{mw,ie} & 0 & 0 & \cdots & -(k_{ie,nm} + k_{ie,mw}) \\
k_{mw,ie} & 0 & 0 & \cdots & 0 \\
0 & 0 & 0 & \cdots & 0 \\
0 & 0 & \cdots & \cdots & 0 \\
0 & 0 & \cdots & \cdots & 0 \\
0 & 0 & \cdots & \cdots & 0 \\
\end{bmatrix} \] (3.17)

\[ \mathbf{\Delta} + \mathbf{\Omega}_1(t) = \begin{bmatrix}
0 & 0 & \ldots & \ldots & 0 \\
0 & -W_m(t) & \cdots & \cdots & \cdots \\
0 & \delta & -\delta & -\text{Im}[\omega_1(t)] & \cdots \\
\delta & 0 & \text{Re}[\omega_1(t)] & \cdots & \cdots \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
\text{Im}[\omega_1(t)] & -\text{Re}[\omega_1(t)] & 0 & \cdots & \cdots \\
\delta & 0 & \text{Re}[\omega_1(t)] & \cdots & \cdots \\
\text{Im}[\omega_1(t)] & -\text{Re}[\omega_1(t)] & 0 & \cdots & \cdots \\
0 & \delta & 0 & \text{Re}[\omega_1(t)] & \cdots \\
0 & 0 & \cdots & \cdots & \cdots \\
0 & 0 & \cdots & \cdots & \cdots \\
0 & \delta & 0 & \text{Re}[\omega_1(t)] & \cdots \\
0 & 0 & \cdots & \cdots & \cdots \\
0 & 0 & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \cdots & \cdots \\
\end{bmatrix} \] (3.18)
Chapter 4

Reproducibility of QMTI

4.1 Preface

Cross-sectional studies using the QMTI and QT2 techniques, reviewed in Chapter 2, have revealed specific changes in lesions and NAWM of MS patients; in addition, histological validation of both techniques supports their potential specificity to demyelination [155, 195]. The logical extension is to study WM disease progression with quantitative MRI techniques. This requires that methods be both validated and reproducible. To determine patient sample sizes, or to establish study power, estimates of the variability of the techniques over repeated measurements must to be evaluated. In addition, the impact of strategies for data acquisition and parameter estimation on measurements should be evaluated.

In this chapter, the variability of two-pool MT model parameters is documented. The effect of corrections for RF and static field inhomogeneities on variability is also investigated. A novel technique is proposed for optimization of the sampling of MT-weighted data, and its effect on parameter maps and variability is evaluated. This constitutes the first evaluation of the reproducibility of MT model parameters over repeated measurements. More specifically, the reliability of QMTI parameters $F$, $k_f$, and $T_{2f}$ benefits significantly from field inhomogeneity corrections. A simple, efficient algorithm for reduction of data sampling is presented, we demonstrate that MT contrast sampling can be carefully reduced at relatively low cost in the quality of parameter maps and longitudinal reproducibility.
Reproducibility of quantitative magnetization transfer imaging parameters from repeated measurements


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4.2 Abstract

Quantitative magnetization transfer imaging (QMTI) methods provide *in vivo* estimates of parameters of the two-pool model for magnetization transfer (MT) in tissue. The goal of this study was to evaluate the reproducibility of QMTI parameter estimates in healthy subjects. MT-weighted and $T_1$ relaxometry data were acquired in five healthy subjects at multiple time points, and the variability of the resulting fitted MT parameters was evaluated. The impact of sub-sampling the MT data and correcting field inhomogeneities were also evaluated. The key parameters measured in this study had an average variability, across time points, of 4.7% for the relative size of the restricted pool ($F$), 7.3% for the forward exchange constant ($k_f$), 1.9% for the free pool spin-lattice relaxation constant ($R_{1f}$), 4.5% for the $T_2$ of the free pool ($T_{2f}$), and 2.3% for the $T_2$ of the restricted pool ($T_{2r}$). Our findings show that serial QMTI experiments can be performed reliably with good reproducibility of the model parameter estimates, and demonstrate the reproducibility of acquisition schemes with fewer MT contrasts. This establishes the feasibility of this technique for monitoring patients affected by degenerative white matter diseases, while providing critical data to estimate the statistical power of such studies.

**Key words:** quantitative magnetization transfer, longitudinal, reproducibility, field inhomogeneity correction
4.3 Introduction

The magnetization transfer (MT) effect is now well established as a contrast mechanism that can be exploited in MR imaging [36, 60, 75]. It is most often characterized via the calculation of the MT ratio (MTR), which has proven very useful and reliable in studies of healthy human brain white matter (WM) [223], of WM in multiple sclerosis (MS) [224–227], of grey matter disorders [228], of ageing [229] and others (see [36]). The MTR is quite reproducible [226,230] and correlates strongly with myelination [215,231]; however, it is also sensitive to water content and the $T_1$ of water protons.

Quantitative MT imaging (QMTI) can be performed using off-resonance saturation [137, 139, 141] to yield more detailed information about the MT phenomenon, by estimating the parameters of the two-pool model of MT in tissue [125, 131]. The key model parameters estimated with QMTI are the relative size of the restricted proton pool ($F$), the first-order forward magnetization exchange rate ($k_f$), and relaxation parameters of both pools (free pool: $R_{1f}$ and $T_{2f}$, and restricted pool: $T_{2r}$) [141]. These parameters are fundamental physical quantities with a potentially meaningful biological interpretation. QMTI has been used to study regional variations in healthy controls [151], WM fiber tracts [140], chronic lesions of MS [153], demyelination and axonal damage in lesions and normal-appearing WM of MS [154], and other aspects of MS [138, 152, 214]. In a study of post-mortem MS brain [155], the bound pool fraction (BPF, or $f_b$, which is directly related to $F$) was shown to correlate strongly with myelination, as quantified by the density of Luxol fast blue staining. QMTI parameters provide information about the underlying factors that determine the MT contrast, independent of the imaging technique; thus, these may serve to refine our understanding of the MTR changes observed in diseases such as MS, and could be used on their own to monitor disease progression.

The use of any quantitative technique in longitudinal studies requires an evaluation of the variability of the measurement to establish feasibility and to enable accurate statistical power calculations. The objective of this work was to evaluate the variability of parameter estimates obtained with our QMTI protocol [141]. Scan-rescan differences were evalu-
ated first in phantoms and in six healthy human subjects. A retrospective study was then performed using repeated measures in five subjects to investigate scan-reposition-rescan reproducibility over longer intervals. The impact of field inhomogeneity compensations – included in our standard imaging protocol – on parameter variability was also evaluated. Finally, the impact on variability associated with reducing the number of MT data samples, to reduce the scan time and facilitate inclusion of QMTI in clinical studies, was evaluated and compared to predictions from experiment design theory.

4.4 Methods

4.4.1 Data acquisition

Estimation of the two-pool model parameters requires, in principle, a minimum of 4 different MT-weighted measurements and a $T_1$ measurement [141]. The model parameters can be estimated by fitting an analytical signal equation to the acquired data. To ensure robust parameter estimation and allow verification of the selected model, the protocol used here relied on the acquisition of 60 MT-weighted images.

MT and relaxometry data were acquired according to the single-slice imaging protocol described in [141], on a 1.5 T Sonata (Siemens, Erlangen, Germany), using a quadrature head coil. MT-weighted images were obtained using a spoiled gradient echo sequence, in which a Hanning-windowed Gaussian off-resonance pulse was used to partially saturate the semi-solid components. The complete acquisition used two different protocols, with two saturation pulse powers, and with 20 and 10 offset frequencies respectively, for a total of 60 measurements. A normalization image, without off-resonance saturation, was also acquired for each TR. The complete sequence parameters are listed in Table 4.1 (protocols I and II).

The observed $T_1$ ($T_{1,obs}$) was measured using a Look-Locker pulse sequence with 4 readout blocks [40]. The sequence has TR/TE = 2 s/12 ms, and consists of a non-selective composite inversion pulse ($90^\circ_x$-180$^\circ_y$-90$^\circ_x$), followed after a time $T_1$ by 4 small-angle ($20^\circ$)
Table 4.1: Parameters of the MT-weighted acquisitions for the full, reduced, and optimized protocol.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>TR/TE (ms)</th>
<th>pulse angle</th>
<th>MT pulse angles</th>
<th>MT pulse duration (ms)</th>
<th>logarithmic frequency steps</th>
<th>offset frequencies (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25/4</td>
<td>7°</td>
<td>142°, 710°</td>
<td>10.24</td>
<td>20</td>
<td>0.23 – 80.0</td>
</tr>
<tr>
<td>II</td>
<td>50/4</td>
<td>10°</td>
<td>347°, 1388°</td>
<td>30.72</td>
<td>10</td>
<td>0.23 – 58.5</td>
</tr>
<tr>
<td>reduced</td>
<td>25/4</td>
<td>7°</td>
<td>142°, 710°</td>
<td>10.24</td>
<td>5</td>
<td>0.43 – 17.2</td>
</tr>
<tr>
<td></td>
<td>50/4</td>
<td>7°</td>
<td>710°</td>
<td>–</td>
<td>–</td>
<td>0.8, 1.09</td>
</tr>
<tr>
<td>optimized</td>
<td>25/4</td>
<td>7°</td>
<td>142°</td>
<td>10.24</td>
<td>–</td>
<td>1.5, 2.01, 9.3</td>
</tr>
<tr>
<td></td>
<td>50/4</td>
<td>10°</td>
<td>347°</td>
<td>30.72</td>
<td>–</td>
<td>0.23, 0.8</td>
</tr>
<tr>
<td></td>
<td>50/4</td>
<td>10°</td>
<td>1388°</td>
<td>–</td>
<td>–</td>
<td>0.23, 0.43, 9.3</td>
</tr>
</tbody>
</table>
slice selective excitations and gradient echo readouts at intervals $T_{I_2}$ ($T_{I_1}/T_{I_2} = 15/495$ ms).

Variations in the static and transmit excitation fields were measured in each subject. $B_0$ inhomogeneities were mapped using a two-point phase-difference technique [232], with a gradient echo sequence ($TR/TE/\alpha = 53$ ms/10 ms/31°) and readout time delay set to allow a complete rephasing of the fat signal (4.48 ms at 1.5 T). Separate $B_0$ maps were also acquired based on the MT spoiled gradient-echo sequence (without the saturation pulse) to account for any residual uncompensated eddy currents due to the large crushing gradients in that sequence. A two point method was used to map variations in the $B_1$ field [148], in which a non-selective preparation pulse ($\beta = 33^{\circ}$) is followed by a slice-selective turbo spin echo readout ($TR/TE = 2$ s/15 ms, ETL 7). The angle of the preparation pulse is doubled in the second acquisition, and the ratio of the acquired magnitude images is related to the nominal flip angle of the preparation pulse. Total scan time for the entire protocol was 53 minutes (17 minutes for structural $T_1/T_2/$PD-weighted scans and 36 minutes for all quantitative imaging).

4.4.2 Parameter estimation

All MT-weighted data were analyzed in combination with $T_{1obs}$, obtained from the Look-Locker measurement, using Sled and Pike’s rectangular pulse model of steady-state pulsed MT [126], to yield maps of the estimated model parameters. The solid pool was modeled using a Gaussian lineshape for agar gel and a super-Lorentzian lineshape for subjects [125, 131, 141]. Fitting of the analytical signal equation was performed using a Levenberg-Marquardt non-linear least-squares algorithm implemented in MATLAB (The Mathworks, Natick, MA, USA). For our standard protocol (hereafter called the “full” protocol), and for the reduced and optimized protocols, data analysis was performed with corrections for $B_0$ and $B_1$ field inhomogeneities. The parameter estimation was also performed without these corrections (the “uncorrected” protocol), to evaluate their impact on measurement variability.

Various data reduction and optimization schemes have been proposed [137, 139, 142,
143, 233], and the impact of fitting fewer MT samples on reproducibility was also considered here. Two subsets of the acquired MT contrasts were selected, each with 10 MT contrasts and associated normalization images. The first subset (the “reduced” protocol) was selected to match another published protocol [142] as closely as possible. It consisted of data acquired with protocol I, as listed in Table 4.1, using the $3^{rd}$, $6^{th}$, $9^{th}$, $12^{th}$, and $15^{th}$ offset frequencies and both MT flip angles. Second, an “optimized” 10-point MT sampling scheme was derived from our acquired data, based on average WM parameter values. This optimized sampling was determined by an iterative search based on the standard error of the model parameters estimated from the fit residuals. Starting from the “full” protocol ($N = 60$), each iteration reduced the sampling by one MT data point. By considering all schemes with $(N-1)$ points, and computing the predicted standard error for each new scheme, the offset/flip-angle combination that resulted in the smallest global increase in the predicted standard error was eliminated. This process was repeated until 10 MT contrasts (plus the no-saturation image) were left. The selected offset/flip-angle combinations are listed in Table 4.1.

### 4.4.3 Scan-rescan experiments

Repeated acquisitions were performed in gel phantoms to evaluate the measurement variability in a chemically stable material while eliminating issues related to partial volume contamination, physiological noise, and motion artifacts. Agar gel (Becton-Dickinson, Sparks, MD, USA) was selected due to its well-characterized MT properties [125]. Two phantoms (agar concentration 2% and 4% w/w) were constructed in one-litre plastic bottles (Thermo Fisher Scientific, Waltham, MA, USA) using distilled water. These bottles were placed in the head coil and imaged at the magnet isocentre using the same protocol as for in vivo imaging. Single-session scan-rescan experiments were also performed in six subjects without repositioning (as per Table 4.2) to measure differences due strictly to the technique, while eliminating variability due to long-term scanner drift, repositioning or biological changes.
Table 4.2: Subject group description, including regions-of-interest (ROIs) retained for analysis in each subject. Subject age is at study entry. Number of exams refers to the number of longitudinal time points available for each subject. Scan-rescan indicates that a same day scan-rescan pair of acquisitions was performed in that subject.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Number of exams</th>
<th>Available ROIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>male</td>
<td>26</td>
<td>4 + scan-rescan, Centrum semiovale (CSO), Minor forceps (mF), Corona radiata (CR)</td>
</tr>
<tr>
<td>2</td>
<td>male</td>
<td>38</td>
<td>7 + scan-rescan, mF, Genu and Splenium (Spl) of corpus callosum, Major forceps (MF), Caudate (Cd)</td>
</tr>
<tr>
<td>3</td>
<td>female</td>
<td>24</td>
<td>4 + scan-rescan, CSO, mF</td>
</tr>
<tr>
<td>4</td>
<td>female</td>
<td>32</td>
<td>4 + scan-rescan, CSO</td>
</tr>
<tr>
<td>5</td>
<td>male</td>
<td>31</td>
<td>4 + scan-rescan, CSO</td>
</tr>
<tr>
<td>6</td>
<td>male</td>
<td>31</td>
<td>scan-rescan, mF, Genu, Spl, MF, Cd</td>
</tr>
</tbody>
</table>
4.4.4 Longitudinal study

A retrospective study was performed using repeated QMTI measurements from five healthy individuals serving as control subjects in a separate longitudinal study of MS patients. These control subjects were selected based on age and gender to match specific patients. Data acquisition was performed in a single oblique 7-mm section (details presented in Table 4.2) at multiple time points over a period of three years. Positioning of the quantitative imaging slice varied from subject to subject to suit the needs of the MS study. All subjects were scanned four times, and one was scanned seven times, providing a total of 23 datasets. Inter-scan periods ranged from 11 days to 2.4 years. Care was taken to match the slice positioning for each scan to that from the initial acquisition.

4.4.5 Data analysis

The single-slice geometry of the quantitative images was more amenable to a ROI-based analysis of the variability of each subjects data across time points than a voxel-based analysis. In phantoms, ROIs were defined as the cross-section of each bottle excluding the area approximately 1 cm away from the edge of the bottle. In the subjects, sixteen (16) WM and GM ROIs were manually identified on the initial $T_1$-weighted structural scan of each individual, separating left and right homologous ROIs when possible. ROI labels were propagated to subsequent time points, and re-sampled to the lower resolution of the quantitative MT scans. Label voxels that exhibited obvious partial volume contamination when viewed superimposed on registered structural scans were manually excluded from the ROIs for the analysis. Each ROI was available only for a sub-group of subjects, due to the single-slice acquisition, as listed in Table 4.2. Parameters were normally distributed in each ROI, and the mean from each propagated ROI was used for this analysis.

To compare the variability of estimates introduced by repeated measurements over time to the variability expected from noise, we performed a one-way ANOVA on the voxel data from each combination of subject, ROI, and parameter, treating time as the factor in
the analysis. For ROIs that were labeled separately in the left and right hemispheres, the ANOVA was first performed including a second factor for hemisphere. A threshold of \( p = 0.01 \), corrected for multiple comparisons (\( N = 16 \), the total number of subject-ROI combinations), was selected for significance.

### 4.5 Results

#### 4.5.1 Phantom experiments

Parameter values in the agar gel phantom are presented in Table 4.3: the mean of each parameter across the phantom was computed for each scan, and the values shown in the table are the average of both scans. The error values presented in Table 4.3 are the pooled within-ROI standard deviations of parameters, derived from the spatial standard deviation within each phantom and propagated through the same averaging process as the mean values. Percent differences between scan and rescan are presented in Table 4.3.

Table 4.3: Mean, within-ROI standard deviation, and scan-rescan differences, of parameters in agar gels of 2 and 4%. Mean values are the average of two scans and within-ROI standard deviations are pooled from both scans.

<table>
<thead>
<tr>
<th>agar concentration</th>
<th>2%</th>
<th>scan-rescan</th>
<th>4%</th>
<th>scan-rescan</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI volume (ml)</td>
<td>43.8</td>
<td>-</td>
<td>43.5</td>
<td>-</td>
</tr>
<tr>
<td>( F ) (%)</td>
<td>0.0068 ± 0.0011</td>
<td>3.3%</td>
<td>0.0132 ± 0.0014</td>
<td>1.7%</td>
</tr>
<tr>
<td>( k_f ) (s(^{-1}))</td>
<td>2.13 ± 1.49</td>
<td>5.2%</td>
<td>1.49 ± 0.40</td>
<td>0.3%</td>
</tr>
<tr>
<td>( R_{1f} ) (s(^{-1}))</td>
<td>0.456 ± 0.021</td>
<td>0.6%</td>
<td>0.546 ± 0.020</td>
<td>0.7%</td>
</tr>
<tr>
<td>( T_{2f} ) (ms)</td>
<td>53.3 ± 1.9</td>
<td>0.5%</td>
<td>32.5 ± 1.2</td>
<td>0.9%</td>
</tr>
<tr>
<td>( T_{2r} ) (µs)</td>
<td>16.3 ± 1.8</td>
<td>4.4%</td>
<td>15.5 ± 1.1</td>
<td>1.7%</td>
</tr>
</tbody>
</table>
4.5.2 *In vivo experiments*

Example maps of the pool size ratio $F$ and the magnetization exchange rate $k_f$, produced using all four variations of the protocol (full, uncorrected, reduced, and optimized) in one subject, are shown in Figure 4.1.

For ROIs that were identified as homologous left/right pairs, the 2-way ANOVA (with factors time and hemisphere), revealed no significant effect of hemisphere for any of the ROI-subject combinations (at a level of $p = 0.01/16 = 6.25e-3$). As a result, the data for homologous left/right pairs was pooled and subsequently analyzed with one-way ANOVA for the effect of time.

4.5.3 *Scan-rescan experiments*

The mean parameter values in subject ROIs are listed in Table 4.4: scan and rescan values were first averaged for each subject, and the average across subjects is reported here. The error values presented in Table 4.4 are the pooled within-ROI standard deviations of parameters, derived from the spatial standard deviation within each ROI and propagated through the same averaging process as the mean values. Percent differences between scan and rescan are presented in Table 4.5.

4.5.4 *Longitudinal study*

The variability of the longitudinal MT-weighted data was evaluated, as it is input to the fitting routine and is likely to be the primary source of the variability of the model parameters. The CoV of the MT-weighted data (averaged across all weightings) for this study ranged from 0.4 to 1.5% depending on the subject and region-of-interest, with maximum variability occurring for weightings obtained using near-resonance saturation pulses (range 4 to 18%). This is illustrated in Figure 4.2, which shows a plot of MT-weighted data obtained from the short TR sequence (sequence I), normalized to the data acquired without saturation, in one ROI of subject 2, as a function of offset frequency, for both pulse pow-
Figure 4.1: Maps of the pool size ratio ($F$, top row) and forward magnetization transfer rate ($k_f$, in s$^{-1}$, bottom row) in one subject. Each column (left to right) shows examples of maps produced using the full protocol (column 1), without field inhomogeneity corrections (column 2), with the reduced MT dataset (column 3), and with the optimized MT dataset (column 4). The small patch of signal on the upper left of each map is from a small vial of water doped with manganese chloride. Note that the full, reduced, and optimized protocols correctly result in $F \approx 0$ in water, but that the uncorrected processing results in $F \neq 0$. Note also the diagonal gradient across the uncorrected $k_f$ map (bottom, column 2).
Table 4.4: Mean parameters in WM and GM regions of 6 healthy subjects (ROI descriptions are in Table 4.2), averaged over scan and rescan then over subjects. Also shown is the pooled within-region standard deviation of the given parameter.

<table>
<thead>
<tr>
<th>ROI</th>
<th>$F$</th>
<th>$k_f$ (s$^{-1}$)</th>
<th>$R_{1f}$ (s$^{-1}$)</th>
<th>$T_{2f}$ (ms)</th>
<th>$T_{2r}$ (µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSO (ant.)$^a$</td>
<td>0.16 ± 0.03</td>
<td>4.0 ± 0.9</td>
<td>1.78 ± 0.12</td>
<td>35 ± 3</td>
<td>12.2 ± 0.9</td>
</tr>
<tr>
<td>CSO (mid.)$^a$</td>
<td>0.15 ± 0.03</td>
<td>3.7 ± 0.8</td>
<td>1.76 ± 0.06</td>
<td>37 ± 4</td>
<td>12.3 ± 0.7</td>
</tr>
<tr>
<td>CSO (post.)$^a$</td>
<td>0.15 ± 0.03</td>
<td>3.6 ± 0.6</td>
<td>1.76 ± 0.10</td>
<td>37 ± 4</td>
<td>12.3 ± 0.8</td>
</tr>
<tr>
<td>mF$^a$</td>
<td>0.16 ± 0.03</td>
<td>4.1 ± 0.9</td>
<td>1.83 ± 0.10</td>
<td>34 ± 3</td>
<td>11.8 ± 0.8</td>
</tr>
<tr>
<td>Genu</td>
<td>0.18 ± 0.04</td>
<td>4.8 ± 1.3</td>
<td>1.96 ± 0.12</td>
<td>31 ± 3</td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td>Splenium</td>
<td>0.15 ± 0.03</td>
<td>3.5 ± 0.6</td>
<td>1.75 ± 0.12</td>
<td>36 ± 4</td>
<td>10.8 ± 0.6</td>
</tr>
<tr>
<td>Caudate$^a$</td>
<td>0.07 ± 0.01</td>
<td>1.8 ± 0.7</td>
<td>1.00 ± 0.08</td>
<td>51 ± 7</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td>MF$^a$</td>
<td>0.16 ± 0.03</td>
<td>3.5 ± 0.8</td>
<td>1.79 ± 0.10</td>
<td>36 ± 4</td>
<td>11.9 ± 0.9</td>
</tr>
<tr>
<td>CR$^a$</td>
<td>0.15 ± 0.03</td>
<td>3.8 ± 1.0</td>
<td>1.78 ± 0.08</td>
<td>40 ± 4</td>
<td>12.4 ± 0.9</td>
</tr>
</tbody>
</table>

$^a$ Indicates regions where left and right hemisphere data were combined.
Table 4.5: Single-session scan-rescan percent differences in ROIs from 6 healthy subjects (ROI descriptions are in Table 4.2). Differences were computed between the ROI-averaged values for each subject, and then averaged across subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\Delta F$</th>
<th>$\Delta k_f$</th>
<th>$\Delta R_{1f}$</th>
<th>$\Delta T_{2f}$</th>
<th>$\Delta T_{2r}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSO (ant.)$^a$</td>
<td>1.8%</td>
<td>3.4%</td>
<td>0.3%</td>
<td>1.3%</td>
<td>0.6%</td>
</tr>
<tr>
<td>CSO (mid.)$^a$</td>
<td>2.4%</td>
<td>6.2%</td>
<td>0.1%</td>
<td>3.1%</td>
<td>0.2%</td>
</tr>
<tr>
<td>CSO (post.)$^a$</td>
<td>2.4%</td>
<td>5.8%</td>
<td>1.4%</td>
<td>1.1%</td>
<td>2.6%</td>
</tr>
<tr>
<td>mF$^a$</td>
<td>1.9%</td>
<td>4.8%</td>
<td>1.6%</td>
<td>1.3%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Genu</td>
<td>3.4%</td>
<td>3.8%</td>
<td>2.7%</td>
<td>4.8%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Splenium</td>
<td>5.3%</td>
<td>6.2%</td>
<td>0.4%</td>
<td>0.7%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Caudate$^a$</td>
<td>5.1%</td>
<td>11.5%</td>
<td>2.2%</td>
<td>3.5%</td>
<td>0.4%</td>
</tr>
<tr>
<td>MF$^a$</td>
<td>3.2%</td>
<td>4.0%</td>
<td>0.3%</td>
<td>2.4%</td>
<td>0.8%</td>
</tr>
<tr>
<td>CR$^a$</td>
<td>1.3%</td>
<td>1.3%</td>
<td>1.1%</td>
<td>2.7%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Mean</td>
<td>3.0%</td>
<td>5.9%</td>
<td>1.3%</td>
<td>2.5%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Range across subjects</td>
<td>[0.06, 6.3]</td>
<td>[0.2, 16.3]</td>
<td>[0.02, 4.9]</td>
<td>[0.3, 8.0]</td>
<td>[0.04, 3.7]</td>
</tr>
</tbody>
</table>

$^a$ ROIs where left and right hemisphere data were combined.
Figure 4.2: Sample normalized MT-weighted data obtained using sequence I (TR = 26 ms), from the splenium of subject 2, plotted as a function of frequency offset from resonance. Data are averaged across 7 measurements, and error bars show the standard deviation. Note the greater variability of data near resonance.

ers. The error bars represent the standard deviation across repeated measurements, which is relatively greater for data near resonance.

The CoV of each ROI value across time was computed. The CoVs for each parameter were then pooled across subjects for each region-of-interest, and are presented for the full protocol, along with the range of observed values, in Table 4.6. The CoVs for each parameter pooled across all regions and subjects are also plotted in Figure 4.3 (“full protocol”).

Different slice positioning and orientation across subjects precluded the analysis of inter-subject variability with the data in this study. Inter-subject variability was estimated
Figure 4.3: Average CoV across time for each parameter, averaged over all ROIs and subjects. Parameter estimates were produced with the full protocol (blue), with the optimized MT sampling (green), with the reduced MT sampling (dark red), and without field corrections (orange).
Table 4.6: Mean coefficients of variation (in %) computed longitudinally across time points for each parameter, pooled across 5 subjects by ROI.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CoV ([F])</th>
<th>CoV ([k_f])</th>
<th>CoV ([R_{1f}])</th>
<th>CoV ([T_{2f}])</th>
<th>CoV ([T_{2r}])</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSO (ant.)(^a)</td>
<td>5.2</td>
<td>5.8</td>
<td>1.3</td>
<td>4.4</td>
<td>2.1</td>
</tr>
<tr>
<td>CSO (mid.)(^a)</td>
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<td>0.9</td>
<td>5.3</td>
<td>1.7</td>
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<tr>
<td>CSO (post.)(^a)</td>
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<td>1.9</td>
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<tr>
<td>mF(^a)</td>
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<td>7.3</td>
<td>0.7</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Genu</td>
<td>6.9</td>
<td>7.7</td>
<td>3.1</td>
<td>4.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Splenium</td>
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<td>9.5</td>
<td>1.4</td>
<td>3.3</td>
<td>2.2</td>
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<tr>
<td>Caudate(^a)</td>
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<td>7.4</td>
<td>4.8</td>
<td>4.6</td>
<td>2.7</td>
</tr>
<tr>
<td>MF(^a)</td>
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<td>7.0</td>
<td>3.0</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td>CR(^a)</td>
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<td>6.7</td>
<td>1.0</td>
<td>3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>All ROIs</td>
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<td>1.9</td>
<td>4.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Range</td>
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<td>0.4 - 4.8</td>
<td>1.6 - 9.2</td>
<td>0.2 - 3.9</td>
</tr>
<tr>
<td>Inter-subject CoV, based on [151]</td>
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<td>12</td>
<td>5</td>
<td>(not reported)</td>
<td>4</td>
</tr>
<tr>
<td>Range</td>
<td>8 - 12</td>
<td>8 - 18</td>
<td>3 - 6</td>
<td>2 - 7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) ROIs where left and right hemisphere data were combined.
based on parameter measurements previously reported by our group [151], acquired using the same imaging protocol. The inter-subject variability was computed for each parameter by dividing the standard deviation by the mean value among 4 subjects reported in [151], in WM ROIs: the result is presented in the two bottom rows of Table 4.6.

One-way ANOVA computed for each combination of subject, ROI, and parameter revealed variations across time-points beyond what was accounted for by random variation of the parameter estimates within an ROI. This additional variation, detected significantly ($p = 0.01$, corrected for multiple comparisons) for 30 of 80 subject-ROI-parameter combinations, was on average 140% of the variation in parameter estimates measured within an ROI, and about 300% on average in for combinations where the additional variation over time was significant.

### 4.5.5 Field inhomogeneity corrections

The QMTI parameters were also estimated without field inhomogeneity corrections, using the complete set of MT data. The quality of the fit was evaluated by the normalized root-mean-squared residuals. Overall, these fit residuals were greater for the fits without inhomogeneity corrections: mean residuals of 2.8%, range 2.2 - 3.5%, were observed for uncorrected fits, compared to a mean of 2.3%, range 2.0 - 2.7%, for corrected fits. Parameter values computed without field inhomogeneity compensations were compared to the full protocol: the uncorrected protocol generally resulted in greater estimates of $F$, and lower estimates of $k_f$, $T_{2f}$, and $T_{2r}$, and no difference in $R_{1f}$. The greatest differences were observed for $T_{2f}$ (about 16%) and $k_f$ (about 10%).

The CoVs of uncorrected parameters, for all ROIs combined, are plotted in Figure 4.3 (labeled as “uncorrected”). Parameter variability was greater when fits did not include field corrections. Increases in variability were observed for the parameters $F$ (CoV = 6.7%, range 3.0 - 10.4% for uncorrected vs. 4.7%, range 1.5 - 7.7% for corrected), $k_f$ (CoV = 12.2%, range 4.8 - 20.3% for uncorrected, vs. 7.3%, range 3.5 - 9.9% for corrected), and $T_{2f}$ (CoV = 19.4%, range 4.6 - 42.1% for uncorrected vs. 4.5%, range 1.6 - 9.2% for
corrected). The increased variability in $R_{1f}$ (CoV = 1.9%, range 0.2 - 4.9% for uncorrected vs. 1.9%, range 0.4 - 4.8% for corrected) and $T_{2r}$ (CoV = 2.4%, range 1.2 - 3.9% for uncorrected vs. 2.0%, range 0.2 - 4.0% for corrected) was much smaller.

The one-way ANOVA was repeated for parameter estimates that were not corrected for $B_1$ and $B_0$ field inhomogeneities. For these uncorrected parameters, the additional variability over time was significant in over half of the subject-ROI-parameter combinations (49 of 80), a considerable increase over the full protocol. In this case, the additional variability over time was on average 3.5 times the expected (within-ROI) variability, and more than 5 times greater for combinations where the variation over time was significant. This increase resulted mainly from an increase in the variation between time-points, while the within-ROI variation was only slightly greater than for the full protocol.

### 4.5.6 Reduced MT sampling

Model parameter estimates were estimated using two subsets of the acquired MT data (10 MT-weighted images plus a normalization image): the “reduced” and “optimized” protocols described in the Methods section and Table 4.1. The increase in standard error predicted from fit residuals was computed for both protocol variations, compared to the full protocol. The reduced sampling increased the predicted standard error on $F$, $T_{2f}$, and $k_f$ each by a factor between 2.11 and 2.19, while the predicted error of $T_{2r}$ increased by a factor of 3.16; for the optimized sampling, the predicted standard error increased by a factor between 1.5 and 1.9.

Estimates from fits to the reduced datasets were comparable to those obtained using all available MT data. Parameter values were compared to the full protocol for systematic differences: each parameter was considered separately, and comparisons were performed using a paired T-test. The $F$ estimates from the reduced protocol were greater (around 7%) than those from the full protocol; on the other hand, the $T_{2f}$ values from the reduced protocol were smaller (by about 10%). $R_{1f}$, $T_{2r}$ and $k_f$ values were not systematically different between the full and reduced MT sampling schemes. The CoVs of the parameters
4.6 Discussion

estimated with a reduced MT dataset, pooled across all ROIs, are presented in Figure 4.3 ("reduced"). The reduced MT sampling resulted in increased variability in $F$ ($\text{CoV} = 5.2\%$, range 1.9 - 8.4%), $k_f$ ($\text{CoV} = 8.9\%$, range 3.4 - 15.0%), and in $T_2r$ ($\text{CoV} = 3.7\%$, range 1.2 - 5.6%). Parameter estimates from the reduced protocol had greater within-ROI variability. For most model parameter estimates, the within-ROI variability resulting from the reduced protocol was about double the variability observed with the full protocol.

Estimates from fits to the optimized datasets were also comparable to those obtained using all available MT data. The $F$, $T_2f$, and $T_2r$ values from the optimized protocol were greater than those from the full protocol, while $R_1f$ and $k_f$ values were not systematically different between the full and optimized sampling schemes. The CoVs of the parameters estimated with our optimized MT dataset, averaged across all ROIs, are presented in Figure 4.3 ("optimized"). Interestingly, the optimized MT sampling had reduced variability of $k_f$ ($\text{CoV} = 5.6\%$, range 1.1 - 10.2%) and $T_2f$ ($\text{CoV} = 3.3\%$, range 1.2 - 5.1%), when compared to the full protocol was also reduced versus the full protocol. One-way ANOVA of parameter estimates from the optimized protocol resulted in very similar variability, both within ROIs and between time-points, to the variability observed with the full protocol.

4.6 Discussion

This study sought to establish the reproducibility of estimates of the MT two-pool model parameters from QMTI. The variability of each parameter was evaluated, first by scan-rescan experiments in phantoms and a human subject, and then by repeated measures in a group of healthy controls. Technical aspects of the acquisition and analysis method that may influence the resulting parameters, such as the MT data sampling and compensation for field inhomogeneity effects, were also considered.

The parameter estimates obtained in agar gel phantoms compared well with previously published values from imaging experiments [141]: $F$ values were similar and scaled correctly with agar gel concentration, $k_f$ was somewhat high and variable in 2% agar in this
study, $R_{1f}$ was also high in both phantoms, and finally, $T_{2f}$ and $T_{2r}$ were consistent with previous reports. These small differences from the literature in agar gel MT parameter values are attributed to phantom preparation, and are secondary to the more important aspect of stability over repeated experiments. The parameter values estimated in the human subjects compared well with WM values previously reported by our group [151]: $F$ and $T_{2r}$ were slightly higher in this study, $k_f$ is very similar but slightly more variable, and $R_{1f}$ was very close to previous observations.

Percent differences from single session scan-rescan were similar in agar phantoms and human subjects. The percent differences of $F$ and $k_f$ were similar; free pool relaxation parameters $R_{1f}$ and $T_{2f}$ differences were lower in phantoms, while $T_{2r}$ differences were lower in WM. We speculate that greater differences in free pool relaxation parameters of brain tissue could be due to the presence of multi-component relaxation, not accounted for by the two-pool model. On the other hand, the more stable $T_{2r}$ values in WM might reflect a smaller dynamic range of this parameter in tissue, a weak model dependence on $T_{2r}$ (and thus low amplification of measurement noise), or perhaps better performance of the super-Lorentzian lineshape at describing the behavior of WM. Most importantly, these results demonstrate that the reproducibility of in vivo measurements made with our QMTI method is comparable to the reproducibility seen in phantoms, despite any variability introduced by physiological noise and subject movement during the exam.

Overall, repeated QMTI experiments with our full protocol yielded an average longitudinal variability in ROIs of 4.7% for the relative size of the restricted pool $F$, 7.3% for the forward exchange constant $k_f$, 1.9% for the free pool spin-lattice relaxation constant $R_{1f}$, 4.5% for the $T_2$ of the free pool ($T_{2f}$), and 2.3% for the $T_2$ of the restricted pool ($T_{2r}$). The observed variability of the parameter estimates combines the effects of biological variations, noise and drift in the data acquisition, slice positioning by the radiology technician, and fitting error. Parameters were more variable in certain regions, notably the caudate nuclei and the various regions of the corpus callosum. This higher variability could be due to partial volume contamination by cerebrospinal fluid in the ventricles, bordering
these tissue regions, which can have a very important impact on the measured MT effect within the relatively large quantitative imaging voxels \((2\times 2\times 7 \text{ mm}^3)\). The longitudinal coefficient-of-variability of parameters was comparable to the differences observed in the control scan-rescan experiment. Our observations suggest that the reliability of QMTI measurements depends mainly on the measurement noise and stability of parameter estimation, and that factors such as scanner drift and subject position seem to be secondary effects over the course of a longitudinal study using the full protocol.

For reasons outlined in the Results section, the slice position was different across subjects; as a result, the available ROIs were different for each individual, thus precluding the analysis of inter-subject and inter-region variability. However, comparison to data previously reported by our group using the same imaging protocol \([151]\), indicated that inter-subject differences are generally greater than the intra-subject variability observed in this study, even over long inter-scan periods. This relative intra-subject stability of parameter estimates enables the use of QMTI in serial studies.

In this group of subjects, field inhomogeneity corrections resulted in significantly smaller fit residuals, and in improved reproducibility for the parameters \(F, k_f, \text{ and } T_{2f}\). Without the applied corrections, the variability of these 3 parameters increased to 6.7%, 12.2%, and 19.4% respectively, from 4.7%, 7.3%, and 4.5%. This was also reflected in the considerable increase of the number of subject-ROI-parameter combinations (from 30/80 to 49/80) where variations between time-points were significantly greater than expected from noise alone. This increase in longitudinal variability is most likely attributable to the variability in the static and excitation magnetic fields within the brain, which can vary from session to session due to different subject placement within the scanner. Thus, the scan time used for field mapping, which is relatively short (4.5 minutes for \(B_1\) and all \(B_0\) maps in our protocol) and could potentially be shorter if acquired at a lower resolution, is time well-spent. We speculate that these effects would be even more important when imaging at higher field strengths, where \(B_1\) field inhomogeneity is greater.

Parameter estimation with a subset of the MT data (the “reduced” protocol) introduced
a bias in the parameter values, and increased the variability of certain parameters. In reducing the number of MT samples from 60 to 10, one would expect an average increase of the predicted standard error of each parameter by a ratio of approximately 2.4 \( (= \sqrt{6}) \).

The predicted standard error increased as expected for the reduced sampling; however, the increase for the reduced sampling was not equal for all parameters, likely because of the non-linearity of the model. The increase with the optimized sampling scheme was lower than expected, highlighting the benefit of careful selection of the MT weightings in this non-linear analysis.

The measured variability for the reduced protocol was observed to increase to 5.2%, 8.9% and 3.7%, for \( F \), \( k_f \) and \( T_{2r} \), respectively. The optimized protocol also resulted in different parameter estimates when compared to the full protocol; however, the optimized sampling scheme did not increase the variability of the parameter estimates, and in some cases (\( k_f \) and \( T_{2f} \)) resulted in lower variability than using the full 60-point sampling. We believe that this reduced variability is due in large part to fewer points near resonance in the optimized sampling, where the MT data is more variable (see Figure 4.2) and where our model for pulsed saturation is potentially less accurate [89]. The longitudinal variability and within-ROI variations were smaller than expected from predictions by experiment design theory: we attribute this difference to systematic fit residuals related to the limitations of the two-pool MT model, unaccounted for by the error analysis.

The magnitude of the variability increase was less pronounced when reducing the number of MT contrasts used than when neglecting field inhomogeneity compensation. Most importantly, the reproducibility of the pool size ratio \( F \), a potential marker for myelin in WM, was not significantly affected by the reduction in MT samples in this study. Acquisition of the optimized dataset would decrease the overall scan time to 31 minutes, a substantial difference of 22 minutes, with little increase in parameter variability. Our findings support the careful reduction of the MT data sampling, either by use of sparse or optimized sampling of more extensive protocols as done here, or via optimization techniques such as Cramer-Rao lower bounds as proposed by Cercignani and Alexander [143], to reduce the
exam duration. Note that the reduced MT datasets in this study were processed including corrections for field inhomogeneities.

As a demonstration of the application of these measurements, the sample sizes required to observe specific parameter changes were computed using G*Power 3 [234]. Estimates of the sample size were obtained by combining the average of the parameter estimates and the variability in WM. Calculations were performed for expected changes in the pool size ratio $F$ and the exchange constant $k_f$, based on a two-tailed test, with alpha ($\alpha$) and beta ($\beta$) levels of 0.05 (false positives & false negatives, respectively) and a power of 0.95. The sample sizes required to observe a significant change in $F$ and $k_f$ are plotted in Figure 4.4, versus the expected change. Computations were also performed using the variability measures for parameters obtained from uncorrected fits, and fits performed using the reduced and optimized datasets. These calculations highlight that, for example, the detection of a 15% decrease in $F$ in a longitudinal study would require 12 patients if the field mapping were not performed, versus 8 if it were; obtaining the same observation using the optimized protocol – with field corrections – would also require 8 patients.

In conclusion, this work has demonstrated that longitudinal quantitative MT imaging experiments can produce reliable estimates of the two-pool model parameters, with an average variability ranging from 1.9% to 7.3% depending on the parameter. The exchange rate $k_f$ is the most difficult parameter to constrain, consistent with prior observations by our group and others. Our observations support the importance of compensating for field inhomogeneities to ensure the consistency of parameter estimates over longitudinal studies, and support the reduction of data sampling while maintaining acceptable reproducibility in serial experiments. The findings from this study establish the feasibility of using quantitative magnetization transfer MRI techniques for the monitoring of tissue changes in patients affected by degenerative WM diseases, and provide data on which to base the statistical power of longitudinal studies.
Figure 4.4: Estimates of the sample size required to observe significant changes in $F$ and $k_f$ ($p < 0.05$, power = 0.95), based on average WM parameter values and variability. Measurement protocols are color-coded to match Figure 4.3. Sample sizes for $F$ estimates from the optimized protocol were identical to those from the full protocol, and are omitted from the plot.
4.7 Acknowledgments

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Chapter 5

Reproducibility of multi-component $T_2$

5.1 Preface

In keeping with the use of quantitative MRI techniques in longitudinal studies, in this chapter we report our evaluation of the reproducibility of the QT2 method, as a complement to Chapter 4. Data were collected in the same group of subjects, in same-day sessions and exams repeated at intervals over a period of 2.5 years. This constitutes the first evaluation of the reproducibility of QT2 over repeated measurements outside the pioneering group at the University of British Columbia. In particular, this study demonstrates that while the values of the MWF, $\langle T_2 \rangle$, and $\langle T_{2\text{te}} \rangle$ may depend on the choice of $T_2$ range for the myelin water signal, the reliability of the measurements appears to be independent of the selection. Our observations also indicate that the choice of geometry and repetition time in the data acquisition, selected here to accelerate the acquisition while maintaining SNR, can influence the estimation of the $T_2$ distribution. As a side note, a very simple method was implemented and tested for the evaluation of the MWF using a flexible, or adaptive, MW $T_2$ window, which searched for the local minima between peaks in the $T_2$ distribution. This method did not yield any improvement over the fixed MW range method in this particular, but was not tested over a range of experimental variations, such as the amount of regularization (2.5% here), or the definition of the $T_2$ scale (120 values between 10 ms and 4 s here).
Reproducibility of *in vivo* magnetic resonance imaging based measurement of myelin water

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5.2 Abstract

**Purpose:** To evaluate the reproducibility of multi-component quantitative $T_2$ (QT2) measurements, in particular the myelin water fraction (MWF), to determine the potential role of this method in monitoring myelin changes in longitudinal studies and to provide a basis for correctly powering such studies.

**Materials and Methods:** The *de facto* standard 32-echo spin echo imaging sequence was used throughout, and data were analyzed using regularized non-negative least squares (NNLS) to produce $T_2$ distributions. Three studies were conducted in healthy subjects. First, two acquisition protocols were compared in 10 subjects. Second, variability of QT2 was evaluated over same-day scan-rescan experiments in 6 subjects. Finally, variability was quantified in a longitudinal study of 5 subjects.

**Results:** A within-subject coefficient-of-variation (CoV) of 12% was observed for the MWF in WM ROIs. The geometric mean $T_2$ was more stable, with a longitudinal CoV of about 4%. The choice of the geometry and TR of the acquisition protocol influenced the estimates of the MWF and $T_2$ values. The choice of integration range for the short-$T_2$ component had a significant effect on MWF estimates, but not on reproducibility.

**Conclusion:** The reproducibility of QT2 measurements using existing methods is moderate and the method can be used in longitudinal studies, with careful consideration of the methodological variability and an appropriate group size.

**Key words:** multi-component $T_2$, myelin water fraction, reproducibility
5.3 Introduction

Quantitative magnetic resonance imaging (MRI) techniques sensitive to specific tissue constituents are becoming increasingly important and a technique that could reliably map myelin content and status in vivo would be of significant value. One proposed method of myelin imaging is based on multi-exponential analysis of multi-echo spin-echo data, often referred to as quantitative $T_2$ relaxometry (QT2). In these imaging experiments, the $T_2$ decay is sampled with at least 32 closely-spaced echoes, usually with echo spacing around 10 ms. Data analysis is most often performed with a modified version of the non-negative least-squares (NNLS) fitting technique [178, 179, 235], which transforms the discretely sampled $T_2$ decay curve into a distribution of signal amplitudes versus $T_2$ value. To deal with the presence of measurement noise and with fitting more $T_2$ values than available data points, the NNLS distribution can be regularized to yield a more continuous model and avoid spurious peaks [178]. Regularization is commonly performed by including an energy or curvature term in the minimization cost function [172], with iterative adjustment of the weight of the regularization [180]. Water in different micro-environments can be identified by distinct $T_2$ relaxation times, reflected in the $T_2$ distribution [235]. In brain white matter (WM), three water components are routinely observed [170–172, 236]: (i) myelin water (MW, i.e. water trapped within the myelin structure, $T_2 \approx 15$ ms) (ii) intra/extracellular (IE) water ($T_2 \approx 80$ ms), and (iii) cerebrospinal fluid (CSF, $T_2 \approx 1$ s).

Mackay and colleagues at the University of British Columbia (UBC) have pioneered in vivo QT2 studies with applications to multiple sclerosis (MS) and other diseases [171, 172, 181, 183, 189, 190, 192–195, 204, 209, 235, 237, 238] using a 32-echo single-slice acquisition [162] and multi-exponential analysis. They have defined the myelin water fraction (MWF) as the percentage of the total signal associated with the short-$T_2$ peak and have suggested it is an index of the myelin content. The MWF in WM can range approximately from 6 to 18%, and 2 to 6 % in GM [172, 190, 204]. Regional WM variations in controls are attributed to varying degrees of myelination, and this is supported by observations of reduced MWF in lesions of MS [204]. Histology studies have also shown that the size
of short-$T_2$ component correlates with myelin content as measured with Luxol Fast-Blue (LFB) [194, 195]. In addition to the MWF observations, the geometric mean $T_2$ of the IE peak is prolonged in the normal-appearing WM (NAWM) of MS subjects, suggesting that QT2 is sensitive to subtle MS pathology [189].

While the MWF is a strong candidate for monitoring demyelination and remyelination in patients, knowledge about the variability of the QT2 method over repeated measurements must be established before use in longitudinal clinical studies. The reliability of various quantitative MRI metrics has been evaluated in an other study [238]. The absolute myelin water content (MWC) demonstrated a high reliability coefficient (RC) in 5 subjects scanned 5 times over one year, implying that it is stable in individuals despite the large spread of observations between individuals. However, there has been very limited work published by groups other than the UBC group using the QT2 technique in vivo [187, 214, 239], especially concerning the reproducibility of the measurements. QT2 measurements are used by our group within the context of a broader set of quantitative methods (including others such as $T_1$ relaxation and magnetization transfer imaging) to study CNS pathology.

In the present work, we report the results of two studies aimed at quantifying the variability of QT2, and of one study comparing two data acquisition protocols. First, data was collected from a group of healthy subjects using two imaging protocols. One protocol was identical to that established by the UBC group, and the other was based on an SNR-matched version of the same sequence adapted to fit within the time constraints of broader in vivo quantitative MRI studies in MS patients. Second, a scan-rescan experiment on 6 healthy subjects was performed using our QT2 protocol to estimate measurement reproducibility. Finally, we performed analysis of data acquired longitudinally in 5 healthy subjects using our QT2 protocol, over a period of 3 years.

The assignment of each signal component is trivial if distributions contain consistently well-defined peaks corresponding to the expected components, but this is not always the case. MWF calculations often use a pre-defined MW $T_2$ range cut-off. In the literature, the myelin water range most often used is $T_2 = 10 - 50$ ms [181, 183, 190, 204], although 10
Reproducibility of multi-component T₂

- 40 ms has been recently been used [192, 238]. One study explicitly excluded any CSF signal in the definition of total water signal [209], and others have estimated the MWC using an external water standard and correction for incomplete T₁ relaxation [193, 238]. In our analysis, the impact of the choice of a T₂ range for myelin water was investigated in the protocol comparison and for scan-rescan reproducibility.

5.4 Methods

Sequence and protocol design

The most extensively used in vivo QT2 imaging protocol was developed by the UBC group [171], and most publications since have employed it. This protocol is based on the imaging sequence developed by Poon and Henkelman [162], a single-slice 32-echo spin-echo sequence with non-selective composite (90°-180°-90°) refocusing pulses flanked by decreasing crusher gradients with alternating sign. The “UBC protocol” has a repetition time (TR) of 3 seconds, with voxel dimensions 0.86×1.72×5.0 mm³, matrix size 256×128 (reconstructed to 256×256), square field-of-view of 220 mm, and four averages for a total scan time of approximately 26 minutes. It results in an SNR of ~200 in the first echo when implemented on the 1.5T Siemens scanner at our institute, which is double the minimum SNR suggested by the UBC group [173]. An SNR of 200 should yield a theoretical precision of 25% in the MWF estimates according to simulations [181].

The protocol used for QT2 data collection at our institution (the “MNI protocol”) is based on the same imaging sequence, but is modified for inclusion in a more comprehensive quantitative MRI protocol, used in the study of healthy and diseased WM. Modifications to the protocol were selected to reduce the acquisition time (∼4 min), while maintaining an SNR comparable to the UBC protocol. The number (32) and spacing (10 ms) of echoes are identical for both protocols. The MNI protocol has a TR of 2 seconds and no signal averaging; the rectangular field-of-view is 256×192 mm, with a matrix of 128×96, resulting in a voxel size of 2×2×7 mm³.
5.4 Methods

We have previously validated our implementation of the UBC QT2 technique in a cross-site comparison study [240]. Data were collected and analyzed in the same subject at two sites (at the UBC and the MNI) using the UBC protocol and matched analysis techniques. That study established consistent data analysis between sites, and revealed that data acquired at different sites resulted in somewhat different $T_2$ distributions but consistent MWF estimates (differences $\leq 2\%$).

All data in this work were acquired with a 1.5 T Siemens Sonata (Siemens Medical Systems Erlangen, Germany) using a quadrature head coil. Ethics approval was obtained from the MNI Research Ethics Board, and informed consent was obtained from all subjects.

Multi-component analysis

The regularized NNLS analysis method, widely used for multi-component $T_2$ analysis [171, 178, 180] was employed in our studies. Analysis parameters such as the number, range, and spacing of $T_2$ values, and the type and strength of regularization, were selected based mostly on work by the UBC group [181, 183, 241]. We used 120 $T_2$ values to achieve adequate resolution of the distribution [241]. These ranged from 10 ms, about half the smallest $T_2$ expected in the sample, to 4000 ms, a few times the largest expected $T_2$ [196, 242]. As in most QT2 studies using regularized NNLS, we chose to regularize by minimizing the energy of the distribution [172], setting the weight to allow $\chi^2$ increases of 2 - 2.5% (i.e., $1.02\chi^2_{\text{min}} < \chi^2 < 1.025\chi^2_{\text{min}}$) [181, 183]. All analyses were performed for two MW $T_2$ ranges (10 - 40 ms, actual upper integration limit of 41.0 ms, and 10 - 50 ms, actual limit of 50.1 ms) to investigate how much the MWF depends on the selected range, and because there has been work published using both ranges.

$T_2$ decay data were analysed to estimate distributions on a per-voxel basis. Maps of the MWF, the geometric mean of the whole distribution ($\langle T_2 \rangle$), and of the IE water peak ($\langle T_{2ie} \rangle$, for $T_2 > \text{MW } T_2$ range) were produced. Region-of-interest (ROI) analysis was then performed by averaging the voxel values in each ROI. The same analysis was performed for all parts of the study (protocol comparison, scan-rescan, and longitudinal).
Comparison of two protocols for myelin water imaging in controls

For the cross-sectional protocol comparison, data were acquired using both the UBC and MNI protocols in 10 healthy subjects (5 males and 5 females), mean age of 27 (range 24-31). Images were acquired in a single slice positioned to pass through the thickest part of the genu and splenium of the corpus callosum. The genu and splenium of the corpus callosum, and the major and minor forceps were selected as WM ROIs. The thalamus and head of the caudate nucleus were selected as GM ROIs. All ROIs were conservatively hand drawn on the higher-resolution UBC-protocol image (Figure 5.1) to minimize any contamination from artifacts or unwanted tissue from partial voluming. ROIs labeled on the UBC protocol images contained the same number of voxels across all subjects to maintain similar SNR (median volume 155 mm$^3$, range 96-473 mm$^3$). ROI labels from the UBC images were resampled to the larger voxel size of the MNI images. ROI volumes from the UBC protocol images were approximately preserved in the MNI ROIs. When compared between protocol data sets, the ROIs were on average 30% larger for the MNI protocol than for the UBC protocol (median volume 196 mm$^3$, range 84-1316 mm$^3$). To compare the two protocols, a 2-way ANOVA was performed for each metric (MWF, $\langle T_2 \rangle$, and $\langle T_{2ie} \rangle$), factored by protocol (UBC, MNI) and MW $T_2$ range (10 - 40 ms, 10 - 50 ms).

Scan-rescan and longitudinal reproducibility

Acquisitions for these parts of the study were performed on the same scanner, using the MNI protocol. To evaluate the scan-rescan variability of the QT2 technique, six healthy subjects (4 males, 2 females, median age: 31 years, range: 24-38 years) were scanned twice, on the same day, without repositioning of the subjects in the scanner, or of the imaging slice, between scans. Acquired this way, scan-rescan differences reflect variations due mainly to the technique, physiological noise, and instrumentation noise.

Longitudinal variability over time was evaluated in a retrospective analysis of data acquired in 5 healthy subjects (3 males, 2 females, median age: 31 years, range: 24-31 years) serving as controls in the context of a clinical study, over a span of 3 years. The variability
Figure 5.1: An example of the slice acquired in the first part of the study, which passes through the genu and splenium of the corpus callosum, with labels for the various WM (genu and splenium of the corpus callosum, and major and minor forceps) and GM (head of the caudate nucleus and thalamus) ROIs drawn conservatively to avoid partial volume effects.
measured in this manner is due not only to the technique, but also due to subject repositioning, scanner drift, and potential biological variations. Subjects were scanned at 4 times (one was scanned 7 times), with inter-scan periods ranging from 1 month to 2.4 years. As these data were acquired as controls in the context of an MS study, the slice placement varied from subject to subject according to the needs of the patient study. Care was taken to manually align the slice position to previous exams. High-resolution $T_1$-weighted scans were also acquired in each subject at each time-point, for ROI identification and propagation.

$T_2$ distributions were estimated with the procedure described earlier and ROI-based analysis was used to determine mean parameter values at each time point. Sixteen (16) WM and GM ROIs were manually identified on the initial high-resolution scan of each subject, propagated to subsequent time points, and re-sampled to the lower resolution of the QT2 images. Propagated ROI label voxels with obvious partial volume contamination, when registered to the high-resolution scans, were manually excluded prior to the analysis. Percent differences were calculated between the ROI-mean values from the same-day scans, and within-subject CoVs were computed for the longitudinal data.

To compare the variability of $T_2$ distribution metrics introduced by repeated measurements over time to the variability expected within ROI (due mostly to instrument noise and instability in the analysis), we performed a one-way ANOVA on the voxel data. The ANOVA was computed for each individual combination of subject, ROI, and metric, using time-point as the lone factor in the analysis. For ROIs that were labeled separately in the left and right hemispheres, the ANOVA was first performed including a second factor for hemisphere. A threshold of $p = 0.01$, corrected for multiple comparisons ($N = 16$, the total number of subject-ROI combinations), was selected for significance.
5.5 Results

Protocol comparison

Figure 5.2 compares example data acquired with the UBC and MNI protocol, from the minor forceps of one subject, showing the normalized decay curves and their associated $T_2$ distributions. UBC and MNI protocol data have similar SNR in the 1st echo and small signal differences in late echoes. The $T_2$ distributions are similar: the IE peak’s central $T_2$ is stable, while differences in the short-$T_2$ component are more pronounced.

Examples of a subject’s myelin water map from both protocols (UBC and MNI), using a 40-ms and a 50-ms MW $T_2$ range cut-off are shown in Figure 5.3. The 10 - 50 ms MW $T_2$ range has some slightly brighter areas than the 10-40 ms, as would be expected.

MWF estimates for the ROIs, calculated with different MW $T_2$ ranges and averaged across subjects, are plotted in Figure 5.4 (error bars show $\sigma$, the standard deviation, across subjects). The MWF estimates in the caudate are in very good agreement for the different MW $T_2$ ranges, less so for the thalamus. Larger differences between MW $T_2$ ranges are seen for WM structures than GM structures. The 10 - 50 ms integration window resulted in larger MWF estimates and $\sigma$ than the 10 - 40 ms window. The results from the two protocols overlap but the MNI $\sigma$ are marginally higher in most cases. The ROI MWFs were analyzed using a 2-way ANOVA factored by protocol and MW $T_2$ range, revealing significant differences between MW $T_2$ ranges ($p = 0.001$), but no significant effect of protocol and no interaction effect.

IE water peak $T_2$ values ($\langle T_{2ie} \rangle$) were largely similar between MW $T_2$ ranges, and between the UBC and MNI protocols. $\langle T_{2ie} \rangle$ values were analyzed with a 2-way ANOVA, again factored by MW $T_2$ range and protocol. The effect of MW $T_2$ range was significant ($p < 0.001$), while the effect for protocol was only slightly significant ($p = 0.024$). The UBC and MNI protocols resulted in consistent $\langle T_2 \rangle$ values (data not shown).
Figure 5.2: Comparison of the protocols’ decay curves (top, normalized by the signal in the first echo) and distributions (bottom, normalized by the maximum value in the distribution), in the minor forceps of a single subject. Note the very slight differences in late echoes of the decay curve, and the somewhat unpredictable behavior of the short-$T_2$ peak.
Figure 5.3: Myelin water maps from UBC protocol data (left column) and from MNI protocol data (right column), from a single healthy subject, with a MW $T_2$ range of (top) 10 - 40 ms and (middle) 10 - 50 ms.
Figure 5.4: Summary plot of MWF estimates (mean ± std dev, across subjects) from UBC (left) and MNI (right) protocol data for various MW $T_2$ ranges. MWF estimates computed using a MW $T_2$ range of 10 - 40 ms (blue ×), and 10 - 50 ms (red ⊙). Caud: head of the caudate, Thal: thalamus, Min: minor forceps, Maj: major forceps, Genu: genu, Spl: splenium.
5.5 Results

Scan-rescan reproducibility

Mean values of various $T_2$ metrics, averaged over two scans, from the 6 subjects, are presented in Table 5.1. Although the results of our cross-sectional protocol comparison showed MWF estimates were slightly more uniform when using the 10-40 ms MW $T_2$ range (see Figure 5.4), scan-rescan MWFs were also estimated with a $T_2$ range of 10-50 ms for the purpose of comparison. Percent differences of MWFs in WM ROIs averaged 7.9 % (range 0.6-23.1%), with a MW $T_2$ range of 10-40 ms. MWFs computed with the larger MW $T_2$ range presented very similar scan-rescan differences (mean 7.3 %, range 0.9-18.3%). Much larger differences were observed for the caudate nuclei, (34.4 % for the 10-40 ms MW $T_2$ range, 56.3 % for the 10-50 ms range), perhaps highlighting signal contamination by the neighbouring cerebrospinal fluid (CSF). Average scan-rescan differences for $\langle T_2 \rangle$ and $\langle T_{2ie} \rangle$ were identically 1.5 % (ranging up to 7%), regardless of the MW $T_2$ range.

Longitudinal reproducibility

Examples of decay data from the genu of a single healthy subject at four time-points are plotted in Figure 5.5 (top), along with absolute differences between the first and subsequent scans (middle), and the associated $T_2$ distributions (bottom). Similar to the scan-rescan study, MWF estimates were computed using two MW $T_2$ ranges, 10-40 ms and 10-50 ms. The COV for each metric, computed for each ROI of each subject, then averaged across subjects where possible, are reported in Table 5.2.

The two-way ANOVA with factors time-point and hemisphere revealed no significant effect of hemisphere in homologous ROIs, and data from left and right pairs were combined for the subsequent analysis.

The one-way ANOVA was performed for the MWF, $\langle T_2 \rangle$, and $\langle T_{2ie} \rangle$ from each combination of subject and ROI, and was performed for MWFs estimated using both MW $T_2$ ranges. The ANOVA revealed a significant effect of time-point in 5 of 16 subject-ROI combinations ($p = 0.01$, corrected for multiple comparisons, $N = 16$, corrected $p = 0.01/16 = \ldots$)
Table 5.1: Mean values of MWF, $\langle T_2 \rangle$, and $\langle T_{2ie} \rangle$, from MNI protocol data. Metrics were calculated for healthy subjects from distributions with 2 - 2.5% regularization using two MW $T_2$ ranges. The values were averaged within each ROI of each healthy subject, and then averaged across scans, and subjects when possible. Values in brackets are an estimate of the standard deviation in each ROI, averaged across subjects and both scans.

<table>
<thead>
<tr>
<th>MW $T_2$ range (ms)</th>
<th>MWF (%)</th>
<th>$\langle T_{2ie} \rangle$ (ms)</th>
<th>$\langle T_2 \rangle$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-40</td>
<td>10-50</td>
<td>10-40 $a$</td>
</tr>
<tr>
<td>Centrum semiovale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anterior</td>
<td>8.7 (3.7)</td>
<td>9.8 (4.1)</td>
<td>81.8 (3.8)</td>
</tr>
<tr>
<td>middle</td>
<td>12.0 (5.8)</td>
<td>14.6 (6.6)</td>
<td>90.7 (7.7)</td>
</tr>
<tr>
<td>posterior</td>
<td>10.6 (5.4)</td>
<td>12.6 (6.1)</td>
<td>90.4 (6.4)</td>
</tr>
<tr>
<td>Minor forceps</td>
<td>8.8 (4.3)</td>
<td>10.9 (4.9)</td>
<td>78.8 (4.0)</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genu</td>
<td>10.2 (4.8)</td>
<td>15.0 (6.4)</td>
<td>74.6 (4.7)</td>
</tr>
<tr>
<td>splenium</td>
<td>14.9 (7.6)</td>
<td>20.9 (9.3)</td>
<td>95.4 (13.6)</td>
</tr>
<tr>
<td>Caudate nuclei</td>
<td>2.0 (2.6)</td>
<td>2.5 (2.6)</td>
<td>82.9 (6.2)</td>
</tr>
<tr>
<td>Major forceps</td>
<td>13.2 (4.8)</td>
<td>15.4 (5.4)</td>
<td>92.0 (6.6)</td>
</tr>
<tr>
<td>Corona radiata</td>
<td>14.0 (6.3)</td>
<td>17.0 (6.7)</td>
<td>92.7 (8.7)</td>
</tr>
<tr>
<td>Mean (all WM ROIs)</td>
<td>11.1 (5.2)</td>
<td>13.9 (6.1)</td>
<td>86.5 (6.5)</td>
</tr>
<tr>
<td>Range (all WM ROIs)</td>
<td>6.0 - 15.8</td>
<td>7.7 - 21.6</td>
<td>69.9 - 103.8</td>
</tr>
</tbody>
</table>

$a$ $\langle T_{2ie} \rangle$ differences computed with the 10-50 ms MW $T_2$ range were virtually the same as for the 10-40 ms range.
Figure 5.5: Entire ROI $T_2$ decay curves for MNI protocol data collected on four separate scans in the genu of a single healthy subject (top). Absolute decay curve differences between the first scan and subsequent scans were calculated (middle). $T_2$ distributions (2 - 2.5% regularization) from the ROI-averaged data are also plotted (bottom).
Table 5.2: Coefficients of variation of MWF, $\langle T_2 \rangle$, and $T_2$ of the intermediate peak, from MNI protocol data. Values were calculated from fits of data from normal subjects using a 10-40 ms MW $T_2$ range cut-off from distributions with 2 - 2.5% regularization. Each CoV was computed for each ROI of each subject, then averaged across subjects when possible.

<table>
<thead>
<tr>
<th>MW $T_2$ range (ms)</th>
<th>[MWF]</th>
<th>$\langle T_{2ie} \rangle$</th>
<th>$\langle T_2 \rangle$</th>
<th>CoV (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>10-40</td>
<td>10-50</td>
<td>10-40</td>
<td>10-50</td>
</tr>
<tr>
<td>Centrum semiovale</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anterior</td>
<td>15.7</td>
<td>20.5</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>middle</td>
<td>7.8</td>
<td>7.4</td>
<td>5.5</td>
<td>4.5</td>
</tr>
<tr>
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<td>9.4</td>
<td>7.2</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Minor forceps</td>
<td>23.6</td>
<td>15.7</td>
<td>4.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genu</td>
<td>12.0</td>
<td>17.9</td>
<td>4.4</td>
<td>4.0</td>
</tr>
<tr>
<td>splenium</td>
<td>7.4</td>
<td>4.7</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Caudate nuclei</td>
<td>23.4</td>
<td>28.2</td>
<td>3.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Major forceps</td>
<td>7.9</td>
<td>3.4</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Corona radiata</td>
<td>6.3</td>
<td>7.7</td>
<td>3.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Pooled CoV</td>
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<td>12.1</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Range</td>
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<td>3.4 - 29.7</td>
<td>0.7-6.7</td>
<td>1.0 - 6.0</td>
</tr>
</tbody>
</table>
5.6 Discussion

Our overall goal was to assess the robustness and reliability of the MWF and \( \langle T_2 \rangle \) from QT2. We have previously established acquisitions consistent with those reported by the UBC group [240]. In this study, we have presented results from healthy subjects, first to compare two data acquisition protocols, and second, to evaluate the variability of repeated MWF measurements. This is the first such evaluation of the reproducibility of the QT2 technique over repeated measurements outside of the UBC group, and the first evaluation of a protocol for shorter data acquisition.

The ROI measurements acquired here on 10 subjects, using both the UBC and MNI protocol, showed relatively broad, but similar, MWF estimates to those reported by the UBC group. These ranged from 0 - 20%, whereas values reported by the UBC group typically range over 2 - 18% [174]. Our MWF estimates in WM were generally greater than in the literature, while our MWF estimates in GM were smaller. More specifically, our UBC-protocol MWF estimates in frontal WM (9.6 ± 1.5%) agree closely with another study of 9 healthy subjects using the same protocol (9.4 ± 2.7%) [214]. These overlap with other reports (7.0 ± 2.0% in right minor forceps and 12 ± 4.5% in left minor forceps [241]; 7.3 ± 2.1% in minor forceps [190]). Another study reported clearly lower MWFs in the minor forceps of healthy controls (2.5-4.5% when matching subjects’ ages) [237]. The above suggests that MWFs can be drastically different from one experiment to another with
relatively small differences in the acquisition and analysis. The trend of MWFs across WM structures was consistent with what is reported by the UBC group, but there were some subtle variations, and the trend depended on the choice of MW $T_2$ range. Our measures calculated from the 40 ms cut-off were better matched with values from the literature. An increase in ROI variability $\sigma$ was observed with increased MWF content, behaviour which agrees with the literature.

**Protocol comparison**

The MNI protocol generally resulted in larger mean MWF estimates than the UBC protocol, though these differences were not statistically significant given the large variability. Data from both protocols, with similar SNRs and acquired on the same scanner and subject, showed large between-subject variability in MWFs, generally larger with the MNI protocol. The larger voxels of the MNI protocol entail generally larger ROIs, which may be responsible for higher variability between subjects. The 10-50 ms MW $T_2$ range resulted in significantly larger MWFs and larger variations across brain structures than using a $T_2$ range of 10-40 ms, for both protocols. Our observations show that this was due to encroachment of signal from the IE water peak into the MW $T_2$ range. MWFs from both protocols, with a 50-ms MW $T_2$ cut-off, were larger than those reported in literature, but this difference was not statistically significant, likely due to the large standard deviations. The trend of MWF values was not strictly maintained when comparing results from different MW $T_2$ ranges, but the two protocols behaved consistently for each range.

Our data appeared to have some measure of dependence on MW $T_2$ range. Figure 5.6 shows distributions from an *in vivo* data set analyzed with two levels of regularization (red and green lines) on top of the direct NNLS distribution (blue lines). The example shows how the choice of a 50 ms MW cut-off results in greater MWF and illustrates how crucial MW $T_2$ ranges are in the proper assignment of signal components. It also appears that regularization may not suppress spurious peaks in the NNLS distribution consistently, or may result in incorrect assignment of the signal. From this example, it might appear that
less regularization and/or a lower MW cut-off would solve the problem, but our experience suggest that this is not necessarily the case. Analysis procedures might need to be tailored to such specifics as acquisition parameters and scanner type. MWF measurements may also be impacted by the $T_2$ sampling used in the fits, which may result in different components, and MW $T_2$ ranges that do not coincide exactly with values included in the solution set. This may affect the precise comparison of results.

![Graph showing $T_2$ distribution with different regularization levels and MW $T_2$ range cut-offs.](image)

**Figure 5.6:** A $T_2$ distribution from *in vivo* data with different amounts of regularization showing how MW $T_2$ range cut-offs are crucial in defining MWFs. Additional signal not related to the short-$T_2$ peak (as pointed out with the pink arrow) can be assigned to the MWF when the main $T_2$ peak spills over into the MW $T_2$ range.

As an alternative to a fixed MW $T_2$ range, we attempted “adaptive” separation of the $T_2$ peaks at the appropriate local minimum (possibly zero-valued) of the $T_2$ distribution. Our method performed automated separation of peaks in the $T_2$ distribution based on the local...
minimum nearest to $T_2 = 50$ ms. This intuitively-appealing technique could potentially better identify the different water components. When applied to data from the protocol comparison, adaptive peak separation showed MWF values spanning the combined range observed with the two fixed MW $T_2$ limits, and greater MWF variability between subjects. This technique also resulted in greater scan-rescan differences than the fixed range analyses, yielded little to no improvement over fixed MW $T_2$ ranges, and thus was not considered further.

Scan-rescan and longitudinal reproducibility

Same-day scan-rescan percent differences for the MNI protocol were reasonable, just over 7% on average for the MWF in WM. $\langle T_2 \rangle$ values had much smaller scan-rescan differences (1.5% on average). These measures indicate the variability associated with the instrument noise, physiological noise and subject movement. Given that our analysis was performed on an ROI basis, and that the subjects were not repositioned between scans, we can infer that these differences are more indicative of the technique itself. Inter-scan differences were generally independent of the choice of $T_2$ range for myelin water (10-40 ms or 10-50 ms). Variability in the MWF is likely related to difficulties in acquiring the short-$T_2$ signal; indeed, for a MW $T_2$ of 15 ms and an SNR of 200, only the first 4 or 5 10-ms-spaced echoes contribute to the detection of that component.

The longitudinal variability, reported here as the CoV of the mean values in individual ROIs, were generally greater than scan-rescan differences. This CoV is an indicator of within-subject variability that excludes between-subject and between-ROI differences. This measure is of interest in the case of longitudinal studies following progression in individuals, for example in therapeutic trials. The ANOVA revealed comparable within-ROI and between-time-point variation for the MWF, which suggests that subject repositioning, scanner drift, and potential biological variations introduce variability that is comparable to the noise variability associated with the technique.

A similar longitudinal study using the UBC protocol found that MWC, the MWF cor-
rected for $T_1$ effects and normalized to an external water standard, had a high reliability coefficient (RC) [238]. As a comparison, we estimated the within-subject CoV from that study, in the same manner as our results, using a 10-40 ms MW $T_2$ range: we obtained a mean CoV of 19%, with a range of 7-40%, which is comparable to our observations. Although results were matched for stated MW $T_2$ ranges, the amount of regularization was not stated in that study, thereby making further direct comparisons difficult. In our results, between-subject variation from the ANOVA was generally larger than the longitudinal variation for most ROIs. The latter is driven by measurement variability, while the former also includes biological variability. However the differences between longitudinal and between-subject variation were inconsistent across ROIs: longitudinal variation was much larger (by a factor of 2 to 3) than between-subject variation for the caudate and splenium, larger for major forceps and genu (less than a factor of 2), and similar for the minor forceps. This may indicate certain ROIs with greater biological variability than others, or inconsistent ROI definition. Our observations contrast with the conclusion that differences between subjects were due to biological variability [238].

In an attempt to improve the reproducibility of MWF estimates, we also analyzed MWFs and $T_2$s from distributions estimated using the average signal in each ROI (the “average-invert” method). Because this method starts with higher-SNR data before computing the $T_2$ distribution, it could yield more stable results. Mean metric values obtained from this analysis, using a MW $T_2$ range of 10-40 ms, were virtually identical to those presented in the Results section, obtained using the “invert-average” technique. Scan-rescan differences from the average-invert analysis were slightly larger than from the invert-average analysis for the MWF, and mostly similar for the $T_2$ values. The longitudinal variability of average-invert MWF estimates was considerably greater than that of invert-average MWF estimates. This contradicts the intuition that ROI-averaged data, with higher SNR, should result in more reproducible fits, and may indicate other, unknown, systematic factors that are not taken into account.

In longitudinal studies, the variability within individuals plays an important role in
determining the sample sizes required to show an effect. As an application of our results, we computed sample sizes for \textit{a priori} power analysis. Calculations were performed using G*Power [243], based on our longitudinal results using the 10 - 40 ms MW $T_2$ range. Table 5.3 gives sample sizes required to observe a MWF change of 15% in individuals, with $\alpha$ and $\beta$ of 0.05 (\textit{i.e.}, false positive and false negative discovery rates, respectively) and a power of 0.95. As a result of the large spread in normal MWF estimates due to addition of biological variability, and the potentially larger variability between patients, detection of differences between groups would require even larger sample sizes.

Table 5.3: Calculated sample sizes required to ensure statistical power to observe a MWF decrease of 15% with $\alpha = 0.05, \beta = 0.95$. Sample sizes are computed for MWF estimated using MW $T_2$ ranges of 10-40 ms (top rows) and 10-50 ms (bottom rows).

<table>
<thead>
<tr>
<th>Control MWF</th>
<th>Expected MWF</th>
<th>$\sigma$</th>
<th>Total sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor forceps</td>
<td>9%</td>
<td>7.65%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Genu</td>
<td>10%</td>
<td>8.5%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Splenium</td>
<td>15%</td>
<td>12.75%</td>
<td>0.8%</td>
</tr>
<tr>
<td>WM average</td>
<td>11%</td>
<td>9.35%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Minor forceps</td>
<td>11%</td>
<td>9.35%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Genu</td>
<td>15%</td>
<td>12.75%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Splenium</td>
<td>21%</td>
<td>17.85%</td>
<td>0.7%</td>
</tr>
<tr>
<td>WM average</td>
<td>14%</td>
<td>11.9%</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

On top of fully characterizing $T_2$ distributions using various analysis procedures, there is a need for standardizing procedure for analysis and acquisition protocols to obtain more consistent and accurate MWF estimates. The same protocol implemented at various sites may yield different distributions [244], but very similar MWF estimates. Improved MWF consistency might be achieved by using a calibration phantom. Phantoms with multi-exponential $T_2$ decay, such as demonstrated with dairy cream [245] or urea-water mix-
5.7 Acknowledgments

The authors thank the MRI technicians at the McConnell Brain Imaging Centre of the Montreal Neurological Institute, and the volunteers who participated in the study.
Reproducibility of multi-component $T_2$
Chapter 6

Progression of acute lesions of MS

6.1 Preface

In Chapter 3, the interpretation of pathological changes in WM observed with QMTI and QT2 was explored in a set of simulations. The variability of the of MT and multi-component $T_2$ imaging were reported in Chapters 4 and 5. In particular, the influence of certain primary factors in the data acquisition and analysis has also been investigated. In this final study, the QMTI and QT2 techniques were applied to a dynamic phase of disease in patients with MS. Acute lesions of MS are identifiable on MRI with paramagnetic gadolinium-based contrast agent, due to the temporary opening of the blood-brain barrier (BBB) during the immune system attack on WM in new or active chronic lesions [248,249]. Previous studies using the MTR technique have revealed a certain amount of recovery from tissue injury after the period of enhancement [250, 251].

This study, the first to investigate gadolinium-enhancing lesions with QMTI and only the second with QT2, imaging data were acquired monthly in a longitudinal study of 5 MS patients who presented with acute lesions. Our observations demonstrate the sensitivity of MT parameters to acute tissue damage and recovery in MS lesions, and suggests that the recovery might occur in two stages, by quick resorption of edema and a slower partial recovery of the tissue matrix and myelin content. Acute changes were also detected by the
mean of the $T_2$ distribution, but not by the MWF, likely due to the large variability of the QT2 technique and the small number of subjects.
Quantitative magnetization transfer and myelin
water imaging of the evolution of acute multiple
sclerosis lesions

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6.2 Abstract

Quantitative magnetization transfer (MT) imaging provides in vivo estimates of liquid and semi-solid constituents of tissue, while estimates of the liquid sub-populations, including myelin water, can be obtained from multi-component $T_2$ analysis. Both methods have been suggested to provide improved myelin specificity compared to conventional MRI. The goal of this study was to investigate the sensitivity of each technique to the progression of acute, gadolinium-enhancing regions of multiple sclerosis (MS). MT and $T_2$ relaxometry data were acquired longitudinally over the course of one year in five relapsing-remitting MS patients and in five healthy controls. Parametric maps were analyzed in enhancing lesions and normal-appearing white matter regions. Quantitative MT parameters in lesions were most abnormal at the time of enhancement, and followed a pattern of recovery over subsequent months. Lesion myelin water fraction was abnormal but did not show a significant trend over time. Quantitative MT was able to track the degree and timing of the partial recovery in enhancing MS lesions in a small group of patients, while the recovery was not detected in myelin water estimates, possibly due to their large variability. Our data suggest the recovery is characterized by quick resolution of inflammation and a slower remyelination process.

Key words: quantitative, magnetization transfer, multi-component $T_2$, multiple sclerosis, acute lesions
6.3 Introduction

The magnetization transfer (MT) effect can be exploited to produce contrast in MR imaging (for a review see [60] and [36]), and is sensitive to the principal constituents of myelin in human white matter (WM) [105]. MT can be described using a two-component model, grouping the spins into the free (or liquid) pool, consisting of water protons with long $T_2 (T_{2f} > 10 \text{ ms})$, and the restricted (or semi-solid) pool, consisting of hydrogen atoms residing on large lipids with $T_2$ too short to be imaged directly using MRI ($T_{2r} < 100 \mu s$). Detailed information about these spin populations can be mapped using a variety of off-resonance quantitative MT imaging (QMTI) techniques, with an appropriate model of tissue [131]. The technique of Sled and Pike yields the relative size of the restricted proton pool ($F$), the first-order forward magnetization exchange rate ($k_f$), and most relaxation parameters of the free and restricted pools ($R_{1f}, T_{2f},$ and $T_{2r}$) [141].

Multi-component relaxation behavior of transverse magnetization has been observed ex vivo and in vivo using multi-echo spin echo sequences [170, 171, 173]. From multi-component quantitative analysis of such spin echo data (QT2), a distribution of the $T_2$ species present in each voxel can be estimated. In white and gray matter, these $T_2$ distributions present a few peaks, which have been experimentally assigned to compartmentalized spin populations: a short $T_2$ peak around 20 ms (between 10 and 50 ms) representing the water trapped between the layers of myelin, a second peak around 70-90 ms assigned to both intra- and extra-cellular water, and a third peak with $T_2 > 2 \text{ s}$ most often assigned to CSF signal [170, 173]. The myelin water fraction (MWF) is computed as the fraction of signal below approximately 40 or 50 ms in the $T_2$ distribution, and can be computed voxel-by-voxel to yield parametric maps [171], giving rise to a technique called myelin water imaging.

While QMTI measurements cannot provide absolute specificity to the molecular constituents of tissue, there is strong and convergent evidence that informs the interpretation of MT changes in WM as reflecting changes in myelin content. In a study of fixed post-mortem brain samples, Schmierer et al. showed a strong correlation between the relative
size of the restricted pool shown by QMTI and the myelin lipid content observed with the Luxol fast blue (LFB) stain [155]; in addition, a weaker correlation was observed with axonal density. In a follow-up study, the same authors showed a similar correlation in unfixed post-mortem tissue [156]. In vivo imaging studies have also contributed to the interpretation of the MT in tissue. Variations have been reported in the QMTI parameters of WM of healthy controls [151] that are consistent with tissue myelination, and others have observed that densely myelinated WM fiber tracts are distinguishable in maps of the restricted pool fraction [140]. In multiple sclerosis (MS), the size of the restricted pool ratio has been observed to decrease substantially in chronic lesions [138, 141, 152, 153], while small but significant decreases in $F$ have also been reported in the normal-appearing white matter (NAWM) of MS [138, 154]. The impact of MS pathology on the $T_2$ of the restricted pool ($T_{2r}$) is still a matter for discussion, as both decreases [152] and increases [138] have been reported in the lesions of patients with MS.

Myelin water imaging is also a putative marker of myelin in WM and MS. The MWF and the geometric mean of the overall $T_2$ distribution ($\langle T_2 \rangle$) are significantly altered in lesions and the NAWM of MS [189, 190, 204]. The MWF changes are mainly due to myelin loss, as confirmed by pathology studies using LFB [194, 195], while increases in $T_2$ of the intermediate component (and by extension, $\langle T_2 \rangle$) have been proposed as a marker of inflammation [211]. A recent study of MS lesions did not reveal differences in myelin water content between $T_1$-hypointense and $T_1$-isointense lesions [193].

In a study combining QMTI and myelin water imaging in 9 controls and 19 MS patients [214], Tozer et al. reported significantly decreased restricted pool and myelin water fractions in lesions, and significantly decreased restricted pool fraction in NAWM. These authors concluded that, while lesion pathology affects each measure differently (potentially explained by the presence of inflammation and axonal loss), the restricted pool fraction and the MWF both reflect demyelination to some extent.

Despite the growing number of studies of MS employing these two quantitative techniques, there has been limited work on one of the most dynamic phases of the disease, acute
6.4 Methods

This study followed five (5) patients with relapsing remitting MS, all female, who were recruited based on the presence of an acute relapse and an active lesion as defined by gadolinium enhancement. Inclusion criteria included a diagnosis of relapsing remitting MS, an acute gadolinium-enhancing hemispheric lesion, and age greater than 18. Patients were excluded if they were pregnant, unable to receive gadolinium, or if they had received steroids prior to the baseline scan. Active lesions had to be of sufficient volume to be studied at the resolution of the quantitative imaging techniques (see below). All patients underwent clinical evaluation by a neurologist prior to the baseline scan and at subsequent intervals of 3 months. Expanded Disability Status Scale (EDSS) scores were evaluated for each patient and ranged from 1 to 4 at the time of the baseline scan. One patient was treated with glatiramer acetate, while the remaining four patients were not on any
Progression of acute lesions of MS disease modifying therapy at the time of the initial scan. Steroids were permitted after the baseline scan. Only one patient showed a lesion with gadolinium enhancement that persisted beyond the baseline scan. The enhancement in that lesion subsequently resolved the following month without treatment. MRI exams were performed during the period of lesion enhancement, and at monthly intervals up to 5 months post-enhancement, with follow-up scans at 8 and 11 months, for a total of 34 datasets. Patient age-at-entry and total number of exams were, respectively: 25 years/7, 42 years/7, 56 years/7, 41 years/7, 49 years/6. Data were also acquired in 6 healthy adult controls (2 women, 4 men, aged 26-38 years) during the study. Each control was scanned at least four times, at intervals ranging from 11 days to 2.5 years. Approval for the study was obtained from the Research Ethics Board of the MNI, and each participant provided written informed consent.

6.4.1 Scanning protocol

Three-dimensional $T_1$-weighted data were acquired in each patient with an isotropic voxel size of 1.3 mm. FLAIR, $T_2$-, and PD-weighted data were also acquired in each patient, in addition to a second $T_1$-weighted scan following injection of a 0.2-ml/kg dose of gadolinium (Gd)-DTPA (Magnevist, Bayer HealthCare), for lesion identification and localization, all with a voxel size of $1 \times 1 \times 3$ mm$^3$.

All quantitative data ($MT, B_1, B_0, T_1$, and $T_2$) were acquired in a single, oblique 7-mm section, with 2 mm × 2 mm in-plane voxel size, and a rectangular 96×128 matrix. The slice position for each subject was initially selected to intersect the largest enhancing lesion during the baseline exam, and was carefully reproduced for each subsequent exam.

MT data were acquired according to the single-slice imaging protocol described in [141]. MT-weighted images were obtained from a spoiled gradient echo sequence in which a Hanning-windowed Gaussian off-resonance pulse was used to partially saturate the semi-solid components. The MT-weighted data consisted of two protocols with different repetition times and saturation pulse durations, each using two pulse powers with 20 and 10 offset frequencies, respectively, for a total of 60 measurements. The QMTI protocol se-
sequence parameters are listed in Table 6.1.

Table 6.1: Parameters of the MT-weighted pulse sequences.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>TR/TE (ms)</th>
<th>pulse angle</th>
<th>MT pulse angles</th>
<th>MT pulse duration</th>
<th>logarithmic frequency steps</th>
<th>offset frequency range</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25 / 4</td>
<td>7°</td>
<td>142°, 568°</td>
<td>10.24 ms</td>
<td>20</td>
<td>234 Hz-80 kHz</td>
</tr>
<tr>
<td>II</td>
<td>50 / 4</td>
<td>10°</td>
<td>347°, 1388°</td>
<td>30.72 ms</td>
<td>10</td>
<td>234 Hz-59 kHz</td>
</tr>
</tbody>
</table>

The observed $T_1 (T_{1,obs})$, required for the quantitative analysis of the MT data, was measured using a Look-Locker pulse sequence with 4 readout blocks [40]. This sequence has TR/TE = 2,000/12 ms, and consists of a non-selective composite inversion pulse (90x-180y-90x), followed after a time $T_{I_1}$ by 4 small-angle ($\alpha = 20^\circ$), slice selective excitations and gradient echo readouts at intervals $T_{I_2}$ ($T_{I_1}/T_{I_2} = 15/495$ ms).

To improve the accuracy and reproducibility of $T_1$ and QMTI parameters, the static ($B_0$) and transmit ($B_1$) fields were mapped in each subject. The relative $B_0$ field was obtained using a two-point phase-difference technique [232], with a gradient echo sequence (TR/TE/$\alpha = 53$ ms/10 ms/31°) having a readout time delay chosen to bring the fat and water signals in phase (4.48 ms at 1.5 T). To account for any residual uncompensated eddy currents due to the large crushing gradients in the spoiled gradient-echo sequence used for MT imaging, separate $B_0$ field maps were also acquired for each MT sequence without the saturation pulse, thus resulting in two additional $B_0$ maps. A two-point method was used to map the relative $B_1$ field [148], in which a non-selective preparation pulse ($\beta = 33^\circ$) is applied prior to a slice-selective turbo spin echo readout (TR/TE = 2,000/15 ms, ETL 7): two images are acquired, with the preparation pulse angle doubled for the second acquisition. The ratio of the acquired magnitude images is related to the nominal flip angle of the preparation pulse.
Multi-echo spin echo data were acquired using a 32-echo modified CPMG sequence [171] with a selective 90° excitation pulse, non-selective 90x-180y-90x composite refocusing pulses, and an alternating, decreasing crusher gradient scheme to spoil stimulated echoes [162]. A repetition time of 2 s and echo spacing of 10 ms were used. These data were acquired with the same matrix and voxel size as all other quantitative data.

Total scan time for the entire protocol was 64 minutes (28 minutes for conventional $T_1$/$T_2$/PD-weighted, FLAIR, and post-gadolinium scans, and 36 minutes for all quantitative imaging). All data were acquired on a 1.5 T Siemens Sonata (Erlangen, Germany), using a quadrature head coil.

### 6.4.2 Processing of quantitative parameter maps

All data were exported from the scanner in DICOM format and converted to MINC format for subsequent analysis [257]. MT-weighted data were analyzed in combination with $T_{1\text{obs}}$, obtained from the Look-Locker measurement, using Sled and Pikes rectangular-pulse model of pulsed saturation [141], to yield maps of the estimated model parameters. QMTI analysis was performed including corrections for $B_0$ and $B_1$ field inhomogeneities. The solid pool was modeled using a super-Lorentzian lineshape [131]. Fitting of the analytical signal equation was performed using a Levenberg-Marquardt non-linear least-squares algorithm implemented in MATLAB (The Mathworks, Natick MA).

Multi-component analysis of the spin echo data was carried out using regularized non-negative least squares (NNLS) to estimate the $T_2$ distribution [178] at each voxel. A distribution of 120 $T_2$ values was used, spanning a range of 10 ms to 4 s in logarithmic steps. The distributions were regularized by minimizing their energy, allowing an increase of 2-2.5% in the resulting chi-squared value [181]. The MWF was computed at each voxel by summing the $T_2$ distribution between 10 and 40.9 ms [193, 238, 256]; the choice of this value was confirmed after visual inspection of peak positions in control data. The geometric mean of the $T_2$ distribution ($\langle T_2 \rangle$) was also computed at each voxel [189].
6.4.3 Regional analysis

A regional analysis of the parameters was performed on enhancing lesions and homologous NAWM regions. Enhancing regions were labeled on the high-resolution post-gadolinium $T_1$-weighted scan at the first time point as illustrated by a green outline in Figure 6.1(a). Control regions of NAWM were defined contra-lateral to the lesions in homologous regions free of any visible $T_2$-hyperintense pathology, also illustrated in Figure 6.1(a) by the blue outline. Labels were propagated to subsequent exam time points using software developed at the Montreal Neurological Institute. The label maps were then resampled to the lower resolution of the QMTI scans, retaining only those voxels containing greater than 80% of each label, to limit partial volume contamination by peri-lesional tissue. An example of the resulting label outlines at the first time point is illustrated in Figure 6.1(b).

6.5 Results

Examples of the quantitative MT and $T_2$ maps are displayed in Figure 6.2. The ROIs included in the analysis are also displayed, green for the enhancing lesion, and blue for NAWM. In total, six (6) enhancing lesions were identified (one per patient, and two in patient 1). The mean parameter values were computed, at each time point, for the ROI corresponding to the initially enhancing portion of the lesions, and for the NAWM ROI. Each parameter in enhancing regions and NAWM was analyzed over time using a three-way ANOVA with factors ROI (enhancing and NAWM), subject, and time; in addition, parameter values in enhancing regions at baseline and in the chronic phase, approximately 8 months later (time-point 8), were also compared using a T-test.

6.5.1 Quantitative magnetization transfer results

Key QMTI parameter values from enhancing regions and NAWM are plotted versus time post-enhancement in Figure 6.3, where the error bars indicate the standard error of the mean. WM values acquired in healthy control subjects using the same protocol and scanner
Figure 6.1: (a) Initial post-gadolinium $T_1$-weighted scan of a single patient, with outlines of enhancing region (green) and contra-lateral NAWM (cyan). (b) Lower resolution version of enhancing region and NAWM outlines containing quantitative voxels with at least 80% of the specified tissue type, displayed on a $T_2$-weighted image resampled to the lower resolution of the quantitative maps.
Figure 6.2: QMTI (a) and QT2 (b) parameter maps from the initial scan of one patient. The green outline is enhancing region and the blue outline is NAWM. Units are seconds$^{-1}$ for $k_f$ and $R_{1f}$, and seconds for $T_{2f}$ and $\langle T_2 \rangle$. 
are also plotted as a single point at month zero. The percent difference relative to NAWM was computed for each patient, and the average of the changes for all parameters is plotted in Figure 6.4. The overall trends observed in the patient group were not observed in any individual patients.

Figure 6.3: Plots of QMTI parameters, averaged across subjects, for each month, starting at initial Gd enhancement. Error bars indicate the standard error of the mean across subjects.

$F$, $k_f$ and $R_{1f}$ were substantially reduced in enhancing regions and followed a general recovery pattern after resolution of enhancement, stabilizing around 2 to 3 months post-enhancement. $T_{2f}$ was dramatically increased in enhancing regions, and quickly decreased in the month following enhancement. $T_{2r}$ was stable in lesions following enhancement, with a maximum change of -6% (data not shown). The ANOVA revealed significant effects ($p < 0.05$) of subject and ROI for all QMTI parameters but one; the $k_f$ was the notable
6.5 Results

Figure 6.4: Percent change in each parameter relative to NAWM. Note the relative stability of $T_{2r}$.

exception, showing significant effects for ROI only. Significant ROI-by-time interactions were observed for $F$ and $R_{1f}$. None of the abnormal QMTI parameters returned to NAWM levels during the course of this study, but significant recoveries of all parameters were observed in enhancing regions ($p < 0.05$), at 8 months post-enhancement.

6.5.2 Multi-component $T_2$ analysis results

The MWF and $\langle T_2 \rangle$ of enhancing regions and NAWM ROIs are plotted versus time post-enhancement in Figure 6.5. Control WM values acquired in 5 healthy control subjects using the same protocol and scanner are plotted as a single point at month zero.

The MWF appeared to be reduced in enhancing regions compared to NAWM, but the difference was not statistically significant according to our ANOVA results; on the other hand, the ANOVA revealed a significant effect of subject for the MWF. No clear time evolution was observed following enhancement. $\langle T_2 \rangle$ values were greater in enhancing
regions than in NAWM, a difference that was significant in the ANOVA ($p < 0.05$), and largest at initial enhancement. Partial recovery of $\langle T_2 \rangle$ was observed post-enhancement, and this recovery was very significant in the ANOVA (as effects of time and ROI-by-time interactions, $p \leq 0.008$), and when comparing baseline and month 8 ($p < 0.05$). The $\langle T_2 \rangle$ in enhancing regions did not return to baseline during the course of this study.

Since both the MWF and $F$ have been proposed as markers of myelin content, we computed the Spearman’s rank correlation coefficient between them. The Spearman’s rank correlation coefficient ($\rho_s$) was also evaluated for $\langle T_2 \rangle$ and $T_{2f}$, to evaluate the extent to which the MT-protocol-estimated $T_{2f}$ reflects the average $T_2$ of the observable water protons. $F$ and MWF were not significantly correlated in Gd-enhancing regions or NAWM ROIs ($p \geq 0.15$), but were very weakly correlated when Gd-enhancing and NAWM regions were pooled ($\rho_s = 0.25$, $p = 0.018$). The correlation between $\langle T_2 \rangle$ and $T_{2f}$ was modest in enhancing regions ($\rho_s = 0.61$, $p < 0.0001$), and weaker in NAWM regions ($\rho_s = 0.33$, $p = 0.037$); moreover, the strength of the correlation between $\langle T_2 \rangle$ and $T_{2f}$ increased when Gd-enhancing and NAWM ROIs were pooled ($\rho_s = 0.76$, $p < 0.0001$).
6.6 Discussion

Despite the high sensitivity of MRI to the detection of WM lesions in MS, a robust and specific method of mapping demyelination and remyelination remains elusive. Potential candidates such as myelin water imaging and quantitative magnetization transfer imaging each have their strengths and limitations. The magnetization transfer effect can only provide partial specificity to the macromolecular components of myelin, as it is also sensitive to other non-myelin semi-solids. Myelin water imaging, on the other hand, probes myelin indirectly by measuring the water trapped within it, and relies on the assumption that variations in myelin water content equate to variations in myelin content. Furthermore, our experience shows that myelin water imaging suffers from relatively high variability, which stems mainly from the ill-posed nature of the constrained NNLS analysis and the limited number of samples of the decay curve. While three-dimensional implementations of QMTI [142] and myelin water imaging [164] have recently been reported, single-slice acquisitions remain the norm. Furthermore, both techniques require somewhat long scan times.

This study presents longitudinal quantitative MRI measurements of magnetization transfer and myelin water content in gadolinium-enhancing regions of 5 patients with relapsing-remitting MS and 5 healthy controls. Myelin water imaging was performed using the well-established technique of multi-component $T_2$ analysis using regularized NNLS analysis [173]. Magnetization transfer model parameter maps were obtained from pulsed MT images according to the technique of Sled & Pike [141]. Data were collected monthly for at least 5 months following the appearance of a gadolinium-enhancing lesion. To our knowledge this is the first longitudinal, comparative study of QMTI and multi-component $T_2$ parameters in gadolinium-enhancing lesions of MS patients.

The QMTI parameter changes that we observed in acute lesions all exceeded the range of longitudinal variability reported in WM of healthy controls [258]. When compared with previous reports from our group [153, 154], the $F$, $k_f$, and $T_2r$ of the enhancing lesions in this study are slightly closer to normal than in chronic lesions; this can likely be attributed
to less severe tissue damage in the acute lesions than in older chronic lesions.

Enhancing regions had significantly lower restricted-to-free proton pool ratio ($F$), along with significantly longer free pool relaxation times ($T_{2f}$) than in homologous NAWM. These lesions presented with more heterogeneous parameter estimates compared to NAWM, as would be expected, due to greater pathological heterogeneity. Deviation from NAWM values in the QMTI parameters were greatest in these lesions at the time of enhancement, consistent with acute demyelination, inflammation, and edema, that resolved over two to three months. $T_{2r}$ was significantly altered in these acute lesions, as has been reported in chronic lesions [152], but by a smaller margin (a decrease of 6% here, and approximately 13% in chronic lesions). This suggests that the structural integrity of the myelin is also affected in acute, enhancing lesions, lending support to the proposed pathologic specificity of $T_{2r}$ [152]. Perhaps the initial inflammatory tissue injury associated with acute lesions results in less severe changes in semi-solid constituents, resulting in intermediate changes in $T_{2r}$, in contrast to more advanced lesions with more severe destruction of myelin.

It is also interesting to note the differences in the timing of recovery of the various QMTI parameters. We suggest that the quick drop in $T_{2f}$ between the first and the second month post-enhancement primarily reflects the resolution of inflammation, while the slower recovery in $F$ and $k_f$ reflects the combination of remyelination and further resolution of inflammation.

Since changes in the restricted pool ratio ($F$) can result from a decrease in the restricted proton population, or an increase in the free proton population, we evaluated the water increase in each lesion voxel relative to the contra-lateral homologous NAWM ROI. We used a method similar to that proposed in [193, 238], in which the total spin echo signal was extrapolated to TE = 0 ms, corrected for incomplete $T_1$ recovery, and normalized to the corresponding value from the NAWM ROI. On average, we observed an increase of 9% in water content in enhancing regions when compared to NAWM. From this, we can derive that the overall decrease (of about 60%) in $F$ observed here would require a 50% decrease in macromolecular content, relative to NAWM. Alternatively, if our observed changes in
F were driven solely by edema, the water content would have to increase by about 125%, which is physically impossible. In short, water increases alone cannot explain the change in the ratio of restricted pool protons.

The average $T_2$ estimated from multi-echo spin-echo data was sensitive to changes in the enhancing lesions, and to the post-enhancement partial recovery. We believe that this change in the average $T_2$ is mostly indicative of the resolution of inflammation, as the increase in $T_2$ is much too large to be driven only by myelin water fluctuations. Furthermore, $\langle T_2 \rangle$ was significantly correlated with $T_{2f}$.

The MWF was not significantly different between lesions and NAWM, and was unable to detect changes during the partial recovery of enhancing regions. A recent study examining three acute enhancing lesions [256] at 1 month and 6 months also found MWF unchanged over this period. Differences between the MWF of lesion and NAWM have been reported [190, 204], suggesting the present study was under powered to detect this difference.

In this study, the MWF was the most variable metric considered, and we believe that this is the reason why no clear conclusions can be drawn as to the comparison of MWF between NAWM and acute lesions, and why no clear trend was observed over time following resolution of enhancement. Based on an ROI analysis of our control data, we evaluated the coefficient-of-variation of the MWF over time in individual subjects to be 13% (range 5-25%), which is comparable to what has been reported in 5 controls by Vavasour et al. [238]. Using the results published in that study, we computed the average coefficient-of-variation over time (averaged across subjects) to be 19%, with a range of 7-40%, over WM ROIs. Possible causes for the variability of the myelin water imaging results include 1) an SNR that is too low to allow reliable multi-component analysis of the spin-echo data, 2) the voxel size of our MWF images (larger than many other publications) perhaps resulting in too much partial volume contamination; 3) the low number of patients/lesions studied leading to a study power too low for the detection of MWF changes; and finally, 4) the ill-posed nature of the multi-exponential analysis of spin echo data [259]. With respect to
SNR, care was taken to maintain the SNR to the same level as other investigators when designing the study: our protocol yielded an SNR in the range of approximately 200 in the first echo, which is similar to the value we compute for the 32-echo imaging protocol used by MacKay and colleagues. Concerning points 2) and 3), similar conditions were imposed on the QMTI acquisition, yet these measurements were sensitive to the acute lesion progression while the MWF was not. Finally, the difficult nature of multi-exponential analysis, while attenuated by the regularization process, likely contributes to variability in the MWF estimates. It is most likely that this inherent variability of the MWF estimation method is responsible for the negative results in this case. In an attempt to improve the analysis of our multi-echo spin-echo data, these data were also processed after averaging the raw spin echo signal within the ROIs (as suggested by Graham et al. [180]) in contrast to the voxel-wise process; unfortunately, this did not result in any appreciable improvement in our measurements. Other models have been proposed for the analysis of such data, for instance a log-Gaussian $T_2$ distribution [211], but their application and comparison is beyond the scope of this study.

Despite the variability of MWF estimates, correlation coefficients were computed for lesions and NAWM, in particular between \( F \) and MWF, and between $\langle T_2 \rangle$ and $T_{2f}$. The restricted pool ratio and the MWF were not significantly correlated when considering the enhancing regions or the NAWM ROIs. This observation contrasts with prior reports that the subject-mean restricted pool and myelin water fractions were significantly correlated in the NAWM of patients [214] (no correlations were observed within individuals). This study also reported a positive correlation between F and MWF in lesions for combined between- and within-subject measurements. While the larger number of subjects in that study ($N = 19$) was more likely to yield significant correlations, we suggest that the very weak correlation in this study is due to the high variability of MWF estimates. Alternatively, the weak correlation between \( F \) and the MWF could indicate that they provide largely independent and complementary information as suggested by Tozer et al. [214]. The average $T_2$ (from multi-component $T_2$) and free pool $T_2$ (from QMTI), while not equal, were moderately but
6.7 Acknowledgments

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Chapter 7

Conclusion

7.1 Summary

This work sought to reconcile the quantitative MRI techniques of QMTI and QT2, and to advance the understanding of their in vivo use and observations. This was achieved first through theoretical work based on a model of WM that combines the characteristics of multi-component $T_2$ relaxation and MT, based on published in vitro observations, and plausible modifications inspired by pathology. This was followed by the implementation of both techniques and their evaluation for the purpose of longitudinal studies. Finally, QMTI and QT2 were applied in vivo to track the progression of post-enhancement recovery in acute lesions of MS. In general, these techniques provide complementary information, and can be used jointly. We have demonstrated that, with current methods, obtaining stable measurements of the MWF is more difficult than for $F$, indicating the latter as the potentially better choice for evaluation of WM damage. In addition, other parameters measured with QMTI, such as $k_f$, $T_{2f}$, and $T_{2r}$, may provide information regarding the degree of edema or tissue damage not captured by $F$. The MWF remains an attractive quantity, because of its intuitive appeal and implied specificity to myelin, but reliable estimation of the MWF remains elusive.

The QMTI and QT2 techniques provide information about WM structure that can be
used to study disease in vivo; however, each is based on a distinct model that provides only a limited view of WM. This four-pool model of WM that results from the merger of the models for MT and $T_2$ relaxation currently provides the most complete picture. It has previously been useful in the analysis of in vitro experiments that combine MT and relaxation measurements. In this work, this model of WM was adopted to explore the sensitivity and limitations of the measurements performed using QMTI and QT2. The results, presented in Chapter 3, revealed that $F$ is a robust estimate of semi-solid spin population, despite the existence of multiple water and semi-solid compartments. Other parameters estimated in QMTI, such as $k_f$ and $T_{2f}$, are potentially specific to WM changes beyond semi-solid pool content. This suggests that these parameters should not be neglected, despite some reported difficulties in estimating $k_f$ [137], and in contrast to proposed approximations that fix the value of $T_{2f}$ [140]. Compartmental water exchange in the model of bovine WM appears to be fast on the MT time-scale and intermediate on the $T_1$ time-scale. While baseline water exchange is slow on the $T_2$ time-scale, increasing exchange due to pathology can confound MWF estimates. Despite this limitation, stable QT2 measurements appear to have the ability to separate myelin damage from edema, as suggested previously [211].

Variability of estimated parameters of QMTI and QT2 over repeated measurements was investigated, and reported in Chapters 4 and 5. Our observations indicate that both techniques are amenable to use in longitudinal studies, albeit with different requirements in terms of subject group sizes. Each parameter displayed a different degree of variability over repeated measurements. Relaxation parameters from the QMTI experiments ($R_{1f}$, and $T_{2r}$) were the most stable (up to 2% variation), with the exception of $T_{2f}$. $T_{2f}$, along with mean $T_2$ metrics from QT2 ($\langle T_2 \rangle$ and $\langle T_{2e} \rangle$), proved more variable over repeated measurements ($\approx 4\%$). Estimates of $F$ were slightly more variable than relaxation parameters (just under 5%), but much less so than MWF estimates (just over 12%), which was consistently the most variable parameter throughout our simulations and experiments.

As highlighted in Chapter 4, corrections for static and RF field inhomogeneities are essential to achieve better uniformity of QMTI parameter maps and significantly more re-
producing parameter estimates, with current commercially-available MRI apparatus. The effect was very important for $T_{2f}$ and $k_f$, and to a lesser extent for $F$. The relative maps of the $B_0$ and $B_1$ fields required for these corrections can be obtained using relatively simple and efficient techniques and require only very simple image post-processing. The importance of inhomogeneity corrections likely extends to the comparison of measurements across sites and scanner manufacturers.

Data acquisition for QMTI can be optimized by a simple data reduction technique, using error analysis at each optimization step to eliminate the “least-important” data point. This technique successfully produced an optimized 10-point sub-sampling scheme, from the acquired 60-point data, that maintained the quality of parameter maps and the longitudinal reproducibility. In fact, the 6-fold reduction in the number of MT samples increased the variability of QMTI parameter maps by 50-80% and produced no appreciable increase in the longitudinal variability. The optimized sampling introduced only small deviations in average parameter values versus those from the more complete acquisition. This technique is similar to a previously published optimization of QMTI data acquisition [143], but is conceptually simpler. Our method also avoids the pitfall of optimized sampling designs with repeated samples, i.e. when the optimization routine recommends that specific combinations of saturation pulse angle and offset frequency be acquired more than once in the experimental protocol. Our optimization results support the use of two protocols with different TRs to acquire QMTI data [141], in contrast to the suggestion made by others that this practice affords no advantage and may be detrimental [150].

The technique introduced to optimize QMTI acquisitions was used to sub-sample the existing 60-point datasets down to 10-point sets, which were then tested in the context of longitudinal reproducibility. This evidently implies a very limited initial search-space for the reduction algorithm. The method is eminently flexible and is easily expanded to a larger search space; moreover, the optimization can be performed for one or more sets of expected model parameters, for example, to match the intended tissue (WM, GM, muscle) or specific pathology of interest. Preliminary results not included in this thesis indicate that
7.1 Summary

the technique can reliably reduce initial search-spaces with over 300 points to converge on optimized sampling schemes that are physically reasonable, and that agree with previous results [143].

As reported in Chapter 5, basic factors in the QT2 acquisition protocol, such as image geometry and TR, can impact the output of the multi-component analysis, and this must be kept in mind when using this method and comparing data from different studies. In processing the data, the choice of myelin water $T_2$ range affects the MWF estimates and their variability across subjects, but has little effect on reproducibility across repeated measurements. It is possible that the current implementation of the QT2 technique is inherently limited by the difficulties of fitting 100-plus parameters to 32 or 48 data samples, despite the use of a non-negative constraint and of regularization.

In the last study, in a small group of MS patients in the early phase of the disease and presenting active, gadolinium-enhancing lesions, the QMTI technique was sensitive to the dynamic changes of myelin content and inflammation. Results suggested a relatively quick partial resorption of the inflammation and edema, accompanied by a slower, incomplete recovery of tissue membrane integrity, likely reflecting partial remyelination. Mean $T_2$ measures from QT2 also reflected the resolution of edema during post-enhancement recovery, behaving very similarly to the $T_{2f}$. The MWF did not reveal any significant trends, perhaps due to the variability of the measurement and the relatively small number of patients.

The inference made in Chapter 6 that $F$ and $T_{2f}$ provide largely specific information about the timing of recovery in these lesions is generally supported by observations from Chapter 3. We reported that changes in $F$ are reliably indicative of the semi-solid to liquid ratio, while $T_{2f}$ increases reflect the increase of intra/extracellular $T_2$ associated inflammation, and that these two variations are essentially decoupled. The reduced $F$ (-60%) and increased $T_{2f}$ (+100%) seen at the time of enhancement, in Chapter 6 is consistent with a combination of the important pathological features of demyelination and edema. The values seen one month post-enhancement ($\Delta F$: -40%, $\Delta T_{2f}$: +60%) are consistent with
edema that is largely resolved and persistent demyelination. This recovery would appear to continue into the second month post-enhancement. In addition, we observed consistent behaviour for the simulations and in vivo observations with regards to the behaviour of $T_{2f}$ and $\langle T_{2ie} \rangle$: a given increase in the intra/extracellular $T_2$ was reflected, in theory and experimentally, by a relatively larger increase in $T_{2f}$ than in $\langle T_{2ie} \rangle$. Unfortunately, the MWF estimates could not be used to support or contradict our MT observations, as the number of patients examined in the MS study was too low to overcome measurement variability and reveal corroborative information.

### 7.2 Future Work

The observations presented in this thesis suggest that it would be ideal to combine both techniques in the study of MS, as they provide complementary measures. QMTI and QT2 would potentially be applied in clinical research on a larger scale, as they provide more specific pathological information than their respective “predecessors”, MTR and $T_2$-weighted imaging. The following discusses aspects of constructing such an experimental protocol. From the outset, these quantitative techniques must be acquired with some minimum SNR: a value of 100 is often quoted for 32-echo QT2 [173], while the minimum has not been evaluated for QMTI. Resolution and volume coverage are other major obstacles to routine use. Evolving implementations must also carefully balance these constraints with exam duration.

The increasing field strength of MRI scanners can supply part of the desired SNR. Our preliminary measurements comparing QMTI measurements at 3 T and 1.5 T, in a single subject, were very promising. Parameter estimates showed very good correspondance, as expected from in vitro experiments [88] and from experiments performed to optimize MTR acquisitions at 3 T [260]. The approximately linear increase in SNR with field strength resulted in a visible improvement in the quality of our parameter maps. Imaging at higher field strengths suffers from greater inhomogeneities in the transmit $B_1$ field, underlining
the importance of observations presented in Chapter 4 concerning corrections for field inhomogeneities. Energy deposition by the RF $B_1$ fields is another limiting factor for MRI techniques, and this increases with the square of the applied field strength. At 3 T, the energy deposition, or specific absorption rate (SAR), limit is reached more quickly than at 1.5 T, restricting the amount of saturation power that can be used in MT experiments. Fortunately, this limitation is counterbalanced by the slower $T_1$ recovery at higher field strength, and thus sufficient saturation can be produced to observe magnetization transfer [261].

Use of the QT2 technique has been demonstrated at 3 T [188], highlighting the benefits of higher SNR; however, measurements showed some discrepancies with reports at 1.5 T. Energy deposition is also an issue for QT2. The composite pulses used to refocus the magnetization and create the series of spin echoes quickly add up to exceed the SAR limit in certain subjects. This issue is most conveniently resolved by reducing the peak power of each pulse, which in turn increases the lowest achievable echo spacing. The longer $T_1$ values at 3T also conspire against fast data acquisitions by imposing longer repetition times.

Volumetric coverage is essential for both techniques achieve widespread use, but has been slow to evolve. The QMTI techniques has been extended by others to provide whole-brain coverage [139, 142], and preliminary experiments in our group indicate that volumetric coverage can be provided with our technique while maintaining SNR. Volumetric acquisitions of QMTI will benefit tremendously from optimized acquisition schemes, as reported [260] or as presented in Chapter 4, to select the best combination of MT contrasts to acquire in the available scan time. As mentioned in the previous section, the optimization algorithm can be used to taylor the QMTI acquisition to one or more combinations of expected model parameters. Optimization of the sampling scheme will be applied in future development of the methods. Whole-brain coverage is more difficult to achieve with the QT2 technique, but 3D acquisitions with partial brain coverage have been reported by the group at UBC [164]. Alternatively, a $T_2$-prepared fast-imaging technique has also been used to produce MWF maps [187]. Consideration of optimized echo spacing may also help
improve the quality of QT2 measurements [262].

Multiple sclerosis was the clinical application motivating the work presented in this thesis. These MRI methods have been, or could be, applied in the study of brain development, and to conditions and diseases other than MS. The characterization of brain development in early childhood, previously investigated with $T_2$-weighted imaging [263], could be studied in greater detail. Other applications include neurodegenerative diseases and psychiatric disorders, were the sensitivity of the methods to tissue structure and composition can be exploited. For example, QMTI has been applied to Alzheimer disease to reveal some sensitivity to hippocampal pathology [264], and QT2 techniques have been applied in schizophrenia [237] and phenylketonuria (PKU) [192].

Emerging alternatives have been proposed to perform the QMTI and QT2 measurements discussed in this work, more specifically using steady-state imaging sequences and multi-component models. These techniques are generally faster and provide much better image resolution, but require more sophisticated analysis methods and are insensitive to certain parameter estimates or to make assumptions on their value. QMTI has been demonstrated using balanced steady-state free-precession sequences [265]. Multi-component relaxation has been observed with a combination of balanced steady-state free-precession and spoiled gradient-echo sequences [56]. These methods will need to be validated, either against the established QMTI and QT2 techniques, or directly to in vitro or postmortem histology measurements as the others have been. Once optimized, these methods could easily be combined to yield parallel observations of MT and multi-component relaxation in vivo. With further work, these quantitative MRI methods based on steady-state sequences may also have the potential to explore the four-pool model of WM. While this model is complex and has many free parameters, it might be possible to combine a carefully-selected set of steady-state observations with a few judicious approximations in the model to produce estimates of the parameters, in a manner similar to published in vitro experiments.
Appendix A

Approval for *in vivo* MRI studies

Studies involving human subjects require review and approval by the appropriate board. Ethical approval for the quantitative MRI study of multiple sclerosis, involving patients and control subjects, has been maintained continuously with the Research Ethics Board of the Montreal Neurological Institute since the initial approval (1995). The most recent letter of re-approval (2009-10) from the MNI-REB is reproduced on the next page.
Centre universitaire de santé McGill
McGill University Health Centre

February 19, 2009

Dr. Bruce Pike
McConnell Brain Imaging Centre
Montreal Neurological Institute/Hospital
WB2/WB3

Re: 5.f. PIKB 1995/2
Quantitative Magnetization Transfer Imaging of Multiple Sclerosis
Pt: Dr. Bruce Pike
- Submission letter dated February 3, 2009
- Application for Continuing Review signed February 3, 2009
- Consent Form, English & French version January 30, 2009

Dear Dr. Pike,

Thank you for submitting your Application for Continuing Review for the above-cited research protocol.

The above submission, reviewed by the full REB at the February 17, 2009 meeting #5.f., was found to be acceptable for continuation at the McGill University Health Centre (MUHC). This was entered accordingly into the minutes of the REB meeting.

The re-approval of the study is valid until March 6, 2010.

All research involving human subjects requires review at recurring intervals. To comply with the regulation for continuing review of at least once per year, it is the responsibility of the investigator to submit an Application for Continuing Review to the REB prior to expiry. However, should the research conclude for any reason prior to approval expiry, you are required to submit a Termination Report to the board once the data analysis is complete to give an account of the study findings and publication status.

The Research Ethics Boards (REBs) of the McGill University Health Centre are registered REBs working under the published guidelines of the Tri-Council Policy Statement, in compliance with the Plan d’action ministériel en éthique de la recherche et en intégrité scientifique (M555, 1998) and the Food and Drugs Act (2001.06.07), acting in conformity with standards set forth in the (US) Code of Federal Regulations governing human subjects research and functioning in a manner consistent with internationally accepted principles of good clinical practice.

Should any revision to the study or other development occur prior to the next required review, you must advise the REB without delay. Regulation does not permit initiation of a proposed study modification prior to REB approval of the amendment.

We trust this will prove satisfactory to you. Thank you for your consideration in this matter.

Yours very truly,

Eugene Bereza, MD CM, CCFP
Chair, MNH/I Research Ethics Board
EB/ah

Meeting of February 17, 2009 #5.f.

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