Quantitative Magnetic Resonance Imaging of the Brain

Applications for Tissue Segmentation and Multiple Sclerosis

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Cover: White matter, grey matter, and CSF segmentation in a healthy subject.

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*Man måste förstå att man inte förstår det mesta. Fast man kan leta efter förklaringar.
Men inte förklara med Astrologi och annat trams.*

- Skalman

ABSTRACT

Magnetic resonance imaging (MRI) is a sensitive technique for assessing white matter (WM) lesions in multiple sclerosis (MS), but there is a low correlation between MRI findings and clinical disability. Because of this, other pathological changes are of interest, including changes in *normal appearing white matter* (NAWM) and *diffusely abnormal white matter* (DAWM). Even so, the mechanisms leading to permanent disability in MS remain unclear.

In contrast to conventional MRI, *quantitative MRI* (qMRI) is aimed at the direct measurement of the physical tissue properties, such as the relaxation times, T1 and T2, as well as the proton density (PD). QMRI is promising for characterising and quantifying changes in MS and for brain tissue segmentation.

The present work describes a novel method of qMRI for the human brain (QMAP), and a segmentation method based on this. The developed methods were validated in control subjects and MR phantoms. Furthermore, an application in diseased human brain was demonstrated in MS patients. In all, 50 healthy controls and 35 MS patients were scanned with qMRI in a total of 225 acquisitions.

One major finding of this work was that qMRI was able to detect and quantify changes in the MS disease that were not visible using conventional MRI. In particular, it was found that DAWM appears to constitute an intermediate between focal white matter (WM) lesions and NAWM. These changes may be caused by pathological processes that are not entirely attributable to Wallerian degeneration.

This study showed that the QMAP method had high accuracy and relatively high precision, within a clinically acceptable time. This work also demonstrated that qMRI could be used for brain tissue segmentation and volume estimation of the whole brain, using pre-defined tissue characteristics. The results showed that brain tissue segmentation had high repeatability, which was somewhat lower when different geometries were acquired or different field strengths used. In particular, small differences were found between 1.5 T and 3.0 T in deep brain structures, the cerebellum and the brain stem.

This work leads the way for early clinical applications of qMRI, and the challenge for the years to come is to understand the connection between qMRI properties of the brain and underlying biology.

SAMMANFATTNING

Bildtagning med magnetresonanstomografi (MRT) är en teknik som kan användas för att upptäcka lesioner i vit substans hos patienter med multipel skleros (MS), men sambandet mellan lesioner och klinisk funktionsnedsättning är svagt. På grund av detta har intresset för andra patologiska processer i hjärnan ökat. Exempel är förändringar i vit substans som ser normal ut vid MRT (NAWM) och även så kallad diffus vit vävnad (DAWM). Det är emellertid fortfarande oklart vilka mekanismer i MS som leder till klinisk funktionsnedsättning.

Med kvantitativ MRT (qMRT) kan fysiologiska egenskaper i vävnaden, som till exempel relaxationstiderna (T1 och T2) samt protontäthet (PD), mätas. qMRT kan användas för att mäta förändringar i hjärnan hos MS patienter och dessutom för segmentering av hjärnvävnad vid neurodegenerativa sjukdomar.

I detta arbete beskrivs en ny metod för qMRT applicerat på den mänskliga hjärnan (QMAP) och en segmenteringsmetod som baserades på denna. Metoderna validerades både i friska kontroller och i MR fantom. Slutligen användes qMRT för att undersöka hjärnan hos MS patienter. I studierna inkluderades 50 friska kontroller och 35 MS patienter, där totalt 225 bildtagningar med QMAP utfördes.

Ett viktigt resultat var att qMRT kunde användas för att upptäcka och mäta förändringar i hjärnan hos MS patienter som inte var synliga vid konventionell MRT. DAWM utgjorde en intermediär mellan NAWM och lesioner i vit vävnad. Resultaten pekade mot att dessa förändringar inte endast orsakades av Wallerisk degeneration.

QMAP metoden hade hög noggrannhet och relativt hög precision samt kunde användas med en kliniskt relevant tid för bildtagningen. Genom att använda förhandsdefinierade vävnadsegenskaper kunde qMRT tekniken även användas för segmentering av hjärnvävnad och för att beräkna volymer. Segmenteringen hade hög repeterbarhet men den minskade något när olika geometrier eller fältstyrkor användes. Små skillnader mellan 1.5 T och 3.0 T detekterades framför allt i djupa hjärnstrukturer, lillhjärnan och hjärnstammen.

I detta arbete demonstrerades två applikationer av qMRT för hjärnan. Den största utmaningen för kommande år blir att förstå och förklara sambanden mellan qMRT och underliggande biologiska egenskaper.

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LIST OF PAPERS

This thesis is based on the following four papers, in the text referred to by their roman numerals:

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Rapid Magnetic Resonance Quantification on the Brain: Optimization for Clinical Usage
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- II J. West, J. B. M. Warntjes, P. Lundberg
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- III J. West, I. Blystad, M. Engström, J. B. M. Warntjes, P. Lundberg
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Absolute Quantification of T1, T2, PD and B1 on Patients with Multiple Sclerosis,
Covering the Brain in 5 Minutes
ISMRM, Toronto, (2008)
2. J. West, J. B. M. Warntjes, P. Lundberg, A. M. Landtblom
Accurate Estimation of Tissue Volumes by means of Quantitative MR on patients
with Multiple Sclerosis
ISMRM, Honolulu, (2009)
3. J. West, J. B. M. Warntjes, P. Lundberg
Segmentation and Volume Estimation on a Sub-voxel Basis using Quantitative
MR: *A Validation Study*
ISMRM, Stockholm, (2010)
4. J. B. M. Warntjes, J. West, O. Dahlqvist Leinhard, G. Helms, A. M. Landtblom, P.
Lundberg
Absolute quantification of myelin in the brain using quantitative MRI
ISMRM, Stockholm, (2010)
5. J. B. M. Warntjes, J. West, R. Birgander, P. Lundberg
Semi-automatic Brain Ventricle Segmentation using Partial Volume Measure-
ment of CSF based on Quantitative MRI
ISMRM, Stockholm, (2010)
6. J. B. M. Warntjes, J. West, O. Dahlqvist-Leinhard, G. Helms, A. M. Landtblom,
P. Lundberg
Estimation of total myelin volume in the brain
ISMRM, Montréal, (2011)
7. J. West, P. Lundberg
Quantitative Magnetic Resonance Imaging: *Sensitivity to Acquisition Parameters*
ESMRMB, Leipzig, (2011)
8. J. West, J. B. M. Warntjes, P. Lundberg
Using Quantitative Magnetic Resonance Imaging to Generate Disease Images
of Multiple Sclerosis
ESMRMB, Leipzig, (2011)
9. J. B. M. Warntjes, A. Tisell, J. West, A. M. Landtblom, P. Lundberg
Fully Automatic Brain Tissue Mapping on Multiple Sclerosis Based on Quantita-
tive MRI
RSNA, Chicago, (2011)

10. J. West, A. Aalto, J. B. M. Warntjes, O. Dahlqvist Leinhard, A. M. Landtblom, Ö. Smedby, P. Lundberg
Characterizing Normal Appearing and Diseased White Matter in Multiple Sclerosis Using Quantitative MRI
ISMRM, Melbourne, (2012)
11. J. West, I. Blystad, M. Engström, J. B. M. Warntjes, P. Lundberg
On Fully Automatic Whole-Brain Tissue Segmentation at 1.5 T and 3 T based on Quantitative MRI
ISMRM Workshop on Multiple Sclerosis as a Whole-Brain Disease, London, (2013)
12. J. West, A. Aalto, A. Tisell, O. Dahlqvist Leinhard, A. M. Landtblom, Ö. Smedby, P. Lundberg
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1. SyMRI Suite (CE), **2009**
2. SyMRI Diagnostics (CE), **2012**

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1

INTRODUCTION

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1.1 | Human Brain

The brain is the centre of the central nervous system (CNS) and consists mainly of neurons, glia cells and cerebro-spinal fluid (CSF). Neurons are cells that mediate signals through chemically-induced electric *action potentials*, and glia cells are non-neuronal cells in the brain that maintain homeostasis, and provide support and protection for the neurons. CSF is a clear liquid that carries oxygen and chemicals from the blood to the neurons and glia cells, and protects the brain from injury. CSF surrounds the brain and resides in ducts inside the brain, such as the *ventricles*.

Figure 1.1: Illustration of a neuron, with dendrites and a myelin wrapped axon (*Re-printed from Wikimedia commons, Villarreal M R, 2007*).

Each neuron receives electrochemical stimulation from other neurons through *dendrites* and communicates the signal through the *axon*. The site of signal transmission between neurons is called the *synapse*. When a pulse of electricity reaches a synapse it causes a chemical, called a *neurotransmitter*, to be released, which binds to receptors on the other cell, thereby transmitting the signal. The axon can be wrapped in isolating *myelin* sheets, which increase the propagation speed of the electric signals. The myelin is created by glia cells called *oligodendrocytes*.

Figure 1.1 shows an illustration of a neuron with dendrites and a myelinated axon. Regions of the brain that are myelinated have a white colour and are therefore termed *white matter* (WM), while unmyelinated parts of the brain are termed *grey matter* (GM). Grey matter forms a *cortex* around the myelinated WM as well as structures inside the WM, called *deep GM*.

1.1.1 | Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic disease of the CNS. The pathophysiological characteristics of MS are inflammation, demyelination, and the formation of multiple lesions. Recent studies, however, suggest that the spectrum of MS pathology is much broader and heterogeneous (1).

The pathogenesis of MS involves the clonal expansion of auto-reactive T lymphocytes (T cells) in peripheral lymph nodes and the spleen, and the migration of these cells into the CNS via the blood-brain barrier (BBB). Once inside the CNS the T cells are re-activated by resident immune cells, and subsequently attack the myelin and initiate an inflammation, with destruction of myelin and axonal transection (2). B lymphocytes (B cells) are also involved, which can be observed in the production of oligoclonal bands in the CSF. MS has been considered to be an autoimmune disease where the primary reaction is the immune response against self-antigens causing inflammatory lesions (3). This is supported by animal models of MS where experimental allergic encephalomyelitis (EAE) was induced by the injection of a myelin-derived protein into rodents (4). The rodents developed an immune response where T cells migrated through the BBB and attacked myelin structures, similar to the auto-immune response in MS. In the early stages of MS the lesions are often partially re-myelinated by the oligodendrocytes and a remission may take place. However, the lesions may not be fully restored, and with time there is an accumulation of neuroaxonal damage. The final result of tissue breakdown in the lesions is CSF filled cavities.

In addition to the autoimmune inflammatory response in MS, a process of neurodegeneration is crucial. An association between focal demyelinating lesions and axonal damage has been suggested in histopathologic studies, showing that axonal transection is common in lesions and was related to inflammation (2). However, recent studies have also shown evidence of axonal damage independent of focal WM lesions (5–7), suggesting that there may be a component of ongoing neurodegeneration in MS that do not depend on focal inflammatory lesions (8).

There are also secondary effects caused by axonal transections within the lesions. The part of the axon not connected to the neuronal body will disintegrate in time, leaving empty myelin sheets. This axonal destruction, which may be distant from

the actual lesion, is termed *Wallerian degeneration* (5) and has been suggested to partially explain axonal loss in non-lesional WM without inflammation. The exact relationship between inflammation and neuro-degeneration, however, remains unclear.

The clinical presentation of MS is commonly divided into three separate disease phases (9); (1) the preclinical phase, (2) the *relapsing-remitting* (RR) phase where attacks with full or partial remission occur, and (3) the progressive phase where the disease progress with no or limited remission. Most MS patients follow this disease course, in which case the third phase is termed *secondary-progressive* (SP). In some patients the RR phase is skipped; this is termed a *primary-progressive* (PP) disease (10). In early MS inflammation in the CNS is high and lesions are common, but with time as the disease moves into the progressive phase the focal lesions become less common and axonal loss without clinical relapse increases (9). The different phases of MS are illustrated in Figure 1.2.

Figure 1.2: The three phases of MS, (A) the preclinical phase without clinical attacks and disease progression, (B) the relapsing-remitting phase where attacks with remission occur, and (C) the progressive phase without remission. The figure also indicates the first clinically isolated syndrome (CIS), before the diagnosis of MS is made.

MS is the most common neurological disease in young adults, and is about twice as common in females as in males (11). The worldwide prevalence of MS is estimated at between 1.1 and 2.5 million cases (11), and the disease is most common in northern Europe, North America, and Australia, and less common in regions around the equator (12). The prevalence of MS in Sweden is high with about 188 cases per 100.000 (13). The causal factors of MS are not fully clarified, but there is evidence that a combination of genes (especially HLA) and environmental factors (especially Epstein Barr virus, vitamin D deficiency and smoking) are of importance (14). There is so far no cure for MS, but there are immunomodulating treatments that

reduce the number of MS relapses in RR MS, and there is also observations that this can lead to reduced disease progression (15).

1.1.1.1 | Clinical Assessment of MS

MS is diagnosed by demonstrating dissemination in time and space of MS lesions while excluding other diseases (16). The diagnose is typically based on clinical symptoms, MR imaging and laboratory testing of CSF. One single relapse typical of MS is termed a clinically isolated syndrome (CIS) (17), and the diagnosis of MS can only be confirmed after a second attack separated in time and space (*i.e.* affecting a different functional region of the brain). The second attack can be either clinical or radiological. MS is diagnosed using the McDonald criteria, of which the latest revision was in 2011 (18–20).

Disability caused by MS is often assessed using the *Expanded Disability Status Scale* (EDSS) (21). This scale measures the disability in eight *functional systems* of the brain, such as the cerebellar, brain-stem, sensory- and visual systems. The result is given on a scale from 0.0 to 10.0 where 0.0 is a normal neurological examination and 10.0 is death due to MS. Although EDSS measures the accumulated disability it does not reflect the disease activity. In an older MS patient, a high EDSS score may not indicate a rapid progression, whereas in a younger patient it would. To overcome this limitation, a measure of disease progression has been suggested, the *MS Severity Score* (MSSS) (22, 23). The MSSS is calculated from the EDSS and the disease duration, so that the progression speed is taken into consideration.

CSF samples, obtained from a lumbar puncture, is tested for intrathecal immunoglobulin G production, which is measured by IgG-synthetesis index and oligoclonal bands. These are, however, not detected in all MS patients (24).

1.2 | Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive medical imaging modality that images soft tissue *in vivo*. Magnetic Resonance (MR) is the physical phenomenon which is utilised to generate and acquire radiofrequency (RF) signals from tissue.

MRI is a versatile technique with many applications in medical imaging, among others to image tissue properties, blood-flow, dynamic processes and tissue diffusion. MRI generally images the water content in tissue, contrasted with different tissue characteristics such as molecule mobility, water compartments and flow.

Figure 1.3: Sample contrast MRI images from a healthy 25-year-old male subject (at 3.0 T) (A-E) and a 45-year-old female MS patient (at 1.5 T) (F). The top row shows T2-weighted images in three orientations, (A) axial, (B) coronal, and (C) sagittal. The bottom row shows two different contrast weightings, where different tissue properties are highlighted; (D) fluid attenuated inversion recovery (FLAIR) and (E) T1-weighted image. Finally (F) shows one axial FLAIR slice of the MS patient with typical WM lesions visible.

Sample contrast images, from a healthy 25-year-old male subject and a 45-year-old female MS patient, are shown in Figure 1.3.

The MR signal was discovered in 1946 by two separate groups, Bloch *et al.* (25) and Purcell *et al.* (26). For their discovery, Bloch and Purcell were awarded the Nobel prize in physics in 1952. The technique for generating images using spatial encoding with magnetic field gradients was discovered by Lauterbur (27) and Mansfield *et al.* (28). For this Lauterbur and Mansfield were awarded the Nobel prize in medicine in 2003.

1.3 | Spin Physics

MR is based on the interaction between nuclear *spins* and externally applied static and dynamic magnetic fields. A measurable MR signal is generated when the *thermal equilibrium* of spins, in the presence of a static magnetic field, is perturbed by a radiofrequency (RF) pulse. In order to model the phenomenon of MR a combination of classical mechanics and quantum mechanics is generally applied. Quantum mechanics describes the properties of nuclear spins and their interactions, whereas classical mechanics describes the behaviour of spin *isochromates* and macroscopic magnetisation.

This section gives a brief account of MR Physics. For a more comprehensive description refer to the excellent books by Levitt (29) and Haacke *et al.* (30).

1.3.1 | Spins

In quantum mechanics the nuclear particles are described in terms of their quantum mechanical properties that determine their interactions with the surroundings. One of these properties is the *spin*, s . Nuclear particles which possess spin can be referred to as *spins*. For protons $s = \frac{1}{2}$, this is referred to as a spin-half system. Furthermore, each spin is a magnetic dipole with a magnetic polarisation.

When placed in an external magnetic field, B_0 , spins split up into distinct energy states. In a spin-half system there are two energy states, *spin-up* and *spin-down*, where spin-up is the lower energy state, aligned with the external magnetic field. In the transverse plane, perpendicular to B_0 , the angular momentum causes the spins to precess around B_0 . The frequency of precession, the Larmor frequency, ω , is dependent on the *gyromagnetic ratio*, γ , and the external magnetic field, and is given by:

Figure 1.4: A single spin with a precession, ω , around a static magnetic B_0 -field.

$$\omega = -\gamma B_0. \quad (1.1)$$

The quantum model of a single precessing spin is shown in Figure 1.4

1.3.2 | Macroscopic Magnetisation

Spins constantly flip between energy states with a slightly higher probability of favouring the lower energy state. Therefore, there will be a small abundance of spins in the spin-up state. When the ratio of spin-up and spin-down remains fixed, the spin-system is in *thermal equilibrium*. The spin excess in such a system is predicted by the Boltzmann equation:

$$\text{spin excess} \approx p_0 \frac{\hbar\gamma}{2kT} B_0, \quad (1.2)$$

p_0 is the number of spins in the measured volume, $\hbar$ is the reduced Planck's constant ($\hbar = h/2\pi$), k is Boltzmann's constant and T is the absolute temperature in units of Kelvin.

Since all spins possess an intrinsic magnetic polarisation, this produces a small macroscopic magnetisation, M_0 , aligned with the external B_0 -field. The macroscopic magnetisation is several orders of magnitude smaller than the static magnetic field and cannot be detected in the same direction as the B_0 -field. A large collection of spins that generate a macroscopic magnetisation is referred to as a *spin isochromate*. The thermal equilibrium magnetisation is found by multiplying the spin excess (Eq. 1.2) by the magnetic moment component in each spin, in the direction of B_0 , which is $\gamma\hbar/2$:

$$M_0 = p_0 \frac{\hbar^2\gamma^2}{4kT} B_0. \quad (1.3)$$

1.3.3 | Radiofrequency Pulse

In order to detect the magnetisation in a spin isochromate it is necessary to *flip* the magnetisation to the transverse plane, where it can be detected perpendicular to B_0 . A second magnetic field, B_1 , is applied orthogonally to B_0 , driving the spins to precess around both of these fields. Even though spins are precessing at the same frequency they have different phases, and to achieve persistent magnetisation it is also necessary to create coherence between the individual spins. This is done by rotating B_1 at the Larmor frequency, causing the spins to precess coherently. The frequency of B_1 is in the radiofrequency domain, and therefore B_1 is referred to as a radiofrequency (RF) pulse. By selecting the Larmor frequency associated with the nuclei of interest, coherence is only created for this nuclei.

An RF pulse is expressed in terms of its flip angle. A $\pi/2$ -pulse flips the magnetisation completely to the transverse plane and a π -pulse inverts the magnetisation.

In practice the flip angle of the RF pulse is difficult to establish and it varies depending on the spatial location. This has consequences for the MR signal and is especially crucial in quantitative MR, where the B_1 -field must often be measured. The evolution of the macroscopic magnetisation during the application of three different RF pulses is illustrated in Figure 1.5.

Figure 1.5: Evolution of macroscopic magnetisation during the RF pulse for (A) a $\pi/2$ -pulse, (B) an α -pulse with lower flip angle, and (C) an off-resonance pulse not resulting in transverse magnetisation.

1.3.4 | MR Signal

The macroscopic magnetisation of a spin isochromate, where the spins have been made to precess coherently in the transverse plane, induces a measurable current in a receiving coil. This current is the MR signal, and is termed the *free induction decay* (FID).

After the application of the RF pulse the FID quickly diminishes through two separate relaxation processes which are characteristic of the material measured. The return to thermal equilibrium is termed the T_1 relaxation, and is caused by the exchange of energy with the surroundings. The de-phasing of the spins is termed the T_2 relaxation and is mainly caused by fluctuations in the local magnetic field. Whereas T_2 relaxation causes an irreversible decay of the transverse coherence, there is also decay which can be reversed. The combination of reversible and irreversible transverse coherence decay is termed T_2^* relaxation.

The FID oscillate at the Larmor frequency, but by applying a rotating frame of reference these oscillations are removed. In MR experiments the z-axis is placed in the direction of the B_0 -field and the x- and y-axes are placed in the transverse plane, rotating at the Larmor frequency. The magnetisation in the direction of B_0

is labelled M_z and the magnetisation in the transverse plane is labelled M_x and M_y respectively.

In the MR experiment the signal is acquired by repeatedly applying RF pulses and sampling the resulting FID at specific time-points. The time between subsequent repetitions of the RF pulses is termed the *repetition time*, T_R , and the time from the generation of the FID to the acquisition of the MR signal is termed the *echo time*, T_E . If the magnetisation is not allowed to return to thermal equilibrium in between subsequent repetitions, the spin system reaches a *steady-state*.

In order to generate an image it is necessary to use spatial encoding. This is done by application of magnetic gradients in the B_0 -field, inducing spatial dependency on the Larmor frequency. These variations can be decoded as variations in phase and frequency in the measured MR signal. For a detailed explanation of spatial encoding, refer to (31). The combination of RF pulses and magnetic field gradients is termed the *MR pulse sequence*.

The influence of the relaxation processes on the measured signal and on the generated image is dependent on the specific pulse sequence used and the MR scanner settings. For example, when the FID is sampled after the application of one RF pulse in each repetition (using a $\pi/2$ -pulse in steady-state), it is called a *gradient echo*. In this experiment the signal can be modelled by the following equation:

$$S = C_i * M_0 * (1 - \exp(-T_R/T_1)) \exp(-T_E/T_2^*). \quad (1.4)$$

In this equation, S is the signal measured in the receiving coil, and C_i is a scaling constant depending on many different effects, such as the receiver hardware. The T_1 and T_2 relaxation processes will be discussed in detail in Sections 1.4.2 and 1.4.3.

1.3.5 | Spin Echo

The T_2^* decay can be re-phased, only leaving the irreversible T_2 relaxation. This is called the *spin echo* (32).

The spin echo pulse sequence is initiated with a $\pi/2$ -pulse, flipping the M_z magnetisation to the transverse plane. In the transverse plane, spins begin to de-phase with T_2^* relaxation. At another later time-point, $T_E/2$, a π -pulse is applied that flips the magnetisation from M_x to $-M_x$. When the magnetisation is inverted, the precession reverses, and spins start to re-phase again. After the time T_E the spins have re-phased and the effects of T_2^* relaxation are removed, only leaving the non-reversible T_2 relaxation. Since the refocusing occurs at exactly the time T_E ,

the signal can be modelled using the same equation as for the gradient echo (Eq. 1.4, also in steady-state), changing $T2^*$ to $T2$:

$$S = C_i * M_0 * (1 - \exp(-T_R/T1)) \exp(-T_E/T2). \quad (1.5)$$

The spin evolution of the spin echo sequence is illustrated in Figure 1.6 and the magnetisation is shown in Figure 1.7.

The application of π -pulses can be repeated several times, creating multiple spin echoes after one $\pi/2$ -pulse. When this is done, π -pulses are applied at times of $n * T_E/2$ causing the spins to re-phase at each $n * T_E$ time-point where a new spin echo can be acquired. This sequence can be repeated as long as the non-reversible $T2$ relaxation permits. This experiment is, however, prone to imperfect flip angles where the spins are not completely inverted, causing an accumulative error for each additional application of a π -pulse.

Another way to perform the multiple spin echo experiment is to apply the π -pulses in quadrature with the $\pi/2$ -pulse, *i.e.* rotating the magnetisation around the M_x axis instead of inverting the magnetisation (rotation around y-axis). This is termed the Carr-Purcell-Meibom-Gill (CPMG) sequence (33, 34). In this way, the spin precession changes direction, but the spin magnetisation is not inverted. By alternating the sign of the π -pulses in the multi-echo experiment the accumulative error is removed and each second echo results in the true signal with $T2$ relaxation.

1.4 | Quantitative Magnetic Resonance Imaging

Conventional MRI is based on the acquisition of *contrast images*. These images are affected by many different contrast mechanisms, such as the MR pulse sequence, the MR scanner settings, B_0 - and B_1 -field inhomogeneities, as well as the different tissue properties¹. In conventional MRI the MR scanner settings are chosen to highlight or saturate tissue properties (for example in Eq. 1.5), resulting in *e.g.* $T1$ -weighted or $T2$ -weighted images.

Conversely, *quantitative MRI* (qMRI) is aimed at the direct measurement of physical tissue properties, such as the relaxation times, $T1$ and $T2$, as well as the proton density (PD). These properties are, in theory, independent of acquisition method and system imperfections.

There are three main reasons that qMRI is not commonly used. First, the acquisition time to date has been too long to be practical. Second, visualisation of qMRI

Figure 1.6: The spin evolution during the spin echo experiment. The pulse sequence is initiated with a $\pi/2$ -pulse, which flips the spins from the z-axis (A) to the transverse plane (B) where the spins begin to de-phase (C). After some time, $T_E/2$, the spins have de-phased (D) and a second π -pulse is applied (E). Now the spins will instead re-phase (F) and at the time T_E a spin echo can be acquired (G) (Re-printed from Wikimedia commons, Filler A, 2010).

Figure 1.7: Magnetisation during the spin echo experiment with six repetitions. After some time, $T_E/2$, the phase is reversed with a π -pulse, refocusing the magnetisation at the time-point T_E . The echo is not fully refocused because of the irreversible T2 relaxation.

properties is not trivial, and third, clinical use and clinical evidence for qMRI are in many cases sparse or lacking.

Only recently have new qMRI techniques has been developed that allows fast quantification by using methods to speed up the acquisition (e.g. FLASH, EPI and Compressed Sensing) (35–40), interleaved slice acquisition (41,42), or steady-state acquisition (43–45).

One way to visualise qMRI tissue properties - one that is important in many neurological applications - is the classification (or segmentation) of tissue types and calculation of tissue volumes. This will be discussed in Section 1.6. Another way of visualisation is that of *synthetic MRI*² (46–51), where contrast images are calculated (or synthesised) by using the qMRI tissue properties in signal equations (e.g. Eq. 1.5, could be used to generate synthetic spin echo images). Synthetic MRI is particularly straightforward, as it generates images similar to conventional MRI images, with the added benefit that any contrast image can be synthesised without the need for additional MR scans. However, the synthetic MRI images are not identical to conventional contrast images, and clinical validation is mandatory (52–54). In particular *fluid attenuated* images (e.g. *FLAIR*) are difficult to generate from qMRI data because of partial volume effects (55). There have also been other attempts to visualise qMRI data such as *color MRI* (56,57) and *vector MRI* (57), but these methods have not been widely applied.

In the following sections the qMRI tissue properties PD and the relaxation times, T1 and T2, will be discussed. The physiological basis, principles of measurements and gold standard methods for acquisition will be presented for each property.

1.4.1 | Proton Density

Protons are often used in MRI because they are abundant in the body. The proton density (PD) is the number of protons in each imaged volume element. By using RF pulses at the Larmor frequency associated with protons (Eq. 1.1) only protons are made to precess coherently; thus a signal is generated only from these nuclei. Therefore, the number of spins in the thermal equilibrium equation (Eq. 1.3) can be changed to PD, and the specific gyromagnetic ratio for protons can be inserted:

$$M_0 = PD \frac{\hbar^2 \gamma_p^2}{4kT} B_0. \quad (1.6)$$

The thermal equilibrium magnetisation, M_0 , is in this way only related to the PD, the external magnetic field, B_0 , and the absolute temperature, T , apart from known constants, $C_{const} = \hbar^2 \gamma_p^2 k^{-1} / 4$:

$$M_0 = C_{const} * PD * \frac{B_0}{T}. \quad (1.7)$$

In MR the measured PD originates from water as these protons have sufficiently long relaxation times to generate a measurable FID. These protons are therefore referred to as *MRI-visible* and PD is sometimes used as a measure of tissue water contents. There are also *MRI-invisible* protons that are bound in e.g. lipids, proteins, nucleic acids, cellular structures etc. The amount of protons that is included in the MRI experiment is determined by how fast the FID can be acquired.

PD is often measured in percentage units (pu)³ of water PD at the same temperature. So that water is set to the value of 100 pu.

1.4.1.1 | Measuring Proton Density

Proton density is in principle derived directly from image intensity corrected for all secondary effects in the signal, such as weightings caused by the relaxation times, distortions of the static magnetic field, B_0 , and the RF pulses, B_1 . In practice, this often means removal of T1- and T2(*)-weightings (39, 58–60).

In the spin echo equation (Eq. 1.5), there is a contribution of both T1 and T2 to the measured signal. One way to remove this is to measure T1 and T2 and then extrapolate a PD image where $T_E \rightarrow 0$ and $T_R \rightarrow \infty$:

$$\begin{aligned} \lim_{T_E \rightarrow 0, T_R \rightarrow \infty} S &= C_i * M_0 * (1 - \exp(-T_R/T1)) \exp(-T_E/T2) = \\ &= C_i * M_0. \end{aligned} \quad (1.8)$$

However, the signal S is not equal to the equilibrium magnetisation, and M_0 is not only dependent on PD. Combining Eq. 1.8 and Eq. 1.7 results in:

$$S = C_i * C_{const} * PD * \frac{B_0}{T}. \quad (1.9)$$

In order to extract PD from this equation it is necessary to find a value for C_i . This can be done by including a reference with known PD (61). This can be either an internal reference, such as CSF for brain imaging (62), or an external reference e.g. a water phantom (60). If an external reference is used the temperature difference must be accounted for. Another approach to finding a value for C_i is to compensate for all known scaling factors (39):

$$C_i = C_{coil} * C_{load} * \dots * C_n. \quad (1.10)$$

This approach is often impractical as it demands the measurement of several scaling factors with high precision, and even so there may still remain an unknown scaling factor, C_n , which must be empirically determined.

Furthermore, when the constant C_i has been determined the signal is also dependent on the external magnetic field, B_0 , and the applied RF pulses, B_1 . These fields are not homogeneous, as discussed in Section 1.3.4; therefore it is necessary to measure and compensate for local variations (39, 60, 61).

PD has also been found to strongly correlate with T1 in healthy brain tissue (63–66). Therefore another approach to measuring PD is to measure the T1 relaxation and then calculate the PD:

$$PD \approx A + \frac{B}{T1}. \quad (1.11)$$

In this equation A is an offset and B a scaling factor, analogous with C_i in Eq. 1.9, which can be determined iteratively. This relationship holds only in healthy tissue in some anatomies, such as the brain. The relationship does not necessarily hold in pathological tissue or after the application of a contrast agent. One way to circumvent this limitation is to exclude regions of pathological tissue in the determination of A and B (66), but then include these regions again when PD is calculated.

1.4.2 | T1 Relaxation

T1 relaxation⁴ is the process where a spin system returns to thermal equilibrium. Another notion that is sometimes used is the R1 relaxation *rate*, where $R1 = 1/T1$. The relaxation is dependent on the spin energy exchange with the environment, mainly in the form of thermal vibrations causing the spins to flip between the spin-up and spin-down energy states. T1 relaxation is often modelled by the Bloch equation:

$$\frac{dM_z(t)}{dt} = \frac{M_0 - M_z(t)}{T1}. \quad (1.12)$$

If the longitudinal magnetisation immediately after an RF pulse is given by M_z^+ then the return to thermal equilibrium can be described by solving the above equation:

$$M_z(t) = M_0 + (M_z^+ - M_0) \exp(-t/T1). \quad (1.13)$$

Typical T1 relaxation curves for human brain tissue are shown in Figure 1.8. The longitudinal magnetisation after an RF pulse, M_z^+ , can be calculated by using the flip angle of the RF pulse according to:

$$M_z^+ = M_z^- \cos(\alpha), \quad (1.14)$$

where M_z^- is the magnetisation before the RF pulse. For example, by applying a perfect $\pi/2$ -pulse on a spin system in thermal equilibrium, the longitudinal magnetisation after the RF pulse will be 0 and the return to thermal equilibrium simply becomes:

$$M_z(t) = M_0(1 - \exp(-t/T1)). \quad (1.15)$$

This is how the T1 relaxation is modelled in the signal equation for the spin echo sequence (Eq. 1.5).

The single component Bloch equation is only valid when the T1 relaxation is mono-exponential. This is usually sufficient and the T1 relaxation can then be described by a single value. In real tissue, however, multi-exponential T1 behaviour has been detected (67), and it may be more accurate to model T1 using several exponents:

$$M_z(t) = M_{0,1}(1 - \exp(-t/T1_1)) + \dots + M_{0,i}(1 - \exp(-t/T1_i)). \quad (1.16)$$

1.4.2.1 | Measuring T1 Relaxation

In principle, T1 relaxation is measured by sampling the T1 relaxation curve at multiple time-points and fitting these to a mono-exponential decay curve. This can be done in any pulse sequence, where T1 can be separated, by modelling the T1 relaxation using Eqs. 1.13 and 1.14. There are three main approaches to estimating T1; signal measurements at different time points after preparation pulses (35,37,68), several signal measurements after one preparation pulse (36,38,39,41,69), and signal measurements using various flip angles (43–45).

When measuring T1 relaxation, either the repetition times must be long or a steady-state acquisition must be applied. In the latter case the signal at the beginning and end of each repetition is equal, but not equal to M_0 . Generally, a *crushing* or

spoiling gradient is also necessary. This removes remaining transverse magnetisation between subsequent pulses.

1.4.2.2 | Inversion and Saturation Recovery

The gold standard method to measure T1 relaxation is the *inversion recovery* experiment. In this experiment the magnetisation is prepared with a π -pulse and then one acquisition is performed after a specific *inversion time*. This procedure is repeated after full relaxation with various inversion times, collecting several time-points on the T1 relaxation curve. The signal equation for the inversion recovery experiment is:

$$S = C_i * M_0(1 - (1 - \cos(\Theta)) \exp(-T_I/T_1)). \quad (1.17)$$

Here T_I is varied in several measurements and M_0 , T_1 and the inversion Θ -pulse are included in a three-parameter fit. It is also possible to use a separately acquired B_1 map to determine Θ , and apply a two parameter fit for M_0 and T_1 .

The inversion recovery method can be acquired faster using a lower flip angle for Θ , in which case the sequence is termed *saturation recovery* (68). When a lower flip angle than π is used it is necessary to employ crushing gradients to remove transverse relaxation and to avoid spurious echoes.

It is possible to create fast multi-slice saturation recovery sequences by using time efficient slice ordering (41,42). In this case, slice n is prepared using the saturation pulse, whereas acquisition is performed on another slice, m . By alternating the order of n and m the T1 relaxation takes place in one slice while another slice is acquired. In principle this means that an equal number of slices and time-points on the T1 relaxation curve can be acquired.

1.4.2.3 | Look-Locker

Look-Locker sequences measure several time-points on the same T1 relaxation curve after an initial Θ -pulse (36,38,39,41,69). In this experiment the spin system is prepared by the Θ -pulse, then several *fast low angle shots* (FLASH) are applied, sampling the magnetisation at several time points using a low α flip angle.

The Look-Locker sequence is modelled using the inversion recovery Eq. 1.17. However, the apparent relaxation, $T1^*$, is shorter than the actual T1 relaxation because the FLASH pulses perturb the longitudinal magnetisation. A two- or three-parameter fit is then applied in the same manner as for the inversion recovery

experiment to acquire $T1^*$, M_z and Θ . The actual $T1$ relaxation can then be calculated from:

$$T1 = \frac{\tau}{\tau/T1^* + \ln(\cos(\alpha))}, \quad (1.18)$$

where τ is the inter-pulse interval and α is the angle of the FLASH pulses. This equation is derived from Eqs. 1.13 and 1.14.

1.4.2.4 | Spoiled Gradient Echo

The *spoiled gradient echo* (SPGR) is a gradient echo sequence where crushing of transverse magnetisation is applied between subsequent repetitions (43–45). When this sequence is used to measure $T1$, a steady-state approach with variable flip angles is applied. The signal equation for this sequence is:

$$S = C_i * M_0 \sin(\alpha) \frac{1 - \exp(-T_R/T1)}{1 - \exp(-T_R/T1) \cos(\alpha)}. \quad (1.19)$$

By making several measurements with T_R constant and increasing α a curve characterised by $T1$ is generated. This equation can then be linearised, $Y = mX + b$, according to:

$$\frac{S}{\sin(\alpha)} = \frac{S}{\tan(\alpha)} * \exp(-T_R/T1) + M_0(1 - \exp(-T_R/T1)). \quad (1.20)$$

Finally, M_0 and $T1$ can be found from the slope and intercept of this curve using linear regression:

$$\begin{cases} T1 &= -T_R / \ln(m) \\ M_0 &= b / (1 - m). \end{cases} \quad (1.21)$$

1.4.3 | T2 Relaxation

$T2$ relaxation⁵ is the process where a spin system that has been made to precess coherently in the transverse plane by the application of an RF pulse, loses coherence and de-phases, causing the MR signal to diminish. Another notion that is commonly used is the $R2$ relaxation *rate*, where $R2 = 1/T2$. In a spin isochromate the individual spins interact, and since spins are magnetic dipoles they create variations in

the local magnetic field. This results in local inhomogeneities in the B_0 -field which cause spins to precess at slightly different Larmor frequencies and thus the spins de-phase. Since spins rapidly change location because of Brownian motion, the local magnetic fields constantly change and therefore the spin-spin interaction is *dynamic*. There are also *static* relaxation processes caused by macroscopic inhomogeneities in the B_0 -field. These can be reversed using the spin echo sequence, as discussed in Section 1.3.5. The irreversible relaxation is the T2 relaxation and the combination of static and dynamic relaxation is termed T2* relaxation. T2 relaxation is often modelled by the Bloch equations:

$$\begin{cases} \frac{dM_x(t)}{dt} = \omega M_y(t) - \frac{M_x(t)}{T2} \\ \frac{dM_y(t)}{dt} = -\omega M_x(t) - \frac{M_y(t)}{T2} \end{cases} \quad (1.22)$$

These equations can be simplified in the rotating frame of reference (assuming that the RF pulse rotates the magnetisation around the y-axis, resulting in transverse magnetisation in M_x):

$$\frac{dM_x(t)}{dt} = -\frac{M_x(t)}{T2}. \quad (1.23)$$

If the transverse magnetisation immediately after an RF pulse is given by M_x^+ then the loss of coherence can be described by solving the above equation:

$$M_x(t) = M_x^+ \exp(-t/T2). \quad (1.24)$$

Typical T2 relaxation curves for human brain tissue are shown in Figure 1.8. The transverse magnetisation after an RF pulse, M_x^+ , can be calculated by using the flip angle of the applied RF pulse according to:

$$M_x^+ = M_z^- \sin(\alpha), \quad (1.25)$$

where M_z^- is the longitudinal magnetisation before the RF pulse, this equation together with Eq. 1.14 describe how much of the magnetisation is converted from M_z to M_x using an α -pulse. For example, after application of a $\pi/2$ -pulse to a spin system in thermal equilibrium all magnetisation will be converted to transverse magnetisation and the T2 relaxation can then be modelled by:

$$M_x(t) = M_0 \exp(-t/T2). \quad (1.26)$$

This is how the T2 relaxation is modelled in the signal equation for the spin echo sequence, see Eq. 1.5. The same equation is also used to model T2* relaxation, by changing T2 for T2*.

T2 relaxation is often thought to be multi-exponential in real tissue (70,71). When a single T2 component is modelled it is strongly dependent on the echo times of the sequence, echo spacing and the number of echoes. Another approach is to model either several T2 relaxation components (analogous to multi-exponential T1 relaxation, Eq. 1.16) or a continuous relaxation spectrum:

$$M_x(t) = M_{0,1} \exp(-t/T2_1) + \dots + M_{0,i} \exp(-t/T2_i). \quad (1.27)$$

1.4.3.1 | Measuring T2 Relaxation

T2 relaxation is estimated using several acquisitions of a pulse sequence where T2 can be extracted, generally acquiring several different echo times and modelling the signal using Eqs. 1.24 and 1.25. T2 relaxation is either estimated in a mono-exponential fitting or several relaxing compartments are included. There are three main approaches to estimating T2; signal measurements at different echo times (26), signal measurements at multiple echo times after the same RF pulse (39,68,70), or signal measurement using various flip angles (43,44).

1.4.3.2 | Spin Echo

The gold standard method to measure T2 relaxation is to use the CPMG spin echo sequence, discussed in Section 1.3.5 (33,34). One spin echo is acquired in each acquisition using sufficiently long repetition time to remove dependency on T1 relaxation. The signal equation in this case becomes:

$$S = C_i * M_0 \exp(-t/T2). \quad (1.28)$$

It is possible to acquire the T2 relaxation curve faster using a multi-echo approach where multiple spin echoes are acquired after one excitation pulse, using refocusing pulses (39,68,70). In this case, as discussed in Section 1.3.5, each second echo reflects the true T2 relaxation, whereas odd echoes include errors caused by RF

pulse imperfections. It is therefore necessary to either discard odd echoes, or include empirical correction factors.

In principle it is sufficient to acquire two echoes in order to fit a mono-exponential T2 decay curve. This, however, results in poor reproducibility and large variability in the T2 measurements, mostly due to noise (61). Whittall *et al.* (72) noted that dual-echo mono-exponential T2 measurements with varying echo spacing, in WM, created artificial statistically significant differences, where in fact there were none. Dual-echo T2 measurements should be used with caution.

1.4.3.3 | Steady-State Free Precession

The *steady-state free precision* (SSFP) experiment is similar to the SPGR experiment for T1 estimation, but without crushing gradients (43,44). By omitting the crushing gradients a steady-state is achieved for both the longitudinal and the transverse magnetisation. Extending the signal equation for the SPGR experiment (Eq. 1.19) to also include T2 relaxation the following signal equation is obtained:

$$S = C_i * M_o \sin(\alpha) * \frac{1 - \exp(-T_R/T1)}{1 - \exp(-T_R/T1) \exp(-T_E/T2) - (\exp(-T_R/T1) - \exp(-T_E/T2)) \cos(\alpha)} \quad (1.29)$$

By holding T_R and T_E constant, but increasing the RF flip angle, α , acquisitions depend on both T1 and T2. The equation can be linearised in the same manner as the SPGR sequence (Eq. 1.20). Finally, if T1 is known, for example from a previous SPGR acquisition, T2 and M_0 can be found using linear regression:

$$\begin{cases} T2 &= -T_R / \ln \left(\frac{m - \exp(-T_R/T1)}{m \exp(-T_R/T1) - 1} \right) \\ M_0 &= b * \frac{\exp(-T_R/T1) \exp(-T_E/T2) - 1}{1 - \exp(-T_R/T1)} \end{cases} \quad (1.30)$$

1.5 | MR Relaxation in Brain Tissue

The T1 and T2 relaxation processes are directly influenced by the local biophysical structure and biochemical environment. In particular T1 and T2 in the brain are dependent on tissue water mobility, macromolecules, composition of proteins and lipids, iron concentration and the paramagnetic environment. T1 in the brain is mainly influenced by water concentration (63, 64, 73–75), but also by iron concentration (76–79). T2 in the brain is mainly affected by different tissue water environments (70, 71), such as water inside cells, extracellular water and water between the myelin sheets, but T2 is also affected by iron concentration (79–82). The relative sizes of each tissue water environment, as well as exchange rate among these, affects the T2 relaxation, especially when only one T2 component is estimated. Generally, several processes concurrently affect T1 and T2, making it challenging to separate specific biological processes. For example myelin sheets contain lipids and trap water in close proximity to larger molecules. The myelin is created by oligodendrocytes which contain iron, affecting both T1 and T2. The deep GM structure also accumulates iron, and CSF, which consists mainly of free water, infiltrates tissue, leading to partial volume effects and prolongation of T1 and T2. Manifestations of these processes are seen in the contrast between WM and GM, where myelin and iron create a difference in T1 and T2 (75). Typical qMRI values and relaxation curves for the human brain are shown in Figure 1.8.

All of the concurrent processes affecting T1 and T2 lead to low specificity for qMRI. One way to tackle this is to create tissue-specific models where simulations of healthy and diseased tissues can be performed. Two approaches for such models are outlined below.

1.5.1 | Four-Pool Model

One way to model the relaxation properties of brain tissue is to model different tissue compartments (or pools) that contribute to the MR signal, and the interaction among these. One such tissue model, suggested by Levesque *et al.* (83), was created with two liquid compartments and two semi-solid compartments. The liquid pools described water environments, the first consisting of intra/extracellular water, and the second consisting of water in close proximity to lipids, trapped between myelin sheets around the axons. The semi-solid pools described non-aqueous environments, the first consisting of myelin semi-solids and the second consisting of non-myelin semi-solids, such as macromolecules. Figure 1.9 shows a schematic of the four-pool model with relevant model parameters.

WM			GM			CSF		
T1 (ms)	T2 (ms)	PD (pu)	T1 (ms)	T2 (ms)	PD (pu)	T1 (ms)	T2 (ms)	PD (pu)
600	80	70	950	100	78	4500	2200	100

Figure 1.8: Typical qMRI values (at 1.5 T) and mono-exponential relaxation curves approximating to WM, GM and CSF in the human brain. T1 relaxation (A) shows the return to thermal equilibrium and T2 relaxation (B) shows the diminishing of the signal, in the transverse plane, due to de-phasing. T1 values were obtained using the IR sequence (30), T2 values were obtained using the CPMG sequence (30), and PD values for WM and GM were obtained using the multi-component T2 analysis method of Whittall *et al.* (71). PD for CSF was set to the reference value of 100.

In this model qMRI properties, volume fractions, and exchange rates between compartments are parameters and Bloch equations are used to simulate the characteristics of the tissue. This model has been used to predict changes in qMRI properties induced by different diseases or tissue characteristics in specific MR pulse sequences (84).

1.5.2 | Myelin Water Fraction

Another approach to modelling the relaxation behaviour in the brain is to estimate several different exponents in the relaxation (Eqs. 1.16 and 1.27), either on a continuous spectrum or using discrete distributions (67, 70, 71, 85). These *in vivo* models have been created with two (70, 71, 86) or three (87) separate water environments. By assuming relaxation times of different water compartments, the relative sizes of the water pools can be estimated. Although a few groups have attempted to separate intra- and extracellular water compartments (88, 89) this is normally difficult to achieve and the intra/extracellular water is considered as one compartment (70). A typical T2 distribution spectrum for brain tissue is shown in Figure 1.10.

Figure 1.9: Illustration of the four-pool model of brain tissue with relevant model parameters. $T1_{mw}$, $T2_{mw}$, $T1_{ie}$, $T2_{ie}$, $T1_m$, $T2_m$, $T1_{nm}$, and $T2_{nm}$ are the relaxation times for each compartment respectively, and R_D , R_m , R_{nm} are the exchange rates between the compartments. Also, the volume fraction of each compartment is included in the model, these are not shown in the figure.

Figure 1.10: Histogram of typical T2 relaxation spectra for brain tissue with three separate water environments, with different T2 times. The intra- and extracellular water are challenging to separate and are often considered as one component.

This technique has mostly been used in multi-exponential T2 relaxation (70, 71, 90, 91), but also in T2* relaxation (85), and T1 relaxation (67). By estimating the relative sizes of the different components this has been used as a surrogate measure of myelin content in tissue, the *myelin water fraction* (MWF):

$$MWF = \frac{\sum M_{myelin}}{\sum M_{total}}. \quad (1.31)$$

MWF measurements have been applied *in vivo* to assess changes in myelin contents in diseased tissue. This will be discussed further in section 1.7.3.

1.5.3 | Brain Iron

Another process that affects both T1 and T2 relaxation in the brain is the iron concentration (76–82). Deep GM structures are particularly rich in iron (79, 81, 92). Furthermore, iron accumulates with age (80, 93) and is abnormal in diseases such as Alzheimer’s disease (94), Hallevorden-Spatz syndrome (95), Parkinson’s disease (96–98), and MS (99–104).

Since iron is paramagnetic it creates fluctuations in the local B_0 -field affecting the Larmor frequencies of surrounding spins. This affects both the static and dynamic field inhomogeneities. The effect of iron is greater at higher field strengths, and the differences in T1 and T2 between iron-rich and iron-depleted regions are enhanced in higher fields (79, 81).

1.6 | Brain Tissue Segmentation

Brain segmentation (or classification) is the assignment of voxels to one or more class. The classes can either be based on tissue type, or anatomical region. Tissue types commonly used in brain tissue segmentation include white matter (WM), grey matter (GM) and CSF. In some methods a fourth tissue type is included, either for unknown tissue or for partial volume.

Tissue segmentation is insensitive to biological changes in tissue; instead it is aimed at grouping similar tissues, and estimating volumetric information. Segmentation methods find similarities in the MR data and classify voxels according to this. Diffuse alterations in tissue properties, from *e.g.* disease, are generally not detected by segmentation methods. Instead, global and regional volume changes are recognised, such as ventricle enlargement or atrophy.

Brain tissue segmentation has been suggested for analysing several neuro-degenerative diseases such as multiple sclerosis (105–107), Alzheimer’s disease (108), and other forms of dementia (109–111), as well as for assessing normal ageing (110, 112), quantitative patient follow-up (108), and for monitoring the effects of therapy and rehabilitation (113).

There are two general approaches to brain tissue segmentation, either each voxel is assigned to one specific tissue class (114–119), or each voxel is assigned a volume fraction of more than one tissue class (120–123). Allowing more than one tissue class in each voxel is sometimes referred to as *fuzzy segmentation*, and the resulting voxels with tissue fractions are termed *mixels*. This type of segmentation has advantages since voxels with more than one tissue are common in the brain, even at high-resolution imaging, especially close to tissue borders and around the cortex where in many cases GM and CSF or WM and GM are mixed in voxels. Furthermore, calculating tissue fractions in the voxels reduces the need for high-resolution imaging which may allow larger voxels with higher signal-to-noise ratio (SNR).

Most brain tissue segmentation methods are based on conventional contrast-weighted MRI (115, 116, 118–122). Although these images provide high anatomical detail, segmentation is complicated by the inhomogeneities and the arbitrary grey-scaling of the contrast images. As discussed in Section 1.3.4, the MR signal is dependent on the MR pulse sequence, MR scanner settings and hardware. The complexity of the acquired signal in the contrast images complicate brain tissue segmentation, and generally the contrast differences in the images are used, instead of absolute pixel intensity. Different methods to normalise the images or filters (118, 122) may be necessary to compensate for variations in the signal across the imaged volume.

One way to increase the specificity of brain tissue segmentation is to use multi-channel MR data (114, 117, 118, 120, 121), e.g. T2-weighted and PD-weighted images. These different images form dimensions in n -dimensional feature space, where tissue can be segmented based on several different contrast differences.

Conversely to conventional contrast images, qMRI, in principle, remove the scanner dependency and allows brain tissue segmentation based on physiological tissue properties. QMRI also allows segmentation in multi-feature space based on the different qMRI properties of the tissue.

1.6.1 | Brain Tissue Volumes

The determination of brain tissue volumes is complicated for several reasons. The delineation between WM and GM is not clear, and partial volume is common. Furthermore, the qMRI properties of WM and GM vary across the brain, depending on local differences such as myelination. For example, the parietal WM appears brighter than the frontal WM. For these reasons the method for separation of WM and GM is still unreliable and a gold standard method does not exist (61).

The delineation between brain WM/GM and CSF is less complex, since CSF is a liquid with very distinct qMRI properties and higher uniformity across the brain. Furthermore, CSF is contained mostly in larger ducts, inside the brain, and partial volume is therefore limited (61). For this reason it may be beneficial to segment brain tissue and CSF without separating WM and GM.

Another complication is the relative brain sizes of different subjects. Although it is known that brain volumes decrease with ageing, and in disease it is difficult to establish a standard to compare subjects, since the variation in brain volumes between individuals is high. One way to counter this is to normalise the brain volumes relative to the total skull size. This estimates tissue fractions, rather than absolute volumes, such as WM fraction (WMF), GMF and CSFF. In order to overcome the problem with WM/GM separation it is possible to calculate the *brain parenchymal fraction* (BPF) which is the relation of WM+GM to total skull size (124, 125). When calculating brain tissue fractions, however, the result is not only dependent on the brain tissue segmentation, but also on the *skull stripping* algorithm used (126, 127).

When using brain tissue fractions, the fractional volumes cannot be interpreted as absolute volumes. Since all tissue fraction volumes include the volumes of the other tissues in the calculations they are not independent variables. For example, the calculation of WMF includes the volumes of WM, GM and CSF:

$$WMF = \frac{WM}{WM + GM + CSF}. \quad (1.32)$$

This creates arithmetically-induced correlations between the different tissue fractions. For example, if subject A has higher GMF than subject B, it is probable that subject A also has a lower WMF, inducing a negative correlation between GMF and WMF that has no biological basis.

Figure 1.11: Illustration of brain tissue clusters in the R1-R2-PD space with projections on the R1-PD plane.

1.6.2 | R1-R2-PD Space

Healthy brain tissue exhibits narrow ranges of T1, T2 and PD values (117,128–130), whereas pathological tissue exhibits significantly different tissue characteristics (131). The observed *in vivo* qMRI tissue properties can be used in a multi-parametric space, where each tissue property represents one dimension. Instead of directly using the relaxation times to construct such a space, the use of relaxation rates, R1 and R2, allows greater separation of tissue components (117). The observed R1, R2 and PD values can then be used as coordinates in a 3D feature space, the R1-R2-PD space (117, 130). Acquisition noise and anatomical variation causes random variations in the measured R1, R2, and PD values which results in tissue clusters with a mean position and finite distribution. This parametric space can readily be used for brain tissue segmentation, *e.g.* using clustering or threshold methods. Figure 1.11 shows an illustration of brain tissue clusters in the R1-R2-PD space.

1.6.3 | Effects of Field Strength

The MR relaxation rates of tissue depend on the main magnetic field strength, and this has consequences for brain tissue segmentation (132–135). The R1 and R2 parameters generally decrease when the main magnetic field strength is increased depending on many factors, such as iron content and water mobility.

Because of this, longitudinal studies and quantitative patient follow-ups can be seriously limited by system changes from lower to higher field strengths, and for this reason, combining data acquired at different field strengths has not been endorsed.

1.7 | MRI in MS

MRI is a sensitive technique for assessing WM lesions *in vivo*, and T2-weighted imaging is commonly used to monitor and diagnose MS (136). In the latest revision of the McDonald criteria for Multiple Sclerosis (20) the use of MRI has been emphasised, and in some circumstances dissemination in space and time can be established from a single MRI scan. The McDonald criteria describe MRI findings for dissemination in time and space, including gadolinium-enhancing lesions and T2-weighted lesions in regions typical of MS.

There is low correlation between focal WM lesions, detected with T2-weighted imaging, and clinical disability, a phenomenon that has been known for many years and is referred to as the *clinikoradiologic paradox* (137). Some lesions, when viewed on T1-weighted images, appear hypo-intense compared to surrounding WM, and the inclusion of T1-weighted imaging may increase the correlation with clinical disability, but only to a limited degree (138, 139). Factors believed to explain this discrepancy include cortical reorganisation (140), where other parts of the brain may compensate for focal injury; brain plasticity (141, 142), where signal transmissions may use alternative pathways; and the consideration of strategic lesion location (143, 144). Subsequently, other pathological changes have been of interest in recent years, including lesions in cortical GM (145, 146), changes in deep GM (147, 148) and changes in *normal appearing white matter* (NAWM) (5, 149–153). Even so, the mechanisms leading to permanent disability in MS need further exploration.

1.7.1 | Diffusely Abnormal White Matter

In addition to NAWM and WM lesions there are diffuse signal intensity changes that are seen on conventional T2-weighted images, but these changes are unaccounted for in the radiological criteria for MS. These regions, which have fuzzy borders and a signal intensity that is intermediate between NAWM and focal WM lesions, are referred to as *diffusely abnormal white matter*, or *dirty-appearing white matter* (DAWM) (154–158). DAWM is believed to be either a separate pathological entity (155, 157, 158) or caused by Wallerian degeneration (156). Histopathological examinations have shown that DAWM has reduced axonal density as well as reduced myelin, but to a lesser extent than focal lesions (149, 156, 157, 159).

Regions of DAWM are difficult to characterise in conventional T2-weighted imaging because of their diffuse nature, and NAWM is indistinguishable from WM in healthy subjects. Recent studies, however, suggest that these changes may be important markers of disease progression (157, 158). Technological developments have therefore been suggested to characterise and quantify DAWM, as well as to detect changes in NAWM. These techniques include *diffusion tensor imaging* (DTI) (157, 158, 160), *quantitative magnetisation transfer imaging* (qMTI) (84, 155, 158), *quantitative MR spectroscopy* (qMRS) (161, 162), and qMRI (84, 157, 158).

1.7.2 | MRI Negative MS

In a minority of MS patients very few focal WM lesions are detected in the brain using conventional MRI, even after a disease period. These patients constitute a subgroup of MS (162–164) that is of interest for investigating changes in NAWM and DAWM without WM lesions, and with probably less Wallerian degeneration. In this group of MS patients the clinical investigation must be extraordinarily thorough before the diagnose of MS can be established, especially concerning the radiological investigation (163).

1.7.3 | QMRI in MS

In MS pathology several separate processes lead to increased T1 and T2 (decreased R1 and R2) and increased PD (83, 165, 166). There are three main pathological processes in MS that affect qMRI properties; **(1)** increased water contents and changes in the relative sizes of the water compartments, **(2)** demyelination and decreased myelin density, and **(3)** axonal loss and decreased axonal density. Evaluations of qMRI properties of tissue pathology are complicated by the fact that pathological processes often occur concurrently. A further complicating factor may

be the accumulation and presence of iron as a result of the MS disease, influencing the T1 and T2 times (99–104).

In acute WM lesions T1, T2 and PD are all substantially increased (73, 84, 167–169) which, during the following months, decreases towards slightly elevated T1, T2 and PD seen in chronic lesions (84, 170). The main pathological process in the initial increase is attributed to inflammatory oedema, causing increases in both T1 and T2, as well as PD. Then, as the oedema resolves the increase due to excess water is reduced and other pathological processes affecting T1 and T2 become apparent.

In particular the initial increase in T1 has been attributed to the increased water content in the free water pool, with long T1 relaxation (71, 73). After the initial oedema resolves, lesions which remain elevated in T1 have shown lower levels of N-acetyl-aspartate (NAA), which is considered a marker of neuronal integrity, and increased concentration of cholin, which is considered a marker of cell membrane turnover (138, 171–175). These correlations suggest that a persisting elevation of T1 may be related to axonal damage in the lesion (138). In histopathological studies T1 in chronic lesions has been shown to correlate with axonal density (176, 177), but not with myelin density (177). This suggests that T1 in chronic lesions may be a marker of axonal integrity. Furthermore, assessments of T1-weighted chronic lesions have been shown to correlate with disease progression (178, 179).

The initial increase in T2 of the lesion has also been attributed to increased water content due to the oedema (84, 168). Over time, T2 decreases to that of chronic lesions (84, 139, 170), similar to the behaviour of T1. One study detected mono-exponential relaxation in acute WM lesions, which became bi-exponential during remission, and finally returned to mono-exponential as the lesion turned into a chronic lesion (168). This suggests that the free water pool is the main source of T2 relaxation during the initial increase, whereas during the remission the water trapped between the myelin sheets remains for some time, resulting in bi-exponential relaxation. When the lesion turns into a liquid CSF-filled cavity the compartmentalisation of water breaks down and the myelin water disappears.

Since T1 and T2 may describe different characteristics of the lesion development, where T1 is mainly influenced by axonal integrity and T2 is mainly influenced by myelin contents, the PD may provide additional discriminating power, as it can be used to determine the amount of oedema remaining in the lesion. During remyelination of the lesion, T1 and T2 levels decrease towards that of WM, but never fully recover (170) and finally as the lesion turns into a liquid CSF-filled cavity the T1, T2 and PD levels approach that of CSF.

In NAWM both T1 (151, 180–187) and T2 (169, 180, 188, 189) have been shown to increase compared to WM in healthy controls. Studies have shown globally

increased T1 affecting all WM, consistent with axonal loss to some degree independent of focal lesions (149, 172, 190, 191). It has, however, also been suggested that the abnormalities in NAWM is due to increased glia cell numbers, without evidence for axonal loss (192). The increase in T1 was more pronounced in supratentorial WM, compared to infratentorial WM. In other studies the increase of T1 depended on the distance to focal WM lesions (193, 194) and the subtype of MS (SP MS compared to PP MS) (193). The mean T1 of NAWM showed higher correlation to disability than focal WM lesions (184). In a similar manner, T2 has been found to be shifted globally in NAWM (169, 183, 188, 189), where the genu and splenium of the corpus callosum had the highest increase (169, 189). Furthermore, regions in NAWM that developed lesions had pre-lesional elevated T2 (170). Also the increase of T2 depended on MS subtype (189), similar to the increase in T1.

In DAWM, T1 and T2 were prolonged to a greater extent than in NAWM (156, 157). Also, T1 of DAWM differed between PP MS and SP MS (158).

Multi-component measurements of T1 (67) and T2 (70, 71) have been used as surrogate measures of myelin in MS in order to demonstrate demyelination (195–200). This is especially interesting as the bi-exponential behaviour varies during the different phases of the lesion development. When the MWF is calculated, however, caution should be exercised when interpreting the data. Even when there is no myelin loss the process of inflammation, which results in increased free water, produces a decrease in the relative amplitude of the short T2 component, resulting in reduced MWF. One way to circumvent this limitation is to include a separate water pool for the free water and subsequently remove this in the calculation (87).

As has been outlined in this section the dynamic processes in the lesions and heterogeneity lead to a wide range of qMRI properties in focal lesions, in NAWM, and in DAWM. This makes it challenging to separate the different pathological processes underlying the change in qMRI properties. The precise identification of the pathological processes that occur in MS is of considerable importance to increase accuracy of diagnosis and to understand the mechanisms and dynamics leading to tissue degeneration. The ability to differentiate the processes is enhanced when several qMRI properties are combined, *e.g.* PD may be used to separate oedema from axonal loss and demyelination, assessed with T1 and T2. Furthermore, multi-component analysis may discriminate demyelination from inflammation (201, 202).

1.8 | Aims

The aims of this work were to develop and investigate methods for qMRI and in particular the visualisation of qMRI properties in the human brain. The main method of visualisation was *tissue segmentation* (or *classification*). The developed methods were validated in healthy subjects and an application of qMRI was investigated in MS.

The aim of Paper I was to develop a novel qMRI method optimised to accommodate clinical use, especially concerning acquisition time. This method was then applied in Paper II, where the aim was to develop and validate a fully automatic brain tissue segmentation method based on qMRI that was insensitive to partial volume effects. The segmentation method was validated further in Paper III, where the aim was to compare the acquisition and calculation of brain tissue volumes at two field strengths. Finally, in Paper IV one application of the qMRI method in diseased human brain was explored in subjects with MS. In this work, the qMRI properties of brain tissue were compared among two groups of MS patients and one group of healthy controls.

In summary, this work has considered the qMRI acquisition on the MR scanner and two different applications; the first being brain tissue segmentation in healthy subjects, and the second the direct application of qMRI in diseased human brain.

Notes

¹Sometimes the notion *tissue parameters* is used instead of *tissue properties*. This, however, indicates that the 'parameters' can be altered, which is generally not the case for *in vivo* tissue. The term 'parameter' may be preferred when discussing tissue models.

²The terms *synthetic MRI* and *quantitative MRI* are sometimes confused, and are used to describe the same phenomenon. Quantitative MRI is the obtaining of qMRI properties, whereas synthetic MRI is the generation of synthetic contrast images.

³Percentage units (pu) is used instead of percent (%) to make clear that the percentage is not in absolute units, but rather relative to a temperature dependent reference. Which in this case is the PD of water at the same temperature (This notion is further described by Tofts (61), Chapter 4).

⁴T1 relaxation is also referred to as *spin-lattice relaxation* or *longitudinal relaxation*.

⁵T2 relaxation is also referred to as *spin-spin relaxation* or *transverse relaxation*.

2

MATERIALS AND METHODS

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2.1 | Implementation of qMRI for Brain Imaging

A rapid multi-slice, multi-echo and multi-saturation recovery qMRI method was implemented (Paper I). The method was called *quantification of relaxation times and proton density by multi-echo acquisition of a saturation-recovery using spin-echo readout* (QMAP) and was designed to quantify T1, T2 and PD with B_1 -field inhomogeneity correction within a clinically acceptable time.

2.1.1 | Pulse Sequence Design

The qMRI pulse sequence consisted of interleaved saturation pulses and a CPMG acquisition. The pulse sequence was divided into two different phases that were executed on different slices, using slice-selective magnetic field gradients. The first phase was the *saturation phase*, which was applied on slice n ; the second phase was the *acquisition phase*, which was applied on a different slice, m . During the saturation phase a saturation pulse with flip angle Θ was applied, followed by crushing of the transverse magnetisation, leaving only magnetisation in the z direction. In the acquisition phase an excitation pulse with flip angle α was applied, followed by a CPMG multi-echo acquisition. The complete pulse sequence with both saturation and acquisition was repeated several times, with different orders of the slices n and m with respect to each other. In this way several acquisitions with different saturation delay times were performed. The pulse sequence is shown in Figure 2.1.

Figure 2.1: The QMAP pulse sequence, with two phases. First, in the *saturation phase*, a slice-selective saturation pulse was applied to slice n . In the second *acquisition phase* a slice-selective excitation pulse was applied followed by acquisition of multiple echoes using a CPMG acquisition with refocusing pulses, on a different slice m . In this figure the magnetic field gradients are omitted.

By acquiring multiple echoes during the CPMG acquisition several time-points on the T2 relaxation curve were acquired, and by performing several repetitions with different saturation delays, several time-points on the T1 relaxation curve were acquired. Furthermore, by using a saturation pulse it was possible to determine the B_1 -field inhomogeneity, as will be detailed below.

The acquisition could be accelerated by reading several image encoding lines in each acquisition, a technique called *echo planar imaging* (EPI), or by using *sensitivity encoding* (SENSE), a technique in which the complete image data is not acquired.

2.1.2 | Data Fitting

When the qMRI sequence was repeated several times with a different order of the slices n and m , several acquisitions with different saturation delay times, T_D , were acquired. The magnetisation, M_{TD} , at any specific delay time was modelled using the Bloch equations (Eqs. 1.13 and 1.14). By including both the saturation RF pulse and the excitation RF pulse, the magnetisation during the entire repetition time, T_R , was modelled:

$$\begin{cases} M_{TD} &= M_0 - (M_0 - M_{TR} \cos(\Theta)) \exp(-T_D/T1) \\ M_{TR} &= M_0 - (M_0 - M_{TD} \cos(\alpha)) \exp(-(T_R - T_D)/T1), \end{cases} \quad (2.1)$$

where M_0 was the thermal equilibrium magnetisation and M_{TR} was the steady-state magnetisation at the end of a repetition, just before the subsequent saturation pulse, in the next acquisition. Θ was the flip angle of the saturation pulse, and α was the flip angle of the excitation pulse. Combining these equations gave the magnetisation for each saturation delay time, as a function of T_D and T_R :

$$M_{TD} = M_0 \frac{1 - (1 - \cos(\Theta)) \exp(-T_D/T1) - \cos(\Theta) \exp(-T_R/T1)}{1 - \cos(\Theta) \exp(-T_R/T1) \cos(\alpha)}. \quad (2.2)$$

This equation was included in a least-square fit to determine T1 and M_0 , using several different measurements with varying T_D . Furthermore, from the determined T1 relaxation curve it was possible to determine the effective local saturation flip angle, Θ_e , and the influence of the local B_1 -field inhomogeneities. This was done by measuring the ratio between the magnetisation just before the saturation pulse, M_{TR} , and just after, M_{T0} (in a similar manner as in e.q 1.14):

$$\Theta_e = \arccos(M_{T0}/M_{TR}). \quad (2.3)$$

In order to relate Θ_e to the effective excitation flip angle, α_e , a Bloch simulation was performed of the complete pulse sequence, where the pulse envelopes for each of the RF pulses were obtained from the MR scanner software. In the simulation the relative effects of Θ_e and α_e were estimated and this estimation was subsequently used to recalculate T1 using the effective flip angles. The RF pulse profiles of the QMAP sequence, exported from the MR scanner, are detailed in Figure 2.2.

Fig.	RF Pulse	Flip angle* (rad)	Duration (μs)	Max amp. (μT)	Gradient (mT/m)	Dir.
(A)	saturation	$2/3 * \pi$	7418	5.7804	5.2845	x
(B)	excitation	$1/2 * \pi$	2701	6.2712	4.8930	x
(C)	refocusing	π	3002	26.9283	5.8717	y

* Nominal flip angle unaffected by B_1 -field inhomogeneities and other effects.

Figure 2.2: Details of the RF pulses included in the QMAP acquisition, with the associated gradient field strengths. The pulses were (A) the saturation pulse, (B) the excitation pulse, and (C) the refocusing pulse.

The data fitting algorithm was performed in six steps:

1. The images were acquired as *phase* and *modulus* images after a saturation pulse, with a higher flip angle than $\pi/2$. Because of this, the sign of each voxel in the images was restored using the phase images with longest saturation delay time, assuming that these images only had positive magnetisation. This resulted in *real* images.
2. All images with the same echo time, but different saturation delay times were summarised to increase SNR. A least-square mono-exponential fit for T2 was then performed on these *echo images*.
3. The T2 information, retrieved in step 2, was used to extrapolate all images to $T_E = 0$, thus removing the effect of T2 relaxation. Then all images with the same saturation delay time were summarised, generating *dynamic images*. These images were used to perform a least-square mono-exponential fit for T1 and M_0 using Eq. 2.2.

4. The B_1 -field was estimated using Eq. 2.3.
5. T1 and M_0 was recalculated using the effective flip angles, Θ_e and α_e .
6. Finally, PD was calculated by scaling M_0 using several empirically determined scaling factors, analogous to Eq. 1.10.

A summary of the acquired data, and operations to estimate the T1 and T2 relaxation curves are shown in Figure 2.3. The fitting algorithm was implemented in C++ as part of the SyMRI software (Synthetic MR AB, Linköping, Sweden, 2007).

2.2 | Implementation of Brain Tissue Segmentation

A brain tissue segmentation method based on qMRI was implemented (Paper II). The segmentation method required prior knowledge of **(1)** 'pure tissue clusters' in the R1-R2-PD space and **(2)** the characteristics of partial volume voxels in this space. The pure tissue cluster positions and variances were measured using region of interest (ROI) measurements in healthy subjects at both 1.5 T (Papers I and III) and 3.0 T (Paper III), which is described in Section 2.3 on data acquisition. The characteristics of partial volume voxels were assessed using simulations where *synthetic tissue* was created with mixtures of two tissue types. The simulations were performed using a Bloch simulator, with which the complete qMRI acquisition was simulated.

2.2.1 | Bloch Simulator

A numerical Bloch simulator was implemented to simulate the signal from one single voxel, with specified R1 and R2 properties, during the qMRI acquisition. The simulated voxel contained equidistantly aligned spin isochromates in the direction of the external magnetic field, causing each spin isochromate to experience slightly different effects of RF pulses and magnetic field gradients.

The spin isochromates were implemented as column vectors containing the magnetisation in x, y and z directions separately, according to $\mathbf{M} = [M_x, M_y, M_z]^\top$. The initial equilibrium state magnetisation for all isochromates was set to $\mathbf{M} = [0, 0, 1]^\top$. The T1 and T2 relaxation processes were simulated in time-steps of $\Delta t = 1$ ns according to:

Figure 2.3: Summary of all the acquired data for one slice of the QMAP sequence. Different echo times, T_E , in combination with different saturation delay times, T_D , resulted in a matrix of images. The different images at different saturation delay times were combined to form the *echo images*, and the images at different echo times were combined to form the *dynamic images*. These were then used to estimate T2 and T1 relaxation respectively.

$$\begin{aligned} \mathbf{M}_{n+1} = & \begin{bmatrix} \exp(-R2\Delta t) & 0 & 0 \\ 0 & \exp(-R2\Delta t) & 0 \\ 0 & 0 & \exp(-R1\Delta t) \end{bmatrix} * \mathbf{M}_n + \\ & + \begin{bmatrix} 0 \\ 0 \\ 1 - \exp(-R1\Delta t) \end{bmatrix}. \end{aligned} \quad (2.4)$$

During the application of RF pulses and magnetic field gradients the evolution of the magnetisation was simulated in time-steps of $\Delta t = 1 \mu s$ using rotation matrices:

$$\mathbf{M}_{n+1} = \mathbf{R}_{RF}(t) * \mathbf{R}_{GR}(d) * \mathbf{M}_n, \quad (2.5)$$

where $\mathbf{R}_{RF}$ described the rotation around the x- or y-axis caused by the RF pulse, and $\mathbf{R}_{GR}$ described the rotation around the z-axis caused by the magnetic gradient field strength. The rotation angle, α , of $\mathbf{R}_{RF}$ depended on the amplitude of the RF pulse, $A_{pulse}(t)$, at each time-point, and the rotation angle, β , of $\mathbf{R}_{GR}$ depended on the gradient field strength, $S_{gradient}(d)$, at each position:

$$\begin{aligned} \alpha &= A_{pulse}(t) * 2\pi * \gamma \Delta t, \\ \beta &= S_{gradient}(d) * 2\pi * \gamma \Delta t. \end{aligned} \quad (2.6)$$

The RF pulses and gradients (detailed in Figure 2.2) as well as the timings for the qMRI sequence were implemented as a script for the Bloch simulation. For each specific combination of saturation delay time, T_{Di} , and echo time, T_{Ej} , the vector sum of all isochromates was recorded, forming a magnetisation state matrix, $\mathbf{E}$:

$$\mathbf{E} = \begin{bmatrix} \mathbf{M}_{TD1,TE1} & \mathbf{M}_{TD1,TE2} & \cdots & \mathbf{M}_{TD1,TEj} \\ \mathbf{M}_{TD2,TE1} & \mathbf{M}_{TD2,TE2} & \cdots & \mathbf{M}_{TD2,TEj} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{M}_{TDi,TE1} & \mathbf{M}_{TDi,TE2} & \cdots & \mathbf{M}_{TDi,TEj} \end{bmatrix}. \quad (2.7)$$

This matrix was formatted similarly to *in vivo* data (see Figure 2.3) and the fitting procedure, described in Section 2.1.2, was applied to calculate T1 and T2 values. R1 and R2 were calculated by inverting T1 and T2 ($R1 = 1/T1$ and $R2 = 1/T2$).

All simulations were performed in Matlab R2008a (MathWorks, 2008).

2.2.2 | Partial Volume Simulations

The Bloch simulator was used to estimate the observed R1, R2 and PD values for synthetic partial volume voxels with two different tissue types in the combinations WM+GM, WM+CSF and GM+CSF, for 1.5 T (Papers II and III) and 3.0 T (Paper III). The tissues were combined with different volume fractions to cover a range of 0 to 100% in incremental steps of 0.1%, so that the sum of the two tissue fractions always resulted in 100%.

By assuming that each tissue generated a signal independently of the others, and that there was no interaction between the tissues, then the synthetic partial volume voxel signal was approximated as the sum of the signals from the two tissues independently. The Bloch simulator with the qMRI sequence was therefore executed once for each pure tissue in order to simulate the resulting magnetisation states for each tissue separately, $\mathbf{E}_{WM}$, $\mathbf{E}_{GM}$ and $\mathbf{E}_{CSF}$. For each specific partial volume mixture of two tissues the total voxel signal from the combinations was estimated by weighting $\mathbf{E}_{tissue1}$ and $\mathbf{E}_{tissue2}$ with the respective PD values and volume fractions, α :

$$\mathbf{E}_{combined} = \alpha * PD_{tissue1} * \mathbf{E}_{tissue1} + (1 - \alpha) * PD_{tissue2} * \mathbf{E}_{tissue2}. \quad (2.8)$$

The presence of noise was simulated by including the synthetic partial volume voxels in Monte Carlo simulations where noise was simulated by adding a matrix, $\mathbf{N}$, containing random white noise in the interval $[-1, 1]$ scaled with an SNR factor:

$$\mathbf{E}_{combined,noise} = \mathbf{E}_{combined} + \mathbf{N}/SNR. \quad (2.9)$$

This procedure was performed for noise levels of $SNR = 20$ (Papers II and III), $SNR = 10$ and $SNR = 40$ (Paper II). Each Monte Carlo simulation was repeated 10,000 times.

In order to retrieve the R1, R2 and PD values of the synthetic tissue mixture, the QMAP fitting procedure was applied to $\mathbf{E}_{combined,noise}$. The different combinations of two synthetic tissues are illustrated in Figure 2.4.

As tissue fractions were altered in steps of 0.1% in three partial volume transitions this resulted in 3,000 multi-variate tissue clusters in the R1-R2-PD space. These simulations were performed three times in total, once for 1.5 T (Paper II) and for both 1.5 T and 3.0 T separately (Paper III). The multi-variate tissue clusters

Figure 2.4: Two simulated tissues are combined to form several synthetic partial volume voxels in a partial volume transition, covering 100% down to 0% of each tissue.

were tested for normal distribution using skewness and kurtosis tests. Skewness is a measure of the asymmetry of a distribution, and kurtosis is a measure of the concentration of data in the distribution. For normal distribution, skewness and kurtosis are zero, but any value below two is a strong indication that the data may be normally distributed (203). For normally distributed data, covariance matrices were calculated and these were used as prior knowledge to describe the characteristics of partial volume voxels in the segmentation procedure.

2.2.3 | Brain Tissue Segmentation

Once the prior knowledge about pure tissue clusters and the partial volume transitions in the R1-R2-PD space had been established, lookup grids in R1-R2-PD space were constructed. The lookup grids translated *in vivo* qMRI data into tissue fractions of WM, GM and CSF.

The lookup grids were constructed using the *95% prediction ellipses*, which were calculated from the covariance matrices of simulated partial volume voxels. The 95% prediction ellipses were defined as tilted ellipses in the R1-R2-PD space encompassing 95% of the simulated data, such that measured qMRI values were expected to fall inside the prediction for 95% of measurements. The lookup grids were constructed such that voxels within the 95% prediction ellipses of the pure tissue clusters were mapped to 100% of that tissue. All the remaining clusters, with different simulated tissue fractions, were overlaid on the lookup grids, and voxels within any of these clusters were mapped to the closest simulated tissue mixture. The remaining voxels outside the pure tissue and partial volume clusters were defined as unknown tissue and termed Non-(WM/GM/CSF) (NON).

Where different combinations of qMRI properties led to identical coordinates in R1-R2-PD space these ambiguous voxels were determined using the neighbouring voxels. The two most common tissue types among the surrounding 48 voxels (7x7 kernel, except the middle voxel) were used to determine which partial volume transition was most probable.

The total volumes of WM, GM, CSF and NON in the brain were calculated by summation of all volume fractions from each voxel, multiplied by the voxel volume. The brain parenchymal volume (BPV) was calculated as the sum of WM, GM and NON. The intracranial volume (ICV) was calculated as the sum of BPV and CSF, and finally the brain parenchymal fraction (BPF) was calculated as the ratio of BPV to ICV (BPV/ICV). The tissue segmentation was implemented in C++ as part of SyMRI.

Skull-stripping, to remove tissue outside of the brain, was automatically performed by an existing function in SyMRI and was not part of this work.

2.3 | Subjects and Data

This work included subjects in four different cohorts, where the first three cohorts (i, QMAP, ii, QMR-Segment, and iii, QMR-3T) were healthy subjects included for validation of qMRI and brain tissue segmentation (Papers I-III). The fourth cohort (iv, QMR-MS) included patients diagnosed with MS as well as age- and sex-matched healthy controls. These were included to investigate qMRI in MS (Paper IV) and to exemplify the application of qMRI and segmentation on MS patients (Papers I and II). In total, 50 healthy controls and 35 MS patients were scanned with qMRI in a total of 225 acquisitions. Of these, 185 acquisitions were performed on a Philips Achieva 1.5 T scanner (Philips Medical Systems, Best, The Netherlands) and 40 were performed on a Philips Ingenia 3.0 T scanner. The different subject

cohorts and data acquisition protocols are summarised in Table 2.1. Furthermore, phantom measurements were performed on two phantoms on four MR scanners, for validation of qMRI.

Quantitative tissue maps of T1 (or R1), T2 (or R2) and PD were calculated for all cohorts, using the fitting algorithm described in Section 2.1.2. Region of Interest (ROI) analysis was performed in cohort **i** and **iii** in order to determine the normal values for brain tissues, and these were then applied as prior knowledge in the brain tissue segmentation. ROI analysis was also done on cohort **iv** to investigate qMRI tissue characteristics in MS patients. For all acquisitions in cohort **ii** and **iii** brain tissue maps and total volumes of WM, GM, CSF, and NON were calculated. Furthermore, the ICV, BPV and BPF were calculated for these subjects.

All studies and MR acquisitions were approved by the regional ethical review board in Linköping⁶.

2.3.1 | Cohort i (QMAP)

In this cohort, *in vivo* measurements were performed with a Philips Achieva 1.5 T scanner on 10 healthy subjects (eight male, two female, mean age 29). The qMRI acquisition settings were: repetition time (T_R) = 2600 ms with five echoes in intervals of 20 ms. Voxel size was $4 \times 0.8 \times 0.8$ mm³ in 20 slices, and no slice gap was applied. Scan time was 9 min per acquisition. In addition, in one subject, standard measurements were performed with inversion recovery (IR) to determine T1 and multi-echo spin echo to determine T2. The acquisition settings for the IR sequence were $T_R = 10$ s, inversion times = 100/400/700/1500/5000 ms. The acquisition settings for the spin echo sequence were $T_R = 3000$ ms with 15 echoes in intervals of 15 ms.

2.3.2 | Cohort ii (QMR-Segment)

In vivo measurements were performed with a Philips Achieva 1.5 T scanner on 10 healthy subjects (five male, five female, aged 27 (22-39) (median (range)) years). Six axial acquisitions were performed where the in-plane resolution varied among 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mm, leaving all other parameters constant. Also, one coronal and one sagittal acquisition were performed at 2.0-mm resolution. Changing the voxel size or the geometry introduced variations in the partial volume effects. The qMRI acquisition settings were: $T_R = 2600$ ms with five echoes in intervals of 20 ms. The number of slices varied in the range of 26-29 in order to cover the whole brain, and slice thickness was 5 mm with no slice gap. The scan

Cohort	Subjects Sex (M/F), age (median (range))	Field	Geometry	QMRI Acquisition Settings Slices (thickness + gap), resolution, FOV T_R , T_E Analysis SW	Paper
i, QMAP	10 Healthy subjects 8/2	1.5 T	Ax	20 (4+0 mm), 0.8 mm, 215x180 mm ² T_R = 2600 ms, T_E = 20*5 ms <i>in-house</i> <i>Analysis SW</i>	I
ii, QMR-Segment	10 Healthy subjects 5/5, 27 (22-39)	1.5 T	Ax Sag, Cor	26-29 (5+0 mm), 1.0-2.0 mm, 215x180 mm ² T_R = 2600 ms, T_E = 20*5 ms <i>SyMRI 5.0.8 CE</i> 26-29 (5+0 mm), 2.0 mm, 215x180 mm ² T_R = 2600 ms, T_E = 20*5 ms <i>SyMRI 5.0.8 CE</i>	II
iii, QMR-3T	10 Healthy subjects 4/6, 25 (21-29)	1.5 T 3.0 T	Ax Ax	40 (3+0.5 mm), 1.3 mm, 228x181 mm ² T_R = 5000 ms, T_E = 16*6 ms <i>SyMRI 0.9.11</i> 40 (3+0.5 mm), 1.3 mm, 228x181 mm ² T_R = 5000 ms, T_E = 16*6 ms <i>SyMRI 0.9.11</i>	III
iv, QMR-MS	20 Healthy controls 5/15, 48 (27-72) 35 MS patients 7/28, 48 (20-69)	1.5 T 1.5 T	Ax Ax	30 (4+0 mm), 0.8 mm, 215x180 mm ² T_R = 3000 ms, T_E = 14*5 ms <i>SyMRI 0.9.3</i> 30 (4+0 mm), 0.8 mm, 215x180 mm ² T_R = 3000 ms, T_E = 14*5 ms <i>SyMRI 0.9.3</i>	I, II, IV

Table 2.1: Subjects and Data.

time was 7 min for the 1.0 mm resolution, decreasing to 4 min for the 2.0 mm resolution.

2.3.3 | Cohort iii (QMR-3T)

In this cohort *in vivo* measurements were performed with a Philips Achieva 1.5 T scanner and a Philips Ingenia 3.0 T scanner on 10 healthy subjects (four male, six female, aged 25 (21-29) years). Health status was assessed using a questionnaire where brain disorders were ruled out. The qMRI acquisition settings were: $T_R = 5000$ ms with six echoes in intervals of 16 ms. The voxel size was $3 \times 1.3 \times 1.3$ mm³ in 40 slices and a slice gap of 0.5 mm was applied in order to reach full brain coverage. The sequence was identical on 1.5 T and 3.0 T and the scan time was 6 min. In addition, conventional T2-weighted images were acquired. For each of the two scanners all subjects were scanned in two separate sessions, and the subject was removed from the scanner room between sessions. Furthermore, two acquisitions were performed in each session. This resulted in a total of eight acquisitions for each subject (two scanners * two sessions * two acquisitions).

2.3.4 | Cohort iv (QMR-MS)

A total of 35 clinically definite MS (CDMS) patients and 20 healthy subjects were included in this cohort. Of these, 15 MS patients had shown two or fewer T2-hyperintense WM lesions on a previous clinical MR examination. These were included in the low-lesion MS group, *MRIneg*. Some of these patients could have developed lesions before the present examination, but were included in the *MRIneg* group nevertheless. The remaining 20 MS patients fulfilled the 'Barkhof-Tintoré criteria' as defined in (18), and these were included in the MS group with typical WM lesions, *MRIPos*. All patients, in both MS groups, fulfilled the Poser criterion; with at least two relapses, separated in space and time (204). All patients had been confirmed positive for oligoclonal bands in the CSF. Table 2.2 lists the clinical parameters of the subjects. The qMRI acquisition settings were: $T_R = 3000$ ms with five echoes in intervals of 14 ms. The voxel size was $4 \times 0.8 \times 0.8$ mm³ in 30 slices and no gap was applied.

In addition, the qMRI acquisition was part of a more extensive investigation including clinical imaging sequences, as well as measurements of WM metabolite concentrations assessed using *quantitative MR spectroscopy* (qMRS) (161, 162). The qMRS acquisition was performed using the *point-resolved spectroscopy sequence* (PRESS) (205), with $T_R = 3000$ ms, $T_E = 30$ ms and 128 transients were averaged. Two MRS volumes of interest (VOIs) were placed bi-laterally in NAWM,

Parameter	HC	MRIp _{os}	MRIn _{eg}
Number of subjects	20	20	15
Age, (median (range))	48 (27-72)	46 (20-66)	57 (32-69)
Sex (M/F)	5/15	6/14	1/14
MS type (RR/SP/PP)	N/A	12/7/1	10/3/2
EDSS (median (range))	N/A	3.25 (1.0-8.5)	2.50 (0.0-6.5)
MSSS (median (range))	N/A	3.74 (0.45-9.57)	3.65 (0.05-9.38)
Disease duration years (median (range))	N/A	13 (2-35)	16 (2-44)
Number of MS lesions (median (range))	N/A	16 (3-30)	1 (0-20) † † †
BPF (mean±SD)	0.881±0.035	0.806±0.046 ***	0.868±0.024

Table 2.2: Clinical parameters of MS patients and healthy controls in cohort iv. The MS patients were divided in two groups; the *MRIneg* showed two or fewer T2-hyper-intense WM lesions, on a previous clinical MR examination, whereas the *MRIp_{os}* fulfilled the 'Barkhof-Tintoré criteria' as defined in (18). *** $p < 0.001$ compared to control group (2-sample t-test), † † † $p < 0.001$ compared to *MRIp_{os}* (Mann-Whitney U test).

and absolute aqueous fraction concentrations of creatine (tCr-Aq), myo-Inositol (mIns-Aq), glutamate and glutamine (tGlx-Aq), N-acetyl aspartate (tNA-Aq), and cholin (tCho-Aq) were calculated using the procedure described in (161).

2.3.5 | Phantom Acquisitions

Phantom measurements were performed on two phantoms in four MR scanners. The scanners were a Philips Achieva 1.5 T, a Philips Ingenia 3.0 T, a GE 450W 1.5 T (GE Healthcare, Waukesha, USA) and a GE 750 3.0 T. In all MR scanners the QMAP measurements were repeated five times for each phantom.

Two phantoms were prepared with test-tubes, the first phantom for simultaneous measurements of T1 and T2 within the clinical range, and the second for PD measurements. Test-tubes were placed in random order in a water-bath. The T1-T2 phantom consisted of 24 test-tubes where water was mixed with solutions of Agarose (Sigma-Aldrich, St. Louis, USA) and Magnevist (Bayer-Shering, Leverkusen, Germany), in order to lower water T1 and T2 respectively. The PD phantom consisted of 33 test-tubes where water was mixed with deuterium oxide (D₂O)⁷ (Cambridge Isotope Laboratories Inc, Tewksbury, USA). Deuterium has no MR visible protons and was therefore used to saturate the MR signal. Each test-tube had a volume of 40 mL, and micro-pipettes were used to dilute the solutions. Concentrations were prepared in fractions of 0%-100% in steps of 10%, and each fraction was prepared in three test-tubes. Figure 2.5 shows annotated synthetic T2-weighted images of the central slices of each of the two phantoms.

The T1-T2 phantom was scanned using IR and a multi-echo spin echo sequence, at Philips 1.5 T and Philips 3.0 T, to determine reference values for both field

Figure 2.5: Synthetic T2-weighted images from (A) the T1-T2 phantom and (B) the PD phantom (at 1.5 T).

strengths. QMAP scans were subsequently performed on all four MR scanners in five repetitions. Table 2.3 lists the MR scanner settings used for each MR pulse sequence. QMAP data fitting for Philips data was performed using SyMRI 0.9.10, and for GE data it was done using SyMRI 0.14.9 (which supported the GE DICOM format). Multi-echo spin echo data was fitted using SyMRI 0.9.10 and IR data was fitted using an in-house built IDL application.

2.3.6 | ROI for Quantitative Imaging Analysis

In order to measure brain qMRI values for different tissue types, standardised ROIs were positioned manually in the subjects of cohort **i**, **iii** and **iv**. In cohort **i** the ROIs were placed by a technician, in cohort **iii** by a neuro-radiologist, and finally in cohort **iv** by a radiologist. In all cohorts the ROI size was 3x3 voxels (corresponding to 2.4 mm² in cohort **i** and **iv** and 3.9 mm² in cohort **iii**). ROIs were placed on synthetic T2-weighted images, with conventional T2-weighted images as visual references where available. The synthetic T2-weighted images were generated from the same data as the qMRI maps and were therefore perfectly registered, removing the need for additional data registration. The average $\pm$ SD for T1 (or R1), T2 (or R2) and PD of all measured tissue types was calculated from the voxels in the ROIs.

MR Scanner	Sequence	Acquisition Settings
		Slices (thickness + gap), resolution T_R , T_E
Philips 1.5 T	QMAP	32 (4+0 mm), 1.1 mm $T_R = 4500$ ms, $T_E = 6*15$ ms
	IR	9 (5+0 mm), 1.0 mm $T_R = 8$ s, $T_I = 100/200/400/800/1600/3200/5000$ ms
	ME	9 (5+0 mm), 0.8 mm $T_R = 3000$ ms, $T_E = 20*10$ ms
Philips 3.0 T	QMAP	32 (4+0 mm), 1.0 mm $T_R = 4500$ ms, $T_E = 5*17.5$ ms
	IR	9 (5+0 mm), 1.0 mm $T_R = 10$ s, $T_I = 100/200/500/1000/2000/5000$ ms
	ME	9 (4+0 mm), 1.0 mm $T_R = 5000$ ms, $T_E = 20*10$ ms
GE 1.5 T	QMAP	30 (4+0 mm), 0.8 mm $T_R = 4000$ ms, $T_E = 31/94$ ms
GE 3.0 T	QMAP	30 (4+0 mm), 0.5 mm $T_R = 4000$ ms, $T_E = 32/97$ ms

Table 2.3: Acquisition settings for phantom measurements.

2.3.6.1 | ROIs in Cohort i

ROIs were placed in the structures of WM, GM and CSF. WM was measured in the frontal WM, in the occipital WM, and in the genu and splenium of the corpus callosum. GM was measured both in the cortex, and in the deep GM structures of the thalamus, putamen, and in the head of the caudate nucleus. CSF was measured in the ventricles.

2.3.6.2 | ROIs in Cohort iii

ROIs were placed on 1.5 T and 3.0 T data in the same session to minimise systematic differences in positioning between the field strengths. ROIs were placed in both data-sets from the first session of each subject in each scanner, resulting in four data-sets for each subject (two for each field strength). ROIs were placed on the structures of WM, GM and CSF. WM was measured peri-trigonally (left and right), frontally (left and right), parietally in the centrum semiovale (left and right) and in the genu and splenium of the corpus callosum. GM was measured para-sagittally in the frontal lobe, anterior to the genu of the corpus callosum where the cortex is thick (left and right). CSF was measured in the frontal horns (left and right). For WM, each tissue region was measured separately, as was an average of all eight

ROIs. Sample ROI placements for both field strengths, in one subject, are shown in Figure 2.6.

Figure 2.6: Sample ROI placements in a healthy 25-year-old male subject. Only the ROIs in the current slice are shown. The ROIs for the 1.5 T image are (A) WM in the splenium of the corpus callosum, (B) GM in the frontal lobe, and (C) CSF in the frontal horns. The ROIs for the 3.0 T image are (A) WM in the frontal lobe, (B) WM in the genu of the corpus callosum, (C) WM in the splenium of the corpus callosum, (D) GM in the frontal lobe, and (E) CSF in the frontal horns.

2.3.6.3 | ROIs in Cohort iv

In the healthy controls, two ROIs were placed in the frontal and in parietal WM, in the centrum semiovale (left and right). Similarly, in the MS patients two ROIs were placed in the NAWM (left and right), frontally and parietally in the centrum semiovale, making sure that DAWM and WM lesions were excluded. Two ROIs were also placed in areas of DAWM, and two additional ROIs were placed in focal WM lesions, whenever present. The radiological DAWM definition was adapted from (157), and it was defined as a diffuse uniform, non-focal area in WM, preferentially periventricular and of increased signal intensity in the T2-weighted image. Compared with the signal intensity of WM lesions, DAWM was observed as subtly, but distinctly increased signal intensity. The DAWM signal intensity decreased towards the border with NAWM, leading to a relatively poorly defined border of DAWM areas, compared to focal WM lesions. Typical ROI placements are shown in Figure 2.7.

2.3.6.4 | ROIs in Phantoms

For each of the phantoms, ROIs were placed in each test-tube in one of the central slices, where every test-tube was clearly visible and surrounded by the water bath. For Philips data, the ROI size was 5x5 voxels (corresponding to 5 mm²), and for GE, which was acquired at higher resolution, the ROI size was 10x10 voxels (corresponding to 8 mm² at 1.5 T and 5 mm² at 3.0 T).

Figure 2.7: Typical ROI placements in MS patients and a healthy control (at 1.5 T); (A) WM in a healthy control, (B) NAWM in MRIneg, (C) DAWM in MRIneg, (D) WM lesion in MRIpos, (E) NAWM in MRIpos, (F) DAWM in MRIpos. Healthy control was a 42-year-old female, MRIneg subject was a 43-year-old female and MRIpos subject was a 66-year-old female.

2.4 | Statistical Analysis

Statistical analyses were performed to validate the QMAP method (Paper I), in order to validate the brain tissue segmentation (Papers II and III), and to investigate qMRI in MS patients (Paper IV). Furthermore, validations were performed to compare QMAP to gold standard qMRI sequences.

2.4.1 | Validation of qMRI

QMRI measurements were compared to gold standard measurements in one subject (Paper I).

For the phantom measurements, the accuracy of QMAP was estimated using root-mean-square (RMS) error, and precision was estimated using inter-measurement standard deviation (SD). For each test-tube in each experiment with the T1-T2 phantom, the mean T1 and T2 values were compared to gold standard measurements, and inter- and intra-measurement variability were calculated. For each test-tube in each experiment with the PD phantom, the mean PD was compared to the known reference value and inter- and intra-measurement variability were calculated.

Statistical Analyses performed in SPSS Statistics 19 (IBM, USA, 2010).

2.4.2 | Validation of Brain Tissue Segmentation

For all acquisitions in cohort **ii** and **iii**, the volumes of WM, GM, CSF, NON, ICV, BPV and BPF were calculated. The within-subject standard deviation, sw , was estimated as the square-root of the mean within-subject variance. The repeatability was calculated as:

$$\text{repeatability} = 1.96 * \sqrt{2} * sw \approx 2.77 * sw, \quad (2.10)$$

as suggested by Bland and Altman (206). Using this definition of repeatability, any two measurements for the same subject are expected to be less than this value for 95% of pairs of observations. Furthermore, the repeatability of each tissue type was related to the complete ICV as repeatability/ICV. Finally, the within-subject coefficient of variation (CV) was calculated. In cohort **ii** these calculations were performed for (1) only the axial acquisitions at different resolution, and (2) only the 2.0-mm acquisitions at different geometries. For cohort **iii** these calculations were performed for the first acquisition in each session for each field strength

separately. In cohort **ii** Kendall's tau-b rank correlation coefficients were calculated to check whether the standard deviations were unrelated to the magnitude of the measurements (Paper II). In cohort **iii** Shapiro-Wilk's test was used to test volume measurements for normal distribution (Paper III).

Statistical Analyses performed in SPSS Statistics 19 (IBM, USA, 2010).

2.4.2.1 | Effects of Field Strength

Analyses were performed to assess both the global and regional effects of field strength on the brain tissue segmentation in cohort **iii** (Paper III).

A general linear model (GLM) analysis was performed to examine the effects of field strength on the total brain tissue volumes. In the analysis; field strength, subject and session were included as fixed effects. Analyses of main effects were performed where significance was found.

Regional differences in brain tissue volume estimation between 1.5 T and 3.0 T were determined using voxel-wise t-tests of the tissue maps. Before calculating the statistics, maps of WM, GM, and CSF were normalised to a standard stereotactic space in Montreal Neurological Institute (MNI) coordinates using the T2-weighted template available in SPM8. Synthetic T2-weighted images, which were smoothed with an 8 mm Gaussian kernel to reduce the individual anatomical details, were used as source images to calculate the transformation matrix. A 12-parameter (translation, rotation, shear, zoom) affine regularisation was used to transform the synthetic T2-weighted images of each subject to the template. The resulting transformation matrices were then applied to the WM, GM, and CSF maps. The resulting maps were re-gridded to $2 \times 2 \times 2 \text{ mm}^3$ voxel size to obtain an isotropic data-set. Voxel-wise differences between maps acquired at 1.5 T and 3.0 T were estimated by one-tailed paired t-tests of each tissue type. The resulting t-maps were initially thresholded at $p=0.001$, uncorrected for multiple comparisons. Based on those initially thresholded maps, ROI analyses were made of regions representing the different divisions of the brain: the frontal, temporal, parietal, occipital, and the limbic lobes; the latter including the cingulate cortex and the hippocampal formation; as well as the cerebellum (anterior and posterior lobes), pons, medulla, midbrain, *i.e.* the upper brain stem, and the sub-lobar regions comprising the thalamus and the basal ganglia. The results are reported as significant if cluster $p < 0.001$, Family Wise Error (FWE) corrected, and cluster size was at least 20 voxels.

Voxel-wise t-tests were performed using SPM8 (Wellcome Department of Imaging Neuroscience, London, UK, 2009).

2.4.3 | Analysis of qMRI MS Data

In cohort **iv** three different sets of statistical tests were performed (Paper IV). First, descriptive statistics were calculated for qMRI measurements of the tissue types WM in healthy controls, NAWM in both groups of MS patients, DAWM in both groups of MS patients, and WM lesions in both groups of MS patients, where available. Comparisons were performed using two separate general linear models (GLM1 and GLM2) where subject and ROI were included as random effects in the analysis. In GLM1, the tissue types NAWM, DAWM, and WM lesions were compared to WM in healthy controls. Analyses were performed for each group of MS patients separately. In GLM2, the same tissue type was compared between the two MS groups (e.g. DAWM in MRIpos compared to DAWM in MRIneg).

Second, correlations were calculated between the qMRI measurements in NAWM and DAWM and a battery of clinical parameters (see Table 2.2). Correlations were also calculated between qMRI measurements and NAWM metabolite concentrations in the same subjects, previously reported in (162). In these subjects a second qMRI measurement was also performed inside the MRS VOIs, and correlations between the qMRI measurements and WM metabolite concentrations were also calculated in the same VOIs. Initially, Pearson correlations were calculated for all variables, and Spearman correlations were subsequently calculated for EDSS and MSSS scores, if significant, to verify the findings.

Third, regression analyses were performed in both MS groups combined to assess associations between qMRI measurements and BPF, EDSS and MSSS. The analyses were reported in Paper IV, except associations between qMRI measurements and EDSS, which are previously unpublished.

Statistical Analyses performed in SPSS Statistics 21 (IBM, USA, 2013).

Notes

⁶The reference number for the approval was **Dnr: M88-07 T93-08**.

⁷99.9% D₂O, $\rho = 1.107$ g/mL at 25 °C.

3

RESULTS

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3.1 | QMRI of the Brain

Absolute quantification of T1, T2, and PD was performed on all subjects in all cohorts, using the QMAP sequence. Representative qMRI maps of one slice in a healthy subject from cohort ii are shown in Figure 3.1 Furthermore, synthetic contrast images were calculated for the same slice, shown in Figure 3.2.

Figure 3.1: Sample qMRI maps from a healthy 25-year-old male subject (at 3.0 T); (A) T1-map, (B) T2-map, (C) PD-map, (D) R1-map, (E) R2-map, (F) reference synthetic T2-weighted image, calculated from the qMRI maps (*Images generated with SyMRI 0.9.12*).

Table 3.1 summarises the pure brain tissue clusters, measured in cohort i, for various anatomies of the brain. These values corresponded well with the literature. The average values of WM varied across the brain and T1 was shorter in the frontal WM than in the occipital WM, while T2 was slightly higher in the central WM. The thalamus showed varying values across the tissue and the values of its centre are reported. Most voxels containing cortical GM were affected by partial volume

Figure 3.2: Sample synthetic MR images compared to conventional images, from a healthy 25-year-old male subject (at 3.0 T); (A,D) T2-weighted images, (B,E) FLAIR images, and (C,F) T1-weighted images. The top row, (A-C) shows the synthetic images, and the bottom row, (D-F) shows the conventional images. The lower imaging resolution of the synthetic images can clearly be seen (*Synthetic images generated with SyMRI 0.9.12*).

effects with CSF or WM, and the absolute GM value was therefore difficult to retrieve. The PD of CSF appeared higher than the expected 100 pu. Even so, the average pure brain tissue values for the healthy subjects in cohort *i* were within a narrow range.

The QMAP sequence results from a single slice of the brain in one of the healthy subjects were compared to the gold standard measurements of T1 and T2. The gold standard measurements of T1 and T2 were also combined to estimate PD, and the results were compared to the PD estimated from the QMAP sequence. Results from these comparisons are reported in Paper I, Figure 5. The mean difference for the measured T1 values, comparing QMAP with IR, was 6.2%. The mean difference for the measured T2 values, comparing QMAP with multi-echo spin echo was 5.2%. The mean difference of the values for PD was 12.1%.

Anatomy	T1 (ms)	T2 (ms)	PD (pu)
Frontal WM	561 ± 12	73 ± 2	66 ± 1
Occipital WM	589 ± 19	78 ± 3	67 ± 4
Genu	556 ± 15	72 ± 4	64 ± 3
Splenium	598 ± 11	83 ± 7	66 ± 3
Cortex	1048 ± 61	94 ± 6	88 ± 4
Thalamus	738 ± 39	76 ± 2	79 ± 2
Putamen	832 ± 25	75 ± 3	88 ± 3
Head of caudate nucleus	917 ± 43	82 ± 2	91 ± 2
CSF	3940 ± 340	1910 ± 520	103 ± 3

Table 3.1: Pure brain tissue clusters, measured in the 10 healthy subjects of cohort i. Average ± SD are reported for T1, T2 and PD of the average from each ROI.

MR Scanner	RMS Error			SD		
	T1 (%)	T2 (%)	PD (%)	T1 (%)	T2 (%)	PD (%)
Philips 1.5 T	4.34	13.55	13.12	0.84	1.39	6.07
Philips 3.0 T	12.71	7.96	15.43	2.55	2.61	5.98
GE 1.5 T	5.66	20.65	19.32	1.68	2.08	3.56
GE 3.0 T	11.13	9.96	46.64	1.08	1.40	4.02

Table 3.2: Root-mean-square (RMS) error and inter-subject SD for phantom measurements at four different MR scanners.

3.1.1 | Phantom Measurements

Phantom measurements were performed in order to compare T1 and T2 values from the QMAP sequence with gold standard measurements and to compare PD values to known reference values. Measurements were performed on four MR scanners at two different field strengths, and repeated five times. RMS error and inter-subject SD for all measurements in all MR scanners are reported in Table 3.2. Bland-Altman plots of T1 and T2 compared to the gold standard, as well as regression plots of PD compared to reference values are shown in Figure 3.3.

3.2 | Brain Tissue Segmentation

Brain Tissue segmentation was performed on all subjects in cohorts ii and iii. Representative results of WM, GM, CSF and NON segmentation *in vivo* performed with different geometries are shown in Figure 3.4. Representative results for brain tissue segmentation at the two field strengths, 1.5 T and 3.0 T, are shown in Figure 3.5.

Figure 3.3: Comparisons of T1 and T2 to gold standard measurements and PD to reference values in four different MR scanners. T1 and T2 results are shown as Bland-Altman plots where the limits of agreement and measurements mean (bias) are shown as dashed lines. PD results are shown as regression plots. Note the different scales on the y-axes in the Bland-Altman plots.

Figure 3.4: Segmentation results from axial (A, D, G, J), coronal (B, E, H, K), and sagittal (C, F, I, L) acquisitions from a healthy 21-year-old female subject (at 1.5 T) in cohort ii. The top row (A-C) shows WM in blue, the second row (D-F) shows GM in green, the third row (G-I) shows CSF in purple, and the last row (J-L) shows NON in yellow overlaid on synthetic T2-weighted images.

Figure 3.5: Segmentation results from one axial slice from a healthy 29-year-old-female subject in cohort **iii** comparing acquisitions at 1.5 T and 3.0 T. The top row **(A-D)** shows the 1.5 T results and the bottom row **(E-H)** shows the 3.0 T results. From left to right; **(A, E)**, synthetic T2-weighted images, **(B, F)** WM in blue, **(C, G)** GM in green, and **(D, H)** CSF in purple. All segmentation maps were overlaid on synthetic T2-weighted images.

3.2.1 | Prior Knowledge

The presented brain tissue segmentation method required prior knowledge of pure brain tissue cluster positions and the characteristics of partial volume voxels in the R1-R2-PD space. Pure tissue clusters for the segmentation with different axial resolutions and geometries were copied from the QMAP validation (Paper II) and measured using ROIs (Paper III). Results from these ROI measurements at both 1.5 T and 3.0 T are reported in Table 3.3. The pure tissue cluster positions were used in the subsequent partial volume simulations and in order to create the partial volume lookup grids.

The partial volume simulations resulted in 3,000 multivariate clusters in the R1-R2-PD space, one for each specific mixture of two tissues in the transitions WM $\leftrightarrow$ GM, WM $\leftrightarrow$ CSF and GM $\leftrightarrow$ CSF. The simulations were performed with three different SNR settings, SNR = 10, SNR = 20 and SNR = 40, at 1.5 T. The procedure was also performed for 1.5 T and 3.0 T separately. In these simulations the SNR was set to 20. The results of the simulations with different SNR settings and field strengths are shown in Figure 3.6. The indicated ellipses enclose 95% of the R1-R2-PD estimates from the Monte Carlo simulations. The clusters are symmetrical in the

	Anatomy	n ROIs	R1 (s ⁻¹)	R2 (s ⁻¹)	PD (pu)
1.5 T	Frontal WM	2	1.67 ± 0.09	13.55 ± 0.63	64.86 ± 2.04
	Peritrigonal WM	2	1.64 ± 0.12	12.66 ± 0.90	62.03 ± 3.07
	Centrum semiovale	2	1.51 ± 0.08	11.80 ± 0.56	68.53 ± 1.75
	Genu	1	1.78 ± 0.14	14.23 ± 0.73	59.88 ± 2.26
	Splenium	1	1.58 ± 0.12	12.46 ± 0.79	65.33 ± 2.92
	Average WM	8	1.62 ± 0.14	12.84 ± 1.09	64.51 ± 3.77
	Frontal cortex	2	0.81 ± 0.08	10.29 ± 0.59	88.06 ± 3.76
	CSF	2	0.24 ± 0.01	0.87 ± 0.55	103.76 ± 3.54
3.0 T	Frontal WM	2	1.39 ± 0.06	14.49 ± 0.57	66.31 ± 1.46
	Peritrigonal WM	2	1.41 ± 0.08	13.57 ± 0.89	63.14 ± 2.90
	Centrum semiovale	2	1.31 ± 0.04	12.77 ± 0.56	68.27 ± 1.51
	Genu	1	1.46 ± 0.07	15.29 ± 0.58	63.72 ± 1.46
	Splenium	1	1.38 ± 0.09	13.11 ± 0.83	65.61 ± 2.37
	Average WM	8	1.38 ± 0.08	13.76 ± 1.10	65.60 ± 2.84
	Frontal cortex	2	0.77 ± 0.08	10.69 ± 0.76	86.88 ± 2.86
	CSF	2	0.32 ± 0.09	0.79 ± 0.59	94.40 ± 6.59

Table 3.3: Pure brain tissue clusters, measured in the 10 healthy subjects of cohort **iii**, at both 1.5 T and 3.0 T field strengths. Average ± SD are reported for R1, R2 and PD from each ROI.

R1-R2 plots, indicating a low correlation between R1 and R2. In the R1-PD and R2-PD plots, however, the clusters are tilted, which indicates correlation of R1 and R2 with PD. Skewness and Kurtosis measurements indicated that the tissue clusters were normally distributed in the R1-R2-PD space.

The lookup grids, which were subsequently used in the brain tissue segmentation, were created from the measured pure tissue clusters and the partial volume simulations. When the 95% prediction ellipses of the 3,000 simulated partial volume clusters were joined together in the R1-R2-PD space, curved bands were formed. In Figure 3.7 these lookup grids, two for 1.5 T, and one for 3.0 T are shown. The lookup grids were defined so that 95% of the *in vivo* brain voxels containing WM, GM and CSF, acquired at a noise level of SNR = 20, were expected to fall within the indicated regions. Figure 3.7 also shows simulation results without added noise. These results are shown as curves between the mean values of the pure brain tissue clusters.

3.2.2 | Volume Measurements

The average ± SD as well as within-subject standard deviation, *sw*, repeatability and coefficients of variation (CV) for volume measurements are listed in Table 3.4. Results from cohort **ii** are reported for all the axial acquisitions at different resolutions, as well as only the 2.0-mm acquisitions at different geometries. Results

Figure 3.6: Simulated pure tissue clusters WM, GM, CSF, and the mixtures of 50% WM/GM, 50% WM/CSF and 50% GM/CSF shown in the R1-R2-PD space. Projections on (A, D) R1-R2, (B, E) R1-PD, (C, F) R2-PD planes. The top row, (A-C), shows simulations at the different noise levels, SNR = 10, SNR = 20 and SNR = 40. The lowest SNR gave the widest ellipses. The bottom row, (D-F), shows simulations at 1.5 T and 3.0 T. Coloured clusters are for 3.0 T and corresponding grey clusters are for 1.5 T. Contour lines show the 95% prediction ellipses.

from cohort **iii** are reported for 1.5 T and 3.0 T separately. In cohort **ii** Kendall's τ indicated that no relation existed between the magnitude and standard deviation of the measurements ($\tau < 0.25$, $P > 0.3$). In cohort **iii** the Shapiro-Wilk test indicated that tissue volumes were normally distributed.

3.2.2.1 | Field Strength

In cohort **iii**, further analysis was performed using the GLM to compare 1.5 T and 3.0 T segmentation. The results revealed statistically significant differences in total volume between the field strengths for WM ($p < 0.001$), GM ($p < 0.001$), CSF ($p < 0.001$), BPV ($p < 0.001$), and BPF ($p < 0.001$) but not for NON and ICV. Analyses of main effects showed that WM was underestimated, while GM and CSF were overestimated, on 1.5 T compared to 3.0 T. The differences are detailed in Table 3.5. Bland-Altman plots comparing the results for each tissue volume at the

Figure 3.7: Lookup grids formed when joining the 3,000 simulated partial volume clusters appear as dashed lines. The transition GM $\leftrightarrow$ WM is shown in green, WM $\leftrightarrow$ CSF in red and GM $\leftrightarrow$ CSF in blue. The top panels (A-C) show the lookup grid used to segment images in cohort ii, the middle panels (D-F) show the lookup grid used to segment the 1.5 T images in cohort iii and the bottom panel (G-I) show the lookup grid for 3.0 T images in cohort iii. The solid lines show simulations without added noise, where each diamond represents a change of 20% tissue fractions, and each dot represents a change of 10% tissue fractions. Projections on (A, D, G) the R1-R2 plane, (B, E, H) the R1-PD plane, and (C, F, I) the R2-PD plane.

	Subject	WM (mL)	GM (mL)	CSF (mL)	NON (mL)	ICV (mL)	BPV (mL)	BPF (%)
cohort ii axial 1.0-2.0 mm	F38	705±14	619±9	121±2	14±6	1444±9	1323±7	91.65±0.12
	F26	732±16	663±16	127±2	12±1	1522±7	1395±6	91.68±0.10
	F24	546±6	495±9	162±2	14±2	1203±4	1041±4	86.51±0.15
	F23	556±8	633±4	113±4	9±1	1301±9	1188±6	91.33±0.23
	F21	553±36	674±37	149±5	10±3	1375±9	1226±6	89.17±0.32
	M32	796±18	714±18	127±8	15±4	1637±22	1510±14	92.23±0.41
	M29	623±8	625±11	159±2	16±1	1407±6	1248±6	88.72±0.11
	M28	622±18	667±19	149±13	15±1	1439±15	1289±4	89.63±0.81
	M26	778±10	715±7	171±1	15±2	1664±10	1493±9	89.73±0.06
	M23	678±12	704±11	169±3	13±2	1552±11	1382±8	89.09±0.12
	<i>sw</i>	16.67	16.62	5.58	2.81	11.29	7.54	0.33
	rep.	46.18	46.05	15.46	7.78	31.27	20.87	0.90
	CV	0.023	0.021	0.030	0.18	0.0069	0.0052	0.0027
cohort ii ax, cor, sag	F38	716±41	621±42	110±11	13±2	1445±7	1334±11	92.38±0.77
	F26	759±68	647±72	115±13	14±2	1521±12	1406±10	92.44±0.79
	F24	512±35	537±46	162±3	13±2	1211±10	1049±12	86.63±0.36
	F23	516±44	681±48	101±14	8±0	1298±8	1197±6	92.19±1.01
	F21	587±27	647±32	131±15	10±3	1366±14	1234±10	90.39±1.03
	M32	768±46	742±45	109±19	15±4	1619±22	1510±3	93.26±1.11
	M29	627±9	633±17	512±8	18±2	1412±13	1260±15	89.26±0.55
	M28	596±48	699±61	133±8	18±2	1429±6	1296±12	90.68±0.60
	M26	736±52	771±55	159±13	21±7	1666±8	1507±5	90.47±0.74
	M23	656±47	742±53	158±12	13±3	1555±6	1398±9	89.85±0.76
	<i>sw</i>	44.30	49.08	12.44	3.02	11.63	9.83	0.80
	rep.	122.71	135.96	34.45	8.35	32.21	27.24	2.23
	CV	0.064	0.070	0.094	0.17	0.0073	0.0072	0.0085
cohort iii 1.5 T	F29	540±3	697±4	155±1	13±0	1405±2	1250±2	88.95±0.07
	F27	448±3	713±4	156±0	10±0	1328±2	1171±1	88.20±0.00
	F26	452±8	723±9	131±2	8±0	1314±1	1183±1	90.10±0.14
	F25	564±1	768±1	166±3	12±1	1511±1	1345±1	89.00±0.14
	F24	505±3	774±0	104±0	13±1	1396±2	1292±2	92.55±0.07
	F21	383±0	679±2	80±0	9±0	1151±2	1071±2	93.05±0.07
	M25	568±0	840±1	177±0	12±0	1598±1	1420±1	88.90±0.00
	M23	556±3	839±0	170±0	15±0	1580±2	1410±2	89.20±0.00
	M23	596±1	911±3	206±1	22±1	1735±0	1530±2	88.15±0.07
	M21	521±7	824±7	130±1	11±0	1486±1	1357±1	91.25±0.07
	<i>sw</i>	3.75	4.23	1.19	0.50	1.66	1.72	0.08
	rep.	10.38	11.73	3.29	1.39	4.59	4.76	0.22
	CV	0.0057	0.0043	0.0059	0.0329	0.0011	0.0013	0.0007
cohort iii 3.0 T	F29	611±5	659±8	127±9	12±2	1409±8	1282±1	91.00±0.57
	F27	509±4	684±2	127±9	9±1	1329±4	1202±6	90.45±0.64
	F26	517±17	680±4	107±0	8±0	1312±13	1205±13	91.85±0.07
	F25	620±3	741±8	135±4	11±0	1508±1	1373±5	91.05±0.21
	F24	564±7	746±14	81±2	10±2	1402±3	1321±6	94.20±0.14
	F21	438±6	644±4	57±1	9±0	1149±4	1092±2	95.00±0.14
	M25	633±9	807±13	137±0	8±0	1586±5	1448±4	91.35±0.07
	M23	645±25	763±6	142±6	18±1	1568±13	1426±20	90.95±0.49
	M23	671±1	871±20	167±15	23±3	1732±2	1565±17	90.35±0.92
	M21	592±4	769±6	104±1	14±0	1480±11	1375±10	93.00±0.00
	<i>sw</i>	10.59	9.95	6.78	1.34	7.83	10.26	0.44
	rep.	29.33	27.57	18.77	3.71	21.70	28.42	1.21
	CV	0.0141	0.0111	0.0371	0.0716	0.0045	0.0061	0.0036

Table 3.4: Segmentation results for the 20 healthy subjects in cohort ii and cohort iii. The columns list the average $\pm$ SD for each subject. The last rows of each section tabulate the within-subject SD, *sw*, the repeatability and the coefficient of variation (CV). The first section shows segmentation results for cohort ii when all the axial acquisitions with resolutions 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mm were considered. The second section shows segmentation results for cohort ii when only the 2.0-mm acquisitions in the three planes; axial, coronal and sagittal, were considered. The last two sections show results for cohort iii at 1.5 T and 3.0 T separately.

Tissue	Mean Diff. 1.5 T - 3.0 T (mL)	RMSE (mL)	Mean Diff. (%) of 1.5 T	95% CI of Diff. (mL)
WM	-66	68	-12.85	[-72, -61]
GM	40	43	5.15	[34, 47]
CSF	29	30	19.66	[26, 33]
NON	0	2	0.00	[-1, 1]
ICV	3	7	0.21	[-1, 7]
BPV	-26	27	-2.00	[-31, -22]
BPF (%)	-1.99	2.00	-2.21	[-2.18, -1.79]

Table 3.5: Results from pair-wise comparisons of tissue volumes between the 1.5 T and 3.0 T segmentation in cohort **iii**.

two field strengths are shown in Figure 3.8.

The voxel-wise comparisons of tissue maps at 1.5 T and 3.0 T revealed regional differences in WM, GM and CSF volume estimations. In general, differences occurred in deep brain structures where tissue classified as mostly GM at 1.5 T was instead classified as mostly WM at 3.0 T. Statistically significant differences for CSF were only found in one small region close to the midline anterior cingulate

Figure 3.8: Bland-Altman plots comparing volumes between 1.5 T and 3.0 T measured in cohort **iii**. Volumes of (A) WM, (B) GM, (C) CSF, (D) NON, (E) ICV and (F) BPV.

gyrus in the limbic lobe (cluster size 26 voxels). Statistically significant regional differences in WM and GM estimation between 1.5 T and 3.0 T were found in bilateral and symmetrical regions in the following structures: the cerebellum, the anterior parts of the hippocampal formation, dorsal parts of the medulla and pons, midbrain structures comprising the thalamus, substantia nigra, red nucleus, and the dorsal midbrain tegmentum, as well as in several clusters in sub-lobar WM and GM including the putamen. Figure 3.9 shows the results of the voxel-wise t-test where the WM estimate was significantly greater at 3.0 T compared to 1.5 T, the regions where GM estimates were significantly larger at 1.5 T were virtually symmetrical.

3.2.3 | Application in MS

One MS patient from cohort **iv** was segmented using the lookup-grid created from the QMAP pure tissue cluster measurements ((**A-C**) in Figure 3.7). One example slice from the brain tissue segmentation of WM, GM, CSF and NON is shown in Figure 3.10. A synthetic T2-weighted image was included for visual guidance. Partially liquefied periventricular lesions are visible in the T2-weighted image. In the tissue segmentation these were attributed to NON and CSF. The tissue volumes of the MS patient were estimated to be: WM 517 mL, GM 485 mL, CSF 380 mL, NON 24 mL, ICV 1407 mL, BPV 1026 mL and the BPF was 73.0%. The ICV was comparable to that of healthy subjects. The WM and GM volumes, however, were lower, and CSF volume was higher. The resulting BPF was substantially lower than that of healthy subjects. NON was higher in the MS patient than in any of the healthy subjects from cohort **ii** and **iii**.

3.3 | QMRI in MS

NAWM was present, and measured, in all MRIpos and MRIneg patients. DAWM was present in all MRIpos patients, and in 12 of the MRIneg patients. WM lesions were found in all MRIpos patients, and in nine of the MRIneg patients. No WM lesions at all were observed in six of the MRIneg patients, and DAWM was not observed in three of the MRIneg patients.

Descriptive statistics and results of the group comparisons, both comparing MS patients to healthy controls and also comparing MRIneg to MRIpos, are presented in Table 3.6. BPF was significantly lower in the MRIpos group compared to the healthy controls, but this was not observed in the MRIneg group (see Table 2.2). Details of the qMRS measurements are reported elsewhere, see Tisell *et al.* (162).

Figure 3.9: Results from voxel-wise t-tests comparing brain tissue segmentation from all 10 subjects in cohort iii between 1.5 T and 3.0 T. Statistically significant differences are indicated where WM volume is estimated to be larger in the 3.0 T system.

Figure 3.10: Segmentation results from a 45-year-old female MS patient in cohort *iv* (at 1.5 T). Segmentation maps are shown in (A-D), where (A) shows WM in blue, (B) shows GM in green, (C) shows CSF in purple and (D) shows NON in yellow. A synthetic T2-weighted image is shown for reference in (E). Several partially liquefied lesions are visible (red arrows in (E)). These were segmented as NON with a CSF centre. There are also some GM misclassifications visible around the lesions.

Group	Tissue	n subjects	R1 (s ⁻¹)	R2 (s ⁻¹)	PD (pu)
HC	WM	20	1.65 ± 0.10	12.87 ± 0.66	65.83 ± 1.66
MRIpos	NAWM	20	1.61 ± 0.13	12.74 ± 1.01	66.60 ± 3.04
	DAWM	20	1.22 ± 0.19***	8.80 ± 1.30***	76.91 ± 5.08***
	Lesion	20	1.09 ± 0.11***	7.97 ± 0.91***	82.16 ± 3.78***
MRIneg	NAWM	14	1.57 ± 0.10*	12.64 ± 0.66	68.59 ± 2.23***†
	DAWM	12	1.25 ± 0.15***	9.24 ± 0.70***	75.63 ± 4.21***
	Lesion	9	1.18 ± 0.11***	9.46 ± 0.82***†††	78.69 ± 3.36***†

Table 3.6: Descriptive statistics and group comparisons for tissue types in cohort **iv**. Stars indicate p-levels of comparisons to WM in healthy controls, and daggers indicate p-levels of comparisons of the same tissue type in MRIpos- and MRIneg groups. * p<0.05 compared to WM in healthy controls, ** p<0.01 compared to WM, *** p<0.001 compared to WM. † p<0.05 compared to the same tissue type in MRIpos, †† p<0.01 compared to the same tissue type in MRIpos, ††† p<0.001 compared to the same tissue type in MRIpos.

3.3.1 | Normal Appearing White Matter

NAWM ROIs could be placed parietally in the centrum semiovale, similarly to placements of WM ROIs in the healthy controls.

Statistical analysis revealed that NAWM in the MRIneg group had significantly lower R1 and higher PD than WM in the healthy controls. Moreover, NAWM in the MRIneg group had significantly higher PD than in the MRIpos group. NAWM in the MRIpos group, on the other hand, was not significantly different from WM in the healthy controls.

No correlations between the qMRI properties of NAWM and clinical parameters were observed in the MRIneg group. In contrast, R1 and R2 in the MRIpos group were negatively correlated with EDSS (p<0.05) and MSSS (p<0.05), and these correlations were confirmed when the Spearman correlations were calculated. Furthermore, a significant positive correlation between R1 of NAWM in MRIpos and tCho-Aq concentration (p<0.05) was also observed. Correlation analysis of qMRI measurements and qMRS measurements inside the MRS VOIs revealed negative correlations between R1 and R2 of NAWM in the MRIneg group and tNA-Aq concentration (p<0.05). In contrast, such correlations were not observed in the MRIpos group. Positive correlations were also obtained between R1 of NAWM in MRIpos and tCho-Aq concentration (p<0.05). Finally, a positive correlation was detected between R2 of WM in healthy controls and mIns-Aq (p<0.05), and a negative correlation was detected between PD of WM in healthy controls and mIns-Aq (p<0.05).

Regression analyses revealed no associations between any qMRI properties of NAWM and BPF, MSSS or EDSS when both MS groups were pooled.

3.3.2 | Diffusely Abnormal White Matter

DAWM was mostly detected in periventricular WM, and only rarely in the centrum semiovale. DAWM was observed in proximity to focal WM lesions, but also in areas with no visible WM lesions. DAWM was detected in the majority of the MRIneg patients with very few or no focal WM lesions. Such DAWM usually extended over large areas, and the border with NAWM was not clearly distinguishable. DAWM ROIs could be placed avoiding the borders with NAWM, as has been suggested by Seewann *et al.* (157).

Statistical analysis revealed that DAWM was significantly different from WM in healthy controls, in both MS groups for all qMRI measurements. Conversely, no statistically significant difference in DAWM was detected between the two MS groups.

When correlating qMRI properties of DAWM with clinical parameters, a significant negative correlation was found between age and R2 of DAWM in the MRIneg group ($p < 0.05$). No other significant correlations were detected.

Regression analyses revealed an association between R2 of DAWM and BPF ($\beta = 0.038$, $p < 0.05$). No similar association was observed between the qMRI properties of DAWM and MSSS or EDSS.

4

DISCUSSION

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4.1 | Present Work

In the present work a method for qMRI, capable of quantifying the complete brain in about 5-8 minutes, was developed. This method was applied in two separate applications; the first was the visualisation and volume calculation of brain tissues in healthy subjects, and the second was the measurement and investigation of qMRI properties in MS patients. The current work is summarised in Figure 4.1. The qMRI method and the brain tissue segmentation were thoroughly validated in control subjects and MR phantoms.

The most important findings of this work were that qMRI could be used for brain tissue segmentation with high repeatability (Papers II and III), and that qMRI could detect and quantify changes in MS that were not discernible using conventional MR imaging (Paper IV).

Figure 4.1: The current work consisted of three parts, the first was the development of a qMRI sequence for full brain quantification in a clinically acceptable time, the second part was the development of a brain segmentation method based on qMRI. Finally, the qMRI method was applied in MS patients to investigate qMRI properties in diseased brain tissue.

4.2 | QMRI for Brain Imaging

A novel qMRI method (QMAP) was developed and optimised to accommodate clinical requirements for brain imaging. The method was a 2D multi-slice, multi-echo and multi-saturation recovery MR sequence. The QMAP data was fitted with an algorithm based on least-squares optimisation. The method was capable of quantifying the clinically relevant properties T1 (or R1) relaxation, T2 (or R2)

relaxation and proton density (PD), with intrinsic B_1 -field correction. The main challenge in qMRI, which has persisted for many years, has been the acquisition time, which is too long to be clinically acceptable. The presented method performed quantification of the full brain in a mere 5-8 minutes.

The *in vivo* QMAP quantification corresponded well with gold standard qMRI measurements and literature references. Brain tissue was heterogeneous throughout the brain and varied depending on region, both for WM and GM. CSF on the other hand, was less heterogeneous. Even so, healthy brain tissues exhibited a narrow range of qMRI properties, agreeing with previous studies on qMRI of the brain (117, 129, 130).

QMAP was further validated using phantom measurements, in which it was found that the RMS error of the qMRI measurements was in the order of 5-20% in four separate MR scanners of two vendors (Philips and GE) at two field strengths (1.5 T and 3.0 T). One of the measurements, PD measured at the GE 3.0 T system, did show a larger RMS error. Conversely, this was not detected in either the Philips 3.0 T system or the GE 1.5 T system, indicating that this may have been related to the specific scanner. Bland-Altman analysis revealed systematic errors in T1 and T2 measurements for long relaxation times, compared to gold standard measurements. This was detected in all systems for relaxation times which are longer than those of healthy WM and GM, but not longer than relaxation of CSF. One reason for this may be that QMAP is based on steady-state acquisition where full relaxation does not have time to occur. This effect is normally outside the clinically relevant range, but caution should be exercised when longer relaxation times are of interest, such as e.g. in oedema where the water contents of the tissue increase and the relaxation times are prolonged. Within the clinical range the phantom measurements showed that QMAP had high accuracy and precision.

The relatively short acquisition time combined with high accuracy and precision makes QMAP an interesting method for clinical applications. A second hurdle, which was also addressed in this work, is the visualisation of qMRI properties in a clinically relevant way. One straightforward approach to maintain the common conventional MR images while performing quantification is to utilise synthetic MR. This approach, which was suggested in the eighties (46), allows the calculation of synthetic contrast images mimicking the appearance of conventional MRI. There are, however, differences in conventional MR images and synthetic contrast images that should be considered. Although the images are deceptively similar, some studies have reported higher contrast in synthetic images (53, 54), deviations in FLAIR images (55), and high levels of noise (54). Synthetic MRI has the potential to reduce the overall examination time by synthesis of multiple contrast images from the same qMRI acquisition. A standard MR imaging protocol may be as long

as 15 minutes, compared to the 8 minutes for QMAP.

A more intuitive approach is that of brain tissue segmentation, where real physiological tissue maps and volumes are calculated from the qMRI properties. This subject is discussed later on.

4.2.1 | Limitations

Even though qMRI has several advantages compared to conventional MRI, it also presents new challenges. In particular the implementation of qMRI has greater requirements of specificity and precision than conventional MRI because of the desire to acquire real absolute quantitative measurements. In qMRI, many of the sources of inhomogeneities and imperfections in the MR scan are of great concern, even though these do not matter as much when acquiring contrast images. In particular, the inhomogeneity of the RF pulses, B_1 -field, must be considered and compensated for. Also, MR artefacts, such as spurious echoes caused by off-resonance spins, and cross-talk between slices in 2D acquisition have a greater effect on qMRI than on conventional MRI.

Furthermore, it is well known that brain tissues exhibit multi-exponential T1 (67) and T2 relaxation (70, 71), reflecting the underlying water compartments in the microscopic environment. When a single mono-exponential relaxation is assumed, as is done in QMAP, the actual estimated values of T1 and T2 depend to some degree on the specific number of echoes used, the number of saturation delays, the echo times, and the saturation delay times. Shorter times favour short relaxation components whereas long times favour the longer relaxation components, resulting in shifts of the estimated T1 and T2 values. Few time-points and mono-exponential fits are therefore limited (61). While increasing the SNR, by more acquisition time for each raw image, this is at the expense of quantitative value.

4.3 | Brain Tissue Segmentation

In this work a method for automated whole brain tissue segmentation and volume estimation on a sub-voxel basis was implemented. The acquisition strategy was based on qMRI and the segmentation procedure was carried out in the R1-R2-PD space, similar to the approach of Alfano *et al.* (117). The segmentation procedure required prior knowledge of pure tissues and partial volume voxels. This was determined using ROI measurements and Bloch simulations. The prior knowledge was subsequently used to construct lookup grids which related R1, R2 and PD values of *in vivo* voxels to tissue fractions of WM, GM and CSF. The segmentation

procedure was implemented for both 1.5 T and 3.0 T scanners and validated in 20 healthy controls. All controls were scanned on the 1.5 T and 10 were scanned on both the 1.5 T and 3.0 T.

There are significant advantages in using qMRI for brain tissue segmentation. First, the qMRI acquisition is quantitative and therefore the segmentation procedure is based on real physical properties of the tissue itself and not the arbitrary grey-scale of conventional MRI images. This, in principle, reduces the dependency on the MR scanner hardware and settings and allows segmentation based on the properties of the brain tissue itself. One major advantage is the reproducibility across subjects, MR scanners and even across different sites. Furthermore, the sub-voxel segmentation reduces the dependency on MR scanner acquisition resolution, and allows for a significant decrease in scan time or, alternatively, an increase in SNR. This was evident in the validation where acquisition at 1.0 mm in-plane resolution gave similar results to acquisitions at the 2.0 mm in-plane resolution.

Even so, there is one dependency that is not removed in the qMRI acquisition. That is the dependency on main magnetic field strength, which directly affects the measured R1 and R2 rates (79, 81, 135). In general, R1 and R2 decrease when the main magnetic field is increased; this relation is complex and depends on tissue composition. Furthermore, in some cases the R1, R2 and PD values were similar for different tissue mixtures. This generally occurred close to the ventricles where partial volume voxels with WM and CSF were common. These voxels showed similar characteristics to GM voxels in the R1-R2-PD space, and in the present segmentation procedure a geometrical iteration was performed to replace these voxels with the correct partial volume transition. This was challenging as there are deep GM structures close to the ventricles, such as the caudate nucleus, which should not be classified as WM $\leftrightarrow$ CSF. Some misclassifications occurred in these regions.

The SNR is difficult to determine in qMRI since the SNR of the acquired raw images is not directly related to the SNR in the qMRI maps. For example some level of noise may cause the fitting algorithm to become unstable. In the present work the level of noise was empirically established by comparing the three simulated noise levels of SNR = 10, SNR = 20 and SNR = 40 to *in vivo* acquired qMRI data. The noise level of SNR = 20 most closely resembled *in vivo* data, and therefore this level was chosen in the lookup grids. The same noise levels were used for 1.5 T and 3.0 T. In general, the noise may be less on the 3.0 T scanner, but instead the dynamic range of R1 and R2 values decreased, leading to similar segmentation precision, and even lower repeatability than in the 1.5 T system.

Some differences in segmentation results were found across field strengths and geometries. The differences were less or comparable to those reported in other

popular segmentation methods. Pfefferbaum *et al.* (135) found regional volume differences between 1.5 T and 3.0 T, where some structures were measured as being up to 15% larger at 3.0 T, while other structures were instead down to 19% smaller, when using an SRI24 atlas-based approach. In another study, Jovicich *et al.* (133) found, when using the Freesurfer tool that both the magnitude and signs of regional volume differences depended on brain structure and field strength. The volume differences between field strengths in the current method were about 5-20% for each tissue type and about 2% for the total brain volume. Interestingly, it seemed that the volume estimations had some dependency on the angle of the image plane, leading to differences in volume estimation between the axial, coronal and sagittal acquisitions. A similar effect has recently been reported for T2* measurements of WM where T2* depended on the angle of WM fibres to the B_0 -field (207).

The high precision of the presented method, together with the fast acquisition time and sub-voxel classification, are promising for clinical applications, and this was exemplified in one MS patient. In particular, the BPF was shown to be less in this patient, which is consistent with other studies (124, 125).

4.3.1 | Limitations

In practice there are limitations to qMRI, as discussed in section 4.2.1, and these also affect the brain tissue segmentation. In particular, the mono-exponential fitting results in a dependency on the qMRI acquisition settings, such as number of echoes and timings. Because of this it was essential to use the same QMAP sequence and scanner settings in all steps of the brain tissue segmentation, *i.e.* in the determination of pure brain tissue clusters, in the Bloch simulations, and finally in the acquisition of *in vivo* data.

Furthermore, one limitation of the segmentation procedure was the assumptions involved in the creation of the lookup grids. These were (1) that there are at most two tissues present in each voxel and (2) there is no interaction between the tissues. In real brain tissue, on the other hand, several different tissues and tissue compartments are present in the voxels, and the water protons are rapidly exchanged between these compartments. This is highlighted in the four-pool tissue model (83) and could be incorporated in the brain tissue segmentation in future developments.

Since the R1, R2 and PD differences in tissue characteristics between the two field strengths, 1.5 T and 3.0 T, were considered, using separate lookup grids, it was expected that tissue volumes would be similar at both field strengths, but some variations were found. These differences could to some degree be caused by the complex relationship between R1 and R2 values and field strength, in different

regions of the brain. When the bulk WM, GM and CSF is considered, as was done in the presented segmentation method, regional information was lost. Regional tissue characteristics may vary between 1.5 T and 3.0 T in a non-linear manner, and this has been attributed to iron deposits in different anatomical regions (79,81). Schenck *et al.* (81) found that the R2 dependence on field strength is greatest in regions which are rich in iron concentration, and pointed out that the substantia nigra and the red nuclei are prominent indicators of this phenomenon. One study found that the dependence of R2 on field strength for ferritin, which accumulates in deep brain nuclei, was linear (208), whereas another *in vitro* study found the relation to be non-linear (209). These studies were, however, performed on low field strengths. Furthermore, it has been shown that the increase of T1 and T2 (decrease of R1 and R2), which is dependent on the main magnetic field strength, is higher in the globus pallidus, the red nucleus, substantia nigra, the putamen, the thalamus and the splenium, compared with relatively iron-depleted regions of the mid brain (81). In the present study, regional voxel-wise t-tests were performed to investigate the differences in brain tissue segmentation at 1.5 T and 3.0 T of the whole brain, and the voxels which were differently classified closely resembled these regions.

Finally, in the validation of the brain tissue segmentation a low number of young and healthy subjects were investigated. The included subjects did not show any signs of the degeneration or disease in the brain that would be expected in an elderly group or patient group. Such degeneration includes changes due to tissue atrophy and small unspecific age-induced changes in the WM. The segmentation procedure was exemplified in one MS subject, and it was shown that the WM lesion was classified as NON and CSF, which was expected. Other diffuse changes in MS, such as the increase of PD in WM may result in misclassification between WM and GM, which can be difficult to detect.

4.4 | QMRI in MS

We used qMRI to investigate diffuse WM changes in two groups of clinically definite MS (CDMS) patients. One of the MS groups, *MRIneg*, showed very few focal WM lesions in a previous clinical examination; this group was useful for investigating WM in the absence of focal lesions. In this group of patients the clinical investigation was thorough before a diagnosis of CDMS was made.

We included normal appearing white matter (NAWM), diffusely abnormal white matter (DAWM), and focal WM lesions in the investigations. Comparisons were performed both between *MRIneg* and typical MS patients with focal lesions, *MRIp*os,

as well as with WM in healthy controls. Furthermore, correlation analyses were performed between qMRI measurements and a battery of clinical parameters, and metabolite concentrations in NAWM. The latter were measured using quantitative magnetic resonance spectroscopy (qMRS), reported previously in (162). Finally, regression analyses were performed to determine the associations between qMRI measurements and BPF, MSSS and EDSS.

4.4.1 | Normal Appearing White Matter

Changes found in NAWM have previously been attributed to axonal disruption (149, 172, 190, 191), but also to increased glia cell numbers, without evidence for axonal loss (192). In the present study we did not observe any significant difference between NAWM in MRIpos and WM in healthy controls, but NAWM in the low-lesion MRIneg group was characterised both by significantly higher PD and lower R1 than in normal subjects.

In the MRIpos group it was found that R1 and R2 of NAWM were correlated with both EDSS and MSSS, something which may suggest that R1 and R2 are related to brain atrophy.

A positive correlation was found between the tCho-Aq concentration of NAWM and R1 in MRIpos patients. Since tCho has previously been shown to be a marker for membrane turnover (174, 175) this suggests that R1 may be related to this process. Furthermore, the mIns-Aq concentration of WM in healthy controls correlated negatively with R2 and PD. This could be due to an effect of age (162).

The MRS VOIs were large compared to the qMRI ROIs, and mean qMRI values were also measured inside these volumes, and subsequently correlated with qMRS measurements in the same volumes. An interesting observation was that there was a negative correlation between R1 and R2 in the MRS VOIs with tNA-Aq concentration in the MRIneg patients. This indicates that the tNA-Aq concentration in these VOIs actually increased as R1 and R2 decreased. Since tNA is considered a marker of neuronal density (138, 172–174) this result suggests that there was a process of demyelination, indicated by decreased R1 and R2, without neuronal loss in these patients. The neuronal density of the tissue (per volume) may in fact increase as myelin diminishes, caused by a net shrinkage of the tissue, leading to more axons inside the VOI. The correlation was not observed in the MRIpos patients, indicating that these patients suffered from axonal loss as well in the NAWM. This could be attributed to Wallerian degeneration, which is considered to be secondary to focal WM lesions (149, 156, 159).

4.4.2 | Diffusely Abnormal White Matter

Previously, changes in DAWM have been attributed mainly to Wallerian degeneration. Seewann *et al.* (157) showed in their comprehensive histopathological study that decreased R1 and R2 in DAWM were associated with axonal loss and decreased myelin. In our present study, DAWM was observed in both the MS group with typical MR presentation, and in the MS group with a low number of focal lesions. Moreover, the qMRI properties of DAWM were similar in both groups. Since DAWM was found to a large extent also in the group with a low number of lesions, this might suggest that DAWM is not exclusively due to Wallerian degeneration, but that it is also a process in the WM which to some extent is independent of the presence of focal WM lesions.

One major finding of this study was that DAWM appeared to constitute an intermediary between focal WM lesions and NAWM for all qMRI measurements, in both groups of MS patients. This is consistent with previous findings where Vrenken *et al.* (158) showed that DAWM was an intermediary in T1. This may reflect the relative increase of the extracellular water of the tissues, where focal lesions have the highest increase, DAWM has a less pronounced increase, and NAWM only has a slight increase. This is supported by previous studies which have shown that decreased R1 and R2 are linked to increased water contents due to increased size of extracellular spaces caused by a combination of axonal loss and demyelination (156, 157, 171, 190). This is also consistent with the observed increases of PD. Conversely, the WM lesions detected in the MRIneg group were significantly different from the lesions in the MRIpos group, the lesions in the MRIneg group seem to constitute an intermediate between the MRIpos lesions and DAWM.

Another important finding was that DAWM was common in both groups of MS patients. The MRIneg group had similar disability and clinical presentations as the MRIpos group, even though the number of WM lesions was low and the BPF was unaffected. Furthermore, focal WM abnormalities detected in the MRIneg group were significantly different from the WM lesions detected in the MRIpos group. These abnormalities were more like those in DAWM, and some of them may in fact have been small regions of DAWM misinterpreted as focal lesions.

4.4.3 | Limitations

This study was cross-sectional and as such it was not possible to investigate changes of qMRI properties over time, nor their relation to disease progression. Nevertheless, associations between measurements and clinical parameters as well as metabolite concentrations were observed.

In the present study a single component of R1 and R2 was measured. In this way the different compartments were averaged together and it was therefore difficult to establish whether decreased R1 and R2 rates were due to loss of myelin or increased water content.

4.5 | Future Perspective

The application of qMRI and the concept of absolute quantification of tissue properties are truly elegant. As MRI moves into the next decade it is time for a paradigm shift from the qualitative to the quantitative. In order to facilitate this shift emphasis must be on clinical evidence.

In the past decade the challenges of qMRI were those of acquisition speed, uncertainty and reproducibility. New emerging sequences, such as the QMAP sequences presented in this work, have bridged the gap to clinically acceptable times. Now, as the qMRI sequences are in place and the accuracy and specificity have been demonstrated, a second challenge is of increasing interest. Now that qMRI can be performed in a clinical setting, the question is, what do these qMRI properties mean? Do they relate to pathophysiological changes in disease? And most importantly how can they influence clinical research, diagnosis and in the end patient care?

Figure 4.2: Future work should combine the concepts of brain tissue segmentation, developed in the present work, with knowledge of qMRI in MS, in order to create the brain tissue segmentation for the MS disease. This may well give further insights into the diffuse changes in MS and show correlation with disease progression and disease status, something which is much sought after today.

The gap in clinical practice has moved from the technique to the clinical validation and application. In this work, two applications of qMRI are demonstrated; brain tissue segmentation and application in MS subjects. A future step that should be taken based on this work is to combine the concepts of brain tissue segmentation and the lessons learned on qMRI in MS, and extend the brain tissue segmentation to MS patients, see Figure 4.2. Even though (as exemplified in Paper II), the brain tissue segmentation was able to demonstrate focal WM lesions in MS, there are, as we know, diffuse processes which may relate better to disease status and disease progression. The brain tissue segmentation for MS patients should consider these diffuse changes and must incorporate a model for the disease, similar to the four-pool model. This holds great promise to supply surrogate measures for disease progression; something which is much sought after today.

Moreover, this is only one of the possible applications of qMRI. As qMRI sequences are becoming readily available in clinical settings, work should be carried out for clinical validations and visualisations in brain disorders such as hydrocephalus, dementia's, brain tumours, and in normal ageing. Also, other anatomies could be investigated with qMRI.

With all this said it is important to know the limitations of qMRI and keep the focus on clinical evidence. The main challenge for the years to come is to understand the connection between the qMRI properties of the tissue and underlying biology.

5

CONCLUSIONS

Magnetic resonance imaging (MRI) is a sensitive technique to assess white matter (WM) lesions in Multiple Sclerosis (MS), but there is a low correlation between MRI findings and clinical disability. To date, mechanisms leading to permanent disability in MS remain unclear and new techniques to assess changes in MS as well as methods for brain tissue segmentation in neuro-degenerative diseases are much sought after.

The present work describes one novel method of quantitative MRI (qMRI) for the human brain (QMAP) and a segmentation method based on this. Furthermore, one application in diseased human brain was demonstrated in MS patients.

One major finding of this work was that qMRI was able to detect and quantify changes in the MS disease that were not visible using conventional MRI. In particular it was found that diffusely abnormal white matter (DAWM) appears to constitute an intermediate between focal WM lesions and normal appearing white matter (NAWM). Furthermore, the results suggest that diffuse changes in WM can be caused by pathological processes that are not entirely attributable to Wallerian degeneration.

The presented methods for qMRI and brain tissue segmentation are highly promising for application in different MR systems from different vendors and with different field strengths, but the differences should preferably be considered before combining field strengths in the same study or in clinical practice.

In conclusion, this study showed that the QMAP method had high accuracy and relatively high precision, within a clinically acceptable time. This work also demonstrated that qMRI could be used for brain tissue segmentation and volume estimation of the whole brain, using pre-defined tissue characteristics. The results showed that brain tissue segmentation had high intra-scanner repeatability, which was somewhat lower when different geometries were acquired, or different field strengths used. In particular, differences were found between 1.5 T and 3.0 T in deep brain structures, the cerebellum and the brain stem. The difference in total

brain volume was, however, less than that of individual tissues, since differences in WM and GM had different signs.

This work leads the way for early clinical applications of qMRI, but much work remains. The challenge for the years to come is to understand the connection between the qMRI properties of the brain and underlying biology.

REFERENCES

1. Confavreux C, Lassman H, McDonald I, Miller D, Noseworthy J, et al. (2006) *McAlpine's Multiple Sclerosis*. Elsevier, forth edition edition. ISBN 0-4430-7271-X.
2. Trapp D B, Peterson J, Ransohoff M R, Rudick R, Mörk S, et al. (1998) Axonal transection in the lesions of multiple sclerosis. *The New England Journal of Medicine* 338: 278-285.
3. Hemmer B, Archelos J J, Hartung P H (2002) New concepts in the immunopathogenesis of multiple sclerosis. *Nature Reviews Neuroscience* 3: 291-301.
4. Zamvil S S, Steinman L (1990) The T lymphocyte in experimental allergic encephalomyelitis. *Annual Review of Immunology* 8: 579-621.
5. Bjartmar C, Kinkel P R, Kidd G, Rudick A R, Trapp D B (2001) Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology* 57: 1248-1252.
6. Miller H D, Barkhof F, Frank A J, Parker J G, Thompson J A (2002) Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain* 125: 1676-1695.
7. De Stefano N, Bartolozzi L M, Guidi L, Stromillo L M, Federico A (2005) Magnetic resonance spectroscopy as a measure of brain damage in multiple sclerosis. *Journal of the Neurological Sciences* 233: 203-208.
8. Trapp D B, Nave A K (2008) Multiple sclerosis: an immune or neurodegenerative disorder? *Annual Review of Neuroscience* 31: 247-269.
9. Trapp D B, Ransohoff M R, Fisher E, Rudick A R (1999) Neurodegeneration in multiple sclerosis: Relationship to neurological disability. *Neuroscientist* 5: 48-57.
10. Miller H D, Leary M S (2007) Primary-progressive multiple sclerosis. *Lancet Neurology* 6: 903-912.
11. Miller E (2012) Multiple sclerosis. *Advances in Experimental Medicine and Biology* 724: 222-238.
12. Rosati G (2001) The prevalence of multiple sclerosis in the world: an update. *Neurological Sciences* 22: 117-139.
13. Ahlgren C, Odén A, Lycke J (2011) High nationwide prevalence of multiple sclerosis in sweden. *Multiple Sclerosis* 17: 901-908.
14. Ebers G (2013) Interactions of environment and genes in multiple sclerosis. *Journal of the Neurological Sciences* 334: 161-163.
15. Tedeholm H, Skoog B, Hillert J, Runmarker B, Stawiarz L, et al. (2007) Tidig immunterapi vid MS minskar risk för senare handikapp. *Läkartidningen* 104: 1684-1688.
16. Miller H D, Weinshenker G B, Filippi M, Banwell L B, Cohen A J, et al. (2008) Differential diagnosis of suspected multiple sclerosis: a consensus approach. *Multiple Sclerosis* 14: 1157-1174.
17. Miller H D, Chard T D, Ciccarelli O (2012) Clinically isolated syndromes. *Lancet Neurology* 11: 157-169.

18. McDonald I W, Compston A, Edan G, Goodkin D, Hartung P H, et al. (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. *Annals of Neurology* 50: 121-127.
19. Polman H C, Reingold C S, Edan G, Filippi M, Hartung P H, et al. (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Annals of Neurology* 58: 840-846.
20. Polman H C, Reingold C S, Banwell B, Clanet M, Cohen A J, et al. (2011) Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology* 69: 292-302.
21. Kurtzke F J (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33: 1444-1452.
22. Roxburgh H R, Seaman R S, Masterman T, Hensiek E A, Sawcer J S, et al. (2005) Multiple sclerosis severity score: using disability and disease duration to rate disease severity. *Neurology* 64: 1144-1151.
23. Pachner R A, Steiner I (2009) The multiple sclerosis severity score (MSSS) predicts disease severity over time. *Journal of the Neurological Sciences* 278: 66-70.
24. Imrell K, Landtblom M A, Hillert J, Masterman T (2006) Multiple sclerosis with and without CSF bands: clinically indistinguishable but immunogenetically distinct. *Neurology* 67: 1062-1064.
25. Bloch F, Hansen L W, Packard M (1946) The nuclear induction experiment. *Physical Review* 70: 474-485.
26. Purcell M E, Torrey C H, Pound R V (1946) Resonance absorption by nuclear magnetic moments in a solid. *Physical Review* 69: 37-38.
27. Lauterbur PC (1973) Image formation by induced local interactions: Examples employing nuclear magnetic resonance. *Nature* 242: 190-191.
28. Mansfield P, Grannell PK (1973) NMR 'diffraction' in solids? *Journal of Physics C: Solid State Physics* 6: 422-426.
29. Levitt M H (2008) *Spin Dynamics Basics of Nuclear Magnetic Resonance*. Wiley, second edition edition. ISBN 978-0-470-51117-6.
30. Haacke M E, Brown R W, Thompson M R, Venkatesan R (1999) *Magnetic Resonance Imaging Physical Principles and Sequence Design*. Wiley. ISBN 0-471-35128-8.
31. Matt B A, Kevin K F, Xiaohong Z J (2004) *Handbook of MRI Pulse Sequences*. Wiley. ISBN 978-0-12-092861-3.
32. Hahn EL (1950) Spin echoes. *Physical Review* 80: 580-594.
33. Carr Y H, Purcell M E (1954) Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Review* 94: 630-638.
34. Meiboom S, Gill D (1958) Modified spin-echo method for measuring nuclear relaxation times. *Review of Scientific Instruments* 29: 688-692.
35. Ordidge R J, Gibbs P, Chapman B, Stehling K M, Mansfield P (1990) High-speed multislice T1 mapping using inversion-recovery echo-planar imaging. *Magnetic Resonance in Medicine* 16: 238-245.
36. E H, McKinnon G, Lee Y T, Rutt K B (1999) A fast 3D look-locker method for volumetric T1 mapping. *Magnetic Resonance Imaging* 17: 1163-1171.

37. Clare S, Jezard P (2001) Rapid T(1) mapping using multislice echo planar imaging. *Magnetic Resonance in Medicine* 45: 630-634.
38. Deichmann R (2005) Fast high-resolution T1 mapping of the human brain. *Magnetic Resonance in Medicine* 54: 20-27.
39. Warntjes B J, Dahlqvist O, Lundberg P (2007) Novel method for rapid, simultaneous T1, T*2, and proton density quantification. *Magnetic Resonance in Medicine* 57: 528-537.
40. Ma D, Gulani V, Seiberlich N, Liu K, Sunshine L J, et al. (2013) Magnetic resonance fingerprinting. *Nature* 495: 187-192.
41. Shah J N, Zaitsev M, Steinhoff S, Zilles K (2001) A new method for fast multislice T(1) mapping. *Neuroimage* 14: 1175-1185.
42. Zhu C D, Penn D R (2005) Full-brain T1 mapping through inversion recovery fast spin echo imaging with time-efficient slice ordering. *Magnetic Resonance in Medicine* 54: 725-731.
43. Deoni C S, Rutt K B, Peters M T (2003) Rapid combined T1 and T2 mapping using gradient recalled acquisition in the steady state. *Magnetic Resonance in Medicine* 49: 515-526.
44. Deoni C S, Peters M T, Rutt K B (2005) High-resolution T1 and T2 mapping of the brain in a clinically acceptable time with DESPOT1 and DESPOT2. *Magnetic Resonance in Medicine* 53: 237-241.
45. Deoni C S (2007) High-resolution T1 mapping of the brain at 3T with driven equilibrium single pulse observation of T1 with high-speed incorporation of RF field inhomogeneities (DESPOT1-HIFI). *Journal of Magnetic Resonance Imaging* 26: 1106-1111.
46. Riederer J S, Suddarth A S, Bobman A S, Lee N J, Wang Z H, et al. (1984) Automated MR image synthesis: feasibility studies. *Radiology* 153: 203-206.
47. Hylton M N, Crooks E L, Feinberg A D, Kaufman L, Ortendahl A D (1985) Analytical techniques for post-imaging evaluation of NMR tissue contrast. *IEEE Transactions on Nuclear Science* NS-32: 803-806.
48. Riederer J S, Lee N J, Farzaneh F, Wang Z H, Wright C R (1986) Magnetic resonance image synthesis. clinical implementation. *Acta Radiologica Supplementum* 369: 466-468.
49. Bobman A S, Riederer J S, Lee N J, Tasciyan T, Farzaneh F, et al. (1986) Pulse sequence extrapolation with MR image synthesis. *Radiology* 159: 253-258.
50. Deoni C S, Rutt K B, Peters M T (2006) Synthetic T1-weighted brain image generation with incorporated coil intensity correction using DESPOT1. *Magnetic Resonance Imaging* 24: 1241-1248.
51. Maitra R, Riddles J J (2010) Synthetic magnetic resonance imaging revisited. *IEEE Transactions on Medical Imaging* 29: 895-902.
52. Peterson E (2008) Synthetic MRI for visualization of quantitative MRI. Master's thesis, Linköpings Universitet. ISRN LIU-IMH/RV-A-10/001-SE.
53. Helmersson T (2010) Evaluation of Synthetic MRI for Clinical Use. Master's thesis, Linköpings Universitet. ISRN LIU-IMH/RV-A-10/002-SE.
54. Blystad I, Warntjes B J, Smedby O, Landtblom M A, Lundberg P, et al. (2012) Synthetic MRI of the brain in a clinical setting. *Acta Radiologica* 53: 1158-1163.

55. Gulani V, Schmitt P, Griswold A M, Webb G A, Jakob M P (2004) Towards a single-sequence neurologic magnetic resonance imaging examination: multiple-contrast images from an IR TrueFISP experiment. *Investigative Radiology* 39: 767-774.
56. Alfano B, Brunetti A, Ciarmiello A, Salvatore M (1992) Simultaneous display of multiple MR parameters with "quantitative magnetic color imaging". *Journal of Computer Assisted Tomography* 16: 634-640.
57. West J (2007) Synthetic MRI Visualization Principles and Application. Master's thesis, Linköpings Universitet. ISRN LITH-IMT/MI20-EX-07/454-SE.
58. Bakker J C, de Graaf N C, van Dijk P (1984) Derivation of quantitative information in NMR imaging: a phantom study. *Physics in Medicine and Biology* 29: 1511-1525.
59. Wehrli W F, Breger K R, MacFall R J, Daniels L D, Haughton M V, et al. (1985) Quantification of contrast in clinical MR brain imaging at high magnetic field. *Investigative Radiology* 20: 360-369.
60. Neeb H, Zilles K, Shah J N (2006) A new method for fast quantitative mapping of absolute water content in vivo. *Neuroimage* 31: 1156-1168.
61. Tofts P (2004) Quantitative MRI of the Brain Measuring Changes Caused by Disease. Wiley. ISBN 0-470-84721-2.
62. Gasparovic C, Neeb H, Feis L D, Damaraju E, Chen H, et al. (2009) Quantitative spectroscopic imaging with in situ measurements of tissue water T1, T2, and density. *Magnetic Resonance in Medicine* 62: 583-590.
63. Fatouros P P, Marmarou A (1990) Experimental studies for use of magnetic resonance in brain water measurements. *Acta Neurochir Suppl* 51: 37-38.
64. Fatouros P P, Marmarou A, Kraft A K, Inao S, Schwarz P F (1991) In vivo brain water determination by T1 measurements: effect of total water content, hydration fraction, and field strength. *Magnetic Resonance in Medicine* 17: 402-413.
65. Fatouros P P, Marmarou A (1999) Use of magnetic resonance imaging for in vivo measurements of water content in human brain: method and normal values. *Journal of Neurosurgery* 90: 109-115.
66. Volz S, Nöth U, Jurcoane A, Ziemann U, Hattingen E, et al. (2012) Quantitative proton density mapping: correcting the receiver sensitivity bias via pseudo proton densities. *Neuroimage* 63: 540-552.
67. Labadie C, Lee H J, Rooney D W, Jarchow S, Aubert-Frécon M, et al. (2013) Myelin water mapping by spatially regularized longitudinal relaxographic imaging at high magnetic fields. *Magnetic Resonance in Medicine* : doi: 10.1002/mrm.24670. [Epub ahead of print].
68. Andersen C, Jensen T F (1994) Precision, accuracy, and image plane uniformity in NMR relaxation time imaging on a 1.5 T whole-body MR imaging system. *Magnetic Resonance Imaging* 12: 775-784.
69. Look D, Locker D (1970) Time Saving in Measurement of NMR and EPR Relaxation Times. *Rev Sci Instr* 41: 250-251.
70. MacKay A, Whittall K, Adler J, Li D, Paty D, et al. (1994) In vivo visualization of myelin water in brain by magnetic resonance. *Magnetic Resonance in Medicine* 31: 673-677.
71. Whittall P K, Mackay L A, Graeb A D, Nugent A R, Li K D, et al. (1997) In vivo measurement of T2 distributions and water contents in normal human brain. *Magnetic Resonance in Medicine* 37: 34-43.

72. Whittall P K, MacKay L A, Li K D (1999) Are mono-exponential fits to a few echoes sufficient to determine T2 relaxation for in vivo human brain? *Magnetic Resonance in Medicine* 41: 1255-1257.
73. Kamman L R, Go G K, Brouwer W, Berendsen J H (1988) Nuclear magnetic resonance relaxation in experimental brain edema: effects of water concentration, protein concentration, and temperature. *Magnetic Resonance in Medicine* 6: 265-274.
74. MacDonald L H, Bell A B, Smith A M, Kean M D, Tocher L J, et al. (1986) Correlation of human NMR T1 values measured in vivo and brain water content. *British Journal of Radiology* 59: 355-357.
75. Koenig H S, Brown Dr R, Spiller M, Lundbom N (1990) Relaxometry of brain: why white matter appears bright in MRI. *Magnetic Resonance in Medicine* 14: 482-495.
76. Ye Q F, Martin R W, Allen S P (1996) Estimation of brain iron in vivo by means of the interecho time dependence of image contrast. *Magnetic Resonance in Medicine* 36: 153-158.
77. Gelman N, Ewing R J, Gorell M J, Spickler M E, Solomon G E (2001) Interregional variation of longitudinal relaxation rates in human brain at 3.0 T: relation to estimated iron and water contents. *Magnetic Resonance in Medicine* 45: 71-79.
78. Rooney D W, Johnson G, Li X, Cohen R E, Kim G S, et al. (2007) Magnetic field and tissue dependencies of human brain longitudinal $1H_2O$ relaxation in vivo. *Magnetic Resonance in Medicine* 57: 308-318.
79. Hocq A, Brouette N, Saussez S, Luhmer M, Gillis P, et al. (2009) Variable-field relaxometry of iron-containing human tissues: a preliminary study. *Contrast Media & Molecular Imaging* 4: 157-164.
80. Schenker C, Meier D, Wichmann W, Boesiger P, Valavanis A (1993) Age distribution and iron dependency of the T2 relaxation time in the globus pallidus and putamen. *Neuroradiology* 35: 119-124.
81. Schenck F J (1995) Imaging of brain iron by magnetic resonance: T2 relaxation at different field strengths. *Journal of the Neurological Sciences* 134 Suppl: 10-18.
82. Hardy A P, Gash D, Yokel R, Andersen A, Ai Y, et al. (2005) Correlation of R2 with total iron concentration in the brains of rhesus monkeys. *Journal of Magnetic Resonance Imaging* 21: 118-127.
83. Levesque R I, Pike B G (2009) Characterizing healthy and diseased white matter using quantitative magnetization transfer and multicomponent T(2) relaxometry: A unified view via a four-pool model. *Magnetic Resonance in Medicine* 62: 1487-1496.
84. Levesque R I, Giacomini S P, Narayanan S, Ribeiro T L, Sled G J, et al. (2010) Quantitative magnetization transfer and myelin water imaging of the evolution of acute multiple sclerosis lesions. *Magnetic Resonance in Medicine* 63: 633-640.
85. Lenz C, Klarhöfer M, Scheffler K (2012) Feasibility of in vivo myelin water imaging using 3D multigradient-echo pulse sequences. *Magnetic Resonance in Medicine* 68: 523-528.
86. Deoni C S, Rutt K B, Arun T, Pierpaoli C, Jones K D (2008) Gleaning multicomponent T1 and T2 information from steady-state imaging data. *Magnetic Resonance in Medicine* 60: 1372-1387.

87. Deoni C S, Matthews L, Kolind H S (2013) One component? two components? three? the effect of including a nonexchanging "free" water component in multicomponent driven equilibrium single pulse observation of T1 and T2. *Magnetic Resonance in Medicine* 70: 147-154.
88. Does D M, Gore C J (2002) Compartmental study of T(1) and T(2) in rat brain and trigeminal nerve in vivo. *Magnetic Resonance in Medicine* 47: 274-283.
89. Lancaster L J, Andrews T, Hardies J L, Dodd S, Fox T P (2003) Three-pool model of white matter. *Journal of Magnetic Resonance Imaging* 17: 1-10.
90. Laule C, Vavasour M I, Mädler B, Kolind H S, Sirrs M S, et al. (2007) MR evidence of long T2 water in pathological white matter. *Journal of Magnetic Resonance Imaging* 26: 1117-1121.
91. Laule C, Vavasour M I, Kolind S H, Traboulsee A L, Moore G R, et al. (2007) Long T2 water in multiple sclerosis: What else can we learn from multi-echo T2 relaxation? *Journal of Neurology* 254: 1579-1587.
92. Hare J D, Lee K J, Beavis D A, van Gramberg A, George J, et al. (2012) Three-dimensional atlas of iron, copper, and zinc in the mouse cerebrum and brainstem. *Analytical Chemistry* 84: 3990-3997.
93. Hallgren B, Sourander P (1958) The effect of age on the non-haemin iron in the human brain. *Journal of Neurochemistry* 3: 41-51.
94. Quintana C, Bellefqih S, Laval Y J, Guerquin-Kern L J, Wu D T, et al. (2006) Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. *Journal of Structural Biology* 153: 42-54.
95. Szumowski J, Bas E, Gaarder K, Schwarz E, Erdogmus D, et al. (2010) Measurement of brain iron distribution in Hallevorden-Spatz syndrome. *Journal of Magnetic Resonance Imaging* 31: 482-489.
96. Dexter T D, Wells R F, Lees J A, Agid F, Agid Y, et al. (1989) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *Journal of Neurochemistry* 52: 1830-1836.
97. Dexter T D, Carayon A, Vidailhet M, Ruberg M, Agid F, et al. (1990) Decreased ferritin levels in brain in Parkinson's disease. *Journal of Neurochemistry* 55: 16-20.
98. Lewis M M, Du G, Kidacki M, Patel N, Shaffer L M, et al. (2013) Higher iron in the red nucleus marks Parkinson's dyskinesia. *Neurobiology of Aging* 34: 1497-1503.
99. Craelius W, Migdal W M, Luessenhop P C, Sugar A, Mihalakis I (1982) Iron deposits surrounding multiple sclerosis plaques. *Archives of Pathology & Laboratory Medicine* 106: 397-399.
100. Paling D, Tozer D, Wheeler-Kingshott C, Kapoor R, Miller H D, et al. (2012) Reduced R2' in multiple sclerosis normal appearing white matter and lesions may reflect decreased myelin and iron content. *Journal of Neurology, Neurosurgery & Psychiatry* 83: 785-792.
101. Mehta V, Pei W, Yang G, Li S, Swamy E, et al. (2013) Iron is a sensitive biomarker for inflammation in multiple sclerosis lesions. *PloS ONE* 8: doi: 10.1371/journal.pone.0057573.
102. Bagnato F, Hametner S, Welch B E (2013) Visualizing iron in multiple sclerosis. *Magnetic Resonance Imaging* 31: 376-384.
103. Walsh J A, Blevins G, Lebel M R, Seres P, Emery J D, et al. (2013) Longitudinal MR imaging of iron in multiple sclerosis: An imaging marker of disease. *Radiology* : doi: 10.1148/radiol.13130474 [Epub ahead of print].

104. Al-Radaideh M A, Wharton J S, Lim Y S, Tench R C, Morgan S P, et al. (2013) Increased iron accumulation occurs in the earliest stages of demyelinating disease: an ultra-high field susceptibility mapping study in Clinically Isolated Syndrome. *Multiple Sclerosis* 19: 896-903.
105. Filippi M (2001) In-vivo tissue characterization of multiple sclerosis and other white matter diseases using magnetic resonance based techniques. *Journal of Neurology* 248: 1019-1029.
106. Wu Y, Warfield K S, Tan L I, Wells M I W, Meier S D, et al. (2006) Automated segmentation of multiple sclerosis lesion subtypes with multichannel MRI. *Neuroimage* 32: 1205-1215.
107. Rovira A, Leon A (2008) MR in the diagnosis and monitoring of multiple sclerosis: an overview. *European Journal of Radiology* 67: 409-414.
108. Simmons A, Westman E, Muehlboeck S, Mecocci P, Vellas B, et al. (2009) MRI measures of Alzheimer's disease and the AddNeuroMed study. *Annals of the New York Academy of Sciences* 1180: 47-55.
109. Jack R J C, Slomkowski M, Gracon S, Hoover M T, Felmlee P J, et al. (2003) MRI as a biomarker of disease progression in a therapeutic trial of milameline for AD. *Neurology* 60: 253-260.
110. Bozzali M, Cercignani M, Caltagirone C (2008) Brain volumetrics to investigate aging and the principal forms of degenerative cognitive decline: a brief review. *Magnetic Resonance Imaging* 26: 1065-1070.
111. Barnes J, Ourselin S, Fox C N (2009) Clinical application of measurement of hippocampal atrophy in degenerative dementia. *Hippocampus* 19: 510-516.
112. Guttmann R C, Jolesz A F, Kikinis R, Killiany J R, Moss B M, et al. (1998) White matter changes with normal aging. *Neurology* 50: 972-978.
113. Moncrieff J, Leo J (2010) A systematic review of the effects of antipsychotic drugs on brain volume. *Psychological Medicine* 40: 1409-1422.
114. Vannier M W, Butterfield L R, Jordan D, Murphy A W, Levitt R G, et al. (1985) Multispectral analysis of magnetic resonance images. *Radiology* 154: 221-224.
115. Kikinis R, Shenton E M, Gerig G, Martin J, Anderson M, et al. (1992) Routine quantitative analysis of brain and cerebrospinal fluid spaces with MR imaging. *Journal of Magnetic Resonance Imaging* 2: 619-629.
116. Jackson F E, Narayana A P, Falconer J C (1994) Reproducibility of nonparametric feature map segmentation for determination of normal human intracranial volumes with MR imaging data. *Journal of Magnetic Resonance Imaging* 4: 692-700.
117. Alfano B, Brunetti A, Covelli M E, Quarantelli M, Panico R M, et al. (1997) Unsupervised, automated segmentation of the normal brain using a multispectral relaxometric magnetic resonance approach. *Magnetic Resonance in Medicine* 37: 84-93.
118. Andersen A H, Zhang Z, Avison J M, Gash M D (2002) Automated segmentation of multispectral brain MR images. *Journal of Neuroscience Methods* 122: 13-23.
119. Fischl B (2012) FreeSurfer. *Neuroimage* 62: 774-781.
120. Choi S H, Haynor R D, Kim Y (1991) Partial volume tissue classification of multichannel magnetic resonance images-a mixel model. *IEEE Transactions on Medical Imaging* 10: 395-407.

121. Bonar C D, Schaper A K, Anderson R J, Rottenberg A D, Strother C S (1993) Graphical analysis of MR feature space for measurement of CSF, gray-matter, and white-matter volumes. *Journal of Computer Assisted Tomography* 17: 461-470.
122. Shattuck W D, Sandor-Leahy R S, Schaper A K, Rottenberg A D, Leahy M R (2001) Magnetic resonance image tissue classification using a partial volume model. *Neuroimage* 13: 856-876.
123. Noe A, Gee C J (2001) Partial volume segmentation of cerebral MRI scans with mixture model clustering. *Lecture Notes in Computer Science* 2082: 423-430.
124. Rudick A R, Fisher E, Lee C J, Simon J, Jacobs L (1999) Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. *Neurology* 53: 1698-1704.
125. Vågberg M, Lindqvist T, Ambarki K, Warntjes B J, Sundström P, et al. (2013) Automated determination of brain parenchymal fraction in multiple sclerosis. *American Journal of Neuroradiology* 34: 498-504.
126. Fennema-Notestine C, Ozyurt B I, Clark P C, Morris S, Bischoff-Grethe A, et al. (2006) Quantitative evaluation of automated skull-stripping methods applied to contemporary and legacy images: effects of diagnosis, bias correction, and slice location. *Human Brain Mapping* 27: 99-113.
127. Ambarki K, Lindqvist T, Wåhlin A, Pettersson E, Warntjes J M, et al. (2012) Evaluation of automatic measurement of the intracranial volume based on quantitative MR imaging. *American Journal of Neuroradiology* 33: 1951-1956.
128. Bottomley A P, Foster H T, Argersinger E R, Pfeifer L M (1984) A review of normal tissue hydrogen NMR relaxation times and relaxation mechanisms from 1-100 MHz: dependence on tissue type, NMR frequency, temperature, species, excision, and age. *Medical Physics* 11: 425-448.
129. Breger K R, Wehrli W F, Charles C H, MacFall R J, Haughton M V (1986) Reproducibility of relaxation and spin-density parameters in phantoms and the human brain measured by MR imaging at 1.5 T. *Magnetic Resonance in Medicine* 3: 649-662.
130. Fletcher M L, Barsotti B J, Hornak P J (1993) A multispectral analysis of brain tissues. *Magnetic Resonance in Medicine* 29: 623-630.
131. Bottomley A P, Hardy J C, Argersinger E R, Allen-Moore G (1987) A review of ¹H nuclear magnetic resonance relaxation in pathology: are T1 and T2 diagnostic? *Medical Physics* 14: 1-37.
132. Han X, Jovicich J, Salat D, van der Kouwe A, Quinn B, et al. (2006) Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *Neuroimage* 32: 180-194.
133. Jovicich J, Czanner S, Han X, Salat D, van der Kouwe A, et al. (2009) MRI-derived measurements of human subcortical, ventricular and intracranial brain volumes: Reliability effects of scan sessions, acquisition sequences, data analyses, scanner upgrade, scanner vendors and field strengths. *Neuroimage* 46: 177-192.
134. Keihaninejad S, Heckemann A R, Fagiolo G, Symms R M, Hajnal V J, et al. (2010) A robust method to estimate the intracranial volume across MRI field strengths (1.5T and 3T). *Neuroimage* 50: 1427-1438.
135. Pfefferbaum A, Rohlfing T, Rosenbloom J M, Sullivan V E (2012) Combining atlas-based parcellation of regional brain data acquired across scanners at 1.5 T and 3.0 T field strengths. *Neuroimage* 60: 940-951.

136. Paty W D, Oger JJ, Kastrukoff F L, Hashimoto A S, Hooge P J, et al. (1988) MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. *Neurology* 38: 180-185.
137. Barkhof F (2002) The clinico-radiological paradox in multiple sclerosis revisited. *Current Opinion in Neurology* 15: 239-245.
138. Brex A P, Parker J G, Leary M S, Molyneux D P, Barker J G, et al. (2000) Lesion heterogeneity in multiple sclerosis: a study of the relations between appearances on t1 weighted images, T1 relaxation times, and metabolite concentrations. *Journal of Neurology, Neurosurgery & Psychiatry* 68: 627-632.
139. Minneboo A, Uitdehaag M B, Jongen P, Vrenken H, Knol D, et al. (2009) Association between MRI parameters and the MS severity scale: a 12 year follow-up study. *Multiple Sclerosis* 15: 632-637.
140. Rocca A M, Colombo B, Falini A, Ghezzi A, Martinelli V, et al. (2005) Cortical adaptation in patients with MS: a cross-sectional functional MRI study of disease phenotypes. *Lancet Neurology* 4: 618-626.
141. Audoin B, Guye M, Reuter F, Au Duong V M, Confort-Gouny S, et al. (2007) Structure of WM bundles constituting the working memory system in early multiple sclerosis: a quantitative DTI tractography study. *Neuroimage* 36: 1324-1330.
142. Harirchian H M, Rezvanizadeh A, Fakhri M, Oghabian A M, Ghoreishi A, et al. (2010) Non-invasive brain mapping of motor-related areas of four limbs in patients with clinically isolated syndrome compared to healthy normal controls. *Journal of Clinical Neuroscience* 17: 736-741.
143. Kincses T Z, Ropele S, Jenkinson M, Khalil M, Petrovic K, et al. (2011) Lesion probability mapping to explain clinical deficits and cognitive performance in multiple sclerosis. *Multiple Sclerosis* 17: 681-689.
144. Sbardella E, Petsas N, Tona F, Prosperini L, Raz E, et al. (2013) Assessing the correlation between grey and white matter damage with motor and cognitive impairment in multiple sclerosis patients. *PLoS ONE* 8: doi: 10.1371/journal.pone.0063250. Print 2013.
145. Kidd D, Barkhof F, McConnell R, Algra R P, Allen V I, et al. (1999) Cortical lesions in multiple sclerosis. *Brain* 122: 17-26.
146. Kutzelnigg A, Lucchinetti F C, Stadelmann C, Brück W, Rauschka H, et al. (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128: 2705-2712.
147. Vercellino M, Masera S, Lorenzatti M, Condello C, Merola A, et al. (2009) Demyelination, inflammation, and neurodegeneration in multiple sclerosis deep gray matter. *Journal of Neuropathology & Experimental Neurology* 68: 489-502.
148. Hagemeyer J, Weinstock-Guttman B, Heininen-Brown M, Poloni U G, Bergsland N, et al. (2013) Gray matter SWI-filtered phase and atrophy are linked to disability in MS. *Frontiers in bioscience (Elite edition)* 5: 525-532.
149. Evangelou N, Esiri M M, Smith S, Palace J, Matthews M P (2000) Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. *Annals of Neurology* 47: 391-395.
150. Allen V I, McQuaid S, Mirakhur M, Nevin G (2001) Pathological abnormalities in the normal-appearing white matter in multiple sclerosis. *Neurological Sciences* 22: 141-144.

151. Vrenken H, Geurts J J, Knol L D, van Dijk N L, Dattola V, et al. (2006) Whole-brain T1 mapping in multiple sclerosis: global changes of normal-appearing gray and white matter. *Radiology* 240: 811-820.
152. Moll M N, Rietsch M A, Thomas S, Ransohoff J A, Lee C J, et al. (2011) Multiple sclerosis normal-appearing white matter: pathology-imaging correlations. *Annals of Neurology* 70: 764-773.
153. Mezer A, Yeatman D J, Stikov N, Kay N K, Cho J N, et al. (2013) Quantifying the local tissue volume and composition in individual brains with magnetic resonance imaging. *Nature Medicine* : doi: 10.1038/nm.3390. [Epub ahead of print].
154. Chen C S, Chung W H, Liou M (2003) Measurement of volumetric lesion load in multiple sclerosis: moving from normal- to dirty-appearing white matter. *American Journal of Neuroradiology* 24: 1929-1930.
155. Ge Y, Grossman I R, Babb S J, He J, Mannon J L (2003) Dirty-appearing white matter in multiple sclerosis: volumetric MR imaging and magnetization transfer ratio histogram analysis. *American Journal of Neuroradiology* 24: 1935-1940.
156. Moore R G, Laule C, Mackay A, Leung E, Li K D, et al. (2008) Dirty-appearing white matter in multiple sclerosis: preliminary observations of myelin phospholipid and axonal loss. *Journal of Neurology* 255: 1802-1811, discussion 1812.
157. Seewann A, Vrenken H, van der Valk P, Blezer L E, Knol L D, et al. (2009) Diffusely abnormal white matter in chronic multiple sclerosis: imaging and histopathologic analysis. *Archives of neurology* 66: 601-609.
158. Vrenken H, Seewann A, Knol L D, Polman H C, Barkhof F, et al. (2010) Diffusely abnormal white matter in progressive multiple sclerosis: in vivo quantitative MR imaging characterization and comparison between disease types. *American Journal of Neuroradiology* 31: 541-548.
159. Evangelou N, Konz D, Esiri M M, Smith S, Palace J, et al. (2000) Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain* 123: 1845-1849.
160. Filippi M, Cercignani M, Inglese M, Horsfield A M, Comi G (2001) Diffusion tensor magnetic resonance imaging in multiple sclerosis. *Neurology* 56: 304-311.
161. Tisell A, Leinhard D O, Warntjes B J, Lundberg P (2013) Procedure for quantitative (1) H magnetic resonance spectroscopy and tissue characterization of human brain tissue based on the use of quantitative magnetic resonance imaging. *Magnetic Resonance in Medicine* 70: 905-915.
162. Tisell A, Leinhard D O, Warntjes B J, Aalto A, Smedby, et al. (2013) Increased concentrations of glutamate and glutamine in normal-appearing white matter of patients with multiple sclerosis and normal MR imaging brain scans. *Plos ONE* 8: doi: 10.1371/journal.pone.0061817.
163. Thorpe W J, Kidd D, Moseley F I, Thompson J A, MacManus D G, et al. (1996) Spinal MRI in patients with suspected multiple sclerosis and negative brain MRI. *Brain* 119: 709-714.
164. Gustafsson C M, Dahlqvist O, Jaworski J, Lundberg P, Landtblom M A (2007) Low choline concentrations in normal-appearing white matter of patients with multiple sclerosis and normal MR imaging brain scans. *American Journal of Neuroradiology* 28: 1306-1312.
165. MacKay L A, Vavasour M I, Rauscher A, Kolind H S, Mädler B, et al. (2009) MR relaxation in multiple sclerosis. *Neuroimaging Clinics of North America* 19: 1-26.

166. Deoni C S (2010) Quantitative relaxometry of the brain. *Topics in Magnetic Resonance Imaging* 21: 101-113.
167. Ormerod E I, Bronstein A, Rudge P, Johnson G, Macmanus D, et al. (1986) Magnetic resonance imaging in clinically isolated lesions of the brain stem. *Journal of Neurology, Neurosurgery & Psychiatry* 49: 737-743.
168. Larsson B H, Frederiksen J, Petersen J, Nordenbo A, Zeeberg I, et al. (1989) Assessment of demyelination, edema, and gliosis by in vivo determination of T1 and T2 in the brain of patients with acute attack of multiple sclerosis. *Magnetic Resonance in Medicine* 11: 337-348.
169. Whittall P K, MacKay L A, Li K D, Vavasour M I, Jones K C, et al. (2002) Normal-appearing white matter in multiple sclerosis has heterogeneous, diffusely prolonged T(2). *Magnetic Resonance in Medicine* 47: 403-408.
170. Goodkin E D, Rooney D W, Sloan R, Bacchetti P, Gee L, et al. (1998) A serial study of new MS lesions and the white matter from which they arise. *Neurology* 51: 1689-1697.
171. van Walderveen A M, Barkhof F, Pouwels J P, van Schijndel A R, Polman H C, et al. (1999) Neuronal damage in T1-hypointense multiple sclerosis lesions demonstrated in vivo using proton magnetic resonance spectroscopy. *Annals of Neurology* 46: 79-87.
172. van Waesberghe H J, Kamphorst W, De Groot J C, van Walderveen A M, Castelijns A J, et al. (1999) Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Annals of Neurology* 46: 747-754.
173. Bjartmar C, Battistuta J, Terada N, Dupree E, Trapp D B (2002) N-acetylaspartate is an axon-specific marker of mature white matter in vivo: a biochemical and immunohistochemical study on the rat optic nerve. *Annals of Neurology* 51: 51-58.
174. Li S B, Regal J, Soher J B, Mannon J L, Grossman I R, et al. (2003) Brain metabolite profiles of T1-hypointense lesions in relapsing-remitting multiple sclerosis. *American Journal of Neuroradiology* 24: 68-74.
175. de Graaf A R (2007) *in vivo NMR Spectroscopy Principles and Techniques*. Wiley, second edition. ISBN 978-0-470-02670-0.
176. Brück W, Bitsch A, Kolenda H, Brück Y, Stiefel M, et al. (1997) Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. *Annals of Neurology* 42: 783-793.
177. van Walderveen A M, Kamphorst W, Scheltens P, van Waesberghe H J, Ravid R, et al. (1998) Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. *Neurology* 50: 1282-1288.
178. Truyen L, van Waesberghe H J, van Walderveen A M, van Oosten W B, Polman H C, et al. (1996) Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. *Neurology* 47: 1469-1476.
179. van Walderveen A M, Barkhof F, Hommes R O, Polman H C, Tobi H, et al. (1995) Correlating MRI and clinical disease activity in multiple sclerosis: relevance of hypointense lesions on short-TR/short-TE (T1-weighted) spin-echo images. *Neurology* 45: 1684-1690.
180. Ormerod E I, Johnson G, MacManus D, du Boulay P E, McDonald I W (1986) Relaxation times of apparently normal cerebral white matter in multiple sclerosis. *Acta radiologica Supplementum* 369: 382-384.

181. Miller H D, Johnson G, Tofts S P, MacManus D, McDonald I W (1989) Precise relaxation time measurements of normal-appearing white matter in inflammatory central nervous system disease. *Magnetic Resonance in Medicine* 11: 331-336.
182. Barbosa S, Blumhardt D L, Roberts N, Lock T, Edwards H R (1994) Magnetic resonance relaxation time mapping in multiple sclerosis: normal appearing white matter and the "invisible" lesion load. *Magnetic Resonance Imaging* 12: 33-42.
183. Stevenson L V, Parker J G, Barker J G, Birnie K, Tofts S P, et al. (2000) Variations in T1 and T2 relaxation times of normal appearing white matter and lesions in multiple sclerosis. *Journal of the Neurological Sciences* 178: 81-87.
184. Parry A, Clare S, Jenkinson M, Smith S, Palace J, et al. (2002) White matter and lesion T1 relaxation times increase in parallel and correlate with disability in multiple sclerosis. *Journal of Neurology* 249: 1279-1286.
185. Srinivasan R, Henry R, Pelletier D, Nelson S (2003) Standardized, reproducible, high resolution global measurements of T1 relaxation metrics in cases of multiple sclerosis. *American Journal of Neuroradiology* 24: 58-67.
186. Vrenken H, Rombouts A S, Pouwels J P, Barkhof F (2006) Voxel-based analysis of quantitative T1 maps demonstrates that multiple sclerosis acts throughout the normal-appearing white matter. *American Journal of Neuroradiology* 27: 868-874.
187. Davies R G, Hadjiprocopis A, Altmann R D, Chard T D, Griffin M C, et al. (2007) Normal-appearing grey and white matter T1 abnormality in early relapsing-remitting multiple sclerosis: a longitudinal study. *Multiple Sclerosis* 13: 169-177.
188. Larsson B H, Frederiksen J, Kjaer L, Henriksen O, Olesen J (1988) In vivo determination of T1 and T2 in the brain of patients with severe but stable multiple sclerosis. *Magnetic Resonance in Medicine* 7: 43-55.
189. Grenier D, Pelletier D, Normandeau M, Newitt D, Nelson S, et al. (2002) T2 relaxation time histograms in multiple sclerosis. *Magnetic Resonance Imaging* 20: 733-741.
190. Vavasour M I, Li K D, Laule C, Traboulsee L A, Moore R G, et al. (2007) Multi-parametric mr assessment of T(1) black holes in multiple sclerosis : evidence that myelin loss is not greater in hypointense versus isointense T(1) lesions. *Journal of Neurology* 254: 1653-1659.
191. Howell W O, Rundle L J, Garg A, Komada M, Brophy J P, et al. (2010) Activated microglia mediate axoglia disruption that contributes to axonal injury in multiple sclerosis. *Journal of Neuropathology & Experimental Neurology* 69: 1017-1033.
192. Vrenken H, Barkhof F, Uitdehaag M B, Castelijns A J, Polman H C, et al. (2005) MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. *Magnetic Resonance in Medicine* 53: 256-266.
193. Castriota-Scanderbeg A, Fasano F, Filippi M, Caltagirone C (2004) T1 relaxation maps allow differentiation between pathologic tissue subsets in relapsing-remitting and secondary progressive multiple sclerosis. *Multiple Sclerosis* 10: 556-561.
194. Vrenken H, Geurts J J, Knol L D, Polman H C, Castelijns A J, et al. (2006) Normal-appearing white matter changes vary with distance to lesions in multiple sclerosis. *American Journal of Neuroradiology* 27: 2005-2011.
195. Webb S, Munro A C, Midha R, Stanisz J G (2003) Is multicomponent T2 a good measure of myelin content in peripheral nerve? *Magnetic Resonance in Medicine* 49: 638-645.

196. Laule C, Vavasour M I, Moore G R, Oger J, Li D B K, et al. (2004) Water content and myelin water fraction in multiple sclerosis. *Journal of Neurology* 251: 284-293.
197. Laule C, Leung E, Li K D, Traboulsee L A, Paty W D, et al. (2006) Myelin water imaging in multiple sclerosis: quantitative correlations with histopathology. *Multiple Sclerosis* 12: 747-753.
198. Laule C, Kozlowski P, Leung E, Li K D, Mackay L A, et al. (2008) Myelin water imaging of multiple sclerosis at 7 T: correlations with histopathology. *Neuroimage* 40: 1575-1580.
199. Vavasour M I, Laule C, Li K D, Oger J, Moore R G, et al. (2009) Longitudinal changes in myelin water fraction in two MS patients with active disease. *Journal of the Neurological Sciences* 276: 49-53.
200. Laule C, Vavasour M I, Zhao Y, Traboulsee L A, Oger J, et al. (2010) Two-year study of cervical cord volume and myelin water in primary progressive multiple sclerosis. *Multiple Sclerosis* 16: 670-677.
201. Stanisz J G, Webb S, Munro A C, Pun T, Midha R (2004) MR properties of excised neural tissue following experimentally induced inflammation. *Magnetic Resonance in Medicine* 51: 473-479.
202. Odrobina E E, Lam Y T, Pun T, Midha R, Stanisz J G (2005) MR properties of excised neural tissue following experimentally induced demyelination. *NMR in Biomedicine* 18: 277-284.
203. Groeneveld A R, Meeden G (1984) Measuring skewness and kurtosis. *Journal of the Royal Statistical Society Series D (The Statistician)* 33: 391-399.
204. Poser M C, Paty W D, Scheinberg L, McDonald I W, Davis A F, et al. (1983) New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Annals of Neurology* 13: 227-231.
205. Bottomley A P (1987) Spatial localization in NMR spectroscopy in vivo. *Annals of the New York Academy of Sciences* 508: 333-348.
206. Bland M J, Altman G D (1996) Measurement error. *BMJ* 313: 744.
207. Lee J, van Gelderen P, Kuo W L, Merkle H, Silva C A, et al. (2011) T2*-based fiber orientation mapping. *Neuroimage* 57: 225-234.
208. Vymazal J, Brooks A R, Zak O, McRill C, Shen C, et al. (1992) T1 and T2 of ferritin at different field strengths: effect on MRI. *Magnetic Resonance in Medicine* 27: 368-374.
209. Gomori M J, Grossman I R (1988) Mechanisms responsible for the MR appearance and evolution of intracranial hemorrhage. *Radiographics* 8: 427-440.