

Quantitative T2 imaging of whole post-mortem brains in ALS

Feng Qi

St Catherine's College
University of Oxford

*A thesis submitted for the degree of
Doctor of Philosophy*

Trinity 2019

Abstract

Quantitative MRI has promise to provide precise measures for assessing neurological disorders such as amyotrophic lateral sclerosis (ALS). While MRI is very sensitive to pathological changes in tissue, it lacks the specificity needed for biological interpretability. One way to address this is to compare MRI to more direct measurements, such as histopathology. In this thesis, I present methods that enable direct comparison of quantitative T2 imaging to histopathology in the same post-mortem tissue samples, with the aim of better understanding alterations to T2 in ALS. This requires that we address several challenges to acquiring T2 maps in post-mortem brains, particularly in the context of our study at 7 tesla. First, 7T MRI exhibits considerable spatial inhomogeneity of the B1 field, leading to non-exponential signal evolution. To more accurately model the signal, we utilize the extended phase graph (EPG) model and derive voxel-wise maps of B1, T2 and S0 simultaneously. We show that EPG fitting provides improved T2 mapping under inhomogeneous B1 field. A second issue for quantitative T2 mapping in post-mortem brains is the influence of formalin fixation, which can dramatically reduce the T2 value in proportion with the formalin concentration distribution. We propose a kinetic tensor (KT) model to simulate 4D formalin flux under Fick's diffusion law. Based on the KT prediction of formalin concentration, we propose a simple linear correction of quantitative T2 maps. Combining EPG fitting with the KT correction, we demonstrate that quantitative T2 map values in motor cortex can distinguish ALS brains from the control group, and that quantitative T2 value is significantly correlated to the tissue ferritin content. This work provides evidence that quantitative T2 mapping may provide a non-invasive marker of cortical iron deposition in ALS.

Quantitative T2 imaging of whole post-mortem brains in ALS



Feng Qi
St Catherine's College
University of Oxford

A thesis submitted for the degree of
Doctor of Philosophy
Trinity 2019

Acknowledgements

I would like to thank all the people who have accompanied me through my DPhil. stage. I could not make the glorious achievement without the invaluable academic and family support from them.

Firstly, I am very grateful to my supervisor, Professor Karla Miller for her priceless support and guidance throughout my DPhil. Karla is the best scientist in MRI physics, she has taught me how to be a great professional researcher in academic areas with her deep insight in MRI and considerable encouragement. I appreciate all her contributions of time, ideas, and funding to make my DPhil. productive. I would also like to thank my co-supervisors Dr. Benjamin Tandler and Dr. Samuel Hurley. They are professional postdoctoral researchers and the source of good advice and collaboration. They are always available to discuss projects and provide help at any time and any aspect of my research.

Secondly, I would like to thank many people in FMRIB for contributing immensely to my research project and thesis. I have had my greatest time study and work here. Especially, Wenchuan Wu is my best friend. I appreciate all his precious discussions in the thesis and all other aspects.

Thirdly, I wish to thank my Mom and Dad. They have endured a very hard life to give me financial support on my DPhil. study. There is no word that can describe my gratitude for them. Also my sister JiaJia and DaPeng, without their critical help, I may have already sold out my house for the tuition. And my son, no matter what difficulty I encounter, when saw him, I will always be full of joy and hope.

Lastly and the most importantly, I wish to thank my wife. For these four years, she has worked hard to support the whole family and given me help in every aspect of life. It is the most thankful thing to have you in my life. I love you!

Abstract

Quantitative MRI has promise to provide precise measures for assessing neurological disorders such as amyotrophic lateral sclerosis (ALS). While MRI is very sensitive to pathological changes in tissue, it lacks the specificity needed for biological interpretability. One way to address this is to compare MRI to more direct measurements, such as histopathology. In this thesis, I present methods that enable direct comparison of quantitative T2 imaging to histopathology in the same post-mortem tissue samples, with the aim of better understanding alterations to T2 in ALS. This requires that we address several challenges to acquiring T2 maps in post-mortem brains, particularly in the context of our study at 7 tesla. First, 7T MRI exhibits considerable spatial inhomogeneity of the B1 field, leading to non-exponential signal evolution. To more accurately model the signal, we utilize the extended phase graph (EPG) model and derive voxel-wise maps of B1, T2 and S0 simultaneously. We show that EPG fitting provides improved T2 mapping under inhomogeneous B1 field. A second issue for quantitative T2 mapping in post-mortem brains is the influence of formalin fixation, which can dramatically reduce the T2 value in proportion with the formalin concentration distribution. We propose a kinetic tensor (KT) model to simulate 4D formalin flux under Fick's diffusion law. Based on the KT prediction of formalin concentration, we propose a simple linear correction of quantitative T2 maps. Combining EPG fitting with the KT correction, we demonstrate that quantitative T2 map values in motor cortex can distinguish ALS brains from the control group, and that quantitative T2 value is significantly correlated to the tissue ferritin content. This work provides evidence that quantitative T2 mapping may provide a non-invasive marker of cortical iron deposition in ALS.

Contents

1 Introduction.....	1
1.1 Motivation.....	1
1.2 Research Summary	2
1.3 Thesis Outline	3
1.4 Reference	4
2 Background.....	5
2.1 MRI Physics.....	7
2.1.1 MR signal.....	7
2.1.2 Longitudinal relaxation time, T1.....	8
2.1.3 Transverse relaxation time, T2.....	9
2.1.4 Bloch description	10
2.2 MR imaging formation.....	11
2.3 Pulse sequences.....	18
2.3.1 Basic pulse sequence	18
2.3.2 Measuring relaxation times.....	19
2.3.2.1 Gradient echo sequence	20
2.3.2.2 Spin echo	21
2.3.2.3 Fast spin echo	22
2.4 Diffusion Weighted MRI.....	24
2.4.1 Diffusion Physics.....	24
2.4.2 Measuring diffusion with MR.....	27
2.5 MRI of amyotrophic lateral sclerosis.....	32
2.6 Reference.....	35
3 Quantitative T2 mapping with Extended Phase Graph Theory...	40
3.1 Background.....	41
3.1.1 Quantitative T2 in ALS project.....	41
3.1.2 The fast spin echo sequence.....	42
3.2 Method	45
3.2.1 The EPG theory.....	45
3.2.2 B1 influence on FSE.....	53
3.2.3 T1 influence on FSE.....	55
3.2.4 Fitting: general approach	56

3.2.5 Two-stage fitting.....	60
3.2.5.1 Median filter.....	61
3.2.5.2 Regularisation	64
3.2.6 Experimental parameters	66
3.3 Results and Discussion.....	67
3.3.1 Quality of fit	67
3.3.2 Normalisation.....	69
3.3.3 Formalin influence.....	72
3.4 Conclusion.....	74
3.5 Reference.....	75
4 Kinetic Tensor Model for Quantitative T2 Correction	81
4.1 Background	80
4.1.1 Formalin fixation and brain preparation.....	80
4.1.2 Histological evidence of formalin influence.....	81
4.1.3 Physics interpretation of formalin influence on T2.....	83
4.2 Theory	86
4.2.1 Analytical solution of formalin distribution.....	87
4.2.2 Distance model.....	89
4.2.3 Kinetic Isotropic model.....	90
4.2.4 Kinetic Tensor model.....	91
4.2.5 T2 correction	98
4.3 Methods.....	99
4.3.1 Masks and segmentations	100
4.3.2 Hyperintensity artefact in diffusion data	104
4.3.3 Scan parameters and processing scripts.....	106
4.4 Results.....	108
4.5 Conclusion	114
4.6 References.	115
5 Quantitative T2 in post-mortem brain: ALS vs controls.....	120
5.1 Background.....	120
5.2 Method	122
5.2.1 Processing pipeline	122
5.2.2 Region of interest.....	123

5 Quantitative T2 in post-mortem brain: ALS vs controls.....	120
5.1 Background.....	120
5.2 Method	122
5.2.1 Processing pipeline	122
5.2.2 Region of interest.....	123
5.2.3 Quantitative T2 map processing.....	124
5.2.4 Histological sampling.....	125
5.2.5 Cohort information.....	126
5.3 Results and Discussion.....	128
5.3.1 Histological data.....	128
5.3.2 Quantitative T2 statistics.....	131
5.3.3 Ferritin and PLP versus T2 measures.....	132
5.4 Discussion.....	134
5.5 Conclusion.....	135
5.6 Reference.....	136
 6 Summary and Future Work.....	 141
6.1 Thesis Summary.....	141
6.2 Future Works	143
6.2.1 Quantitative T1 map.....	143
6.2.2 Computation efficiency.....	145
6.2.3 EPG fitting with AFI B1.....	147
6.3 Conclusion	148
6.4 Reference.....	148

Chapter 1

Introduction

Contents

1.1 Motivation.....	1
1.2 Research Summary	2
1.3 Thesis Outline	3
1.4 Reference	4

This DPhil thesis presents methods for quantitative T2 mapping in whole, post-mortem human brain, with application to amyotrophic lateral sclerosis (ALS). Magnetic resonance (MR) relaxometry is a non-invasive measurement that reflects tissue pathology and which ideally can be directly compared across subjects and scanners. Relaxometry is becoming widely used in clinics as a standard tool to study in-vivo diseases, but which requires validation in the same post-mortem tissue as is subjected to gold-standard histopathology. This Chapter describes the motivation of the research and an outline of the thesis.

1.1 Motivation

Most MR imaging (MRI) modalities suffer from one common problem: measurements are usually qualitative rather than quantitative, with measured values varying according to scanner hardware and acquisition parameters (Ooi *et al.*, 1999). This weakness precludes direct comparison across subjects and even within the same subject. Quantitative MRI modalities aim to enable scans to be compared in a robust and reliable manner. Transverse relaxation time, T2, is one of the quantitative measures that can be obtained from MRI and is the primary focus of

this thesis. While T2 is known to be altered in pathology, it is a property of the MR signal rather than a biological property of direct interest. For this reason, it is important to be able to compare T2 to more biologically interpretable measurements, such as histopathological stains. In general, this is ideally done in the same post-mortem tissue that is then subjected to histopathology. However, MRI of post-mortem tissue is not a trivial extension of in-vivo imaging, and often requires bespoke MRI methods, including both acquisition and signal processing.

1.2 Research Summary

In the following chapters, we address several key issues in quantitative T2 measurement of post-mortem brains. The resulting correction methods provide ‘clean’ quantitative T2 maps that distinguish ALS brains from the control group, which are then correlated to histopathology in the same tissue, suggesting iron deposition as a driver of these signal changes.

The first obstacle impeding the accurate measurements of quantitative T2, particularly at ultra-high magnetic field: inhomogeneity in the transmit field (B1) (Uğurbil *et al.*, 1988). The B1 field is used to generate radio frequency (RF) pulses to manipulate nuclei’s spins during T2 measurement. The inhomogeneous B1 strength across the brain causes the signal to deviate from the expected behavior, confounding quantification. This is particularly a problem for the acquisitions conducted at ultra-high fields, such as the 7T data analysed in this thesis (Ibrahim *et al.*, 2001). We utilize a T2 quantification method based on an extended phase graph (EPG) representation of the signal (Weigel *et al.*, 2015). EPG is a multivariate model that takes B1 and T2 as input variables and produces serial echo signals accordingly. Instead of supposing B1 is spatially homogeneous, we treat B1 as a voxel-wise variable across the whole brain, in a two-stage fitting process that also enforces smoothness of the B1 map.

The second obstacle impeding quantitative T2 in post-mortem brains: the effect of formalin fixation (Shepherd *et al.*, 2009). In most post-mortem brain studies, it is necessary to fix the brain tissue before histological staining in order to preserve the tissue state as well as possible. However, the fixative solution is usually made up of formaldehyde molecules, which are known to dramatically decrease the tissue T2 measurement (Shepherd *et al.*, 2009). It has previously been demonstrated that the process of “immersion fixation” used in human brains, in which fixative passively enters the brain through diffusion, can be simulated through basic diffusion physics. Since our brain samples were scanned with diffusion tensor imaging (DTI) (Behrens *et al.*, 2003) protocols, we propose a diffusion tensor model to simulate the formaldehyde molecules’ distribution and correct their influence on T2 accordingly. We demonstrate that the use of the full diffusion tensor (which we term the ‘kinetic tensor’ model) provides a better characterization of the patterns of T2 across the brain than previously proposed models (the ‘kinetic isotropy’ model) (Dawe *et al.*, 2009), as indicated by the overall homogeneity of the T2 maps. To our knowledge, this is the first attempt to *correct* for fixation in T2 maps, whereas previous studies aimed to simply *characterize* the effect of fixation.

Using the quantitative T2 measures of the motor cortex, we are able to differentiate the ALS brains from the control group. In addition, we compare these T2 quantifications with iron (ferritin) and myelin (PLP) staining in the same tissue, demonstrating that in ALS, alterations in T2 in the motor cortex appear to predominantly relate to changes in iron, with little evidence for changes to myelin. These results suggest that quantitative T2 may provide a biologically meaningful marker of tissue changes in ALS-related pathology.

1.3 Thesis Outline

This thesis is structured as follows:

Chapter 2 provides a brief introduction of MRI physics, including image formation principles, pulse sequences, and post-processing algorithms. We also outline specific methods relevant to this thesis, including MR relaxometry and diffusion-weighted MRI, and the state-of-the-art in imaging of ALS.

In Chapter 3, we present the EPG model as a solution to B1 inhomogeneity confounds in T2 mapping. We demonstrate the advantage of the EPG fitting method as compared with the conventional exponential decay model in the presence of the inhomogeneous B1 field. However, we demonstrate that the EPG-fitted T2 quantifications are insufficient to identify differences between ALS and control brains.

In Chapter 4, we overview existing models for simulating the dynamics of the fixation process and then introduce our kinetic tensor model that leverages diffusion tensor measurements to better calculate formalin distribution. We present a novel T2 correction based on the calculated formalin distribution. We evaluate multiple corrections based on these models in terms of corrected homogeneity within both white and grey matter regions.

In Chapter 5, we present a study of quantitative T2 in ALS. We demonstrate how quantitative T2 maps produced by the EPG-fitting and kinetic models from Chapters 3 and 4 improve the discrimination of ALS from controls in ALS-related cortical regions. We also correlate the derived T2 quantifications against iron (ferritin) and myelin (phospholipid protein) stains in the same brains, which suggest that iron is the primary driver of the observed signal differences.

In Chapter 6, we summarize the research work in this thesis and discuss possible future research directions.

1.4 Reference

Behrens, T. E., Woolrich, M. W., Jenkinson, M., Johansen-Berg, H., Nunes, R. G., Clare, S., ... & Smith, S. M. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, 50(5), 1077-1088.

Dawe, R. J., Bennett, D. A., Schneider, J. A., Vasireddi, S. K., & Arfanakis, K. (2009). Postmortem MRI of human brain hemispheres: T2 relaxation times during formaldehyde fixation. *Magnetic Resonance in Medicine*, 61(4), 810-818.

Ibrahim, T. S., Lee, R., Abduljalil, A. M., Baertlein, B. A., & Robitaille, P. M. L. (2001). Dielectric resonances and B1 field inhomogeneity in UHFMRI: computational analysis and experimental findings. *Magnetic Resonance Imaging*, 19(2), 219-226.

Pallebage-Gamarallage, M., Foxley, S., Menke, R. A., Huszar, I. N., Jenkinson, M., Tendler, B. C., ... & Ansorge, O. (2018). Dissecting the pathobiology of altered MRI signal in amyotrophic lateral sclerosis: A post mortem whole brain sampling strategy for the integration of ultra-high-field MRI and quantitative neuropathology. *BMC Neuroscience*, 19(1), 11.

Ooi, G. C., Chen, F. E., Chan, K. N., Tsang, K. W. T., Wong, Y. H., Liang, R., & Ngan, H. (1999). Qualitative and quantitative magnetic resonance imaging in haemoglobin H disease: screening for iron overload. *Clinical Radiology*, 54(2), 98-102.

Shepherd, T. M., Thelwall, P. E., Stanis, G. J., & Blackband, S. J. (2009). Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*, 62(1), 26-34.

Uğurbil, K., Garwood, M., Rath, A. R., & Bendall, M. R. (1988). Amplitude-and frequency/phase-modulated refocusing pulses that induce plane rotations even in the presence of inhomogeneous B1 fields. *Journal of Magnetic Resonance*, 78(3), 472-497.

Weigel, M. (2015). Extended phase graphs: dephasing, RF pulses, and echoes-pure and simple. *Journal of Magnetic Resonance Imaging*, 41(2), 266-295.

Chapter 2

Background

Contents

2.1 MRI Physics.....	7
2.1.1 MR signal.....	7
2.1.2 Longitudinal relaxation time, T1.....	8
2.1.3 Transverse relaxation time, T2.....	9
2.1.4 Bloch description	10
2.2 MR imaging formation.....	11
2.3 Pulse sequences.....	18
2.3.1 Basic pulse sequence	18
2.3.2 Measuring relaxation times.....	19
2.3.2.1 Gradient echo sequence	20
2.3.2.2 Spin echo	21
2.3.2.3 Fast spin echo	22
2.4 Diffusion Weighted MRI.....	24
2.4.1 Diffusion Physics.....	24
2.4.2 Measuring diffusion with MR.....	27
2.5 MRI of amyotrophic lateral sclerosis.....	32
2.6 Reference.....	35

2.1 MRI Physics

This thesis considers magnetic resonance imaging (MRI) techniques that target the hydrogen nucleus, ^1H . In this first section, I outline the source and behavior of this MRI signal.

2.1.1 MRI signal

The hydrogen nuclei (protons) are charged particles with ‘spin’ and the magnetic moment μ serves as the signal source of MRI. These spinning protons can be regarded as tiny magnets (Fig. 2.1.A). When there is no magnetic field applied, these spins are randomly oriented with no net magnetic moment observed (Fig 2.1.B). However, in the presence of magnetic field B_0 , these spins have a natural tendency to align toward the external field direction (Fig 2.1.C), and thus, a net magnetic moment could be measured in terms of magnetization M . The above-mentioned process where protons align, in an external magnetic field, to create magnetization is called polarization.

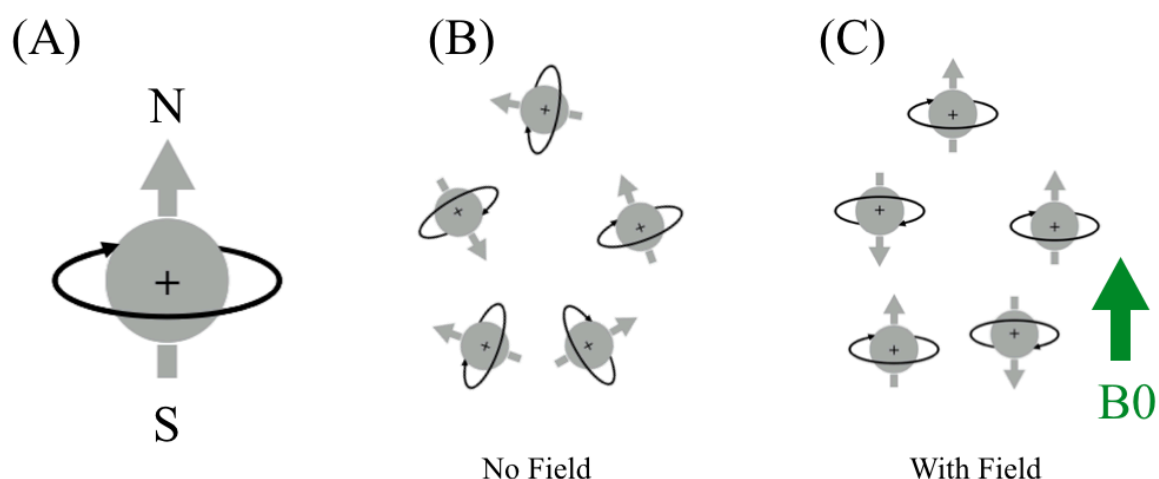


Figure 2.1: The polarization process. (A) The spinning hydrogen nucleus could be treated as ‘tiny magnets’. (B) Spins randomly orient when there is no external magnetic field applied. (C) Spins tend to align with the external field to create net magnetization.

The polarised magnetization oscillates around the magnetic field $\mathbf{B}_0$ with a characteristic frequency (also called Larmor frequency) ω_0 following a linear relationship as,

$$\omega_0 = \gamma \mathbf{B}_0 \quad (2.1)$$

where γ is the gyromagnetic ratio, a constant whose value depends on the type of nuclei. For hydrogen nucleus, $\gamma = 42.58 \times 10^6$ Hz/T; while for Carbon-13 (^{13}C), $\gamma = 10.71 \times 10^6$ Hz/T.

If external electromagnetic field $\mathbf{B}_1$ (Radio Frequency, RF) oscillates at the Larmor frequency, it could tip sample magnetization away from $\mathbf{B}_0$, i.e. the process of excitation. After that, under the torque given by the external field, the magnetization will rotate around the $\mathbf{B}_0$ in Larmor frequency in terms of magnetization precession, which induces a current in receiver coils as the detected MRI signal.

2.1.2 Longitudinal relaxation time, T1

Longitudinal relaxation (also called spin-lattice relaxation) is the mechanism by which magnetization along the main magnetic field $\mathbf{B}_0$ reaches thermodynamic equilibrium under the condition of vibrational and rotational magnetic field given by environmental ‘lattice’ (Markley *et al.*, 1971; Lu *et al.*, 2004). In general, after application of a 90° RF pulse at $t=0$, we describe the relaxation process by a time constant T1 (namely, the longitudinal relaxation time) as,

$$M_z(t) = M_{z,eq}(1 - e^{-t/T1}) \quad (2.2)$$

where $M_{z,eq}$ is the longitudinal magnetization in the equilibrium state.

T1 is a decay constant, that defines the time needed by magnetization to recover to 63% of its equilibrium value. Different tissue has different T1 value. For example, in our 7 tesla study, human cerebrospinal fluid has the longest T1 value of approximately 4000 ms, white matter and grey matter have T1 values of 2000 ms and 1200 ms, respectively (Franconi *et al.*, 2000;

Spijkerman *et al.*, 2018, and <http://www.mritoolbox.com/ParameterDatabase.html>). This also allows us to use T1 as a quantitative measure to differentiate pathological tissue from the normal tissue, and also provides a way to adjust scanning parameters to enhance pathology contrast by different MRI modalities.

	T1 (ms)	T2 (ms)
Cerebrospinal Fluid	4000	900
Grey Matter	2000	60
White Matter	1200	45

Table 2.1: Relaxation time (T1 and T2) at 7 tesla. Data comes from (Franconi *et al.*, 2000; Spijkerman *et al.*, 2018) and (<http://www.mritoolbox.com/ParameterDatabase.html>).

2.1.3 Transverse relaxation time, T2

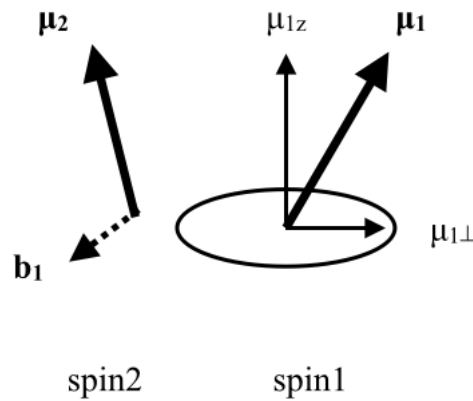


Figure 2.2: The magnetic moment from one spin, μ_1 , generates a rotating magnetic field that is experienced by a second spin, μ_2 .

The main magnetic field B_0 establishes the coordinate system by defining the longitudinal axis, denoted z, and the transverse plane, conventionally indicated as x-y or ‘ $\perp$ ’. So, the magnetic moment from a spin μ_1 can be decomposed into longitudinal and transverse components (μ_{1z} and $\mu_{1\perp}$). $\mu_{1\perp}$ rotates around B_0 , at resonant frequency ω_0 in the transverse plane (Fig. 2.2).

Such rotating $\mu_{1\perp}$ generates a magnetic field $\mathbf{b}_I$ in the nearby space, which can alter the precession rate of a neighboring spin, μ_2 . This spin-spin interaction causes spins to lose phase coherence (dephasing), and reduces the net transverse magnetization $M_{\perp}$, as,

$$M_{\perp} = M_0 e^{-t/T_2} \quad (2.3)$$

Where M_0 is the longitudinal magnetization in the fully relaxed (equilibrium) state.

We define this process as transverse relaxation (also called spin-spin relaxation) (Goldman *et al.*, 1966; Bloembergen *et al.*, 1961) and characterize it with a time constant T_2 , which indicates a time duration that the transverse magnetization could fall to roughly 37% of its original value. The T_2 value is considerably shorter than T_1 . In our 7 tesla study, the in-vivo grey and white matter have T_2 values of 60 ms and 45 ms, respectively, while the cerebrospinal fluid has the longest T_2 of 900 ms. Note that, our post-mortem study does not have the cerebrospinal fluid. The detailed information of the T_2 value can be referred to Table 2.1.

2.1.4 Bloch description

This classical description of the NMR phenomenon was introduced by Bloch in terms of the net magnetization vector (Bloch, 1946). In an external magnetic field $\mathbf{B}$, the magnetization $\mathbf{M}$ is subject to a torque force $\mathbf{L} = \mathbf{M} \times \mathbf{B}$ that rotates the magnetization vector according to nutation equation:

$$\frac{d\mathbf{M}(t)}{dt} = \gamma \mathbf{M}(t) \times (\mathbf{B}_0 + \mathbf{B}_1(t)) \quad (2.4)$$

If $\mathbf{B}_I$ is added and rotates at the resonant frequency ω_0 , $\mathbf{M}$ would precess around $\mathbf{B}_0$ away from its equilibrium state, and the transverse component $M_{\perp}$ would come into being. After application of $\mathbf{B}_I$, $\mathbf{M}$ would recover to its equilibrium state $M_z \rightarrow M_0$ and $M_{\perp} \rightarrow 0$ according to longitudinal and transverse relaxation mechanisms (Eq. 2.5) in differential equation form,

$$\frac{dM_z}{dt} = -\frac{M_z - M_0}{T_1} \quad (2.5)$$

$$\frac{dM_{\perp}}{dt} = -\frac{M_{\perp}}{T_2}$$

In the Bloch description, the magnetization vector $\mathbf{M}$ is governed by (1) the torque given by the external magnetic field $\mathbf{B}$ and (2) the relaxation mechanisms:

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times (\mathbf{B}_0 + \mathbf{B}_1) - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} - \frac{M_z - M_0}{T_1} \mathbf{k} \quad (2.6)$$

where $\mathbf{i}$, $\mathbf{j}$, and $\mathbf{k}$ are the unit vectors along x, y, and z. If we adopt a frame of reference that is also rotating at the resonance frequency ω_0 (where x' , y' , and z' indicate the axes in rotating coordinates), the Bloch equation can be simplified as:

$$\frac{\partial \mathbf{M}}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}_1 - \frac{M_{x'} \mathbf{i} + M_{y'} \mathbf{j}}{T_2} - \frac{M_{z'} - M_0}{T_1} \mathbf{k} \quad (2.7)$$

Equation 2.7 provides a fundamental description of the magnetic resonance signal.

2.2 Image Formation

Localization of magnetization to form an image utilizes the third type of magnetic field, the gradient field $\mathbf{G}$. Gradients vary linearly over space so that the total magnetic field at one end of the magnet is high, while the other end is low (Fig. 2.3). This position-encoded magnetic gradient field $\mathbf{G}$ provides us a way to modulate the voxel resonant frequency as,

$$\omega = \gamma(\mathbf{B}_0 + \Delta \mathbf{B}) \quad (2.8)$$

$$\Delta \mathbf{B} = \mathbf{G} \cdot \mathbf{r} \quad (2.9)$$

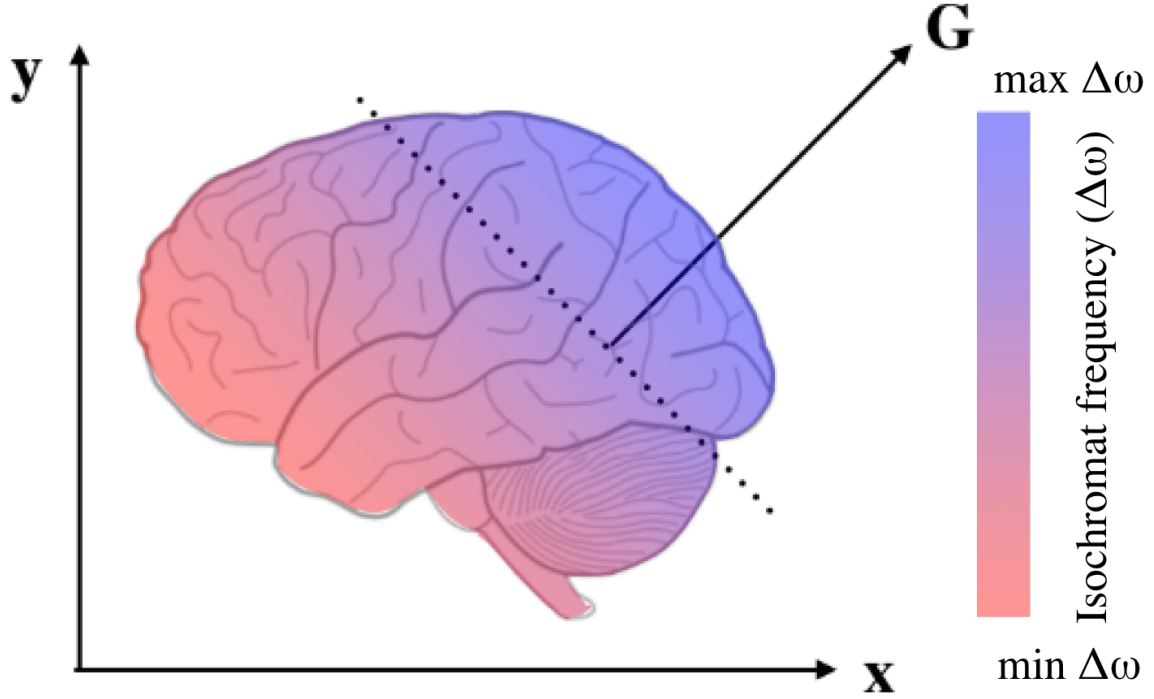


Figure 2.3: The use of a magnetic gradient field $\mathbf{G}$ causes the resonance frequency to vary across space (indicated by different colors). The gradient field enables us to encode tissue location information. For example, a single frequency RF pulse could selectively excite only one imaging slice indicated by the dashed line, which is the basis for most modern 2D MR imaging.

One example is shown in Fig. 2.3, where the gradient field $\mathbf{G}$ modulates the resonant frequency of tissue voxels according to their location $\mathbf{r}$, so that only voxels within the isochromatic plane defined by resonance frequency $\omega = \gamma(\mathbf{B}_0 + \mathbf{G} \cdot \mathbf{r})$ could be excited by a single frequency RF pulse at frequency ω , while magnetization at other locations have a different resonance frequency and are not excited. In reality, instead of exciting an infinitely thin plane of voxels by single RF frequency, we use an RF pulse consisting of a band of frequencies to excite a thicker plane. To create a 3D volume, a series of imaging slices could be excited sequentially by temporally changing the excitation frequency corresponding to each desired isochromatic plane, which is called, 2D multi-slice imaging. Indeed, many MRI scans are acquired in this way, such as functional MRI (fMRI) (Barry *et al.*, 2012; Todd *et al.*, 2016) and diffusion

weighted MRI (dwMRI) (Chen *et al.*, 2013; Chilla *et al.*, 2015; Seehaus *et al.*, 2012). Having excited a selected slice, we now need to localize the received signal within the plane to form an image.

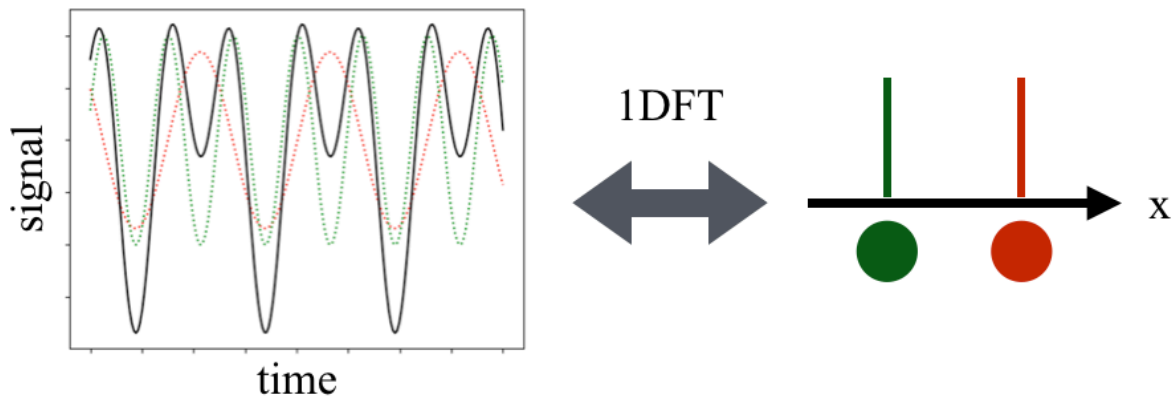


Figure 2.4: Imaging with Fourier transformation. For the 1D case, green and red objects (water tubes) have different resonant frequency encoded by the external gradient field. 1D FT could transform measured signals into a position-encoded spectrum. The total signal received by an RF coil is indicated by the black curve.

For in-plane spatial encoding, we use additional gradient fields that are orthogonal to the slice selection gradient. These gradients encode in-plane voxel location by modulating its precession frequency during signal acquisition (Eq. 2.8-2.9). The receiver coil hears the signal from all voxels simultaneously, and then, the Fourier Transformation (FT) could differentiate signals from different locations encoded by different frequencies. Fig. 2.4 illustrates the signal localization process with 1D-FT: (1) In the presence of gradient field, the red and green circles generate MRI signal with different frequencies depending on their locations. (2) The 1D Fourier transformation could derive the 1D spectrum from the measured total signal (indicated by the black curve). (3) In the spectrum shown in Fig. 2.4, the position is encoded by the spectrum frequency, and the hydrogen density is presented by the spectrum strength. In sum, the gradient field enables us to encode voxels position, and the FT allows us to determine the

signal intensity at each specific position. Based on this concept, in the later part, we will discuss how to manipulate the gradient to form 2D and even 3D MRI images.

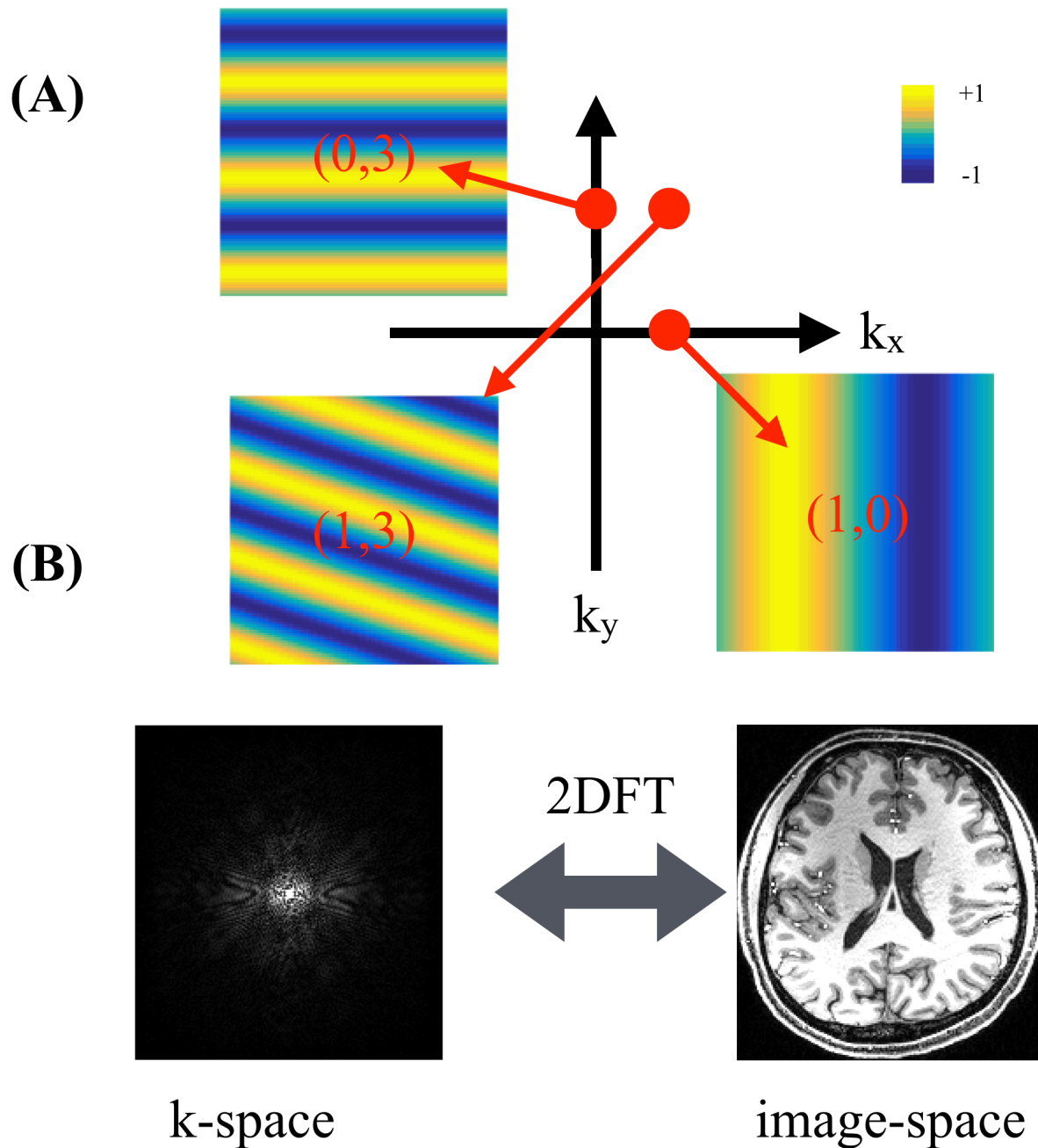


Figure 2.5: 2D Fourier transformation. (A) The 2D spatial frequency is denoted in the form of (k_x, k_y) . Here, we illustrate three spatial patterns with corresponding representations in the spectrum. The 2D spectrum (k-space) presents the contribution of each spatial frequency. (B) 2D images can be decomposed into the spatial frequency spectrum. Inversely, the image could be formed as a weighted sum of these spatial patterns via the 2D Fourier transformation.

Image formation is based on measurement of individual spatial frequencies (Fig. 2.5), which are used to build up a representation of the image. We denote a given 2D spatial frequency using two parameters, k_x and k_y , that indicate how many cycles exist across a given spatial extent along the x and y directions respectively. We can consider these parameters as coordinates (k_x, k_y) in a two-dimensional space, called k-space. We construct the image based on measurements that cover k-space. Like temporal waves, the phase component of structural spatial waves also plays a key role in multi-dimensional position encoding.

After exciting, the magnetization is coherent across space (has the same phase in the transverse plane). At this point, the introduction of gradient $\mathbf{G}$ causes the magnetization to precess according to its local field $\mathbf{B}_0 + \mathbf{G} \cdot \mathbf{r}$. The spatial linearity of the gradient field means that this precession accumulates to form spatial frequencies along the direction of the gradient $\mathbf{G}$. At any given moment in time, the magnetization reflects a single spatial frequency, and the measured signal represents the contribution of this spatial frequency to the object being imaged. Modern gradient field techniques allow us to modulate $\mathbf{G}$ direction and magnitude, and acquire the k-space signal by point by point sampling. Finally, the image could be formed as a weighted sum of these spatial frequencies via the 2D Fourier transformation (Fig. 2.5).

So we need to sample finite signals in k-space, normally arranged in a grid, then FT could be used to reconstruct the images. There are two key acquisition parameters: the sampling interval Δk_x , and the highest sampling range $k_x^{\max}$. The sampling frequency or interval Δk_x determines the field of view (FOV) of the final reconstructed image, and under-sampled k-space data (usually in phase encoding direction) could cause the aliasing effect. On the other hand, the highest sampling range $k_x^{\max}$ indicates how much high-frequency spatial component is involved, which determines the resolution of the reconstructed image.

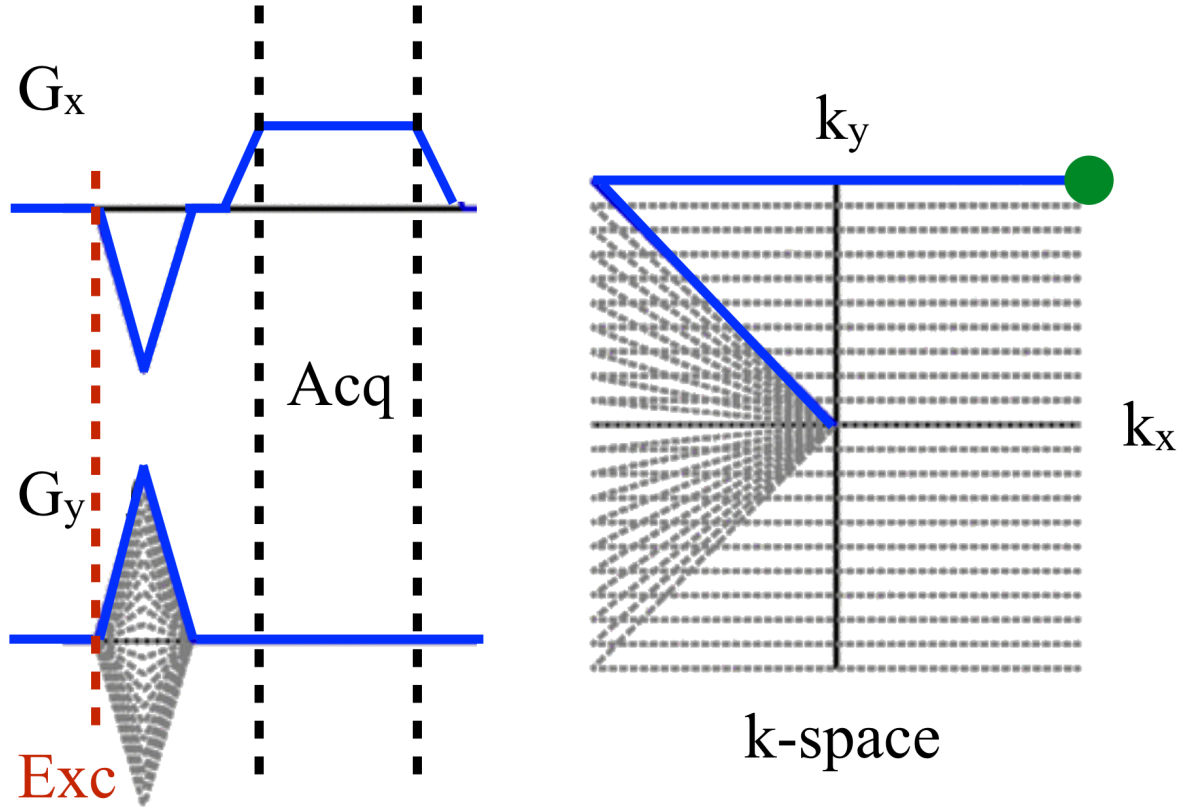


Figure 2.6: 2DFT (spin-warp or raster scan) acquisition and its k-space trajectories. G_x is the read-out gradient, while G_y is the phase gradient whose encoding direction is perpendicular to that of G_x . The application of the two gradients enables the k-space data acquisition. Note, data is acquired only when the read-out gradient is on. Courtesy of Karla Miller adapted from the FMRIB physics course.

In general, the MRI acquisition is a k-space filling problem, where we manipulate gradient fields to control the path or trajectories through the whole k-space. The commonly used 2D k-space data collection methods include spin-warp, echo-planar imaging (EPI), spiral, and radial acquisition approaches.

Nobel Laureate Paul Lauterbur was the first to use magnetic gradients to encode spatial information in vivo, giving rise to modern MR imaging techniques (Lauterbur, 1973).

Lauterbur's experiment was conducted in 2D, superimposing a gradient field $\mathbf{G} = \left[\frac{\partial B_x}{\partial x}, \frac{\partial B_y}{\partial y} \right] = [G_x, G_y]$ on $\mathbf{B}_0$. The gradient fields are varied on the timescale of milliseconds to obtain a desired waveform, under the constraints of the gradient hardware (dictating some maximum

achievable gradient strength and rate of field change over time). The k-space location can be calculated as,

$$k_x(t) = \gamma \int_0^t G_x(\tau) d\tau \quad (2.10)$$

$$k_y(t) = \gamma \int_0^t G_y(\tau) d\tau \quad (2.11)$$

Traditionally, we define G_x as frequency encoding gradient to shift frequency along x axis, and G_y as phase encode gradient to shift phase along y axis. Figure 2.6 illustrates how the k-space data is acquired. After application of one or multiple excitations (magnetization are tipped to the transverse plane), a strong negative G_x followed by a positive gradient G_x is applied, which enables us to first offset the pulse trajectory to the left most end ($-k_x^{max}$), and then to move toward the right end k_x^{max} . On the other hand, G_y is turned on and off with varying strength from one excitation to the next, which enables us to achieve a series of parallel acquisition lines. In practice, only during the parallel line scanning (we call it acquisition time), do we acquire the k-space data for processing. Finally, the corresponding image could be derived by inverse 2DFT as,

$$\rho(\mathbf{r}) = \iint_{-\infty}^{+\infty} S(\mathbf{k}) e^{-i2\pi\mathbf{k}\cdot\mathbf{r}} d^2\mathbf{k} \quad (2.12)$$

Where $S(\mathbf{k})$ is the acquired 2D k-space data, and $\rho(\mathbf{r})$ is the reconstructed image.

2.3 Pulse Sequences

2.3.1 Basic Pulse Sequence

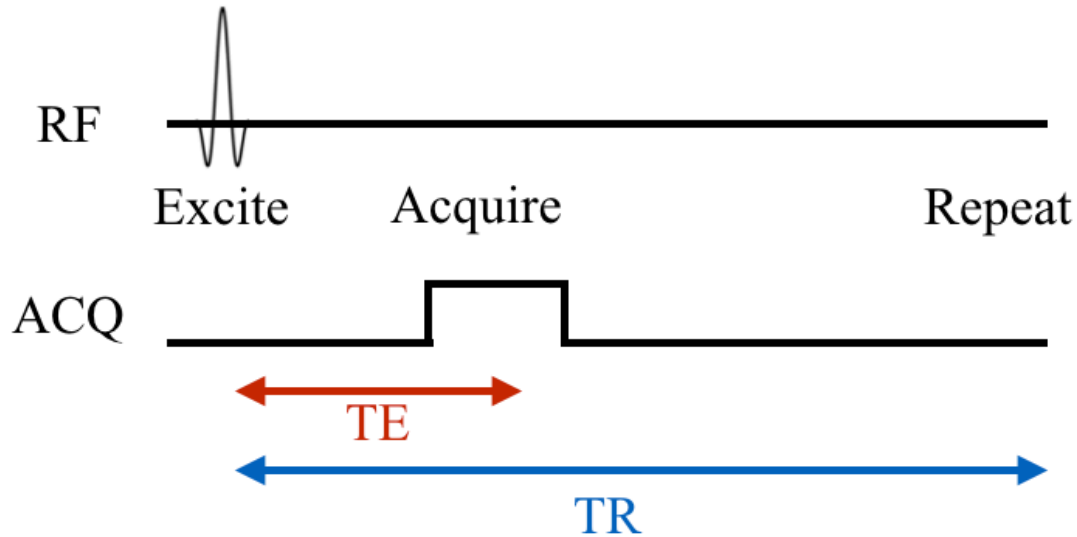


Figure 2.7: Basic MRI pulse sequence. The transmitting RF pulse excites magnetization into the transverse plane. Receive coil acquire MR signal at echo time TE. After repetition time TR, the whole process loop back again.

The diagram in Fig. 2.7 provides a very simple pulse sequence illustrating how the MR signal is acquired. The transmit RF pulse excites part of the magnetization from z-direction (along the main magnetic field B_0) to the x-y plane, where the signal can be detected. After that, the magnetization simultaneously experiences two relaxation processes i.e. longitudinal and transverse relaxation characterized by time constants T_1 and T_2 . Considering the inhomogeneity of the magnetic field, the dephasing speed of transverse magnetization is usually much faster than T_2 decay, which is usually characterized by the time constant T_2^* . The receive RF coil acquires MR signal in an acquisition window that is typically centred around the “echo time” (TE, the time between excitation and readout, specifically defined as the time of k-space centre) which is a user controllable parameter to govern the signal magnitude. Another important parameter is repetition time (TR, the time between repeated

excitations) which determines the recovery of longitudinal magnetization toward z-direction before the next excitation.

2.3.2 Measuring relaxation times

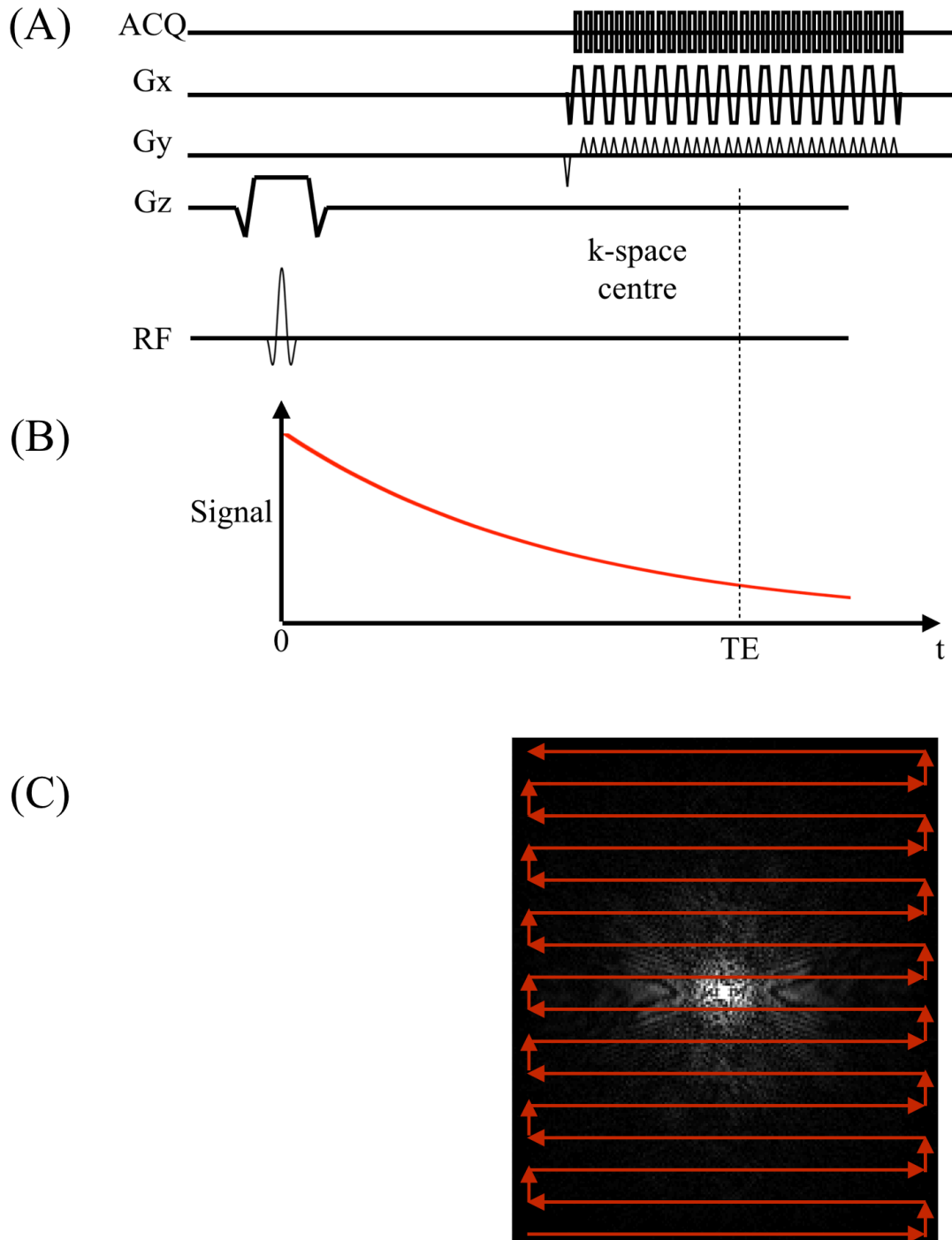


Figure 2.8: GRE pulse sequence with EPI acquisition. (A) The acquisition, gradient and RF pulse temporal profile of the 2D GRE_EPI sequence. (B) The signal evolution of the 2D GRE_EPI sequence. (C) the k-space trajectory of the 2D GRE_EPI sequence.

The main topic of this thesis is the measurement of T2 relaxation in the post-mortem brain. In this section, I will first overview the main methods employed for relaxometry.

2.3.2.1 Gradient echo sequence

To measure the transverse relaxation in the post-mortem brain, the simplest method is of using the 2D Gradient Echo (GRE) pulse sequence as illustrated in Fig. 2.8. First, we introduce some MR concepts for the pulse sequence diagram: (1) three gradients [G_x , G_y , G_z] encode frequency, phase, and slice, separately; (2) the RF pulse excites the magnetization; (3) ACQ represents the signal data acquisition for the k-space graph. In the 2D GRE sequence of Figure 2.8, we first excite the magnetization in the desired slice by applying the corresponding G_z gradient and a 90° RF pulse. The MR signal attributed from the excited spins begins to decay due to its magnetic field perturbation induced by its neighbors and by the inhomogeneous magnetic field (T2* decay) etc. During the decay process, we use a fast data acquisition technique called EPI to collect all of the k-space for the excited slice. This EPI technique is first introduced by Sir. Peter Mansfield in 1977, which could obtain individual MR slice within 100 msec. For the GRE sequence with EPI acquisition shown in Fig. 2.8, the frequency encoding gradient G_x moves the scanning point left and right across the horizontal scanning lines, while the phase encoding gradient G_y provides ‘blipped’ phase increments to shift the parallel scanning lines in the k-space. After filling the k-space graph (Fig.2.8.C), the corresponding 2D image of the desired slice could be reconstructed by 2D Fourier Transformation of Eq.2.12. Note that this reconstructed intensity image is the relaxation weighted image denoted by echo time TE. So after acquiring the k-space data and reconstructing the corresponding images, we can calculate the T2* map for the slice with Eq. 2.13

$$M_{TE_i} = M_0 e^{-TE_i/T2^*} \quad (2.13)$$

where TE_i is a series of echo time (the centre of scanning lines in k-space).

2.3.2.2 Spin Echo (SE)

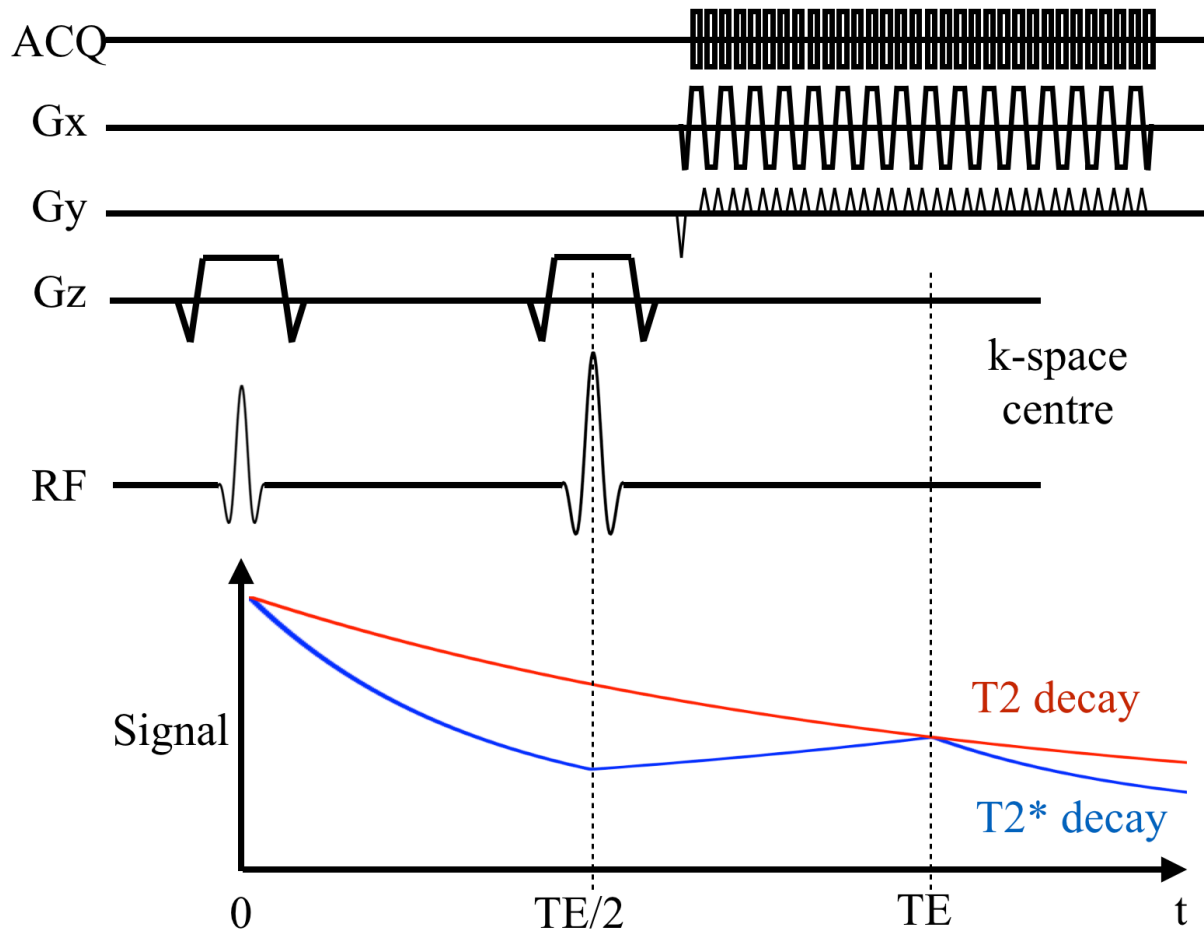


Figure 2.9: Spin echo pulse with EPI acquisition. One 90° pulse excites the magnetization into the transverse plane, followed by a transverse relaxation or dephasing process denoted by T2. At time point $TE/2$, a 180° pulse refocuses the phase of all magnetization, so that the spin echo could be measured at echo time TE . The red curve indicates the T2 decay in the perfect homogeneous magnetic field, while the blue curve indicates the T2* decay in the imperfect magnetic field.

To acquire an accurate T2 map that isn't influenced by T2*-related field inhomogeneities, we have to cancel out the influence of local field inhomogeneity. Instead of using a GRE sequence, which is sensitive to T2*, we use the spin echo sequence to measure the T2 relaxation time. Fig. 2.9 briefly demonstrates how the spin echo is formed. First, one 90° pulse is used to tip the spins into the transverse plane. Because of the magnetic fields experienced by spins are

different, the dephasing process could be observed in terms of T2 signal decay after excitation. Then, one 180° pulse is applied to flip all magnetization in the transverse plane, so that the accumulated spin phase order is inversed. After the flip at TE/2, the faster spins begin to chase the slower spins and reach them at the echo time TE. The 180° pulse is commonly called the refocusing pulse, because it can refocus the signal decay caused by the local inhomogeneous magnetic field. In general, we can vary the refocusing time TE/2 and measure the signal strength at a series of echo time TE_i , and then the transverse relaxation time T2 can be derived by the fitting.

$$M_{TE_i} = M_0 e^{-TE_i/T_2} \quad (2.14)$$

2.3.2.3 Fast Spin Echo (FSE)

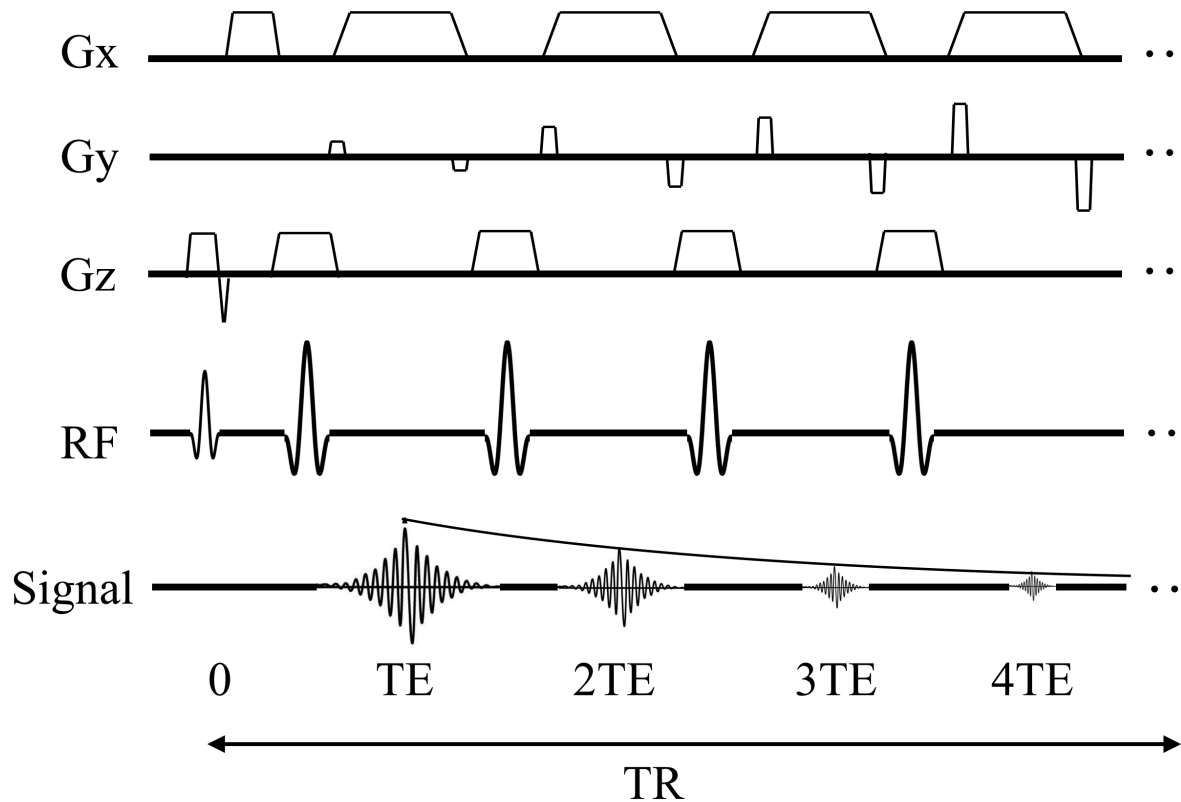


Figure 2.10: Fast spin echo sequence diagram for quantitative T2 measurement. Note after each echo a rewinding gradient (same magnitude but reverse direction) is applied to compensate for the phase shift generated by the phase encode gradient. At time point $n \cdot TE$, we measure the decayed MR signals for each k-space line.

For T2 imaging, people commonly used a fast spin echo sequence (Mugler *et al.*, 2003). This sequence was first proposed by (Hennig *et al.*, 1986) as a modification of the CPMG sequence, but in each RF shot, multiple 180 RF pulses were used to excite echoes with different phase encoding information (Fig. 2.10), and subsequently filled several k-space lines. This enables the imaging to be accelerated by n_e (the number echoes acquired after each excitation) times as compared with the standard spin echo.

The number of echoes refocused in each shot is called the echo train length (ETL), which is normally in the range of 2~16. The temporal gap between adjacent echoes is called echo spacing (ESP). Images acquired with FSE do not have a well-defined echo time, since different k-space lines are acquired at different TEs. A standard approximation is to define an effective echo time (T_{eff}) as corresponding to the echo at the center of k-space. This is because image contrast is predominantly reflected by the signal at the center of k-space, which is by definition the average signal over the reconstructed image. However, this is only an approximation and the high spatial frequencies contain different T2 weighting.

The original spin-echo (Carr and Purcell, 1954) introduced this sequence (90_x° - $TE/2$ - 180_x° - TE - 180_x° - TE - ...), which refocuses a train of echoes after each excitation. After the 90_x° excitation, magnetization vectors are tipped to the y-axis and begin to dephase. At $t = \tau$, a 180_x° refocusing pulse is applied that creates an echo at $t = 2\tau$. Then, magnetization again dephases until $t = 3\tau$ when another 180_x° refocusing pulse is applied, creating an echo at $t = 4\tau$. These operations are repeated N times to acquire an echo train at $T_E = 2\tau, 4\tau, 6\tau \dots 2N\tau$. The measured signal can in theory then be fit to a simple model,

$$M_{y'} = M_0 e^{-2n\tau/T2} \quad (2.15)$$

One key challenge with the use of FSE is that there is, in general, some imprecision to the 180° refocusing pulse. For example, if this pulse instead achieves 170° , then the next 180° pulse will cause magnetization to be oriented at 340° instead of 360° in the $z'y'$ plane. This deviation of elevation angle of magnetization population will accumulate as subsequent 180_x° are applied. To address this issue, Meiboom and Gill modified the sequence to instead use $(90_x^\circ - TE/2 - 180_{y'}^\circ - TE - 180_{y'}^\circ - TE - \dots)$ (Meiboom and Gill, 1958), where the 180° pulse is applied to the y' axis, and thus imperfect 180° pulses do not accumulate elevation angle deviation (Song, 2002).

2.4 Diffusion weighted MRI

Although diffusion-weighted MRI is not a primary focus of this thesis, we do utilize MRI-based measurements of diffusion to build models of the in- and out-flux of fixative in post-mortem tissue. In this section, we therefore overview the physics of diffusion and its measurement using MRI.

2.4.1 Diffusion physics

To describe the diffusion phenomenon, we can use Fick's laws:

$$J = -D\nabla c \quad (2.16)$$

$$\frac{\partial c}{\partial t} = -\nabla \cdot J = \nabla \cdot (D\nabla c) = D\nabla^2 c \quad (2.17)$$

Where c is the molecule concentration, D is the diffusion coefficient, J is the molecule flow, ∇ is the gradient operator and ∇^2 is the Laplacian operator. Eq. 2.16 demonstrates molecule flow

depends on concentration gradient and diffusion speed characterized by D . The negative sign indicates that molecules flow run from high concentration to low. Eq. 2.17 is the continuity law of molecule flow. If the initial condition is $c(\mathbf{r}, t) = \delta(\mathbf{r}-\mathbf{r}_0)$, that is if we consider a group of molecules at a given location, then we can solve Fick's equation in time t , as follows:

$$c(\mathbf{r}, t) = \left(\frac{1}{\sqrt{4\pi Dt}} \right)^3 \exp \left(\frac{-(\mathbf{r} - \mathbf{r}_0) \cdot (\mathbf{r} - \mathbf{r}_0)}{4Dt} \right) \quad (2.18)$$

For water molecules in the absence of a diffusion gradient, this corresponds to random motion due to thermal energy, and is termed self-diffusion. Considering a random walk of a large number of molecules, the population displacement follows a Gaussian distribution, namely, an isotropic motion with zero mean displacement. For a given molecule, its mean displacement over a given time t depends on its diffusion coefficient D according to:

$$\begin{aligned} \text{1D free diffusion:} \quad & \langle x(t)^2 \rangle = 2Dt \\ \text{3D free diffusion:} \quad & \langle \mathbf{r}(t)^2 \rangle = 6Dt, \quad \mathbf{r}^2 = x^2 + y^2 + z^2 \end{aligned} \quad (2.19)$$

Water molecules have diffusion coefficient $D = 2.2 \cdot 10^{-3} \text{ mm}^2/\text{s}$. Over a diffusion time of $t=100\text{ms}$, this implies that of 32% of water molecules displace by more than $25\mu\text{m}$, and 5% displace by more than $40\mu\text{m}$.

Water molecules randomly walk inside the brain tissue, and their paths are restricted by various tissue boundaries. Therefore, by measuring the diffusion signal of water probes, we could derive some microstructural properties of brain tissue. As is shown in Fig. 2.11, we use a diffusion tensor to approximate the diffusion displacement of water molecules as ellipsoid, where eigenvectors $\mathbf{e}_1$, $\mathbf{e}_2$, and $\mathbf{e}_3$ represent three axes of the diffusion ellipsoid, from which the orientation of the brain tissue can be derived, while three scalar diffusivities of l_1 , l_2 , and l_3 are used to represent the lengths of the three axes of the diffusion ellipsoid, which imply the size

of the tissue. For example, the white matter fibre has an intrinsic orientation, and the molecules diffuse more freely along tracts than the perpendicular direction, so the diffusion signal of water molecules can serve as a marker to allow us to track the white matter directionality and diffusivity.

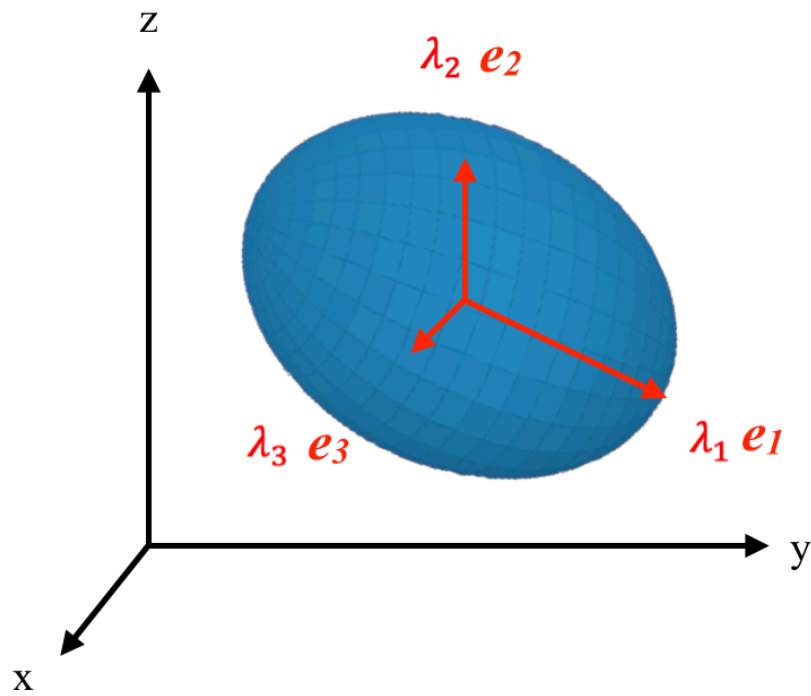


Figure 2.11: The diffusion ellipsoid. In the diffusion tensor, an ellipsoid is used to represent the diffusion properties of sampled tissue. The eigenvectors $\mathbf{e}_1$, $\mathbf{e}_2$, and $\mathbf{e}_3$ represent the direction of the investigated molecules, while the scalar values of λ_1 , λ_2 , and λ_3 represent the diffusivity strength.

In the diffusion tensor model, we consider three diffusion properties: (1) Apparent diffusion coefficient (ADC), counts the mean diffusivity across all directions, so it can provide tissue integrity information. (2) Fractional anisotropy (FA), measures how the diffusion ellipsoid is elongated, so it can provide information about the white matter fibre tract. (3) Principal diffusion direction (PDD), describes the most preferential direction along which the water molecules tend to diffuse. Fig. 2.12 illustrates one brain example processed using the diffusion tensor model. We can see the corticospinal fluid in ventricles has a relatively high ADC value

because its diffusion is unrestricted by tissue boundaries. The FA reflects the variance of diffusivity across different orientations within a tissue voxel, where the fibre bundle with strong preferential orientations has high value, such as that of the corpus callosum (Fig. 2.12). The fibre orientation where the greatest diffusion occurs, could be seen from the PDD vector map of Fig. 2.12.C, which is commonly used for fiber tracking.

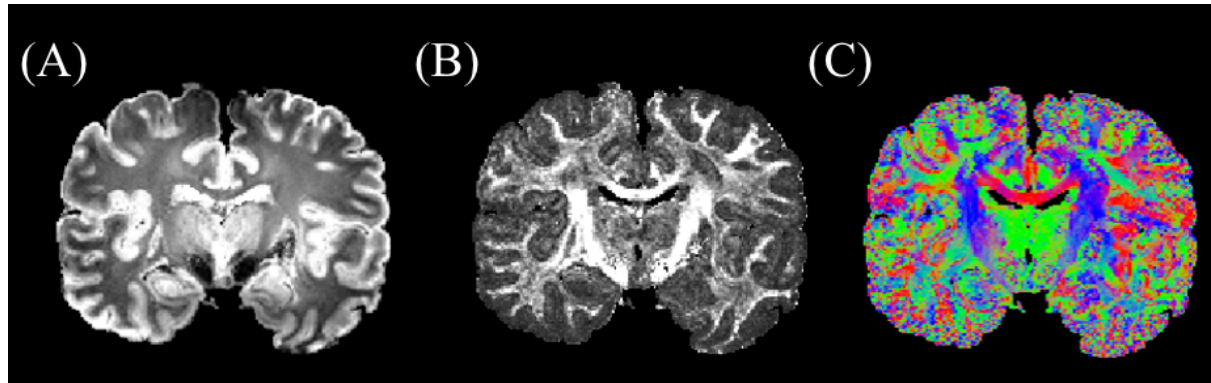


Figure 2.12: Diffusion tensor MRI. (A) The ADC map. The corticospinal fluid in ventricles is high in ADC value. (B) is the FA map. White matter tracts such as corpus callosum and corticospinal tract have relatively high FA values. (C) the PDD map, where red indicates left and right orientation, green indicates anterior-posterior, and blue indicates top-down directions.

3.4.2 Measuring diffusion with MR

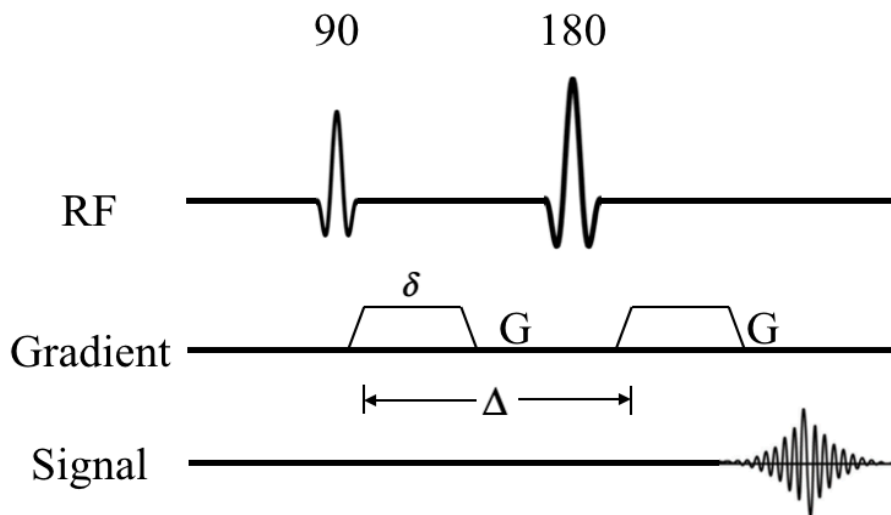


Figure 2.13. Diffusion measurement by a spin echo sequence. Two diffusion gradients are placed on either side of the 180° RF pulse. The 180° RF pulse can inverse the dephasing effect caused by the gradient field, while moving molecules will have reduced signal depending on their diffusion properties. The time interval is Δ and gradient magnitude is G , width is δ .

In this section, we overview how water diffusion can be measured with magnetic resonance. These measurements will be used in Chapter 4 of the proposed kinetic tensor model to model the concentration of fixative in brains when scanned for T2 measurements.

As described above, a water sample in a magnetic field gradient $B_z = B_0 + \mathbf{G} \cdot \mathbf{r}$ will experience a resonance frequency $\omega = \omega_0 + \gamma \mathbf{G} \cdot \mathbf{r}$. Assuming no displacement over a time interval δ during which this gradient is on, the magnetization will accumulate phase:

$$\varphi(\delta) = \int_0^\delta \gamma \mathbf{G} \cdot \mathbf{r} dt \quad (2.20)$$

To measure diffusion, we conduct a spin-echo experiment with two diffusion gradients added, which we here assume without loss of generality to be the x axis (Fig. 2.11). If the spin is at the location $\mathbf{x}_1$ during the first diffusion gradient, it would experience a phase shift:

$$\varphi_1 = \int_0^\delta \gamma G x_1 dt = \gamma G \delta x_1 \quad (2.21)$$

For this spin echo sequence, the magnetization would then experience a 180° RF pulse, which will negate the phase to become $-\varphi_1$. If the magnetization at location $\mathbf{x}_2$ during the second diffusion gradient, it would experience another phase shift:

$$\varphi_2 = \int_\Delta^{\Delta+\delta} \gamma G x_2 dt = \gamma G \delta x_2 \quad (2.22)$$

So the net phase shift is:

$$\Delta\varphi = \varphi_2 - \varphi_1 = \gamma G \delta (x_2 - x_1) \quad (2.23)$$

If the water molecule is static ($x_1 = x_2$), the diffusion gradients would not generate a net phase shift. However, for diffusive water molecules, the net phase shift depends on its motion. Fig. 2.14 demonstrates the diffusion weighted signal under the spin-echo sequence of Fig. 2.13. The signal loss mainly follows the exponential decay form with respect to diffusion b-value:

$$S = S_0 e^{-bD} \quad (2.24)$$

It reflects that the signal loss is caused by water molecules diffusion. The faster the water molecules diffuse, the more signal loss is observed. The gradient field enables to us measure diffusion by acquiring measurements at different b values and solving for D in Eq. 2.24. Note that the higher the b -value, the greater the diffusion sensitivity. The b -value is primarily governed by the gradient strength G and application duration δ .

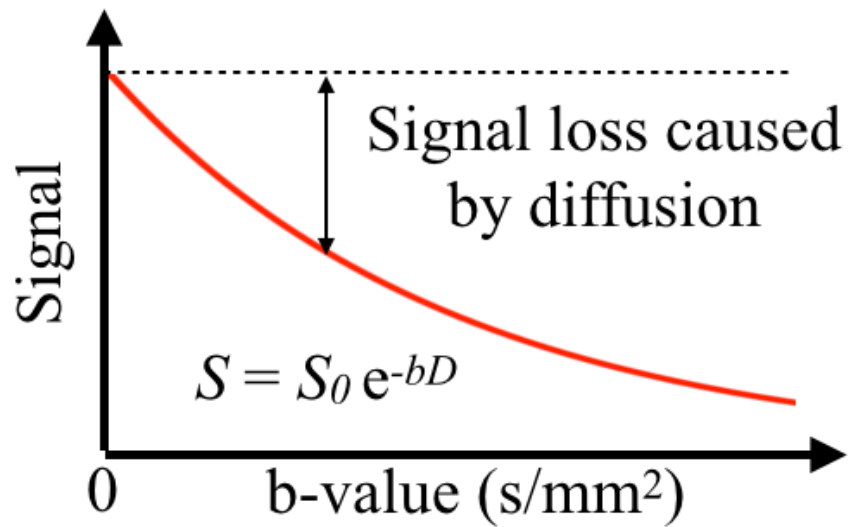


Figure 2.14. The diffusion-weighted signals. The diffusion gradient causes the signal loss which follows the exponential decay form depending on b -values. By measuring several diffusion weighted signals, the diffusion coefficient D and undiffused signal S_0 could be derived.

To measure diffusion coefficient D , we simply take two measurements with diffusion gradients (normally, set one diffusion gradient to zero). Then, we have:

$$S_1 = S_0 \exp(-TE/T_2) e^{-b_1 D} \quad (2.25)$$

$$S_2 = S_0 \exp(-TE/T_2) e^{-b_2 D}$$

by dividing the two signals, we have:

$$D = \frac{\ln(\frac{S_1}{S_2})}{b_2 - b_1} \quad (2.26)$$

As we know, the diffusion induced signal attenuation is significant along the diffusion gradient, we can estimate diffusion along with multiple directions by multiple diffusion experiments with different directions of diffusion gradient. For example, if the white matter tract orients along with diffusion gradient direction, then we could observe greater signal loss due to the faster diffusion.

Now, we can write out the diffusion tensor in matrix form (Basser *et al.*, 1994; Le Bihan, 2003) as,

$$\bar{\bar{D}} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix} \quad (2.27)$$

The diffusion tensor represents the covariance of diffusion induced molecule displacements.

So the diffusion tensor matrix is symmetric.

The diffusion effect can also be considered in to Bloch equation (Torrey, 1956),

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times (\mathbf{B}_0 + \mathbf{B}_1) - \frac{M_x \mathbf{i} + M_y \mathbf{j}}{T_2} - \frac{M_z - M_0}{T_1} \mathbf{k} + \nabla \cdot (\bar{\bar{D}} \nabla \mathbf{M}) \quad (2.28)$$

By solving the Bloch Eq. 2.28, we obtain

$$S(TE) = S(0) \exp(-TE/T_2) \exp(-b \mathbf{g}^T \bar{\bar{D}} \mathbf{g}) \quad (2.29)$$

Where $\mathbf{g}$ is the unit vector of the diffusion gradient.

There are only six independent components in the diffusion tensor $\bar{\bar{D}}$, each measured based on the attenuation of the signal $S(0)$. Therefore, we need at least seven diffusion weighted measurements (one with $\mathbf{G}_d = 0$, and the other six non-collinear $\mathbf{G}_d$). Normally, to increase the tensor imaging precision, 10-30 diffusion weighted imaging scans are conducted with prescribed diffusion gradients (Assaf and Pasternak, 2008; Price *et al.*, 2008; Davis *et al.*, 2009; Asato *et al.*, 2010). In many experiments, images with diffusion weighting along far more

orientations are collected, but in general this is when one aims to fit a more complicated orientation model (beyond the diffusion tensor).

After calculating the diffusion tensor, we can calculate its three orthogonal eigenvectors $\mathbf{e}_i$ and eigenvalues λ_i (all are positive, $i = 1, 2, 3$) by

$$\bar{D}\mathbf{e}_i = \lambda_i\mathbf{e}_i, \quad i = 1, 2, 3 \quad (2.30)$$

$$\bar{D}\mathbf{E} = \mathbf{E}\mathbf{\Lambda}, \quad \mathbf{\Lambda} = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \quad (2.31)$$

$\mathbf{E}$ is the eigenvector matrix indicating the direction of three orthogonal diffusion axes; $\mathbf{\Lambda}$ is the diagonal eigenvalue matrix indicating corresponding diffusion intensities. Then we can define a diffusion ellipsoid (at time τ_d) as

$$\left(\frac{x - x_0}{\sqrt{2\lambda_1\tau_d}}\right)^2 + \left(\frac{y - y_0}{\sqrt{2\lambda_2\tau_d}}\right)^2 + \left(\frac{z - z_0}{\sqrt{2\lambda_3\tau_d}}\right)^2 = 1 \quad (2.32)$$

The mean diffusivity (MD) can be defined as,

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (2.33)$$

and fraction anisotropy (FA) as,

$$FA = \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}} \quad (2.34)$$

FA reflects the diffusion anisotropy of a given voxel, with the value ranging from 0 to 1.

MD and FA reflect the microscopic properties of tissue diffusion, with MD summarizing how far molecules move and FA summarizing the degree of anisotropy. Perfect isotropic diffusion has a diffusion sphere (i.e. $\lambda_1 = \lambda_2 = \lambda_3$, and $FA = 0$), while voxels with FA value close to 1 would represent diffusion purely along a single direction.

The orientation of diffusion is summarized by the principal diffusion direction (PDD), defined as the direction of the eigenvector corresponding to the largest eigenvalue. This is the most elongated direction of the tensor, or equivalently the direction with the strongest diffusivity. One very useful application of PDD is fibre tractography, where the PDD is used as an estimation of fibre orientation in each voxel and structural connections between different brain regions are constructed by tracking the white matter fibre pathways.

2.5 MRI of amyotrophic lateral sclerosis

The work in this thesis focuses on a post-mortem MRI dataset obtained in post-mortem brains of individuals diagnosed with the neurological disease amyotrophic lateral sclerosis (ALS). Here, we provide a brief overview of ALS and the current literature on the use of quantitative MRI to study ALS.

ALS is a progressive motor neuronal disease (Andersen and Al-Chalabi, 2011; Swinnen and Robberecht, 2014) that still lacks effective markers for diagnosis and treatment assessment (Turner *et al.*, 2009; Stagg *et al.*, 2013). The key feature of ALS is the loss of neurons in motor sub-regions of the central nervous system. Pathologists can gain certain direct histological evidence for ALS after patients' death, however, the difficulty of straight assessment (namely, tissue assessment) of the in-vivo brain pathologies circumvent much of the progress in ALS research. Histological studies on density and physiological state of axon, cell, myelin, microglia and even iron ion concentration are currently the reliable measures for disease assessment. An important advance in our understanding of ALS is the discovery of accumulation of phosphorylated TAR DNA-binding protein 43 (pTDP43) (Lagier-tourenne and Cleveland, 2009; Wegorzewska *et al.*, 2009; Wils *et al.*, 2010), which is common to almost all ALS sub-variants.

The use of neuroimaging, and in particular MRI, provides a complementary window into the disease as it progresses in-vivo (Turner *et al.*, 2009). While early work focused on conventional structural measures (Hecht *et al.*, 2002; Iwata, 2008), later studies have focused on techniques like diffusion imaging (Ellis *et al.*, 1999; Agosta *et al.*, 2009, Iwata, 2008). In addition, quantitative multimodal MRI measures could reveal physiological characteristics that could directly link to ALS pathologies (Hardiman *et al.*, 2011; Grossman, 2019). However, the observation of signal changes in MRI has only rarely been accompanied by histopathological investigation of the source of these signal changes.

Post-mortem or ex-vivo MRI has several advantages: (1) validation of MRI by correlation with histology; (2) higher quality neuroimaging data to investigate pathological phenotypes using long scan duration; (3) explicitly bridging the gap between in-vivo MRI and pathology in the same patients. The data used in this project is part of a larger project in post-mortem imaging of ALS that has acquired multiple MRI measures (morphology, diffusion, relaxography, and susceptibility-based contrast) and aims to relate these to a broad range of histopathological features (neurodegeneration, proteinopathy, myelin, iron, inflammation, etc.). This thesis focuses on how to make an accurate quantitative measure of transverse relaxometry time T2 in post-mortem brains, and whether quantitative T2 could provide valuable indicators related to ferritin fraction and finally differentiation ALS from the controls.

In our ALS project, there are thirteen ALS (Age = 64 ± 13 years) and three age-matched control brains (Age = 65 ± 7.5 years) were recruited for this study (Table 2.2). These datasets are used throughout this thesis and form the basis of our analyses in Chapters 3-5.

ID	Age	Sex	Post Mortem Interval (days)	Scan Interval (days)	Fixative	Site of Onset
MND12	60	F	4	173	10%Formalin	N/A
MND13	77	F	3	114	10%Formalin	Right upper limb
MND14	27	M	N/A	228	20%NBF	N/A
MND15	66	M	7	137	10%Formalin	Right lower limb
MND16	65	M	4	94	10%Formalin	Right upper limb
MND17	76	M	3	158	10%Formalin	Right upper limb
MND18	68	M	1	35	10%NBF	Left lower limb
MND19	54	M	N/A	77	N/A	N/A
MND20	65	M	N/A	96	10%Formalin	N/A
MND21	68	M	2	87	10%NBF	N/A
MND22	64	M	2	283	10%Formalin	N/A
MND23	71	M	3	N/A	10%NBF	N/A
MND24	79	F	2	N/A	10%NBF	Bulbar
ALS Mean(SD)	64(13)	-	3.1(1.7)	134(72)	-	-
CTL10	61	M	3	115	10%NBF	N/A
CTL11	74	M	3	45	10%NBF	N/A
CTL12	61	M	3	48	10%NBF	N/A
CTL Mean(SD)	65(7.5)	-	3(0)	69(40)	-	-

Table 2.2: Details of the postmortem brains used within this study. Here, SD is the Standard deviation, CTL represents the control group, while MND represents the ALS group (where MND stands for motor neuron disease); N/A indicates that data is unavailable; M is for male and F is for female; NBF represents neutral buffered formalin.

2.6 Reference

- Agosta, F., Rocca, M. A., Valsasina, P., Sala, S., Caputo, D., Perini, M., ... & Filippi, M. (2009). A longitudinal diffusion tensor MRI study of the cervical cord and brain in amyotrophic lateral sclerosis patients. *Journal of Neurology, Neurosurgery & Psychiatry*, 80(1), 53-55.
- Andersen, P. M., & Al-Chalabi, A. (2011). Clinical genetics of amyotrophic lateral sclerosis: what do we really know?. *Nature Reviews Neurology*, 7(11), 603.
- Assaf, Y., & Pasternak, O. (2008). Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. *Journal of Molecular Neuroscience*, 34(1), 51-61.
- Asato, M. R., Terwilliger, R., Woo, J., & Luna, B. S. (2010). White matter development in adolescence: a DTI study. *Cerebral Cortex*, 20(9), 2122-2131.
- Barry, R. L., Strother, S. C., & Gore, J. C. (2012). Complex and magnitude-only preprocessing of 2D and 3D BOLD fMRI data at 7 T. *Magnetic Resonance in Medicine*, 67(3), 867-871.
- Basser, P. J., Mattiello, J., & LeBihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophysical Journal*, 66(1), 259-267.
- Bloch, F. (1946). Nuclear induction. *Physical Review*, 70(7-8), 460.
- Bloembergen, N., & Morgan, L. O. (1961). Proton relaxation times in paramagnetic solutions. Effects of electron spin relaxation. *The Journal of Chemical Physics*, 34(3), 842-850.
- Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Review*, 94(3), 630.
- Chen, F., De Keyzer, F., Feng, Y. B., Cona, M. M., Yu, J., Marchal, G. (2013). Separate calculation of DW-MRI in assessing therapeutic effect in liver tumors in rats. *World Journal of Gastroenterology*, 19(47), 9092.
- Chilla, G. S., Tan, C. H., Xu, C., & Poh, C. L. (2015). Diffusion weighted magnetic resonance imaging and its recent trend—a survey. *Quantitative Imaging in Medicine and Surgery*, 5(3), 407.
- Davis, S. W., Dennis, N. A., Buchler, N. G., White, L. E., Madden, D. J., & Cabeza, R. (2009). Assessing the effects of age on long white matter tracts using diffusion tensor tractography. *Neuroimage*, 46(2), 530-541.

Ellis, C. M., Simmons, A., Jones, D. K., Bland, J., Dawson, J. M., Horsfield, M. A., ... & Leigh, P. N. (1999). Diffusion tensor MRI assesses corticospinal tract damage in ALS. *Neurology*, 53(5), 1051-1051.

Goldman, M., & Shen, L. (1966). Spin-spin relaxation in La F3. *Physical Review*, 144(1), 321.

Grossman, M. (2019). Amyotrophic lateral sclerosis—a multisystem neurodegenerative disorder. *Nature Reviews Neurology*, 15(1), 5.

Franconi, F., Lemaire, L., Marescaux, L., Jallet, P., & Le Jeune, J. J. (2000). In vivo quantitative microimaging of rat spinal cord at 7T. *Magnetic Resonance in Medicine*, 44(6), 893-898.

Hardiman, O., Van Den Berg, L. H., & Kiernan, M. C. (2011). Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nature Reviews Neurology*, 7(11), 639.

Hecht, M. J., Fellner, F., Fellner, C., Hilz, M. J., Neundörfer, B., & Heuss, D. (2002). Hyperintense and hypointense MRI signals of the precentral gyrus and corticospinal tract in ALS: a follow-up examination including FLAIR images. *Journal of the Neurological Sciences*, 199(1-2), 59-65.

Hennig, J., Nauerth, A., & Friedburg, H. R. (1986). RARE imaging: a fast imaging method for clinical MR. *Magnetic Resonance in Medicine*, 3(6), 823-833.

Iwata, N. K., Aoki, S., Okabe, S., Arai, N., Terao, Y., Kwak, S., ... & Ugawa, Y. (2008). Evaluation of corticospinal tracts in ALS with diffusion tensor MRI and brainstem stimulation. *Neurology*, 70(7), 528-532.

Lagier-Tourenne, C., & Cleveland, D. W. (2009). Rethinking ALS: The FUS about tdp-43. *Cell*, 136(6), 1001-1004.

Lauterbur, P. C. (1973). Image formation by induced local interactions: examples employing nuclear magnetic resonance. *Nature*, 242, 190–191.

Le Bihan, D. (2003). Looking into the functional architecture of the brain with diffusion MRI. *Nature Reviews Neuroscience*, 4(6), 469.

Lu, H., Clingman, C., Golay, X., & Van Zijl, P. C. (2004). Determining the longitudinal relaxation time (T1) of blood at 3.0 tesla. *Magnetic Resonance in Medicine*, 52(3), 679-682.

Markley, J. L., Horsley, W. J., & Klein, M. P. (1971). Spin-lattice relaxation measurements in slowly relaxing complex spectra. *The Journal of Chemical Physics*, 55(7), 3604-3605.

Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear relaxation times. *Review of Scientific Instruments*, 29(8), 688-691.

Mugler, J. P., Meyer, H., & Kiefer, B. (2003, July). Practical implementation of optimized tissue-specific prescribed signal evolutions for improved turbo-spin-echo imaging. In *Proceedings of the Eleventh Meeting of the International Society for Magnetic Resonance in Medicine*. (Vol. 203).

Price, G., Cercignani, M., Parker, G. J., Altmann, D. R., Barnes, T. R., Barker, G. J., ... & Ron, M. A. (2008). White matter tracts in first-episode psychosis: a DTI tractography study of the uncinate fasciculus. *Neuroimage*, 39(3), 949-955.

Seehaus, A. K., Roebroek, A., Chiry, O., Kim, D. S., Ronen, I., Bratzke, H., ... & Galuske, R. A. (2012). Histological validation of DW-MRI tractography in human postmortem tissue. *Cerebral Cortex*, 23(2), 442-450.

Song, Y. Q. (2002). Categories of coherence pathways for the CPMG sequence. *Journal of Magnetic Resonance*, 157(1), 82-91.

Spijkerman, J. M., Petersen, E. T., Hendrikse, J., Luijten, P. R., & Zwanenburg, J. J. (2018). Mapping of cerebrospinal fluid at 3T and 7T MRI. *Quantitative MRI*, p85.

Stagg, C. J., Knight, S., Talbot, K., Jenkinson, M., Maudsley, A. A., & Turner, M. R. (2013). Whole-brain magnetic resonance spectroscopic imaging measures are related to disability in ALS. *Neurology*, 80(7), 610-615.

Swinnen, B., & Robberecht, W. (2014). The phenotypic variability of amyotrophic lateral sclerosis. *Nature Reviews Neurology*, 10(11), 661.

Todd, N., Moeller, S., Auerbach, E. J., Yacoub, E., Flandin, G., & Weiskopf, N. (2016). Evaluation of 2D multiband EPI imaging for high-resolution, whole-brain, task-based fMRI studies at 3T: sensitivity and slice leakage artifacts. *Neuroimage*, 124, 32-42.

Torrey, H. C. (1956). Bloch equations with diffusion terms. *Physical Review*, 104(3), 563.

Turner, M. R., Kiernan, M. C., Leigh, P. N., & Talbot, K. (2009). Biomarkers in amyotrophic lateral sclerosis. *The Lancet Neurology*, 8(1), 94-109.

Wegorzewska, I., Bell, S., Cairns, N. J., Miller, T. M., & Baloh, R. H. (2009). TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proceedings of the National Academy of Sciences*, 106(44), 18809-18814.

Wils, H., Kleinberger, G., Janssens, J., Pereson, S., Joris, G., Cuijt, I., ... & Kumar-Singh, S. (2010). TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proceedings of the National Academy of Sciences*, 107(8), 3858-3863.

Chapter 3

Quantitative T2 mapping with Extended Phase Graph Theory

Contents

3.1 Background.....	41
3.1.1 Quantitative T2 in ALS project.....	41
3.1.2 The fast spin echo sequence.....	42
3.2 Method	45
3.2.1 The EPG theory.....	45
3.2.2 B1 influence on FSE.....	53
3.2.3 T1 influence on FSE.....	55
3.2.4 Fitting: general approach	56
3.2.5 Two-stage fitting.....	60
3.2.5.1 Median filter.....	61
3.2.5.2 Regularisation	64
3.2.6 Experimental parameters	66
3.3 Results and Discussion.....	67
3.3.1 Quality of fit	67
3.3.2 Normalisation.....	69
3.3.3 Formalin influence.....	72
3.4 Conclusion.....	74
3.5 Reference.....	75

In this chapter, I will describe a method to derive quantitative T2 maps in whole, fixed, postmortem human brain samples using an extended phase graph (EPG) framework from data obtained with a multi-echo FSE sequence. The EPG model to describe signal evolution was adopted due to the observation that T2 maps generated via a traditional mono-exponential signal model in our data led to artefacts in the form of hyper-intense T2 values as we approach the boundary of the brain. The signal evolution, due to a combination of the FSE sequence and B1 inhomogeneity at 7T, cannot be adequately modeled with a mono-exponential model. EPG, a model more suited to match the signal evolution observed within regions of low B1 was used to derive quantitative T2 maps, in addition to S0 and B1 maps within each postmortem brain sample. Here, I will talk about the physics, mathematical formulation and implementation of EPG.

When implementing the EPG model to the acquired postmortem data, we found that the resulting B1 map derived from the EPG fit contained artefacts that are not consistent with the expected smoothness of the B1 field. To account for this, a two-step algorithm was introduced, where the first step ensures that the calculated B1 map is smooth, whilst the second step computes the quantitative T2 map based on the smoothed B1 field. In the peripheral areas where both B1 and signal are low, regularization was introduced into the fitting process to prevent hyper-intense T2 values.

Sixteen post mortem brains have been processed with this EPG framework to derive quantitative T2 maps, which were compared with mono-exponential T2 maps. An initial analysis of differences in the T2 maps derived with the EPG framework between amyotrophic lateral sclerosis (ALS) and control (CTL) brains over the motor cortex was performed. Finally, we identify the influence of formalin (both fixation type and concentration distribution) as a

further confound to these T2 maps that ideally would be removed. This motivates us to develop the kinetic tensor model study in the next chapter.

3.1 Background

3.1.1 Quantitative T2 mapping in the ALS project

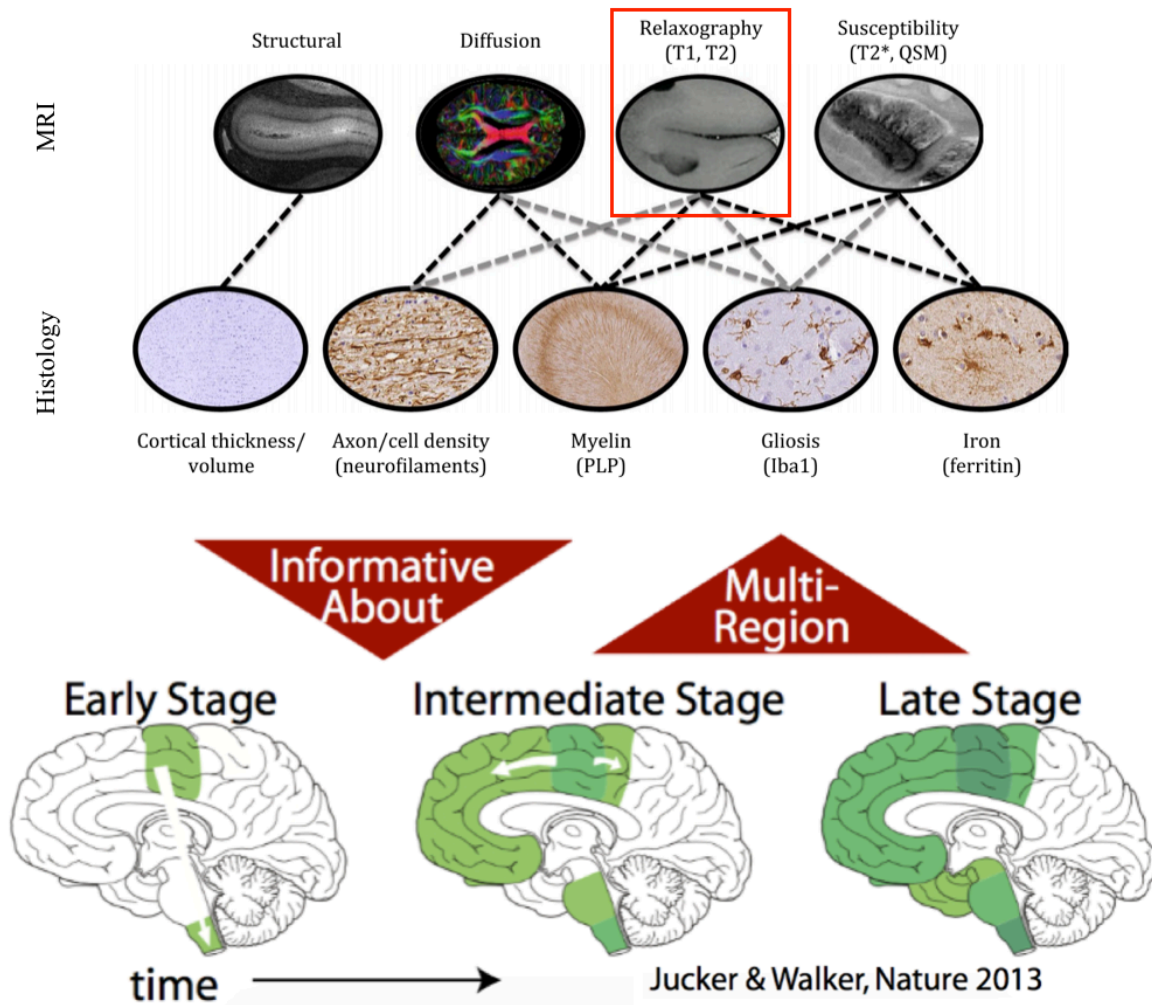


Figure 3.1: The quantitative T2 mapping study presented in this chapter forms part of a wider project investigating ALS pathology in the human brain (Pallebage Gamarallage et al., 2018). In this project, whole postmortem brain samples of patients who had been diagnosed with ALS during their lifetime are examined with a combined multimodal MRI and histopathology protocol. With this, the project aims to map the relationship between the quantitative MRI measures and histology within the same tissue sample.

In Chapter 2, we have presented the importance of MRI as a potential biomarker for monitoring disease progression and/or treatment response in ALS. Based on these perspectives, this work,

as part of the ALS project (Fig. 3.1), focuses on how to make accurate quantitative measurements of the transverse relaxometry time, T2, in post-mortem brains (Fabes *et al.*, 2017; Melhem, 2018), and whether this imaging approach could provide valuable indicators related to ALS pathologies such as iron and myelin, and as an indirect marker of pTDP43 accumulation (Foerster *et al.*, 2013; Gamarallage *et al.*, 2018). Cortical pathology in ALS is generally thought to follow a known series of stages (typically four stages are defined), progressively appearing in specific brain regions. Imaging is very well suited to this approach, by capturing all of these brain regions in a single snapshot. By strategically taking samples from a broad range of brain regions, we can compare postmortem MRI to histopathology in a way that captures different disease stages and therefore captures how MRI signals reflect these different stages.

3.1.2 The FSE Sequence

To quantitatively measure T2 in the whole, post-mortem brain samples, we used a commercially available FSE (Hennig and Nauerth, 1986) pulse sequence (displayed in Fig. 2.10), where a series of 180°-refocusing pulses are applied after a single 90°-pulse to generate a train of echoes, as $90^\circ - (180^\circ - \text{echo})_{\text{ETL}}$. The role of the 180° pulses is to refocusing dephased magnetization in the x-y plane to generate measurable echo signals (Bloch, 1946). After scanning samples with the FSE sequence, a simple exponential decay model can, in theory, be used for fitting the measured data and derive T2 values within each voxel.

Within our experimental data, we observed the phenomenon that at the edge of the tissue, namely in the area of low B1, the T2 weighted intensities are not in shape of exponential decay, but rather appear to initially rise before decaying in subsequent echoes (Fig. 3.3). However, voxels close to the center of the brain do not initially appear to follow a similar signal evolution.

Though the FSE and mono-exponential signal model are simple and powerful tools for T2 mapping, this fitting may not be accurate in the presence of B1 field inhomogeneity, i.e. inhomogeneous field used to rotate magnetization into the transverse plane (Deene *et al.*, 2000). This is particularly problematic at 7T, where this work is performed, where the B1 radiofrequency wavelength is on the order of a postmortem brain. Fig. 3.2 demonstrates the distribution of B1 intensity inhomogeneity in a sagittal view, which leads to an artefactual increase in apparent T2 on the edge of the cerebellum in presence of low B1 (Lebel *et al.*, 2010; McPhee *et al.*, 2017; Milford *et al.*, 2018). Here an inadequate transmit field strength at the brain boundary could not provide sufficient rotation for the 90° and 180° pulses, resulting in the error accumulation of individual nuclei orientation and measured signals (Ahola, 2006). This leads to multiple signals forming pathways over the echo train (further details provided in the EPG theory section), causing the estimation of implausibly high T2 values that do not reflect the underlying anatomy of the tissue. Fig. 3.2 highlights (red box) such an area of low B1 that leads to anomalously high T2 in the mono-exponential fit, which could prevent the detection of pathology in these regions. In particular, the motor cortex, a region of substantial pathological burden in ALS, lies at the boundary of the brain where B1 is very low. Therefore, it is extremely important to use a more complete signal model that can provide accurate quantitative T2 measurements.

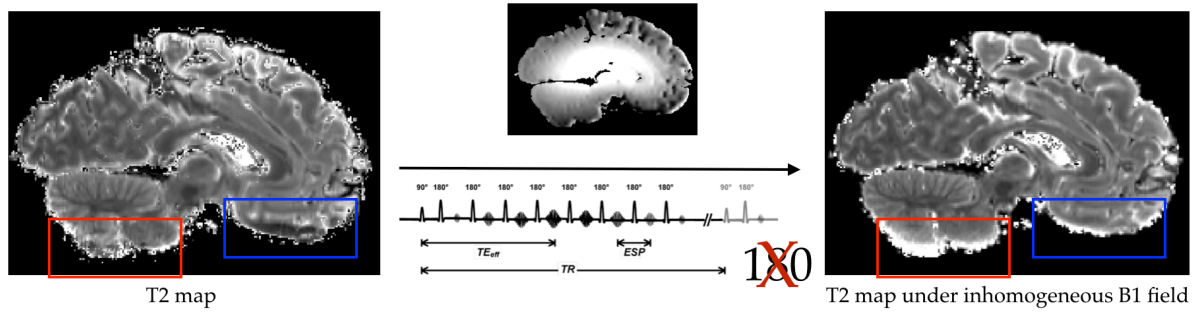


Figure 3.2: B1 inhomogeneity and the resulting artefacts within quantitative T2 maps. Spatial B1 inhomogeneity, particularly at ultra-high field, leads to diminished B1 strength in voxels with increased distance from the centre of the brain. If B1 is accurately calibrated at the centre of the brain, it will be under-calibrated at the edge and signal error will accumulate over the echo train. This leads to B1-dependent T2 estimates that are biased towards higher values. We simulated the effect within this figure as following: Assuming the brain on the left is the original T2 map; then under an inhomogeneous B1 field, shown in the middle, we calculated the signal evolution via the EPG forward signal model. A mono-exponential fit was then used to compute the T2 map based on the EPG signal evolution as shown on the right; Comparing the two T2 maps, we observe accurate T2 values in the centre of the brain, but hyper-intense T2 values in regions of low B1 at the periphery.

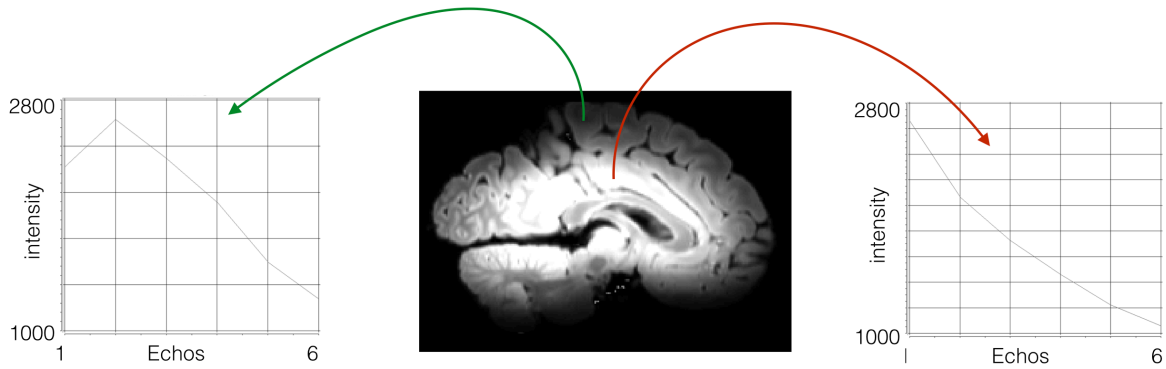


Figure 3.3: The abnormal signal evolution observed at the periphery of the brain. From the data acquired with an FSE sequence close to the brain center (red arrow), the T2 weighted decay signals follow an expected mono-exponential signal decay. However, at the edge of the brain (green arrow), non-mono-exponential (and indeed non-monotonic) signal evolution is observed.

In this chapter, we use a signal formalism perspective to improve the T2 fitting. We confirm that observed signal evolution is induced due to B1 inhomogeneity, and show that it can be addressed with EPG-based fitting.

3.2 Method

3.2.1 EPG theory

EPG is an effective tool for depicting and understanding the evolution of magnetization evolution under various MR sequences. It represents a spin system in terms of a magnetization vector $\mathbf{M} = (M_x, M_y, M_z)^T$ which can be modified by matrix operations related to the specifications of gradient fields, RF pulses, relaxation, off-resonance, flow, diffusion etc. (Malik et al., 2012; Weigel, 2015). EPG theory provides a simple way to calculate what type of echoes are formed and when, disentangling the complicated signal evolution that can develop after a series of RF pulses and gradients.

First, the magnetization vector can be manipulated by RF pulses. Assuming equilibrium magnetization along the z-axis, according to the Euler's rotation theorem, any RF pulse of flip angle α with azimuthal phase φ relative to x-axis could be described using the rotation matrix composition $\mathbf{R}_\varphi(\alpha) = \mathbf{R}_Z(\varphi)\mathbf{R}_X(\alpha)\mathbf{R}_Z(-\varphi)$

$$\mathbf{R}_X(\alpha) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & -\sin\alpha \\ 0 & \sin\alpha & \cos\alpha \end{bmatrix} \quad (3.1)$$

$$\mathbf{R}_Z(\varphi) = \begin{bmatrix} \cos\varphi & -\sin\varphi & 0 \\ \sin\varphi & \cos\varphi & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (3.2)$$

Where $\mathbf{R}_X(\alpha)$ represents a rotation by α about the x-axis and $\mathbf{R}_Z(\varphi)$ represents a rotation by φ about the z-axis. The effect of an RF pulse can thus be simply represented by applying $\mathbf{R}_\varphi(\alpha)$ to the state vector $\mathbf{M}$.

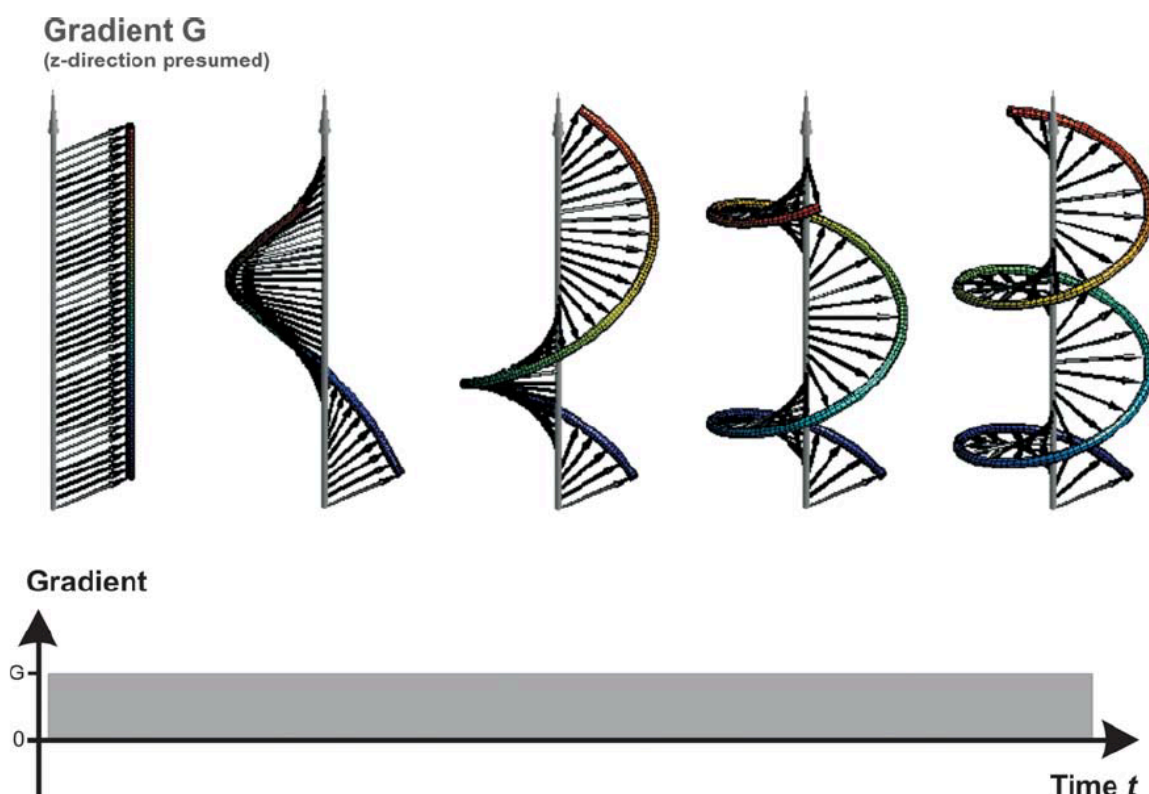


Figure 3.4: Magnetization vectors evolving under a gradient field that is applied along the vertical direction over an extended time period. At time $t=0$, the magnetization is aligned, immediately after excitation. Over time, the magnetization will develop a helical form with a denser helix as time progresses. The number of helical wraps can be represented by a wave vector $\mathbf{k}$. The image comes from (Weigel, 2015).

Gradient fields can manipulate transverse magnetization components M_x and M_y by introducing phase offsets. Most commonly, the EPG framework assumes that signal is being calculated at a voxel-wise level, and that the primary effect of a gradient is to introduce dephasing, although EPG can also be used to represent bulk phase offsets from gradients as well. Fig. 3.4 illustrates an example of how G_z dephases magnetization over time. The gradient field labels the voxel at position $\mathbf{r}$ with an additional longitudinal magnetic field $\Delta B_0 = \mathbf{G} \cdot \mathbf{r}$. As time goes by, the phase of the magnetization evolves according to its position $\mathbf{r}$ and forms a helix shape that can be represented by a single wave vector $\mathbf{k}$ to indicate the spatial frequency

of the magnetization distribution. Summation over the entire population gives the net signal that can be measured over time in the presence of the applied gradient field.

We define the angular wave vector as,

$$\mathbf{k}(t) = \gamma \int_0^t \mathbf{G}(t') dt' \quad (3.3)$$

where $\mathbf{G}$ is the gradient field, t is the duration, and γ is the gyromagnetic ratio. For a voxel at position $\mathbf{r}$, the x and y components of magnetization are expressed as,

$$M_x(\mathbf{r}) = M \cos(\mathbf{k} \cdot \mathbf{r}) \quad (3.4)$$

$$M_y(\mathbf{r}) = M \sin(\mathbf{k} \cdot \mathbf{r}) \quad (3.5)$$

Where M is the magnitude of the voxel's magnetization.

To describe transverse magnetization in a simplified way, the spatially varying transverse magnetization under gradients can be decomposed into dephasing M_+ and rephasing M_- components, respectively. The transformation between $[M_x, M_y]$ and $[M_+, M_-]$ are defined by (Jaynes, 1955) as,

$$M_+(\mathbf{r}) = M_x(\mathbf{r}) + iM_y(\mathbf{r}) = M e^{i\varphi(\mathbf{r})} = M e^{i\mathbf{k} \cdot \mathbf{r}} = (M_-)^* \quad (3.6)$$

$$M_-(\mathbf{r}) = M_x(\mathbf{r}) - iM_y(\mathbf{r}) = M e^{-i\varphi(\mathbf{r})} = M e^{-i\mathbf{k} \cdot \mathbf{r}} = (M_+)^*$$

Note that a 180° -pulse around the x-axis can flip between M_+ and M_- . In the presence of the same gradient, the dephased M_+ will continue to dephase to a higher wave vector, whilst the phase reversed M_- magnetization will rephase to a lower wave vector (and could refocus under the gradient field if this causes it to reach $\mathbf{k} = 0$).

Integrating all isochromats along the gradient's direction represents the net magnetization. Considering the integral over a volume V (Weigel, 2015) where various helices of different spatial frequencies are present, leads to the relation,

$$\begin{aligned}\tilde{F}_+(\mathbf{k}) &= \int_V \left(M_x(\mathbf{r}) + iM_y(\mathbf{r}) \right) e^{-i\mathbf{k}\mathbf{r}} d^3r = \int_V M_+(\mathbf{r}) e^{-i\mathbf{k}\mathbf{r}} d^3r \\ \Leftrightarrow M_x(\mathbf{r}) + iM_y(\mathbf{r}) &= M_+(\mathbf{r}) = \int_V \tilde{F}_+(\mathbf{k}) e^{i\mathbf{k}\mathbf{r}} d^3k\end{aligned}\tag{3.7}$$

In Eq. 3.7, transverse magnetization integrates complex spatial harmonics (helices) with different wave vectors $\mathbf{k}$. $M_+(\mathbf{r})$ and $F_+(\mathbf{k})$ are a Fourier transformation pair, where $F_+(\mathbf{k})$ depicts the amplitude and phase of each helix of wave vector $\mathbf{k}$ under the gradient field (Eq. 3.3). Similar transformations could also be obtained for $F_-(\mathbf{k})$ and the longitudinal component $Z(\mathbf{k})$ in Eqs. 3.8-3.9.

$$\begin{aligned}\tilde{F}_-(\mathbf{k}) &= \int_V \left(M_x(\mathbf{r}) - iM_y(\mathbf{r}) \right) e^{-i\mathbf{k}\mathbf{r}} d^3r = \int_V M_-(\mathbf{r}) e^{-i\mathbf{k}\mathbf{r}} d^3r \\ \Leftrightarrow M_x(\mathbf{r}) - iM_y(\mathbf{r}) &= M_-(\mathbf{r}) = \int_V \tilde{F}_-(\mathbf{k}) e^{i\mathbf{k}\mathbf{r}} d^3k\end{aligned}\tag{3.8}$$

$$\begin{aligned}\tilde{Z}(\mathbf{k}) &= \int_V M_z(\mathbf{r}) e^{-i\mathbf{k}\mathbf{r}} d^3r \\ \Leftrightarrow M_z(\mathbf{r}) &= \int_V \tilde{Z}(\mathbf{k}) e^{i\mathbf{k}\mathbf{r}} d^3k\end{aligned}\tag{3.9}$$

Note that M_z is purely real and so in practice $Z(-\mathbf{k})=Z^*(\mathbf{k})$.

We can conceive of F_- , F_+ , and Z as representing different features of the magnetization distribution. The F states represent transverse magnetization, with $+$ and $-$ indicating right- and left-handed helices. The Z states represent longitudinal magnetization with a sinusoidal modulation. As we will see, this representation enables us to independently manipulate and track each of these states using relatively simple operations to nevertheless describe very complicated distributions of magnetization.

Moving from the conventional magnetization representation in x,y,z to the new configuration state coordinate system requires a simple similarity transformation $\mathbf{S}$ matrix, defined as,

$$\begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} \xleftrightarrow{S \text{ or } S^{-1}} \begin{bmatrix} M_+ \\ M_- \\ M_z \end{bmatrix}, \quad \mathbf{S} = \begin{pmatrix} 1 & +i & 0 \\ 1 & -i & 0 \\ 0 & 0 & 1 \end{pmatrix}, \quad \mathbf{S}^{-1} = \frac{1}{2} \begin{bmatrix} 1 & 1 & 0 \\ -i & +i & 0 \\ 0 & 0 & 2 \end{bmatrix} \quad (3.10)$$

From this, the rotation matrix for an RF pulse can be written as $\mathbf{T} = \mathbf{S}\mathbf{R}\mathbf{S}^{-1}$, leading to,

$$\begin{bmatrix} M_+ \\ M_- \\ M_z \end{bmatrix}^{after} = \mathbf{T} \begin{bmatrix} M_+ \\ M_- \\ M_z \end{bmatrix}^{before} = \begin{bmatrix} \cos^2 \frac{\alpha}{2} & e^{2i\varphi} \sin^2 \frac{\alpha}{2} & -ie^{i\varphi} \sin \alpha \\ e^{-2i\varphi} \sin^2 \frac{\alpha}{2} & \cos^2 \frac{\alpha}{2} & ie^{-i\varphi} \sin \alpha \\ -\frac{i}{2} e^{-i\varphi} \sin \alpha & \frac{i}{2} e^{i\varphi} \sin \alpha & \cos \alpha \end{bmatrix} \begin{bmatrix} M_+ \\ M_- \\ M_z \end{bmatrix}^{before} \quad (3.11)$$

The superscripts signify the notation of magnetization ‘before’ and ‘after’ the RF pulse was applied. Eq. 3.11 is extremely important because it illustrates how RF pulse mixes three components (dephasing transverse magnetization M_+ , rephasing transverse magnetization M_- and longitudinal magnetization M_z). Combining with Eq.3.7-3.9, we can describe how RF pulses influence the configuration states as follows,

$$\begin{bmatrix} \tilde{F}_+(k) \\ \tilde{F}_-(-k) \\ \tilde{Z}(k) \end{bmatrix}^{after} = \begin{bmatrix} \cos^2 \frac{\alpha}{2} & e^{2i\varphi} \sin^2 \frac{\alpha}{2} & -ie^{i\varphi} \sin \alpha \\ e^{-2i\varphi} \sin^2 \frac{\alpha}{2} & \cos^2 \frac{\alpha}{2} & ie^{-i\varphi} \sin \alpha \\ -\frac{i}{2} e^{-i\varphi} \sin \alpha & \frac{i}{2} e^{i\varphi} \sin \alpha & \cos \alpha \end{bmatrix} \begin{bmatrix} \tilde{F}_+(k) \\ \tilde{F}_-(-k) \\ \tilde{Z}(k) \end{bmatrix}^{before} \quad (3.12)$$

Then the effect of RF pulses can be clearly interpreted as in Fig. 3.5. First, RF pulse can only exchange magnetization among $F_+(k)$, $F_-(k)$, and $Z(k)$ states with equal dephasing order k . When $k = 0$, RF pulses can either excite longitudinal magnetization (corresponding to $Z(0)$) into the transverse plane to generate the $F(0)$ component, or store the magnetization components $F(0)$ into the longitudinal state $Z(0)$. For $k \neq 0$ cases, the RF pulse causes three configuration states to mix in a manner governed by Eq. 3.12. We can see $F_+(k)$ and $F_-(k)$ can be flipped to $Z(k)$, or the $Z(k)$ component can be transformed to the $F_-(k)$ state for echo measurements, or to the $F_+(k)$ state for future dephasing. In these cases, we are transferring

magnetization from longitudinal to transverse, or vice versa, and the RF pulse is in effect behaving like a 90° tip. Fig. 3.5 also demonstrates that the RF pulse can mix certain proportions of $F_+(k)$ and $F_-(k)$. Noting that these two components correspond to the opposite phase (right- vs left-handed helices), we can consider this transition to behave like a 180° pulse. Finally, some magnetization in a given state will remain in that state; that is, it behaves as if no RF pulse occurred (a 0° pulse). Hence, it is often noted that the EPG formalism is in effect taking an RF pulse of arbitrary flip angle α and splitting it into 0° , 90° , and 180° components.

While RF pulses only mix within the same wave vector k , the dephasing gradients act to evolve the transverse components between wave vectors. The F_- states will, in general, evolve to lower wave vectors ($k \rightarrow k - \Delta k$), and the F_+ states will evolve to higher wave vectors ($k \rightarrow k + \Delta k$).

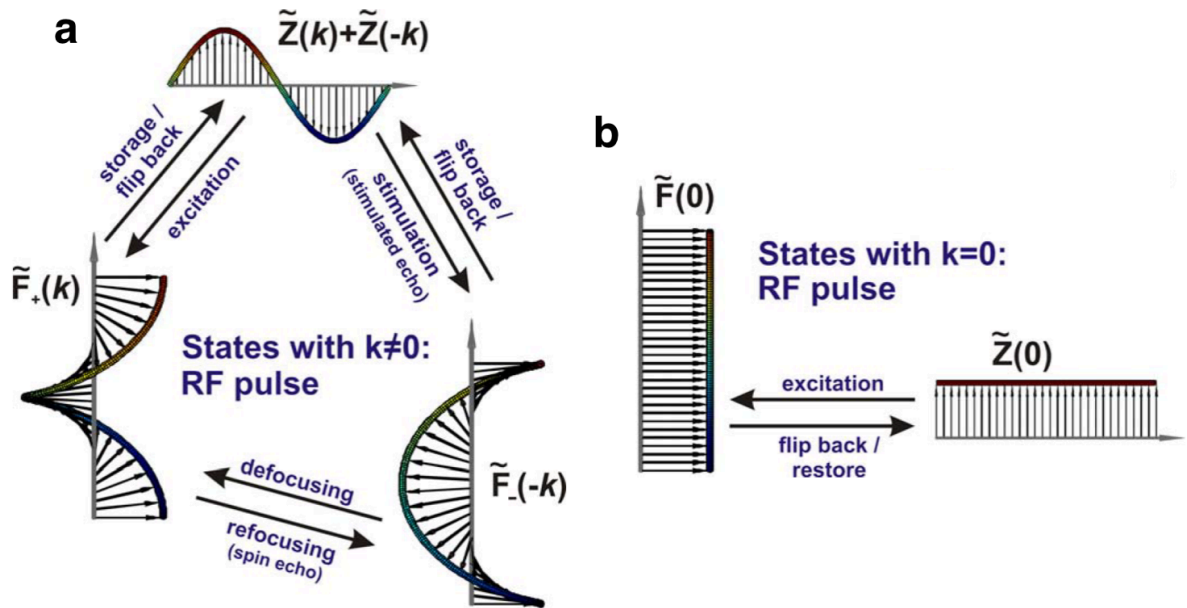


Figure 3.5: Transition among three configuration states. (a) For $k \neq 0$ case, illustrated here as $k=1$, the transition is governed by Eq. 3.12, and the component contents after the RF transition determines whether a spin echo or a stimulated echo ($F_-(k)$), continued dephasing ($F_+(k)$), or storage of coherent magnetization ($Z(k)$) will be generated. (b) For the $k = 0$ case, both $F_+(k)$ and $F_-(k)$ have no imaginary component and $F(0)$ is used to represent them together. RF can exchange $F(0)$ and $Z(0)$ contents, where the former will induce a free induction decay (FID) signal, and the later will be stored as longitudinal magnetization. The image comes from (Weigel, 2015).

For periodic sequences like FSE and steady-state free precession (SSFP), repeatedly applying the same RF pulses will restrict the phase order k to discrete values. In this case, we use a dephasing angle θ instead of spatial coordinate r for the Fourier decomposition (Hayes et al., 1985). Then Eq.3.12 can be rewritten into the discrete form Eq.3.13,

$$\begin{aligned}
M_+(\theta) &= M_x(\theta) + iM_y(\theta) = \sum_{k=-\infty}^{\infty} \tilde{F}_k \cdot e^{ik\theta} \\
M_-(\theta) &= M_x(\theta) - iM_y(\theta) = \left(\sum_{k=-\infty}^{\infty} \tilde{F}_k \cdot e^{ik\theta} \right)^* = \sum_{k=-\infty}^{\infty} \tilde{F}_{-k}^* \cdot e^{ik\theta} \\
M_z(\theta) &= \sum_{k=-\infty}^{\infty} \tilde{Z}_k \cdot e^{ik\theta} = \text{Real} \left(\sum_{k=0}^{\infty} \tilde{Z}_k \cdot e^{ik\theta} \right)
\end{aligned} \tag{3.13}$$

where $\tilde{F}_k, \tilde{F}_{-k}, \tilde{Z}_k$ are the discretized versions of $F_+(k), F_-(k), Z(k)$, defining,

$$\begin{bmatrix} \tilde{F}_{+k} \\ \tilde{F}_{-k}^* \\ \tilde{Z}_k \end{bmatrix}^{after} = \begin{bmatrix} \cos^2 \frac{\alpha}{2} & e^{2i\varphi} \sin^2 \frac{\alpha}{2} & -ie^{i\varphi} \sin \alpha \\ e^{-2i\varphi} \sin^2 \frac{\alpha}{2} & \cos^2 \frac{\alpha}{2} & ie^{-i\varphi} \sin \alpha \\ -\frac{i}{2} e^{-i\varphi} \sin \alpha & \frac{i}{2} e^{i\varphi} \sin \alpha & \cos \alpha \end{bmatrix} \begin{bmatrix} \tilde{F}_{+k} \\ \tilde{F}_{-k}^* \\ \tilde{Z}_k \end{bmatrix}^{before} \tag{3.14}$$

In order to effectively simulate the FSE pulse sequence, it is necessary to add relaxation terms.

$$\mathbf{E}(\tau, T1, T2) = \begin{bmatrix} E_2 & 0 & 0 \\ 0 & E_2 & 0 \\ 0 & 0 & E_1 \end{bmatrix} \tag{3.15}$$

where $E_1 = \exp(-\tau/T1)$ and $E_2 = \exp(-\tau/T2)$, with $T1$ and $T2$ being the relaxation times and τ being the relaxation duration.

$T1$ recovery is described as,

$$\begin{bmatrix} F_+ \\ F_- \\ Z \end{bmatrix}^{after} = \begin{bmatrix} E_2 & 0 & 0 \\ 0 & E_2 & 0 \\ 0 & 0 & E_1 \end{bmatrix} \cdot \begin{bmatrix} F_+ \\ F_- \\ Z \end{bmatrix}^{before} + \begin{bmatrix} 0 \\ 0 \\ M_0(1 - E_1) \end{bmatrix} \tag{3.16}$$

Then, the state matrix can be written as

$$\Omega = \begin{bmatrix} \tilde{F}_0 & \tilde{F}_1 & \tilde{F}_2 & \tilde{F}_3 & \tilde{F}_4 & \tilde{F}_5 & \dots \\ \tilde{F}_0^* & \tilde{F}_{-1}^* & \tilde{F}_{-2}^* & \tilde{F}_{-3}^* & \tilde{F}_{-4}^* & \tilde{F}_{-5}^* & \dots \\ \tilde{Z}_0 & \tilde{Z}_1 & \tilde{Z}_2 & \tilde{Z}_3 & \tilde{Z}_4 & \tilde{Z}_5 & \dots \end{bmatrix} \quad (3.17)$$

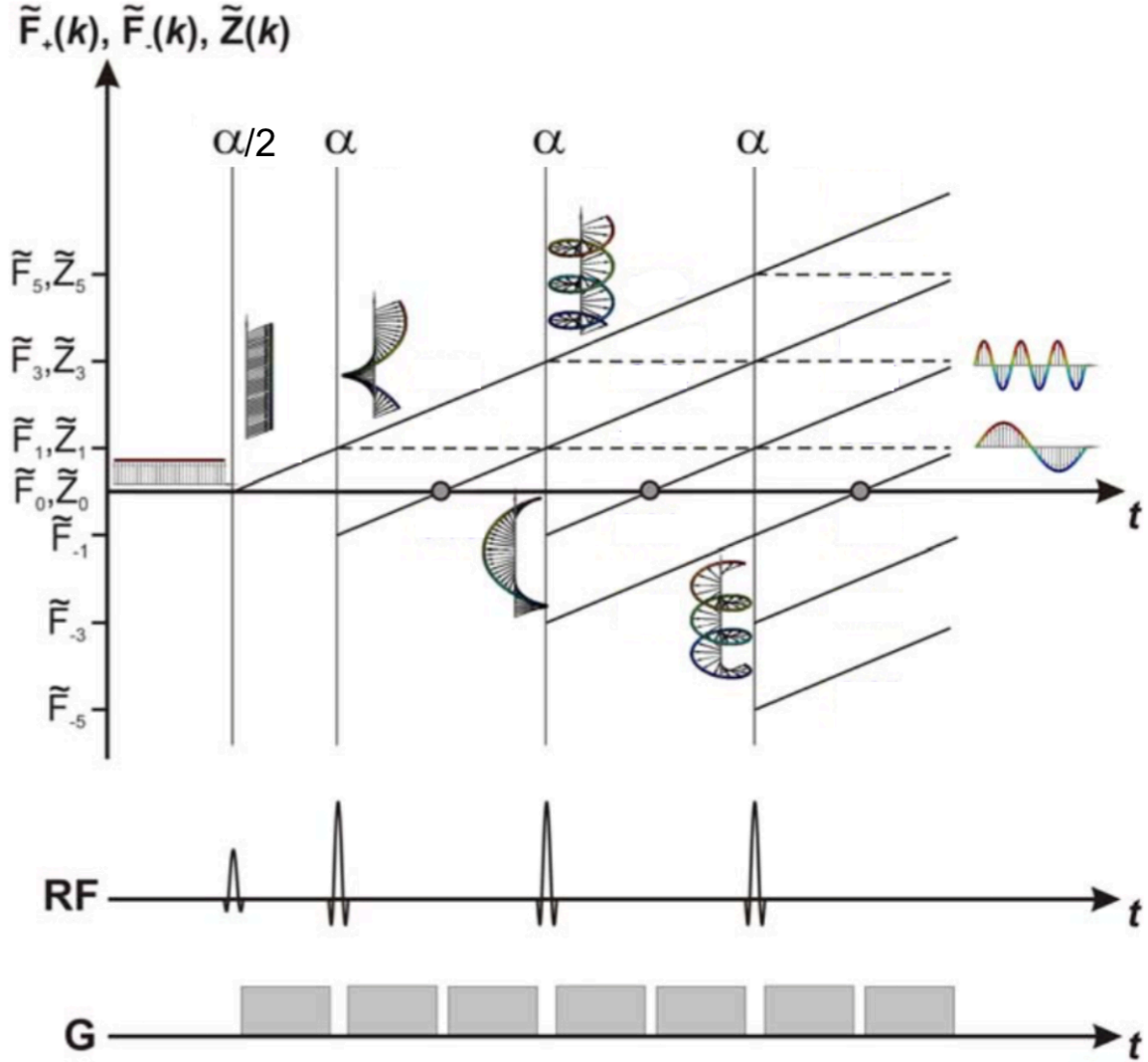


Figure 3.6: EPG graph for the FSE pulse sequence. The x-axis indicates the time of the pulse sequence, whereas the y-axis represents the evolution of the configuration state components. As RF and gradients are applied to the brain, hydrogen nuclei magnetization change configuration accordingly, with helix-shaped distribution of magnetization presented at each time point. Gradients manage the signal evolution represented by the solid sloped line, RF pulses partition or branch the configuration states (Kaiser *et al.*, 2001) horizontal dashed lines represent the storage of Z magnetization and echoes occur when the growth of F₊ intersects the x-axis. Image modified from (Weigel, 2015)

The EPG graph for an FSE sequence is drawn in Fig. 3.6 in a way that accounts for B1 inhomogeneity, with flip angles of $\alpha/2$ and α rather than the prescribed 90° or 180° . The magnetization begins in state Z_0 , and then an $\alpha/2$ pulse tips magnetization into the transverse plane, populating the F_0 state. Gradients for spatial encoding and spoiling cause the magnetization to evolve into the F_1 state. The subsequent α pulses decompose and rearrange the dephasing, rephasing and longitudinal magnetization as in Eq. 3.14. Echoes occur when a component of the longitudinal magnetization completely rephases, corresponding to an F state crossing the $k=0$ line, indicated in Fig. 3.6 by the gray circles. The signal is given by the amount of magnetization in that state at each echo time.

Using the EPG formalism for this FSE sequence, the signal at the n^{th} echo can be calculated as:

$$\Omega(nTE) = K_{TE/2} T_\alpha (K_{TE} T_\alpha)^{n-1} K_{TE/2} T_{\alpha/2} \Omega(0) \quad (3.18)$$

Where T_α represents an RF pulse with flip angle α as in Eq.3.14, the matrix K_T describes the evolution of magnetization due to relaxation and gradients over a time period T . Specifically, the product of a relaxation matrix as in Eq 3.16 and a matrix describing the transfer of magnetization between states due to gradients ($\tilde{F}_k \rightarrow \tilde{F}_{k+\Delta k}, \tilde{F}_k^* \rightarrow \tilde{F}_{k-\Delta k}^*, \tilde{Z}_k \rightarrow \tilde{Z}_k$).

3.2.2 B1 influence on FSE

We implemented the EPG signal calculation (based on code from Weigel, 2015) given input variables of flip angle, tissue T2, T1, initial signal intensity S_0 , echo time (TE) and echo train length (ETL). The simulation in Fig. 3.7 demonstrates that flip angle or B1 intensity has a major effect on FSE signal evolution. For flip angles in the range $[170^\circ, 190^\circ]$, the MR signal follows a mono-exponential evolution. When the flip angle is in the range of $[150^\circ, 170^\circ]$ (or $[190^\circ, 210^\circ]$), we observe small signal fluctuations deviating from the pure exponential decay model. When the flip angle falls in range $[100^\circ, 150^\circ]$ (or $[210^\circ, 260^\circ]$), the FSE signal exhibits an initial increase and peaks at the second echo, and then drops for subsequent echoes, similar

to what we observed at the periphery of the brain in our data (Fig. 3.3). At very low flip angles, there can even be a gradual increase in the signal, as indicated in Fig. 3.7. Were we to model the signal evolution with a mono-exponential decay, these results indicate that the tolerance for B1 inhomogeneity would cause fitting to fail over large regions of the brain. We instead propose to derive the relevant parameters (T_2 , α , S_0 , T_1) voxel-wise using the EPG model to obtain the best fit to the measured echoes signals i.e. FSE:

$$[T_2, \alpha, S_0, T_1] = \arg \min_{T_2, \alpha, S_0, T_1} \|FSE - EPG(T_2, \alpha, S_0, T_1)\|_2 \quad (3.19)$$

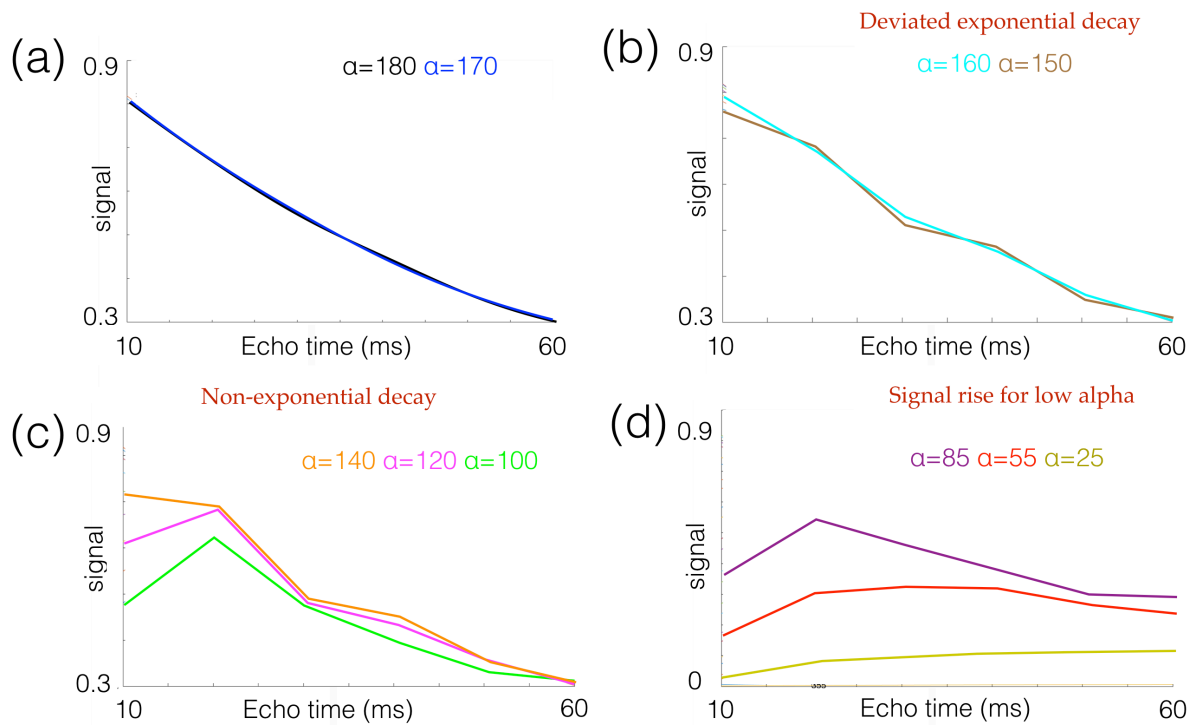


Figure 3.7: Simulation of the FSE signal evolution under various flip angle conditions using the EPG signal calculation framework. (a) When the refocusing angle is close to 180° , the RF pulse is still capable of refocusing MR signals leading to mono-exponential decay. (b) As the flip angle is slightly reduced, the signal begins to deviate slightly from mono-exponential decay. (c-d) When the flip angle is far from 180° , the signal is not even monotonically decreasing, similar to what we measured in the peripheral areas of post mortem brains (Fig. 3.3). For this simulation, sequence parameters were matched to our measurements ($TE = [11 \ 23 \ 34 \ 46 \ 57 \ 69]$ ms, $TR=1s$) and tissue parameters were chosen to be similar to post-mortem tissue $S_0 = 1$, $T_2 = 40$ ms and $T_1 = 450$ ms. Note that (Lebel *et al.*, 2010; McPhee *et al.*, 2017; Milford *et al.*, 2018) demonstrated similar results. Note that (Lebel *et al.*, 2010; McPhee *et al.*, 2017; Milford *et al.*, 2018) demonstrated similar results of how inhomogeneous B1 influence the signal evolution (i.e. the signal could rise in the initial period and then decay under imperfect B1 fields).

3.2.3 T1 influence on FSE

The estimation of 4 parameters from FSE data from only six echoes (Eq.3.19) is expected to be challenging, particularly in light of the subtle differences in signal features in Fig. 3.7. In this section, we consider whether differences in T1 are expected to affect the evolution of signal with echo time. If not, T1 could be fixed, rather than included in the fitting process of Eq.3.19. Using the EPG framework, we investigated the influence of T1 over a wide range of different T2s and flip angles α , as is shown in Fig. 3.8. T1 variations are presented along the x-axis, while the y-axis is signal strength of the sixth echo, which will have the strongest influence from T1. The most striking feature in the figure is that, outside of the combination of very low α and T1, the curves are horizontal, indicating that the signal is largely independent of T1. Therefore, we assigned T1 to a fixed value of 450ms (taken from previous work indicating $T1 \in [320, 580]$) (Qi *et al*, 2016)). This allows us to simplify our fitting equation:

$$[T2, \alpha, S0] = \arg \min_{T2, \alpha, S0} \|FSE - EPG(T2, \alpha, S0)\| \quad (3.20)$$

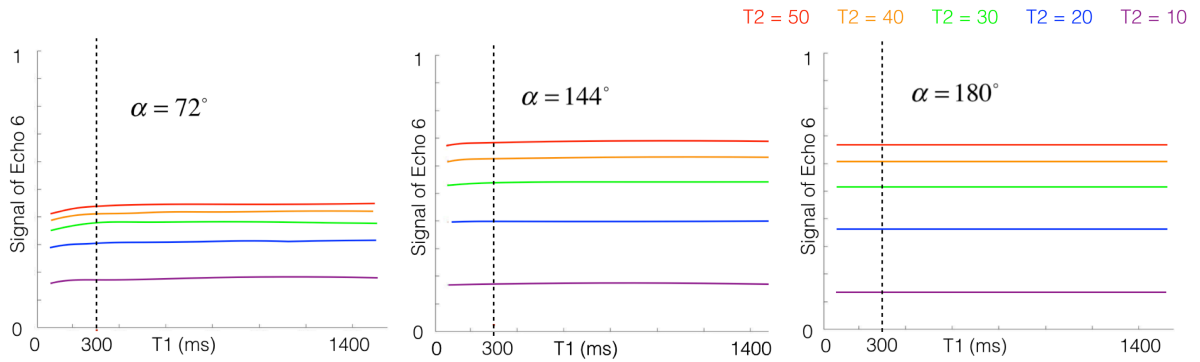


Figure 3.8: The influence of T1 on FSE signals. In this figure array, the x-axis represents the T1 value, the y-axis represents the signal measured at the 6th echo, each color-coded curve represents a specified T2 value, and each subplot represents the flip-angle used in the simulation.

3.2.4 Fitting: general approach

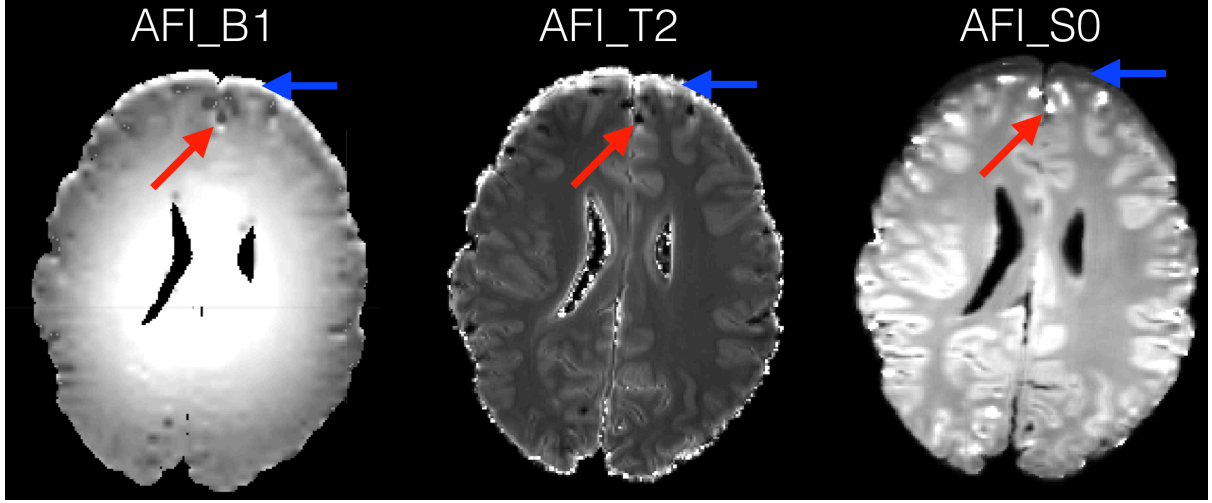


Figure 3.9: EPG fitting with AFI B1. Since the AFI B1 map contains both a hyperintensity band (blue arrows) and hypointensity pits (red arrows) in the frontal region, the derived T2 and S0 suffer abnormal values in the corresponding regions.

In this section, we describe the general fitting algorithm, in the context of the post-mortem data acquired with the protocol described in section 3.2.6. Note that we additionally acquired B1 maps via the actual flip-angle imaging (AFI) sequence, (Yarnykh, 2007) and made an initial attempt to perform S0 and T2 mapping by EPG fitting with these maps. However, as shown in the results in Fig. 3.9, we observed that the AFI B1 map contains a hyperintensity band (blue arrows), in addition to hypointensity pits (red arrows) in the frontal region, and the derived T2 and S0 maps contain abnormal values in the corresponding regions. As the B1 maps generated from the AFI sequence were not sufficiently accurate for fitting B1 and generating quantitative T2 maps, we calculated the B1 value as part of the fitting. In future work (section 6.2.3), we propose two ways to involve the AFI sequence in the EPG fitting framework.

Using Eq.3.20, we can simultaneously fit values of T2, α and S0. This requires an iterative search algorithm running until convergence for approximately 6 million voxels (within the brain mask), implying a high computational cost. We tested several approaches including simulated annealing (Kirkpatrick et al., 1983; Corana et al., 1987), genetic algorithms (Whitley, 1994; Zhang, 2015), Levenberg–Marquardt (Marquardt, 1963; More, 1977), and Nelder Mead

algorithm (Lagariasl, 1998) (NMA). Considering both accuracy and computation speed, NMA was finally selected as the solver for Eq.3.20, as implemented by the *fminsearch* function in MATLAB (version R2017b, Mathworks, US).

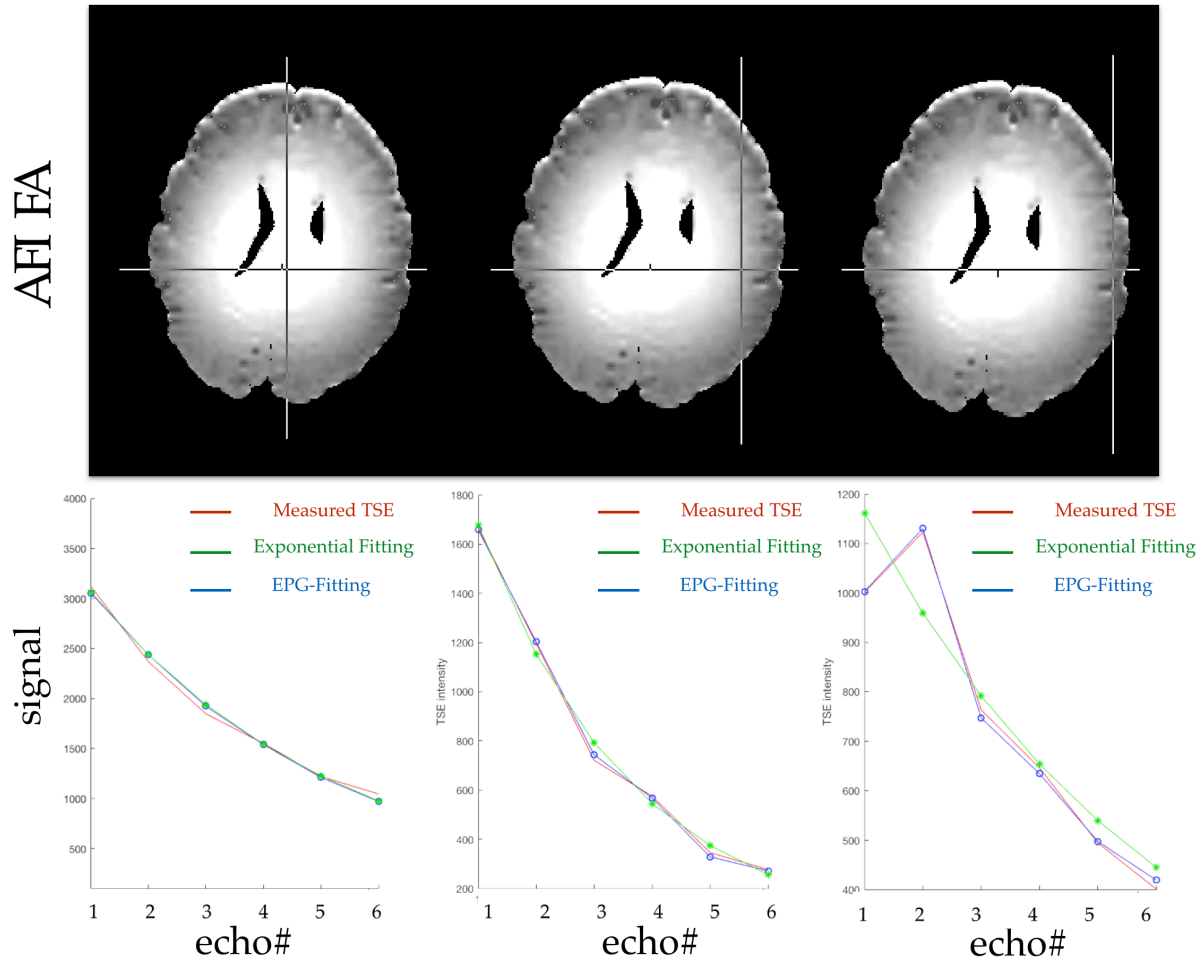


Figure 3.10: Accuracy comparison between the EPG fitting and traditional exponential fitting. Three voxels with different B1 fields (producing 180°, 150°, and 100° flip angle respectively, as measured by AFI) were selected for comparison. EPG produced accurate tissue parameters (T2, S0, and flip angle) for all B1 cases, whilst for the exponential model, the further B1 is away from 180° pulse, the worse the echo fitting. For the example voxel in the periphery, the fitted exponential is unable to capture signal behavior and the derived T2 is unreliable.

To evaluate the quality of fit, we predicted the MR signal for each echo time from the fitted T2, S0 and α for each voxel for both the EPG fitting and mono-exponential model. Fig. 3.10, compares the measured and fitted signals. Three voxels with flip angle 180°, 150°, and 100° (measured by the AFI, (Yarnykh, 2007)) were selected for comparison. The signal reconstruction based on the EPG model and associated fit (blue) gives excellent agreement

with the experimentally determined values (red) across all three points. In contrast, the exponential fitting worked relatively well at a high flip angle, but less well at a lower flip angle. The most striking failure of the mono-exponential fit is at the periphery ($\alpha=100^\circ$) where the exponential model completely fails to describe the experimental signal evolution. This is consistent with our expectation that the exponential model is unable to address B1 inhomogeneity. EPG can address this problem, though more computational resources are required. In our implementation, 3 hours are required with 176 Sun Grid Engine (SGE) computational cores working simultaneously to complete one T2 map calculation.

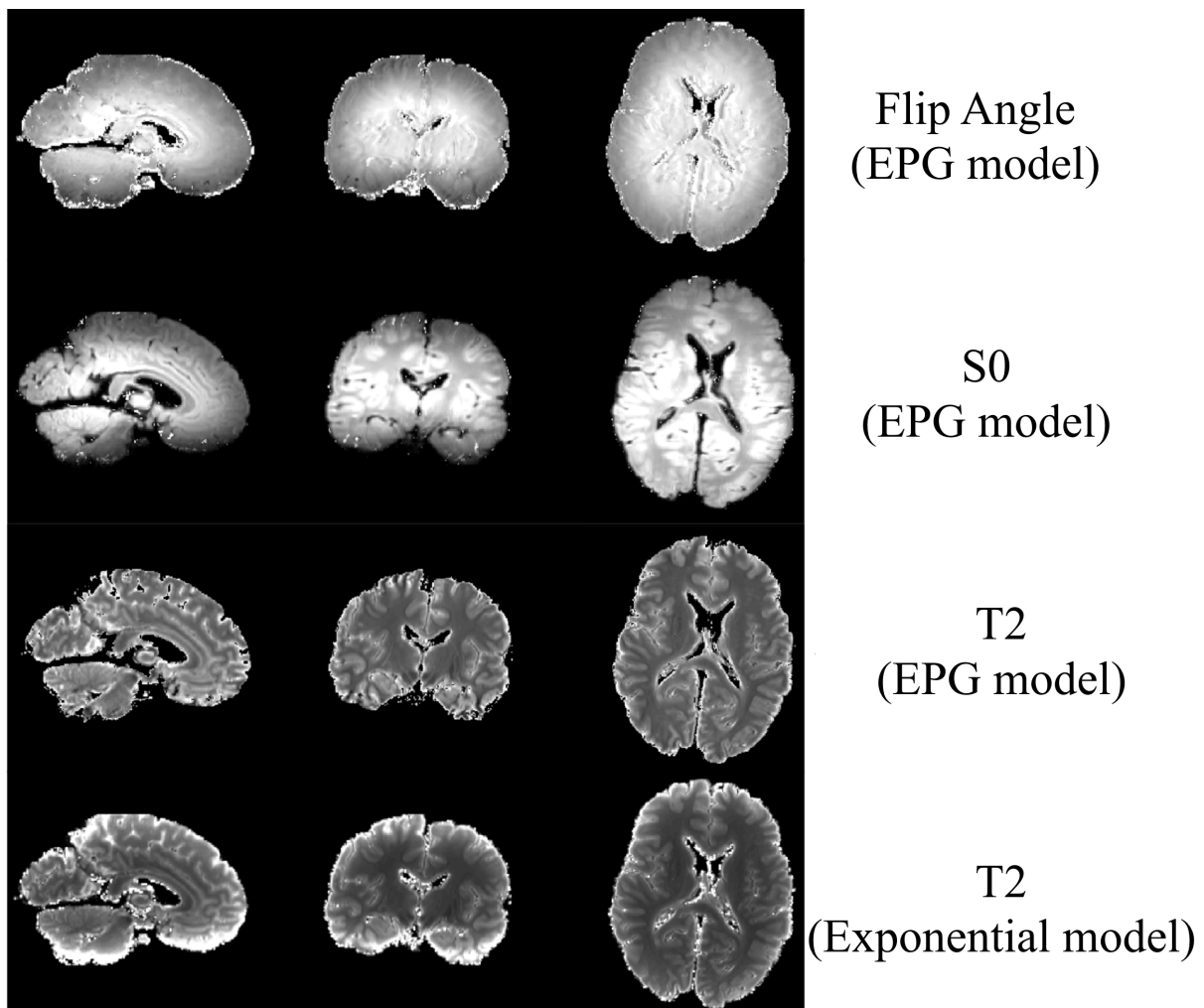


Figure 3.11: Flip angle map, hydrogen density map and quantitative T2 map computed by EPG fitting, and a T2 map calculated by mono-exponential fitting (Exp_T2). Flip angle map presents a pattern of higher values (close to 180°) in the center and lower values on the edge. By fitting with EPG, the exponential-fitting artefacts on the cerebellum and inferior frontal cortex were removed.

Fig. 3.11 shows fitted maps from the mono-exponential and EPG fits, including the S0 and B1 maps for the EPG fitting. The low-B1 induced artefacts in the exponential model have been removed, resulting in a more homogeneous T2 map over the brain with more pronounced gray-white matter contrast. The flip angle map from the EPG follows the general pattern expected, with high B1 at the center of the brain and significantly attenuated B1 at the periphery. However, this B1 map also contains a clear anatomical contrast with high spatial frequency boundaries, whereas the true B1 field should be spatially smooth (Cheng and Huang, 2006). The lack of smoothness suggests overfitting to B1 that may affect T2 quantification. We therefore refined our fitting procedure to use a 2-step optimization process, with the B1 field included in the first stage of fitting (including parameters T2, S0, and B1), after which this B1 map is subsequently smoothed and fixed in the second stage of fitting (including parameters T2 and S0).

To further demonstrate the B1 dependence of fitting errors over the acquired echo times, Fig. 3.12 illustrates FSE images at six echo times, reconstructed images and error maps based on the exponential model, and EPG reconstructed images and error maps based on the EPG fits. We can clearly see that the EPG fitting performs well across the brain and at all echo times, whilst the exponential fitting has high, spatially varying errors, particularly at the first two time points and at the periphery. The error maps for the exponential fit have a pattern that is consistent with B1 inhomogeneity, and with the kind of echo-time variability in the signal shown at the voxel-wise level in Fig. 3.10.

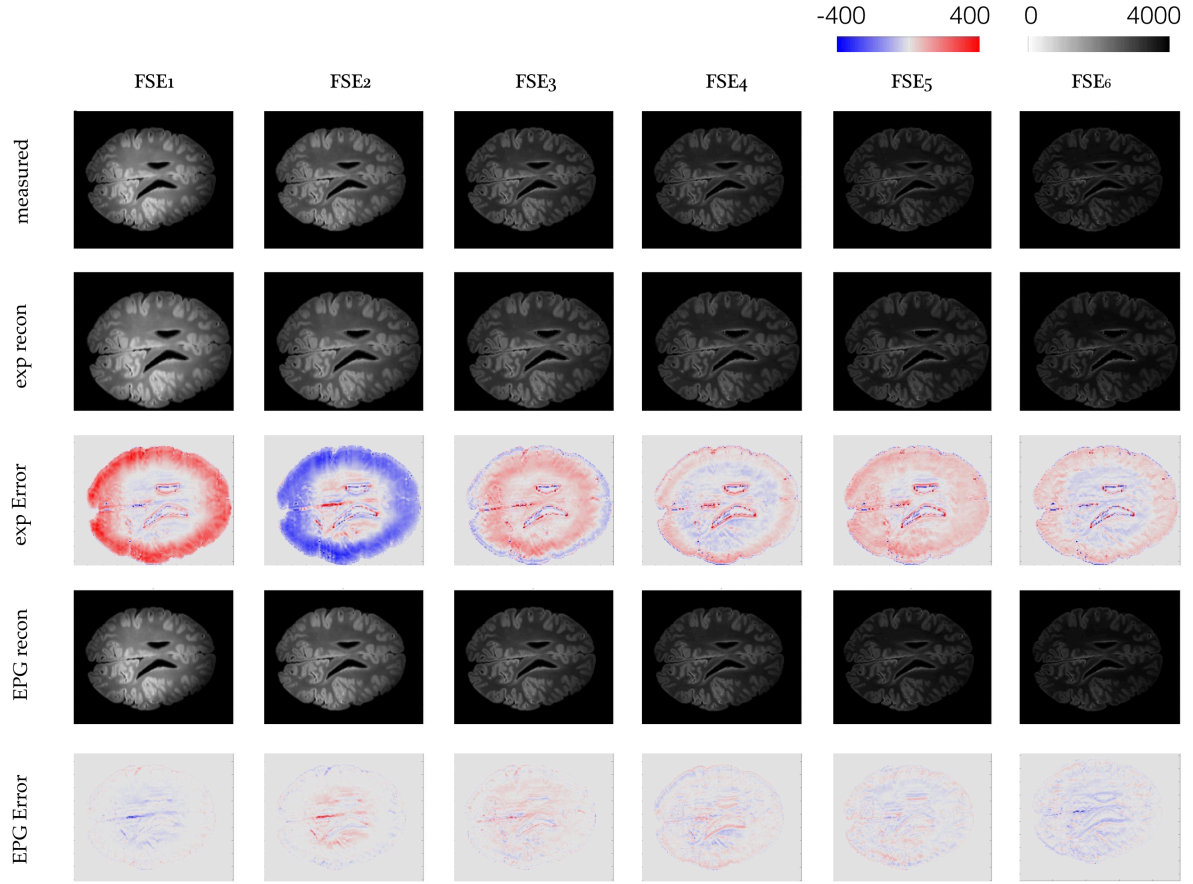


Figure 3.12: Error comparison between the EPG and exponential model fits. Measured and predicted axial slices for the exponential and EPG models for all 6 echoes, alongside their error maps. EPG fitting demonstrated a reduced residual in comparison to the exponential model in all 6 echoes.

3.2.5 Two-stage Fitting

It is well established that the B1 field should be spatially smooth (Lutti et al., 2010), but the EPG-derived B1 map clearly exhibits structure reflecting anatomical boundaries (Fig. 3.11). To address this issue, we adopted a two-stage fitting. In the first stage, the B1 map was fit alongside T2 and S0 as described above. The resulting B1 map was then smoothed using a median filter to eliminate local variance while preserving the low-frequency B1 distribution. We then repeat the fitting to estimate T2 and S0 with this smooth B1 map as a fixed voxel-wise parameter. Hence, our final fitting has fixed T1 and B1, making it better conditioned to our data with only six echo times.

3.2.5.1 Median filtering

A few additional alterations to this basic approach were critical to achieving good results throughout the brain, specifically with respect to the median filtering:

(1). As shown in Fig. 3.13a-b, naïve application of the median filter can yield a non-zero value in background voxels and shrink the object boundary. We, therefore, exclude background voxels from the median filter and remove isolated voxels from main tissue by the MATLAB command *bwconncomp* (Fig. 3.13c-d.)

(2). As shown in Fig. 3.13e, the median filter produces a dark band at the edge of the cerebellum where the RF receiver coils have low sensitivity. The resultant flip angle maps after median filtering have implausibly low B1 field. To compensate this, *fminsearchbd* was used with bounds $[100^\circ, 180^\circ]$ during the first stage of EPG fitting, as shown in Fig. 3.13f. In addition, by forcing the flip angle to be less than 180° , we overcome the degeneracy of the cost function where values of $180^\circ + \theta$ fit equally well to $180^\circ - \theta$. While this degeneracy is not problematic for signal modeling, it can cause problems for median filtering.

(3) Fig. 3.14 shows the kernel size impact on image processing, where small kernels (<7 voxels) still result in images with some anatomical structure, whereas large kernels (>19 voxels) produce a smooth map but introduce large-scale intensity bias (reducing field variance). Based on these evaluations, we selected a kernel size of 11 voxels as providing a plausibly smooth B1 field with no clear bias.

(4) The computational cost for median filtering is another concern. In our case of kernel size = 11 with an image size of 210x240 voxels per slice, the most straightforward implementation of median filtering must store $(11+11+1)^3 \cdot 210 \cdot 240 = 850,000$ values. Using 176 cores on our compute cluster and with slice-wise parallelization, it took approximately 2 hours to process each brain.

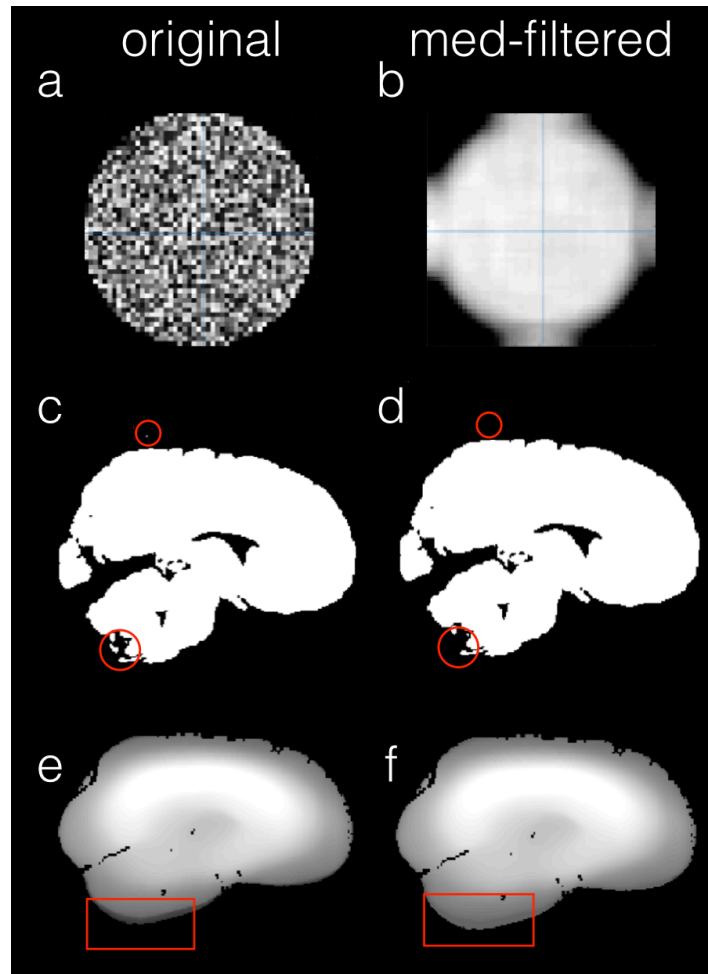


Figure 3.13: Preprocessing procedures preceding the application of median filters. (a-b) demonstrate the experimental simulation of the background effect on the median filter. The shape and intensity of tissue can be modified by background voxels. For example, after filtering, the originally spherical region of signal becomes non-spherical. (c-d) Isolated voxels are also influenced by voxels outside, resulting in the modification of boundaries. (e-f) Low flip angle at the bottom of the cerebellum cannot be fixed by the median filter that could be fixed by bounding the searching algorithm like `fminsearchbd` of Matlab (D’Errico, 2014).

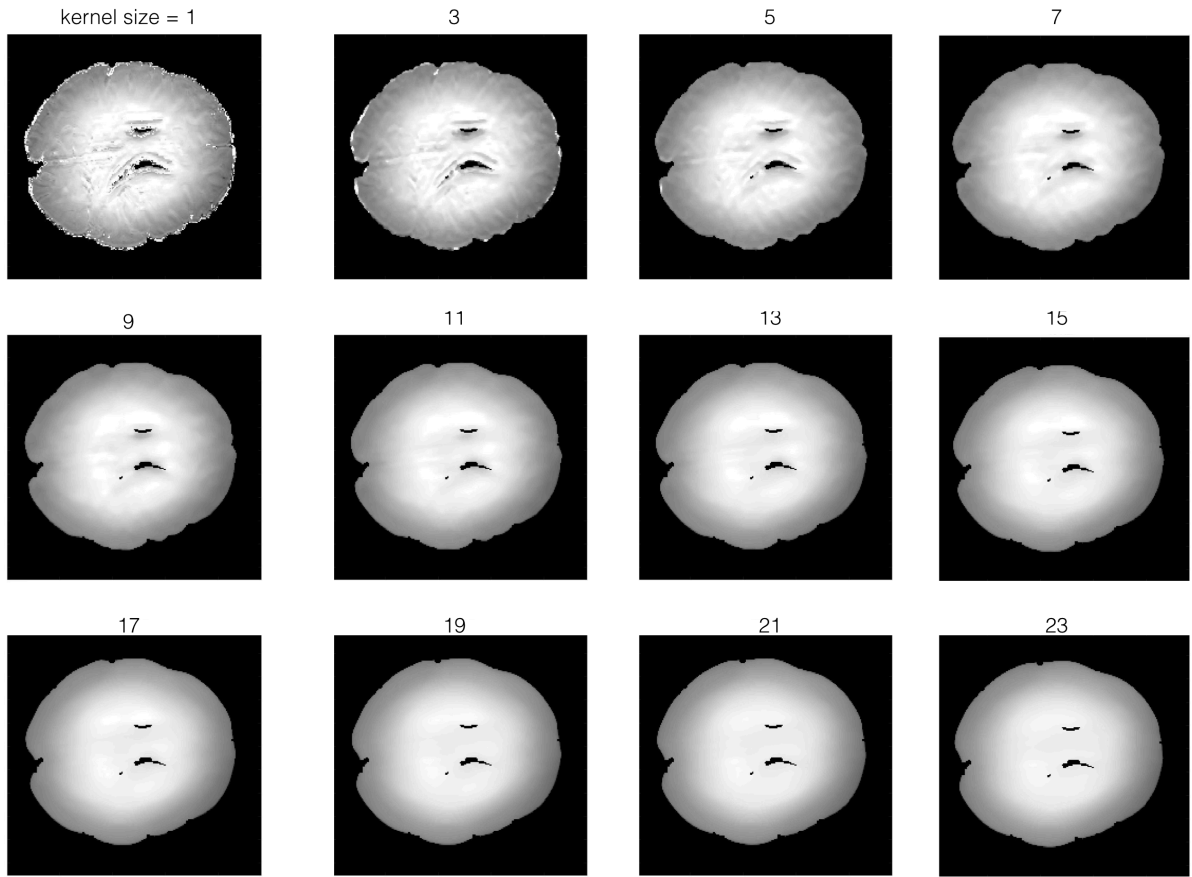


Figure 3.14. The flip angle map after median filtering with a range of kernel sizes.

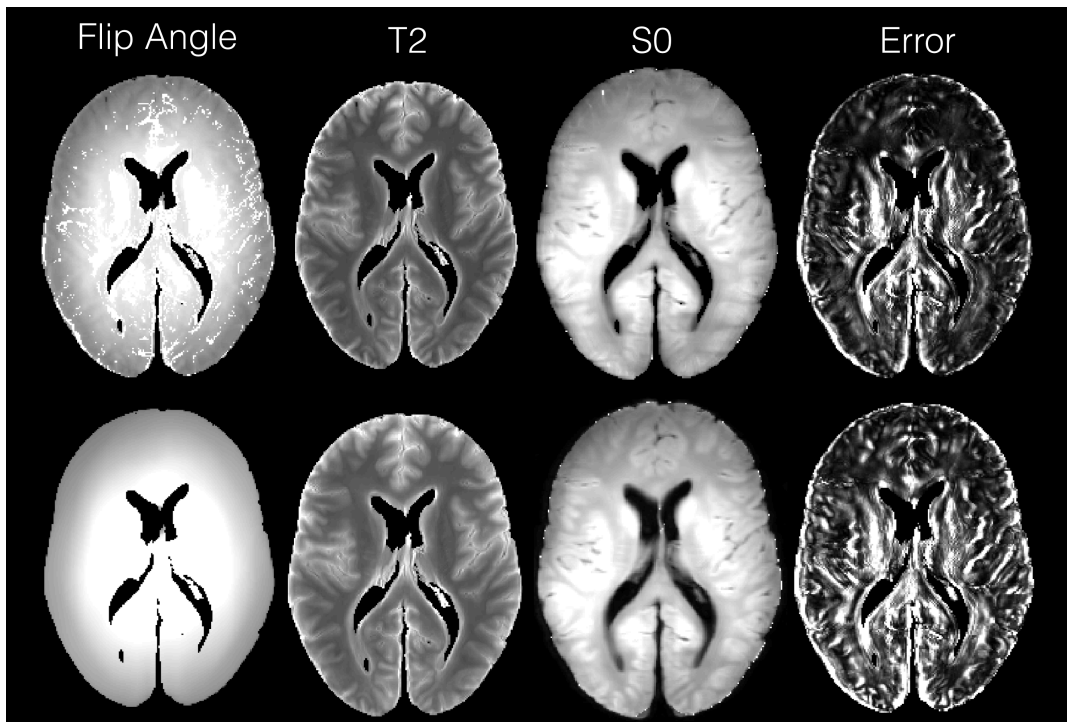


Figure 3.15: Results of the first (top) and second (bottom) stage of EPG fitting.

The optimization for this second stage of fitting, with both T1 and B1 fixed, becomes:

$$[T2, S0] = \arg \min_{T2, S0} \|FSE - EPG(T2, S0)\| \quad (3.21)$$

Example results from the two fitting stages are displayed in Fig. 3.15, high frequency spikes and anatomical structure in B1 from the first stage (top row) have been removed by median filtering for the second stage of fitting (bottom row). The T2 and S0 map do not yield major visible changes as compared with the first fitting stage. As expected, the second stage of fitting corresponds to higher error than the first stage of fitting, but given the non-physical nature of the original estimated B1 map, this likely reflects over-fitting in the first stage.

3.2.5.2 Regularization

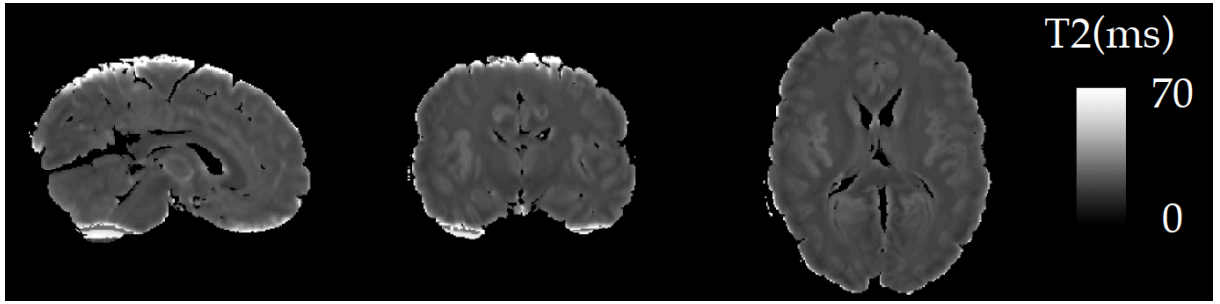


Figure 3.16: White ring artefact on the top and bottom of the quantitative T2 maps (MND12). These artefacts occur significantly in nine brains, including six ALS and three CTL brains. In particular, the artefact lies in the motor cortex and inferior temporal cortex, both of which are regions of known ALS pathology. Therefore, a correction is required to address this issue. Note that the bounded search approach can only limit T2 to the upper bound value but cannot place it at a reasonable value in the middle range.

The quantitative T2 maps in several brains revealed a bright ring artefact at the top and bottom of the brain, regardless of the fitting stage. The artefact occurs only on the top and bottom of the brains, but not on left or right edges. Referring to the raw FSE data, we found that these artefacts were located in regions of particularly low B1 field, and that a certain amount of noise may bias the estimated flip angle towards a high value. We addressed this issue by adding a

regularization term that penalizes implausibly high T2, with the degree of regularization controlled by parameter λ ,

$$L = \arg \min_{T2, S0} \left[\left(\sum_i (FSE_i - EPG_i(T2, S0))^2 / 6 \right) + \lambda \cdot T2^2 \right] \quad (3.22)$$

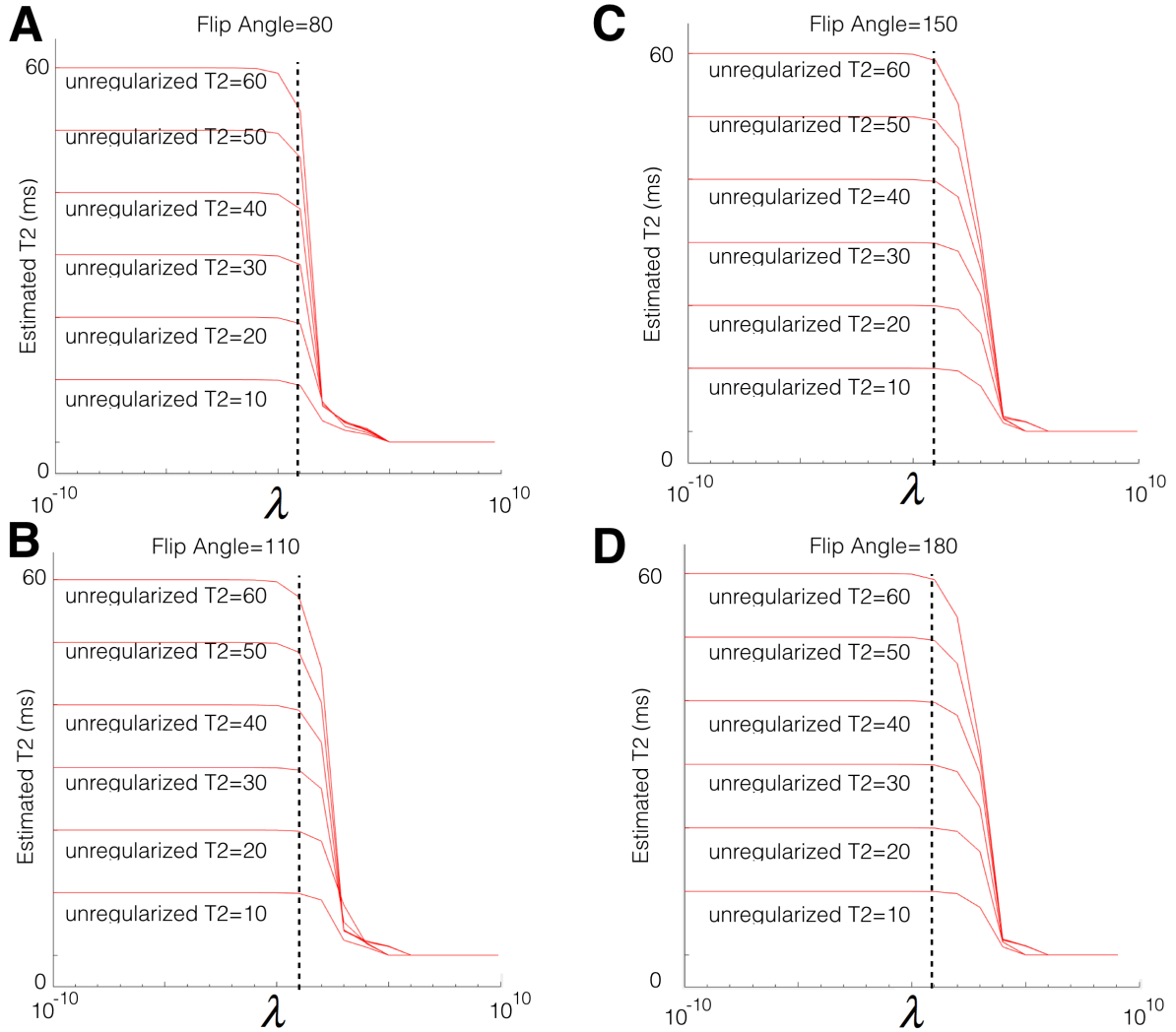


Figure 3.17: Simulations for λ selection for a regularized T2 fitting algorithm. For each pair of T2 (10:10:60 ms, within the range expected for post-mortem tissue at 7T) and flip angle (80°, 110°, 150°, 180°), we simulated the corresponding FSE signals at each echo point. Then the regularized Eq.3.22 was used to compute the estimated T2 with different regularization parameters λ . Here, we display curves to show how the regularization parameter λ influences T2 estimation. T2 can be accurately estimated with $\lambda \leq 10$ (indicated by the dashed line), for flip angles greater than 110° (Fig. C-D); for lower flip angles, regularization reduces the value of T2 from high intensity (Fig. A).

We aim to set the regularization parameter, λ , to avoid the observed hyperintensities in T2 without affecting regions where the fitting is performing well. We evaluated the setting of λ through simulation, as displayed in Fig. 3.17. The simulation explores a range of T2 (10:10:60) and flip angle (80,110,150,180) values and uses EPG to simulate the signals at 6 echo times. Using Eq.3.22, we derive T2 estimates for a range of λ values (displayed on the x-axis). Fig. 3.17 presents the simulation results, which displays four flip angle cases and a range of T2 values. For each case, if λ is smaller than 10, the estimated T2 does not deviate from its original value, and beyond this, the estimated T2 will rapidly drop to zero due to regularization. Based on these simulations, we set $\lambda=10$, such that at low flip angle the constrains the fitting algorithm to avoid T2 over-estimation. Figure 3.18 demonstrates how the control parameter $\lambda = 10$ reduces the T2 hyperintensities at the edge of the brain. There is little associated change to the resulting S0 maps by this additional regularization term.

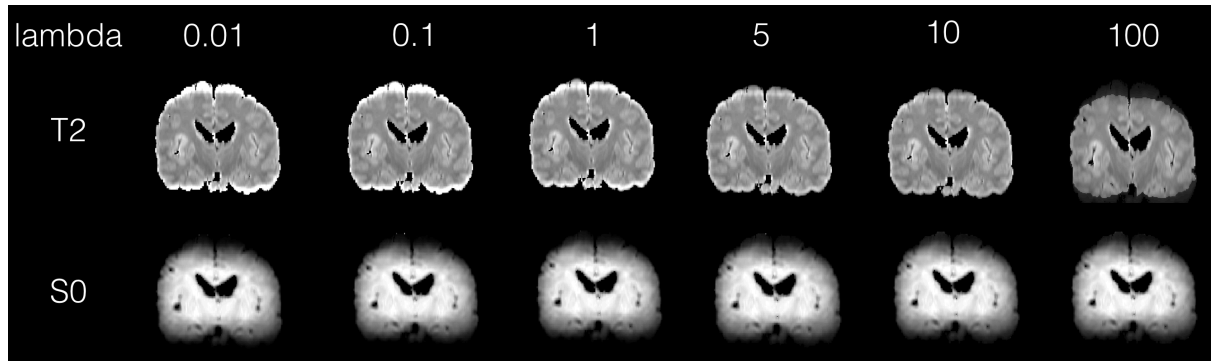


Figure 3.18: Quantitative T2 map and S0 map for different settings of the regularization parameter λ . As λ increases, the T2 hyperintensities are reduced with little change to T2 in the rest of the brain or to the S0 map.

3.2.6 Experimental Parameters

Thirteen ALS and three control post mortem human brains (age 62 ± 12 years, post mortem interval 1-7 days, scan interval 40-356 days) were imaged on a Siemens 7T scanner using a 32 channel receiver head coil. The FSE pulse sequence was applied with TE = [11 23 34 46 57

69] ms, TR = 1000 ms, echo train length (ETL) =6, slice thickness = 1.3mm, FOV=208*156 mm².

3.3 Results and Discussion

3.3.1 Quality of fit

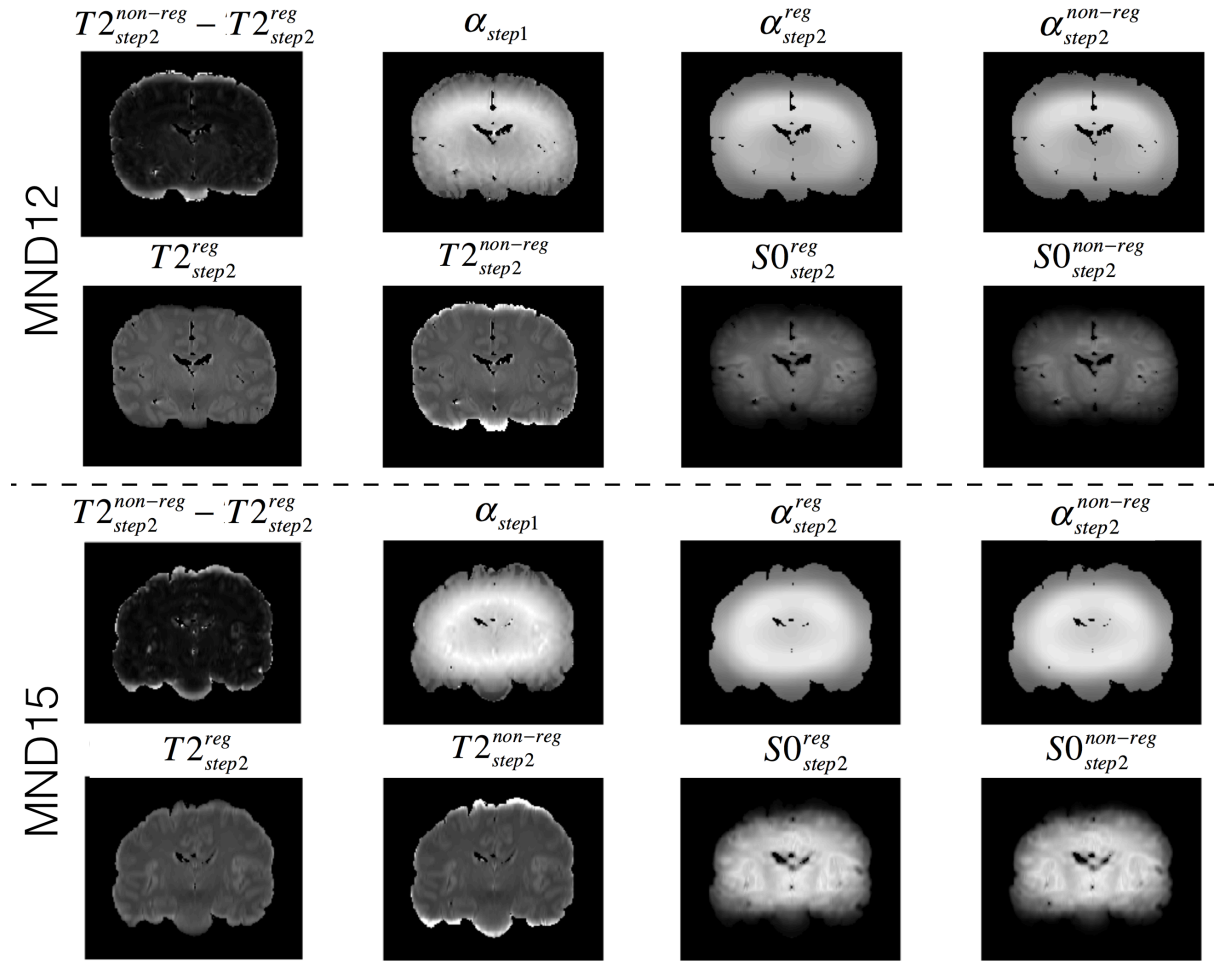


Figure 3.19: Comparison between regularization and non-regularization EPG fitting in brains MND12 and MND15. Adding regularization terms had little effect on the S0 and flip angle maps, but was able to effectively reduce the T2 hyperintensities.

We applied the two-stage processing to all ALS and control brains. Fig. 3.19 demonstrates two brains with severe T2 hyperintensities in non-regularized estimation (indicated as “non-reg”), which is removed by adding the regularization term (indicated as “reg”). The regularization described in Eq.3.22 reduces the observed hyperintensities at the periphery without altering the regions where fitting was already working well, which is indicated by the difference image

(difference between regularized and un-regularized T2 maps). The regularization worked well in all seven brains that were observed to exhibit the T2 hyperintensity artefact, and had little effect on the brains that did not exhibit clear T2 hyperintensities.

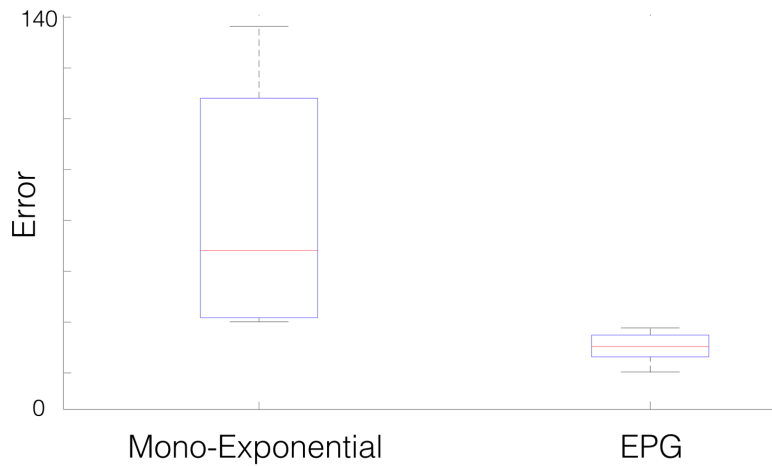


Figure 3.20: The reconstruction error comparison between mono exponential decay model and EPG model ($\text{Err}^{\text{Exp}} = 63.8 \pm 39.5$; $\text{Err}^{\text{EPG}} = 10.1 \pm 6.2$; $P = 0.024$).

Figure 3.20 compares the reconstruction error between fits using the mono-exponential decay model and the two-step EPG algorithm. The two-step EPG fit is significantly more accurate in reconstructing FSE signals than the mono-exponential decay model ($\text{Err}^{\text{EPG}} = 10.1 \pm 6.2$ vs $\text{Err}^{\text{Exp}} = 63.8 \pm 39.5$, $P = 0.024$).

The final two-step fitted T2 maps are displayed in Fig. 3.21 for the full cohort of post mortem brains, including 13 ALS brains and 3 control (CTL) brains. We observe that the T2 intensity, shown as brightness, is correlated with fixative solution (i.e. brains fixed with 10% formalin have relative low T2, whilst brains fixed with 10% neutral buffered formalin have relative high T2), and in the next chapter we will tackle this issue with the proposed kinetic tensor model.

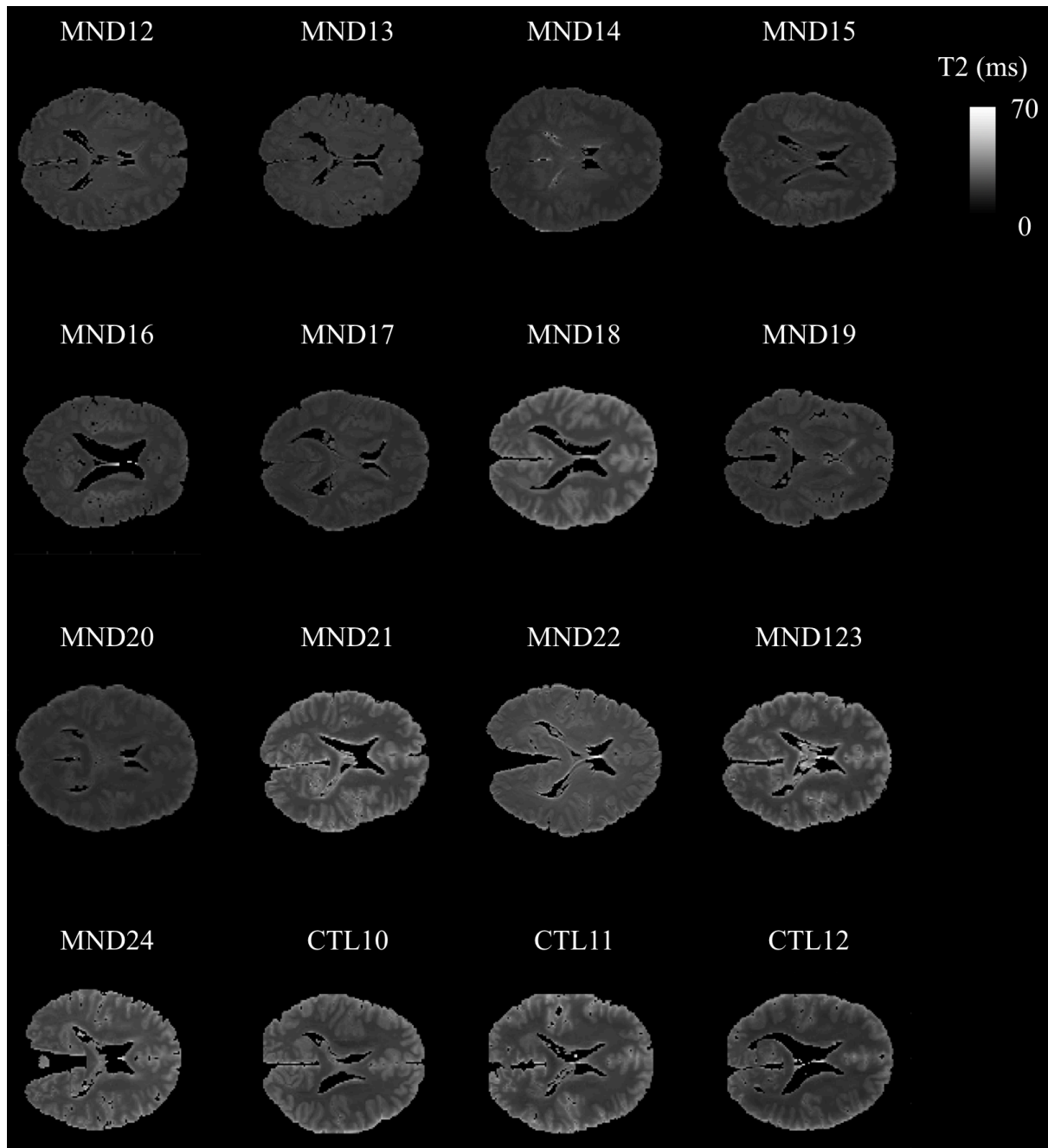


Figure 3.21: The final two-step EPG fitted T2 maps for all cohorts.

3.3.2 Normalization

Our primary aim with respect to ALS was to consider whether we can detect changes in T2 compared to controls in cortical regions that are known to be involved in disease. As illustrated in Fig. 3.22, we used region-of-interest masks corresponding to left and right motor cortex for leg, hand and face regions (red, green and blue), alongside V2 visual cortex (yellow) that serves

as a control region that is expected to be relatively unaffected by pathology. All masks were manually drawn by a neuroanatomist.

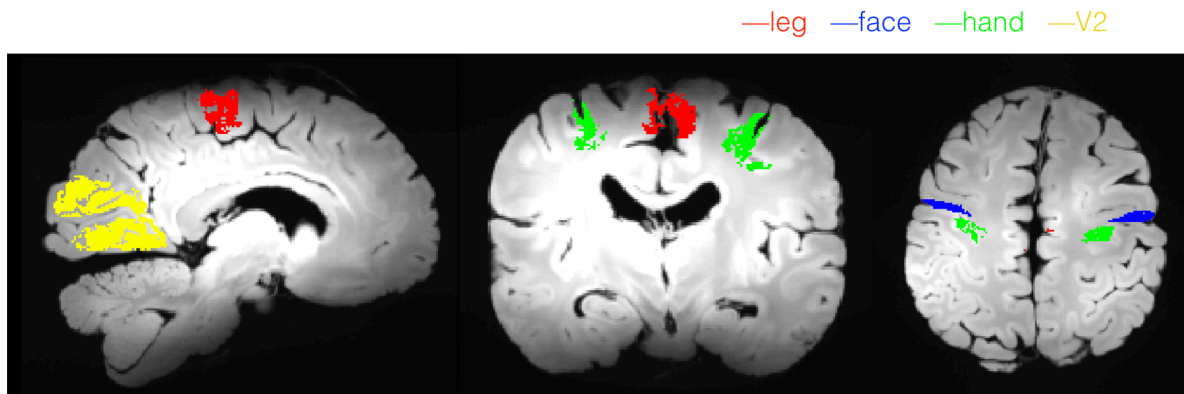


Figure 3.22: Pathological masks of cortical regions including left and right motor cortex leg (red), face (blue), hand (green) areas, and V2 (yellow) to be used for further statistical studies. The masks are manually drawn by neurologists for each brain, also corresponding histological data are available for correlation studies.

Figure 3.23 compares the average T2 value for the three motor regions (including separate values for left and right hemispheres) between ALS and control group. The last two boxes pool across motor regions. While there is a trend for slightly higher T2 in controls (blue) compared to ALS (red), we do not observe any statistically significant T2 differences between the ALS and CTL groups.

One possible factor influencing our results is the fixation, which will, in general, vary from brain to brain due to differences in timing, type of fixative and other confounding factors. In particular, the presence of formalin is known to alter the T2 (Dawe, 2009) and differences in the degree of fixation or concentration of formalin could obscure true differences between the two groups. One simple way to compensate for variation in formalin between brains is to normalize T2 in the motor cortex by T2 in a control region of the same brain, here chosen to be V2 (Qi *et al*, 2016). As illustrated in Fig. 3.24, however, even with this normalization, T2 values are still unable to differentiate between ALS and CTL for any region.

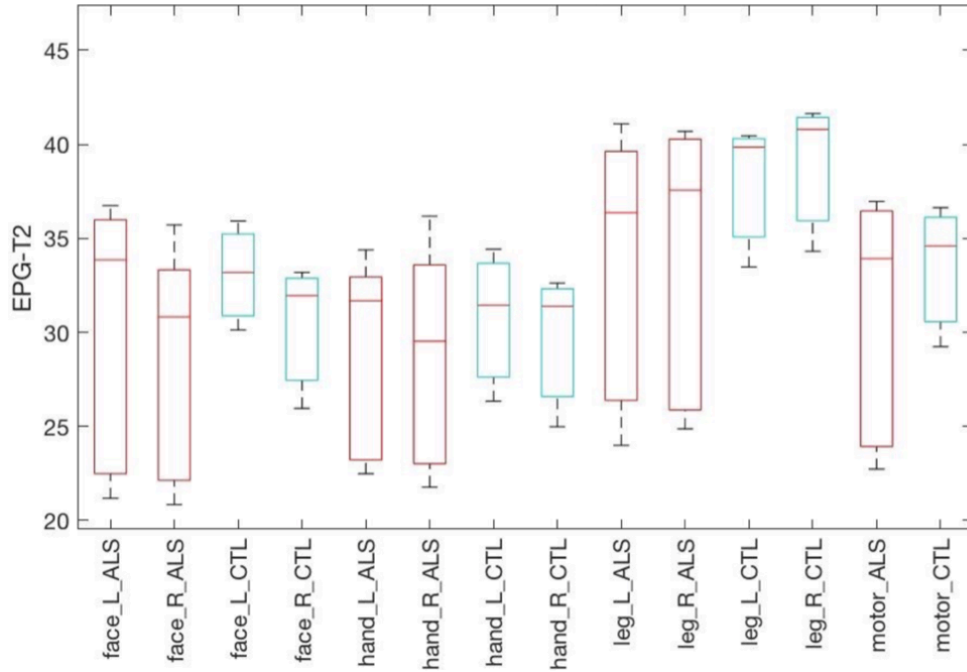


Figure 3.23: Box plots displaying quantitative T2 comparison between ALS and control groups, illustrating the T2 estimates across the three motor cortex regions and the motor cortex as a whole. There is no significant difference in any of these regions, as assessed by two-sample t-test with unequal variance ($P_{\text{face}} = 0.37$, $P_{\text{hand}} = 0.56$, $P_{\text{leg}} = 0.12$ and $P_{\text{Motor}} = 0.53$). Note, red indicates ALS group while cyan indicates control group.

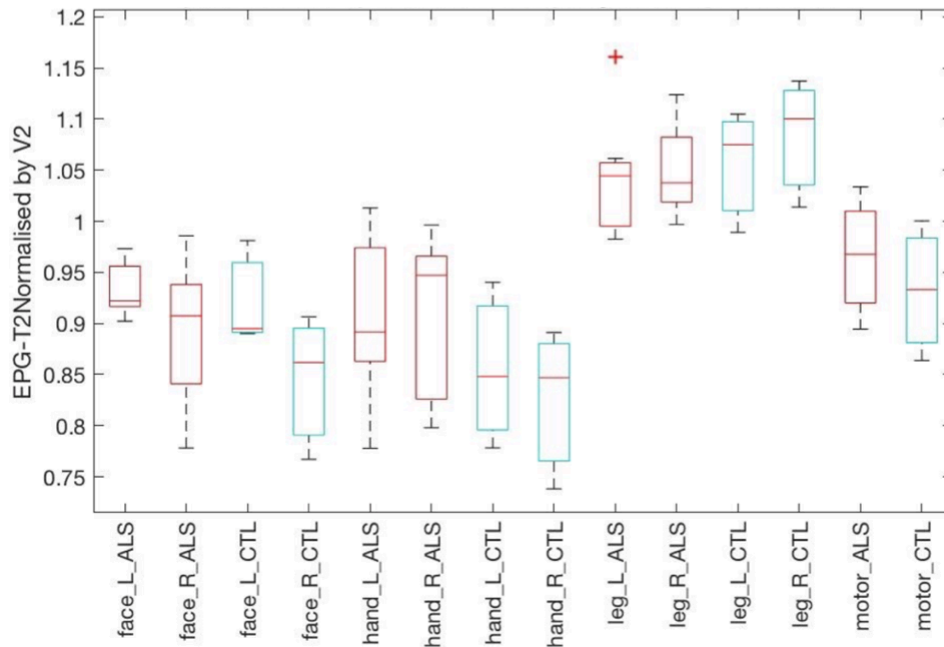


Figure 3.24: Similar box plot as shown in Fig.3.23 for normalized T2 comparison between ALS and CTL groups. There is still no significant difference observed between ALS and CTL groups. This might be due to normalization by V2, that still cannot consider account for the strong variation and influence of formalin concentration in different regions. $P_{\text{face}} = 0.34$, $P_{\text{hand}} = 0.09$, $P_{\text{leg}} = 0.39$ and $P_{\text{Motor}} = 0.44$.

3.3.3 Formalin influence

Figure 3.25 demonstrates that fixation type can influence the T2 intensity, with 10% neutral buffered formalin (NBF, in blue) clustered together. Note that this group includes both ALS and CTL brains, where the other cluster consists entirely of ALS brains. What's more, these clusters suggest that two brains where the fixative is not known to us (Fig. 3.25) were likely to be fixed in 10% formalin, which is the more common preparation method. From the figure, we also note that grey matter has relatively high T2 value as compared with white matter in the same brain (Wansapura *et al.*, 1999), and that formalin concentration can raise or lower both white matter and grey matter T2 together. Though T2 values are elevated by changing fixation to 10%NBF, the variation of T2 value is significantly increased as well across all brains and tissue types (both white matter and grey matter) as compared with 10% formalin. These results suggest that the correction of formalin influence will be necessary in order to compare quantitative T2 value between post-mortem brains.

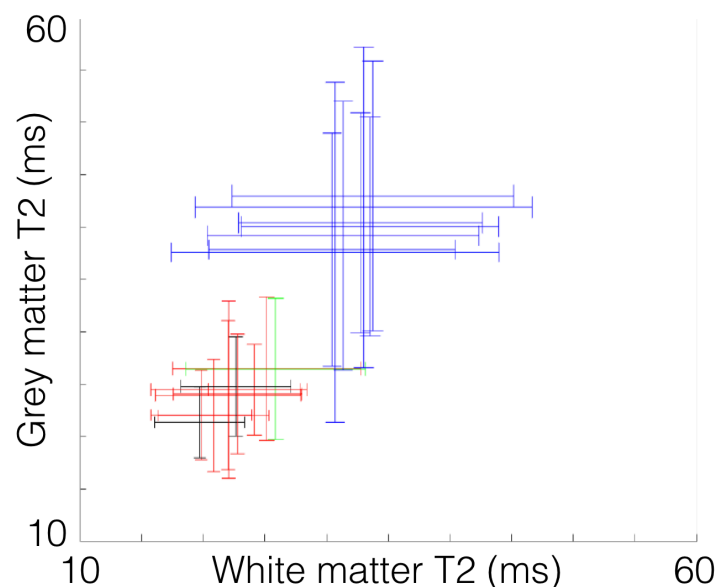


Figure 3.25: Formalin influence on the quantitative T2 measurement. Brains were segmented into white matter and grey matter (excluding subcortical grey matter) by *FSL FAST*. The mean and standard deviation for each brain's gray and white matter are indicated by the bars, and each brain is color-coded with respect to fixation type (red presents 10% formalin, blue presents 10% NBF, green presents 20% NBF, and black has no fixation type recorded). The

brains clearly cluster by fixation type, where 10% NBF fixation separates out from the other fixatives.

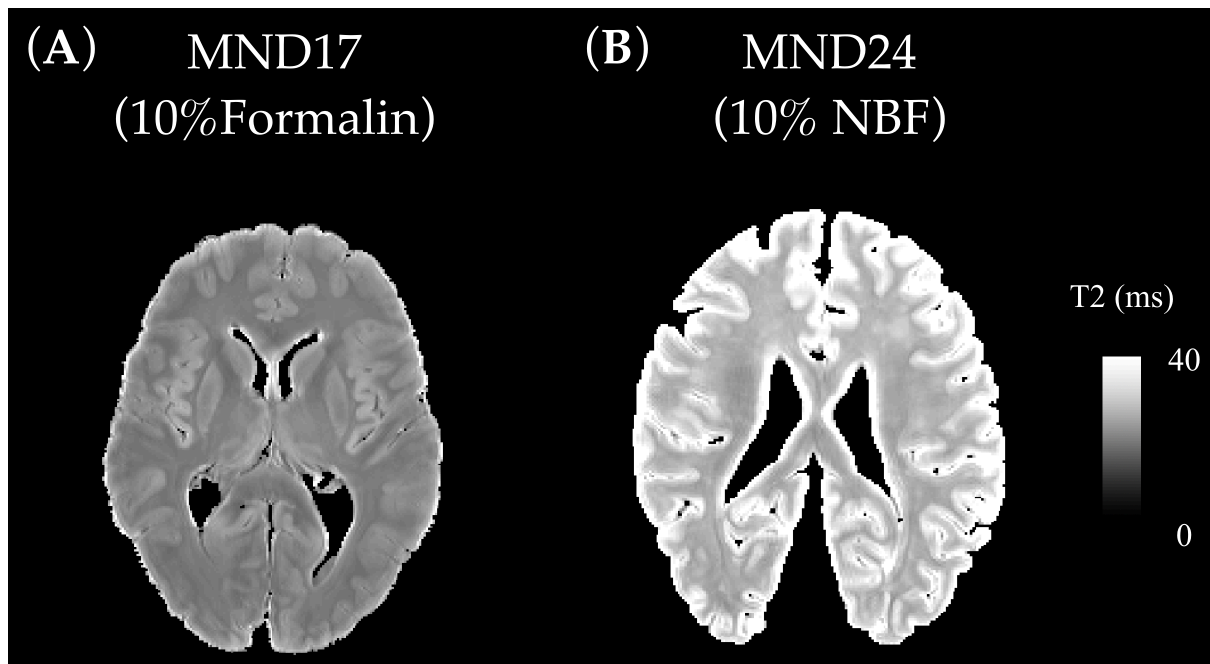


Figure 3.26: Formalin influence on quantitative T2 map with respect to fixation type and concentration. MND17 fixed with 10% formalin and MND24 fixed with 10% NBF have obvious disparity among their image brightness, reflecting the difference of T2 strength due to the formalin types.

Figure 3.26 shows T2 maps for typical brains that prepared using the two fixation types. In addition to having significantly different T2 intensity, we note that the brain prepared with 10% formalin also has some residual heterogeneity with a tendency for higher T2 at the edge of the brain. One interesting possibility, given that brains are taken out of formalin for scanning, is that formalin leaches out of the brain. Since formalin reduces T2, it is possible that the associated reduction formalin concentration at the periphery leads to an increase in T2. In the next chapter propose a model for calculating the spatial pattern of formalin concentration, that we then use as the basis of a correction for the effect of fixation.

3.4 Conclusion

Quantitative T2 measurement has the potential to provide precise measures of pathology in neurodegeneration such as iron deposition and demyelination (Nishijima et al., 2012; Foerster et al., 2013). Here, we implemented the FSE pulse sequence to acquire multi-echo data for quantitative T2 computation. However, the inhomogeneous B1 field causes signal evolution that deviates from the traditional mono-exponential model. To tackle the B1 inhomogeneity issue, we embedded the EPG signal model into the Nelder Mead fitting algorithm to derive maps of flip angle, T2 and S0 simultaneously. This method follows a two-stage fitting, with the first stage generating a B1 map that is smoothed and fixed in a second stage. We present additional algorithm improvements, including median filtering and regularization. We demonstrate that this approach is able to fit the FSE signal with much lower error than an exponential fit and that it produces plausible B1 maps. However, these quantitative T2 maps do not identify group differences in ALS and controls. We found there appears to be a substantial influence on T2 from formalin, specifically fixative type. In the next chapter, we propose a correction to account for the concentration of fixative.

3.5 Reference

- Ahola, S., Perlo, J., Casanova, F., Stapf, S., & Blümich, B. (2006). Multiecho sequence for velocity imaging in inhomogeneous rf fields. *Journal of Magnetic Resonance*, 182(1), 143-151.
- Bloch, F. (1946). Nuclear induction. *Physical Review*, 70(7-8), 460.
- Cheng, H., & Huang, F. (2006). Magnetic resonance imaging image intensity correction with extrapolation and adaptive smoothing. *Magnetic Resonance in Medicine*, 55(4), 959-966.
- Corana, A., Marchesi, M., Martini, C., & Ridella, S. (1987). Minimizing multimodal functions of continuous variables with the “simulated annealing” algorithm Corrigenda for this article is available here. *ACM Transactions on Mathematical Software (TOMS)*, 13(3), 262-280.
- D’Errico, (2014). <https://www.mathworks.com/matlabcentral/fileexchange/8277-fminsearchbnd-fminsearchcon>
- Dawe, R. J., Bennett, D. A., Schneider, J. A., Vasireddi, S. K., & Arfanakis, K. (2009). Postmortem MRI of human brain hemispheres: T2 relaxation times during formaldehyde fixation. *Magnetic Resonance in Medicine*, 61(4), 810-818.
- De Deene, Y., De Wagter, C., De Neve, W., & Achten, E. (2000). Artefacts in multi-echo T2 imaging for high-precision gel dosimetry: II. Analysis of B1-field inhomogeneity. *Physics in Medicine & Biology*, 45(7), 1825.
- Fabes, J., Matthews, L., Filippini, N., Talbot, K., Jenkinson, M., & Turner, M. R. (2017). Quantitative FLAIR MRI in amyotrophic lateral sclerosis. *Academic Radiology*, 24(10), 1187-1194.
- Foerster, B. R., Welsh, R. C., & Feldman, E. L. (2013). 25 years of neuroimaging in amyotrophic lateral sclerosis. *Nature Reviews Neurology*, 9(9), 513.
- Hayes, C. E., Edelstein, W. A., Schenck, J. F., Mueller, O. M., & Eash, M. (1985). An efficient, highly homogeneous radiofrequency coil for whole-body NMR imaging at 1.5 T. *Journal of Magnetic Resonance* (1969), 63(3), 622-628.
- Hennig, J., Nauerth, A., & Friedburg, H. R. (1986). RARE imaging: a fast imaging method for clinical MR. *Magnetic Resonance in Medicine*, 3(6), 823-833.
- Lagarias, J. C., Reeds, J. A., Wright, M. H., & Wright, P. E. (1998). Convergence properties of the Nelder--Mead simplex method in low dimensions. *SIAM Journal on Optimization*, 9(1), 112-147.
- Jaynes, E. T. (1955). Matrix treatment of nuclear induction. *Physical Review*, 98(4), 1099.
- Jucker, M., & Walker, L. C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), 45.
- Kaiser, R., Bartholdi, E., & Ernst, R. R. (1974). Diffusion and field-gradient effects in NMR Fourier spectroscopy. *The Journal of Chemical Physics*, 60(8), 2966-2979.

- Kirkpatrick, S., Gelatt, C. D., & Vecchi, M. P. (1983). Optimization by simulated annealing. *science*, 220(4598), 671-680.
- Lebel, R. M., & Wilman, A. H. (2010). Transverse relaxometry with stimulated echo compensation. *Magnetic Resonance in Medicine*, 64(4), 1005-1014.
- Lutti, A., Hutton, C., Finsterbusch, J., Helms, G., & Weiskopf, N. (2010). Optimization and validation of methods for mapping of the radiofrequency transmit field at 3T. *Magnetic Resonance in Medicine*, 64(1), 229-238.
- Malik, S. J., Padormo, F., Price, A. N., & Hajnal, J. V. (2012). Spatially resolved extended phase graphs: modeling and design of multipulse sequences with parallel transmission. *Magnetic Resonance in Medicine*, 68(5), 1481-1494.
- Marquardt, D. W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *Journal of the society for Industrial and Applied Mathematics*, 11(2), 431-441.
- McPhee, K. C., & Wilman, A. H. (2017). Transverse relaxation and flip angle mapping: evaluation of simultaneous and independent methods using multiple spin-echoes. *Magnetic Resonance in Medicine*, 77(5), 2057-2065.
- Melhem, E. R. (2017). MR Imaging Biomarkers in Amyotrophic Lateral Sclerosis. *Academic Radiology*, 24(10), 1185-1186.
- Milford, D., Bendszus, M., & Heiland, S. (2018). A novel method for T2 quantification in presence of B1 inhomogeneities. *Zeitschrift für Medizinische Physik*, 28(1), 63-72.
- Moré, J. J. (1978). The Levenberg-Marquardt algorithm: implementation and theory. In *Numerical analysis* (pp. 105-116). Springer, Berlin, Heidelberg.
- Nishijima, H., Tomiyama, M., Suzuki, C., Kon, T., Funamizu, Y., Ueno, T., ... & Mori, F. (2012). Amyotrophic lateral sclerosis with demyelinating neuropathy. *Internal Medicine*, 51(14), 1917-1921.
- Pallebage-Gamarallage, M., Foxley, S., Menke, R. A., Huszar, I. N., Jenkinson, M., Tendler, B. C., ... & Ansorge, O. (2018). Dissecting the pathobiology of altered MRI signal in amyotrophic lateral sclerosis: A post mortem whole brain sampling strategy for the integration of ultra-high-field MRI and quantitative neuropathology. *BMC Neuroscience*, 19(1), 11.
- Qi F. et al. (2016). Whole-brain mapping of QMRI parameters in post-mortem brains : towards histological validation in MND. *OHBM16*, 2: 2–6.
- Wansapura, J. P., Holland, S. K., Dunn, R. S., & Ball Jr, W. S. (1999). NMR relaxation times in the human brain at 3.0 tesla. *Journal of Magnetic Resonance Imaging*, 9(4), 531-538.
- Weigel, M. (2015). Extended phase graphs: dephasing, RF pulses, and echoes-pure and simple. *Journal of Magnetic Resonance Imaging*, 41(2), 266-295.
- Whitley, D. (1994). A genetic algorithm tutorial. *Statistics and Computing*, 4(2), 65-85.

Yarnykh, V. L. (2007). Actual flip-angle imaging in the pulsed steady state: a method for rapid three-dimensional mapping of the transmitted radiofrequency field. *Magnetic Resonance in Medicine*, 57(1), 192-200.

Zhang, X., Wang, J., Hong, C., Luo, W., & Wang, C. (2015). Design, synthesis and evaluation of genistein-polyamine conjugates as multi-functional anti-Alzheimer agents. *Acta Pharmaceutica Sinica B*, 5(1), 67-73.

Chapter 4

Kinetic Tensor Model for Quantitative T2 Correction

Contents

4.1 Background	80
4.1.1 Formalin fixation and brain preparation.....	80
4.1.2 Histological evidence of formalin influence.....	81
4.1.3 Physics interpretation of formalin influence on T2.....	83
4.2 Theory	86
4.2.1 Analytical solution of formalin distribution.....	87
4.2.2 Distance model.....	89
4.2.3 Kinetic Isotropic model.....	90
4.2.4 Kinetic Tensor model.....	91
4.2.5 T2 correction	98
4.3 Methods.....	99
4.3.1 Masks and segmentations	100
4.3.2 Hyperintensity artefact in diffusion data	104
4.3.3 Scan parameters and processing scripts.....	106
4.4 Results.....	108
4.5 Conclusion	114
4.6 References.	115

In this chapter, I will discuss a newly proposed kinetic tensor model to correct for the influence of formalin fixation on quantitative T2 mapping. In the first part, I will talk about why fixation is critical for post-mortem brain imaging and histological studies, followed by a discussion about why the fixation process may influence T2 maps from both histological evidence and physics interpretations. Then, I will discuss the theory of formalin dynamics simulation, starting with the analytical solution of Fick's diffusion equation, followed by a numerical solution incorporating a brain-specific boundary. Finally, I propose the kinetic tensor model, which considers an anisotropic diffusion tensor measured from diffusion tensor imaging (DTI). With an accurate formalin concentration distribution, a simple regression model can be used to correct the variation in T2 values caused by formalin. Finally, a comparison study will demonstrate how these models improve the quality of T2 maps step by step, to provide an accurate quantitative measure for future clinical use.

4.1 Background

4.1.1 Formalin fixation and brain preparation

Fixation is usually the first stage in post mortem brain histopathological studies, in order to preserve biological tissue from decay due to autolysis or putrefaction. It stops tissue decomposition and enhances tissue mechanical strength and stability (Kiermnan 2000; Fox *et al.*, 1985; Pinto *et al.*, 2008). Tissue fixation is an essential step in the preparation of both histological and MRI scan samples, which requires both the basic structure and molecular composition to be largely unchanged in order to reliably obtain correct conclusions and insights from pathology specimens.

The fixative most commonly used in histology is formaldehyde (CH_2O), usually as a 10% solution in water referred to as formalin. Another solution called 10% neutral buffered formalin (NBF), which is approximately 3.7%–4.0% formaldehyde in phosphate-buffered saline, has come into wider usage due to its better capacity for preservation. In this study (Mollink *et al.*, 2017; Pallegage-Gamarllage *et al.*, 2018), both types of fixation have been used for histological staining studies and MRI scans. Here, postmortem human brains were extracted from the skull within 72 h after death and fixed in 10% formaldehyde or NBF for at least 2 months (with formalin solution changed at least four times) prior to scanning. Before scanning, the brains were removed from the formalin bucket and placed into a 3D printed holder filled with a fluorocarbon-based liquid (Fluorinert, 3M Corporation) to match susceptibility for imaging quality concerns. It is important to note that immersing brain samples in the Fluorinert solution will cause an outflow of formalin and a change in the distribution of formalin concentration during MRI scans.

4.1.2 Histological evidence of formalin influence

The fixation process is a necessity for keeping brain tissue from decomposing, however, this process can introduce significant variations to quantitative T2 values (Kamman *et al.*, 1985; Thickman *et al.*, 1983). While the EPG fitting discussed in the previous chapter reduces the influence of B1 inhomogeneity on T2 estimates, brains prepared with different fixative types (e.g. 10% formalin vs, 10% NBF) or the same fixative type but different concentration (Shepherd *et al.*, 2009) may show significant discrepancies in quantitative T2 estimates, as is demonstrated in Fig. 4.1. So precisely measuring or computing the formalin distribution and carefully correcting the EPG T2 maps to remove the effect of formalin may improve the accuracy of quantitative T2 mapping, and make it a more reliable tool for elucidating disease processes or discovering related pathologies. In order to do this, I will first address how formalin influences T2 measurements.

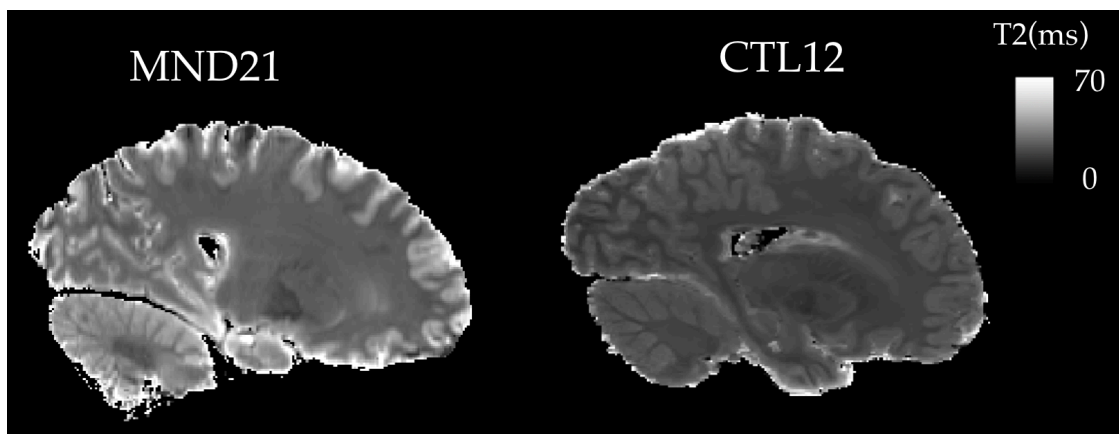


Figure 4.1: Significant differences in T2 estimates observed with different postmortem brain samples (left: MND12, 10% formalin, right: CTL12, 10% NBF), mainly driven by the variation of fixative type and concentration. It is necessary to correct these influences before a precise and quantitative T2 estimate can be obtained.

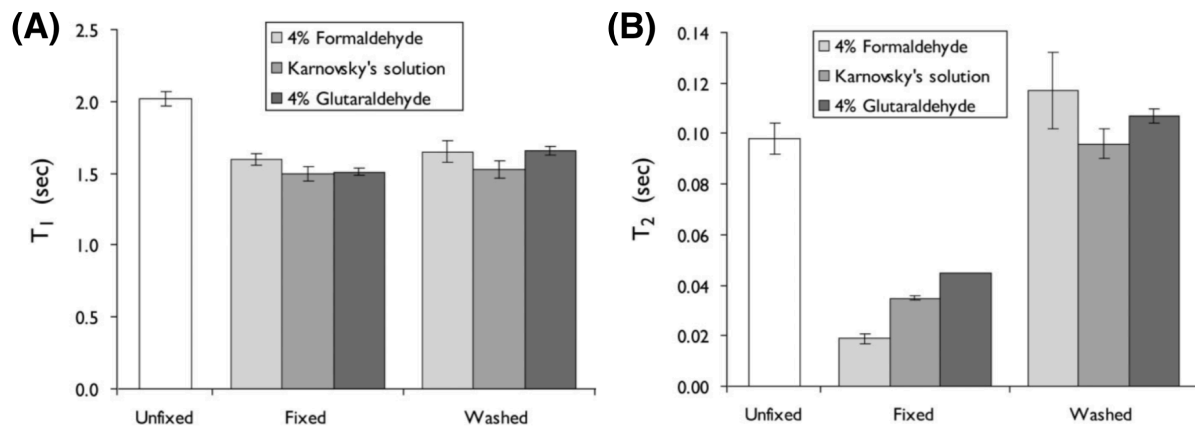


Figure. 4.2. Bar graphs demonstrating the effect of different chemical fixation on T1 (A) and T2 (B) relaxation properties. Shepherd used 4% formaldehyde, 4% glutaraldehyde, and Karnovsky's solution (2% formaldehyde and 2% glutaraldehyde) in experiments. The fixations reduced T1 significantly, which could not be reversed by washing out the fixative solution. In contrast, the fixations also reduced T2 values even more significantly, however, the removal of free fixatives significantly reverses the observed T2 value, which suggests that the change in T2 is the effect of the presence of fixative solution, rather than due to protein cross-linking of tissue molecules. Image from Shepherd et al. 2009.

Shepherd has reported the influence of formalin on quantitative T1 and T2 measurements (Shepherd *et al.*, 2009) by studying brain tissue blocks immersed in three types of fixative, 4% formaldehyde, 4% glutaraldehyde, and Karnovsky's solution (2% formaldehyde and 2% glutaraldehyde) and measuring the corresponding change in T1 and T2 relaxation times with MRI. The results of this study are summarized in Fig. 4.2. For the influence of formalin on T1, two observations were made: (1) All three types of formalin solutions significantly decrease tissue T1 to a similar degree. (2) Washing out formalin with phosphate-buffered saline solution does not recover the T1 value as compared with non-fixed tissue. These observations suggest that formalin fixation is an irreversible process and that the change of molecular structure (protein cross-linking) cannot be restored by the washing process. For the influence of formalin on T2, three substantially different observations were witnessed: (1) Adding formalin solutions significantly decreases tissue T2 values, and the degree of change depends on the fixative type (formaldehyde shows a larger change than glutaraldehyde). (2) Washing out the formalin solution restores T2 to the original value. This suggests that T2 change is not driven by

microstructure alteration (e.g. protein cross-linking), but rather significantly depends on the concentration of unbound formalin molecules. (3) More importantly, the decrease in T2 is linearly correlated with increasing formaldehyde concentration (10-15 milliseconds per 2%). Given this linear correlation, the influence of formalin may be regressed out from T2 maps given a voxel-wise estimation of formalin concentration, leaving only pathology related markers in T2 maps.

4.1.3 Physics interpretation of formalin influence on T2

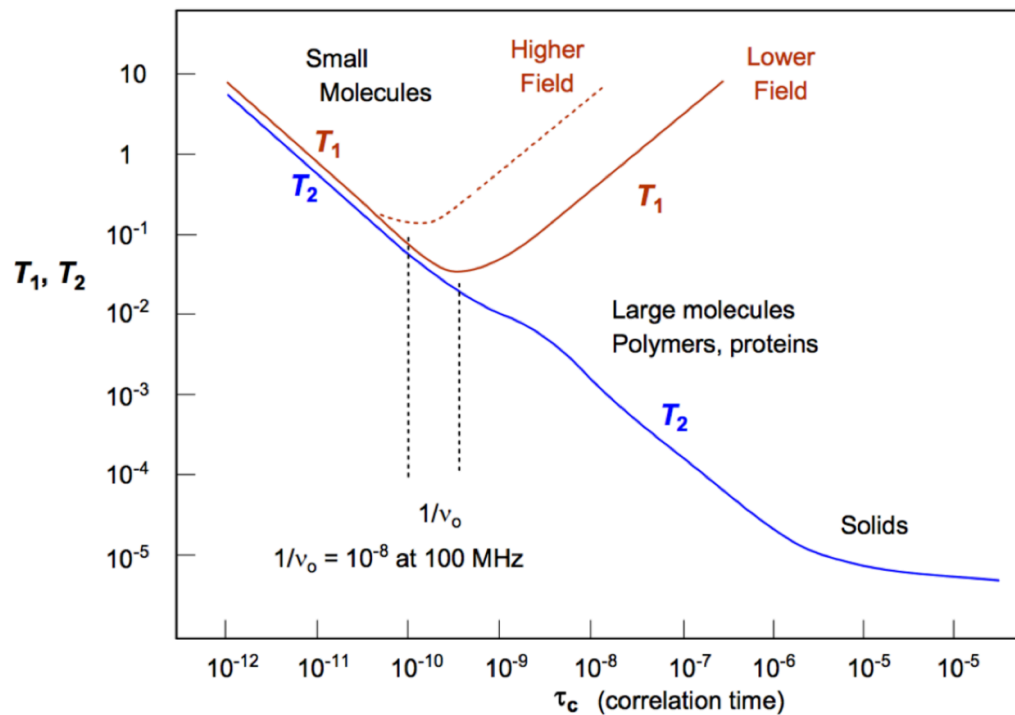


Figure 4.3: T1 and T2 with respect to molecular correlation time (or molecular size). Small molecules have a short correlation time (fast rotation), so their intrinsic magnetic field is not efficient in manipulating magnetization, which leads to a slow relaxation for both T1 and T2 (i.e. long longitudinal and transverse relaxation times). On the other hand, large molecules with a relatively stable state (i.e. rotating much slower) provide inhomogeneous local fields effective to dephase transverse magnetization and shorten T2. For T1, the most effective exchange of energy (and thus fastest relaxation rate) occurs at the resonant frequency; rotation that is either too fast or slow will lead to longer longitudinal relaxation times. So T1 relaxation time has a minimum value (i.e. the minimum of the red plot) determined by the resonant frequency, and this value can be modulated by the main magnetic field B0. (Figure adapted from Bloembergen *et al.*, 1948)

Before attempting to correct for the effect of formalin concentration on relaxation, we introduce a physics interpretation of how formaldehyde molecules change the T2 values of tissue during the fixation process. First, as observed in previous studies, the presence of formaldehyde causes shortening of longitudinal relaxation time T1, due to a change in the surrounding environment, which will simultaneously decrease transverse relaxation time T2. Specifically, the fixation process causes cross-linking of proteins, which makes the characteristic frequency of the macromolecular lattice closer to proton resonant frequency (Fig. 4.3), and makes the exchange of energy between spins and lattice more efficient. Thus, as the fixation process progresses T1 is reduced accordingly. The mechanism that causes fixative to influence T2 is quite different. In addition to factors that shorten T1, the local inhomogeneous field ΔB has a role in dephasing transverse magnetization, which is the primary source of transverse relaxation. From this perspective, free formaldehyde, an electrically polarized molecule, effectively disturbs the local magnetic field, causing increased dephasing of transverse magnetization that cannot be reversed by a 180-degree refocusing pulse, as is illustrated in Fig. 4.4.

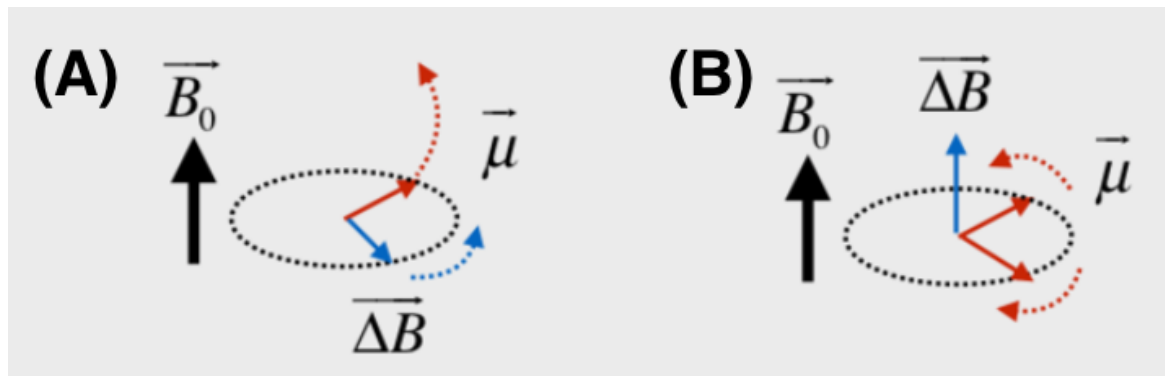


Figure 4.4: Influencing mechanisms on T2 (B) as compared with T1 (A). An external field with resonant frequency and direction perpendicular to B_0 can increase longitudinal relaxation. In contrast, the external field that is parallel to B_0 can facilitate the transverse relaxation. For example, rotating formaldehyde molecules disrupt the local magnetic field and result in the irreversible reduction of T2 value.

In summary, the influencing factors that change the measured value of T2 in the presence of formaldehyde include shortening of T1 relaxation times, leading to shorter T2, and local magnetic fields induced by fast-moving formaldehyde molecules that increase the dephasing process, resulting in transverse decay which cannot be reversed by 180 degree refocusing pulses.

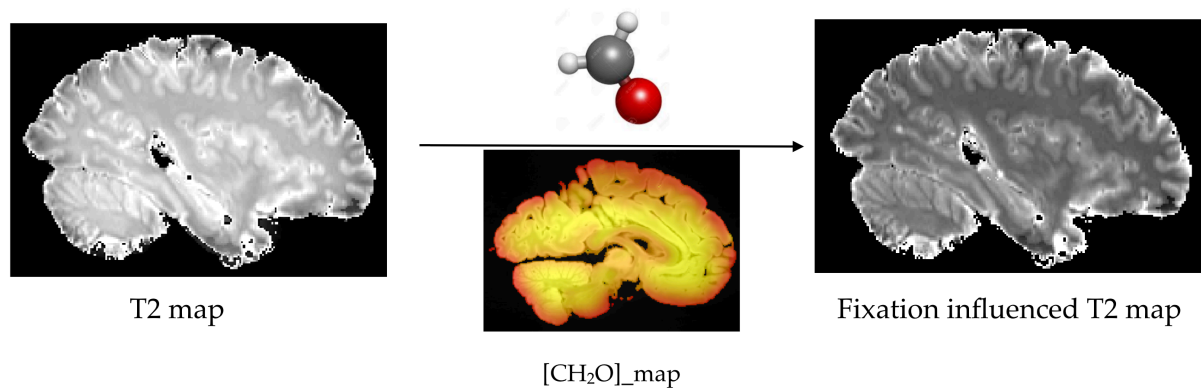


Figure 4.5: A hypothesized mechanism for fixation-induced T2 inhomogeneity. In addition to the presence of fixative generally reducing T2, if brains have a variable concentration of formalin (bright yellow, middle panel), this will lead to subtle variations in T2 over space in proportion to the local concentration of fixative (right panel). Therefore, obtaining a precise estimate of the distribution of formalin, for example by (Dawe *et al.*, 2009) simulation, will remove the effect of formalin to enable accurate and precise estimates of T2.

Knowing how formaldehyde molecules decrease transverse relaxation time (T2) from both histological experiments and a physics interpretation gives us a better understanding of why our quantitative T2 map appears to have low values in the deep tissue and high values on the surface. Fig. 4.5 illustrates our hypothesized effect of fixative on T2 maps. When brains are transferred from formalin into Fluorinert, we create a concentration boundary at the surface of the brain that is likely to cause formaldehyde molecules to diffuse out of the brain. This would mean that fixed brains that are scanned have high formalin concentration in the deep tissue but low concentration in the peripheral tissue, leading to relatively higher T2 values near the surface. This could, for example, lead to higher T2 in superficial regions like the motor cortex

than deep cortical regions like parts of V2 that lie deep in a gyrus. Given the established reduction in T2 value due to formalin, we can estimate the formalin distribution in order to apply a correction to reduce this confound in quantitative T2 mapping.

4.2 Theory

Many groups have developed mathematical models for the influx of formalin into post-mortem brain MRI over the last two decades. These studies are primarily motivated by achieving a better understanding of the process of fixation and in some cases its influence on MRI parameters. These approaches generally evolved from an analytical solution of the diffusion equation to numerical solutions considering the brain-specific boundaries. We propose a “kinetic tensor” (KT) model that builds on this previous work, with the primary novelty that the KT model takes into account the anisotropic diffusion properties of each individual tissue voxel. In this section, we will discuss the development of the KT model.

To our knowledge, there is no previous work using these models to correct for the effect of fixation on post-mortem images. Moreover, previous methods have aimed to characterize the *influx* of fixative during immersion fixation. Based on our observations during the development of the methods presented here, the primary effect that requires correction in T2 mapping is the *outflux* of low-T2 fixative from tissue due to brains being immersed in Fluorinert.

4.2.1 Analytical solution of formalin distribution

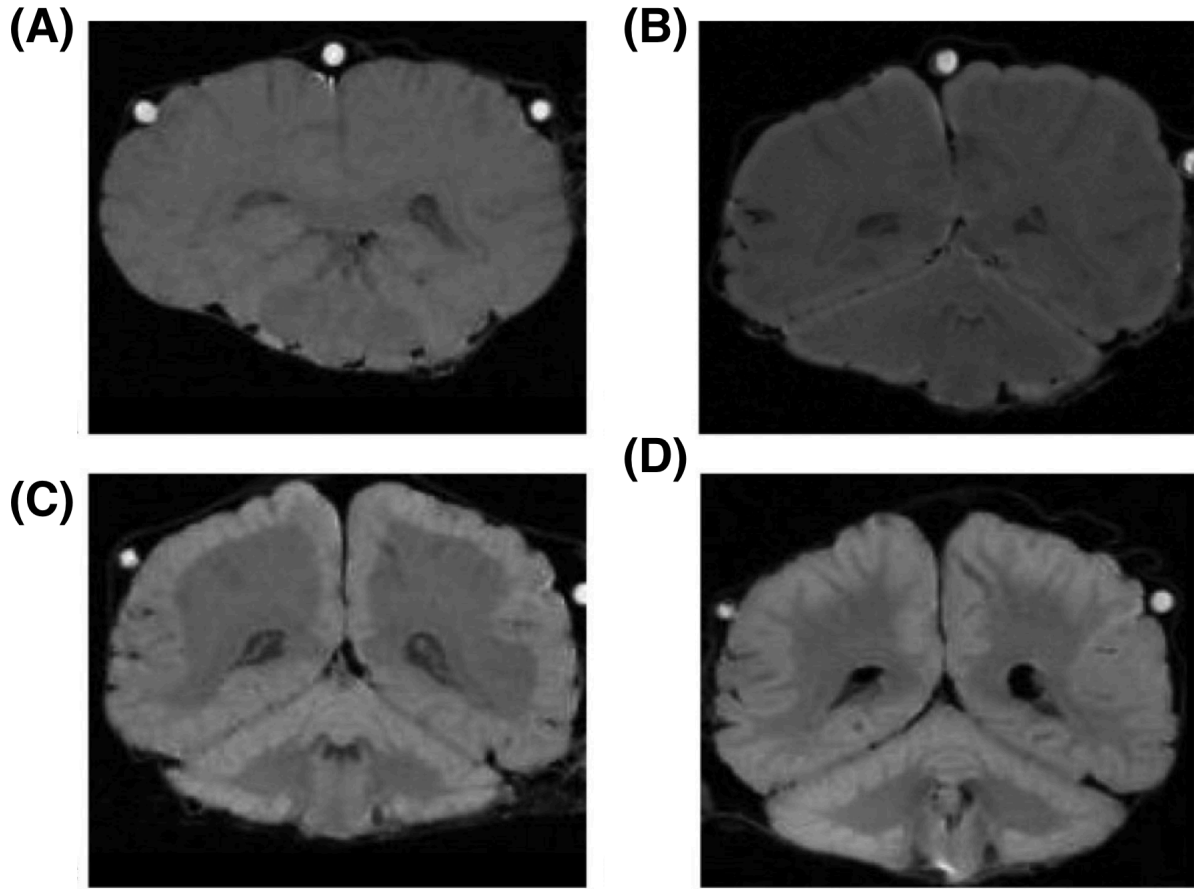


Figure 4.6: T1-weighted images of formalin diffusion process. (a) before formalin fixation and at (b) 18 h, (c) 90 h, and (d) 518 h. The formalin fixed tissue is visible as a light band that becomes progressively thicker with time. Figure adapted from Yong-Hing *et al.*, 2005.

Studies of the influence of Formalin fixation on T1 and T2 properties of the brain have been ongoing since approximately 2000, when investigators realized the significant influence of formalin on multimodality MRI measurements (Yong-Hing *et al.*, 2005; Sun *et al.*, 2003). Initial studies of formalin dynamics based on Fick's diffusion law (Eq. 4.1) were performed considering a low temporal and spatial resolution and were able to provide insights into experimental observations such as the observed signal intensity changes in T1 weighted imaging as a function of depth from the surface on formalin-fixed samples.

$$\mathbf{J} = -D\nabla C \quad (4.1)$$

The influence of formalin diffusing into tissue on T1-weighted images is shown in Fig. 4.6 (Yong-Hing *et al.*, 2005) where over time, formalin moves from the outer surface of the brain into the deep tissue. Note that, this hyperintensity on T1 weighted images does not exactly match the formalin solution distribution but rather is a nonlinear function of pulse-sequence related contrast dynamics. However, the measured intensity can still be used to fit Fick's diffusion equation (Eq. 4.1) to describe the contrast evolution and predict future contrast dynamics.

Yong-Hing and colleagues proposed a simple model that assumes each brain hemisphere is approximately a sphere (Yong-Hing *et al.*, 2005). Fick's diffusion law can be re-expressed in spherical coordinates as

$$\frac{1}{D} \frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial^2 r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \quad (4.2)$$

where D is the diffusion coefficient, c is the concentration of formalin (estimated from T1 weighted intensity in (Yong-Hing *et al.*, 2005)), and r is the voxel radius, the distance of the voxel to the hemispherical center. Under the spherical assumption, the concentration distribution dynamics $c(r, t)$ should follow the standard expansion form of spherical harmonics as,

$$c(r, t) = c_1 + \frac{2Rc_1}{\pi} \sum_{nr}^{\infty} \frac{(-1)^n}{nr} \sin\left[\frac{n\pi r}{R}\right] \exp\left[\frac{-n^2\pi^2 D}{R^2} t\right] \quad (4.3)$$

By setting $R=1$, the distance of voxel in the ideal spherical brain is normalized. By setting $c_1 = 1$, the formalin concentration estimates are in normalized units. Finally, by setting $t_l = t/D$ (and dropping the subscript on the scaled t), then Eq. 4.3 can be simplified as,

$$c(r, t) = 1 + \frac{2}{\pi} \sum_{nr}^{\infty} \frac{(-1)^n}{nr} \sin[n\pi r] \exp[-n^2\pi^2 t] \quad (4.4)$$

and by substituting Eq.4.4 back into the Eq.4.2, the first and second terms can be expanded in the form of Eq. 4.5 and 4.6:

$$\frac{\partial c}{\partial r} = \frac{2}{\pi} \sum_{n=1}^{\infty} - \frac{(-1)^n \sin[n\pi r] \exp[-n^2 \pi^2 t]}{nr^2} + \frac{(-1)^n \cos[n\pi r] \pi \exp[-n^2 \pi^2 t]}{r} \quad (4.5)$$

$$\begin{aligned} \frac{\partial^2 c}{\partial^2 r} = & \frac{2}{\pi} \sum_{n=1}^{\infty} - \frac{2(-1)^n \sin[n\pi r] \exp[-n^2 \pi^2 t]}{nr^3} \\ & - \frac{2(-1)^n \cos[n\pi r] \pi \exp[-n^2 \pi^2 t]}{r^2} \\ & - \frac{(-1)^n \sin[n\pi r] n \pi^2 \exp[-n^2 \pi^2 t]}{r} \end{aligned} \quad (4.6)$$

By fitting to the measured data of normalized inner boundary of the formalin band, the model coefficients can be obtained and the temporal dynamics of concentration distribution $c(r,t)$ can be expressed.

4.2.2 Distance model

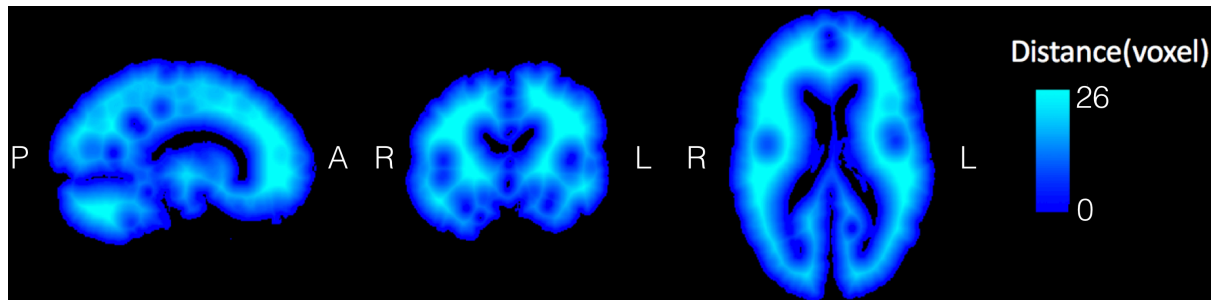


Figure 4.7: The distance map was calculated with the FSL-distancemap command based on the fraction anisotropic (FA) map. The color represents the distance to the nearest non-brain voxels, rendered by the FSL blue-lightblue color-map.

While a useful first step in understanding the effects of fixation on MRI contrast, Yong-Hing's assumption that each brain hemisphere is a sphere is unlikely to provide an estimate of formalin distribution that is sufficiently accurate to be used in correcting quantitative T2 measurements. To accurately estimate the formalin distribution likely requires more detailed information of brain properties such as its morphology, fine structural orientation, and connectivity, in addition to chemical and physical properties of the applied fixative solutions. From the quantitative T2 map acquired from EPG fitting, we observed that voxels on the peripheral surface (including the ventricle surface) have a higher estimated T2 than that of the deep tissue, which is quite similar to the pattern observed in Yong-Hing's study (Fig. 4.6). Therefore, a potentially simple solution to the problem was investigated based on the observation that the distance to surface serves as a predominant factor governing the influence of formalin on T2. Voxel-wise distance to the nearest non-tissue voxel was computed using the FSL (FMRIB Software Library, University of Oxford) *distancemap* command. An example result is shown in Fig. 4.7, illustrating that the peripheral voxels have low distance values, while deep tissues have high values, and the distance map has a similar pattern of distribution as the formalin in the tissue. Using this map and the voxel-wise EPG T2 fitted values, we can examine the relationship between these two parameters. This simple approach will be included alongside more sophisticated models in the following results and discussion sections.

4.2.3 Kinetic isotropic model

With the development of finite element analysis (FEA) (Hughes et al., 1989) and diffusion-weighted MRI (Le Bihan 2003), the diffusion equation can be more accurately solved. Dawe discarded the spherical constraints (Dawe *et al.*, 2009) assumed by Yong-Hing, and took account of the natural morphology of the post-mortem brain as boundary constraints to solve the formalin diffusion problem. This approach assumes the formalin diffusion coefficient D to

be identical to that of water $8.0 \times 10^{-7} \text{ cm}^2/\text{s}$, which fell in the range of previously reported values (Underhill 1983; Start *et al.*, 1992). The results of this model (Dawe *et al.*, 2009) are reproduced in Fig. 4.8, from which we can see that more refined distribution dynamics in the formalin diffusion simulation than the spherical model used in Yong-Hing's study. Because this model incorporates brain morphology into a kinetic simulation assuming isotropic diffusion, we refer to this as the “kinetic isotropy” (KI) model.

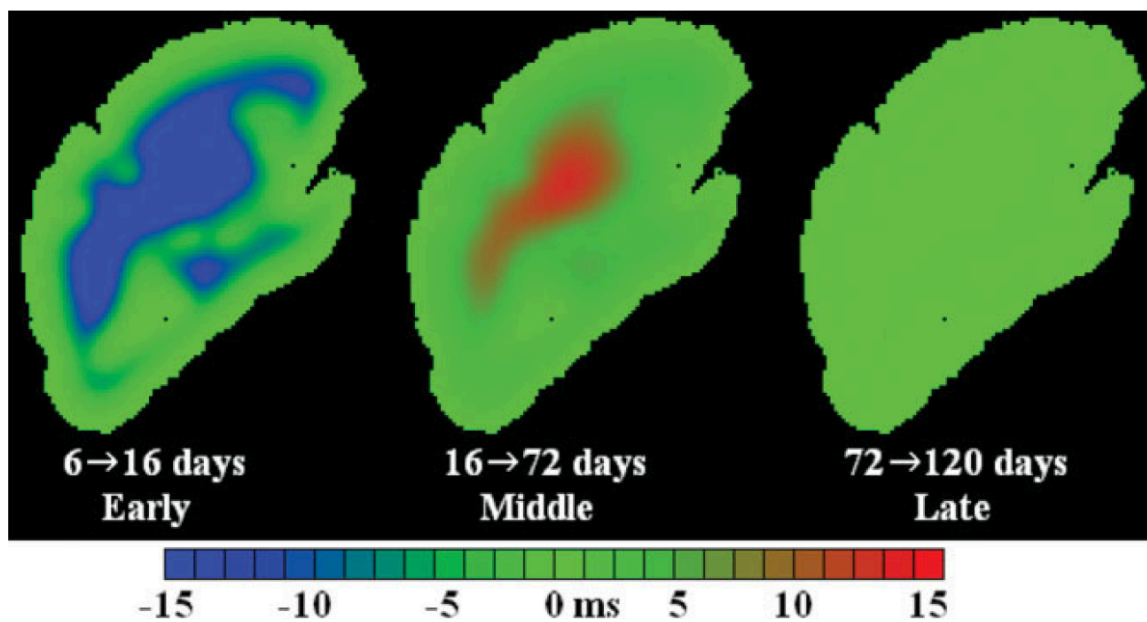


Figure 4.8: T2 difference maps (on left hemisphere of post mortem brain) generated by numerical simulation of the fixation process, which has a much higher spatial resolution than that of the analytical analysis. Note that the initial (6 $\rightarrow$ 16 days) decrease of T2 in deep tissue is due to the formalin fixation, and the further (16 $\rightarrow$ 72 days) increase of T2 is due to the water content growth (from Dawe *et al.*, 2009).

4.2.4 Kinetic tensor model

Our study in ALS brains includes the acquisition of diffusion-weighted MRI measurements in the same post-mortem brains samples (Miller *et al.*, 2011, 2012; Mollink *et al.* 2016; Kolasinski *et al.* 2012). These data could provide valuable information for a more accurate simulation of formalin diffusion dynamics. It is based on this diffusion information that we propose the

“kinetic tensor” (KT) model. Where Dawe’s KI model assumed isotropic diffusion, our KT model uses the diffusion tensor to capture the hindrance of diffusion by cellular membranes, which should be similar for formalin to water. With knowledge of the tensor, Fick’s law in Eq. 4.1 can be rewritten in tensor form:

$$\frac{\partial c}{\partial t} = \nabla \cdot (\mathbf{D} \cdot \nabla c) \quad (4.7)$$

Fick’s law requires the initial conditions $c(0, \mathbf{x}) = c_0(\mathbf{x})$, which describe the distribution of formalin across the whole brain when the simulation starts at $t = 0$. The boundary conditions depend on the processes by which the brain was fixed:

(1) The formalin fixation process: We assume that the concentration of formalin solution in the container that brains are immersed in during fixation was constant (the formalin solution was refreshed at least four times in the interval between extraction and the MRI scan to guarantee the fixation quality). According to the concentration gradient and the diffusion tensor $\mathbf{D}$, the formalin would gradually invade the brain tissue from the outside. The minimum period of fixation before scanning (one month) has been confirmed histologically to correspond to full fixation of deep tissues.

(2) The Fluorinert immersion process (prior to scanning): Approximately 24 hours prior to scanning, brains are transferred from formalin into a 3D printed holder filled with Fluorinert. While in this holder, formalin flows out from brain tissue into the Fluorinert. Here, we assume the formalin concentration outside the brain tissue is zero at the start of scanning. This assumption is reasonable for such a short duration, during which only formalin close to the brain surface could flow out. The concentration gradient and diffusion tensor $\mathbf{D}$ will govern the simulation of formalin outflow from brain tissue into the Fluorinert solution.

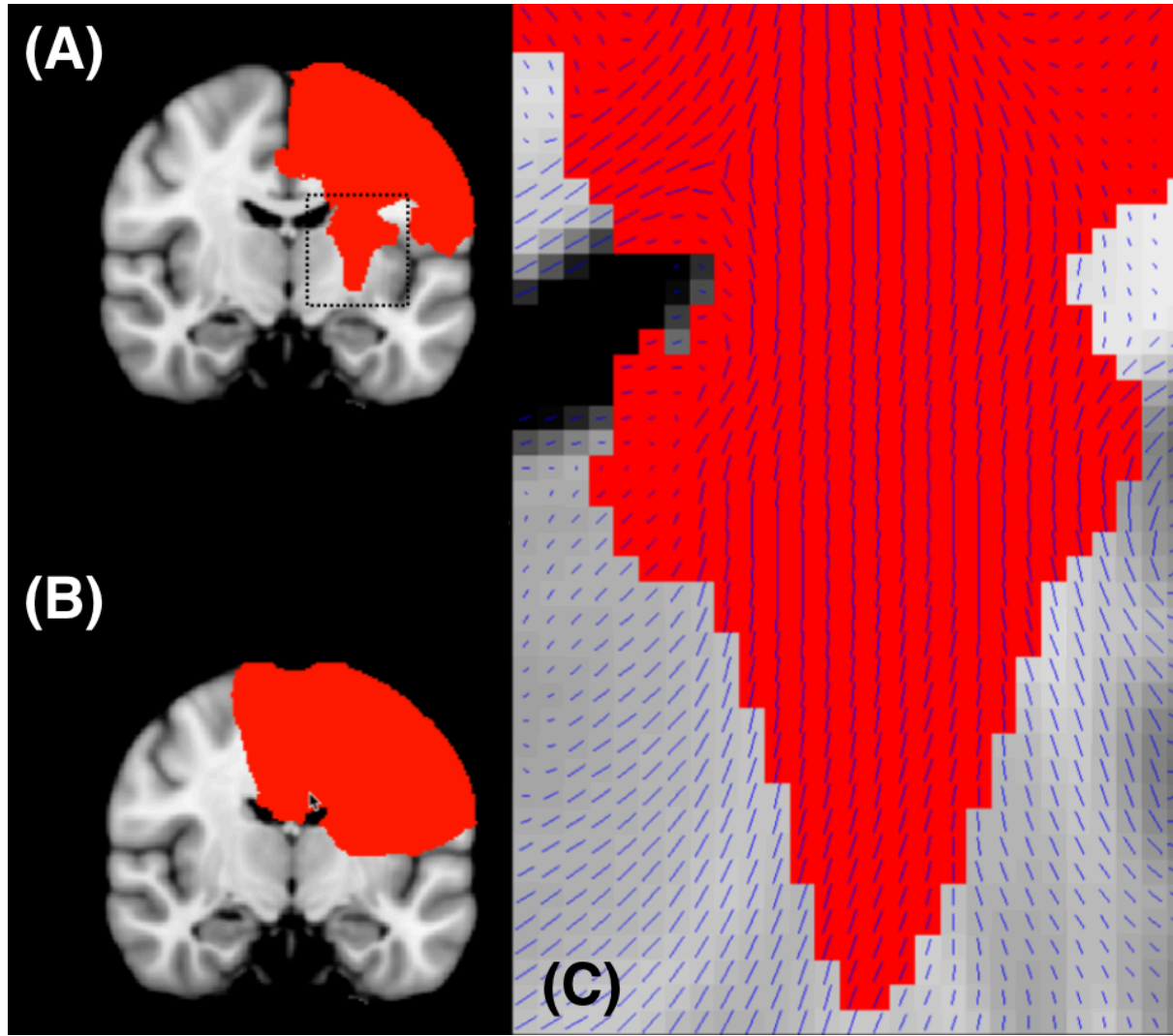


Figure 4.9: (a) Anisotropic diffusion simulated by the tensor (KT) model as compared with (b) the isotropic (KI) model, where (c) is the enlarged image of (a) highlighted by the dashed box. The kinetic tensor model uses the diffusion tensor information from each voxel measured using diffusion weighted MRI. This voxel-wise diffusion tensor provides detailed tissue structural properties that can contribute to a more precise simulation of formalin dynamics. In this simulation, formalin “tracer” is seeded in the right motor cortex. After simulation of the diffusion process, this formalin tracer flow is more concentrated along the corticospinal tract than in perpendicular directions.

The diffusion tensor $\mathbf{D}$ contains much more information about the tissue microstructure than the isotropic diffusion coefficient D , so the KT simulation of formalin diffusion dynamics is expected to be more accurate than Dawe’s KI simulation. For example, by using a voxelwise tensor $\mathbf{D}$, our simulation could differentiate between tissue types of white matter and gray matter while taking into account their diffusivity value. Furthermore, since the diffusion tensor

D contains information about how white matter is oriented, the simulated formalin could flow along the white matter fibre structure rather than isotopically. As illustrated in Fig. 4.9, virtual formalin molecules seeded at the motor cortex of the right hemisphere flow preferentially along the cortical spinal tracts governed by the voxel-wise tensor **D**, calculated from its diffusion tensor imaging measurements. Furthermore, we observe that a certain amount of formalin tracer could diffuse across white matter tracts, reflecting hindered (rather than truly restricted) diffusion.

To simulate the flow dynamics of formalin, it is necessary to discretize the diffusion equation (Eq.4.7) and then use temporal finite difference (Jbabdi *et al.*, 2005; Harpold *et al.*, 2007) techniques to compute the formalin distribution iteratively (update the spatial distribution at each time step). Fick's equation (Eq. 4.7) can be rewritten in the expanded form of Eq. 4.8 with tensor **D**, and gradient of concentration ∇c as,

$$\frac{\partial c}{\partial t} = \nabla \cdot (\mathbf{D} \cdot \nabla c) = \left(\frac{\partial}{\partial x} \frac{\partial}{\partial y} \frac{\partial}{\partial z} \right) \left[\begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{pmatrix} \begin{pmatrix} \frac{\partial}{\partial x} \\ \frac{\partial}{\partial y} \\ \frac{\partial}{\partial z} \end{pmatrix} c \right] \quad (4.8)$$

and then, with matrix multiplication operations, we can obtain,

$$\frac{\partial c}{\partial t} = \sum_{i,j}^3 D_{ij} \frac{\partial^2 c}{\partial x_i \partial x_j} + \sum_{i=1}^3 \tilde{D}_i \frac{\partial c}{\partial x_i} \quad (4.9)$$

$$\tilde{D}_i = (\mathbf{D} \nabla)_i = \sum_{j=1}^3 \frac{\partial D_{ij}}{\partial x_j} \quad (4.10)$$

Denoting $C_{i,j,k}^n$ to be the concentration at voxel location (i, j, k) for the n^{th} iterative step, then the temporal derivative of formalin concentration can be discretized as,

$$\frac{\partial c}{\partial t} = \frac{C_{i,j,k}^{n+1} - C_{i,j,k}^n}{\Delta T} \quad (4.11)$$

The exact form of first and second order spatial derivatives of formalin concentration can be written as,

$$\frac{\partial c}{\partial x} = \frac{C_{i+1,j,k}^n - C_{i-1,j,k}^n}{2\Delta X} \quad (4.12)$$

$$\frac{\partial^2 c}{\partial x^2} = \frac{C_{i+1,j,k}^n - 2C_{i,j,k}^n + C_{i-1,j,k}^n}{\Delta X^2} \quad (4.13)$$

$$\frac{\partial^2 c}{\partial x \partial y} = \frac{C_{i+1,j+1,k}^n + C_{i-1,j-1,k}^n - C_{i+1,j-1,k}^n - C_{i-1,j+1,k}^n}{4\Delta X \Delta Y} \quad (4.14)$$

where $\Delta X, \Delta Y, \Delta Z$ are the spatial discretizing intervals, and ΔT is the temporal interval.

Putting Eq. 4.11-14 into Eq. 4.9, we can get the final discretized form of Fick equation as,

$$\begin{aligned} \frac{C_{i,j,k}^{n+1} - C_{i,j,k}^n}{\Delta T} = & D_{11} \frac{C_{i+1,j,k}^n - 2C_{i,j,k}^n + C_{i-1,j,k}^n}{\Delta X^2} \\ & + D_{22} \frac{C_{i,j+1,k}^n - 2C_{i,j,k}^n + C_{i,j-1,k}^n}{\Delta Y^2} \\ & + D_{33} \frac{C_{i,j,k+1}^n - 2C_{i,j,k}^n + C_{i,j,k-1}^n}{\Delta Z^2} \\ & + D_{12} \frac{C_{i+1,j+1,k}^n + C_{i-1,j-1,k}^n - C_{i+1,j-1,k}^n - C_{i-1,j+1,k}^n}{4\Delta X \Delta Y} \\ & + D_{13} \frac{C_{i+1,j,k+1}^n + C_{i-1,j,k-1}^n - C_{i+1,j,k-1}^n - C_{i-1,j,k+1}^n}{4\Delta X \Delta Z} \\ & + D_{23} \frac{C_{i,j+1,k+1}^n + C_{i,j-1,k-1}^n - C_{i,j+1,k-1}^n - C_{i,j-1,k+1}^n}{4\Delta Y \Delta Z} \\ & + \widetilde{D}_1 \frac{C_{i+1,j,k}^n - C_{i-1,j,k}^n}{2\Delta X} + \widetilde{D}_2 \frac{C_{i,j+1,k}^n - C_{i,j-1,k}^n}{2\Delta Y} \\ & + \widetilde{D}_3 \frac{C_{i,j,k+1}^n - C_{i,j,k-1}^n}{2\Delta Z} \end{aligned} \quad (4.15)$$

With the initial condition $c(t=0, x)$, a boundary condition of an infinite reservoir of 10% formalin with diffusivity identical to water, as previously described in section 4.2.3, and the diffusion tensors computed from diffusion data acquired within the same postmortem brain and processed with the FSL diffusion toolbox (FDT), Eq. 4.15 can be used to iteratively compute the time evolution of formalin concentration. For the forthcoming comparison studies, we simulated three scenarios: kinetic isotropic model “without hole” (where the ventricles have the same isotropic diffusivity as white and grey matter), kinetic isotropic model “with hole” (where the ventricle is filled with formalin or Fluorinert, but white matter and grey matter have the same isotropic diffusivity), and kinetic tensor model “with hole” (all tissue voxels computed using diffusion tensor $\mathbf{D}$ derived from the separate DWI scan, while we assume the “hole” or ventricle is filled with formalin or Fluorinert).

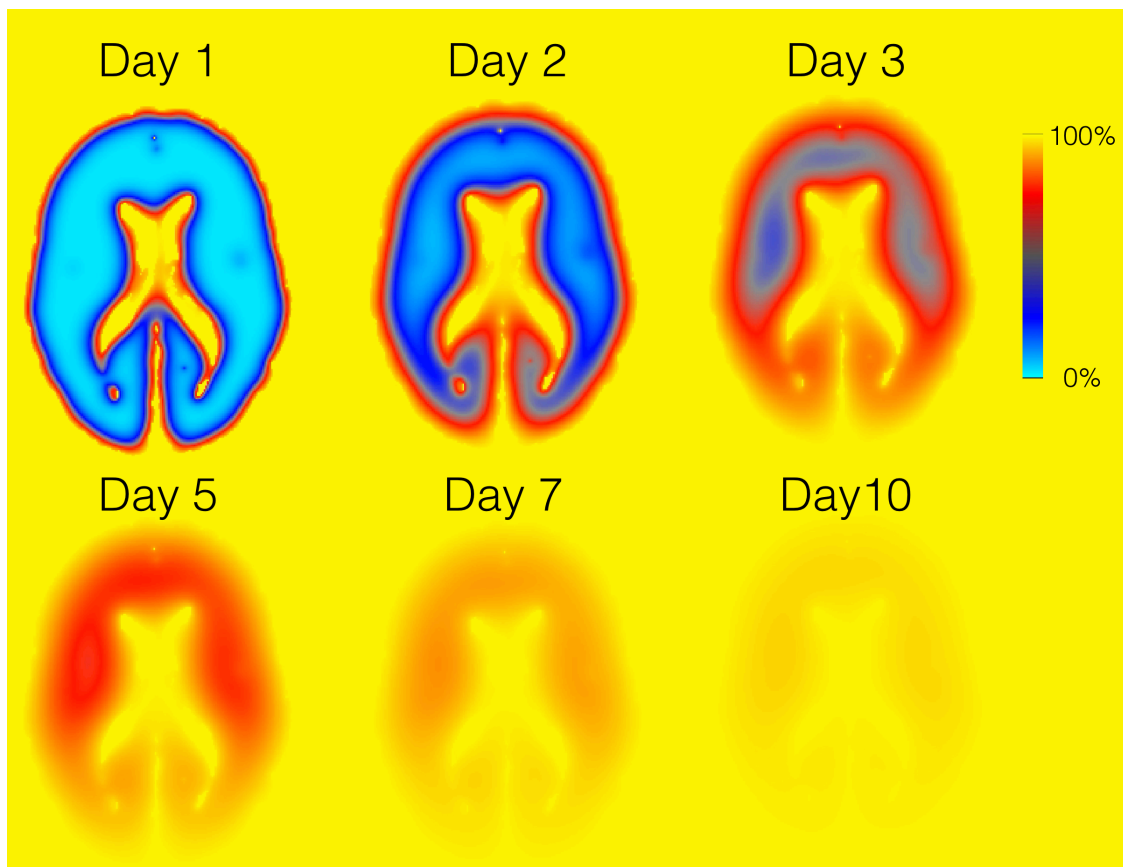


Figure 4.10: The formalin inflow process during brain immersion in the 10% formalin solution. (A-C) show the formalin concentration distribution at day 0, day 5 and day 10. The simulation shows that after 17 days, all voxels are saturated with a concentration greater than 95%; while after 25 days, all voxels are saturated with a concentration greater than 99%.

Fig. 4.10 demonstrates the results of simulating the formalin inflow process in the postmortem brain during immersion fixation from day 0 to day 10. The simulations indicate that after 17 days of fixation, all the tissue voxels are saturated with formalin concentration greater than 95%, while after 25 days of fixation, the concentration for all voxels is greater than 99%. These observations are consistent with previous histological studies (Kiernan *et al.*, 2000; Srinivasan *et al.*, 2002; Howart *et al.*, 2014) showing that deep brain tissues were found to have substantial changes in stiffness after two weeks. All postmortem brains in this study were fixed for at least one month before MRI scans were acquired (scan interval - range from 32 to 128 days). Therefore, we assume that the brains scanned in this work have 100% formalin concentration immediately before being immersed in the Fluorinert solution for scanning.

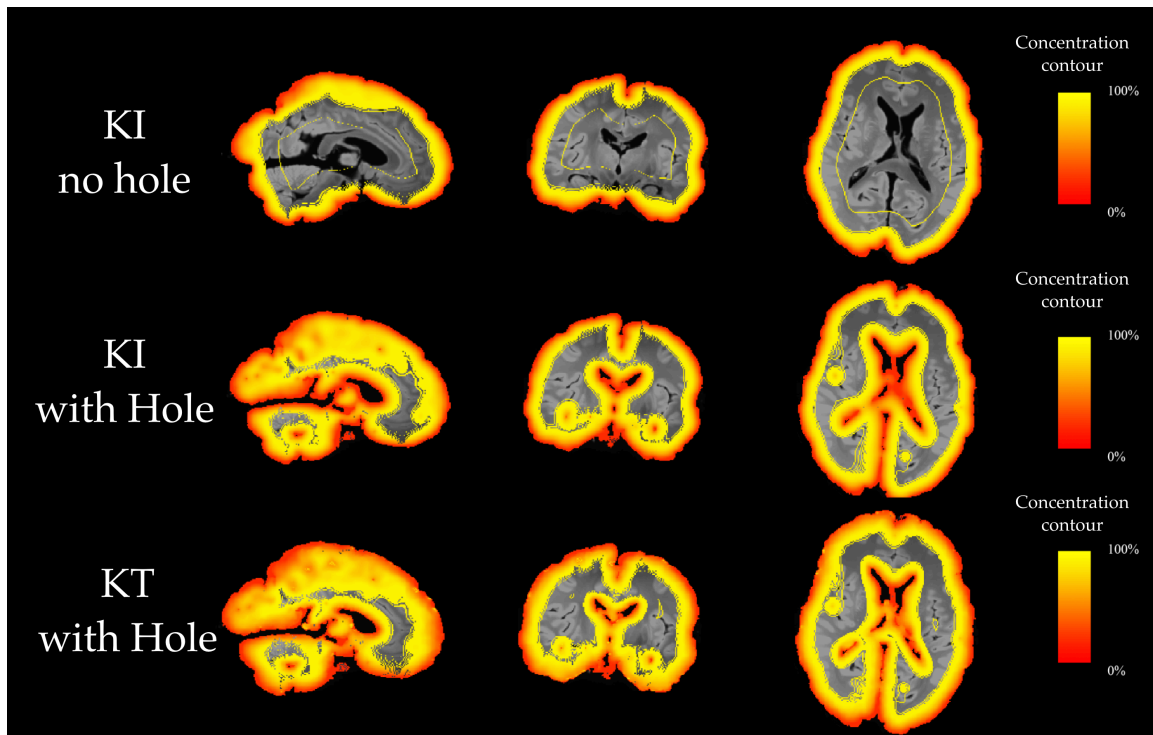


Figure 4.11: Formalin concentration distribution after two days flow out simulation for three scenarios, namely KI without hole, KI with hole, and KT with hole. The red-yellow color-map is used to present formalin concentration contours, where yellow represents high concentration while red represents low concentration. As we can observe that the KI without hole case has formalin flow-out from the surface only, whilst the KI/KT with hole cases both experienced formalin leakage from boundaries of the surface and ventricle. Note that the KI model is computed using the mean diffusivity from the diffusion tensor.

Putting fixed brains into the Fluorinert solution for MRI scans will cause formalin to flow out from the brain tissue, along the concentration gradient. Brains prepared for imaging in this study are immersed in Fluorinert and have Fluorinert inserted into the ventricles prior to imaging, so to model this effect, we simulated a two-day formalin outflow process. The results of this simulation using the three diffusion scenarios previously outlined are presented in Fig. 4.11. From the final concentration contours of the KI no hole model, we can see that formalin flowed out of tissue isotropically from the brain surface, while the deep tissues still contain high concentration of formalin. However, from the simulated results of the KI and KT with hole models, the formalin flows out from both the brain surface and the ventricles. This is expected to be in much better agreement with reality, especially when we consider that, as part of sample preparation, Fluorinert was inserted into the ventricles to match susceptibility prior to imaging.

4.2.5 T2 correction

The simulated concentration maps are then used to perform a simple correction to the EPG fitted T2 maps. The work by Shepherd suggests that reduction in T2 is (approximately) linearly related to formalin concentration (Shepherd *et al.*, 2009). Specifically, a 2% increase in formaldehyde concentration results in a 10 - 15 milliseconds decrease in the measured T2 value. For the predicted formalin concentration maps for each post mortem brain, a simple linear regression model is fitted to the EPG-fitted T2 map (a single global parameter for each brain), as is illustrated by Fig. 4.12.

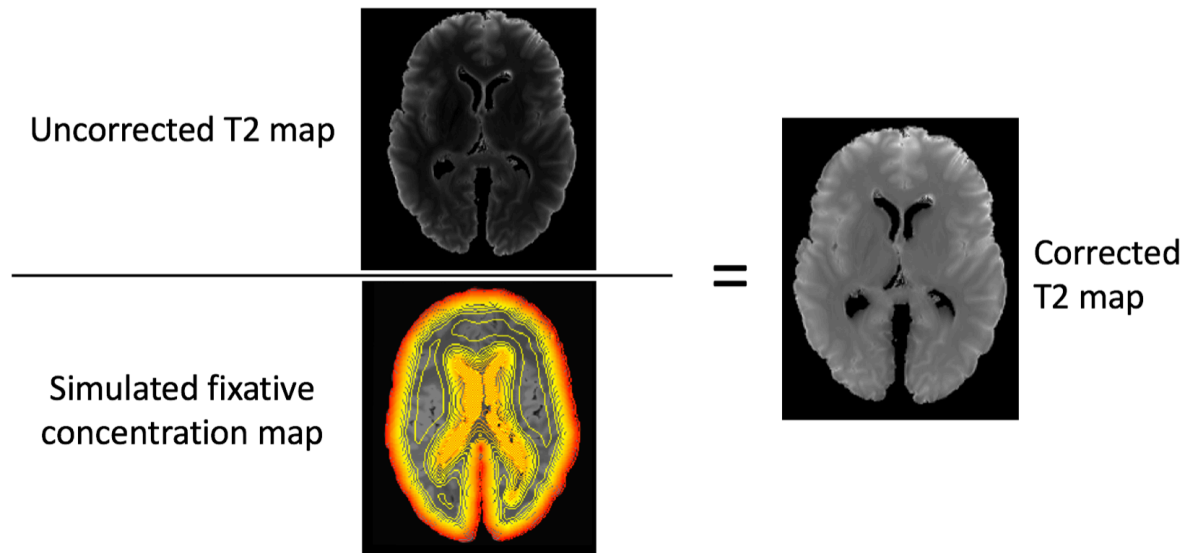


Figure 4.12: Illustration of the simple linear model used to calculate and correct the influence of formalin on T2 measures. The map used in the denominator can be any of the models presented here, including the kinetic isotropy/tensor models, and the distance-to-surface map. A single coefficient is fit to the entire brain, such that the overall shape of the concentration map drives the fit.

4.3 Methods

Postmortem brains were scanned with multiple pulse sequences, including-steady state free precession (SSFP) for structural images (providing strong grey-white contrast similar to T1 weighted structural images obtained in-vivo brain, but with inverted contrast leading to higher signal in gray matter), FSE (Miyazaki *et al.*, 1996; Lichy *et al.*, 2005) for quantitative T2 mapping, and diffusion-weighted SSFP for diffusion tensor estimation (Miller *et al.*, 2010a, 2010b). After acquiring these raw data, a standard pipeline was used for processing the cohort of brains.

4.3.1 Masks and Segmentations

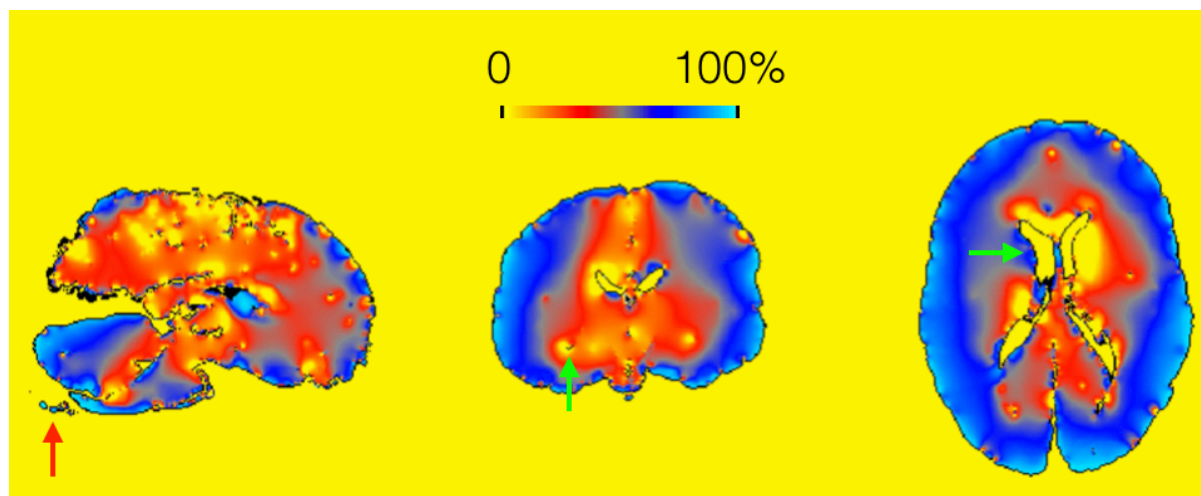


Figure 4.13: Ripple artefact (green arrows) and isolated tissue islands (red arrows). The ripple effect occurs in formalin simulations in voxels with extremely low diffusivity values. Isolated tissue islands or voxels are not of interest in our study, and could cause problems in subsequent statistical analysis, and are removed prior to formalin dynamic simulation.

Creation of a precise mask is important for the simulation of formalin diffusion dynamics, as poor masking can influence both the *distancemap* computation and boundary conditions for kinetic tensor and kinetic isotropic models (Eq. 4.8). Given that postmortem brains are imaged, brain extraction to remove the skull and non-brain tissue is not required. Principal eigenvalue (L1) diffusion maps exhibit artefactual isolated voxels with extremely low diffusivity, which appear black on images. These “holes” need to be filled in, otherwise, they will act as impermeable barriers to diffusion propagation and induce a ripple-like artefact in the output of dynamic formalin simulations, as is shown in Fig. 4.13. There are also typically some isolated tissue voxels or islands in the brain mask (Fig. 4.13) that may cause problems in statistical analysis. To remove these isolated voxels, we used the MATLAB function *bwconncomp* (The Mathworks, Natick, MA, USA). As the FSE and diffusion images were acquired at different spatial resolution, registration was performed to align the FSE image into diffusion space using FSL FLIRT (Jenkinson *et al.*, 2001, 2002;) with a six degrees of freedom (DOF) affine transformation. An intersection operation was then applied on both the diffusion mask and the

transformed FSE mask to generate a mask that is guaranteed to contain nonzero values in both modalities. Fig. 4.14 illustrates the final mask for one postmortem brain.



Figure 4.14: Tissue mask created from the intersection of DTI images and EPG T2 maps. Because these two images are acquired in different spaces, after registering the T2 map into diffusion space, the mask needs to contain nonzero values of both primary diffusivity and T2.

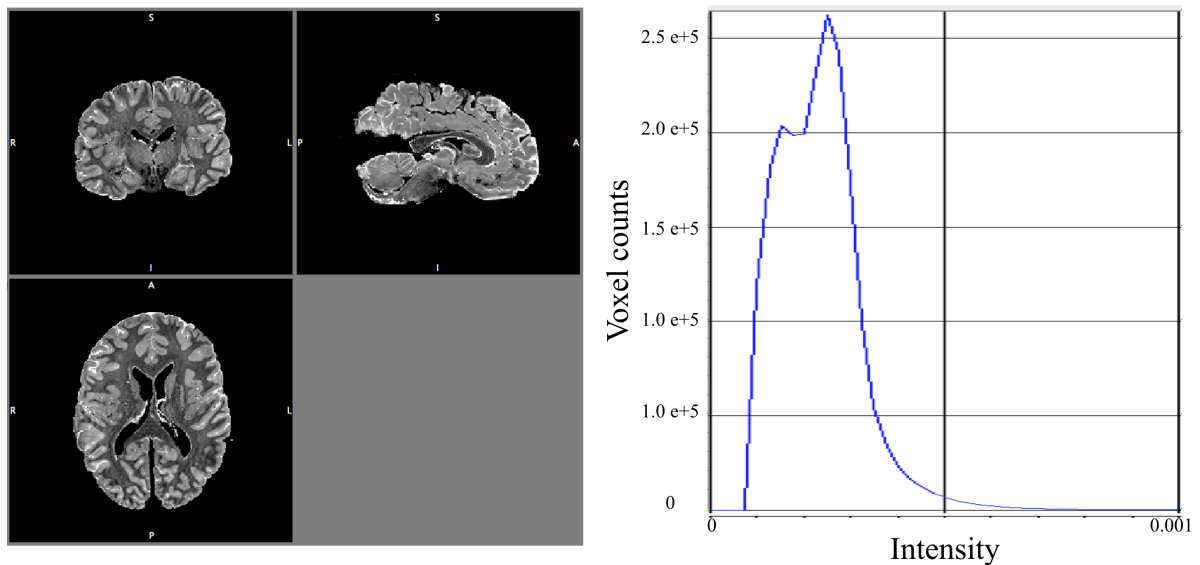


Figure 4.15: mean diffusivity map and its intensity histogram. We found there is no clear threshold to segment white matter from grey matter. A segmentation with the default parameter did not give reliable segmentation for these postmortem brains.

Tissue segmentation with three labels (white matter, grey matter, and ventricle fluid) is also critical for this study in order to assess the accuracy of quantitative T2 values in various cortical regions. Mean diffusivity (MD) images exhibit strong grey-white contrast and were used for FAST segmentation (Zhang *et al.*, 2001). A variety of parameters were investigated to optimize segmentation accuracy, as shown in Fig. 4.15-17. It was initially found that default FAST parameters, which are optimized for in-vivo imaging with T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) scans, do not provide reliable white matter and grey matter masks for our data. The intensity histogram for a typical MD image is presented on the right panel, which shows no clear boundary between white matter and grey matter. Figure 4.16 (n=2, R=0.3) shows the FAST segmentation with default processing parameters, clearly demonstrating that many grey matter voxels are wrongly classified as white matter, e.g. in the cerebellum. Modifications to the default parameters were investigated, including spatial smoothness R ranging from 0.1 to 0.7, and the number of classes (tissue labels/categories). No significant difference was found changing R, so the default value R = 0.3 was chosen. For the number of classes, it was found that using 5 classes, and combining label 4 and 5 together to represent grey matter, resulted in the most accurate classification. We also compared the results of using the mean diffusivity (MD) map alone as an input to FAST, or using multi-channel segmentation with both MD and structural images. The results indicate that the use of two modalities segmentation results in a more accurate segmentation and depiction of detailed fine structures in grey matter.

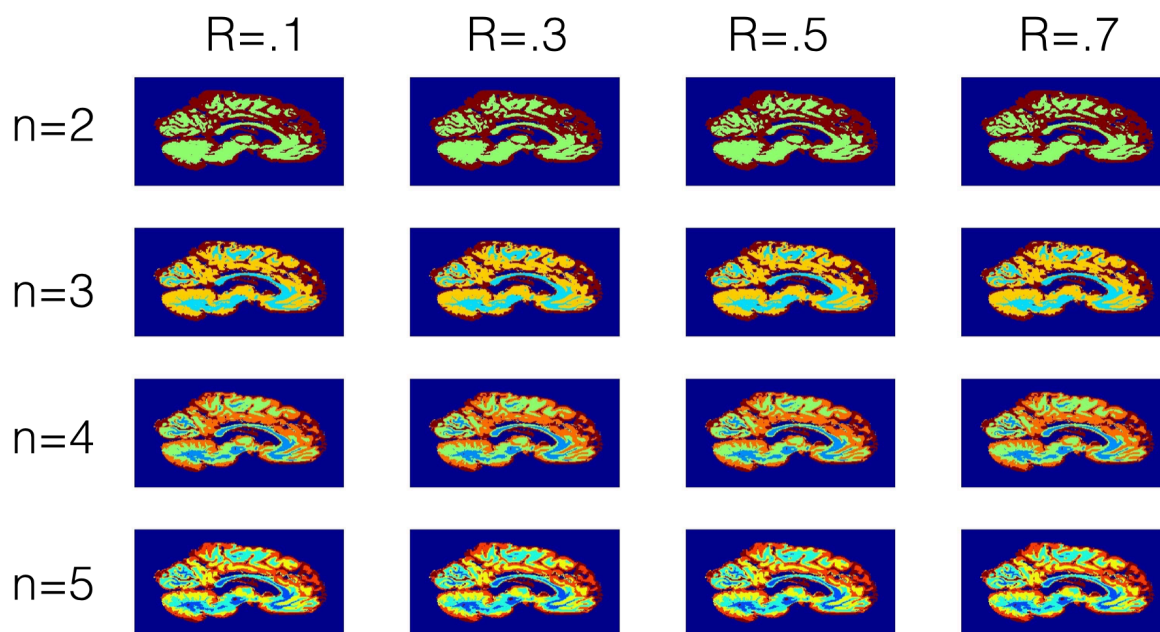


Figure 4.16: Segmentation of white matter and gray matter. The FSL-FAST command was used to segment brain tissue types, and a range of parameters were tested to optimize segmentation results. (Top) number of tissue classes ($n \in [2, 5]$) and spatial smoothness parameter ($R = 0.1: 0.2: 0.7$) are two important parameters to control the segmentation results.

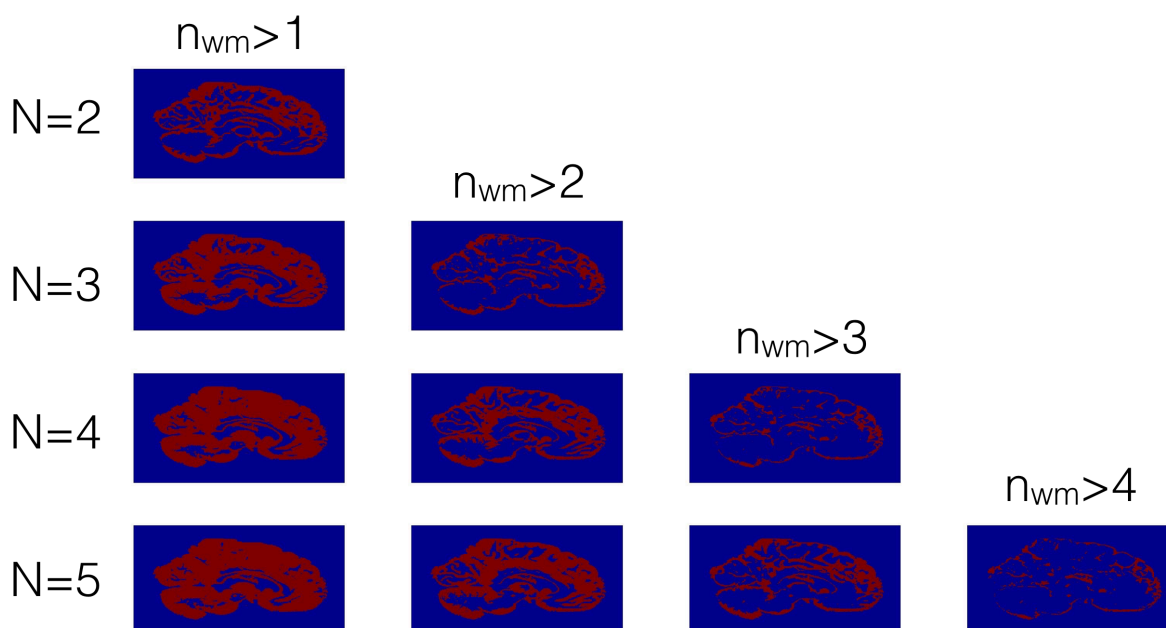


Figure 4.17: Segmentation of white matter and gray matter by combining multiple tissue classes. Note that both mean diffusivity and structural images were used for this segmentation, and that $n_{wm} > m$ indicates that $m+1, \dots, N$ classes are combined to make the displayed white matter mask.

4.3.2 Hyperintensity artefact in diffusion data

A major problem discovered in the diffusion tensor data was an artefactually high diffusivity on the L1 diffusivity map, as illustrated in Fig. 4.18 (yellow voxels in middle row), likely due to RF field inhomogeneity. These artefacts would cause us to simulate faster formalin dynamics on the edge of the brain and accumulate to less accurate predictions of fixative concentration. However, it was observed that structural images exhibit no such hyperintensity. Given that the structural images had a higher spatial resolution than diffusion images in this study, we implemented a “Good Neighbor” (GN) algorithm, outlined below, to transfer information from structural images to diffusion images to fix the white ring artefact. This algorithm is applied to the diffusion data and only affects quantification through the predicted concentration map. Given that a relatively small region is affected and the correction uses to a global (single coefficient) fit to these concentration maps, we felt that this somewhat ad hoc correction was justified.

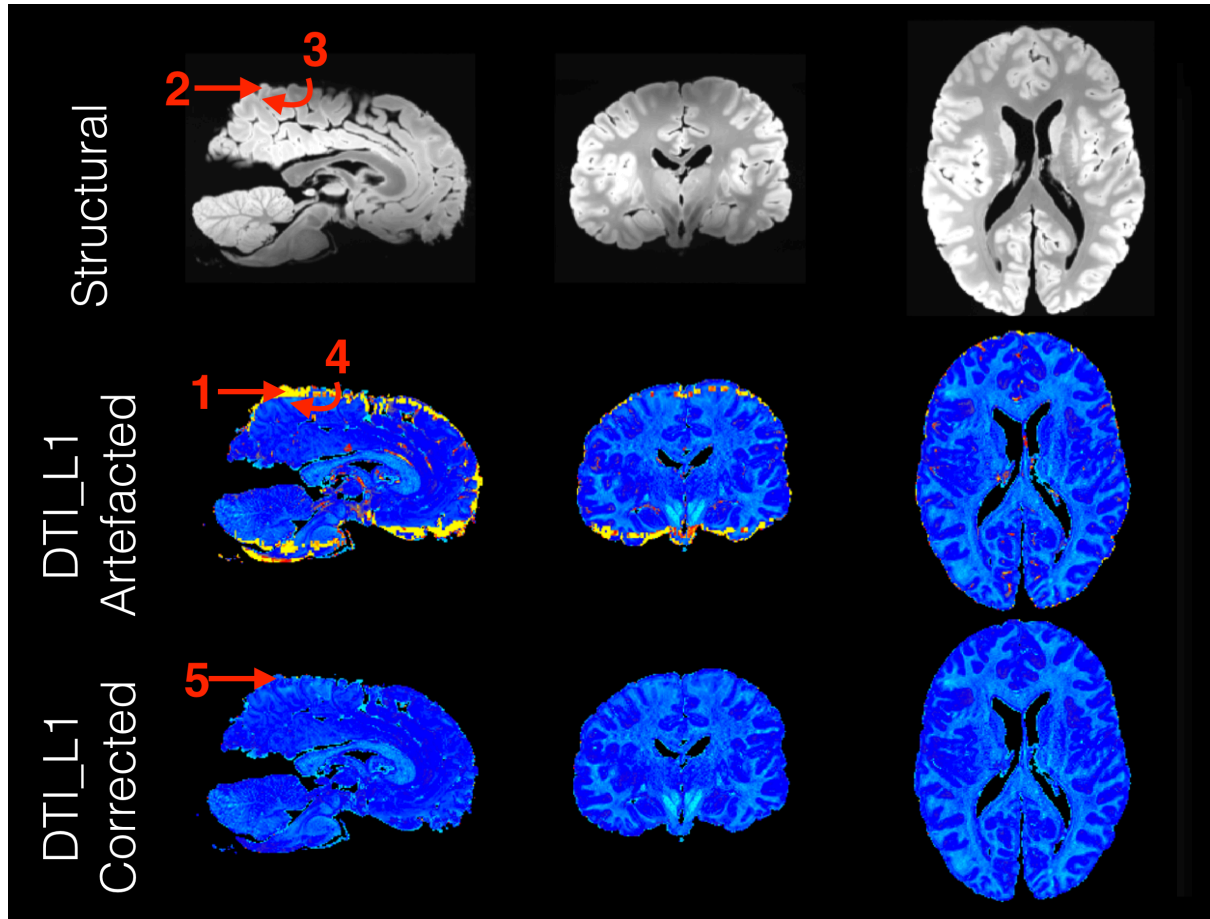


Figure 4.18: White ring artefact on L1 and good neighbor method correction. High-resolution structural image without white ring influence (top row). DTI_L1 map that suffered from white ring effects on the top and bottom of the brain (middle row). DTI_L1 graph after Good Neighbor correction which removed the white ring artefact while keeping the structure and texture unchanged during the correction (bottom row). The method works as: follows (1) Find voxels with artefact, (2) extract its structural intensity, (3) among the surrounding voxels without artefact, find the voxel with the closet structural intensity, (4) extract the L1 value of that neighbor voxel, (5) use its L1 value to replace that of the artefact voxel. Note that the artefact voxels are represented by a yellow mask with a threshold of $4E-4$. The hyperintensity ring may be caused by residual eddy-current which may result in distortion effects including scaling shearing and shift, leading to a final misregistration of the diffusion-weighted images (Mori et al., 2014).

The Good Neighbor correction procedure:

0. L1 diffusivity maps can suffer from a hyperintensity artefact, as shown in Fig. 4.18 (middle row).
1. Threshold high-intensity voxels ($\text{thr} = 4E-4$) to generate an artefact mask (Fig 4.18, yellow voxels).

2. For each voxel in the artefact mask:
 - a. Identify the intensity value from the corresponding voxel in the structural image.
 - b. From the nearest neighbor voxels (outside the artefact mask), find the voxel that has the closest structural intensity.
 - c. Replace the L1 value of the artefact voxel with the L1 value of the identified “good neighbor” voxel.

4.3.3 Scan parameters and processing scripts

This section describes acquisition parameters for each MR sequence and the overall data processing pipeline. Thirteen post-mortem human brains (age: 56 ± 18 years old; post-mortem interval < 72 hrs; scan interval: $> two$ months) with diagnosed ALS, and three healthy controls (age: 61, 61, and 74 years, no diagnosed neurodegenerative disorder) were imaged using a Siemens 7T scanner with a one channel transmit and 32 channel receive head coil. Brains were immersed in Fluorinert for susceptibility matching. Structural images were acquired with a 3D fully balanced SSFP (bSSFP) with flip angle = 35° , bandwidth = 296 Hz/pixel, resolution = $0.35 * 0.35 * 0.40 \text{ mm}^3$, and 180° RF phase cycling. T1 maps were obtained using a turbo inversion recovery (TIR) sequence with flip angle = 180° , bandwidth = 200 Hz/pixel, TE = 12 ms, TR = 2000 ms, TI = [32, 64, 125, 250, 500, 850] ms, FOV = $208 * 156 \text{ mm}^2$, 96 slices, and resolution = $1.0 * 1.0 * 1.4 \text{ mm}^3$. T2 maps were obtained from a FSE sequence (flip angle = 180° , bandwidth = 130 Hz/pixel, TR = 1000 ms, TE = [11, 23, 34, 46, 57, 69] ms, FOV = $208 * 156 \text{ mm}^2$, slices = 96, resolution = $1.0 * 1.0 * 1.4 \text{ mm}^3$) and DTI data were acquired using DW-SSFP with 0.85mm isotropic resolution, two flip-angles (24° and 94°), 120 directions per flip angle ($q = 300 \text{ cm}^{-2}$, TR = 28 ms).

FSL FAST was used for tissue segmentation, FSL DTIFIT for tensor and scalar metric calculation, custom MATLAB code for the GN method to correct for L1 hyperintensities, custom MATLAB code based on nonlinear optimization (fminsearch) to perform EPG T2 and flip angle (or B1) map fitting, custom MATLAB code and FSL *distancemap* for distance-to-surface and custom KI and KT models for formalin dynamics simulation. Finally, exponential and linear regression models were used for T2 corrections, as described below. Statistical results comparing ALS subjects to controls will be presented in Chapter 5.

The final processing pipeline is written in MATLAB, and is parallelized to run on a cluster using a Sun Grid Engine. Fig. 4.19 illustrates the whole processing pipeline, which comprises of 4 components. Data preprocessing, prior to formalin dynamic simulations, includes fixing (1) L1 hyperintensities using the GN method, (2) ripple artefact correction, (3) mask creation, (4) tissue segmentation and (5) registration of diffusivity images to standard space (MNI152) using ANTs (Avants *et al.*, 2014). The other three components represent three previously introduced simulation models (KI_noHole, KI_withHole, and KT_withHole), which can be run concurrently. Each model will run (1) formalin dynamic simulation of the Fluorinert immersion process, i.e. the formalin outflow process, (2) simulation of formalin concentration distribution at the scan time point, (3) fitting a linear regression model for each brain to correct T2 values, and (4) correction of T2 for the effect of formalin concentration using the model. Finally, a comparison study between the original exponential T2 map, EPG-fitted T2 map, and formalin corrected, EPG-fitted T2 maps is performed.

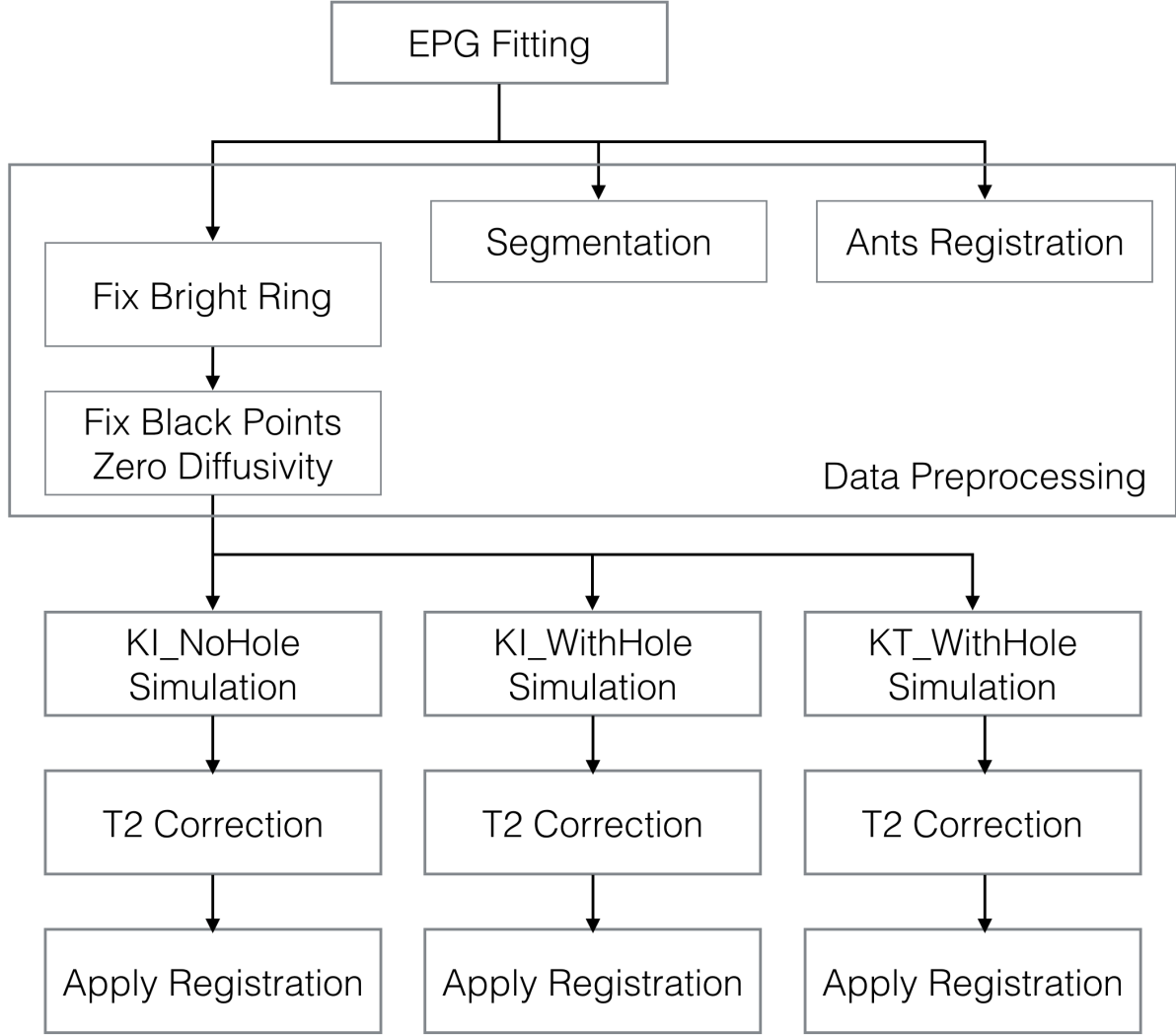


Figure 4.19 Processing pipeline for formalin dynamic simulations and subsequent T2 correction. To prepare data for formalin dynamics simulation, we first apply the GN method to fix the ring of high diffusivity values on L1 maps, and fix low diffusivity and isolated tissue island issues. Nonlinear registration is performed using ANTs to compute a transform from subject to MNI152 space. Then, the simulation of formalin dynamics is performed using the KI_noHole, KI_withHole, and KT_withHole models. Finally, we perform the linear T2 correction with the calculated formalin distribution and register the corrected T2 map to the MNI152 for future statistical analysis.

4.4 Results

After MR acquisition and data processing, comparison studies were performed among the four aforementioned models: distance-to-surface, kinetic isotropic without hole, kinetic isotropic with hole, and kinetic tensor with hole modes. In Fig. 4.20, different formalin concentration

distributions derived from these models are displayed, where each point represents one voxel with its concentration or distance on the x-axis, and T2 value on the y-axis. The average T2 values (bin size = 1% of formalin concentration) are displayed as star points, where red represents white matter, blue grey matter, black represents entire tissue voxels, green represents the fitted regression models (equations shown on the top), and open circles represent the corrected T2 values. From the results of the distance-to-surface model (Fig 4.20a), we found for both white matter and grey matter an approximately exponential relationship between distance and quantitative T2 value. This is reasonable given the assumption that outflow of formalin occurs first at surface tissue voxel, while deep tissue voxels have postponed outflow according to Fick's diffusion law (left part of Eq. 4.1 and 4.2), where concentration gradient governs outflow speed. This explains why after simulating two-day outflow, the tissue voxels with a large distance to the surface still have high formalin concentration, as is displayed in Fig. 4.10. A simple exponential decay model was used to fit all tissue voxels (green), and then used to regress out the influence of formalin on T2 (Fig. 4.20a). For the various kinetic models, we plot the formalin concentration on the x-axis, such that voxels on the left side of the plot with low formalin concentration are voxels close to surface, such as the brain cortical surface or ventricle surface, whilst voxels on the right side of the plot are voxels in deep tissue whose concentration were high until the outflow simulation finished. The KI-noHole fitting (Fig. 4.20b) follows a linear relationship that is consistent with Shepherd's histological conclusion, motivating a linear regressor to remove the fixative influence. The KI-withHole curve (Fig. 4.20c) resembles that of KI-noHole case. Interestingly, for the KI-withHole model, averaged T2 curves of both white matter (*) and grey matter (*) are remarkably flatter in comparison to the KI-noHole modeling, which suggests that incorporating the contribution of ventricles during formalin outflow simulation may provide a more precise estimate of the distribution of the formalin. Finally, KT-withHole's fitting is similar to that of KI-withHole; below, we

investigate whether the more detailed information in the KT model provides a more accurate correction as indicated by the T2 homogeneity within each tissue type.

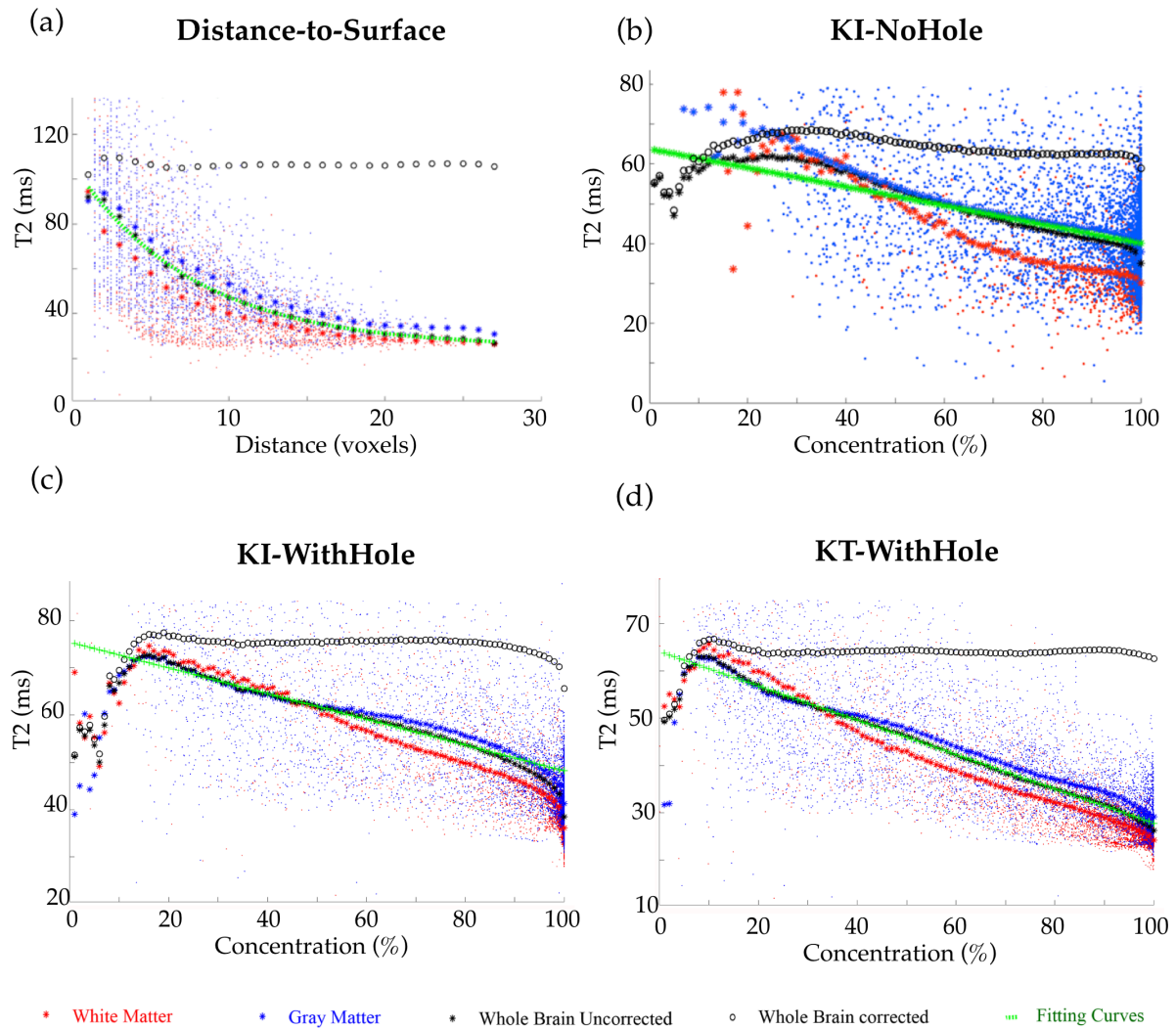


Figure 4.20. Formalin correction based on the four models, the distance-to-surface model, KI-noHole model, KI-withHole model, and the KT-withHole model. The small red background points represent T2 values of white matter tissue voxels, and large points represent the averaged T2 values, whilst blue points represent grey matter. The black points represent averaged values for all brain voxels, green lines represent linear (for KI and KT model) or exponential (for distance-to-surface model) regression lines, and black circles represent the corrected averaged T2 value. Note that, we manually scaled the y-axis to present the curves in the centre of the plot. We hypothesize that the initial rise (concentration from 0% to 15%) in T2 value might be caused by the decomposition of protein cross-link during the formalin outflow, because tissue decomposition causes T2 increase (Dawe, 2009).

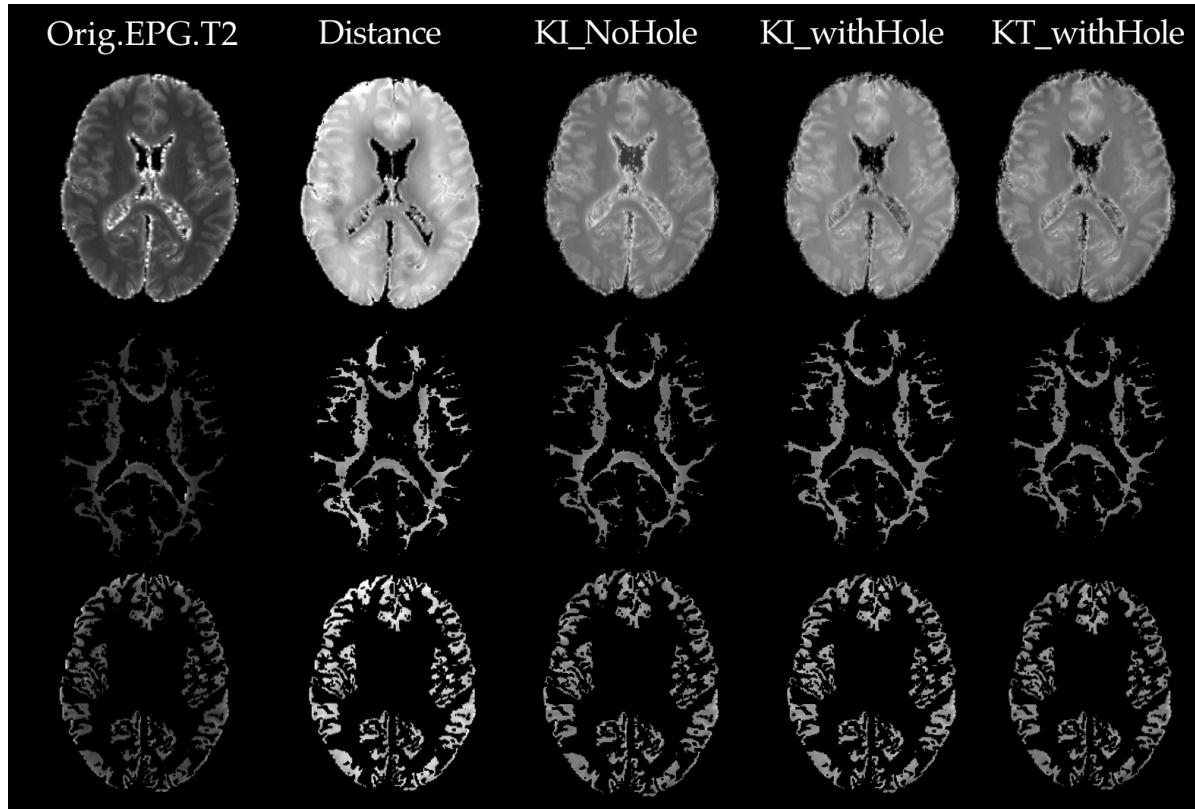


Figure 4.21: T2 comparison among original EPG fitting, distance to surface correction, kinetic isotropic without hole model, kinetic isotropic with hole model, and kinetic tensor with hole model for MND18. The top row is a mid-axial slice for each of these models, while the second and third rows show only values within white matter and cortical grey matter masks.

Inhomogeneity	EPG T2	Distance	KI	KI	KT
(%)	Map	Model	noHole	withHole	withHole
White Matter	14.6	13.5	12.3	10.9	10.2
Grey Matter	19.5	19.2	19.0	17.4	16.9

Table 4.1: Table showing the change in inhomogeneity for different T2 correction models for both white matter and cortical grey matter of MND18.

Mean (SD) %	EPG T2 Map	KI noHole	KI withHole	KT withHole
White Matter	13.3 (4.2)	11.0 (2.9)	8.9 (1.5)	8.4 (1.5)
Grey Matter	18.0 (4.2)	17.1 (4.6)	15.9 (3.5)	15.1 (3.9)
P-Value	-	0.197	0.006	0.001

Table 4.2: Table showing the change in inhomogeneity for different T2 correction models for both white matter and cortical grey matter for all 13 ALS brains and 3 CTL brains. The inhomogeneity is presented in terms of mean (standard deviation). Note that the P-value reflects the significance of T2 homogeneity improvement with the three formalin correction models.

The final comparison of corrected T2 maps among five models including original EPG fitting (no fixation correction), and corrections based on distance-to-surface, KI without hole, kinetic KI with hole, and KT with hole model. These model comparisons in a single brain sample are given in Fig. 4.21 and Table 4.1. First, it can be seen that the uncorrected EPG fit shows remarkably short T2 values, due to the presence of formalin. Using distance to surface as a correction factor for T2 variation can reduce the inhomogeneity caused by formalin, which is demonstrated by the reduction in inhomogeneity from 14.6% to 13.5%, and from 19.5% to 19.2% for white matter and grey matter, respectively. However, residual inhomogeneous regions remain (for example, an obviously shortened T2 in the thalamus and other subcortical grey matter regions). The KI no hole model, as originally implemented by Dawes, provides an improvement in homogeneity over the distance-to-surface model, provided improved homogeneity values of 12.3% and 19.0% in white and grey matter separately than the distance-to-surface model. What's more, the KI-withHole performed even better than the KI-noHole case with 10.9% and 17.4% for white and grey matter. Finally, the KT-withHole model provides the most accurate correction. The results in Fig. 4.21 and Table 4.1 also demonstrate these points, as KT-withHole model can reduce the formalin distribution influence to inhomogeneity of 10.2% and 16.9%, the lowest level for white matter and grey matter. We performed a the statistical analysis of the inhomogeneity across all 13 ALS and 3 CTL brains (Table 4.2), which demonstrates the progressive improvement from EPG model (13.3%, 18.0%), to KI-noHole (11.0%, 17.1%), KI-withHole (8.9%, 15.9%), and finally KT-withHole (8.4%, 15.1%) models for white matter and grey matter, respectively.

The final proposed set of corrections are shown in Fig. 4.22. Compared to the exponential fits, the EPG fitting provides quantitative T2 maps with correction for B1 inhomogeneity but higher T2 estimates on the periphery. After the KT model fitting presented in this chapter, we obtained

quantitative T2 maps with minimal influence from the presence of formalin, and where both deep and superficial tissues are corrected by the model. These two corrections are both critical for quantitative T2 mapping in the post-mortem brain using our FSE-based approach at 7T.

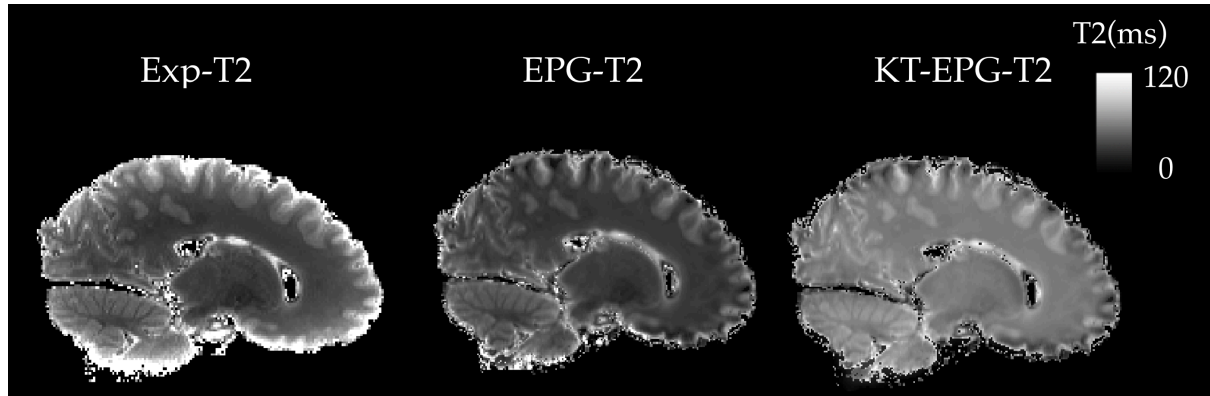


Figure 4.22: Evolution of quantitative T2 maps. Traditionally people use the exponential decay model to derive the T2 maps from raw data measured with a FSE pulse sequence. However, the RF coil cannot provide sufficient B1 homogeneity over the entire brain, with low strength on the edge and subsequent insufficient magnetization flipping, leading to T2 maps that display a bright ring on the edge, including areas such as the primary motor cortex and supplementary motor area. EPG can take into account B1 transmit inhomogeneity and provide a more accurate quantitative T2 map by simultaneously estimating the flip angle and accounting for the B1 inhomogeneity. However, the T2 estimates from EPG are still low because of the influence of formalin. So, in this chapter, we proposed the kinetic tensor model, which could further compensate for the T2 value drop by the computed formalin distribution.

4.5 Conclusion

In this chapter, I discussed why formalin fixation is required in studies of pathology specimens, and how brain preparation is performed in this particular study. Then, I discussed how prior studies, such as Shepherd's histological study, have revealed that both formalin type and concentration are key influential factors that may alter quantitative T2 maps. In the theory section, I discussed in detail the theory of formalin simulation, first providing an analytical derivation using spherical harmonic expansion, then numerical simulations taking brain structure into consideration. The distance-to-surface model was presented as a simple model with limited physical motivation, which employs an exponential relationship between distance and formalin distribution to correct T2 maps. A more physically motivated model, the kinetic isotropy (KI) model, was also considered for the correction of fixation. Boundary conditions within the ventricles were added to the previously proposed KI model, which further increase the simulation accuracy of formalin concentration, especially on subcortical grey matter regions. Finally, a novel approach called the kinetic tensor (KT) method was described for correcting the effect of formalin on quantitative T2 maps. Because it uses the diffusion tensor derived from diffusion weighted MRI, this model is able to provide more accurate estimates of formalin concentration by taking into account the anisotropic properties of tissue. The KT model uses diffusivity estimates on a voxel-wise basis to simulate a more accurate formalin flow inside the brain tissue. As judged by the resulting homogeneity of T2 values after correction, the proposed kinetic tensor model was found to provide the most accurate correction. In the next chapter, we will use the KT derived T2 maps within our post-mortem ALS dataset.

4.5 References

- Avants, B. B., Tustison, N. J., Stauffer, M., Song, G., Wu, B., & Gee, J. C. 2014. The Insight ToolKit image registration framework. *Frontiers in Neuroinformatics*, 8, 44.
- Behrens, T.E., Berg, H.J., Jbabdi, S., Rushworth, M.F. and Woolrich, M.W., 2007. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain?. *Neuroimage*, 34(1), pp.144-155.
- Behrens, T.E., Woolrich, M.W., Jenkinson, M., Johansen-Berg, H., Nunes, R.G., Clare, S., Matthews, P.M., Brady, J.M. and Smith, S.M., 2003. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, 50(5), pp.1077-1088.
- Bloembergen, N., Purcell, E.M. and Pound, R.V., 1948. Relaxation effects in nuclear magnetic resonance absorption. *Physical Review*, 73(7), p.679.
- Dawe, R.J., Bennett, D.A., Schneider, J.A., Vasireddi, S.K. and Arfanakis, K., 2009. Postmortem MRI of human brain hemispheres: T2 relaxation times during formaldehyde fixation. *Magnetic Resonance in Medicine*, 61(4), pp.810-818.
- Fox, C.H., Johnson, F.B., Whiting, J. and Roller, P.P., 1985. Formaldehyde fixation. *Journal of Histochemistry & Cytochemistry*, 33(8), pp.845-853.
- Foxley, S., Jbabdi, S., Clare, S., Lam, W., Ansorge, O., Douaud, G. and Miller, K., 2014. Improving diffusion-weighted imaging of post-mortem human brains: SSFP at 7T. *Neuroimage*, 102, pp.579-589.
- Harpold, H.L., Alvord Jr, E.C. and Swanson, K.R., 2007. The evolution of mathematical modeling of glioma proliferation and invasion. *Journal of Neuropathology & Experimental Neurology*, 66(1), pp.1-9.
- Herneth, A.M., Friedrich, K., Weidekamm, C., Schibany, N., Krestan, C., Czerny, C. and Kainberger, F., 2005. Diffusion weighted imaging of bone marrow pathologies. *European Journal of Radiology*, 55(1), pp.74-83.
- Howat, W.J. and Wilson, B.A., 2014. Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods*, 70(1), pp.12-19.
- Hughes, T.J., Franca, L.P. and Hulbert, G.M., 1989. A new finite element formulation for computational fluid dynamics: VIII. The Galerkin/least-squares method for advective-diffusive equations. *Computer Methods in Applied Mechanics and Engineering*, 73(2), pp.173-189.
- Jenkinson, M. and Smith, S., 2001. A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, 5(2), pp.143-156.

Jenkinson, M., Bannister, P., Brady, M. and Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*, 17(2), pp.825-841.

Jbabdi, S., Mandonnet, E., Duffau, H., Capelle, L., Swanson, K.R., Pélégriani-Issac, M., Guillemin, R. and Benali, H., 2005. Simulation of anisotropic growth of low-grade gliomas using diffusion tensor imaging. *Magnetic Resonance in Medicine*, 54(3), pp.616-624.

Jbabdi, S., Sotiropoulos, S.N., Savio, A.M., Graña, M. and Behrens, T.E., 2012. Model-based analysis of multishell diffusion MR data for tractography: How to get over fitting problems. *Magnetic Resonance in Medicine*, 68(6), pp.1846-1855.

Kamman, R.L., Go, K.G., Stomp, G.P., Hulstaert, C.E. and Berendsen, H.J.C., 1985. Changes of relaxation times T1 and T2 in rat tissues after biopsy and fixation. *Magnetic Resonance Imaging*, 3(3), pp.245-250.

Kiernan, J.A., 2000. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. *Microscopy Today*, 8(1), pp.8-13.

Kolasinski, J., Stagg, C.J., Chance, S.A., DeLuca, G.C., Esiri, M.M., Chang, E.H., Palace, J.A., McNab, J.A., Jenkinson, M., Miller, K.L. and Johansen-Berg, H., 2012. A combined post-mortem magnetic resonance imaging and quantitative histological study of multiple sclerosis pathology. *Brain*, 135(10), pp.2938-2951.

Le Bihan, D., 2003. Looking into the functional architecture of the brain with diffusion MRI. *Nature Reviews Neuroscience*, 4(6), p.469.

Lichy, M.P., Wietek, B.M., Mugler III, J.P., Horger, W., Menzel, M.I., Anastasiadis, A., Siegmann, K., Niemeyer, T., Königsrainer, A., Kiefer, B. and Schick, F., 2005. Magnetic resonance imaging of the body trunk using a single-slab, 3-dimensional, T2-weighted turbo-spin-echo sequence with high sampling efficiency (SPACE) for high spatial resolution imaging: initial clinical experiences. *Investigative Radiology*, 40(12), pp.754-760.

Miller, K.L., 2010a. Asymmetries of the balanced SSFP profile. Part I: theory and observation. *Magnetic Resonance in Medicine*, 63(2), pp.385-395.

Miller, K.L., Smith, S.M. and Jezzard, P., 2010b. Asymmetries of the balanced SSFP profile. Part II: white matter. *Magnetic Resonance in Medicine*, 63(2), pp.396-406.

Miller, K.L., Stagg, C.J., Douaud, G., Jbabdi, S., Smith, S.M., Behrens, T.E., Jenkinson, M., Chance, S.A., Esiri, M.M., Voets, N.L. and Jenkinson, N., 2011. Diffusion imaging of whole, post-mortem human brains on a clinical MRI scanner. *Neuroimage*, 57(1), pp.167-181.

Miller, K.L., McNab, J.A., Jbabdi, S. and Douaud, G., 2012. Diffusion tractography of post-mortem human brains: optimization and comparison of spin echo and steady-state free precession techniques. *Neuroimage*, 59(3), pp.2284-2297.

Miyazaki, T., Yamashita, Y., Tsuchigame, T., Yamamoto, H., Urata, J. and Takahashi, M., 1996. MR cholangiopancreatography using HASTE (half-Fourier acquisition single-shot turbo spin-echo) sequences. *AJR. American Journal of Roentgenology*, 166(6), pp.1297-1303.

Mollink, J., Kleinnijenhuis, M., van Walsum, A.M.V.C., Sotiropoulos, S.N., Cottaar, M., Mirfin, C., Heinrich, M.P., Jenkinson, M., Pallegage-Gamarallage, M., Ansorge, O. and Jbabdi, S., 2017. Evaluating fibre orientation dispersion in white matter: comparison of diffusion MRI, histology and polarized light imaging. *Neuroimage*, 157, pp.561-574.

Mollink, J., van Baarsen, K.M., Dederen, P.J.W.C., Foxley, S., Miller, K.L., Jbabdi, S., Slump, C.H., Grotenhuis, J.A., Kleinnijenhuis, M. and van Walsum, A.V.C., 2016. Dentatorubrothalamic tract localization with postmortem MR diffusion tractography compared to histological 3D reconstruction. *Brain Structure and Function*, 221(7), pp.3487-3501.

Mori S. and Tournier J.D. 2014, Introduction to Diffusion Tensor Imaging. Chapter 6, 39-52. Pallegage-Gamarallage, M., Foxley, S., Menke, R.A., Huszar, I.N., Jenkinson, M., Tendler, B.C., Wang, C., Jbabdi, S., Turner, M.R., Miller, K.L. and Ansorge, O., 2018. Dissecting the pathobiology of altered MRI signal in amyotrophic lateral sclerosis: A post mortem whole brain sampling strategy for the integration of ultra-high-field MRI and quantitative neuropathology. *BMC Neuroscience*, 19(1), p.11.

Pinto, S.H., Pinto, L.M.S., Guedes, M.A., Cunha, G.M.A., Chaves, M.H., Santos, F.A. and Rao, V.S., 2008. Antinociceptive effect of triterpenoid α , β -amyrin in rats on orofacial pain induced by formalin and capsaicin. *Phytomedicine*, 15(8), pp.630-634.

Shepherd, T.M., Thelwall, P.E., Stanis, G.J. and Blackband, S.J., 2009. Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*, 62(1), pp.26-34.

Srinivasan, M., Sedmak, D. and Jewell, S., 2002. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *The American Journal of Pathology*, 161(6), pp.1961-1971.

Start, R.D., Layton, C.M., Cross, S.S. and Smith, J.H., 1992. Reassessment of the rate of fixative diffusion. *Journal of Clinical Pathology*, 45(12), pp.1120-1121.

Sun, S.W., Neil, J.J. and Song, S.K., 2003. Relative indices of water diffusion anisotropy are equivalent in live and formalin-fixed mouse brains. *Magnetic Resonance in Medicine*, 50(4), pp.743-748.

Thickman, D.I., Kundel, H.L. and Wolf, G., 1983. Nuclear magnetic resonance characteristics of fresh and fixed tissue: the effect of elapsed time. *Radiology*, 148(1), pp.183-185.

Underhill, B.M., 1932. V.—The Rate of Penetration of Fixatives. *Journal of the Royal Microscopical Society*, 52(2), pp.113-120.

Yong-Hing, C.J., Obenaus, A., Stryker, R., Tong, K. and Sarty, G.E., 2005. Magnetic resonance imaging and mathematical modeling of progressive formalin fixation of the human brain. *Magnetic Resonance in Medicine*, 54(2), pp.324-332.

Zhang, Y., Brady, M. and Smith, S., 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Transactions on Medical Imaging*, 20(1), pp.45-57.

Zhang, Z., Song, L., Meng, Q., Li, Z., Pan, B., Yang, Z. and Pei, Z., 2009. Morphological analysis in patients with sciatica: a magnetic resonance imaging study using three-dimensional high-resolution diffusion-weighted magnetic resonance neurography techniques. *Spine*, 34(7), pp.E245-E250.

Chapter 5

Quantitative T2 in post-mortem brain: ALS vs controls

Contents

5.1 Background.....	120
5.2 Method	122
5.2.1 Processing pipeline	122
5.2.2 Region of interest.....	123
5.2.3 Quantitative T2 map processing.....	124
5.2.4 Histological sampling.....	125
5.2.5 Cohort information.....	126
5.3 Results and Discussion.....	128
5.3.1 Histological data.....	128
5.3.2 Quantitative T2 statistics.....	131
5.3.3 Ferritin and PLP versus T2 measures.....	132
5.4 Discussion.....	134
5.5 Conclusion.....	135
5.6 Reference.....	136

5.1 Background

ALS is a type of motor neuron disease that is characterized by progressive atrophy and weakness of muscles, leading to a loss of motor function in movement, articulation, and breathing, which is ultimately fatal (Rowland and Shneider, 2009; Kiernan *et al.*, 2011). The disease is characterized by damage of both upper motor neurons and lower motor neurons, with potential causes including genetic deficits (Rakhit *et al.*, 2007; Al-chalabi *et al.*, 2016) and environment factors (Dimos *et al.*, 2008; Y. Yu *et al.*, 2014). The root causes of ALS are still largely (~90%) unknown (Kiernan *et al.*, 2011). There is still no clinical approach to provide early diagnosis of ALS (Kiernan 2011), nor effective medication or therapy to treat ALS (Miller *et al.*, 2007; Carlesi *et al.*, 2011). However, promising research continues within this area, where recently the drug Edaravone, which has been shown to slow the advancement of ALS was approved in the US (Cruz *et al.*, 2018).

With the advance of modern neuroimaging techniques (Foerster *et al.*, 2013), multiple MRI modalities have been investigated to examine ALS neuropathology (Paez-Colasante *et al.*, 2015), distinguish ALS phenotypes (Ravits *et al.*, 2013), and predict treatment efficacy (Van Der Burgh *et al.*, 2017). Foerster first measured the decreased GABA content in the motor cortex of ALS patients via ^1H -MRS (Foerster *et al.*, 2012), which was consistent with a hyperintensity of cortical activity measured by resting motor threshold in the same area (Vucic and Kiernan, 2006) using transcranial magnetic stimulation. Douaud employed both diffusion weighted MRI and resting state functional MRI and discovered (1) ALS patients (compared with healthy controls) had a decreased white matter fractional anisotropy in the cortico-spinal tract and corpus callosum; (2) connected regions (e.g. the premotor, supplementary motor area, etc.) had increased functional connectivity (Douaud *et al.*, 2011). Fabes used T2-weighted MRI

intensity within the cortico-spinal tract as a biomarker, with an increased intensity correlating with a drop of executive function of ALS patients (Fabes *et al.*, 2017). These studies demonstrate that MRI has the potential to make an important contribution to the diagnosis of ALS and the assessment of treatments. These studies demonstrate the potential applications of MRI in ALS, but the interpretation of MRI findings requires comparison to a biological reference, and hence histological examination remains crucial in significance for ALS diagnosis.

A range of histological stains has been used to assess different aspects of ALS pathology. Myelin proteolipid protein (PLP) staining (Li *et al.*, 2012) was introduced to evaluate the status of myelin. Since ALS progression accompanies iron deposition (Yen *et al.*, 2004), the iron carrier protein ferritin was used in the staining process to delineate the distribution of iron deposition across regions of interest (Yiangou *et al.*, 2006; Massignan *et al.*, 2007). Furthermore, ALS is often characterized by the aggregation of phosphorylated 43-kDa trans-actin response DNA-binding protein (pTDP-43) (Braak *et al.*, 2013; Brettschneider *et al.*, 2014; Tan *et al.*, 2015), which can be detected through related staining techniques (Hatanpaa *et al.*, 2010; Mackenzie and Rademakers, 2008; Cykowski *et al.*, 2017). Nowadays, a great many of other molecular and genetic targets such as C21orf (Scott *et al.*, 1998), FUS (Sharma *et al.*, 2016; Kamelgarn *et al.*, 2018) and SOD1 (Bruijn *et al.*, 1998; Turner *et al.*, 2005) have been related to ALS pathologies. Of these histopathological markers, the most likely correlates for quantitative T2 are iron (ferritin) and myelin (PLP) (Ropele *et al.*, 2011; Heath *et al.*, 2018). In this chapter, we will (i) demonstrate the ability to detect differences in quantitative T2 between ALS and controls in the post-mortem brain, and (ii) investigate whether these differences in quantitative T2 correlate with PLP and ferritin staining.

5.2 Method

Neuropathological examination (based on immunohistochemistry) remains the current standard to confirm ALS post mortem, but would require an extremely invasive brain biopsy in vivo. The potential for a quantitative measure of ALS status from MRI is therefore very attractive and determining a correlation between histology and MRI could provide an insight into the underlying mechanisms that lead to signal changes (Assaf, 1997; Wang *et al.*, 2002). Here, we report the results of a correlation study between quantitative T2 and histopathology in ALS.

5.2.1 Processing pipeline

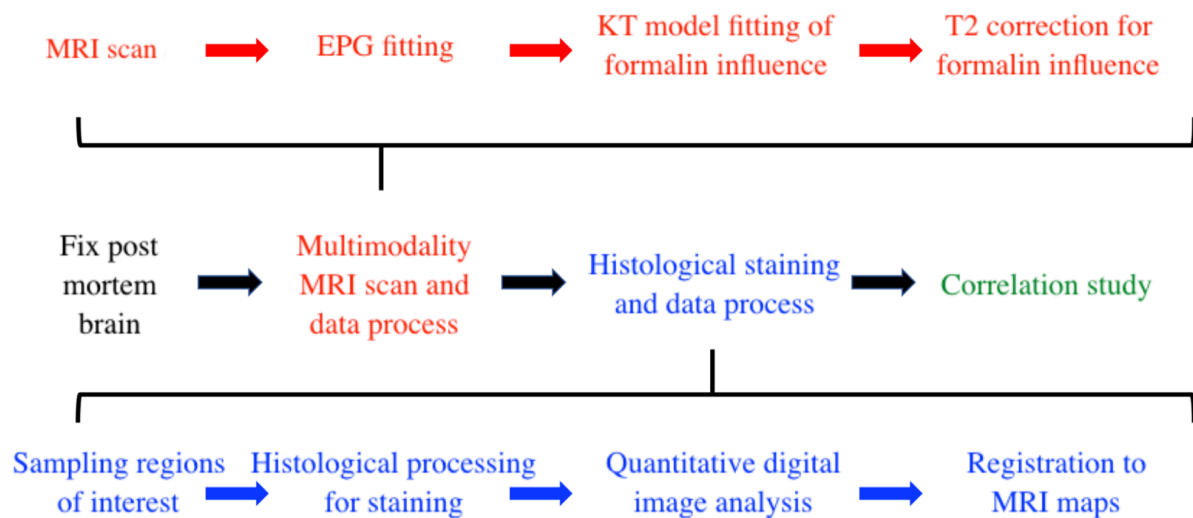


Figure 5.1: Project pipeline. (Middle) Processing pipeline for the ALS post-mortem brain project, which involves tissue fixation, MRI scanning, data processing, histological processing, and a correlation study. (Top) Summary of quantitative T2 map processing which involves an FSE scan, EPG fitting, KT model processing, and T2 correction. (Bottom) Summary of histological processing which involves sampling, staining, image analysis, and registration.

Fig.5.1 demonstrates the whole data pipeline for the ALS post-mortem brain project. After a post-mortem interval ($\text{PMI} = 3.08 \pm 1.44$ days), the brain is extracted and fixed (Howat and

Wilson, 2014; Kiernan, 2016). After 1-4 months (the scan interval), the formalin-fixed brains are packed into an MRI-compatible holder containing a susceptibility-matched fluid (Fluorinert, 3M Corporation). The brains are then scanned (7T, 1ch/32ch Tx/Rx coil) using a multiple modality MRI protocol (Miller *et al.*, 2011, 2012; Foxley *et al.*, 2014) including structural, diffusion weighted, relaxometry (including T1, T2, and T2*), and quantitative susceptibility images. Trained pathologists then dissect the brains according to a standardized scheme and select blocks of interest that are relevant to ALS progression for multiple immunohistochemistry stains (Gamarallage *et al.*, 2018). These data form the basis of this chapter for comparison between MRI and histopathology.

5.2.2 Regions of interest

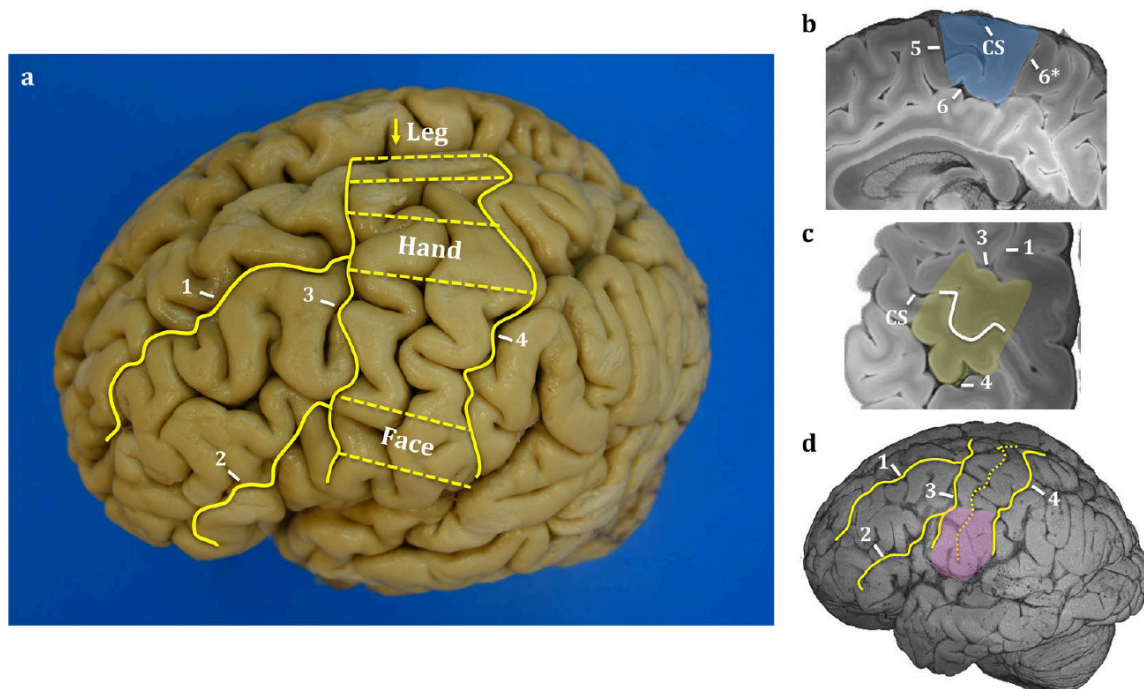


Figure 5.2: Sampling regions of interest. (a) The ALS related motor regions (leg, hand, and face) are defined according to the landmark sulcus. (b) leg region from the medial view. (c) Hand region in omega gyri. (d) The face region is located in the inferior lateral primary motor cortex and somatosensory cortex. Images come from (Gamarallage *et al.*, 2018).

ALS pathology progressively damages several cortical and subcortical regions (Braak *et al.*, 2013; Jucker and Walker, 2013). In this study we focused on the motor cortex (M1), representing early-stage pathology; the anterior cingulate cortex (ACC), which is affected in a later stage; and visual cortex (V2), which is either unaffected or late-stage (Brettschneider *et al.*, 2015). In the primary motor cortex, we separately analyzed the leg, hand and face regions, as is shown in Fig. 5.2. An example ferritin stain is shown in Fig. 5.3.

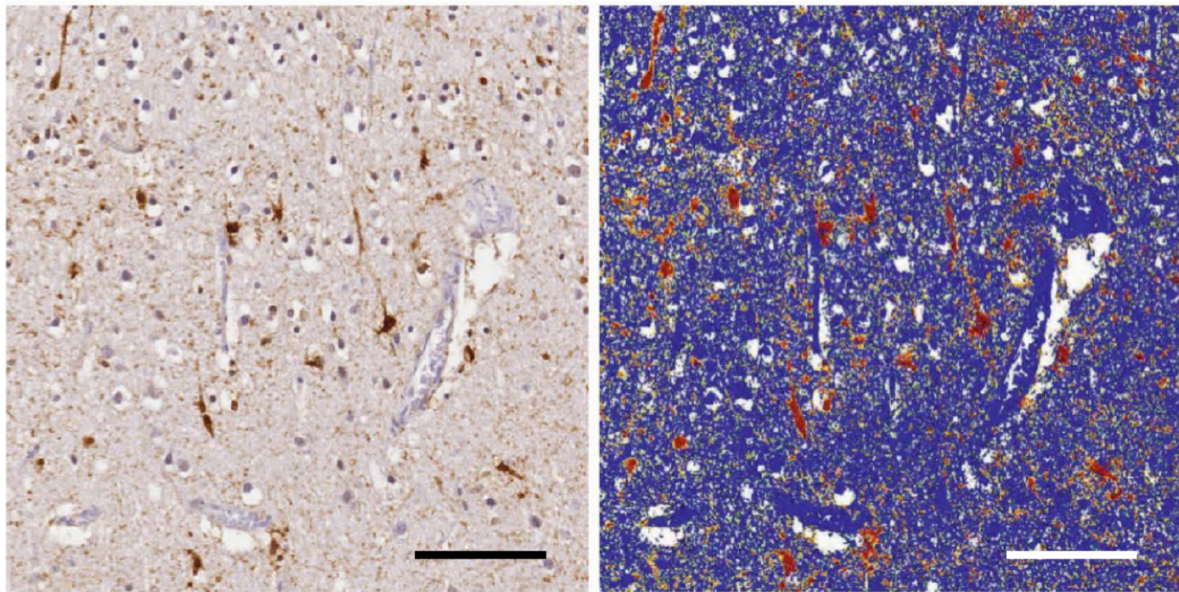


Figure 5.3: An example ferritin stain (left) and the quantitative digital histology image analysis (right). The bar represents 100 μ m. Images come from (Gamarallage *et al.*, 2018).

5.2.3 Quantitative T2 map processing

The processing of the quantitative T2 maps is described in chapters 3 and 4 and summarized in Fig. 5.1. Briefly, FSE data is acquired at six echo times (Barish *et al.*, 1995). The signal is then fit using the extended phase graph signal model (Hennig *et al.*, 2004; Akhondi-Asl *et al.*, 2014) to derive a T2 value in each pixel. Finally, our kinetic tensor (KT) model is used to simulate and correct for the influence of formalin on T2 (Shepherd *et al.*, 2009; Qi *et al.*, 2016).

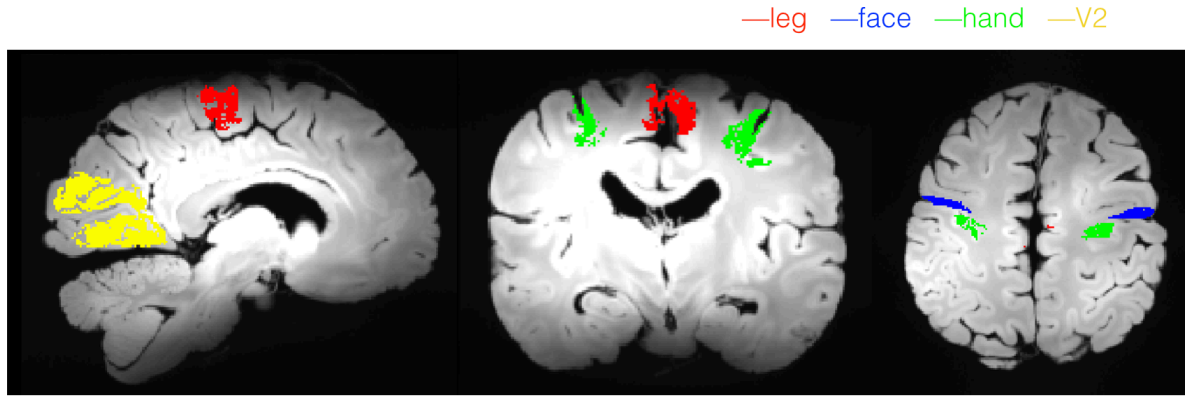


Figure 5.4: Masks of cortical regions, including left and right motor cortex leg (red), face (blue), hand (green) areas and V2 (yellow).

A trained anatomist created masks for the motor cortices (hand, leg, and face areas), secondary visual cortex (V2), and anterior cingulate cortex (ACC), in both hemispheres (example in Fig 5.4). Quantitative T2 values are extracted for each region of interest in every brain and compared between ALS and controls using a two-sample t-test with unequal variance. Based on previous literature, we predict that our data will indicate a reduced T2 in the motor cortex in ALS (Ishikawa *et al.*, 1993; Oba and Araki, 1993) which we statistically evaluated using one-tailed statistics (Bakan, 1966). To perform the analysis in individual regions of the motor cortex, we pooled the left and right hemispheres in each region of the leg, hand, and face in the motor cortex and tested for group differences in ALS vs control for each region separately. To perform a group difference test over the whole motor cortex, we pooled all 3 regions and 2 hemispheres to produce 6 data points for each brain.

5.2.4 Histological sampling

Regions of interest in the post mortem brain were paraffin-embedded, stained for immunohistochemistry, and digitally photographed for quantitative analysis. In this study, we only consider ferritin (Fig. 5.3) and PLP stains, but the broader study includes proteinopathy (TDP-43), glial properties (GFAP, Iba1, CD68) and neurofilaments (SMI-311 and SMI-312).

A region of interest is hand-drawn by an expert that represents the cortical area of interest in each slide. Quantitative digital image analysis was performed using the Aperio color deconvolution algorithm (version 9.1, Leica Biosystems) to separate a given stain from the non-specific background. The images are then thresholded using a consistent manual threshold determined by an expert user, and the stained area fraction within the region-of-interest is used as a quantitative estimate of the given stain. At present, the anterior cingulate, V2 and the leg area of the motor cortex in the left hemisphere have undergone both ferritin and PLP staining. These regions will form the basis of our comparison between quantitative T_2 estimates and histology.

5.2.5 Cohort information

Thirteen ALS (Age = 64 ± 13 years, PMI = 3.1 ± 1.7 days, SI = 134 ± 72 days) and three age-matched control brains (Age = 65 ± 7.5 years, PMI = 3 ± 0 days, SI = 69 ± 40 days) were recruited for this study (Table 5.1). They were fixed with either 10% formalin or neutral buffered formalin (NBF), with one brain fixed with 20% NBF.

ID	Age	Sex	Post Mortem Interval (days)	Scan Interval (days)	Fixative	Site of Onset
MND12	60	F	4	173	10%Formalin	N/A
MND13	77	F	3	114	10%Formalin	Right upper limb
MND14	27	M	N/A	228	20%NBF	N/A
MND15	66	M	7	137	10%Formalin	Right lower limb
MND16	65	M	4	94	10%Formalin	Right upper limb
MND17	76	M	3	158	10%Formalin	Right upper limb
MND18	68	M	1	35	10%NBF	Left lower limb
MND19	54	M	N/A	77	N/A	N/A
MND20	65	M	N/A	96	10%Formalin	N/A
MND21	68	M	2	87	10%NBF	N/A
MND22	64	M	2	283	10%Formalin	N/A
MND23	71	M	3	N/A	10%NBF	N/A
MND24	79	F	2	N/A	10%NBF	Bulbar
ALS Mean(SD)	64(13)	-	3.1(1.7)	134(72)	-	-
CTL10	61	M	3	115	10%NBF	N/A
CTL11	74	M	3	45	10%NBF	N/A
CTL12	61	M	3	48	10%NBF	N/A
CTL Mean(SD)	65(7.5)	-	3(0)	69(40)	-	-

Table 5.1: Details of the postmortem brains used within this study. Here, SD is the Standard deviation, CTL represents the control group, while MND represents the ALS group (where MND stands for motor neuron disease); N/A indicates that data is unavailable; M is for male and F is for female; NBF represents neutral buffered formalin.

5.3 Results and Discussions

5.3.1 Histological Data

	Ferritin stained area fraction		
ROI	Anterior cingulate	Motor cortex - Leg area	Secondary Visual cortex
Block number	8a	3Lb	5La
MND12	0.529	0.728	0.701
MND13	0.732	0.804	0.856
MND14	0.283	0.580	0.636
MND15	0.388	0.699	0.558
MND16	0.450	0.710	0.550
MND17	0.376	0.556	0.530
MND18	0.369	0.715	0.709
MND19	0.328	0.504	0.667
MND20	0.296	0.841	0.489
MND21	0.529	0.692	0.438
MND22	0.671	0.761	0.665
MND23	0.309	0.514	0.731
MND24	0.316	0.511	0.305
ALS mean(SD)	0.43(0.15)	0.66(0.12)	0.60(0.14)
CTL10	0.545	0.538	0.522
CTL11	0.191	0.446	0.436
CTL12	0.706	0.631	0.491
CTL mean(SD)	0.48(0.26)	0.54(0.09)	0.48(0.04)
P-value	0.320	0.054	0.093

Table 5.2: Ferritin staining statistics in the Anterior cingulate, motor leg area, and secondary visual cortex of the ALS and control brains. All the data relates to the region of interest in the left hemisphere. Here, SD is the Standard deviation, CTL represents the control group, while MND represents the ALS group (where MND stands for motor neuron disease).

The results for ferritin quantification are given in Table 5.2. In general, the ferritin content in ALS brains ($\text{Fe}^{\text{ACC}}_{\text{ALS}} = 0.43 \pm 0.15$; $\text{Fe}^{\text{M1}}_{\text{ALS}} = 0.66 \pm 0.12$; $\text{Fe}^{\text{V2}}_{\text{ALS}} = 0.60 \pm 0.14$) is higher than the control brains ($\text{Fe}^{\text{ACC}}_{\text{CTL}} = 0.48 \pm 0.26$; $\text{Fe}^{\text{M1}}_{\text{CTL}} = 0.54 \pm 0.09$; $\text{Fe}^{\text{V2}}_{\text{CTL}} = 0.48 \pm 0.04$), which is consistent with the discovery of iron deposition in these areas (Sadrzadeh and Saffari,

2004; Langkammer *et al.*, 2010; J. Yu *et al.*, 2014). Although there is no significant difference between ALS and controls in terms of ferritin fraction, the motor cortex (leg area) gives the best group difference indicator.

	Myelin proteolipid protein (PLP)		
ROI	Anterior cingulate	Motor cortex - Leg area	Secondary Visual cortex
Block number	8a	3Lb	5La
MND12	0.27	0.60	0.58
MND13	0.22	0.65	0.48
MND14	0.18	0.57	0.50
MND15	0.23	0.45	0.56
MND16	0.26	0.54	0.44
MND17	0.18	0.52	0.58
MND18	0.09	0.37	0.41
MND19	0.17	0.43	0.53
MND20	0.25	0.50	0.39
MND21	0.16	0.43	0.42
MND22	0.14	0.52	0.38
MND23	0.70	0.59	0.42
MND24	0.14	0.57	0.54
ALS mean(SD)	0.23(0.15)	0.52(0.08)	0.48(0.07)
CTL10	0.14	0.34	0.50
CTL11	0.20	0.37	0.49
CTL12	0.15	0.25	0.50
CTL mean(SD)	0.16(0.03)	0.32(0.06)	0.50(0.03)
P-value	0.084	0.006	0.360

Table 5.3: PLP statistics in the Anterior cingulate, motor leg area, and secondary visual cortex of ALS and control brains. All the data relates region of interest in the left hemisphere. Here, SD is the Standard deviation, CTL represents the control group, while MND represents the ALS group (where MND stands for motor neuron disease).

The results for PLP quantification are given in Table 5.3. Similar to the ferritin staining results, the motor cortex (leg area) gives the best group difference indicator, which is able to differentiate between the ALS and control brains ($P = 0.006$).

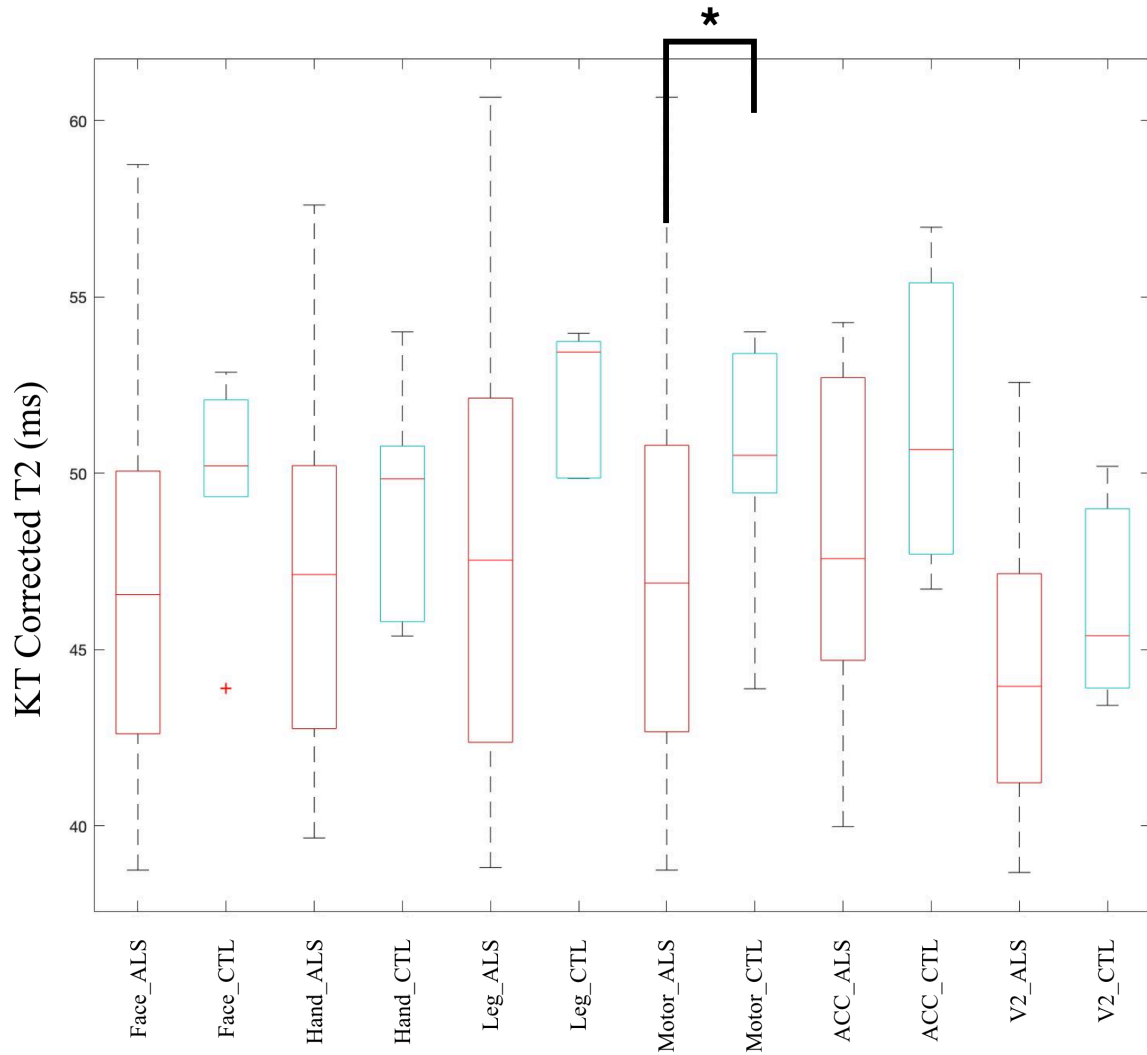


Figure 5.5: Quantitative T2 comparison between the ALS and CTL groups with a formalin correction using the kinetic tensor model. The analysis was performed in the face, hand and leg regions separately, in addition to the motor cortex (pooled across all three regions), ACC and V2 regions, obtaining P-values of 0.147, 0.295, 0.083, 0.011, 0.311, and 0.547 respectively. Red represents ALS while cyan represents the control group. Note, * indicates a significant P-value < 0.05 , and the red bar indicates the median value.

T2 (ms)	Regions of Interest			
	Face	Hand	Leg	Motor
ALS	46.42 (5.22)	46.97 (5.00)	47.71 (6.22)	47.03 (5.46)
CTL	49.76 (3.19)	49.27 (3.25)	52.38 (1.96)	50.47 (3.04)
P-value	0.147	0.295	0.083	0.011

Table 5.4: Quantitative T2 comparison between the ALS and control groups with formalin correction by the kinetic tensor model. Analysis was performed in the face, hand and leg regions separately, in addition to the motor cortex (pooled across all three regions), Note that the data is presented in terms of mean (SD).

5.3.2 Quantitative T2 statistics

Fig. 5.5 and Table 5.4 show the quantitative T2 estimates for the three ROIs in the motor cortex for both the control and ALS groups. We observe that the T2 values in all the motor cortex regions in the ALS cohort are shorter than controls ($T2^{\text{CTL_face}} = 49.76$ ms vs $T2^{\text{ALS_face}} = 46.42$ ms; $T2^{\text{CTL_hand}} = 49.27$ ms vs $T2^{\text{ALS_hand}} = 46.97$ ms; $T2^{\text{CTL_Leg}} = 52.38$ ms vs $T2^{\text{ALS_leg}} = 47.71$ ms) but do not reach the significance threshold of 0.05. We hypothesize that a lack of significance in individual regions reflects the heterogeneity of ALS pathology, which will be further discussed in the following section. If we pool all three regions to form the entire motor cortex, quantitative T2 results reveal a significant difference between the ALS and control cohort ($T2^{\text{CTL_Motor}} = 50.47$ vs $T2^{\text{ALS_Motor}} = 47.03$, $P^{\text{Motor}} = 0.011$).

5.3.3 Ferritin and PLP versus T2 measures

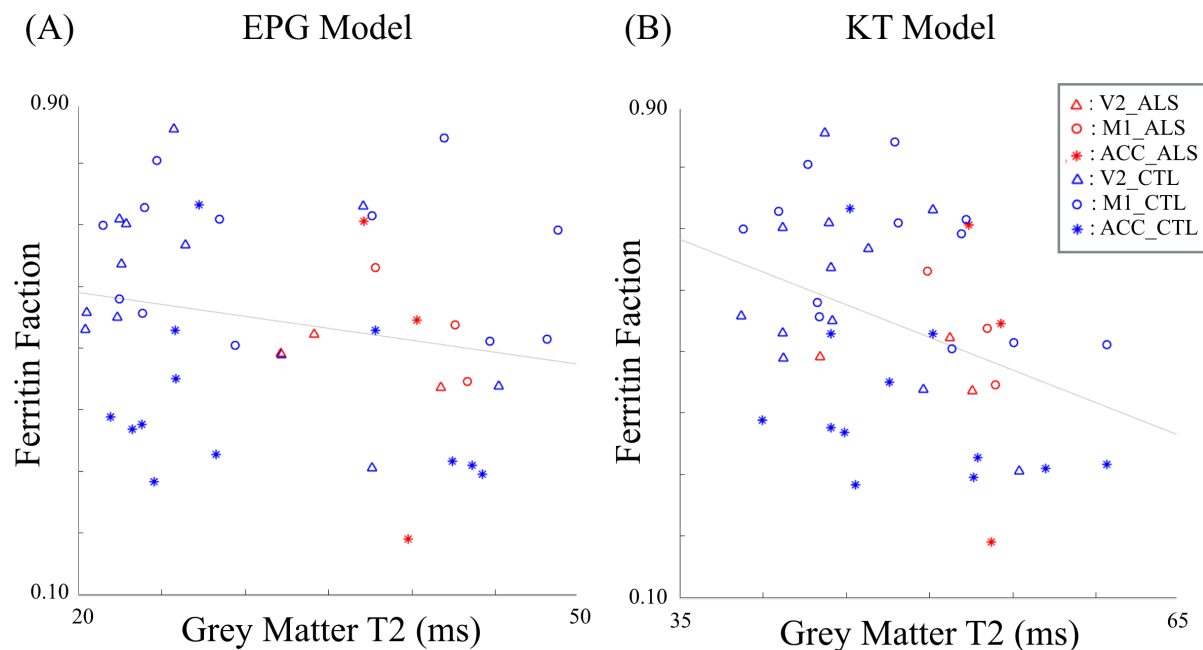


Figure 5.6: The correlation between T2 and ferritin fraction in the V2, motor cortex leg and ACC region. (A) and (B) demonstrate the comparison between the EPG model ($R = -0.21$, $P = 0.16$) and KT model ($R = -0.37$, $P = 0.01$). Red represents control while blue represents ALS brains; triangles represent the V2 region, circles represent the leg region, and stars represent the ACC. Linear regression was used to fit the relationship between the two measures.

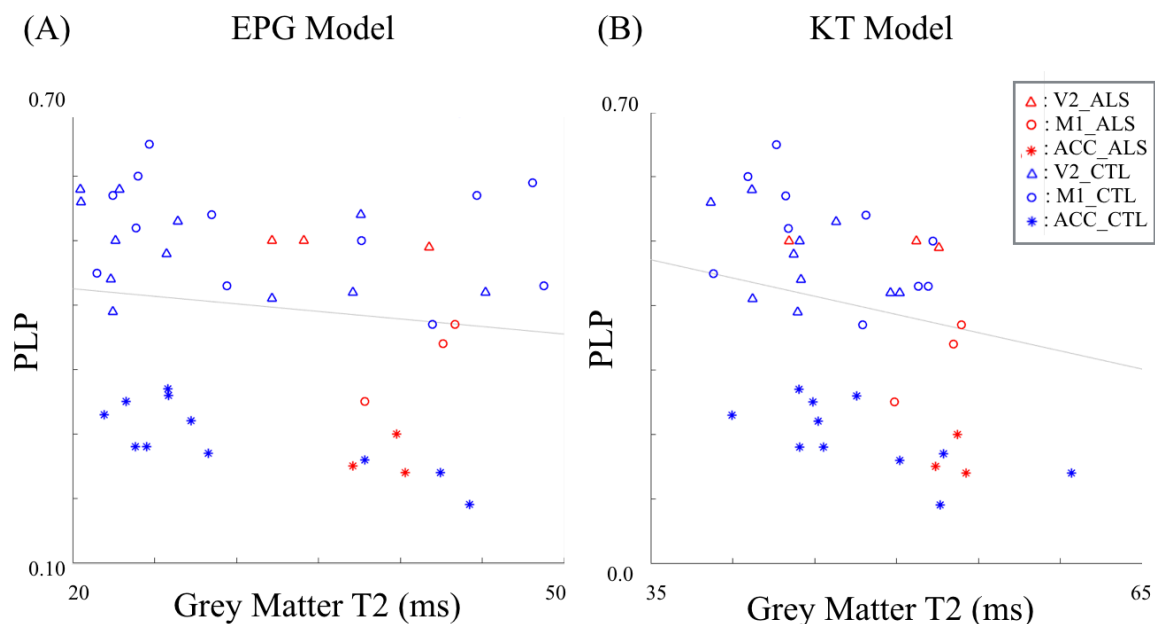


Figure 5.7: The correlation between T2 and PLP fraction in the V2, leg and ACC regions. (A) and (B) demonstrate the comparison between EPG model ($R = -0.07$, $P = 0.69$) and KT model ($R = -0.12$, $P = 0.45$). Red represents control while blue represents ALS brains; triangles represents the V2 region, circles represent the leg regions, and stars represent the ACC. Linear regression was used to fit the relationship between the two measures.

Figure 5.6 displays the relationship between the ferritin fraction and T2 over the regions of interest with and without the KT model correction. Here we observe that the quantitative T2 estimates from EPG fitting without the KT correction do not strongly correlate with ferritin fraction ($R = -0.21$, $P = 0.16$), whereas after correction with the kinetic tensor model, the correlation is considerably improved ($R = -0.37$, $P = 0.01$). Furthermore, if we only consider the motor cortex for our correlation analysis, the linear relationship is estimated as $R = -0.53$, $P = 0.04$ with the KT correction, whereas for the EPG model only, $R = -0.27$, $P = 0.33$. Figure 5.7 demonstrates that the quantitative T2 derived from both EPG and KT models has a negative correlation with PLP content ($R_{\text{EPG}} = -0.07$ and $R_{\text{KT}} = -0.12$, respectively). However, both show no significance ($P_{\text{EPG}} = 0.69$ and $P_{\text{KT}} = 0.45$).

	Result of GLM analysis			
	Beta	SE	t	P-value
Constant	55.86	3.02	18.52	8.11E-22
PLP	-3.16	5.14	-0.61	0.54
Ferritin	-11.89	5.35	-2.22	0.03

Table 5.5: GLM analysis of T2 with PLP and Ferritin (pooled M1, ACC and V2 regions). Where SE is the standard error, and t is the t-statistics.

To analyze the relative contributions of iron and myelin to T2, we constructed a generalized linear model (GLM):

$$\mathbf{T2} = \beta_0 + \beta_1 \cdot \mathbf{Ferritin} + \beta_2 \cdot \mathbf{PLP} \quad (5.1)$$

The results are given in Table 5.5. Both PLP ($\beta_2 = -3.16$) and ferritin ($\beta_1 = -11.89$) negatively correlate with T2 values. However, the correlation between PLP and T2 does not reach the significant threshold ($P^{\text{PLP}} = 0.54$) whereas the correlation between Ferritin and T2 is significant ($P^{\text{Fe}} = 0.03$).

5.4 Discussion

The details of iron deposition as measured by ferritin staining (Table 5.2) do not appear to have a simple relationship to disease stage associated with each region, with M1 expected to have the most severe pathology and V2 the least severe. Both motor regions and V2 have more iron deposition in ALS compared to controls, whilst the ACC has lower. The PLP statistics (Table 5.3) appear to be a more effective histopathological indicator to differentiate ALS from controls, in particular, the PLP in the motor cortex (leg region) where $P=0.006$. Note that, since we have only 3 control post-mortem brains, the statistical tests are likely underpowered. These results are in ongoing discussion with colleagues in neuropathology.

In addition to contributing to the literature on ALS, the results presented here suggest that the KT model presented in Chapter 4 can provide a critical correction to quantitative T2 maps acquired in the post-mortem brain. The results in Fig 5.5 are notably different from the same tests conducted for the EPG fitting on its own (in Fig. 3.21), where we found no statistically significant group differences. After the KT correction, we found significant differences of T2 between patients and controls across the pooled M1 regions ($P\text{-value} = 0.01$). Furthermore, the KT correction has increased the correlation of T2 with a measure of iron content, which is known to reduce T2. This suggests that the KT correction is removing confounding variance in T2 quantification. A small improvement is additionally observed with the PLP results, though the results indicate that T2 measurements have a stronger and more significant negative correlation with Ferritin ($R = -0.37$, $P = 0.01$) than with PLP ($R = -0.12$, $P = 0.45$).

The high variance across brains in measurements of Ferritin, PLP, and quantitative T2 demonstrates the heterogeneous nature of ALS (Beghi *et al.*, 2007), namely that symptomatic

presentation in ALS patients can vary between individuals and ALS pathology can reside in different cortical or subcortical regions and affect different motor behaviors. Since detailed clinical information of each brain is not available, by pooling specific regions (face, hand and leg regions) in both the left and right hemispheres, we are able to enhance the differentiation capacity of the quantitative T2 measures (Last two bars of Fig. 5.5). With this, we observe that T2 measurements in the motor cortex can significantly differentiate ALS from controls ($P^{\text{Motor}} = 0.01$) in our postmortem brain samples.

The GLM results are consistent with the separate correlation tests, suggesting that ferritin is the primary driver of variance in T2 in our data. However, it was useful to include this additional analysis in case a more subtle relationship with PLP could be identified when variance due to ferritin was simultaneously being taken into account, as is the case in this GLM.

5.5 Conclusions

In this chapter, we presented a correlation study between quantitative T2 and histological stains for myelin and iron in a cohort of ALS and control brains. We found that the T2 of the motor cortex differed between ALS and controls, a result that relied on the use of the KT correction developed in Chapter 4. T2 had a significant negative correlation with ferritin content but not PLP, suggesting that the primary driver of these T2 differences is iron deposition.

5.6 Reference

- Akhondi-Asl, A., Afacan, O., Mulkern, R. V., & Warfield, S. K. (2014, September). T 2-Relaxometry for Myelin Water Fraction Extraction Using Wald Distribution and Extended Phase Graph. In *International Conference on Medical Image Computing and Computer-Assisted Intervention* (pp. 145-152). Springer, Cham.
- Al-Chalabi, A., Hardiman, O., Kiernan, M. C., Chiò, A., Rix-Brooks, B., & van den Berg, L. H. (2016). Amyotrophic lateral sclerosis: moving towards a new classification system. *The Lancet Neurology*, 15(11), 1182-1194.
- Assaf, Y., Beit-Yannai, E., Shohami, E., Berman, E., & Cohen, Y. (1997). Diffusion-and T2-weighted MRI of closed-head injury in rats: a time course study and correlation with histology. *Magnetic Resonance Imaging*, 15(1), 77-85.
- Bakan, D. (1966). The test of significance in psychological research. *Psychological Bulletin*, 66(6), 423.
- Barish, M. A., Yucel, E. K., Soto, J. A., Chuttani, R., & Ferrucci, J. T. (1995). MR cholangiopancreatography: efficacy of three-dimensional turbo spin-echo technique. *AJR. American journal of roentgenology*, 165(2), 295-300.
- Beghi, E., Mennini, T., Bendotti, C., Bigini, P., Logroscino, G., Chio, A., ... & Al-Chalabi, A. (2007). The heterogeneity of amyotrophic lateral sclerosis: a possible explanation of treatment failure. *Current Medicinal Chemistry*, 14(30), 3185-3200.
- Braak, H., Brettschneider, J., Ludolph, A. C., Lee, V. M., Trojanowski, J. Q., & Del Tredici, K. (2013). Amyotrophic lateral sclerosis—a model of corticofugal axonal spread. *Nature Reviews Neurology*, 9(12), 708.
- Brettschneider, J., Del Tredici, K., Toledo, J. B., Robinson, J. L., Irwin, D. J., Grossman, M., ... & Kwong, L. (2013). Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Annals of Neurology*, 74(1), 20-38.
- Brujin, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., ... & Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science*, 281(5384), 1851-1854.
- van der Burgh, H. K., Schmidt, R., Westeneng, H. J., de Reus, M. A., van den Berg, L. H., & van den Heuvel, M. P. (2017). Deep learning predictions of survival based on MRI in amyotrophic lateral sclerosis. *NeuroImage: Clinical*, 13, 361-369.
- Carlesi, C., Pasquali, L., Piazza, S., Gerfo, A. L., Ienco, E. C., Alessi, R., ... & Siciliano, G. (2011). Strategies for clinical approach to neurodegeneration in amyotrophic lateral sclerosis. *Archives Italiennes de Biologie*, 149(1), 151-167.

Cykowski, M. D., Powell, S. Z., Peterson, L. E., Appel, J. W., Rivera, A. L., Takei, H., ... & Appel, S. H. (2017). Clinical significance of TDP-43 neuropathology in amyotrophic lateral sclerosis. *Journal of Neuropathology & Experimental Neurology*, 76(5), 402-413.

Cruz, M. P. (2018). Edaravone (Radicava): a novel neuroprotective agent for the treatment of amyotrophic lateral sclerosis. *Pharmacy and Therapeutics*, 43(1), 25.

Dimos, J. T., Rodolfa, K. T., Niakan, K. K., Weisenthal, L. M., Mitsumoto, H., Chung, W., ... & Wichterle, H. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*, 321(5893), 1218-1221.

Douaud, G., Filippini, N., Knight, S., Talbot, K., & Turner, M. R. (2011). Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis. *Brain*, 134(12), 3470-3479.

Fabes, J., Matthews, L., Filippini, N., Talbot, K., Jenkinson, M., & Turner, M. R. (2017). Quantitative FLAIR MRI in amyotrophic lateral sclerosis. *Academic Radiology*, 24(10), 1187-1194.

Foerster, B. R., Callaghan, B. C., Petrou, M., Edden, R. A. E., Chenevert, T. L., & Feldman, E. L. (2012). Decreased motor cortex γ -aminobutyric acid in amyotrophic lateral sclerosis. *Neurology*, 78(20), 1596-1600.

Foerster, B. R., Welsh, R. C., & Feldman, E. L. (2013). 25 years of neuroimaging in amyotrophic lateral sclerosis. *Nature Reviews Neurology*, 9(9), 513.

Foxley, S., Jbabdi, S., Clare, S., Lam, W., Ansorge, O., Douaud, G., & Miller, K. (2014). Improving diffusion-weighted imaging of post-mortem human brains: SSFP at 7 T. *Neuroimage*, 102, 579-589.

Hatanpaa, K. J., Burma, S., Zhao, D., & Habib, A. A. (2010). Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia*, 12(9), 675-684.

Heath, F., Hurley, S. A., Johansen-Berg, H., & Sampaio-Baptista, C. (2018). Advances in noninvasive myelin imaging. *Developmental Neurobiology*, 78(2), 136-151.

Hennig, J., Weigel, M., & Scheffler, K. (2004). Calculation of flip angles for echo trains with predefined amplitudes with the extended phase graph (EPG) - algorithm: principles and applications to hyperecho and TRAPS sequences. *Magnetic Resonance in Medicine*, 51(1), 68-80.

Howat, W. J., & Wilson, B. A. (2014). Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods*, 70(1), 12-19.

Ishikawa, K., Nagura, H., Yokota, T., & Yamanouchi, H. (1993). Signal loss in the motor cortex on magnetic resonance images in amyotrophic lateral sclerosis. *Annals of Neurology*, 33(2), 218-222.

Jucker, M., & Walker, L. C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), 45.

Kamelgarn, M., Chen, J., Kuang, L., Jin, H., Kasarskis, E. J., & Zhu, H. (2018). ALS mutations of FUS suppress protein translation and disrupt the regulation of nonsense-mediated decay. *Proceedings of the National Academy of Sciences*, 115(51), E11904-E11913.

Kiernan, J. A. (2000). Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do. *Microscopy Today*, 8(1), 8-13.

Kiernan, M. C., Vucic, S., Cheah, B. C., Turner, M. R., Eisen, A., Hardiman, O., ... & Zoing, M. C. (2011). Amyotrophic lateral sclerosis. *The Lancet*, 377(9769), 942-955.

Langkammer, C., Enzinger, C., Quasthoff, S., Grafenauer, P., Soellinger, M., Fazekas, F., & Ropele, S. (2010). Mapping of iron deposition in conjunction with assessment of nerve fiber tract integrity in amyotrophic lateral sclerosis. *Journal of Magnetic Resonance Imaging*, 31(6), 1339-1345.

Li, G., Esiri, M. M., Ansorge, O., & DeLuca, G. C. (2012). Concurrent multiple sclerosis and amyotrophic lateral sclerosis: where inflammation and neurodegeneration meet?. *Journal of Neuroinflammation*, 9(1), 20.

Mackenzie, I. R., & Rademakers, R. (2008). The role of TDP-43 in amyotrophic lateral sclerosis and frontotemporal dementia. *Current Opinion in Neurology*, 21(6), 693.

Massignan, T., Casoni, F., Basso, M., Stefanazzi, P., Biasini, E., Tortarolo, M., ... & Bonetto, V. (2007). Proteomic analysis of spinal cord of presymptomatic amyotrophic lateral sclerosis G93A SOD1 mouse. *Biochemical and Biophysical Research Communications*, 353(3), 719-725.

Miller, K. L., McNab, J. A., Jbabdi, S., & Douaud, G. (2012). Diffusion tractography of post-mortem human brains: optimization and comparison of spin echo and steady-state free precession techniques. *Neuroimage*, 59(3), 2284-2297.

Miller, K. L., Stagg, C. J., Douaud, G., Jbabdi, S., Smith, S. M., Behrens, T. E., ... & Jenkinson, N. (2011). Diffusion imaging of whole, post-mortem human brains on a clinical MRI scanner. *Neuroimage*, 57(1), 167-181.

Miller, R. G., Mitchell, J. D., Lyon, M., & Moore, D. H. (2003). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Amyotrophic lateral sclerosis and other motor neuron disorders: official publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases*, 4(3), 191-206.

Oba, H., Araki, T., Ohtomo, K., Monzawa, S., Uchiyama, G., Koizumi, K., ... & Kobayashi, M. (1993). Amyotrophic lateral sclerosis: T2 shortening in motor cortex at MR imaging. *Radiology*, 189(3), 843-846.

Paez-Colasante, X., Figueroa-Romero, C., Sakowski, S. A., Goutman, S. A., & Feldman, E. L. (2015). Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era. *Nature Reviews Neurology*, 11(5), 266.

Pallebage-Gamarallage, M., Foxley, S., Menke, R. A., Huszar, I. N., Jenkinson, M., Tendler, B. C., ... & Ansorge, O. (2018). Dissecting the pathobiology of altered MRI signal in amyotrophic lateral sclerosis: A post mortem whole brain sampling strategy for the integration of ultra-high-field MRI and quantitative neuropathology. *BMC Neuroscience*, 19(1), 11.

Qi F, Foxley S, Thomas A....& Miller K. (2016). Whole-brain mapping of QMRI parameters in post-mortem brains: towards histological validation in MND. *OHBM2016*, 2: 2–6.

Qi F, Hurley S, Gamarallage MP, Ansorge O, Turner MR...& Miller K. (2016). A kinetic tensor approach to correcting for fixation effects on post-mortem T2 measurements. *ISMRM 2016*; 1: 1–6.

Rakhit, R., Robertson, J., Velde, C. V., Horne, P., Ruth, D. M., Griffin, J., ... & Chakrabartty, A. (2007). An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. *Nature Medicine*, 13(6), 754.

Ravits, J., Appel, S., Baloh, R. H., Barohn, R., Rix Brooks, B., Elman, L., ... & McCluskey, L. (2013). Deciphering amyotrophic lateral sclerosis: what phenotype, neuropathology and genetics are telling us about pathogenesis. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 14(sup1), 5-18.

Ropele, S., De Graaf, W., Khalil, M., Wattjes, M. P., Langkammer, C., Rocca, M. A., ... & Fazekas, F. (2011). MRI assessment of iron deposition in multiple sclerosis. *Journal of Magnetic Resonance Imaging*, 34(1), 13-21.

Rowland, L. P., & Shneider, N. A. (2001). Amyotrophic lateral sclerosis. *New England Journal of Medicine*, 344(22), 1688-1700.

Sadrzadeh, S. H., & Saffari, Y. (2004). Iron and brain disorders. *Pathology Patterns Reviews*, 121(suppl_1), S64-S70.

Scott, H. S., Kyriakou, D. S., Peterson, P., Heino, M., Tähtinen, M., Krohn, K., ... & Antonarakis, S. E. (1998). Characterization of a novel gene, C21orf2, on human chromosome 21q22. 3 and its exclusion as the APECED gene by mutation analysis. *Genomics*, 47(1), 64-70.

Sharma, A., Lyashchenko, A. K., Lu, L., Nasrabad, S. E., Elmaleh, M., Mendelsohn, M., ... & Shneider, N. A. (2016). ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nature Communications*, 7, 10465.

Shepherd, T. M., Thelwall, P. E., Stanis, G. J., & Blackband, S. J. (2009). Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*, 62(1), 26-34.

Tan, R. H., Kril, J. J., Fatima, M., McGeachie, A., McCann, H., Shepherd, C., ... & Kiernan, M. C. (2015). TDP-43 proteinopathies: pathological identification of brain regions differentiating clinical phenotypes. *Brain*, 138(10), 3110-3122.

Turner, M. R., Hammers, A., Al-Chalabi, A., Shaw, C. E., Andersen, P. M., Brooks, D. J., & Leigh, P. N. (2005). Distinct cerebral lesions in sporadic and 'D90A'SOD1 ALS: studies with [11C] flumazenil PET. *Brain*, 128(6), 1323-1329.

Turner, M. R., Osei-Lah, A. D., Hammers, A., Al-Chalabi, A., Shaw, C. E., Andersen, P. M., ... & Mills, K. R. (2005). Abnormal cortical excitability in sporadic but not homozygous D90A SOD1 ALS. *Journal of Neurology, Neurosurgery & Psychiatry*, 76(9), 1279-1285.

Vucic, S., & Kiernan, M. C. (2006). Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain*, 129(9), 2436-2446.

Wang, X., De Schepper, A., Vanhoenacker, F., De Raeve, H., Gielen, J., Aparisi, F., ... & Somville, J. (2002). Nodular fasciitis: correlation of MRI findings and histopathology. *Skeletal Radiology*, 31(3), 155-161.

Yen, A. A., Simpson, E. P., Henkel, J. S., Beers, D. R., & Appel, S. H. (2004). HFE mutations are not strongly associated with sporadic ALS. *Neurology*, 62(9), 1611-1612.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., ... & Anand, P. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurology*, 6(1), 12.

Yu, J., Qi, F., Wang, N., Gao, P., Dai, S., Lu, Y., ... & Che, F. (2014). Increased iron level in motor cortex of amyotrophic lateral sclerosis patients: an in vivo MR study. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 15(5-6), 357-361.

Chapter 6

Summary and Future Work

Contents

6.1 Thesis Summary.....	141
6.2 Future Works	143
6.2.1 Quantitative T1 map.....	143
6.2.2 Computation efficiency.....	145
6.2.3 EPG fitting with AFI B1.....	147
6.3 Conclusion	148
6.4 Reference.....	148

6.1 Thesis Summary

This thesis aimed to provide a T2 mapping protocol in post-mortem tissue that can be directly compared to gold-standard histopathological staining in neurological diseases, in particular ALS. Data of the kind presented here provide a critical link between non-invasive MRI scans that can be conducted in vivo and biologically-interpretable but highly invasive neuropathology.

To achieve these goals, we acquired multi-echo FSE data for the quantification of T2 at 7T. These measurements are confounded by inhomogeneous B1 field across the scanned region of interest, resulting in signal evolution that does not behave according to traditional mono-exponential decay. We address this with a proposed EPG fitting method that does not assume the B1 field is spatially homogeneous but takes B1 as a voxel-wise variable to generate the theoretical echo signals. Specifically, we implemented a two-step fitting process, first

calculating a B1 field alongside T2 and proton density, and then fixing a smoothed version of this B1 field to calculate tissue T2 and proton density. The proposed method not only removes visible artefacts in the T2 maps derived from the mono-exponential inversion method but also simultaneously produces a B1 (or flip angle) map of the RF coil.

In addition, quantitative T2 is altered by the brain fixation process. Fixative formaldehyde molecules reduce the tissue transverse relaxation T2, overwhelming changes due to pathology. To correct for fixative concentration, we compare several potential models that each aim to predict formalin distribution. This includes a heuristic method of distance-to-surface model, alongside models that simulate formalin dynamics. The latter models can both simulate the influx of formalin during fixation, and the outflux of formalin when brains are being prepared for scanning. Similarly, brains can be simulated as a solid mass with only an exterior surface (the “no hole” models) or with a ventricular space that communicates with the fluid the brain is immersed in (the “hole” models). To further improve the accuracy of the formalin distribution simulation, we introduced the kinetic tensor model, that incorporates the DTI derived diffusion tensor matrix voxel-wise to account for the anisotropic properties of tissue. Compared with the T2 correction results derived from different models, we found that the kinetic tensor model including ventricular communication provided the best simulation of formalin dynamics as indicated by the highest T2 homogeneity within white and grey matter regions.

Using the EPG-fitting and KT model correction, we arrived at quantitative T2 maps for a cohort of ALS and control post-mortem brains. We found that the KT correction enabled us to distinguish differences in T2 in the motor cortex in ALS compared to the control group.

Comparisons against histopathology suggest that this T2 decrease is driven by iron deposition but with at most a modest contribution from myelin. These results show that the proposed T2 maps could be used as a practical measure for studying cortical pathology in ALS.

6.2 Future Work

In this section, I outline a few directions that may be fruitful to follow up on my doctoral research.

6.2.1 Quantitative T1 maps

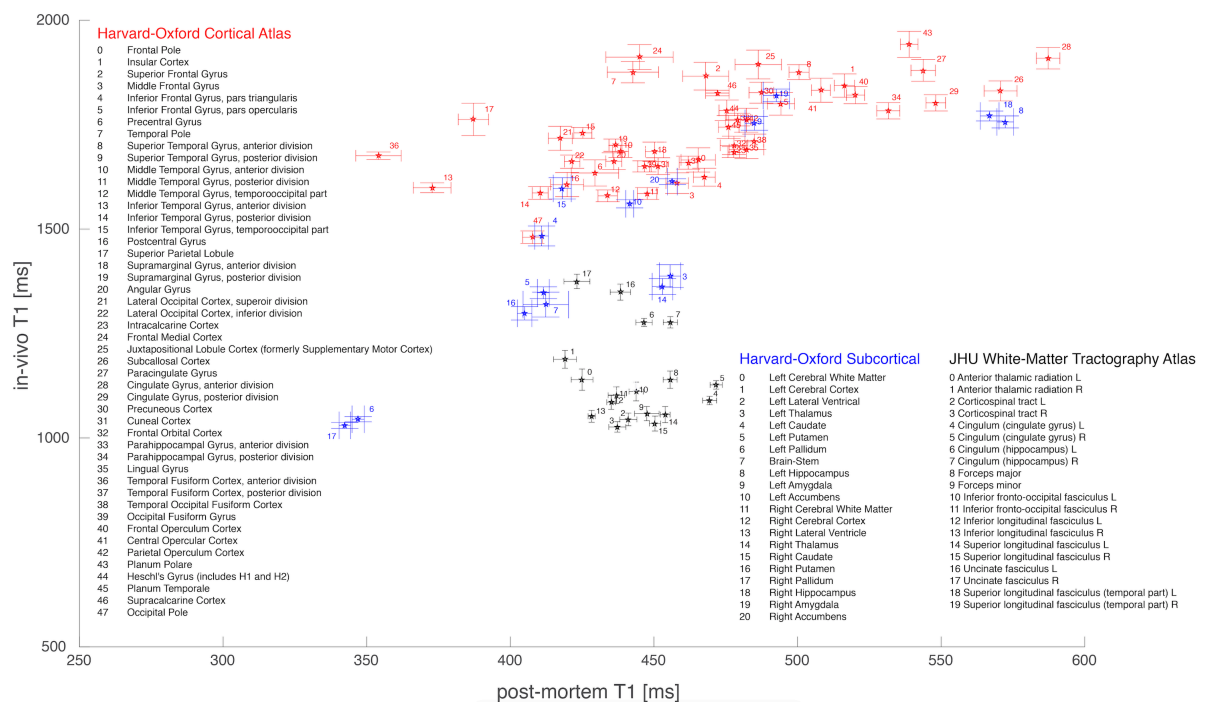


Figure 6.1: Scatter plot comparing T1 in post-mortem brain to in-vivo reference data in cortical (red), subcortical (blue) grey matter regions, and white matter tracts (black) regions. The y-axis presents the in-vivo T1 data from 59 normal subjects (age 33.7 ± 11.9 years. Thanks Professor Alex Mackay for sharing the data), serving as a control value of T1, while the x-axis is the post-mortem T1 data from our ALS brains.

This thesis builds on an existing literature on changes to quantitative T2 in the motor cortex in ALS. Other imaging markers that have been explored for ALS include T2* and QSM, as well as structural alterations as reflected in T1-weighted images. However, T1 mapping has received less attention as a potential marker in ALS.

In addition to the quantitative T2 data presented in this thesis, our post-mortem ALS protocol also acquired multi-inversion data for quantitative T1 mapping. This presents an opportunity to consider whether this might provide additional information about ALS pathology. I have made initial attempts in T1 mapping using this data, with a conventional exponential inversion recovery signal model. Figure 6.1 compares T1 data in healthy, in-vivo brains to the post-mortem ALS brains. This scatter plot provides a simple first exploration as to whether the motor cortex (red region 6) appears to be an outlier in the T1 scatter plot, indicating a deviation from the general relationship between in-vivo and ex-vivo T1, but this is not observed. As with the T2 results presented in this thesis, this could be due to the influence of formalin fixation.

Specific to T1-relevant tissue properties, fixation causes protein cross-linking that could lead to changes in T1 (Shepherd *et al.*, 2009). For T2 mapping, the relevant fixation property was found to be the concentration of fixation in tissue at the time of scanning. If T1 reflects cross-linking, we would instead hypothesize that the relevant aspect of fixation is the effective post-mortem interval (time for the fixative to reach a voxel, which can vary by weeks across the brain), which can also be modeled in the kinetic tensor framework. (Total time in fixative prior to scanning would also likely be relevant to the degree of cross linking, but our scans are conducted after months of fixation that aim to have achieved asymptotic degree of fixation.) In addition, B1 inhomogeneity could introduce spatial variability in the fitted T1, similar to the effects that required the full EPG model presented in Chapter 3. Indeed, the proposed EPG fitting method and kinetic tensor model could be leveraged to address these issues in a similar way as quantitative T2 processing.

As a simpler approach, we considered normalization of these conventional T1 maps in each brain by the average T1 value of the V2 cortex. If V2 is free of ALS pathology but does reflect whole-brain variations in T1, this could put all brains in the same scale for comparison. Fig. 6.2 illustrates the results of the normalization process on one individual brain, the average ALS post-mortem brain and the in-vivo control brains. After normalization, we find there are some significant difference of the normalized T1 in the anterior frontal pole region and the lip region of the motor cortex etc., however, these are preliminary findings that we have not had the opportunity to follow up on, but which will form the basis for future work including comparison with histopathology.

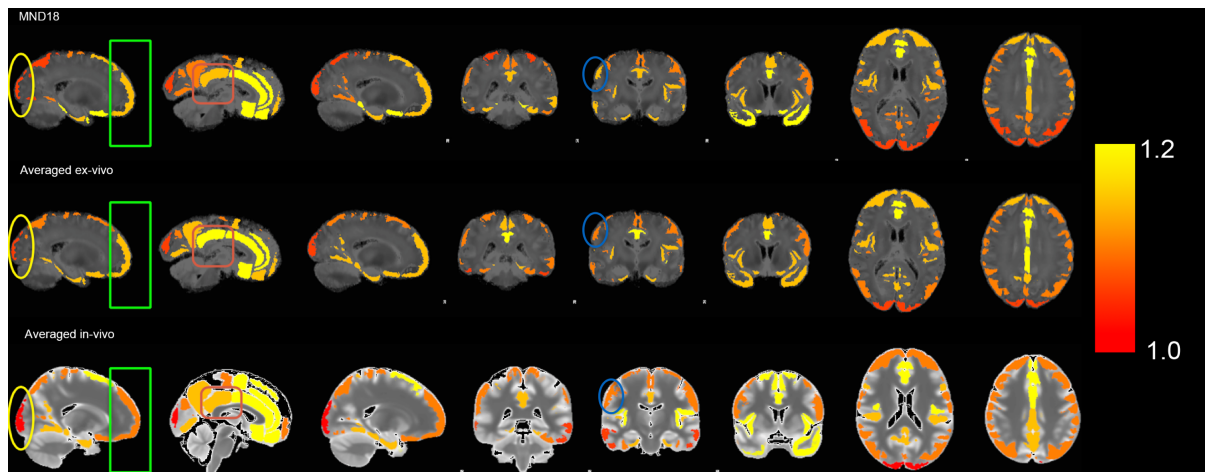


Figure 6.2: The proposed T1 normalization (by average T1 values in the visual cortex, highlighted by yellow circles) method in evaluating ALS pathology. (top) One example of the normalized T1 map for MND18. (middle) The averaged normalized T1 map of the ALS cohort brains. (bottom) The averaged normalized T1 map of the in-vivo control brains. We witness some differences between ALS and controls in anterior frontal pole (green), posterior cingulate cortex (red) and face region of the motor cortex (blue), etc. which need future investigation with colleagues in neuropathology group.

6.2.2 Computation Efficiency

Future work could aim to fit the EPG model simultaneously to multi-echo (T2) and multi-inversion (T1) data to calculate T1, T2, S0 and B1 maps together with higher precision. However, one challenge is the computation cost of the optimization process, which is high in

our implementation of the EPG fitting process. Currently, we separate the brain volume into slices, and each is computed with the EPG fitting model on one single cluster GPU in parallel. Even with this optimization, the fitting takes approximately 3 hours per brain.

Machine learning techniques, such as the deep generative neural network (GNN), can considerably reduce computation times, as is illustrated in Figure 6.3. The EPG model was used to generate a large training dataset corresponding to the FSE signals for different values of B_1 , S_0 , and T_2 . The signals are fed into the GNN, which is trained to predict the tissue parameters. After comparing the predicted parameter set with the real parameter set, we obtain the prediction error, which is used by a gradient descent algorithm to update weights and bias of the GNN to obtain better prediction performance. We built a prototype GNN with one hidden layer, and trained it with 10,000 steps. The trained GNN produced reasonable B_1 , S_0 , T_2 maps, and shortened the computation duration by 20 times. With this particular network architecture, the accuracy may only be good enough to serve as an initialization for a more accurate fitting (e.g. our Nelder-Mead fitting). Alternatively, the GNN might be sufficient to replace the first stage of fitting that is primarily used to estimate the flip angle (B_1) map. In the future, we could add more layers and units to the GNN to make it more accurate in predicting the B_1 , S_0 , T_2 maps.

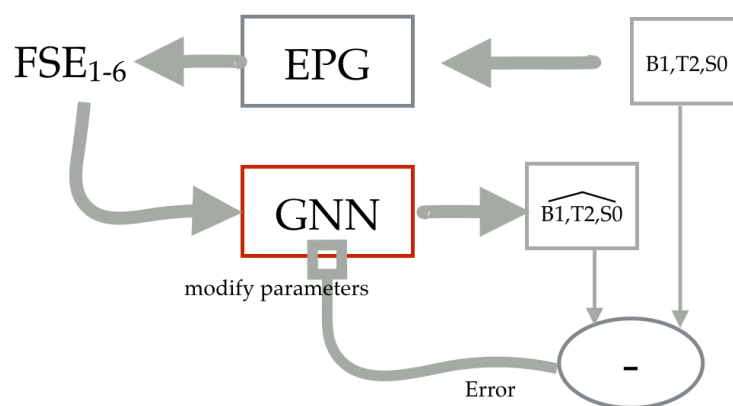


Figure 6.3: A generative neural network (GNN) can be trained to compute the T_2 map directly from the measured FSE signals. Given the parameters of B_1 , T_2 and S_0 , the EPG model can

calculate the corresponding FSE signals. These signals can be used as a training set for GNN network.

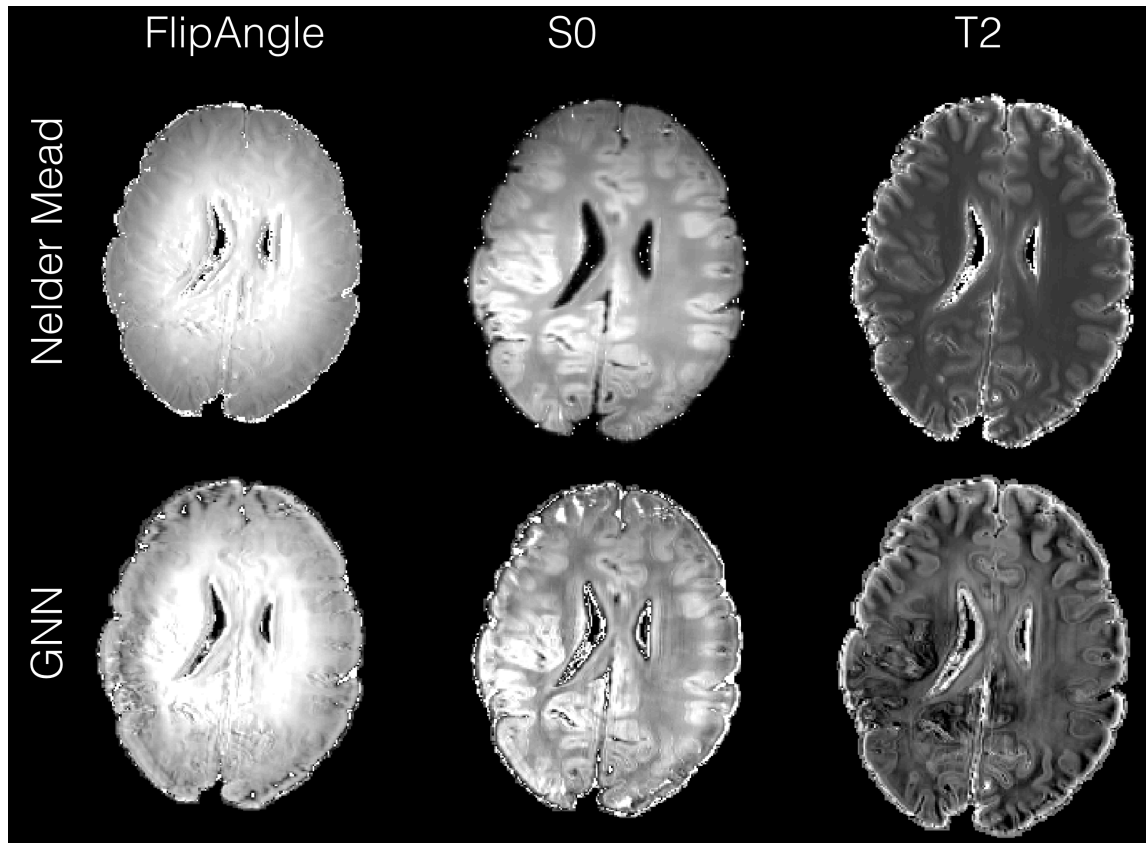


Figure 6.4: A comparison between our current Nelder-Mead fitting algorithm and a simple (one hidden layer) GNN. The GNN can provide a decent prediction on the flip angle, S0 and T2 maps with a 20-fold computational speed up. The GNN maps are unlikely to be of sufficient quality in themselves, but provide a proof of principle of this approach.

6.2.3 EPG fitting with AFI B1

After acquiring the B1 map via the AFI technique(Yarnykh, 2007), we also made an initial attempt for S0 and T2 mapping by EPG fitting with the AFI B1. From the results in Fig. 3.9, we observed that the AFI B1 map suffers from a hyperintensity band (blue arrows) and hypointensity pits (red arrows) in the frontal region, so the derived T2 and S0 contain abnormal values in the corresponding regions. To fix this issue, we propose two methods: (1) Smooth the AFI_B1 map to remove the artefacts in B1, and then calculate T2 and S0 by EPG fitting

with the smoothed AFI_B1 map; (2) Simulate the signal evolutions of both the FSE and AFI, and use Eq.6.1 to optimize the parameters,

$$[T2, S0, B1, T1] = \arg \min_{B1, T2, S0, T1} (\|Msr_{FSE} - Sim_{FSE}(B1, T2, S0, T1)\| + \lambda_1 \|Msr_{AFI} - Sim_{AFI}(B1, T2, S0, T1)\|) \quad (6.1)$$

Where *Msr* represents the measured signal from the MRI scanner, *Sim* represents the simulated signal from EPG, with subscripts AFI and FSE represent the signals related to AFI and FSE sequences. λ_1 is a weighting factor balancing between AFI and FSE.

6.3 Conclusion

In the thesis, we addressed two issues in quantitative T2 mapping of fixed post-mortem brains: (1) using the EPG-fitting method to address B1 inhomogeneity; (2) using the kinetic tensor model to remove the influence of formalin on T2 maps. These two methods can be incorporated into other MRI applications (e.g. for various imaging modalities and neurological disorders) and integrated with many advanced algorithms (e.g. Bayes, convolution network, nonlinear algorithms etc.). With these two methods, we demonstrated that quantitative T2 mapping may provide a non-invasive marker of cortical iron deposition in ALS.

6.4 Reference

Shepherd, T.M., Thelwall, P.E., Stanis, G.J. and Blackband, S.J., 2009. Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*, 62(1), pp.26-34.

Yarnykh, V. L. (2007). Actual flip - angle imaging in the pulsed steady state: a method for rapid three - dimensional mapping of the transmitted radiofrequency field. *Magnetic Resonance in Medicine*, 57(1), 192-200.