

Microstructure imaging with diffusion MRI and microscopy



Amy Howard
Linacre College
University of Oxford

A thesis submitted for the degree of
Doctor of Philosophy

Michaelmas 2020

Acknowledgements

This multi-modal multi-disciplinary thesis has relied on many friends, colleagues and collaborators, to whom I am extremely thankful for their valuable contributions.

First and foremost, I'd like to thank my supervisors, Profs. Karla Miller and Saad Jbabdi. Your curiosity is contagious, and your support has meant the world to me. To get to work with and learn from you is a great privilege. I cannot thank you enough for the countless hours, some of which were tough and confusing, but most of which were great fun. I think you both see beauty in well-written text, so I hope at least a couple of sentences here make you proud.

A huge thanks must go to Jerome Sallet and Rogier Mars for sending the BigMac brain my way and for their ongoing support for this ambitious project. Thanks to all those who have been involved in the BigMac data acquisition: to Alexandre (Sasha) Khrapitchev, for acquiring the MRI data and sticking with me through the mega long scanning session; to Adele Smart, for working tirelessly through the microscopy data collection; to Greg Daubney, for painstakingly sectioning, staining and mounting the hundreds of tissue sections. Furthermore, I would like to thank Connor Scott, Jesper Anderson, Moises Hernandez Fernandez, Paul McCarthy, Michiel Kleinnijenhuis and Menuka Pallegage Gamarallage for their advice at stages throughout this project, and Nicola Sibson for providing access to the MRI scanner.

My thanks also extend warmly to those in CUBRIC Cardiff, especially Prof Derek Jones and Mark Drakesmith, for providing the data for Chapter 3 of this thesis.

Additionally, a massive thanks to all those in the lab. FMRIB is a truly special place because of the amazing people in it. Thank you all for being so welcoming and supportive. A special shout out has to go to the PLI master Jeroen Mollink, the diffusion analysers: Michiel Cottaar and Matteo Bastiani, the registration geniuses: Istvan Huszar and Rick Lange, all the graduate course tutors, the queen of public engagement Carinne Piekema, to Andrew Segerdhal and Maz Aspbury and all those in WIN Pride, to Cristiana Tisca and the incredible WIN Women - may you keep on glowing, and to some incredible friends with pure talent, Nicola, Rick, Sophie

and Kiyana, see you at the White Hart soon.

Last but not least, I am super grateful for my wider friends and family. Thanks to my parents, Jane and David. Amongst all else, you have taught me how learn and to persist until I figure things out - skills I've come to truly value. To Rosie, my sister and absolute best friend. And to Joel, although it has already been seven years, it still feels like the beginning of an adventure. Thank you for reading this thesis, even though you hate thinking about brains. Your brilliance continues to inspire me. I can't wait to see what happens next.

This thesis was written in the summer of 2020 during the COVID pandemic. My final thanks go to all the key workers and frontline staff who are doing incredible work through these difficult times.

Abstract

Diffusion MRI is a powerful, non-invasive tool for microstructure imaging because of its sensitivity to structures on the microscopic scale. By analysing the diffusion signal we can infer the structural connectivity of the human brain or changes in the myelo- and cyto-architecture. This inference requires biophysical signal modelling, where we aim to map macroscopic diffusion signals onto biologically-meaningful tissue parameters. However, this mapping is often ill-posed, with multiple parameter sets fitting the signal equally well, and our parameters are said to be degenerate. Because degenerate parameters lack tissue specificity and are thus difficult to interpret, they present a serious challenge to diffusion modelling and limit the widespread application of diffusion MRI in both neuroscience research and clinical medicine.

This thesis explores various ways in which we can overcome parameter degeneracies to enable more specific microstructural interpretation of the diffusion signal. Consequently, this work spans species (human and macaque), tissue states (in vivo and postmortem), modalities (MRI and microscopy) and signals over four orders of magnitude, from sub-micron microscopy images to MRI at the millimetre scale. In particular, we develop two biophysical models of the white matter that aim to separate the diffusion characteristics from the tissue architecture (i.e. the fibre orientation, dispersion and density) and present a truly unique, multimodal dataset: the BigMac dataset. This dataset combines extensive MRI data with multi-contrast microscopy to characterise a single macaque brain in exquisite detail and will establish an innovative data platform from which we can interconnect microstructural features with MRI signals throughout the brain.

Overall, the models and data presented in this thesis aim to further our understanding of how microscopic tissue features influence the observed diffusion signal. We hope that this work will enhance the biophysical interpretation of diffusion MRI and ultimately advance in vivo quantification of the brain tissue microstructure.

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1

Introduction

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Viewed through a microscopist’s lens, a single cubic millimetre of brain tissue will contain $\sim 100,000$ neurons arranged in a myriad of possible configurations. Depending on the local brain function, neurons vary greatly in shape and size, or “cytoarchitecture”, and patterns of connectivity, or “myeloarchitecture”. Moreover, neurons do not operate in isolation but are interlaced into a multi-scale network; from microcircuits supporting local computation to long-range connections which span the entire brain. Critically, the local tissue structure is coupled to the computational requirements of the tissue, where functionally unique areas have their own microstructural ‘fingerprint’. In the early 1900s, the German anatomist Korbinian Brodmann used this cytoarchitecture to painstakingly subdivide the brain into 52 well-defined areas [1], producing the nomenclature which is still used for brain navigation today.

Microscopy is frequently used to study brain microstructure with $\sim$ micron or

sub-micron resolution. Typically, thin tissue sections are processed to visualise specific cellular structures. Consequently, this high ‘specificity’ approach has applications from basic neuroanatomy to disease mechanisms. Microscopy is however often limited to interrogating small, ex vivo tissue sections and thus has limited applications in vivo.

In recent years, MRI has emerged as a powerful, non-invasive tool for studying whole-brain structure and function, and relating this to cognition, behaviour, and mental health. In particular, diffusion weighted MRI is a fantastic technique that maps the microscopic motion of water molecules as they randomly move through tissue, and which can be used to infer microstructural properties of the brain in vivo. However, this inference requires complex computational signal modelling with many strong assumptions. Crucially, the measurements are averaged over millimetres of tissue and so inference on micrometre resolution processes is hard, if not ill-posed. Hence, although MRI-derived tissue parameters can be highly sensitive to changes in microstructure, they often lack specificity - multiple changes to tissue microstructure may produce the same signal changes, making interpretation challenging. A better understanding of the origins of the MRI signal would greatly advance in vivo quantification of the brain tissue microstructure, with far-reaching implications for both basic neuroscience and clinical medicine.

Through biophysical modelling of the diffusion MRI signal, we aim to extract biologically meaningful tissue parameters such as axon orientation, dispersion, density and the diffusion characteristics, all of which can be indicators of tissue integrity or pathology. Unfortunately, the diffusion signal is often rather unremarkable and the parameter fitting landscape flat [2]. As such the tissue parameters are often degenerate [3], with multiple parameter sets fitting the signal equally well.

These degeneracies represent some of the biggest challenges for the diffusion MRI modelling community. We require models which are *specific* and *sensitive* to the

tissue microstructure, but how can we determine the ‘true’ structure if multiple solutions exist?

1.1 Thesis outline

This thesis looks at three examples of how we may overcome these degeneracies and enable a more specific microstructural interpretability of MR images. It consists of a background chapter (**Chapter 2**) followed by three research chapters, a short description of which is given below.

In **Chapter 3**, we estimate the intra-axonal axial diffusivity in the presence of fibre orientation dispersion. Typically, it is difficult to estimate the fibre orientation dispersion alongside the diffusion properties of the intra- and extra-axonal space. Here, the orientation dispersion and diffusion characteristics are likely degenerate, with multiple combinations explaining the diffusion signal equally well. This study utilises data acquired in the high b-value regime - where the signal can be solely attributed to the intra-axonal compartment - to overcome the degeneracy between the diffusion characteristics of the intra- and extra-axonal space. In this high b-value regime we can then estimate the intra-axonal axial diffusivity and fibre orientation distribution on a voxelwise basis. Using a simple model of dispersed sticks and in vivo, human data, our results show the intra-axonal axial diffusivity to be higher than many previous studies, interestingly with values close to that of free water. Furthermore, our results show how the intra-axonal axial diffusivity varies considerably across the brain. This challenges the assumption of a global intra-axonal axial diffusivity as is used in many current diffusion models.

Chapter 3 tackles the degenerate parameter landscape by utilising data that is only sensitive to a specific subset of tissue parameters. Alternatively, degeneracies may be overcome through the use of additional, orthogonal information provided by other contrasts or modalities. Here the additional information may constrain

certain parameters to either restricted or fixed values. *Chapter 4: Joint modelling of diffusion MRI and microscopy* gives one example of this approach.

In a data-fusion framework, **Chapter 4**, uses orthogonal, complementary information from microscopy to overcome a known degeneracy between fibre dispersion and radial diffusion. Through a generative, joint model, MRI and microscopy data from the same tissue sample inform on the ‘true’ tissue microstructure. Here microscopy-derived fibre orientations act as a soft constraint on the fibre orientation and amount of dispersion within a voxel. This allows us to estimate both the fibre orientation dispersion and diffusion characteristics of the combined intra- and extra-axonal space (described by the ‘fibre response function’) on a voxelwise basis. Our results again show how the diffusion characteristics of the tissue vary both within and between white matter tracts. This contradicts the assumption of a single, brain-wide fibre response function as is used in many current diffusion models where, should the assumed diffusivities be inaccurate, the model outputs may be biased.

The final research chapter, **Chapter 5**, focuses on the acquisition and curation of a truly unique, multi-modal dataset, ‘The BigMac Dataset’. The dataset combines ultra-high angular resolution diffusion MRI with multi-contrast microscopy to characterise a single, whole macaque brain in exquisite detail. The dataset is truly ambitious in its scope, consisting of over 270 hours postmortem MRI scanning and over 1000 hours of microscopy data acquisition. Though few Centres have the resources to acquire data of this kind, many can benefit from its existence where, through publication, we are committed to open dissemination of the data and analysis tools relating to BigMac. In this thesis we detail the multi-faceted data acquisition and curation, and conduct some of the first analyses which demonstrate the unique information provided by this novel resource.

Chapter 6 concludes this thesis with summary remarks and suggested avenues for future research.

Contributions

Please note, my contributions to the work are indicated at the end of each chapter. My supervisors Prof. Karla Miller and Prof. Saad Jbabdi contributed to the conception and development of research ideas throughout; for their direction and endless enthusiasm I am extremely grateful.

2

Background

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This section provides background information for the content of this thesis. First, it gives a brief description of the brain structure on the microscopic and macroscopic level. Second, it describes the physics underpinning diffusion MRI acquisition and analysis. We describe the key concepts behind diffusion MRI signal formation and how, through biophysical modelling of the diffusion signal, we can relate changes in the MRI signal to specific aspects of the tissue microstructure. Finally we describe the two microscopy techniques of this thesis: the histological staining

of tissue and polarised light imaging.

2.1 Brain structure

At the microscopic level, the brain is formed from common cellular components - neurons and glia - arranged in a myriad of configurations. Neurons transmit information around the brain through a series of electrical impulses and chemical signals, whilst glia have wide-ranging function and provide both structural and metabolic support. Notably, the shape and size of the cells, or cellular morphology, is strongly coupled to their function and the broader tissue architecture is designed to support complex neural circuitry.

At the macroscopic level, the brain tissue can be broadly categorised into grey and white matter. Grey matter lines the outer layer of the brain, known as the cerebral cortex, whilst the white matter sits below. Additional grey matter structures such as the thalamus and ‘deep’ cerebral nuclei can be found at the centre of the brain. The grey matter consists predominantly of cell bodies (neurons and glia), a weaved fabric of dendritic trees and short-range intra-cortical (mostly unmyelinated) axons. In comparison the white matter contains long-range, predominantly myelinated axons. These axons span between gyri (the folds of the brain) or cross hemispheres, linking distant and often functionally specific brain areas. Glia can be found in both tissue types but are more numerous in the grey matter.

2.1.1 The neuron structure

The neuronal morphology varies hugely across the brain, with functionally specific neurons given descriptive names such as ‘pyramidal cells’, ‘basket cells’ or ‘spindle cells’. Nonetheless, the basic structure of the neuron (Figure 2.1) remains similar.

Firstly, each neuron has a soma (which contains the nucleus) from which one or more neurite extends. The term neurite is used to describe narrow processes and

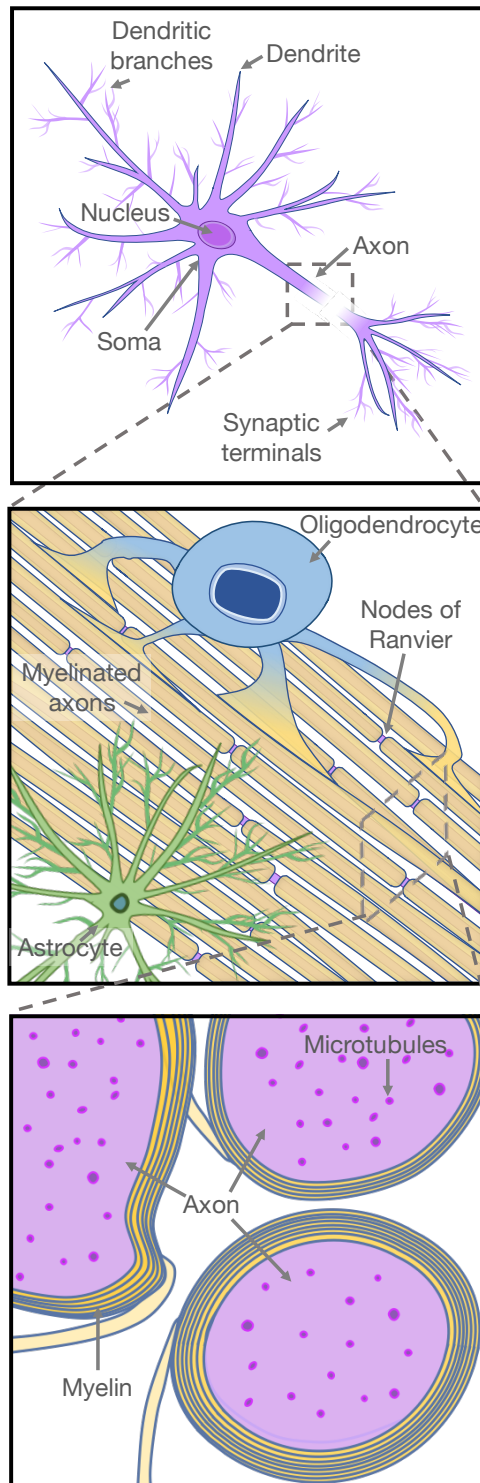


Figure 2.1: Top: A schematic representation of the neuron structure. Middle: The white matter consists of (mostly) myelinated axons and glia (microglia not pictured). Bottom: A cross section of a myelinated axon. Many thanks to Joel Smith for kindly providing this figure.

can refer to either the dendrites, through which information is received, or the axon (or nerve fibre) through which information is transmitted. The dendrites can form beautiful tree-like structures, ‘the dendritic tree’, to receive input from tens or thousands of other cells. The axon is a cable-like projection which carries nerve impulses away from the soma, towards the axon terminals and on to other neurons or an effector organ (e.g. muscles or glands) via the synapse.

The axon

The length of the axon varies substantially: from the intra-cortical short-range connections on the order of millimetres, to corticospinal neurons extending tens of centimetres, linking the cerebral cortex to the base of the spine. Axons have varying calibre (i.e cross-section) and may either appear fairly straight, or be seen to undulate across space. The axon morphology is supported by the cytoskeleton (microtubules) which is also involved in the active transport of organelles through the cytoplasm.

Axon diameter

The axonal conduction speed is closely coupled to both the axon diameter and its degree of myelination [4]. In vertebrates, the axon diameter ranges from ~ 0.1 - $10\text{ }\mu\text{m}$ where, for myelinated fibres, the conduction velocity increases approximately linearly with axon diameter [5, 6]. The corpus callosum has a low-high-low pattern of axon diameter along the anterior-posterior axis, with some of the widest axons ($> \sim 3\text{ }\mu\text{m}$) found in the mid-body of the corpus callosum [7]. Furthermore, wide axons are found in the pyramidal tract [8] where the motor system requires rapid transmission of signals over large distances. However, wide axons are expensive, both metabolically and volumetrically, where the latter limits the number of axons through a given area (i.e. the axon density). Consequently, wide-diameter axons are typically few and far between and the axon diameter distribution in white matter is often described by a gamma distribution - an asymmetric, positively skewed distribution - with a mean axon diameter $\sim 1\text{ }\mu\text{m}$ and a tail describing huge axons [9, 10].

Myelin

Axon myelination can increase the conduction velocity substantially whilst minimising its metabolic expense, where a myelinated axon has a conduction speed around 5 times that of an unmyelinated axon of equal diameter [5]. ‘Myelination’ describes the process where an oligodendrocyte (a type of glial cell) produces a ~ 10 nm thick sheet of the lipid-rich substance myelin, which is then concentrically wrapped around a nearby axon. The myelin can be thought of as an insulating layer as would be employed with electrical cable designs. Notably, there exists a theoretically optimal relationship between the axon diameter and the amount of myelin. This is described by the g-ratio i.e the ratio of the inner and outer (including myelin) diameter of the axon. A g-ratio of ~ 0.6 - 0.7 is theorised to produce maximum conduction velocities [6] where similar g-ratios have also been found in practice.

The myelin does not form a continuous sheath but exists in discontinuous segments (‘internodes’), between which are short unmyelinated sections called ‘nodes of Ranvier’. The myelin segments, together with the nodes of Ranvier, result in saltatory conduction, i.e. where the action potential in effect skips or jumps between the adjacent nodes. Myelin thus acts as a protective sheath - decreasing the capacitance of the axon and increasing the membrane resistance - and facilitates the rapid and efficient transmission of electrical impulses. A loss of myelin, i.e. demyelination through diseases such as multiple sclerosis, can have severe motor, sensory or cognitive impact caused by slow axonal transmission. Unmyelinated, small diameter (typically $< 0.2 \mu\text{m}$, [11]) axons also exist in relative abundance ($< \sim 16\%$ of axons in the mid-sagittal corpus callosum, [7]), though their specific structure or function is relatively unexplored.

2.1.2 The extra-axonal space

Outside of the neuron, the extra-axonal space contains interstitial fluid, vasculature, perivascular space and a plethora of glia. Glia are generally classified into macroglia (oligodendrocytes and astrocytes) and microglia.

As described above, oligodendrocytes produce myelin, support the integrity of the axon structure and promote efficient, saltatory conduction [12]. Oligodendrocytes consist of a small cell body (approximately 13 μm in diameter in the corpus callosum, [9]) from which processes extend radially $\sim 80 \mu\text{m}$ [13] in a fairly symmetric fashion. Due to their close relationship with myelinated axons, oligodendrocytes are mostly found in white matter, oriented in short rows which tend to run parallel to the axon [11].

Astrocytes have a star-like morphology and have many roles which, in general, help to maintain homeostasis through, for example, regulation of extracellular pH, ion concentration and fluid volume [14]. Astrocytic processes extend from the cell soma to axonal nodes of Ranvier or blood vessels where they contribute to the function of the blood-brain barrier. Furthermore astrocytes can together form glial sheets within the white matter which act as a short partition between axonal bundles [15].

Microglia are the immune cells or resident macrophages of the central nervous system. Microglia are small, phagocytic cells which are involved in tissue repair and scarring where they act to engulf waste and dying cells [16]. Notably, the microglia morphology changes in response to injury, a pathologic process, infection or inflammation [17]. These ‘activated’ microglia localise around the injury site and are characterised by a larger soma with fewer and shorter processes [18].

2.1.3 The macroscopic brain: white matter tracts

At the macroscopic level, axons pack together into larger white matter ‘tracts’ or ‘fibre bundles’ which act like highways through the brain, along which axons (or nerve fibres) of similar origin and/or destination travel. Axons within a tract are generally aligned in parallel but with some degree of dispersion or fanning. The axonal packing density along the tract is dependent on both the diameter and myelination of the axons. Example tracts are shown in Figure 2.2. One of the major

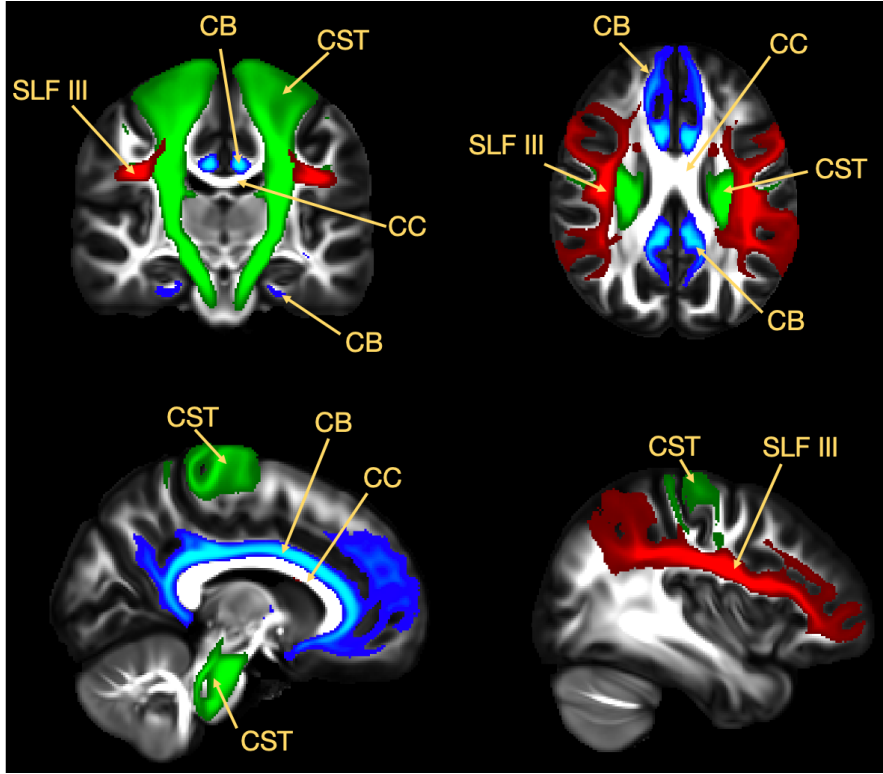


Figure 2.2: Some example white matter tracts are shown: the corpus callosum (CC) links the two hemispheres; the corticospinal tract (CST) relays information from the motor cortex to the spinal chord; the cingulum bundle (CB) wraps around the corpus callosum; and the superior longitudinal fasciculus (SLF) connects the intra-hemispheric frontal, occipital, parietal, and temporal lobes. The tract consists of three distinct components where here we show the SLF-III. The tracts are taken from the XTRACT HCP atlas [20] where the probabilistic tract density mask has been thresholded at 0.05.

tracts, the human corpus callosum, connects the left and right hemispheres of the brain and is estimated to contain between 200 [7] and 800 million [19] axons.

White matter tracts are typically classified into three categories: association, projection or commissural fibres. The commissural fibres connect the two hemispheres of the brain (e.g. the corpus callosum), association fibres connect two regions within the same hemisphere (e.g. the superior longitudinal fasciculus) and projection fibres connect the cortex with lower parts of the brain (or vice versa) or the spinal cord (e.g. the corticospinal tract).

2.1.4 Quantifiable changes in cellular morphology

Tissue development, damage or disease may relate to, or cause, a wide range of morphological changes. For example, there may be axonal loss due to a neurodegenerative disease, cell swelling from cancer or myelination associated with brain development or plasticity. These morphological changes in turn affect quantifiable tissue parameters such as axonal diameter, density, packing, dispersion or myelination. If, by using diffusion MRI, we can estimate changes in these tissue parameters, this will enable the non-invasive, in vivo study of brain development and ageing, or the diagnosis and staging of neurological disease.

2.2 Diffusion MRI

Diffusion MRI is a powerful tool for microstructure imaging due to its sensitivity to structures at the microscopic scale. By mapping the diffusion of water molecules through tissue, diffusion MRI is sensitive to cellular structures on the order of microns such as fibre membranes and macromolecules which restrict free diffusion. When combined into large bundles, neuronal axons result in a macroscopic directional dependence of the diffusion-weighted MR signal. So, by observing variations in MR signal intensity, the underlying tissue microstructure can be inferred.

This section first describes the principle of diffusion and how the observed diffusion is altered inside brain tissue structure. Secondly, we discuss the principles underpinning MRI and describe how diffusion weighting can be added to the MR image. Finally we discuss how through biophysical modelling of the diffusion signal we can estimate micron resolution tissue features from MRI signals on the order of millimetres.

Note that much of this background content is based on the book *Diffusion MRI: From Quantitative Measurement to In-vivo Neuroanatomy*, edited by Johansen-Berg and Behrens [21].

2.2.1 Water diffusion

Water molecules from a drop of the ocean, or inside your brain, are constantly moving around on a random walk through space. This is the phenomenon of self-diffusion or Brownian motion. Although the random walk or *displacement* of a single water molecule is unpredictable, the displacement of a *ensemble* of water molecules is well described by a probability distribution.

In the absence of any barriers, i.e. ‘free diffusion’, the average displacement of water molecules during time Δ in an N -dimensional system (i.e. in a volume $N = 3$) is described by Einstein’s Equation [22, 23]

$$\langle r^2 \rangle = 2ND\Delta. \quad (2.1)$$

Here $\langle r^2 \rangle$ is the mean-squared displacement and the constant of proportionality D is called the diffusion coefficient. Δ is commonly known as the diffusion time. Furthermore, the probability of a particle displacing distance $\mathbf{r}$ in time Δ , i.e. the probability distribution of particle displacements, is described by an N -dimensional Gaussian where, in 1D,

$$P(\mathbf{r}, \Delta) = \frac{1}{(4\pi D\Delta)^{3/2}} e^{-\frac{\mathbf{r}^2}{4D\Delta}}. \quad (2.2)$$

$P(\mathbf{r}, \Delta)$ is commonly known as the ‘diffusion propagator’. Here, there is no directional preference for displacement and the surface of isoprobability traces out a sphere of radius $\sqrt{2D\Delta}$. Note that the diffusion propagator $P(\mathbf{r}, \Delta)$ can be used to describe any form of diffusion. Gaussian diffusion (and thus Equation 2.2) applies to relatively few specialised cases where water diffuses through a homogeneous medium.

Here we should note how the diffusion coefficient is an intrinsic property of the system. It relates to the size of the diffusing molecule, the temperature and the structural features of the environment. In a homogeneous medium,

$$D = \frac{k_B T}{6\pi\eta r}, \quad (2.3)$$

where k_B is the Boltzmann constant, T the temperature, η the viscosity and r the particle's diameter. For example, the diffusion coefficient of free water at 37°C is 3-3.1 $\mu\text{m}^2/\text{ms}$. Substituting this into Equation 2.1, the root mean squared displacement of a freely diffusing water molecule in a cube of water after a time $t=50$ ms (a fairly typical diffusion time in an MRI experiment) is ~ 30 μm .

Sensitivity to tissue microstructure

To understand how water diffuses within the brain, it is instructive to think of particles diffusing within different structures: inside a long, thin axon or dendrite; a fairly spherical cell body; or within the tortuous extra-cellular space. Here we provide a general description of water diffusion within these various compartments.

From Equation 2.1 we see how the diffusion coefficient can be inferred when the displacement of water is measured over time. However Einstein's equation only holds for free diffusion in the absence of barriers. At the length scales probed by diffusion MRI, the motion of water through the brain is impeded by cellular membranes and macromolecules [24]. Notably these barriers can act to 'hinder' (i.e. slow) the diffusion, or 'restrict' (confine) the particles to a space from which they cannot escape. Consequently, the average displacement is reduced and the observed diffusion coefficient is lower than that of free water. As the observed D no longer matches that of free, 'intrinsic' diffusion, it is named the 'apparent diffusion coefficient' or ADC.

The ADC is dependent on both the sample architecture (i.e. the location of the barriers) and the diffusion time Δ (Figure 2.3). The time dependence of the diffusion coefficient can be used to observe the gradual coarse graining of the structure inside a given compartment [2]. To give an example, let us consider water molecules diffusing within the axon (Figure 2.3 left). The axon is myelinated and the membrane is considered impermeable. Inside the axon there are microtubules which form part of the cytoskeleton. At very short Δ , few water molecules have encountered a barrier and the apparent diffusion coefficient is close to that of

free diffusion (not shown). At short Δ , the diffusion is hindered, though mostly due to microtubules. At longer times, where the diffusion length $L(\Delta) \sim \sqrt{D\Delta}$ is much larger than the microtubules, there is an effective coarse graining of the intra-axonal structure. Here, the intra-axonal space can now be considered a homogenised medium with diffusivity $D < D_{free}$ (Figure 2.3 right). Observing the time dependence of the intra-compartmental ADC thus provides information about the structural disorder of the compartment space. Though in practice, probing the effect of structures as small as microtubules would be challenging, a similar description can be used to describe the gradual homogenisation of any compartment. For a detailed review of the diffusion time-dependence and the phenomena of coarse graining, see Novikov *et al.* [2]. Importantly for this thesis, the diffusion propagator is not sensitive to tissue heterogeneity which occurs on length scales much shorter than the diffusion length $L(\Delta)$.

This thesis does not focus on analysing the time dependence of the ADC, but instead analyses data acquired as a single Δ (but across many gradient directions) where we are sensitive to an instantaneous diffusion coefficient $D_{inst} < D_{free}$. By utilising diffusion times of $\Delta \sim 10 - 50$ ms (and thus working in the long diffusion time regime), we probe diffusion lengths $L(\Delta) \sim 10 - 30$ μm . Here the diffusion signal is influenced by parameters such as the axon orientation, dispersion and density, as well as the apparent diffusivities of the intra- and extra-axonal space, making this regime ideal for connectome imaging.

Additionally, the diffusivity may not be constant along each direction but instead will have an angular dependence. In the white matter, anisotropy is typically associated with myelinated axons where, in the long- Δ regime, the axon membrane appears impermeable to water. Along the axon, water can move relatively freely with little impedance. Across the axon, the particles are restricted or confined to the axon, the ADC is reduced and the displacement distribution becomes non-Gaussian. Thus, the diffusion inside axons in the white matter is highly anisotropic, where the axis of

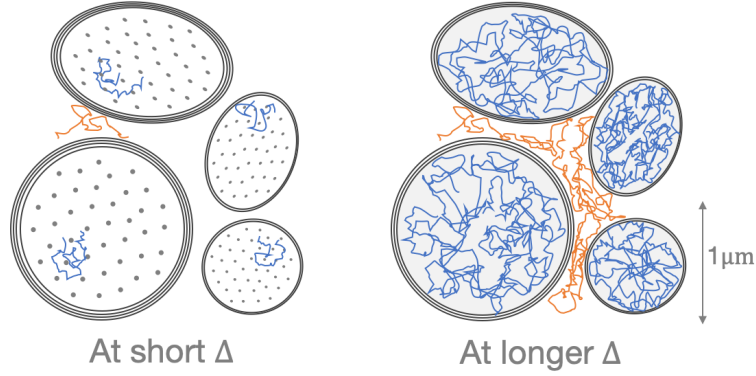


Figure 2.3: Water molecules diffusing in the intra- and extra-axonal space. There is restricted diffusion within the axon (blue lines) and hindered diffusion in the extra-axonal space (orange). At very short diffusion times (left), the diffusivity of the intra-axonal space is sensitive to small structures such as microtubules. However, at longer times (right), the diffusion length $L(\Delta) \sim \sqrt{D\Delta}$ is much larger than the microtubule structure and the intra-compartmental structure is (as far as we can observe it) a homogenised medium.

high diffusion co-aligns with (and so can be used to infer) the orientation of the axon. In comparison, diffusion within cell bodies in the grey matter is fairly isotropic.

In the white matter, hindered diffusion is typically used to describe the tortuous space in and around axons, i.e. the ‘extra-axonal’ space (Figure 2.3, orange). Here the displacement of water particles is impeded and thus the ADC reduced, though the water is not restricted to a certain compartment and in infinite time Δ particles will eventually explore all space. Consequently the diffusion propagator can be reasonably well described by a Gaussian. If axons and processes are somewhat aligned, the extra-axonal space is also often anisotropic, where the degree of anisotropy is affected by the axonal packing and density.

By fixing the diffusion time Δ and estimating the ADC across many orientations, we can map the diffusion propagator in 3D, the shape of which describes the degree of restriction - and thus the presence of barriers - along each axis. Using biophysical modelling of the tissue structure, the diffusion propagator is then related to biologically meaningful tissue parameters. For white matter imaging in the long- Δ regime, this includes the axon orientation, dispersion and density, as well as the diffusion characteristics of the intra- and extra-axonal space.

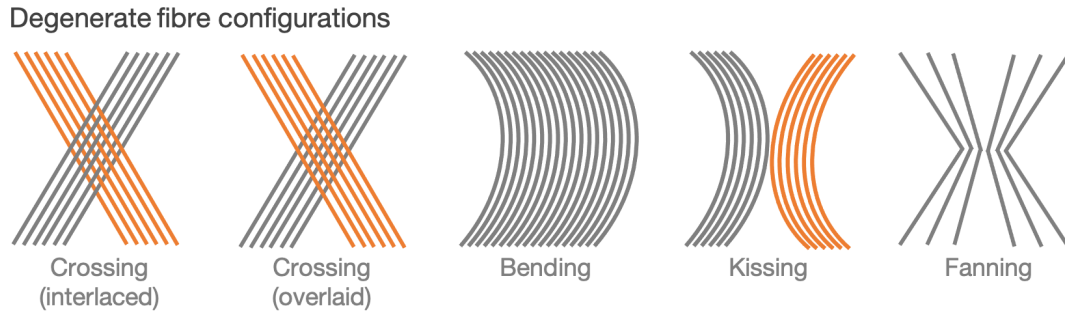


Figure 2.4: All five fibre configurations may produce highly similar diffusion propagators and so would be difficult to distinguish from the diffusion signal without additional information.

Ill-posed diffusion mapping

Through estimation of the diffusion propagator, can we infer the true tissue microstructure? Unfortunately, an additional layer of complexity exists in that the diffusion propagator may not be microstructurally unique. There can exist a many-to-one mapping where many different tissue structures map onto the same single diffusion propagator. The estimation of the tissue structure is thus ill-posed and the tissue parameters are said to be degenerate with multiple parameter sets explaining the data equally well.

This degeneracy can occur between multiple fibre configurations, as shown in Figure 2.4. Here crossing, bending, kissing and fanning fibres all produce the same diffusion propagator.

Degeneracies can also exist between the diffusion characteristics and the tissue architecture [3]. Chapter 4 discusses a known degeneracy between fibre dispersion or fanning and the radial diffusivity. Furthermore, the diffusion properties of the intra- and extra-axonal space can be difficult to separate, though they are likely different (*c.f.* Chapter 3).

In these situations, typical diffusion data is limited in its ability to recover the ‘true’ tissue structure. As such, these degeneracies [3] highlight some of the

biggest challenges currently facing the diffusion MRI community. For example, understanding the structural connectome via tractography requires the ability to discern the different fibre configurations of Figure 2.4 [25], whilst accurate determination of fibre dispersion (a biomarker of development, ageing and various neurological diseases, *c.f.* Chapter 3) requires precise knowledge of the tissue diffusivity. Overcoming these degeneracies typically requires one or some combination of: parameter constraints, usually given prior knowledge of their expected values; sensitising the diffusion signal to only a subset of parameter values through the use of high end hardware and/or bespoke imaging sequences; or complementary ‘orthogonal’ data from other modalities, for example, different MRI contrasts or, as shown in Chapter 4, microscopy.

Postmortem tissue

Chapters 4 and 5 in this thesis consider diffusion MRI data acquired on fixed, postmortem tissue. As the diffusion process is primarily based on Brownian motion - rather than active transport - diffusion occurs in postmortem tissue in a similar way to its in vivo counterpart. The main difference is that the diffusivity of fixed postmortem tissue is considerably reduced when compared to in vivo. Though the anisotropy is mostly preserved [26, 27], postmortem ADCs are approximately 1/3 to 1/4 of their in vivo value [28, 27, 29]. In postmortem tissue, the diffusivity is affected by three main factors: chemical fixation, the postmortem interval, and the reduced tissue temperature.

After death, tissue is typically chemically fixed using formaldehyde where the time between death and fixation (the postmortem interval) is ideally kept to a minimum. At death, there begins autolysis of the tissue and degradation of myelin and cellular membranes [30, 31]. This is problematic as the diffusion contrast relies on the preservation of such membranes and so tissue fixation is usually desirable, though it comes at a cost. Formaldehyde fixation leads to the cross-linking of proteins in both the cytoskeleton and the extra-cellular space, thus altering the

tissue microstructure from its in vivo state. This cross-linking leads to increased tissue rigidity and a reduction in the diffusivity.

In human samples, the brain is typically first extracted from the skull and then immersed in fixative. As such, the postmortem intervals (particularly for deep brain structures) can be relatively long (*c.f.* Chapter 4). In comparison, autolysis effects are minimised for the brains of experimental animals which typically undergo perfusion fixation at death (*c.f.* Chapter 5).

As the diffusion coefficient is inherently linked to temperature (Equation 2.3), the postmortem diffusivity is further reduced when excised samples are scanned at temperatures below 37°C (i.e. in vivo human body temperature). As the relationship between the ADC and temperature is linear, where a 1°C drop in temperature results in a 2.4% reduction in diffusivity [32], this affect can be mostly accounted for in post-hoc analysis. Nonetheless, to avoid fluctuations in the ADC, the postmortem tissue temperature must be carefully controlled and held constant throughout MRI scanning [31].

Though the diffusion properties of in vivo and postmortem tissue are different, as the anisotropy of the diffusion signal is preserved, these issues can be largely addressed during MRI acquisition and analysis, rather than being fundamental, insurmountable problems.

2.2.2 Magnetic resonance imaging

A magnetic resonance image (MRI) is generated through the use of three magnetic fields: the main magnetic field ($\mathbf{B}_0$), a secondary magnetic field in the transverse plane ($\mathbf{B}_1$) and magnetic field gradients [21]. The $\mathbf{B}_0$ and $\mathbf{B}_1$ fields act to produce a measurable Nuclear Magnetic Resonance (NMR) signal, whilst the magnetic gradients add spatial localisation, turning the MR signal into an MR image.

At its essence, MRI measures the magnetic properties of excitable particles - in the brain this is mostly hydrogen nuclei (i.e. protons) in water - as they are placed in, and perturbed from, a large magnetic field. During an MRI scan, the brain is placed inside a strong magnet field typically between 1.5 and 7 Tesla. This static magnetic field is known as $\mathbf{B}_0$ and is designed to be homogenous across the sample.

Magnetic resonance

Due to a quantum mechanical property called nuclear spin, when placed in a large magnetic field, hydrogen nuclei (in MRI often referred to as simply ‘spins’) act like magnetic dipoles with a magnetic moment vector $\boldsymbol{\mu}$. Here the spins exist in two energy states pertaining to the two possible spin states: spin-up or spin-down, where $\boldsymbol{\mu}$ tends to align with or against the main magnetic field respectively. Furthermore the vector $\boldsymbol{\mu}$ precesses about $\mathbf{B}_0$ according to the Larmor frequency,

$$\omega = \gamma B, \quad (2.4)$$

where γ is the gyromagnetic ratio of the nucleus (4257 Hz/gauss for protons) and B the magnitude of the magnetic field. In an ensemble of spins, the proportion of spins in each Eigenstate is given by the Boltzmann distribution,

$$\frac{N_+}{N_-} = e^{\frac{\gamma h B_0}{2\pi k T}}. \quad (2.5)$$

$N_{+/-}$ is the number of spins in the two energy states, N_+ describes the lower energy spin-up state, h is Planck’s constant, k the Boltzmann constant and T the absolute temperature. Equation 2.5 dictates that a small excess of spins tend to align with the main magnetic field, resulting in a net magnetisation of the spin system M_0 along $\mathbf{B}_0$. Here M_0 is proportional to the proton density and can be described by

$$M_0 \approx \frac{\gamma^2 h^2 N_s B_0}{k T}, \quad (2.6)$$

where N_s is the number of spins. Note that at equilibrium, in the transverse plane the spins are randomly oriented such that the transverse magnetisation of the spin system is zero.

Energy can be added to the system through a second magnetic field $\mathbf{B}_1$ which oscillates at, or close to, the resonant frequency of the system (i.e. the Larmor frequency). The $\mathbf{B}_1$ field is generated from an oscillating electromagnetic field or pulse. For $\mathbf{B}_0$ fields of 1-10 T, the proton Larmor frequency (and thus the pulse) resides in the radio-frequency (RF) spectrum. In a typical MRI scan, an RF ‘excitation pulse’ tilts the magnetisation vector $\mathbf{M}$ from its preferred orientation along $\mathbf{B}_0$ into the transverse plane. Instantaneously after a 90° pulse, the magnetisation vector is aligned with the transverse ($x-y$) plane, and precesses about the main magnetic field at the Larmor frequency ω with the spins in coherent phase, i.e. $M_{xy} = M_0$. According to Faraday’s law, the rotating transverse magnetisation induces a current in the receiver coils. This produces the detected NMR signal, S , where the signal magnitude is dependent on the phase coherence of the spin system. The signal magnitude decreases over time as the phase becomes increasingly incoherent due to magnetic field inhomogeneities and dipolar interactions.

Relaxation rates: T_1 , T_2 and T_2^*

In a homogeneous field of static spins, there exists a range of Larmor frequencies, instead of the theoretical single Larmor frequency. This is due to spin-spin or dipole interactions where each precessing spin creates its own magnetic field that interacts with the main magnetic field and those from other spins. Consequently, each spin experiences a somewhat different magnetic field and precesses at a slightly different frequency. This results in gradual dephasing of the spin system in the transverse plane. Here, the transverse magnetisation M_{xy} decays according to time T_2 ,

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2}. \quad (2.7)$$

The T_2 relaxation time is dependent on the frequency of molecular tumbling i.e. the rate of molecular rotations or translations. The T_2 is short for the large, static molecules in tissue, and long for the rapidly tumbling, small molecules in the cerebral spinal fluid (CSF). Hence, in an image with T_2 contrast, the CSF is bright or ‘hyperintense’ and there is little white/grey matter contrast.

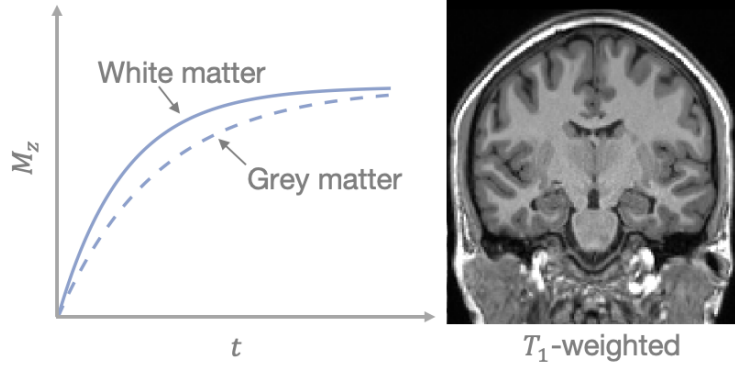


Figure 2.5: After excitation, the longitudinal magnetisation M_z recovers according to T_1 . Due to the tissues difference in T_1 , T_1 -weighted images show good grey/white-matter contrast.

In practice, further spin dephasing is caused by inhomogeneities in the B_0 field. Such inhomogeneities are both inherent to the scan setup (i.e. due to imperfections in the magnet), and result from magnetic susceptibility variations in the sample (e.g. at the air-tissue interface). This additional dephasing causes the transverse magnetisation to decay more rapidly according to T_2^* where $T_2^* < T_2$.

In longitudinal recovery, spins return to their lowest energy state (i.e. thermal equilibrium) by releasing energy to the external environment ('spin-lattice relaxation'). At equilibrium the spin system is re-aligned with the main magnetic field. This occurs according to a time constant T_1 where, after a 90° excitation pulse,

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

M_0 is the bulk magnetisation, z the orientation of main magnetic field $\mathbf{B}_0$. Again, the T_1 is tissue dependent, where the CSF has a much longer T_1 than the grey matter, with the white matter having the shortest T_1 (Figure 2.5). The three tissue types (white/grey matter and CSF) are readily discernable in images with T_1 contrast: the CSF is dark and the white matter hyperintense when compared to the grey matter (Figure 2.5). In the in vivo brain, the T_1 is typically on the order of 1 s and the T_2 is roughly an order of magnitude less (milliseconds).

Though T_1 and T_2 relaxation are inherent properties of the tissue, the T_2^* dephasing due to static field inhomogeneities can be reversed and the signal somewhat recovered. In a spin echo sequence (Figure 2.6), an additional 180 RF pulse, the ‘refocussing pulse’, reverses the precession of the spins to reproduce a signal echo. If τ is the time between the excitation and refocussing pulse, the echo occurs at time $TE = 2\tau$. The refocussing pulse puts the spins with high frequency precession (i.e. the faster spins which have accrued the most phase) behind the slower spins. In a static field, the T_2^* induced dephasing is reversed at time TE . During this time, T_1 and T_2 relaxation still occur and so the refocussed signal has magnitude $S < M_0$ where,

$$S = M_0(1 - e^{-TR/T_1})e^{-TE/T_2}. \quad (2.9)$$

Here the repetition time (TR) is defined as the time between two excitation pulses. The TR is on the order of seconds to allow for T_1 recovery of the magnetisation.

As different tissues have distinct M_0 , T_1 and T_2 , the image contrast can be altered by manipulation of the sequence parameters TE and TR . For example, a long TE results in T_1 contrast, a short TE results in T_2 contrast, and when a short TE is accompanied by a long TR , proton density-weighted images are achieved.

Generating an image: spatial localisation

So far we have discussed the creation of an MR signal, but how does a signal turn into an image? An image is formed when additional magnetic gradients encode the location from which the signal originates. The scanner can produce three orthogonal magnetic gradients which, when played in synchrony, create a linearly varying magnetic field $\mathbf{G}$ along any given axis. Consequently, the magnetic field at position $\mathbf{r}$ in the scanner is given by

$$\mathbf{B} = \mathbf{B}_0 + \mathbf{r} \cdot \mathbf{G}, \quad (2.10)$$

and the Larmor frequency becomes position dependent $\omega(\mathbf{r})$. Note, these same gradients are used to sensitise the signal to diffusion (*c.f. Diffusion weighted sequences, below*).

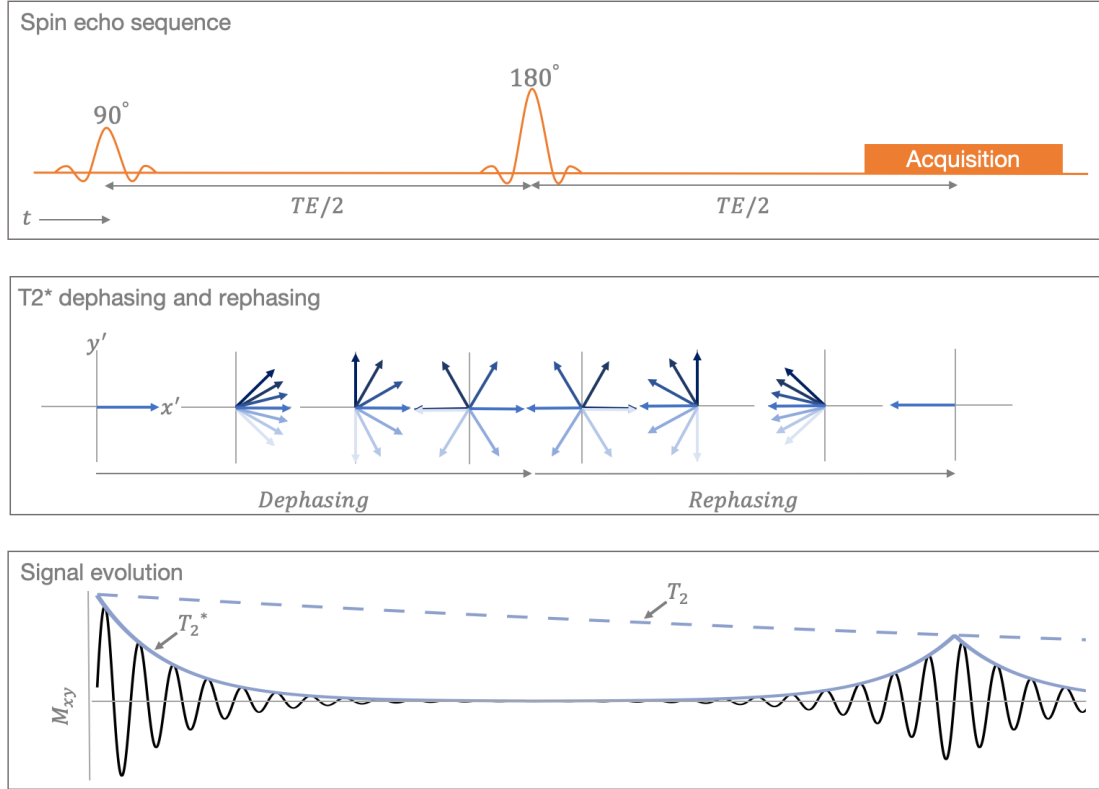


Figure 2.6: Top: A spin echo sequence consists of a 90° excitation pulse and 180° refocussing pulse. Middle: The magnetisation vector M (blue arrow) is tipped into the transverse $x-y$ plane, after which dephasing occurs according to T_2^* . The 180° pulse acts to flip the magnetisation such that the spins with slower precessional frequency (darker blue) are behind those with higher precessional frequency (lighter blue). At time TE , the T_2^* dephasing is rephased to produce an echo. x' , y' indicate the x - y axis in a frame rotating at the Larmor frequency ω_0 . Bottom: Signal evolution (M_{xy}) in the transverse plane. In addition to T_2^* decay, there is also T_2 decay which cannot be reversed.

For the duration of the gradient, the frequency of spin precession varies along the gradient axis. After the gradient, the magnetic field returns to $\mathbf{B}_0$ and the spins precess at the Larmor frequency ω_0 . However, across the sample there now exists a pattern of phase modulation oriented along $\mathbf{G}$ with spatial frequency $|\mathbf{k}|$. This phenomenon is described in Figure 2.7. During the gradient, spins accumulate spatially-dependent phase, $\theta = 2\pi\mathbf{k}$, where $\mathbf{k}$ is the time integral of the gradient,

$$\mathbf{k} = \gamma \int_0^t \mathbf{G}(\tau) d\tau. \quad (2.11)$$

The complex MR signal measured in the receive coil $S(\mathbf{k})$ is then the integrated

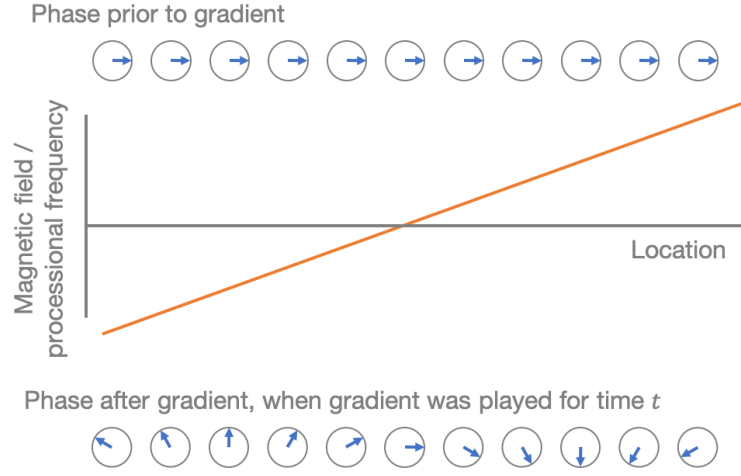


Figure 2.7: During a magnetic gradient, the spin precession frequency depends on the local magnetic field which varies linearly across space. After a gradient (bottom) there exists a spatially-dependent phase distribution across the sample. This concept is integral to both MR image formation and diffusion weighting.

signal from all spins where,

$$S(\mathbf{k}) = \int s(\mathbf{r})e^{i\mathbf{k}\cdot\mathbf{r}}d\mathbf{r}. \quad (2.12)$$

$e^{i\mathbf{k}\cdot\mathbf{r}}$ can be seen as a set of ‘basis functions’ and $S(\mathbf{k})$ the amount of structure in the sample which can be explained by the spatial frequency $\mathbf{k}$. $S(\mathbf{k})$ forms a Fourier pair with $s(\mathbf{r})$, the signal at a given spatial location $\mathbf{r}$. Due to its dependency on $\mathbf{k}$, we call the domain in which we acquire data ‘k-space’. The image is then recovered by taking the inverse Fourier transform of $S(\mathbf{k})$, where

$$s(\mathbf{r}) = \int S(\mathbf{k})e^{-i\mathbf{k}\cdot\mathbf{r}}d\mathbf{k}. \quad (2.13)$$

To form an image, data $S(\mathbf{k})$ must be acquired across multiple points in k-space where the sampling of k-space dictates the image resolution and field of view. Here the extent of k-space dictates the image resolution, Δx , where,

$$\Delta x = \frac{1}{2|\mathbf{k}_{max}|}. \quad (2.14)$$

The distance between points in k-space, Δk , governs the field of view, FOV , where

$$\Delta k = \frac{1}{FOV}. \quad (2.15)$$

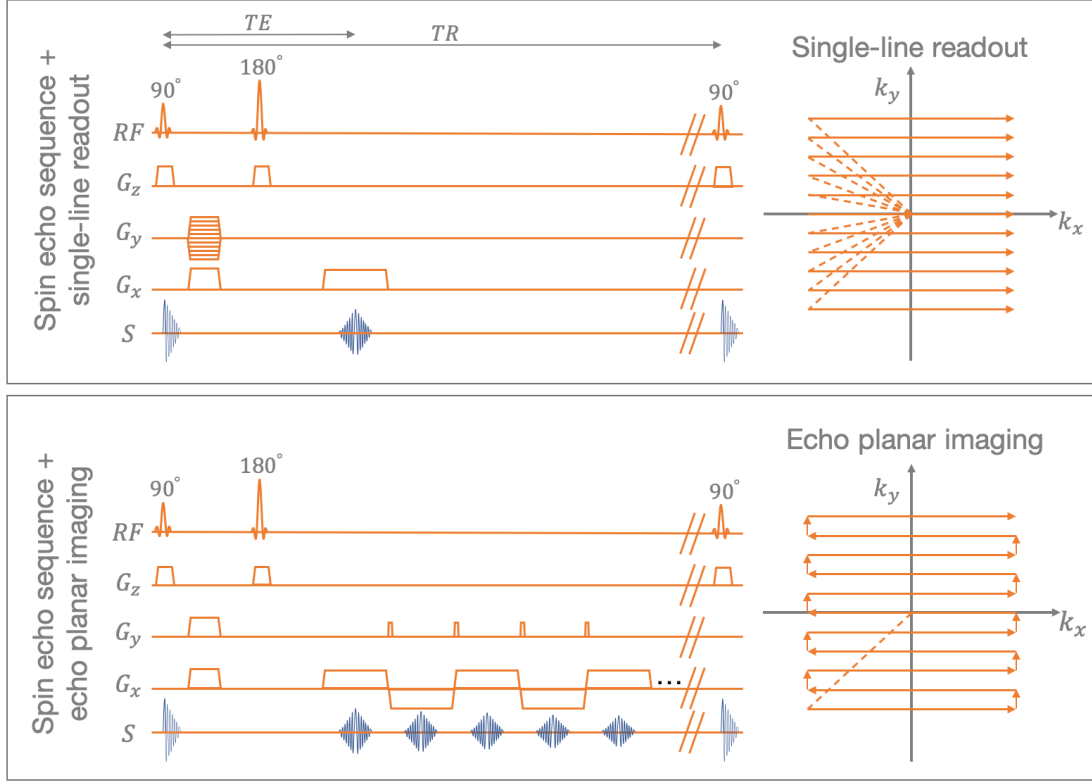


Figure 2.8: The spin echo sequence with either single line readout (top) or echo planar imaging (bottom). Left: The pulse sequence consists of three sets of gradients. Here the slice select gradient is denoted G_z , the phase encoding gradient G_y and the readout gradient G_x . S is the signal where, directly after a 90° radio frequency (RF) pulse we see free induction decay according to T_2^* . The 180° pulse refocusses the dephasing due to T_2^* and produces to produce an echo to the signal at time TE . Right: The readout trajectories through k-space. In single-line readout, a single line of k_x is acquired per repetition time (TR). In comparison, echo planar imaging is a single-shot technique where the entire plane in k-space is acquired within one TR. Here, the trajectory ‘blips’ through k_y whilst oscillating through k_x .

The position in k-space, $\mathbf{k} = [k_x, k_y, k_z]$, is determined by the integrated area of the imaging gradients. By sequentially applying gradients with specific magnitude and orientation, we can map out a sampling trajectory through k-space. Signals acquired at small $|\mathbf{k}|$ relate to patterns of slowly varying phase across the sample or low spatial frequencies. Correspondingly, signals at large $|\mathbf{k}|$ reflect high spatial frequencies and contain details such as the position of sharp boundaries or edges.

To acquire a 3D image, k-space must also be sampled in 3D. However, as T_2 decay occurs on the order of 100 ms, the magnitude of the signal at the receive coil

decreases quickly over time. As such, data is often acquired over multiple excitation pulses or repetition times. Though 3D sampling trajectories exist (i.e. which acquire the full 3D k-space in a single TR), MR images are typically acquired through a series of ‘2D’ slices. Here a magnetic gradient is used to excite a single slice of the tissue, after which additional gradients ‘read’ the signal from that 2D slice. Similarly, the 2D readout may be acquired in a single TR (e.g. echo planar imaging) or over multiple TRs (e.g. line-by-line).

Slice selection occurs when a magnetic gradient is applied during an RF pulse. The gradient acts to create a range of frequencies across the sample. Here the frequency varies linearly along the gradient, but is constant in the perpendicular plane. The RF of the excitation pulse is then tuned to excite only a single slice or plane of spins, where the slice thickness depends on both the pulse bandwidth and the gradient strength. For convenience, here we define the slice selection gradient to be along z and the excited spins lie in the $x - y$ plane, though this notation is arbitrary.

This thesis considers data acquired in a series of 2D slices with two readout strategies: single-line read-out or echo-planar imaging (Figure 2.8). In single line readout, the full extent of k-space along x is acquired per TR. Between TRs the sampling trajectory is moved to a different k_y through short blips of the gradient G_y . Alternatively, echo planar imaging (EPI) blips through the full extent of k_y in a single TR, all the while oscillating back and forth through k_x . This is known as a single-shot readout (i.e. the entire slice is acquired in a single TR). As the EPI readout is very fast and thus less susceptible to motion, it is commonly used for in vivo diffusion MRI (*c.f. Chapter 3*). However, the long EPI trajectory makes it susceptible to artefacts such as distortion, blurring and signal loss. Consequently, time consuming single-line readouts are often favourable for postmortem imaging where the sample is motionless and the scan time less constrained (*c.f. Chapters 4 & 5*).

Diffusion weighted sequences

So far we have described how we can generate an image where the contrast is defined by the relaxation properties of the tissue. These ‘structural’ images are useful in defining tissue boundaries and, in this thesis, are used in the context of image registration. However, to probe the tissue *microstructure*, our images must be sensitive to the diffusion of water molecules which displace on the order of microns i.e. we require diffusion-weighted images. This section will first describe how we add diffusion weighting to the imaging sequence, and secondly how this new image contrast can be related to the tissue microstructure through biophysical diffusion models.

A typical single diffusion weighted sequence - coined the pulse gradient spin echo or PGSE sequence - encodes spin displacement through a pair of diffusion-sensitising gradients. In the presence of diffusion, the MR signal is thus attenuated.

Introduced by Stejskal and Tanner in mid 1960s [24], a typical sequence consists of a pair of gradient pulses defined by three parameters: the gradient amplitude, $|\mathbf{G}|$, duration, δ , and the time between the pulses i.e. the diffusion time, Δ . The b-value is used to summarise the amount of diffusion weighting of a given sequence. For rectangular diffusion gradients the b-value is calculated as [24],

$$b = \gamma^2 |\mathbf{G}|^2 \delta^2 (\Delta - \delta/3), \quad (2.16)$$

where γ is the gyromagnetic ratio. The diffusion gradients can be inserted into almost any pulse sequence. Figure 2.9 shows a typical spin echo diffusion-weighted sequence where the gradients are combined with a 90° excitation pulse followed by a 180° refocussing pulse.

The first ‘encoding’ gradient, $\mathbf{G}$, imparts a phase modulation across the spins. During the gradient, each spin accrues some phase θ_+ , where θ_+ depends on the spin’s position along $\mathbf{G}$. The second ‘decoding’ gradient, $-\mathbf{G}$, imparts an equal yet opposite phase modulation and the spins accrue phase θ_- . Thus at readout, each

spin's net phase (due to the diffusion-encoding gradients) is the sum $\Delta\theta = \theta_+ + \theta_-$. In the case of stationary spins, any phase accrued under the first gradient is exactly reversed by the second, i.e. $\Delta\theta = 0$, and the signal remains coherent. In comparison, diffusing spins - which end up in different locations for the first and second gradient - accrue unequal phase under each gradient, $\Delta\theta \neq 0$. Here the phase difference $\Delta\theta$ is proportional to the spin displacement in the direction of the diffusion weighted gradient. This means that the spin ensemble falls out of phase. This dephasing results in a decrease or attenuation of the MR signal where,

$$S(b, D) = M_0(1 - e^{-TR/T_1})e^{-TE/T_2}e^{-bD}. \quad (2.17)$$

Here, D is the apparent diffusion coefficient and the b-value b equals the time dependent integral of the diffusion weighted gradient, $b = \gamma^2 \int |\mathbf{G}(t)|^2 dt$.

Here the phase dispersion or incoherence is dependent on four factors: i) the apparent diffusion coefficient that describes how likely it is for the spins to displace; the ii) strength and iii) duration of the magnetic gradient that determines the pattern of phase accrual across the sample; iv) the diffusion time dictates the length of time over which the spatial pattern can become disordered.

To estimate the diffusion signal in 3D, the diffusion gradients are applied along multiple different gradient directions across the sphere. How these orientations are distributed determines the angular resolution of the data. Non-diffusion weighted S_0 images are also typically acquired. Here the b-value is set to zero whilst the rest of the sequence, including TE and TR, remains the same. The signal attenuation due to diffusion along the gradient direction $\mathbf{g}$ - accounting for proton density, T_1 recovery and T_2 decay - is thus given by the normalised diffusion signal,

$$\frac{S}{S_0} = e^{-bD}. \quad (2.18)$$

As the application of an imaging gradient causes a traversal in k-space, where the k-space trajectory is determined by the integral under the imaging gradient, the

application of a *diffusion sensitising gradient* results in traversal through ‘q-space’ where (in a similar definition to the $\mathbf{k}$ -vector) the $\mathbf{q}$ -vector is given by,

$$\mathbf{q} = \gamma \int_0^t \mathbf{G}(\tau) d\tau. \quad (2.19)$$

Intuitively, q-space can be seen as an extension of k-space, where the q-space extent is approximately 3 orders of magnitude larger than k-space. Consequently, diffusion MRI constitutes fine scale spatial encoding, on the order of microns [33]. To traverse q-space, diffusion gradients are large in both magnitude ($|\mathbf{G}|$) and duration (δ) when compared to imaging gradients. In the ‘short pulse regime’ where $\delta \ll \Delta$, the magnitude of the $\mathbf{q}$ -vector relates to the b-value where,

$$b \approx |\mathbf{q}|^2 \Delta. \quad (2.20)$$

Furthermore, the orientation of the $\mathbf{q}$ -vector is equal to that of the magnetic gradient $\mathbf{g}$. Thus, when many gradient directions are acquired at a single b-value, we trace out a sphere in q-space, commonly known as a ‘shell’. ‘Multi-shell’ acquisition strategies acquire angular data at two or more b-values (excluding $b = 0$).

As k-space and the image domain form a Fourier pair, q-space forms an similar Fourier pair with spin displacement $\mathbf{r}$. Thus, under the short pulse regime approximation, the MR signal attenuation $E(\mathbf{q}) = S(\mathbf{q})/S_0$ can be described as

$$E(\mathbf{q}) = \int P(\mathbf{r}, \Delta) e^{i\mathbf{q} \cdot \mathbf{r}} d\mathbf{r}. \quad (2.21)$$

The diffusion propagator $P(\mathbf{r}, \Delta)$ can be calculated as the Fourier transform of $E(\mathbf{q})$ and (as discussed above) describes the fraction of spins that have displaced a distance $\mathbf{r}$ in a given diffusion time Δ . This is known as q-space imaging (QSI) [34]. As the Fast Fourier Transform requires Cartesian sampling of q-space, QSI is time intensive and is rarely used in practice. Furthermore, it is often unnecessary to characterise the diffusion propagator in full, rather we wish to interrogate the diffusion process to infer biologically-meaningful features of the tissue. Consequently, most diffusion analyses sparsely sample q-space after which microstructural features are estimated using appropriate approximations and models (*c.f. Diffusion modelling*). Note, the

way in which q-space is sampled (i.e. the b-values and gradient directions probed) strongly determines the models that can be applied to the data.

This thesis focuses on high angular resolution diffusion imaging (HARDI) data, in which multiple ($\sim 30+$) gradient directions are acquired per b-value. The diffusion time Δ , and thus the diffusion propagator, is kept constant across all shells. Furthermore, we acquire data in the long-diffusion time regime ($\Delta =$ many 10's of milliseconds). Here, the diffusion signal can be considered the summation of signals from non-exchanging compartments, where the signal from each compartment is an (often anisotropic) Gaussian.

In postmortem data, the ADC is typically reduced to 1/3 - 1/4 of its in vivo value [28]. Thus to achieve similar diffusion contrast, b-values used in postmortem imaging must be 3 - 4 times that of the in vivo equivalents. To achieve such high b-values postmortem imaging typically relies on small bore scanners fitted with strong imaging gradients. For a review describing the many challenges and practicalities associated with scanning ex vivo tissue samples, please see Roebroek *et al.* [35].

Note, prior to modelling, diffusion MRI data is typically distortion corrected with a particular focus on subject movement, susceptibility-induced and eddy current-induced distortions. For details please see reference [36].

2.2.3 Diffusion modelling

Diffusion MRI modelling aims to relate MRI signal attenuation to specific features of the tissue microstructure. The ideal model is accurate, precise, sensitive and specific. It provides reliable estimates of a biologically meaningful parameter which can be validated against ground truth values obtained independently from other methods. Furthermore, it can detect small microstructural changes and thus differentiate tissue states by, for example, disambiguating axonal loss from demyelination in multiple sclerosis. This must all be achieved whilst inferring micrometer resolution

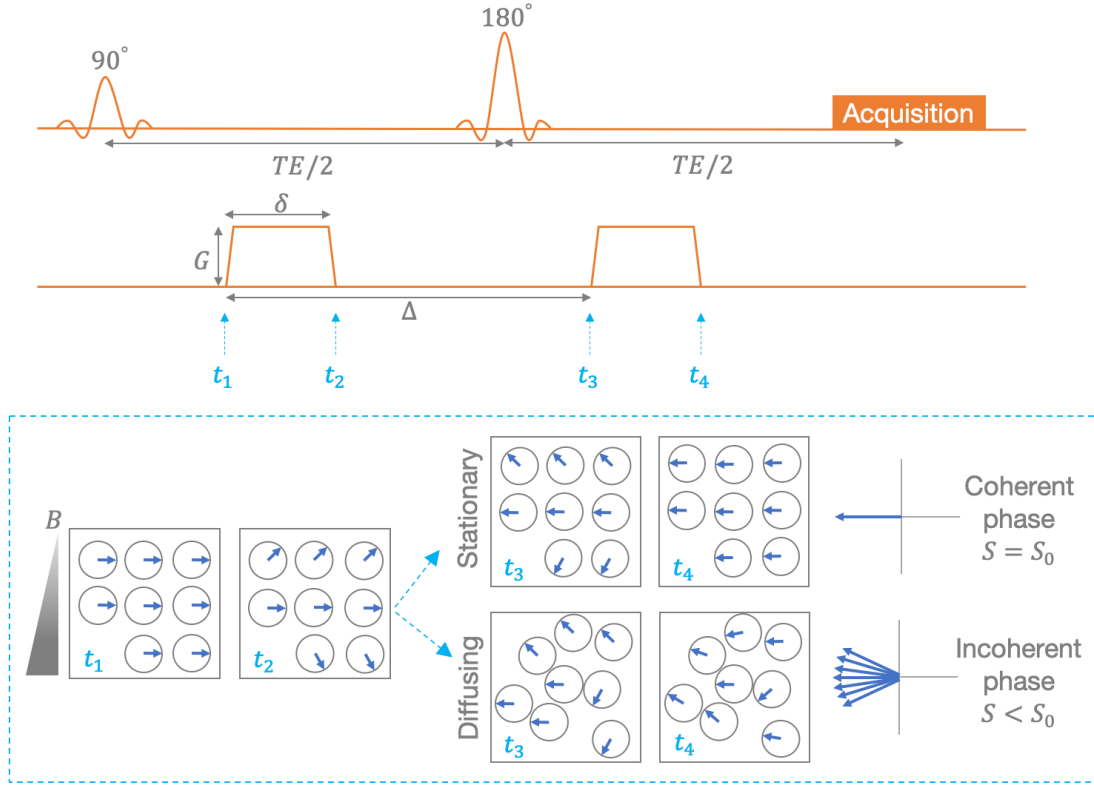


Figure 2.9: Top: A diffusion weighted spin echo sequence. The gradient is of amplitude G , duration δ , Δ is the time between the gradients and TE is the echo time. Bottom: The phase evolution of both stationary and diffusing spins in a voxel. During the first gradient, spins accrue phase according to their position in the sample. For stationary spins, this phase is perfectly reversed under the second gradient. For diffusing spins, after the second gradient the spins remain somewhat dephased and the diffusion signal is attenuated.

processes from MRI signals on the order of millimetres. Thus, diffusion modelling is highly challenging and often contentious, resulting in a plethora of competing diffusion models being proposed (for a review see [37], [2] or [9]).

Nonetheless, if we can analyse the diffusion signal well, the returns can be great. Diffusion MRI can be used to infer the structural connectome of an individual (a connectivity viewpoint) or estimate microstructural parameters such as axon diameter, undulation, density, packing and the degree of myelination (a microstructure viewpoint), all of which affect the diffusion properties of the tissue. Regardless of the viewpoint, such inference requires complex modelling of both the tissue architecture and the resultant diffusion profile.

Convolution

Most diffusion models fall under a general framework which is based on the principle of convolution. Here the diffusion signal attenuation, A , measured along the gradient direction $\mathbf{g}(\theta_0, \phi_0)$ is considered to be the convolution of the fibre orientation distribution (FOD) with the fibre response function (FRF):

$$A(\theta_0, \phi_0) = \iint_{S^2} FOD(\theta, \phi) FRF(\theta_0 - \theta, \phi_0 - \phi) \sin(\theta) d\theta d\phi. \quad (2.22)$$

The fibre orientation distribution describes how the fibres are geometrically oriented within the voxel, and the fibre response function is the diffusion signal from a single fibre.

Following Equation 2.22, the fibre orientation distribution can be estimated from the data by either optimising a generative model [38, 39] or through a process of deconvolution [40]).

Whilst many biophysical diffusion models are based on the principle of convolution, the models differ in how they parameterise both the fibre orientation distribution and the fibre response function. Depending on the complexity of the model, the white matter may be considered to contain multiple independent compartments, each with their own unique diffusion profile.

Note, in the following section we describe compartments of the tissue having a *diffusivity*. This term refers to the apparent diffusion coefficient (ADC, *c.f.* *Water diffusion: sensitivity to microstructure*) as estimated from the data, where the measured diffusivity is inherently less than or equal to the free diffusivity of water.

The standard model

In the long diffusion time regime, the tissue is typically described using the ‘standard model’ [2]. Here axons are considered straight, impermeable cylinders, with intra-axonal axial diffusivity $d_{\parallel, in}$ and radial diffusivity $d_{\perp, in}$. When compared to the axial diffusivity $d_{\parallel, in} \sim 2 - 3 \mu\text{m}^2/\text{ms}$, the radial diffusivity is typically so small ($d_{\perp, in} \sim 0.01 \mu\text{m}^2/\text{ms}$) that, in the normal range of b-values, the radial diffusivity

has an approximately negligible affect on the diffusion signal. Therefore, many diffusion models assume ‘stick-like’ axons with no radial diffusion, an assumption which has been validated experimentally [41, 2]. The fibre response function is then described as,

$$FRF_{in} = e^{-bd_{\parallel, in}(\mathbf{g}^\top \boldsymbol{\mu})^2}. \quad (2.23)$$

The FRF is dependent on the angle between the orientation of the fibre $\boldsymbol{\mu}$ and the diffusion gradient $\mathbf{g}$, with maximum attenuation along the fibre, and minimum attenuation in the perpendicular plane.

The axons are considered to be surrounded by an extra-axonal space, which is typically assumed to be in full exchange with itself (i.e. extra-axonal water can, at infinite times, explore all extra-axonal space). Here we assume that glial cells are in fast exchange with the extra-cellular matrix. The extra-axonal fibre response function is described by an anisotropic Gaussian tensor,

$$FRF_{ex} = e^{-b\mathbf{g}^\top \mathbf{D} \mathbf{g}}. \quad (2.24)$$

The tensor $\mathbf{D}$ is symmetric about the diagonal and so is defined by six unknown values. Using eigenvalue decomposition, $\mathbf{D} = \mathbf{E}^\top \boldsymbol{\Lambda} \mathbf{E}$, the tensor $\mathbf{D}$ is defined by three eigenvectors $\boldsymbol{\epsilon}_{1,2,3}$ and three eigenvalues $\lambda_{1,2,3}$:

$$\mathbf{E} = \begin{bmatrix} \epsilon_{1,x} & \epsilon_{2,x} & \epsilon_{3,x} \\ \epsilon_{1,y} & \epsilon_{2,y} & \epsilon_{3,y} \\ \epsilon_{1,z} & \epsilon_{2,z} & \epsilon_{3,z} \end{bmatrix} \quad \boldsymbol{\Lambda} = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \quad (2.25)$$

Typically the eigenvalues are ordered such that $\lambda_1 > \lambda_2 > \lambda_3$. Often the tensor is assumed to be axially symmetric, where the axial diffusivity $d_{\parallel, ex} = \lambda_1$ and the radial diffusivity $d_{\perp, ex} = \lambda_2 = \lambda_3$. In this case, the extra-axonal fibre response function is,

$$FRF_{ex} = e^{-bd_{\parallel, ex}(\mathbf{g}^\top \boldsymbol{\mu})^2} e^{-bd_{\perp, ex}(1-(\mathbf{g}^\top \boldsymbol{\mu})^2)}. \quad (2.26)$$

The diffusion attenuation in each compartment is then calculated by convolving the compartment-specific fibre response function with the fibre orientation distribution (Equation 2.22).

Finally, some models include an additional isotropic compartment with diffusivity d_{iso} where,

$$A_{iso} = e^{-bd_{iso}}. \quad (2.27)$$

The isotropic compartment is often associated with CSF contributions where typically $d_{iso} = 3 \mu\text{m}^2/\text{ms}$, i.e. the approximate diffusivity of free water.

The diffusion signal attenuation from a white matter voxel is then the weighted sum of signals from each compartment:

$$A = f_{in}A_{in} + f_{ex}A_{ex} + f_{iso}A_{iso} \quad (2.28)$$

where f denotes the signal fraction associated with each compartment and $f_{in} + f_{ex} + f_{iso} = 1$.

Characterisation of this (arguably already reductionist and simplistic) description of the tissue requires the estimation of *many* free parameters: the fibre orientation distribution, as well as the diffusivities and the signal fractions of up to three different compartments. Diffusion MRI is an inherently low signal-to-noise technique and the fitting landscape is typically flat. Consequently, many combinations of the above parameters will fit the diffusion signal equally well and the model parameters are therefore ‘degenerate’. To overcome these degeneracies, diffusion models will either i) constrain some of the parameters, in the extreme fixing them to a predefined value (sometimes zero), or ii) explicitly relate various parameters to one another. For example, the extra-axonal radial diffusivity may be defined using the tortuosity model, $d_{\perp,ex} = f_{ex}d_{\parallel,ex}$. Diffusion models differ in their choice of parameter selection and/or modelling assumptions, resulting in each having unique constraints and limitations.

As it is beyond the scope of this thesis to detail all the different models (for a review see [37], [2] or [9]), below we detail four key models that both inspired the work of, and are used in, this thesis: diffusion tensor imaging [42] (DTI, *c.f.* *Chapters 4 & 5*), the ball and stick model [38] (BAS, *c.f.* *Chapter 5*), neurite orientation

dispersion and density imaging [39] (NODDI, *c.f.* Chapter 3) and constrained spherical deconvolution [40] (CSD, *c.f.* Chapter 4).

Diffusion tensor imaging

Introduced by Basser in 1994, diffusion tensor imaging (DTI) [42] models the diffusion signal using a rank-2 tensor that can be estimated through simple linear regression of the log-transformed signal attenuation. Though it is not typical to think of DTI in terms of the standard model, in the above framework, it would equate to $A = A_{ex}$, where the fibre orientation distribution is described by a delta function. Though the simplicity of the model limits biophysical interpretation of the parameters, it can characterise the anisotropy of the diffusion signal fairly well. Here, the fractional anisotropy (FA) is typically defined as [43],

$$FA = \sqrt{\frac{3}{2} \frac{\sqrt{(\lambda_1 - \hat{\lambda})^2 + (\lambda_2 - \hat{\lambda})^2 + (\lambda_3 - \hat{\lambda})^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}}, \quad (2.29)$$

where $\hat{\lambda}$ is the mean diffusivity,

$$\hat{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}. \quad (2.30)$$

The FA ranges from 0 to 1 and is generally high in the anisotropic white matter. In high FA regions we can assume the direction of highest diffusivity $\mathbf{e}_1$ corresponds to the primary orientation of white matter fibres. Due to its simplicity, DTI is one of the most commonly used diffusion models, where the DTI metrics have been associated with many white matter changes, from brain development and plasticity to numerous neurological conditions [21, 43, 44]. However, DTI is limited in its ability characterise voxels containing crossing fibres, where the DTI metrics should be interpreted with caution. Consequently, multi-tensor approaches exist where the diffusion signal is considered the weighted sum of many tensors (for example, see the Ball and Stick model below).

Ball and stick

As it's name suggests, the ball and stick (BAS) model [38] considers the signal attenuation A to be the weighted contributions from both an isotropic ‘ball’ and an anisotropic ‘stick’ compartment,

$$A = (1 - f)A_{iso} + fA_{aniso}, \quad (2.31)$$

where f is the signal fraction of the anisotropic compartment.

The anisotropic compartment is associated with axons where the FOD is described by a continuous distribution $H(\theta, \phi)$ and the FRF describes axons of zero radius (i.e. sticks) with an axial diffusivity d and no radial diffusivity,

$$A_{aniso} = \int_{S^2} H(\theta, \phi) \cdot \exp[-bd(\mathbf{g}^\top \boldsymbol{\mu})^2] dS. \quad (2.32)$$

The isotropic compartment is simply described by,

$$A_{iso} = \exp[-bd]. \quad (2.33)$$

To estimate f , the diffusivity of the isotropic compartment is considered equal to the anisotropic compartment, $d_{iso} = d$. In single-shell data, these two parameters are otherwise degenerate: a large isotropic diffusion signal could either come from a high d_{iso} or a large isotropic volume $(1 - f)$. Thus, the ball and stick model has 5 free parameters, S_0 , d , f , $\boldsymbol{\mu}(\theta, \phi)$.

The model is typically fitted through a Bayesian framework using a Markov Chain Monte Carlo (MCMC) method. MCMC estimates samples from the posterior distribution and so provides an estimate of uncertainty in each parameter. The spread in the distribution of θ and ϕ is often interpreted as being analogous to the fibre orientation dispersion.

The ball and stick model can be easily extended [45] to account for multiple (N) fibre populations per voxel through the estimation of numerous distributions $H_{i=1:N}$

with signal fractions $f_{i=1:N}$. Here the number of fibre populations is determined by the automatic relevance determination (ARD) which penalises the presence of additional fibre populations that are not supported by the data.

The ball and stick model can be extended to i) account for multi-shell data where d is replaced by a continuous gamma distribution of diffusivities [10], ii) tensor-like intra-axonal diffusion ('ball and zeppelins') or, iii) anisotropic dispersion ('ball and rackets') [46].

Though conceptually the ball and stick model and its variants appear reductionist, the estimated fibre orientations correspond well known neuroanatomy and the dispersion estimates from the ball and rackets model were found to closely resemble those from microscopy [47]. Furthermore, as the ball and stick model can estimate the orientations of multiple fibre populations per voxel - as well as the uncertainty associated with each orientation - it facilitates probabilistic tractography [45, 48] from which we can trace fibre bundles through crossing fibre regions and characterise the uncertainty in our estimated pathways (or 'streamlines') through the brain.

Neurite orientation dispersion and density imaging

Neurite Orientation Dispersion and Density Imaging (NODDI) [39] is a somewhat more biophysical interpretation of the white matter tissue structure. In NODDI, the diffusion attenuation arises from three compartments - isotropic (*iso*), extra-axonal (*ea*) and intra-axonal (*ia*) - each weighted by their own signal fraction,

$$A = f_{iso}A_{iso} + (1 - f_{iso})((1 - f_{in})A_{ex} + f_{in}A_{in}). \quad (2.34)$$

Here the *FOD* is considered a Watson distribution [49] oriented along $\boldsymbol{\mu}$, with symmetric dispersion described by $\kappa \in [0, \infty]$, where for perfectly aligned fibres

$\kappa = 0$. The Watson distribution has a normalising constant,

$$C_W(\kappa) = \sqrt{\frac{4\pi^3}{\kappa}} \operatorname{erfi}(\sqrt{\kappa}), \quad (2.35)$$

where erfi is the imaginary error function. The probability p of finding a fibre along $\boldsymbol{\mu}(\theta, \phi)$ is given by,

$$p(x) = \frac{1}{C_W} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2], \quad (2.36)$$

where $x(\theta, \phi)$ is a unit vector on the sphere.

To aid interpretability, the orientation dispersion κ can be converted into the orientation dispersion index *ODI* [39] ,

$$ODI = \frac{2}{\pi} \arctan(1/\kappa), \quad (2.37)$$

which ranges from 0 for perfectly aligned fibres, to 1 for isotropic dispersion.

The intra-axonal signal attenuation is modelled as the convolution of the Watson *FOD* with an *FRF* for stick-like fibres with diffusivity $d_{\parallel}$,

$$A_{in} = \frac{1}{C_W} \int_{S^2} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2] \cdot \exp[-bd_{\parallel}(\mathbf{g}^\top \mathbf{x})^2] dS. \quad (2.38)$$

The extra-axonal attenuation is considered to be the convolution of the Watson *FOD* with an *FRF* described by an axially symmetric diffusion tensor with axial diffusivity $d_{\parallel}$ and radial diffusivity $d_{\perp}$,

$$A_{ex} = \frac{1}{C_W} \int_{S^2} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2] \cdot \exp[-bd_{\parallel}(\mathbf{g}^\top \mathbf{x})^2] \exp[-bd_{\perp}(1 - (\mathbf{g}^\top \mathbf{x})^2)] dS. \quad (2.39)$$

Here the extra-axonal axial diffusivity is equal to the intra-axonal axial diffusivity, $d_{\parallel,ea} = d_{\parallel,ia} = d_{\parallel}$; the radial diffusivity is given by a simple tortuosity model and is linked to the signal fraction of the extra-axonal compartment where $d_{\perp} = d_{\parallel}(1 - f_{in})$.

The isotropic compartment with diffusivity d_{iso} is simply given by,

$$A_{iso} = \exp[-bd_{iso}] \quad (2.40)$$

Thus, the NODDI model has seven parameters: $f_{in}, \kappa, \mu(\theta, \phi), d_{\parallel}, f_{iso}$ and d_{iso} .

Again, to stop the model being degenerate, the diffusivities are fixed in vivo to $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$ and $d_{iso} = 3 \mu\text{m}^2/\text{ms}$, the latter being the approximate diffusivity of free water at body temperature. The remaining 5 free parameters are estimated from the data.

The degeneracy between the diffusivities of the intra- and extra-axonal space is highlighted by the extended NODDI model, NODDIDA [3] (NODDI with diffusivity assessment). In NODDIDA, the isotropic compartment is assumed to be zero, and the diffusivities are freely estimated from the data. Here the parameter estimation is found to be ill-posed, with two parameter sets fitting to the signal equally well.

NODDI is limited through its assumption of fixed, predefined diffusivities for the intra- and extra-axonal compartments. In Chapter 3, we show the extent to which varying these assumed diffusivities affect the remaining model parameters.

Constrained spherical deconvolution

Constrained spherical deconvolution [40] (CSD) estimates the fibre orientation distribution per voxel and is frequently used in connectivity analyses and tractography. As the *FRF* and *FOD* are described using spherical harmonics, the *FOD* can have a complex shape and describe many fibre populations in a voxel.

Spherical harmonics are a set of basis functions which describe the shape of a deformed sphere. When parametrised in terms of spherical harmonic coefficients, the *FOD* can be determined by a simple matrix inversion [50], making computation easy. Here the *FRF* is constrained to be axially symmetric whilst the *FOD* is symmetric about the origin, otherwise known as ‘antipodal symmetry’.

In CSD, the fibre response function is first either defined analytically or, as is most common, estimated from the data. Typically, the *FRF* is taken to be the average diffusion signal from the ‘most-likely’ single-fibre voxels. Multiple methods exist for

response function estimation [50, 40, 51, 52, 53], which are described on the MRTrix website [54]). In one of the simplest methods, the highest FA voxels are selected. Though multi-tissue CSD has been proposed [51, 52], the tissue-specific fibre response function does not typically differentiate between contributions from the isotropic, intra- or extra-axonal space but instead combines them into a single estimate.

After estimating an appropriate FRF , the FOD s are reconstructed on a voxel-wise basis by deconvolving the FRF from the diffusion signal. As the deconvolution is sensitive to noise, the spherical deconvolution is constrained to minimise physically impossible negative peaks in the reconstructed FOD [40]. To constrain the optimisation, a modified Tikhonov regularisation method [55] is typically employed to iteratively penalise negative amplitudes on the FOD and update the FOD estimation.

Note that additional processing of the FOD is required to extract biologically meaningful parameters such as fibre orientation (e.g. through peak finding) and dispersion. Furthermore, in Chapter 4 we demonstrate that a single, global fibre response function may not be appropriate [56].

2.3 Microscopy

Microscopy techniques visualise specific tissue features in high detail and so are frequently used to provide a ‘gold standard’ description of the brain against which MRI-derived tissue parameters are validated. Using various staining methods and microscopy contrasts, microscopy can be used to examine the detailed morphology of neural components such as glia, neuronal soma, axons or dendrites. This thesis focuses on imaging the myeloarchitecture of the brain i.e. the myelinated axons which make up the structural connectome. Here two microscopy techniques are considered: the histological staining of myelin and polarised light imaging.

2.3.1 Histopathology

Throughout the 19th and early 20th century, much effort went into visualising and documenting neural morphology through a technique known as histology. To this day, histology is used extensively in research to describe tissue microanatomy and in clinical practice for the diagnosis and staging of various diseases through tissue biopsies. This is because histology is viewed as a high-specificity technique: it facilitates the detailed visualisation of a certain cellular structure in thin tissue sections. Typically, excised tissue is sectioned into thin slices between ~ 5 and $100\text{ }\mu\text{m}$ thick. The tissue is then chemically stained and viewed under a microscope with micron or sub-micron resolution. Notably, in some cases only a small percentage of structures in the tissue are stained. Due to the high density of cells in tissue, were every cell to be stained, some structures could not be differentiated under the microscope.

One of the pioneers of modern day histology, Camillo Golgi, made this important realisation when perfecting a silver-staining method which he coined ‘the black reaction’ [57]. The stain had affinity to only a few neurons, but stained their structure - soma, dendrites and axon - in their entirety. In 1906 Golgi and Santiago Ramon y Cajal (who used Golgi’s stain to describe many important elements of the neuronal architecture) received the 1906 Nobel prize in medicine for their seminal work on understanding the nervous system [57].

Nowadays, there exists a vast range of dyes and chemical compounds for the selective staining of neural components. This includes immunohistochemistry techniques where antibodies attach a marker to a structure of interest via an antigen. Because we are interested in the myeloarchitecture, this thesis considers two complementary techniques for the visualisation of myelin: one based on immunohistochemistry (Chapter 4 proteo-lipid protein), the second a silver-staining technique (Chapter 5, Gallyas). After staining, the sections were imaged with sub-micron resolution from which quantitative parameters were extracted.

Structure tensor analysis

After digitising the myelin stained slides, we can use structure tensor analysis [58, 59, 60] to estimate the primary fibre orientation per microscopy pixel. Structure tensor analysis was performed in Chapters 4 & 5, the theory of which is outlined below.

In each histological pixel, we can define the structure tensor matrix J as [59],

$$J = \begin{bmatrix} \langle I_x, I_x \rangle_w & \langle I_x, I_y \rangle_w \\ \langle I_y, I_x \rangle_w & \langle I_y, I_y \rangle_w \end{bmatrix}, \quad (2.41)$$

where I_x , and I_y are the first derivatives of $I(x, y)$ along x and y respectively. The notation $\langle g, h \rangle_w$ describes how the two images g and h are first multiplied together and then convolved ($\otimes$) with a Gaussian kernel w_σ of width σ where,

$$\langle g, h \rangle_w = w_\sigma \otimes [g(x, y) \cdot h(x, y)]. \quad (2.42)$$

In both Chapter 4 & 5 the local neighbourhood of the Gaussian kernel, σ , was equivalent to 10 histological pixels of 0.28 μm .

The primary fibre orientation is then calculated as [59],

$$\theta = \frac{1}{2} \arctan \left(2 \frac{\langle I_x, I_y \rangle_w}{\langle I_y, I_y \rangle_w - \langle I_x, I_x \rangle_w} \right). \quad (2.43)$$

Comparing fibre orientations from MRI and histology

In Chapters 4 and 5 we directly compare the fibre orientations from histology with those from MRI. Diffusion MRI describes the geometric organisation of fibres within a voxel by the fibre orientation distribution. Therefore, for MRI-microscopy comparison, a similar histologically-derived fibre orientation distribution is generated by combining pixels over a local neighbourhood (a ‘superpixel’) into a histogram. Using data from Chapter 4, Figure 2.10 shows example fibre orientation distributions in both the corpus callosum and centrum semiovale.

When the local neighbourhood is equivalent to an MRI voxel, the histologically-derived fibre orientation distribution can be directly compared to an MR equivalent (cf. Chapters 4 & 5). Note, the MRI-derived fibre orientation distribution is 3D

whilst the histologically-derived fibre orientation distribution is typically only 2D. Thus, to facilitate a fair comparison, the 3D fibre orientation distribution must first be projected onto the 2D microscopy plane.

In Chapter 4 we demonstrate how the 3D fibre orientation distribution can be projected onto a 2D plane. Chapter 4 describes one example of how diffusion MRI and microscopy data can be combined to drive a single, joint model. The cost function of the joint model contains two data fidelity terms: one relating to the diffusion signal, the second to the microscopy-derived 2D fibre orientation distribution. To compare the 3D fibre orientation distribution FOD_{3D} (as estimated by the joint model) with the microscopy data, FOD_{3D} was projected onto the 2D microscopy plane. This projection was achieved as follows. We first estimated the 3D fibre orientation distribution for discrete points (θ, ϕ) on the sphere producing $FOD_{3D}(\theta, \phi)$. Due to the rotational symmetry of the 3D fibre orientation distribution, only a hemisphere ($\theta, \phi \in [-\pi/2, \pi/2]$) was required. The histological plane was defined as having an inclination angle $\phi = 0$. The projection of $FOD_{3D}(\theta, \phi)$ onto the 2D plane was thus defined as,

$$FOD_{2D}(\theta) = \sum_{\phi=-\pi/2}^{\pi/2} FOD_{3D}(\theta, \phi) \cos(\phi) \Delta\phi. \quad (2.44)$$

The $FOD_{2D}(\theta)$ was then normalised such that $\sum_{\theta} FOD_{2D}(\theta) = 1$. As FOD_{2D} and the microscopy-derived FOD_{micro} were defined over the same values of θ , the discrete distributions were directly comparable.

2.3.2 Polarised light imaging

Polarised light imaging is a method for estimating the orientation of white matter fibres from thin, unstained tissue sections with micron resolution [61, 62, 63]. The method exploits the negative uniaxial birefringence of myelinated axons - an optical property in which transmitted light experiences a larger refractive index along the ‘optic axis’ than across it. In brain tissue, birefringence occurs

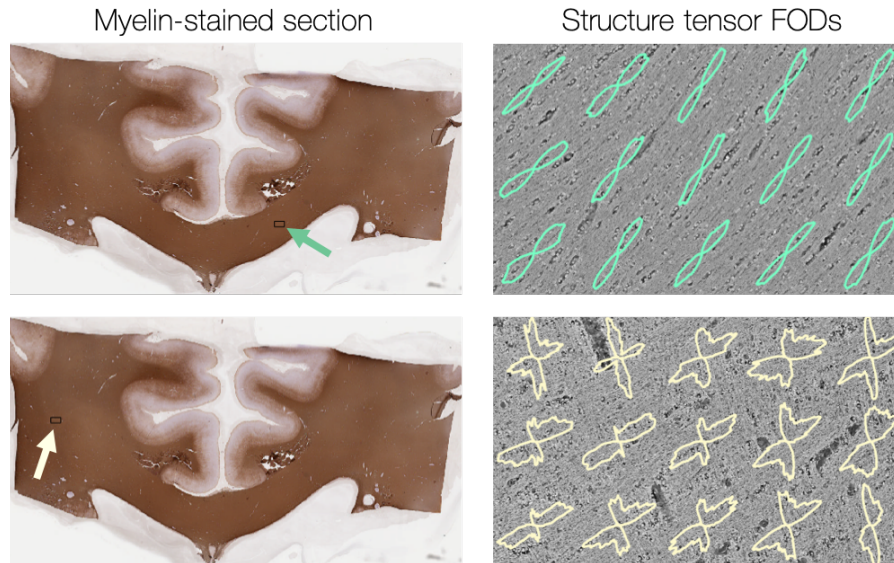


Figure 2.10: Using structure tensor analysis [58, 59, 60] of histological images to derive a 2D fibre orientation distribution. Left: A myelin-stained section (Chapter 4, section 3) was first imaged and then processed using structure tensor analysis. The extracted fibre orientations were then combined into histologically-derived 2D fibre orientation distributions (overlaid). In the corpus callosum (top) we see coherently oriented fibres and low dispersion in the fibre orientation distribution. In comparison, in the centrum semiovale (bottom) we see crossing fibres in the histology image which are reflected in the fibre orientation distribution as multiple, distinct fibre populations and more disperse fibre profiles. In addition, we see small areas of tissue tearing (at the tissue boundary, top right) and degradation (in the cingulum bundle), both of which are artefacts commonly found in histology data.

due to the arrangement of the lipid bilayer in the myelin membrane, where the optic axis aligns with the direction of the fibre [62]. When polarised light is transmitted through myelinated fibres, the polarisation state is changed according to the orientation of the optic axis. Thus, by quantifying this change in polarisation we can infer the local fibre orientation.

A typical confocal microscope can be adapted for PLI through the addition of a linear polariser, a quarter wave plate (oriented at an angle of 45°), and a rotating linear polariser or ‘analyser’ (Figure 2.11 left). We first give a short intuitive description of the setup, followed by a more rigorous mathematical description using Jones calculus.

Briefly, the linear polariser and quarter wave plate create circularly polarised light which is then transmitted through the sample. A birefringent sample will act to

change the state of the polarisation from circular to elliptical. The polarisation state of transmitted light is then measured by the analyser. Here, a number of images are acquired as the analyser is rotated through 180° in the transverse plane. A sinusoid is fitted to the pixelwise image intensity as a function of the analyser rotation (Figure 2.11 right). The peak-to-peak amplitude ΔI and phase φ of the sinusoid is dependent on the quantity and orientation of myelin in the tissue section.

Jones calculus

The PLI microscope can be effectively described by Jones calculus ([64],[65]). Here we need to understand four key principles outlined below.

Firstly, in Jones calculus, polarised light propagating along z is defined by a 2D Jones vector,

$$E = \begin{bmatrix} E_x \\ E_y \end{bmatrix}. \quad (2.45)$$

E is complex where there is some relative phase $\Delta\theta$ between the electric field components E_x and E_y . When $\Delta\theta = 0$ the light has linear polarisation, when $\Delta\theta = 90^\circ$ the light is circularly polarised. For all other $\Delta\theta$, E traces an ellipse in the transverse ($x - y$) plane and is described as having elliptical polarisation.

Secondly, optical elements are described by Jones matrices,

$$A = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}. \quad (2.46)$$

To give two relevant examples: i) a linear polariser along x or y is described by the matrix,

$$P_x = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}, \text{ or } P_y = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} \quad (2.47)$$

respectively, and ii) birefringent material is described as a ‘wave retarder’ which introduces a phase shift δ between the E_x and E_y components,

$$M_\delta = \frac{1}{2} \begin{bmatrix} e^{i\delta/2} & 0 \\ 0 & e^{-i\delta/2} \end{bmatrix}. \quad (2.48)$$

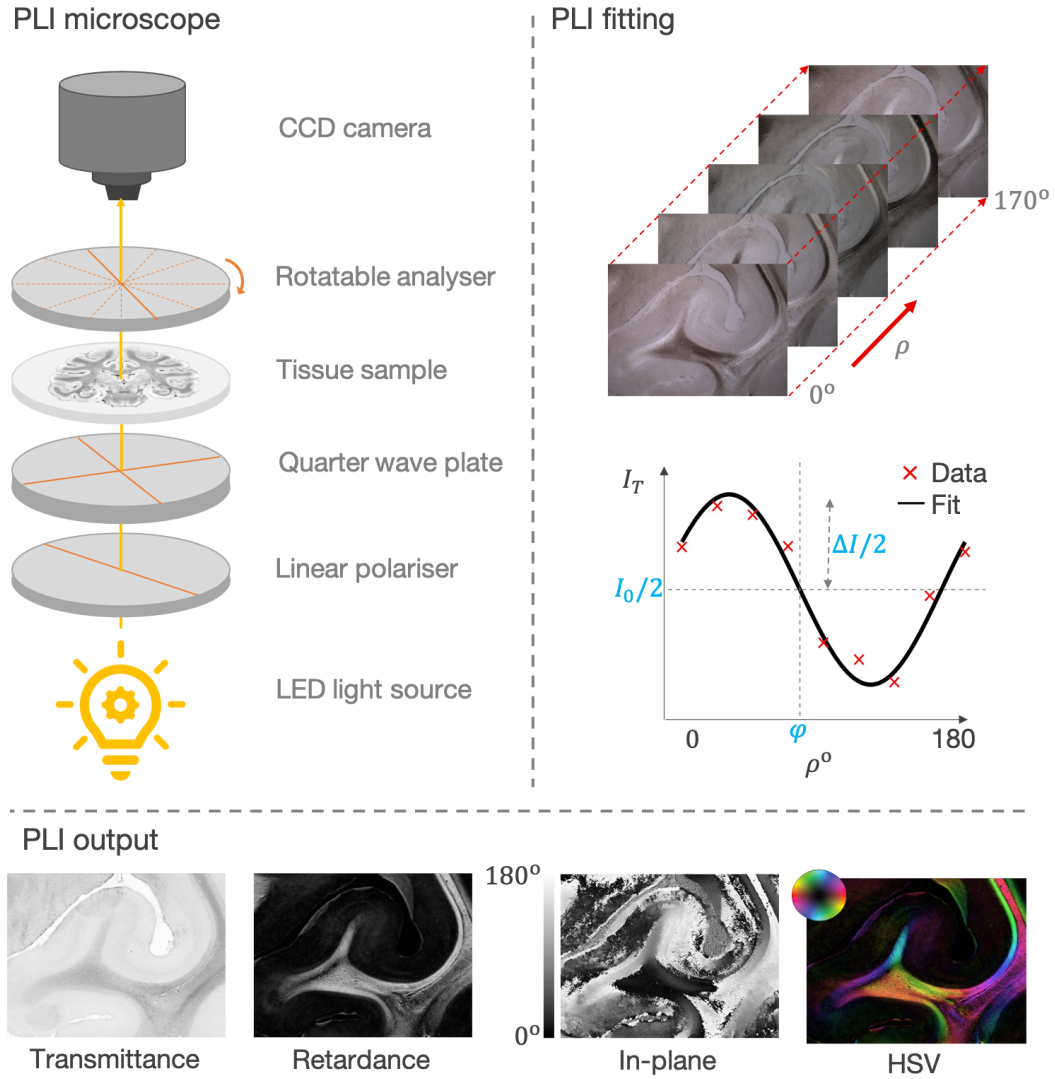


Figure 2.11: Left: A schematic diagram of a polarised light imaging microscope. A confocal microscope is adapted for polarised light imaging by the addition of two linear polarisers and a quarter wave plate at 45° to the first polarisers optic axis (shown in orange). The second polariser is rotatable and typically called the analyser. Right: Images are acquired as the analyser is rotated $\rho = 0 - 180^\circ$. For each pixel, a sinusoid is fitted to the transmitted intensity I_T to extract the transmittance I_0 , the in-plane fibre orientation φ , and the retardance $|\sin \delta|$. Bottom: Example output images. In the hue-saturation-value (HSV) image, the hue is defined by the in-plane angle and the value given by the retardance.

Thirdly, a rotation matrix,

$$R(\psi) = \begin{bmatrix} \cos(\psi) & -\sin(\psi) \\ \sin(\psi) & \cos(\psi) \end{bmatrix} \quad (2.49)$$

is used to describe a rotated optical element A' at angle ψ ,

$$A' = R(\psi) * A * R(\psi)^{-1}. \quad (2.50)$$

Finally, when light is transmitted through multiple optical elements (here $A_{1..3}$), the change of polarisation from input state E_0 to output E_T is given by,

$$E_T = A_3 * A_2 * A_1 * E_0. \quad (2.51)$$

Consequently, the PLI microscope is described in Jones calculus as

$$E_T = A(\rho) * S_\delta(\varphi) * Q_{\lambda/4} * E_0 \quad (2.52)$$

where $A(\rho)$ is the rotatable analyser, $S_\delta(\varphi)$ the sample, $Q_{\lambda/4}$ the quarter wave plate and E_0 the electric field after the first linear polariser. The Jones matrices or vectors related to each optical element are outlined below, beginning with E_0 .

As the Jones matrix formulation can only be applied to polarised light, the first microscope element - the linear polariser - converts the unpolarised light from the light source into linearly polarised light. The output from the linear polariser is described from here on in as E_0 , where

$$E_0 = \begin{bmatrix} 1 \\ 0 \end{bmatrix}, \quad (2.53)$$

Here the light is polarised along x .

The second element, the quarter wave plate, is oriented at 45° to the polariser below and adds a 90° phase shift between the E_x and E_y components. In Jones matrix notation, the quarter wave plate is described as

$$Q_{\lambda/4} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & -i \\ -i & 1 \end{bmatrix}. \quad (2.54)$$

Substituting $Q_{\lambda/4}$ and E_0 into Equation 2.51 we see mathematically how the quarter waveplate converts linearly polarised light into light with circular polarisation,

$$E_T = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & -i \\ -i & 1 \end{bmatrix} * \begin{bmatrix} 1 \\ 0 \end{bmatrix} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ -i \end{bmatrix}, \quad (2.55)$$

where $E_T \sim [1 \ -i]^\top$ describes light with right-handed circular polarisation. For interest, note how the quarter wave plate is a rotated wave retarder (Equation 2.48 and 2.49) where $\delta = 90^\circ$ and $\psi = 45^\circ$.

The third element, the sample, contains birefringent tissue and is described as a rotated wave retarder which introduces an unknown phase, δ ,

$$S_\delta(\varphi) = R(\varphi) * \frac{1}{2} \begin{bmatrix} e^{i\delta/2} & 0 \\ 0 & e^{-i\delta/2} \end{bmatrix} * R(\varphi)^{-1}, \quad (2.56)$$

where φ denotes the optic axis, i.e. the in-plane orientation of the fibre. The phase δ is dependent on the thickness d and the birefringence Δn of the myelin, the wavelength of the input light λ , and the inclination angle of the fibre α where [62],

$$\delta \approx 2\pi \frac{d\Delta n}{\lambda} \cos^2 \alpha. \quad (2.57)$$

α is defined $\alpha \in [-90^\circ, +90^\circ]$ where when $\alpha = 0$ the fibre is oriented within the imaging plane.

Finally, the analyser $A(\rho)$ is a linear polariser rotated through angles $\rho = 0 - 180^\circ$ where,

$$A(\rho) = R(\rho) * \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} * R(\rho)^{-1}. \quad (2.58)$$

When $\varphi = 0$ the polariser is oriented along y .

Putting all of this together and following the Beer-Lambert law $I_T \approx |E_T|^2$, the intensity of light at the detector is described by [61, 62],

$$I_T \approx \frac{I_0}{2} (1 + \sin(2\rho - 2\varphi) \sin \delta), \quad (2.59)$$

where $I_0 \sim |E_0|^2$. I_T is sinusoidal with respect to ρ with an average intensity $I_0/2$, peak-to-peak amplitude $\Delta I = |\sin \delta|$, and a phase dictated by φ .

PLI parameter estimation

To estimate the parameters I_0 , ΔI and φ , PL images are acquired as the analyser is rotated through $\rho = 0 - 180^\circ$. A sinusoid is then fitted to the image intensity per pixel as a function of ρ (Figure 2.11 top right).

PLI thus estimates three parameters:

1. The transmittance, I_0 . I_0 is twice the average intensity of light at the detector and is related to the amount of light extinguished by the sample.
2. The retardance, $\Delta I = |\sin \delta|$. ΔI describes the ellipticity of the polarisation and is determined by both the quantity and birefringence of myelin, as well as its orientation with respect to the imaging plane.
3. The in-plane angle, φ . The optic axis φ describes the azimuth or in-plane angle of the fibre.

To aid interpretation, a composite hue-saturation-value (HSV) image is often generated where the hue is defined by the in-plane angle and the value given by the retardance.

Figure 2.11 (bottom) shows example maps output from PLI. The transmittance is darker in myelinated regions where we see interesting contrast both within the white matter and the cortex. Axer *et al.* [63] compare the transmittance maps to myelin-stained sections and find a striking correspondence in the contrast between the modalities. Furthermore, Axer *et al.* describe how in these transmittance maps, additional myeloarchitectural features such as U-fibre regions can be identified.

We see high retardance across most of the white matter but low retardance in the grey matter and some crossing fibre regions. The retardance is low in crossing fibres regions when the pixel contains multiple, crossing fibre populations stacked on top of one another. A typical PLI section is $\sim 50 \mu\text{m}$ thick whilst a fibre is $\sim$

1 μm diameter. Therefore, when propagating through the tissue, the transmitted light will likely interact with multiple fibres. If the imaging pixel contains two fibre populations of approximately equal weighting oriented at perpendicular angles, the change in polarisation due to the first fibre population is equal and opposite to that of the second. Consequently, the net polarisation change (and thus retardance) is approximately zero and the estimated fibre orientations (both the in-plane and inclination angle) are uncertain or cannot be determined. Interestingly, if one fibre population is proportionally greater than the other, the estimated fibre orientation tends to align with the dominant fibre population [65].

In addition, a map of the fibre inclination angle α can be approximated from the retardance map and Equation 2.57. Due to the $\cos^2(\alpha)$ relationship, the sign of the inclination angle is ambiguous. In a technique coined ‘3D PLI’, Axer *et al.* [63] resolve this ambiguity through the use of a tilting sample stage to reconstruct the PLI fibre orientations in 3D. In Chapter 6 (*c.f. Future work*), we discuss how diffusion MRI could also be used to reconstruct the 3D PLI fibre orientations. Note again how our estimation of α is dependent on the quantity of myelin d . Typically d is assumed constant across the tissue to estimate α , though this is unlikely to be true in practice. Alternatively, Mollink *et al.* [66] compare the hippocampal white matter across subjects and, assuming the inclination angle is fairly consistent between subjects, use the PLI retardance as a measure of myelination.

3

Estimating intra-axonal axial diffusivity in the presence of fibre orientation dispersion

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Foreword

By analysing the diffusion MRI signal, we can infer information about the microscopic structure of the brain. Two parameters of interest - the intra-axonal axial diffusivity and fibre orientation dispersion - are potential biomarkers for very different aspects of the white matter microstructure, yet they are difficult to disentangle. The parameters covary such that, if one is not accurately accounted for, the other will be biased. In this work we use high b-value data to isolate the signal from the intra-axonal compartment and resolve any degeneracies with the extra-axonal compartment. In the high b-value regime, we then use a model of dispersed sticks to estimate the intra-axonal axial diffusivity and fibre orientation distribution on a voxelwise basis. Our results in in vivo, human data show an intra-axonal axial diffusivity of $\sim 2.3 - 3 \mu\text{m}^2/\text{ms}$, where $3 \mu\text{m}^2/\text{ms}$ is the diffusivity of free water at 37°C . The intra-axonal axial diffusivity is seen to vary considerably across the white matter. For example, in the corpus callosum we find high values in the genu and splenium, and lower values in the midbody. Furthermore, the axial diffusivity and orientation dispersion appear negatively correlated, behaviour which we show is consistent with the presence of fibre undulations but not consistent with a degeneracy between fanning fibres and axial diffusivity. Finally, we demonstrate that the parameter maps output from Neurite Orientation Dispersion and Density Imaging (NODDI) change substantially when the assumed axial diffusivity was increased from 1.7 to 2.5 or $3 \mu\text{m}^2/\text{ms}$.

Publications relating to this work

Journal articles:

Howard AFD, Lange FJ, Mollink J, Cottaar M, Drakesmith M, Rudrapatna US, Jones DK, Miller KL*, Jbabdi S*, “Estimating intra-axonal axial diffusivity in the presence of fibre orientation dispersion”, in submission.

Conference proceedings:

Howard AFD, Khrapitchev AA, Mollink J, Mars RB, Sibson N, Sallet, J, Jbabdi S*, Miller KL*, “Estimating intra-axonal axial diffusivity with diffusion MRI in the presence of fibre orientation dispersion”, Proceedings of the 28th Annual Meeting of the ISMRM, online, 2020.

3.1 Introduction

By analysing the diffusion MRI signal, we can infer information about the tissue microstructure to reveal the structural connectivity of the human brain [25], or characterise cellular changes in the myelo- or cyto-architecture [9]. Such inference, however, requires biophysical modelling of the tissue microstructure and accurate knowledge of the resultant diffusion characteristics [37, 2]. Furthermore, the model must be designed to consider the specific microstructural features which influence the diffusion signal.

Two parameters of interest are the intra-axonal axial diffusivity and the fibre orientation dispersion. The intra-axonal axial diffusivity describes the diffusion of water molecules inside the axon as they travel along the primary axis or orientation. Knowledge of the axial diffusivity often underpins our ability to accurately model the diffusion signal. The fibre orientation dispersion describes the extent of fibre fanning or coherence within a voxel. As a measure of white matter organisation, the orientation dispersion is important when determining structural connectivity and is a biomarker of brain development, ageing and various neurological diseases [39]. However, using typical data ($\sim b \leq 3 \text{ ms}/\mu\text{m}^2$) acquired in the long diffusion time limit, it is difficult to simultaneously estimate the intra-axonal axial diffusivity and orientation dispersion for two reasons.

Firstly, when modelling the intra-axonal compartment, both the intra-axonal axial diffusivity and fibre orientation dispersion must be explicitly co-modelled otherwise the parameter estimates will be biased. This is because the intra-axonal axial diffusivity and fibre orientation dispersion covary such that an inaccurate axial diffusivity (if either assumed or estimated from the data) leads to a misestimation of orientation dispersion, and vice versa.

Secondly, we generally receive signal from both intra- and extra-axonal water and therefore need to co-model the diffusion properties of these compartments. Here

there exist two degeneracies [3]: i) between the axial diffusivity of the intra- and extra-axonal compartments, and ii) between the orientation dispersion and the diffusion characteristics of the hindered extra-axonal compartment. For example, in the long diffusion time limit, an apparent radial diffusivity could be attributed to either radial diffusion within the extra-axonal space, or intra-axonal axial diffusion within a disperse fibre bundle.

To address these degeneracies, a common approach is to fix the diffusivities and estimate the fibre orientation dispersion. In constrained spherical deconvolution [40], the diffusion characteristics of the combined intra- and extra-axonal compartment (as described by the fibre response function) are typically first defined from the ‘most likely’ single-fibre voxels, and then assumed constant across the tissue to estimate the fibre orientation distribution. Neurite orientation dispersion and density imaging (NODDI) [39] takes an alternative approach, fixing the intra-axonal axial diffusivity to a global, predefined value (typically $1.7 \mu\text{m}^2/\text{ms}$). The axial diffusivity of the extra-axonal compartment is assumed equal to that of the intra-axonal compartment, and the extra-axonal radial diffusivity is defined by a tortuosity model that describes the hindrance of water in the extra-axonal space. Though these strong modelling assumptions make the problem tractable, numerous studies challenge their accuracy [67, 68, 69, 70, 71] where, if the assumed diffusivities are inaccurate, the estimation of the remaining model parameters will be biased.

In this work we aim to estimate the intra-axonal axial diffusivity by i) only considering signal from the intra-axonal compartment - this is achieved through high b-value, in vivo human data - and ii) explicitly modelling both the orientation dispersion and axial diffusivity on a voxelwise basis. High b-value is crucial because it eliminates the higher-diffusivity extra-axonal water such that only the intra-axonal compartment contributes signal [72, 73, 74, 2, 75, 76, 77], thus overcoming the above described degeneracies and allowing the simultaneous estimation of intra-axonal axial diffusivity and fibre orientation dispersion. We first demonstrate in simulated data the effect of getting some of the model assumptions wrong. For

example, as the model assumes symmetric fibre dispersion, we examine how the parameter estimates behave when the model is applied to simulated data with asymmetric dispersion. The model was then applied to high b-value data from 32 human subjects scanned with ultra-strong gradients. By estimating the axial diffusivity per voxel - rather than fitting to the average signal across many voxels - we demonstrate how the axial diffusivity appears to vary across the brain. Furthermore, we find a negative correlation between the axial diffusivity and orientation dispersion and demonstrate how fibre undulations may explain this behaviour. Finally, we explore how changing the assumed axial diffusivity in the NODDI model biases the remaining estimated parameters.

3.2 Theory

The diffusion signal S can be considered the summation of signal contributions $S_{i=1..N}$ from N compartments, weighted by their respective signal fractions f_i , where

$$S = \sum_i^N f_i S_i. \quad (3.1)$$

For example, in multi-compartment models, a proportion of the signal may be attributed to, inter-alia, the intra-axonal, extra-axonal or CSF compartments respectively. Note here that the signal fractions f_i do not necessarily equate to the volume fractions of the relative compartments [78].

At high b-values, the signal contribution from compartments with highly mobile water becomes negligible [72, 73, 74, 2, 75, 76, 77] and the diffusion signal can be reasonably attributed to arising solely from the intra-axonal compartment,

$$S \approx f_{in} S_{in}. \quad (3.2)$$

3.2.1 The dispersed stick model

The dispersed stick model is specifically designed for high b-value data where the entirety of the diffusion signal can be attributed to the intra-axonal compartment [72, 73, 74, 2, 75, 76, 77]. The model is graphically described in Figure 3.1. In the model, the diffusion signal along gradient direction $\mathbf{g}$ is given by the convolution of the fibre orientation distribution, which is assumed to be a Watson distribution [49, 39], and a fibre response function for stick-like fibres with Gaussian axial diffusion and no radial diffusion:

$$S \approx f_{in} S_0 \frac{1}{C_W} \int_{S^2} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2] \cdot \exp[-bd_{\parallel}(\mathbf{g}^\top \mathbf{x})^2] dS, \quad (3.3)$$

where the integrand is over $\mathbf{x} \in S^2$; f_{in} is the signal fraction of the intra-axonal compartment; $d_{\parallel}$ is the intra-axonal axial diffusivity; $\mathbf{x}$, a unit vector on the sphere S ; b , the b-value and S_0 is the non-diffusion weighted signal $S(b = 0)$. Here the fibre orientation distribution is described by a Watson distribution [49, 39],

$$\frac{1}{C_W} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2] \quad (3.4)$$

with orientation $\boldsymbol{\mu}$, dispersion κ and normalising constant C_W , where,

$$C_W = \int_{S^2} \exp[\kappa(\boldsymbol{\mu}^\top \mathbf{x})^2] dS = \int_{S^2} \exp[\mathbf{x}^\top [\kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top] \mathbf{x}] dS = 4\pi {}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top). \quad (3.5)$$

${}_1F_1(\alpha; \beta; \mathbf{X})$ is the confluent hypergeometric function of the first kind with a matrix argument $\mathbf{X}$.

The orientation dispersion index is defined as $\text{ODI} = 2/\pi \arctan(1/\kappa)$ [39] and ranges from 0 to 1, representing perfectly aligned and isotropic fibre distributions respectively. To aid comparison with previous literature, Appendix A shows how the ODI relates to the fanning angle in degrees.

The model thus has five free parameters: the axial diffusivity, $d_{\parallel}$; the orientation dispersion index, ODI; θ and ϕ which describe the primary fibre orientation of the Watson distribution, $\boldsymbol{\mu}(\theta, \phi)$ and $F = f_{in} S_0$, the scaling parameter. To solve for all

a) The dispersed stick model

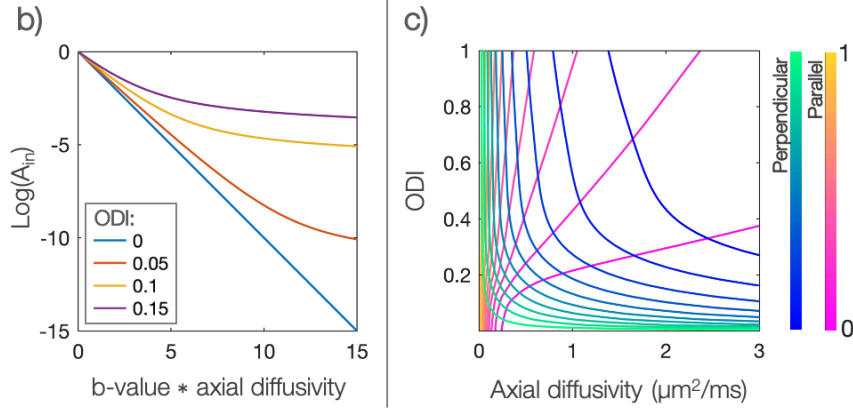
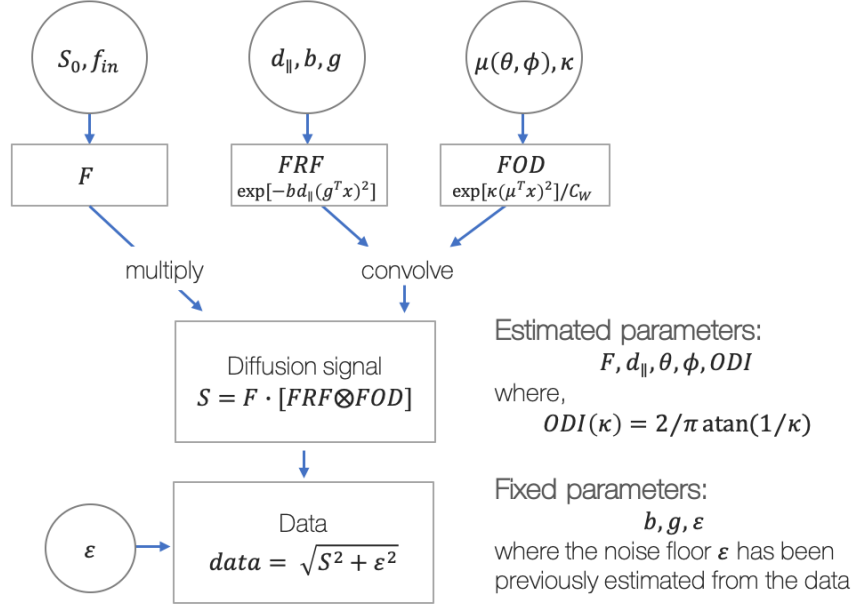


Figure 3.1: The dispersed stick model. a) In the generative model, the diffusion signal is given by the convolution of the fibre response function (FRF) and the fibre orientation distribution, (FOD), which is then scaled by the intra-axonal signal fraction f_{in} and the non-diffusion-weighted signal S_0 . For high b-value data, the FRF is solely attributed to the intra-axonal compartment with axial diffusivity $d_{\parallel}$. The FOD is described as a Watson distribution with primary orientation $\mu(\theta, \phi)$ and dispersion κ . Finally, the signal is combined quadratically with the noise floor ϵ which is first estimated from the data, and then assumed constant across the brain. b) In the presence of fibre orientation dispersion, the diffusion signal appears to vary with b-value. Thus, the orientation dispersion and axial diffusion should be estimated jointly. c) Equation 3.3 can be solved analytically for gradient directions $\mathbf{g}$ both perpendicular (blue-green) and parallel (pink-yellow) to the fibre μ . When analysed separately, each gradient orientation produces a degenerate solution (each line shows combinations of ODI and axial diffusivity which produce the same diffusion signal), but when combined, provide a unique solution (where the lines intersect).

parameters simultaneously and without parameter degeneracy, the dispersed stick model requires data with three b-values or shells, each with negligible contributions from the extra-axonal compartment i.e. $b > \sim 6 \text{ ms}/\mu\text{m}^2$ [41]. These three shells thus enable us to separate the three unknowns: the fibre orientation distribution, $d_{\parallel}$ and F . The $b \sim 0 \text{ ms}/\mu\text{m}^2$ data was not used during optimisation.

Note, the intra-axonal signal fraction with respect to the non-diffusion weighted signal, f_{in} , can be approximated as F/S_0 . As both the intra- and extra-axonal compartment contribute to the S_0 signal, this approximation assumes that the intra- and extra-axonal water has the same T2 properties. As this is unlikely true [79], the approximate f_{in} should be interpreted with caution.

The model is based on four main assumptions:

1. The fibre orientation distribution is accurately described by a Watson distribution.
2. Water experiences axons to be like “sticks” i.e. with no measurable radial diffusion.
3. There is a negligible contribution from extra-axonal water.
4. The voxel contains a single dispersed fibre population.

The latter is enforced for simplicity but could potentially be relaxed in future work. Furthermore, though there is likely a range of diffusivities within a voxel - where axons of different morphology (e.g. caliber or undulation) may have slightly different axial diffusivities - this analysis assumes that each voxel can be described by a single, ‘characteristic’ diffusivity. Finally, as the exchange time has been previously estimated to be considerably larger than typical diffusion times (seconds [80] versus milliseconds) we assume the axons to be impermeable with no slow exchange.

These assumptions require high b-value data which - to efficiently acquire data with high signal-to-noise ratio - are typically acquired using ultra-strong gradient

sets. As these gradient sets are available on only a few human scanners, the assumptions of the model likely limit its widespread application. However, the assumptions do enable the simultaneous estimation of the fibre dispersion and intra-axonal axial diffusion on a voxelwise basis, something that is difficult to do when also accounting for the extra-axonal space. Thus, the model may provide useful insights into quantifying the value of, and variations in, intra-axonal axial diffusivity across the brain. This information can then influence the development of future, more accurate, diffusion models.

Figure 3.1b shows the diffusion attenuation along the fibre ($\mathbf{g} = \boldsymbol{\mu}$) as a function of $b * d_{\parallel}$ for various levels of dispersion. Here we assume the signal arises solely from the intra-axonal compartment, $F = S_0$. With even a small amount of fibre dispersion within an imaging voxel, the intra-axonal axial diffusivity appears to vary with b-value. Note, an ODI of 0.05 or 0.1 relates to a dispersion angle (containing 50% of the fibres) of only 15° or 22° respectively (see Appendix A). If dispersion is ignored, this apparent deviation from Gaussian diffusion (a.k.a. non-zero kurtosis) could be erroneously assigned to a complex diffusion micro-environment other than fibre dispersion (a microstructure properties viewpoint), or it could bias the estimation of the axial diffusivity (a diffusion properties viewpoint). Thus Figure 3.1b highlights the importance of accurately determining the intra-axonal axial diffusivity on a voxelwise basis.

3.2.2 The analytic form

The dispersed stick model has an analytic form. The integral in Equation 3.3 can be written as the confluent hypergeometric function of the first kind with matrix argument $X = \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top - b d_{\parallel} \mathbf{g} \mathbf{g}^\top$, where

$$\begin{aligned} \int_{S^2} \exp[\kappa (\boldsymbol{\mu}^\top \mathbf{x})^2] \cdot \exp[-b d_{\parallel} (\mathbf{g}^\top \mathbf{x})^2] dS &= \int_{S^2} \exp[\mathbf{x}^\top (\kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top - b d_{\parallel} \mathbf{g} \mathbf{g}^\top) \mathbf{x}] dS \\ &= 4\pi {}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top - b d_{\parallel} \mathbf{g} \mathbf{g}^\top) \quad (3.6) \end{aligned}$$

Thus, the diffusion signal attenuation is given by,

$$A_{in} = \frac{{}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top - b d_{\parallel} \mathbf{g} \mathbf{g}^\top)}{{}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top)}, \quad (3.7)$$

and the diffusion signal by,

$$S \cong f_{in} S_0 \frac{{}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top - b d_{\parallel} \mathbf{g} \mathbf{g}^\top)}{{}_1F_1(1/2; 3/2; \kappa \boldsymbol{\mu} \boldsymbol{\mu}^\top)}. \quad (3.8)$$

Two examples of the analytic form are shown in Figure 3.1c: when the gradient is parallel and perpendicular to the fibre. Individual lines in Figure 3.1c represent all pairs of ODI and $d_{\parallel}$ that are consistent with a given diffusion signal. We see how the combined analysis of ≥ 2 unique gradient directions (which don't have the same angle to the fibre orientation $\boldsymbol{\mu}$) provides a unique solution for both the within-voxel fibre dispersion and axial diffusivity, i.e. each blue-green line intersects a pink-yellow line at exactly one point.

3.3 Methods

3.3.1 Model optimisation

During optimisation, the five parameters of the model were bounded such that $d_{\parallel} \in [0, 4] \mu\text{m}^2/\text{ms}$, $\text{ODI} \in [0, 1]$, $F > 0$, as well as θ and ϕ being unbounded. Note that the absolute limit of the axial diffusivity in pure water at 37°C is $\sim 3 - 3.1 \mu\text{m}^2/\text{ms}$, far less than the upper limit of $4 \mu\text{m}^2/\text{ms}$ used here. To reduce computation time, the in vivo data were optimised using the analytic solution and graphical processing units (GPUs) using FSL's CUDA Diffusion Modelling Toolbox, cuDIMOT [81]. The model was optimised using Markov Chain Monte Carlo (MCMC) - which afforded estimation of each parameter's distribution - and Rician noise modelling, both of which are internal functions of cuDIMOT [81]. Following the Ball and Rackets model [46], the hypergeometric function was calculated using the saddlepoint approximation [82] which had been previously implemented in cuDIMOT [81].

The dispersed stick model was applied to multi-shell in vivo human data acquired at b-values of 7.75, 11.6 and 15.5 ms/ μm^2 (data described in full below, *c.f.* 3.3.1 *High b-value data*). Upon inspection, data acquired parallel to the primary fibre orientation approached the rectified noise floor [83] which was subsequently accounted for in the model (Figure 3.2):

$$data = \sqrt{S^2 + \epsilon^2}. \quad (3.9)$$

Prior to model optimisation, the subject-specific noise floor ϵ was estimated directly from the data. Here the noise floor was taken to be the mean signal from voxels located in the ventricles for data with 30 gradient directions and $b = 15.5\text{ms}/\mu\text{m}^2$.

3.3.2 Simulations: model violations

Simulated data were used to examine how deviating from the assumptions 1-4 of section 2.2.1 in turn affected our two main parameters of interest: the axial diffusivity and ODI. Using generative models, data were simulated for conditions which violate our assumptions - asymmetric dispersion, non-zero radial diffusion, extra-axonal contributions and two fibre populations - after which the model was fitted to these data. Each of these simulated violations of our assumptions is described in detail below.

Multi-shell data were simulated using the same b-values and gradient directions as the in vivo data described below (*c.f.* *In vivo data*): 30 gradient directions each for $b = 7.75, 11.6, 15.5\text{ms}/\mu\text{m}^2$ and with $S_0 = 125$. No noise was added to the data. During optimisation the fibre orientation $\boldsymbol{\mu}(\theta = 0, \phi = 0)$ was fixed and the model optimised to estimate the axial diffusivity and ODI. When the simulated data contained contributions from the extra-axonal space, the scaling parameter $F = f_{in} * S_0$ was also fitted. For all other simulated data (i.e. with asymmetric dispersion, non-zero radial diffusion and two fibre populations), the scaling parameter was fixed to the ground truth value $F = 0.5 * S_0$. The ground truth

values for axial diffusivity and ODI were $2.2 \mu\text{m}^2/\text{ms}$ and 0.1 respectively, where the latter is equivalent to a 50% opening angle of 22° (Appendix A). In each case, the model was fitted assuming stick-like fibres described by a Watson distribution with signal contributions arising from only the intra-axonal compartment. The model was optimised using the Metropolis Hastings method [84] where the mean of each parameter distribution is reported.

Violating assumption 1: asymmetric fibre orientation distribution

The model assumes a Watson fibre orientation distribution characterised by symmetric dispersion about a primary fibre orientation [49, 39]. However, the true distribution is likely much more complex. Here we simulated data for a fibre orientation distribution described by a Bingham distribution to depict asymmetric fibre dispersion [85, 46, 86]. The Bingham distribution has two axes of dispersion about the primary fibre orientation μ , with dispersion constants κ_1 and κ_2 . When $\kappa_1 = \kappa_2$, the dispersion is symmetric and the Bingham is again equivalent to the Watson distribution. A Bingham distribution can therefore describe, for example, ‘sheet-like’ fibre distributions which are tightly aligned along one axis, but highly disperse along a second. Data were simulated for Bingham distributions with varying degrees of asymmetry, $\text{ODI}_1 / \text{ODI}_2 = [1, 3, 9]$, whilst keeping the mean dispersion constant.

Violating assumption 2: non-zero radial diffusivity

The model assumes stick-like axons with water diffusion purely along the fibre and no radial diffusivity. Here data were simulated for fibres with non-zero radial diffusivity, where the diffusion profile - characterised by an axially symmetric diffusion tensor with $d_{\parallel} = 2 \mu\text{m}^2/\text{ms}$ and $d_{\perp} = 0, 0.05, 0.01, 0.02 \mu\text{m}^2/\text{ms}$ - was convolved with the disperse fibre orientation distribution.

Violating assumption 3: extra-axonal compartment

The model assumes that, by working in the high b-value regime, the diffusion signal from the extra-axonal space is negligible and thus the signal can be attributed solely to the intra-axonal compartment. Here, data were simulated for a two compartment model: an intra-axonal compartment described by stick-like diffusion, and an extra-axonal compartment described by an axially-symmetric diffusion tensor. In both compartments, the diffusion profile was convolved with the disperse fibre distribution. As in the NODDI model [39], the extra-axonal axial diffusivity was equal to the intra-axonal axial diffusivity, $d_{ex,\parallel} = d_{in,\parallel} = d_{\parallel}$, whilst the extra-axonal radial diffusivity was linked to the volume fraction of the extra-axonal compartment where $d_{ex,\perp} = d_{\parallel}(1 - f_{in})$. The intra- and extra-axonal volume fractions sum to one i.e. $f_{in} + f_{ex} = 1$.

Violating assumption 4: multiple fibre populations

The model assumes that there is a single fibre population in each voxel, though many of the white matter voxels contain more than one fibre population. Here data were simulated for a voxel with two fibre populations of equal weighting or signal fraction. The average fibre orientation was held constant whilst the angle between the fibres was varied, $\gamma = 0 - 60^\circ$. Each fibre population had a ground truth ODI of 0.1.

3.3.3 Simulations: undulations

As we will show in Figure 3.7 (*c.f* 4.2 High b-value, in vivo data), for voxels with low dispersion, there is a negative relationship between intra-axonal axial diffusivity and fibre orientation dispersion. Given that a degeneracy between these parameters would result in the opposite relationship (positive correlation), this cannot be explained due to fitting. Therefore, we questioned whether there was some feature of the data that was unaccounted for in the model, but which resulted

in a signal that our model could only describe through a coupling of low dispersion with high axial diffusivity (and vice versa).

One possibility is if, rather than fanned sticks, fibre undulations lead to apparent dispersion [87, 88, 89, 90, 91]. Though both fibre fanning and undulations are arguably a form of microscopic dispersion, fibre fanning is an effect of pooling over a number of axons and undulations are a feature of individual axons. Whilst both may contribute the total amount of dispersion within a voxel, they may affect the apparent axial diffusivity in different ways. Intuitively, undulations could reduce the apparent intra-axonal axial diffusivity as water diffusing along the primary fibre "axis" would experience hindrance due to interactions with the meandering membrane wall.

To explore how fibre undulations affect the estimated intra-axonal axial diffusivity and ODI, data were simulated for a simple 2D set-up in which the wavelength and amplitude of fibre undulations could be varied. The dispersed stick model was then fitted to these data. The diffusion MRI signal was simulated using a Monte-Carlo simulation of particle displacement according to Brownian motion. To imitate diffusion along an undulating fibre of zero-radius, the particle movements were confined to a single 2D line described by a sinusoid,

$$f(z) = [A \cos(2\pi z/\lambda), 0, z], \quad (3.10)$$

where $f(z)$ defined the particle position when confined to a fibre oriented along the z axis with curvature or undulations along x ; A and λ are the amplitude and wavelength of the undulations respectively.

For an incremental time step $t = 0.1\text{ms}$, each particle stepped tangentially to the curve, where the step size is randomly drawn from a Gaussian distribution $\mathcal{N}(0, \sqrt{2t})$. A pair of diffusion sensitising gradients of strength G , duration δ and spacing (i.e. diffusion time) Δ were applied, during which each spin accrued phase according to its position. The diffusion signal was then calculated as the

magnitude of the sum of signals from all particles. The gradient strength G was dependent on the desired b-value, where

$$G = \frac{\sqrt{(b/(\Delta - \delta/3))}}{\gamma\delta}. \quad (3.11)$$

The acquisition parameters b, Δ , and δ were chosen to mimic those of the in vivo data used in this study.

3.3.4 In vivo human data

High b-value data

MRI data were acquired for 32 healthy participants at the Cardiff University Research Imaging Centre (CUBRIC). The study was approved by the Cardiff University School of Psychology Ethics Committee and written informed consent was obtained from all subjects. Participants were scanned on a 3 T Connectom scanner (Siemens Healthcare, Erlangen, Germany) with an ultra-strong 330 mT/m gradient set [92, 93].

T1-weighted images were used to register each subject to standard space. T1-weighted images were acquired with a Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence and 1 mm isotropic resolution. The T1 images were bias corrected (FSL's FAST [94]) and non-linearly registered to MNI standard space (FSL's FNIRT [95, 96]).

Diffusion-weighted data were acquired with a spin-echo Echo Planar Imaging (EPI) sequence (blipped-CAIPI) [97]: $TR/TE = 3900/80\text{ms}$, $FOV = 220 \times 220$ mm, matrix = 110×110 , 66 slices, 2 mm isotropic voxels and Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) factor = 2. Data were acquired at b-values of 7.75, 11.6 and 15.5 $\text{ms}/\mu\text{m}^2$ where $\delta/\Delta = 7/42$ ms for $b = 11.6 \text{ ms}/\mu\text{m}^2$ and $\delta/\Delta = 7/55$ ms for $b = 7.75, 15.5 \text{ ms}/\mu\text{m}^2$. The different diffusion times were not required for the model, where ideally all data would be acquired with the same Δ .

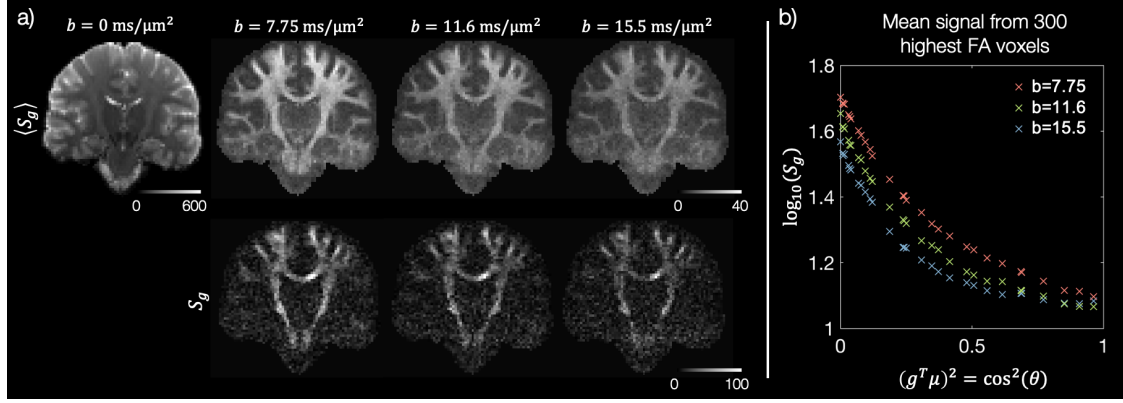


Figure 3.2: a) Example diffusion weighted images from a single subject. Top: the mean image across all gradient directions for $b = 0 \text{ ms}/\mu\text{m}^2$ (12 volumes), $b = 7.75$, 11.6 and $15.5 \text{ ms}/\mu\text{m}^2$ (30 volumes each). Bottom: an example diffusion-weighted image. Here we see how, at very high b-values, we retain considerable signal in the tracts perpendicular to the gradient direction and high diffusion contrast. b) The mean signal intensity from the 300 highest FA voxels is plotted as a function of θ , the angle between the fibres primary axis μ and the gradient direction g .

However, in this long diffusion time regime, the effect of the different Δ is likely small if not negligible. 30 gradient directions were acquired per b-value. An additional 13 volumes were acquired with negligible diffusion weighting ($b \sim 0 \text{ ms}/\mu\text{m}^2$): 12 were interspersed in the diffusion-weighted volumes to track subject motion and the 13th was acquired with reverse phase encoding for susceptibility distortion correction. Note, the $b \sim 0 \text{ ms}/\mu\text{m}^2$ data were not used in the fitting of the dispersed stick model.

The diffusion-weighted data were corrected for susceptibility and eddy current distortions, as well as subject motion (FSL’s TOPUP [98, 99] and EDDY with outlier replacement [100, 101]). The dispersed stick model was optimised on the distortion corrected data in subject space, after which the results were warped to standard space and the mean map across subjects calculated.

Figure 3.2 shows example preprocessed images from the diffusion protocol. In Figure 3.2a we see how the images retain good signal and excellent diffusion contrast at very high b-values. Figure 3.2b shows the mean diffusion signal for each b-value as a function of θ , the angle between the fibre’s primary axis μ and the gradient direction g . Here the 300 highest FA voxels were selected and reoriented to align

with the z axis. Using linear interpolation, the diffusion signal was estimated for each voxel along consistent gradient directions. The mean signal across voxels for each gradient direction is shown. Here we see the signal from all three b-values plateau around $\log_{10}(S_g) \sim 1.08$, indicative of the signal attenuation parallel to the fibre reaching a noise floor of this magnitude.

Human Connectome Project data

As we will show in section 3.4.2 *Results: High b-value in vivo data*, the dispersed stick model estimates an intra-axonal axial diffusivity in the range of $2.3 - 3 \mu\text{m}^2/\text{ms}$ with an average of $2.52 \mu\text{m}^2/\text{ms}$ in the white matter. This is considerably higher than that assumed by one of the most commonly used diffusion models NODDI. To estimate the neurite orientation dispersion and ‘density’, NODDI [39] assumes a predefined value of axial diffusivity which is typically $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$. Due to the discrepancy between the assumed intra-axonal axial diffusivity of NODDI and the intra-axonal axial diffusivity estimates found in this study, we decided to explore how changing the assumed axial diffusivity affected the output of NODDI.

As NODDI models both the intra- and extra-axonal space, we were unable to use the high b-value data above, and so instead utilised preprocessed T1-weighted and diffusion-weighted data for the first 10 subjects of the WU-Minn Human Connectome Project (HCP); for details of the acquisition protocol and preprocessing pipeline, please see [102, 103, 104]. Briefly, the diffusion-weighted data included 90 gradient directions each at b-values of $b = 1, 2$ and $3 \text{ ms}/\mu\text{m}^2$ and 18 interspersed volumes with negligible diffusion weighting. The distortion corrected diffusion-weighted data were linearly registered to each subjects T1-weighted structural scan (FSL’s FLIRT [105, 106]), and the T1 non-linearly registered the MNI standard space (FSL’s FNIRT [95, 96]). The NODDI model was applied to the diffusion data with an assumed axial diffusivity of either $d_{\parallel} = 1.7, 2.5$ or $3 \mu\text{m}^2/\text{ms}$.

3.4 Results

3.4.1 Simulations: violating modelling assumptions

Figure 3.3 explores our sensitivity to the modelling assumptions. In Figure 3.3a, data were simulated for a fibre orientation distribution described by a Bingham distribution with asymmetric dispersion along two axes ($\text{ODI}_{1,2}$). Here the ‘ground truth’ ODI represents the average dispersion across both axes which was kept constant at $\langle \text{ODI} \rangle = 0.1$. We see a negative relationship between the estimated axial diffusivity and ODI where for fibre populations with highly asymmetric dispersion, i.e. ‘sheet-like’ fibres, the orientation dispersion is underestimated and the axial diffusivity overestimated. For lower - and perhaps more realistic - levels of fibre asymmetry, the difference between the estimated and ground truth axial diffusivity and ODI is small.

In Figure 3.3b, axons are considered prolate tensors instead of sticks, with some diffusivity perpendicular to the fibre, $d_{\perp}$, here described by an axially-symmetric diffusion tensor. We see how violation of the stick-model substantially increases the estimated diffusivity, whilst the ODI is relatively unaffected.

In Figure 3.3c, both an intra-axonal and extra-axonal compartment contribute to the simulated diffusion signal. We see how when the signal fraction of the intra-axonal compartment is very large $f_{in} = 0.9$ (so that the extra-axonal compartment is small, $f_{ex} = 1 - f_{in}$), contributions from the extra-axonal compartment increase the estimated axial diffusivity, whilst the ODI remains close to the ground truth value. Furthermore, there is a non-linear relationship between the estimated axial diffusivity and the intra-axonal volume fraction where, when $f_{in} < 0.9$, the overestimation of the axial diffusivity is reduced as f_{in} decreases. These results can be understood if we consider two effects: i) the extra-axonal radial diffusivity is determined by the intra-axonal signal fraction $d_{ex,\perp} = d_{\parallel}(1 - f_{in})$ such that when f_{in} is large, $d_{ex,\perp}$ is small, and ii) at high b-value, the diffusion signal is sensitive to compartments where

the diffusivity *is* small (whilst signal contributions from compartments with higher diffusivities are negligible). Thus, when f_{in} is large (and thus $d_{ex,\perp}$ small), our signal is sensitive to contributions from the extra-axonal space, but when f_{in} increases and thus $d_{ex,\perp}$ decreases, the contributions from the extra-axonal compartment diminish. Notably, in Figure 3.3c, when $0.5 < f_{in} < 0.7$ (i.e. more typically observed intra-axonal signal fractions [37]), the overestimation of the intra-axonal axial diffusivity is relatively small ($d_{\parallel} = 2.2 - 2.3 \mu^2/\text{ms}$).

Finally, Figure 3.3d shows how the estimated ODI is increased from the ground truth and the axial diffusivity reduced when data were simulated for two fibre populations of equal weighting. Here the two fibre populations have a fixed average orientation and variable opening angle $0 \leq \gamma \leq 60$. Note that these details would likely differ when investigating more complex multiple fibre populations where, for example, the fibre populations are unequally weighted or where each has a unique value of axial diffusivity.

3.4.2 Model estimates in high b-value, in vivo data

The dispersed stick model was applied to multi-shell, high b-value data from 32 subjects. Figure 3.4 shows maps of the mean intra-axonal axial diffusivity, ODI and intra-axonal signal fraction across all subjects. In the vast majority of voxels across the brain, the intra-axonal axial diffusivity lies between $\sim 2.3 \mu^2/\text{ms}$ and $3 \mu^2/\text{ms}$ with generally higher axial diffusivity in the white matter than the grey matter ($\langle d_{\parallel} \rangle_{wm} = 2.52 \mu^2/\text{ms}$ and $\langle d_{\parallel} \rangle_{gm} = 2.39 \mu^2/\text{ms}$ respectively). Note, the dispersed stick model may not be a good representation of the tissue structure of the grey matter which comprises cell bodies, dendrites and axons. Though it is typically assumed that stick like diffusion likely represents that from both axons and dendrites where, in the grey matter, the intra-axonal axial diffusivity could better interpreted as an intra-*neurite* axial diffusivity, recent work by Lampinen *et al.* [107] challenges the assumption of stick-like dendrites and we should interpret

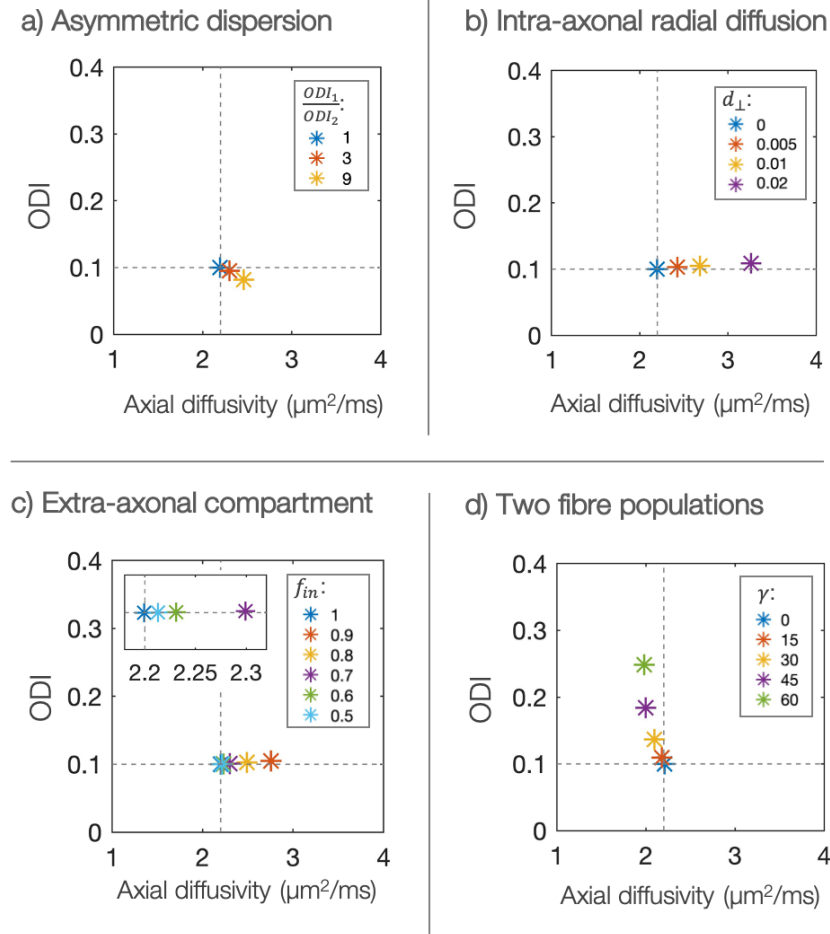


Figure 3.3: Violating the four assumptions of the model. Dashed lines show ground truth values. Data were simulated for a) asymmetric fibre dispersion, b) cylindrical fibres described by an axially symmetric diffusion tensor with intra-axonal radial diffusivity $d_{\perp}$, c) an extra-axonal compartment described by an axially symmetric diffusion tensor with $d_{ex,\parallel} = d_{in,\parallel}$ and $d_{ex,\perp} = d_{\parallel}(1 - f_{in})$ [39], and d) two fibre populations of equal weighting and opening angle γ . In each case, the model was fitted assuming a Watson-like fibre distribution with symmetric dispersion, stick-like diffusion, no extra-axonal contribution and only a single fibre population per voxel. The model was fitted using the Metropolis Hastings method [84] where each point in the Figure shows the mean value of the parameter estimate distribution. Note, so that we can more easily distinguish some of the points more easily, Figure c inset shows the region $2.19 < d_{\parallel} < 2.31$ in greater detail.

these grey matter results with caution. Nonetheless, the maps of ODI and intra-axonal signal fraction are broadly as we would expect: the ODI is low in the white matter and high in the grey matter, whilst the intra-axonal signal fraction is high in the white matter and low in the grey matter. Note, the signal fraction map is somewhat noisy in low f_{in} regions of the grey matter. Finally, the maps in Figure 3.4 demonstrate left-right symmetry and spatial smoothness, matching symmetric and smoothly changing anatomy. From here on in, we focus on the results in the white matter where the orientation dispersion is low and the model likely more representative of the underlying tissue.

Variations across the white matter

In Figure 3.4, the axial diffusivity is particularly high ($d_{\parallel} \geq 2.6 \mu\text{m}^2/\text{ms}$) and ODI particularly low ($\text{ODI} < 0.1$) in regions of the corpus callosum, forceps major, forceps minor, fornix, anterior thalamic radiation, corticospinal tract, optic radiation, inferior fronto-occipital fasciculus, portions of the superior longitudinal fasciculus, as well as regions of the brain stem including the superior cerebellar peduncle, medial lemniscus and pontine crossing tract. Interestingly, the highest values of axial diffusivity ($d_{\parallel} \geq 2.9 \mu\text{m}^2/\text{ms}$) were found mostly in the genu of the corpus callosum, though also to some extent in the body of the corpus callosum, the anterior external capsule, the fornix, the optic radiation and the superior cerebellar peduncle. Here the maps were clipped at $\text{ODI} < 0.1$ and either $d_{\parallel} \geq 2.6$ or $d_{\parallel} \geq 2.9 \mu\text{m}^2/\text{ms}$, and visually compared to the JHU ICBM-DTI-81 white matter labels [108], the JHU white matter tractography atlas [109, 110] and the Juelich histological atlas [111, 112, 113], included in FSLeyes [114] as part of the FSL suite [99].

To check whether these results were being driven by mis-registration of single subject's data to the common template, Figures 3.5 and 3.6 show the output maps from tract-based spatial statistics (TBSS) [115]. TBSS is a method for combining white matter tissue parameters across subjects, whilst avoiding artefacts due to mis-registration to the common template and partial volume effects masquerading

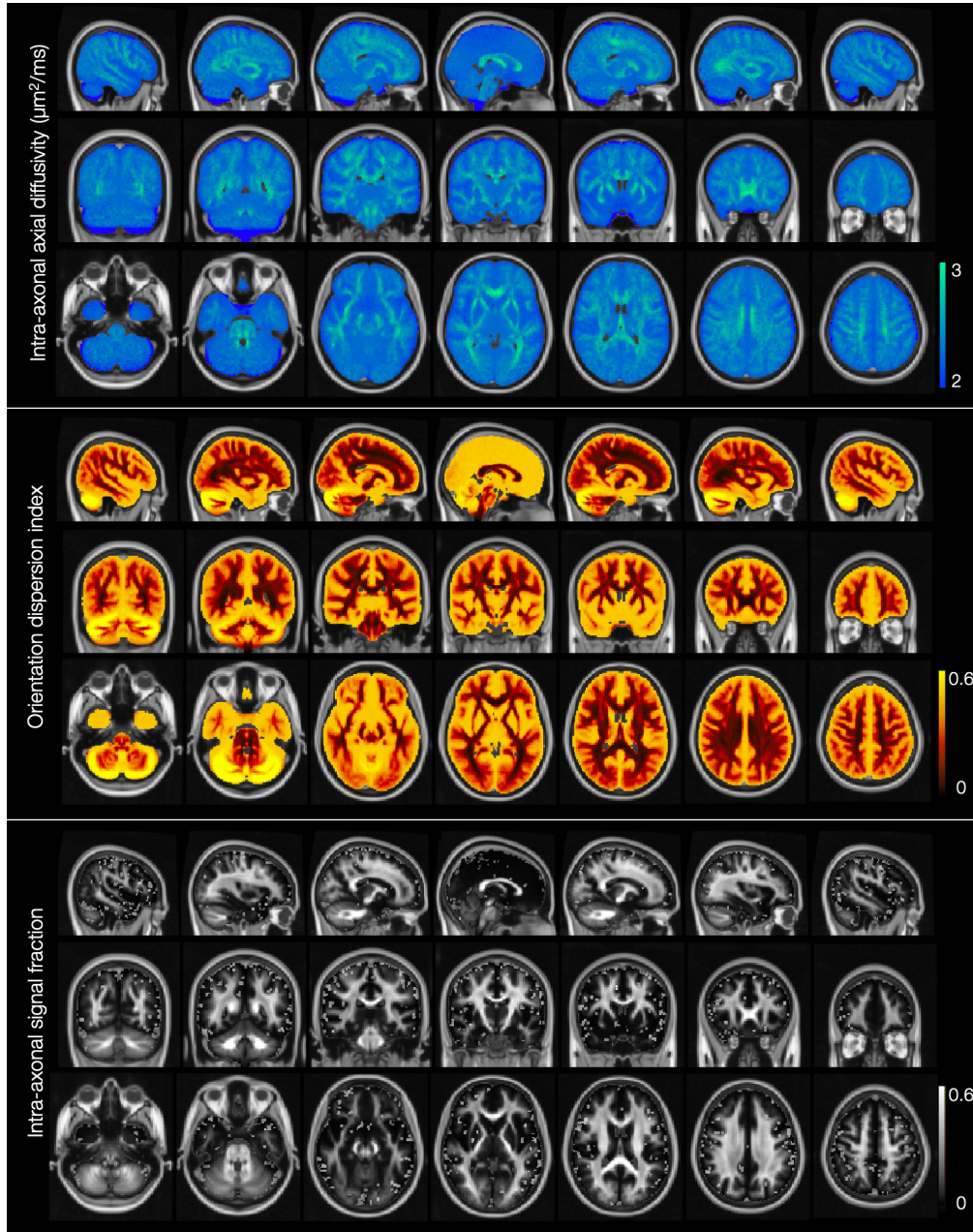


Figure 3.4: The intra-axonal axial diffusivity, orientation dispersion index and intra-axonal signal fraction as estimated from the dispersed stick model. The dispersed stick model was applied to data from 32 subjects and the mean parameter estimate across subjects is shown. The majority of voxels across the brain show an intra-axonal axial diffusivity of $2 < d_{\parallel} < 3 \mu\text{m}^2/\text{ms}$, where the axial diffusivity appears to vary considerably across the brain. As expected, the orientation dispersion index, ODI, is much lower in the white matter than the grey matter, with the lowest ODI values in regions of highly aligned fibres such as the corpus callosum. Assuming the T2 of the intra- and extra-axonal compartments are the same, the approximate intra-axonal signal fraction can be estimated as F/S_0 . The intra-axonal signal fraction is high in the white matter and low in the grey matter.

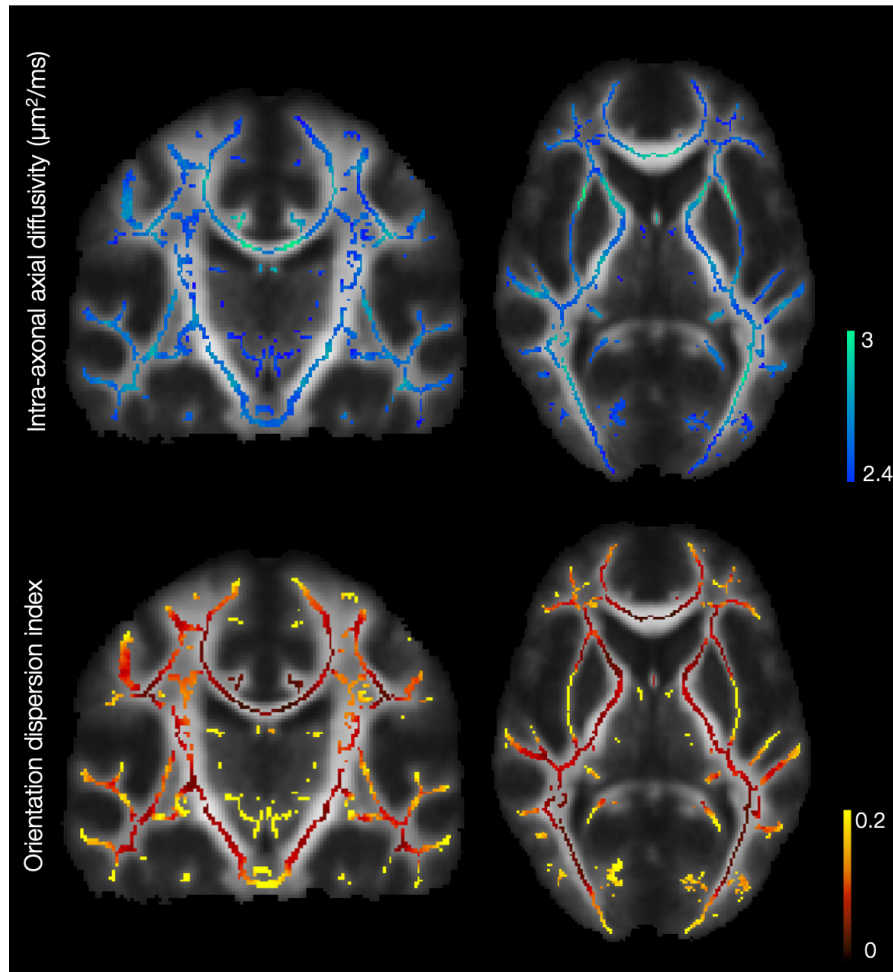


Figure 3.5: The intra-axonal axial diffusivity and ODI maps output from tract-based spatial statistics, TBSS [115]. Note here the change in scale bar for the ODI when compared to Figure 3.4. The axial diffusivity shows clear, symmetric patterns of variation across the white matter, with particularly high values in the internal capsule, external capsule, optic radiation, and in the mid-body of the corpus callosum, with slightly lower values at the midline. The ODI is particularly low in the centre of major tracts such as the corpus callosum, optic radiation and regions of the corticospinal tract.

as changes in diffusion properties. Here TBSS was used to generate a skeleton mask representing the centre of the white matter tracts. For each subject, the axial diffusivity and ODI estimated from the dispersed stick model were then projected onto the skeleton mask, after which the mean value across subjects was calculated. Note here the change in scale bar for the ODI from Figure 3.4. In Figure 3.5 we see many of the same high axial diffusivity regions as described above, giving confidence to our results. Furthermore, Figure 3.5 highlights regions of high axial diffusivity in the mid-body of the corpus callosum when slightly away from the midline.

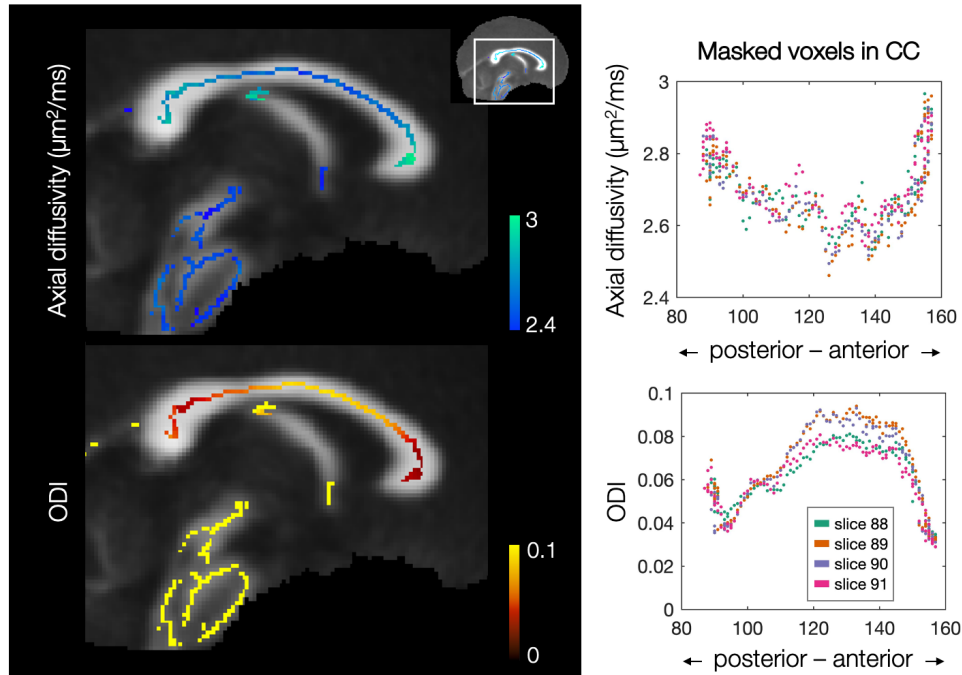


Figure 3.6: Variations in the intra-axonal axial diffusivity (top) and fibre orientation dispersion (bottom) across the corpus callosum. Here we show the mean parameter maps after tract-based spatial statistics, TBSS [115]. For interpretation, the mean parameter estimates (across subjects) are overlaid on a mean FA image. We see a high-low-high pattern of axial diffusivity along the anterior-posterior axis with particularly high values found in the genu (anterior) and splenium (posterior). In comparison, we see a low-high-low pattern of the orientation dispersion. Right: The axial diffusivity and ODI values plotted as a function of coordinate position along the anterior-posterior axis. The colours represent four different sagittal planes or ‘slices’ at the midline of the corpus callosum. Similar trends are seen across all slices where, in the midbody, we see with increased ODI at the midline (slices 89 & 90).

In the corpus callosum (Figure 3.6) we see a high-low-high pattern of axial diffusivity along the anterior-posterior axis with particularly high axial diffusivity in the genu and splenium. Concurrently, we see a low-high-low pattern of dispersion along the anterior-posterior axis where a similar pattern has been previously reported [116, 117]. Furthermore, we see known pattern of higher dispersion at the midline along the left-right axis of the corpus callosum [116, 47], where slices 89 and 90 show elevated ODI in the midbody of the corpus callosum when compared to the more lateral slices 88 and 91.

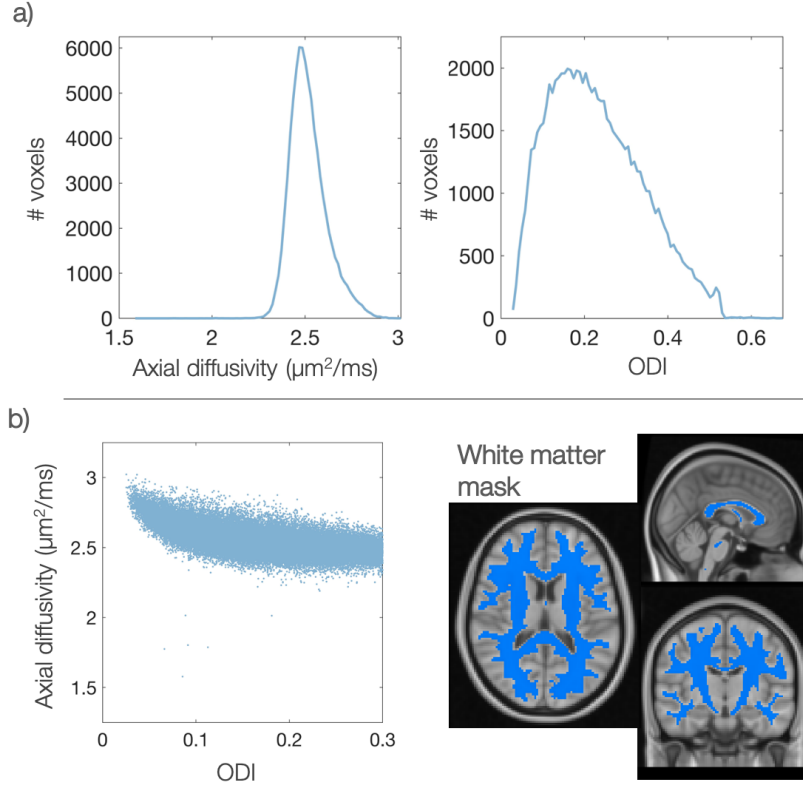


Figure 3.7: Variations in intra-axonal axial diffusivity and orientation dispersion index, ODI, across the white matter. a) Histograms of the mean parameter maps. b) The relationship between the estimated ODI and axial diffusivity. For $\text{ODI} < \sim 0.1$ a negative correlation can be seen between the axial diffusivity and ODI, though in the majority of voxels ($\text{ODI} > 0.1$), the parameters appear fairly uncorrelated.

The relationship between intra-axonal axial diffusivity and ODI

Figure 3.7 shows the distribution of the intra-axonal axial diffusivity and ODI for voxels in the white matter. As the dispersed stick model assumes only a single fibre population per voxel, voxels with $\text{ODI} > \sim 0.3$ should be interpreted with extreme caution and focus should be instead on the most-likely single fibre voxels with low ODI. In highly aligned fibres $\text{ODI} < \sim 0.1$ there is a negative relationship between the axial diffusivity and ODI (Figure 3.7b, $y = -3.14x + 2.90$, $r = -0.61$), which is less pronounced for $\text{ODI} > 0.1$ i.e. the majority of voxels ($y = -0.59x + 2.64$, $r = -0.43$). This behaviour was not observed in simulated data (data not shown).

3.4.3 Undulations

Figure 3.8 explores how fibre undulations - a form of microscopic dispersion - affect our estimated values of axial diffusivity and ODI. Here data were simulated for a simple configuration, where particles displaced along a fibre of zero radius, with undulations of a given wavelength and amplitude. Fibre undulations are seen to increase the estimated ODI and decrease the axial diffusivity from the ground truth values, where the magnitude of this effect is dependent on both the undulation amplitude and wavelength.

Our interpretation of these results is as follows. i) In the dispersed sticks model that is being fitted to data, the only thing that can describe diffusion orthogonal to the principal fibre orientation is dispersion. ii) Since molecules are displacing along a fibre according to the true axial diffusivity, any apparent dispersion has to be accompanied by an apparent reduction in axial diffusivity (since by definition molecules displace less along the "direction" of the fibre). iii) The amount of apparent diffusion in this direction depends on both the undulation amplitude (maximum possible displacement) and wavelength (how far they displace in practice compared to this maximum, given the true diffusivity). iv) In general, higher amplitude increases the apparent dispersion since it appears that there is more diffusion perpendicular to the principal axis. v) This apparent dispersion peaks when the characteristic diffusion length is comparable to a long stretch of an undulation, which corresponds to some "sweet spot" of amplitude and length. If the wavelength is shortened for a given amplitude, the molecules will generally "turn a corner" and displace less on average radially (i.e. less dispersion); if it is longer for that amplitude, they travel less far radially.

Finally, Figure 3.8 generally demonstrates a negative relationship between axial diffusivity and ODI. From Figure 3.8 we can conclude that the ODI parameter captures both the inter-axonal dispersion (e.g. undulations) and intra-axonal dispersion (e.g. fanning), whilst undulations hinder the displacement of water

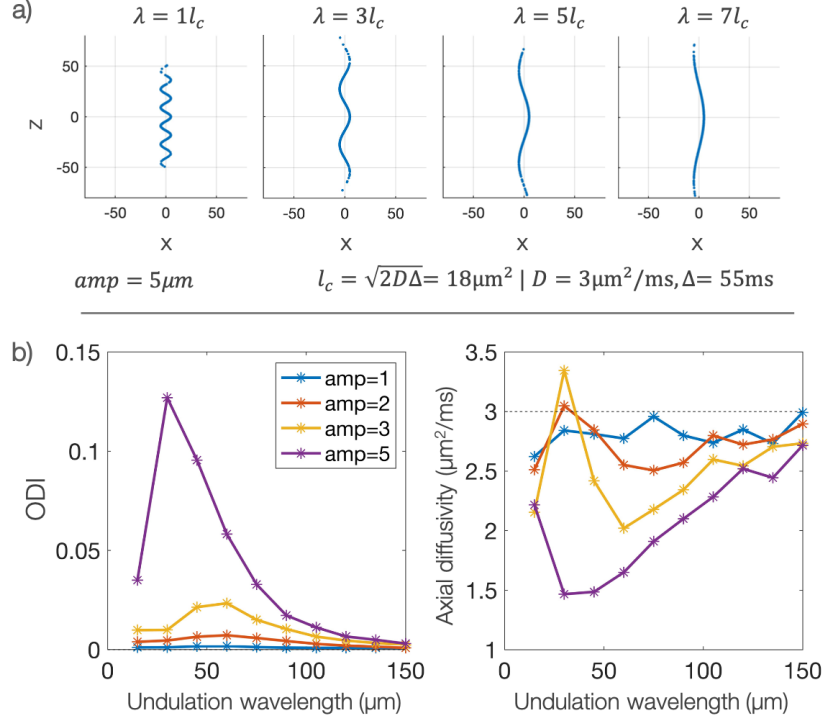


Figure 3.8: The effect of fibre undulations on the estimated axial diffusivity and orientation dispersion index. Here, the diffusion signal was simulated for particles confined to a fibre of zero-radius, with sinusoidal undulations of amplitude amp and wavelength λ . D is the diffusion constant, Δ the diffusion time. a) Example plots of particle displacement along the fibre when $amp = 5\mu\text{m}$. b) In the presence of fibre undulations, the ODI was overestimated whilst the axial diffusivity was underestimated when compared to the ground truth values of macroscopic $\text{ODI}=0$ and $d_{\parallel} = 3\mu\text{m}^2/\text{ms}$ (dashed line).

along the axon, reducing the apparent axial diffusivity. This could help explain the negative correlation between axial diffusivity and ODI in Figure 3.7b when $\text{ODI} < \sim 0.1$. Notably, in this simple experiment, an undulation amplitude of only $\sim 2\mu\text{m}$ can reduce the axial diffusivity from 3 to $\sim 2.5\mu\text{m}^2/\text{ms}$, as is seen in the data (Figure 3.7b).

3.4.4 Impact on existing models: NODDI

To demonstrate how our results may impact the wider modelling community, Figures 3.9 and 3.10 show how the parameters of NODDI [39], one of the most commonly used microstructural models, change when the assumed axial diffusivity is changed from

$d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$ - as is typically assumed - to $2.5 \mu\text{m}^2/\text{ms}$ (the mean axial diffusivity in the white matter) or $3 \mu\text{m}^2/\text{ms}$ (the upper limit of values estimated from the data).

Figure 3.9 shows an increase in the gross signal fraction associated with the intra-axonal compartment and a decrease of the extra-axonal compartment when the assumed axial diffusivity is increased from 1.7 to $3 \mu\text{m}^2/\text{ms}$. Interestingly, when $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$, both the extra-axonal signal fraction and radial diffusivity show two distinct distributions associated with the white and grey matter. The ODI is seen to increase in both the grey and white matter, though the increase is small when $d_{\parallel}$ is increased from $2.5 \mu\text{m}^2/\text{ms}$ to $3 \mu\text{m}^2/\text{ms}$. Finally, the signal fraction associated with isotropic diffusion, f_{iso} is reduced close to zero across most of the brain and particularly in the white matter where we would not expect to find isotropic diffusion.

In Figure 3.10 we see the NODDI parameter maps with axial diffusivity $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$ as a percentage of equivalent maps for $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$. When $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$, the isotropic signal fraction and extra-axonal signal fraction decrease on average to $20 - 40\%$ and $\sim 50\%$ respectively of their value when $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$. Concurrently, the signal fraction associated with the intra-axonal compartment and the ODI increase on average to $\sim 150\%$ and $\sim 120\%$. Here we do not see a global, step change, but rather one which varies across the tissue. For example, the extra-axonal compartment is particularly reduced in the cerebellum with values at $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$ 25% of their value when $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$ ($f_{ex}(d_{\parallel} = 1.7) \sim 0.4$, $f_{ex}(d_{\parallel} = 3) \sim 0.1$). In addition, the ODI is substantially increased in the corticospinal tract as well as by $\leq 250\%$ in areas of the optic radiation and corpus callosum. Interestingly, we see a large ODI change across much of the corpus callosum, though not at the midline along the left-right axis, a known region of increased fibre dispersion. In postmortem macaque data, substantial undulations can be seen off the midline of the corpus callosum in areas similar to the hotspots reported here [67].

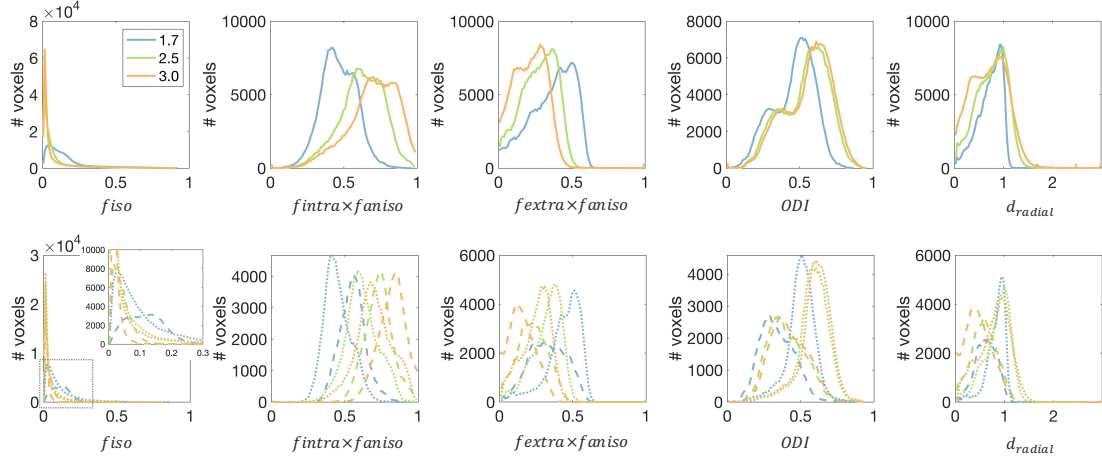


Figure 3.9: The dependence of NODDI parameters on the assumed axial diffusivity. The NODDI model [39] was fitted to 10 subjects from the HCP dataset with various assumed axial diffusivities $d_{\parallel} = 1.7, 2.5, 3 \mu\text{m}^2/\text{ms}$. The distribution of the mean parameters is shown for all voxels in the brain (top) and for the white matter (bottom, dashed) and grey matter (bottom, dotted) separately.

3.5 Discussion

Measurement of the intra-axonal axial diffusivity is typically confounded by both signal contributions from the extra-axonal space and fibre orientation dispersion. If unaccounted for, the former will result in overestimation, and the latter underestimation, of the true axial diffusivity. For example, (from Figure 3.1b) at a b-value of $3 \text{ ms}/\mu\text{m}^2$, if a fibre population with very low dispersion ($\text{ODI}=0.05$) is assumed to be perfectly aligned with zero dispersion, a ground truth axial diffusivity of $2.5 \mu\text{m}^2/\text{ms}$ is reduced to $\sim 2.2 \mu\text{m}^2/\text{ms}$. Therefore, this study utilised high b-value data to isolate the signal arising solely from the intra-axonal compartment and the "dispersed sticks model" to estimate both fibre orientation dispersion and intra-axonal axial diffusivity on a voxelwise basis.

3.5.1 Comparison to previous literature

The model was applied to high b-value in vivo data from 32 subjects where our results suggest that the axial diffusivity lies between ~ 2.3 and 3 in the human brain.

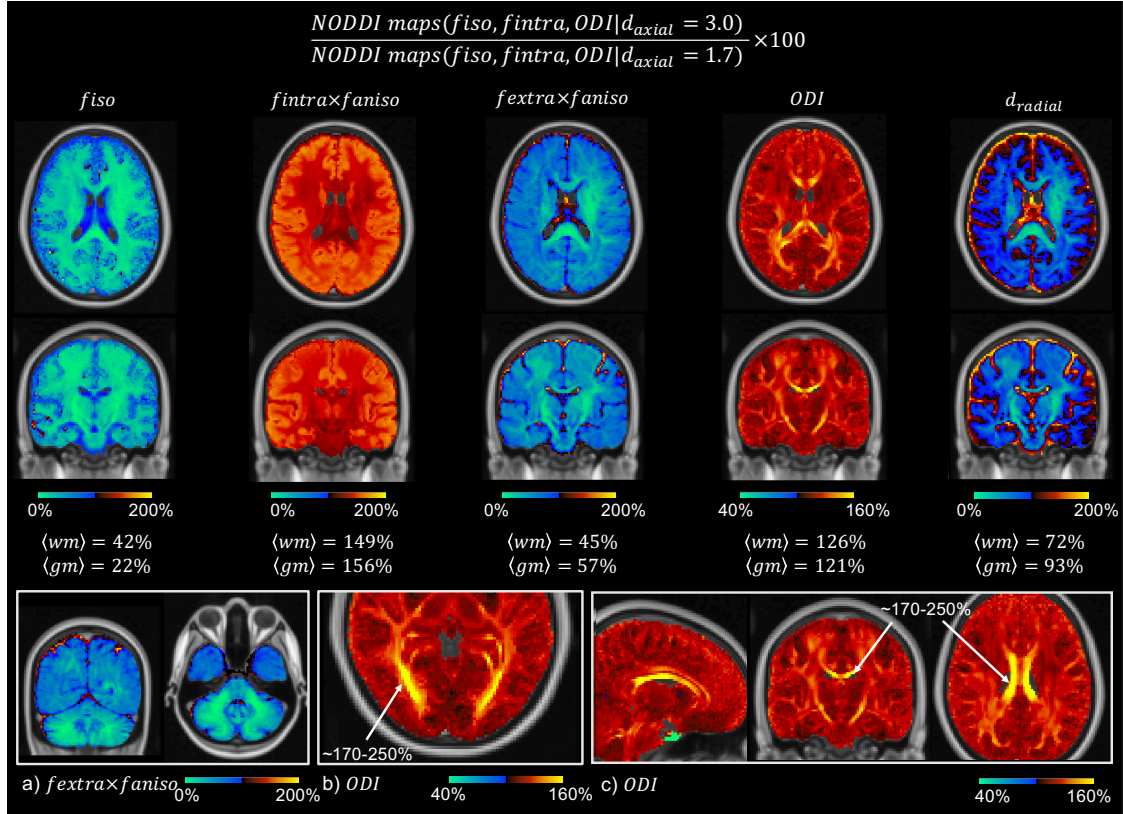


Figure 3.10: Estimated NODDI parameter maps with axial diffusivity $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$ as a percentage of equivalent maps for $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$, as is typically assumed. The signal fraction associated with isotropic diffusivity and the extra-axonal compartment is substantially reduced, as is the radial diffusivity. The intra-axonal signal fraction is largely increased (close to $f_{\text{intra}} \sim 1$) as is the ODI, though to a broadly lesser extent. a) The extra-axonal compartment is reduced substantially in the cerebellum. b,c) The ODI increased to $\sim 170 - 250\%$ in areas of the optic radiation and corpus callosum.

Notably, here the upper limit is close to $\sim 3 - 3.1 \mu\text{m}^2/\text{ms}$, the absolute limit of water diffusion at 37°C , though this limit was never enforced as a constraint during modelling. When compared to previous literature (see Jelescu *et al* [3] for review, as well as [118, 75, 119, 71]), our reported values appear fairly high. Notably, these studies use different methods to achieve compartment specific selectivity and estimate the intra-axonal axial diffusivity to be generally in the range of $\sim 1.7 - 2.4 \mu\text{m}^2/\text{ms}$.

Interestingly, several previous studies have alluded to the importance of accounting for orientation dispersion when estimating axial diffusivity [120, 119]. For example, magnetic resonance spectroscopy is often used to study the diffusion of metabolites

(e.g. NAA). Assuming straight axons, Kroenke *et al.* [121] and Palombo *et al.* [122] measure the diffusivity of NAA to be $0.36 \mu\text{m}^2/\text{ms}$ in the rat and $0.33 \mu\text{m}^2/\text{ms}$ in the mouse brain respectively. In comparison Ronen *et al.* [120] account for the micro and macroscopic curvature of the human corpus callosum to estimate a much higher diffusivity of 0.51. As these studies consider the diffusion of NAA not water (as is reported in this study) the diffusivities should not be directly compared. More, they support our postulation that, when unaccounted for, fibre dispersion artificially lowers the estimate of axial diffusivity.

Higher diffusivities, comparable to those reported here, were previously reported by Kunz *et al.* [70]. Using the injection of a gadolinium(Gd)-based contrast agent to selectively reduce the signal from the extra-axonal compartment, Kunz *et al.* report an intra-axonal axial diffusivity close to $3 \mu\text{m}^2/\text{ms}$ in the genu of the rat corpus callosum. In the work presented here, some of the highest values of axial diffusivity ($> 2.9 \mu\text{m}^2/\text{ms}$) were found in the genu of the corpus callosum, with the genu showing a median value of $2.76 \mu\text{m}^2/\text{ms}$ at the midline (Figure 3.6).

Our work highly complements that by Kaden *et al.* who observe a spatially varying axial diffusivity within a range of $\sim 2 - 2.7 \mu\text{m}^2/\text{ms}$, where the diffusivities are generally slightly lower than those reported here. We utilise high b-value data to remove the effect of extra-axonal water and directly model both the macroscopic orientation dispersion. In comparison, Kaden *et al.* utilise the spherical mean technique to remove the effects of macroscopic dispersion and directly model the extra-axonal space as an axially symmetric diffusion tensor, where the intra- and extra-axonal axial diffusivities are assumed equivalent and so defined by a single parameter. However, recent studies which optimise both the intra- and extra-axonal axial diffusivity independently suggest the values are likely different [3, 123], with intra-axonal axial diffusivity larger than extra-axonal axial diffusivity [69, 79, 119]. This would explain why we report slightly higher diffusivities: if the apparent axial diffusivity measured by Kaden *et al.* represents some weighted mean from both compartments, it would likely be lower than the intra-axonal axial diffusivity

estimated here. Interestingly, Kaden *et al.* report a lower axial diffusivity in the splenium of the corpus callosum than at the midline which contradicts the high-low-high pattern of axial diffusivity in Figure 3.6.

Finally, our average diffusivity across the white matter, $\langle d_{\parallel,wm} \rangle = 2.52 \mu\text{m}^2/\text{ms}$ is not too dissimilar to that reported by McKinnon *et al.* [74] who utilise high b-value data ($b = 6 \text{ ms}/\mu\text{m}^2$) and fibre ball white matter model to estimate an intra-axonal axial diffusivity of $2.46 \mu\text{m}^2/\text{ms}$.

3.5.2 Variations in axial diffusivity across the brain

As we optimise the dispersed stick model on a voxelwise basis - rather than fitting to the average signal across many voxels - our technique shows the extent to which intra-axonal axial diffusivity varies across the brain. The axial diffusivity in the grey matter appears lower than that in the white matter (Figure 3.4) [124], and the intra-axonal axial diffusivity varies considerably across the white matter (Figures 3.4, 3.5 & 3.6). In addition, we report an interesting high-low-high pattern of axial diffusivity across the corpus callosum which qualitatively mirrors (anti-correlates) the previously reported low-high-low patterns in axon diameter [117, 7, 125, 126]. This result is intriguing and possibly opposite to what we would expect (see below discussion on sensitivity to axon diameter), where the relationship between the axial diffusivity and the axon diameter, alongside other aspects of the tissue microstructure, warrant further investigation. Another possibility is that this pattern is driven by variations in dispersion anisotropy across the corpus callosum, with high dispersion anisotropy in the genu and splenium inflating the axial diffusivities and lowering the estimates of the ODI. However, when a modified NODDI-Bingham [86, 46] model was fitted to the data (modified for high b-value data to only consider the intra-axonal compartment), the high-low-high pattern of axial diffusivity persisted (data not shown). This suggests that dispersion anisotropy alone cannot explain the axial diffusivity variations across the corpus callosum.

Furthermore, the observed variations in axial diffusivity directly challenge the assumption of a global axial diffusivity as is assumed by many of the most commonly used diffusion models. Here we give two examples. Firstly, in constrained spherical deconvolution (CSD) [40], typically a global fibre response function is first estimated from the data, and then considered constant across the data to estimate the fibre orientation distribution. In previous work [67] (*c.f Chapter 4*) we combined MRI and microscopy data into a joint model and showed how, when estimated on a voxelwise basis, the fibre response function appeared to vary across the white matter - results which challenge the assumption of a global fibre response function. In this previous study we were unable to separate intra- and extra-axonal contributions or estimate specific microstructural properties such as the intra-axonal axial diffusivity as we do in the work presented here. Secondly, NODDI assumes a global value of axial diffusivity, typically of $1.7 \mu\text{m}^2/\text{ms}$, to estimate the fibre orientation dispersion and ‘density’ i.e. the signal fraction associated with the intra-axonal compartment [39]. In both cases (CSD & NODDI), should the assumed diffusion characteristics be wrong, the estimated parameters may be biased.

Finally, both the observed variations in intra-axonal axial diffusivity, and the sensitivity of our model to detect such fluctuations, suggest the intra-axonal axial diffusivity could act as an interesting biomarker in future studies of tissue health and disease. Indeed, Kaden *et al.* report significant differences in the estimated axial diffusivity in two mouse models of tuberous sclerosis [124], suggesting sensitivity of axial diffusivity to neuropathological processes. Furthermore, our simulations of fibre undulation hint to the estimated axial diffusivity being an indirect measure of fibre undulation, where the biological relevance of fibre undulations remains relatively unexplored.

3.5.3 Negative correlation between axial diffusivity and orientation dispersion

Our results show a negative correlation between axial diffusivity and orientation dispersion (Figure 3.7b), particularly in highly aligned fibre bundles ($ODI < \sim 0.1$). We first questioned whether the behaviour could be due to a degeneracy between the parameters in the model. From Figure 3.1b,c, we see how an underestimation of ODI results in an underestimation of axial diffusivity, suggesting a positive relationship between the variables. Furthermore, through MCMC optimisation [81], we obtain not a single parameter estimate but a parameter distribution and so could examine the covariance of any two parameters in the model. Upon examination we again found a positive correlation between axial diffusivity and ODI (data not shown). Together these results strongly suggests that model degeneracy is not the cause of the negative correlation in the data.

The negative correlation could instead be caused by a feature of the data which is unaccounted for in the model. For example, if one of the 4 modelling assumptions (*c.f.* Section 2.1.1) were violated. One possibility raised by our simulations is sheet-like fibre populations: Figure 3.3a shows how asymmetric dispersion results in a negative relationship between axial diffusivity and ODI. However, this is likely not a good explanation as it implies that low ODI voxels have highly asymmetric dispersion i.e. that we would have ‘sheet-like’ fibres across low ODI regions such as the body of the corpus callosum, the corticospinal tract and the optic radiation. Additionally, the presence of sheet-like fibres in the brain remains a controversial topic [127, 128, 129] and their existence requires concrete validation from non-MRI methods.

Again using simulated data (Figure 3.3), we see how multi-fibre voxels may lead to an underestimation of axial diffusivity and an over estimation of ODI (Figure 3.3d), where the majority of voxels in the white matter likely contain more than one fibre bundle. These results suggest the axial diffusivity could be close to that of free water, and that it appears reduced in white matter regions with multiple

fibre populations per voxel. However, as the depicted relationship did not hold for voxels containing two fibre populations of unequal weighting, it is unlikely that multiple fibre populations fully explain the negative correlation seen in the data. Nonetheless, future work should focus on extending the model to account for multiple fibre populations per voxel.

It is possible that the intra-axonal axial diffusivity is overestimated due to signal contributions from the extra-axonal compartment. At high b-value, signal contributions from highly mobile water (i.e. that with considerable diffusivity) becomes negligible. However, if the extra-axonal compartment is characterised by a *small* radial diffusivity, this could lead to an overestimation of the intra-axonal axial diffusivity (as showed by our simulations in Figure 3.3c). Furthermore, our results from in vivo data show a small negative relationship between the estimated intra-axonal signal fraction $f_{in} = F/S_0$ and the ODI (when $ODI < 0.1$, $f_{in} = -1.21ODI + 0.54$, $r = -0.27$) where the intra-axonal signal fraction is generally higher in low ODI voxels. f_{in} and $d_{||}$ showed no relationship (data not shown). Therefore, if low ODI voxels tend to have a higher intra-axonal signal fraction and thus contain non-negligible contributions from the extra-axonal space (where the radial diffusivity of the extra-axonal space is small), this could lead to an overestimation of the intra-axonal axial diffusivity in low ODI voxels. In this study (Figure 3.4) we estimate an intra-axonal signal fraction of $\sim 0.3 < f_{in} < 0.7$ across the deep white matter. Note, this is only a very approximate estimate as it assumes the T2 of the intra- and extra-axonal space to be the same, though it is likely different [79]. Nonetheless, if these estimates are approximately correct, assuming the extra-axonal space is well described by the tortuosity model of Figure 3.3c, the overestimation of the intra-axonal axial diffusivity due to extra-axonal contributions should be fairly small ($< 5\%$).

Intuitively, the negative correlation could be due to fibre undulations [87, 88, 89, 90, 91] which both act as a form of microscopic dispersion - increasing the amount of dispersion per voxel - and hinder the diffusion of intra-axonal water along the primary fibre axis, decreasing the apparent axial diffusivity. To investigate the

impact of fibre undulations, we simulated data for a simple model with particles displacing along a fibre of zero radius and undulations of varying wavelength and amplitude, to find a negative relationship between the estimated orientation dispersion and axial diffusivity as expected (Figure 3.8). Whilst the microscopic dispersion was accounted for in the estimate of orientation dispersion, the apparent axial diffusivity was lowered with respect to the ground truth value for straight fibres. Interestingly, in this simplistic model, undulations with an amplitude of only $\sim 2 \mu\text{m}$ could lower the axial diffusivity from 3 to $\sim 2.5 \mu\text{m}^2/\text{ms}$, as is seen in the data (Figure 3.7b). In current literature, the impact of undulations often focuses on the measurement of radial diffusion or axon diameter [89, 130, 90], though the effect of undulations on other diffusion characteristics, such as axial diffusion, deserve further investigation. Future work will benefit greatly from more realistic simulations [131, 91, 90] where, for example, the tissue structure is directly inspired by 3D reconstructed surfaces from microscopy images of real tissue [132, 91, 90].

As fibre undulations result in a lowered axial diffusivity, it follows that the observed variations in axial diffusivity may, at least to some extent, be driven by the presence of fibre undulations. Consequently, if undulations vary across the brain, there may exist a more global intra-axonal axial diffusivity. If undulations are the source of this negative correlation, then this would suggest that voxels estimated here with high axial diffusivity - i.e. regions in the corpus callosum (particularly the genu), the external capsule, the fornix, the optic radiation and the superior cerebellar peduncle - should comprise of fairly straight fibres with few undulations. Furthermore, it would imply a low-high-low pattern of undulations along the anterior-posterior axis of the corpus callosum, with large axons experiencing a higher degree of undulation. Note, this remains a highly speculative hypothesis and requires supporting evidence from, for example, maps derived from both MRI and microscopy which show the extent of fibre undulations across the brain.

3.5.4 Impact: NODDI modelling

Neurite Orientation Dispersion and Density Imaging (NODDI) [39] is one of the most commonly used microstructural models in diffusion MR which, due to its clinically feasible scan times, has featured in numerous studies of patient populations [133, 134, 135, 136, 137]. However, to estimate the neurite density or signal fraction, NODDI must assume a fixed, predefined value of the intra-axonal axial diffusivity, which is typically set to $1.7 \mu\text{m}^2/\text{ms}$. If the assumed axial diffusivity is wrong, the remaining NODDI parameters will be biased.

Our results suggest the assumed value of axial diffusivity should be between $\sim 2.5 - 3 \mu\text{m}^2/\text{ms}$. Figures 3.9 and 3.10 demonstrate the substantial impact this would have on the estimated NODDI maps. In Figure 3.10 - when $d_{\parallel} = 3 \mu\text{m}^2/\text{ms}$ is compared to $d_{\parallel} = 1.7 \mu\text{m}^2/\text{ms}$ - we frequently see the parameter estimates rise or fall by $\sim 50\%$, though in extreme cases the parameter may be as little as 20% or as much as 250% of its former value. Notably, in the white matter a substantially larger signal fraction is associated with the intra-axonal compartment, whilst the signal fraction of the extra-axonal compartment is highly reduced and that associated with the isotropic compartment is reduced to ~ 0 . Interestingly, the extra-axonal compartment is decreased from $f_{ex} \sim 0.1$ to $f_{ex} \sim 0$ across much of the corpus callosum as well as various other regions in the white matter. This suggests that the apparent signal attenuation perpendicular to the fibre can be explained solely by the interplay of intra-axonal diffusion and fibre orientation dispersion, without the need for an additional extra-axonal compartment.

We see an overall $\sim 20\%$ increase in the orientation dispersion index, with regions of the corpus callosum and optic tract sometimes doubling, from ~ 0.05 to ~ 0.1 which invites future ODI validation against microscopy gold standards.

Importantly, the changes in the NODDI outputs reported here do not necessarily negate group differences in NODDI parameters, but rather questions the accuracy of the output values. Indeed, Schilling *et al.* [138] found the NODDI ODI to

correlate well with dispersion values derived from histology, though without a one-to-one mapping of the values i.e. the regression line was not the line of unity. In comparison, Grussu *et al.* [139] compared the NODDI outputs to histological data from the human spinal cord to find an approximate one-to-one mapping. The discrepancy between the results from the two studies may indeed be related to the choice of assumed axial diffusivity - a choice that is especially challenging in postmortem tissue where the diffusivities are considerably reduced when compared to their in vivo values. In postmortem spinal cord, Grussu *et al.* assume an axial diffusivity of $1.5 \mu\text{m}^2/\text{ms}$ which was found to optimise the fitting across all samples. Schilling *et al.* imaged postmortem squirrel monkey brain, though the assumed axial diffusivity wasn't reported.

Finally, the NODDI biases are likely affected by the spatial resolution of the data. If the amount of dispersion per voxel tends to decrease as the spatial resolution is increased, any biases associated with the conflation of axial diffusivity with dispersion will be reduced.

3.5.5 Limitations

In this study, the dispersed stick model accounts for only a single fibre population per voxel. Therefore, in our results, areas of known crossing fibres and much of the grey matter should be interpreted with caution and the focus instead placed on the 'most-likely' single-fibre voxels with a low ODI. Notably, we see some of the highest values of axial diffusivity in the low ODI voxels: the mean axial diffusivity is $2.54 \mu\text{m}^2/\text{ms}$ for voxels with $\text{ODI} < 0.3$, but $2.67 \mu\text{m}^2/\text{ms}$ in voxels with $\text{ODI} < 0.1$. In future work, the dispersed stick model could be extended to account for multiple fibre populations per voxel.

Our model is fitted to high b-value data, where much of the diffusion signal is strongly attenuated and the SNR along the fibre is very low. This results in fairly noisy maps of axial diffusivity at the single subject level. Here we report the average

map across 32 subjects which reassuringly shows good smoothness and symmetry. Nonetheless, using the current model, it would be challenging to compare voxelwise changes in axial diffusivity at the level of an individual.

Although the model assumes stick-like axons with no radial diffusion, at such high b-values we may in fact be sensitive to radial diffusion from the extra-axonal space (as described above) or from large diameter axons. From in vivo human data, Veraart *et al.* [41] estimated a radial diffusivity of $\sim 0.01 \mu\text{m}^2/\text{ms}$ across the white matter. Here, using simulated data we found that tensor-like (rather than stick-like) fibres with a radial diffusivity of $\sim 0.01 \mu\text{m}^2/\text{ms}$ led to the over estimation of axial diffusivity, from $d_{\parallel} = 2.2 \mu\text{m}^2/\text{ms}$ to $d_{\parallel} = 2.68 \mu\text{m}^2/\text{ms}$ (Figure 3.3b). Notably, the overestimation of the axial diffusivity increased with increasing radial diffusivity (i.e. increasing axon diameter). Sensitivity to axon diameter could therefore likely explain why our estimates of axial diffusivity are higher than many previous studies. However, this possibility would not be consistent with the high-low-high pattern of axial diffusivity across the corpus callosum. The corpus callosum is known to exhibit a low-high-low pattern of axon diameter [117, 7, 125, 126], from which we would expect a low-high-low pattern of axial diffusivity. The opposite is reported in the data. Nonetheless, the role of axon diameter on the estimated axial diffusivity in high b-value data warrant further investigation.

Finally, the model does not account for a slowly diffusing isotropic compartment, often referred to as the ‘dot compartment’, the existence of which would bias our results. Tax *et al.* [140] recently found evidence for a dot compartment in the cerebellar grey matter accounting for 9.7% of the signal with a diffusivity of $d_{iso} = 0.12 \mu\text{m}^2/\text{ms}$. A smaller contribution was also observed in the white matter of the cerebellum (5.4%, $d_{iso} = 0.16 \mu\text{m}^2/\text{ms}$) and for some voxels in the occipital lobe (2.6%, $d_{iso} = 0.23 \mu\text{m}^2/\text{ms}$). Should a dot compartment exist, the axial diffusivity would be over-estimated. In simulated data with ground truth $d_{\parallel} = 2 \mu\text{m}^2/\text{ms}$, a dot compartment with $d_{iso} = 0.2 \mu\text{m}^2/\text{ms}$ led to an estimated axial diffusivity of $\sim 2.3 \mu\text{m}^2/\text{ms}$ when the dot signal fraction was $f_{dot} = 0.05$ (as

in the cerebellar white matter) and $\sim 2.1 \mu\text{m}^2/\text{ms}$ when $f_{dot} = 0.025$ (occipital white matter) i.e. a dot compartment leads to an overestimation of axial diffusivity, where the degree of overestimation is dependent on the volume fraction. Therefore, in our results the axial diffusivities in the cerebellum and regions of the occipital lobe may be somewhat inflated from their true value. As we do not see a clear bias with large axial diffusivities in these regions - if anything the model estimates lower-than-typical axial diffusivities in these regions - the effect may be small.

3.5.6 Code availability and future work

The dispersed stick model was implemented both in MATLAB and the cuDIMOT framework [81] for GPU acceleration, both of which are available at [link to be added upon publication]. We provide a cuDIMOT implementation of NODDI where the assumed diffusivities $d_{\parallel}$ and d_{iso} are user defined at runtime. Interesting avenues for future work include: the relationship between axial diffusivity and features of the tissue architecture such as axon diameter; the relationship between axial diffusivity and brain development; as well as the estimation of axial diffusivity in postmortem tissue, both in situ and after perfusion or immersion fixation; and how intra-axonal axial diffusivity is affected by tissue degradation or disease. Here it may be interesting to study progressive demyelinating diseases such as multiple sclerosis where accurate estimation of the intra-axonal axial diffusivity may play an important role in determining the stage of demyelination. In a cuprizone mouse model, Xie *et. al* [141] demonstrated how early stage demyelination was associated with increased axonal beading and varicosities, which were again reduced in prolonged demyelination. When compared to DTI, the dispersed stick model can provide axial diffusivity estimates that are not confounded by orientation dispersion (which can be affected by other pathologies such as axonal loss) and so may provide increased sensitivity to these various stages of demyelination. Furthermore, the model could be extended to estimate multiple fibre populations per voxel, thus improving the interpretability of the model in multi-fibre voxels. As it may be difficult to separate

the fibre-specific diffusivities, volume fractions and ODIs, in the first instance, the multi-fibre model would likely need to assume the same axial diffusivity for all fibre populations within the voxel. In addition, extra gradient directions may improve the estimates of the population-specific estimates of orientation and dispersion, particularly when the opening angles between the fibres is small. Estimating population-specific diffusivities may require a modified imaging protocol with additional b-values. Finally, co-registered MRI and microscopy data should be used to validate the orientation dispersion estimates reported here. This will likely require the acquisition of a bespoke postmortem dataset which combines data from 3 shells at ultra-high b-values (accounting for the reduced diffusivity of fixed postmortem tissue) with corresponding microscopy imaging of the white matter fibres.

3.6 Conclusion

Through the simultaneous analysis of orientation dispersion and axial diffusivity we demonstrate substantial variability in the intra-axonal axial diffusivity across the brain. The estimated axial diffusivity ranges from $\sim 2.3 - 3 \mu\text{m}^2/\text{ms}$ with a mean value of $2.52 \mu\text{m}^2/\text{ms}$ in the white matter. This is considerably higher than most of the reported values in current literature, with the upper limit close to the absolute maximum diffusivity of pure water at 37°C , $\sim 3 - 3.1 \mu\text{m}^2/\text{ms}$. Our results show how axial diffusivity varies considerably both across the corpus callosum - with particularly high values found in the genu and splenium - and between white matter tracts. This not only questions the assumption of a global axial diffusivity [39, 40] but also suggests that axial diffusivity could act as an interesting biomarker of disease. Furthermore, we find a negative correlation between axial diffusivity and the orientation dispersion, and suggest this could be explained by the presence of fibre undulations which act to lower the apparent axial diffusivity. Finally, we show how the estimated parameters of the NODDI model [39] changed substantially, when the assumed axial diffusivity was increased from 1.7 to 2.5 or $3 \mu\text{m}^2/\text{ms}$, as our results suggest.

3.7 Contributions

I am hugely grateful to Derek Jones, Mark Drakesmith and Suryanarayana Umesh Rudrapatna for providing the data for this study and to Rick Lange, Jeroen Mollink and Michiel Cottaar for their valuable advice on various topics: Rick Lange advised on GPU optimisation; Jeroen Mollink contributed to early discussions; Michiel Cottaar reviewed the manuscript. My personal contributions span all other aspects of the project: conceptualisation; data preprocessing; design, development and implementation the model; data simulation; analysis and presentation of the results; manuscript writing, editing and review.

3.8 Appendices

3.8.1 Appendix A: converting ODI to degrees

The orientation dispersion can be described in terms of orientation dispersion index (ODI) or degrees, where there does not appear to be consistency throughout the literature. To aid interpretation of our results (quoted in ODI) and facilitate comparison to other literature, Figure 3.11 relates the ODI to the amount of dispersion in degrees: the full width half maximum (FWHM) of the distribution and the angle which contains 50% of all fibres.

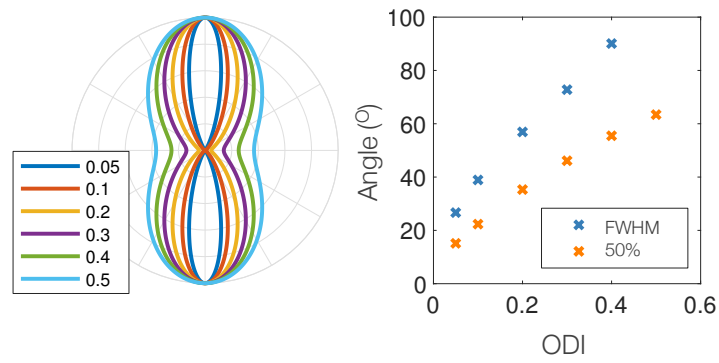


Figure 3.11: How the orientation dispersion index relates to the fibre opening angle in degrees. Left: A 2D plane through the fibre orientation distribution as the ODI is varied from 0.05-0.5. Right: The ODI is plotted against the full width half maximum of the fibre orientation distribution, as well as the angle containing 50% of the fibres.

4

Joint modelling of diffusion MRI and microscopy

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Foreword

The combination of diffusion MRI with microscopy provides unique opportunities to study microstructural features of tissue, particularly when acquired in the same sample. Microscopy is frequently used to validate diffusion MRI microstructure models, addressing the indirect nature of diffusion MRI signals. Typically, these modalities are analysed separately, and microscopy is taken as a gold standard against which diffusion MRI-derived parameters are validated. Here we propose an alternative approach in which we combine diffusion MRI and microscopy data obtained from the same tissue sample to drive a single, joint model. This simultaneous analysis allows us to take advantage of the breadth of information provided by complementary data acquired from different modalities. By applying this framework to a spherical-deconvolution analysis, we are able to overcome a known degeneracy between fibre dispersion and radial diffusion. Spherical-deconvolution based approaches typically estimate a global fibre response function to determine the fibre orientation distribution in each voxel. However, the assumption of a ‘brain-wide’ fibre response function may be challenged if the diffusion characteristics of white matter vary across the brain. Using a generative joint diffusion MRI-histology model, we demonstrate that the fibre response function is dependent on local anatomy, and that current spherical-deconvolution based models may be overestimating dispersion and underestimating the number of distinct fibre populations per voxel.

Publications relating to this work

Journal articles:

Howard AFD, Mollink J, Kleinnijenhuis M, Pallegage Gamarallage M, Bastiani M, Miller KL*, Jbabdi S*, “Joint modelling of diffusion MRI and microscopy”, *Neuroimage* (2019), 201, 11, doi: 10.1016/j.neuroimage.2019.116014.

Conference proceedings:

Howard AFD, Mollink J, Kleinnijenhuis M, Pallegage Gamarallage M, Bastiani M, Miller KL*, Jbabdi S*, “Joint modelling of diffusion MRI and histology”, *Proceedings of the 26th Annual Meeting of the ISMRM, Paris, France, 2018.*

4.1 Introduction

Diffusion MRI is routinely used to study white matter microstructure and connectivity in vivo and non-invasively [142, 143, 25]. Diffusion MRI microstructure models relate variations in the MR signal to microstructural features of interest. Such inference requires biophysical modelling of both the tissue architecture and diffusion process. Although many diffusion MRI models have been proposed, few have been rigorously validated [37, 31], and the link between the observed diffusion signal and the underlying white matter microstructure remains controversial [144, 2].

Microscopy is often considered a ‘gold standard’ technique for the validation of diffusion MRI models. Crucially, microscopy tends to resolve a specific structure of interest (e.g. histological staining of astrocytes or polarised light imaging of myelinated axons) and thus typically provides specificity that is not guaranteed by MRI. In a typical validation study the diffusion MRI and microscopy data are analysed separately, then diffusion MRI-derived tissue parameters (e.g. fibre orientation, myelin density or axon diameter) are compared to microscopy equivalents which are taken to be the ground truth [145, 146, 47, 138]. This is possible due to the complementary nature of the data: both modalities provide information about the same tissue parameters of interest, but each observe them through a different lens. However, by analysing the data separately (rather than simultaneously), such paradigms may not be exploiting the multimodal data to its full potential.

Here we suggest an alternative, data-fusion framework in which we combine diffusion MRI and microscopy data from the same tissue sample into a single joint model. A joint model may be advantageous in three respects. Firstly, by considering both datasets simultaneously, we have access to additional, complementary information about the tissue microstructure and may be able to accurately determine tissue parameters that are currently unobtainable from the diffusion signal alone. A secondary benefit of the data-fusion framework is that the joint model considers both diffusion MRI and microscopy to be informative of the ‘true’ underlying

microstructure, but also that both have sources of uncertainty (Figure 4.1). Crucially, these are unique, modality-dependent sources of noise. Therefore, by using a data-fusion framework we can in theory obtain a higher-precision estimate of the underlying microstructure of interest. Finally, microscopy is typically 2D and may only be sensitive to a subset of the tissue compartments (e.g. myelinated axons or astrocytes). For example, histological staining of the tissue (a gold standard microscopy technique) typically produces 2D images of thin tissue sections, where only the stained microstructure is easily visualised. Thus, the information provided by microscopy only partially informs on the tissue microstructure. The joint model can overcome this limitation by considering the microscopy as a soft constraint on the model, as opposed to a hard constraint or ‘ground truth’ in post-hoc validation. This framework is inspired by a similar data-fusion approach [147] which demonstrated improved brain connectivity analysis when complementary 3T and 7T diffusion MRI data was analysed jointly rather than separately. It should be noted that a similar joint modelling approach could be applied to co-analyse any two datasets which share a common parameter of interest, to obtain a higher-precision estimate of that parameter. The two datasets could be a) intra-modality, such as the two diffusion MRI datasets in the above example by Sotiropoulos *et al.* [147], b) from different MRI techniques (e.g diffusion MRI-relaxometry) or c) inter-modality such as the combination of MRI with positron emission tomography (PET), electro- or magneto-encephalography (E/MEG) [148, 149] or microscopy data, as in the approach presented here.

This report considers one example of how diffusion MRI-microscopy data-fusion allows us to extract tissue parameters which are difficult to obtain from the diffusion MRI data alone. Here we aim to separate out fibre orientation dispersion from radial diffusion i.e. the apparent diffusion coefficient perpendicular to the direction of the fibre. As is illustrated in Figure 4.1 (bottom), a highly dispersed fibre population with low radial diffusivity may produce the same diffusion MRI signal as a single, coherently oriented fibre population with high radial diffusion. This degeneracy is commonly overcome by assuming one can identify a region with

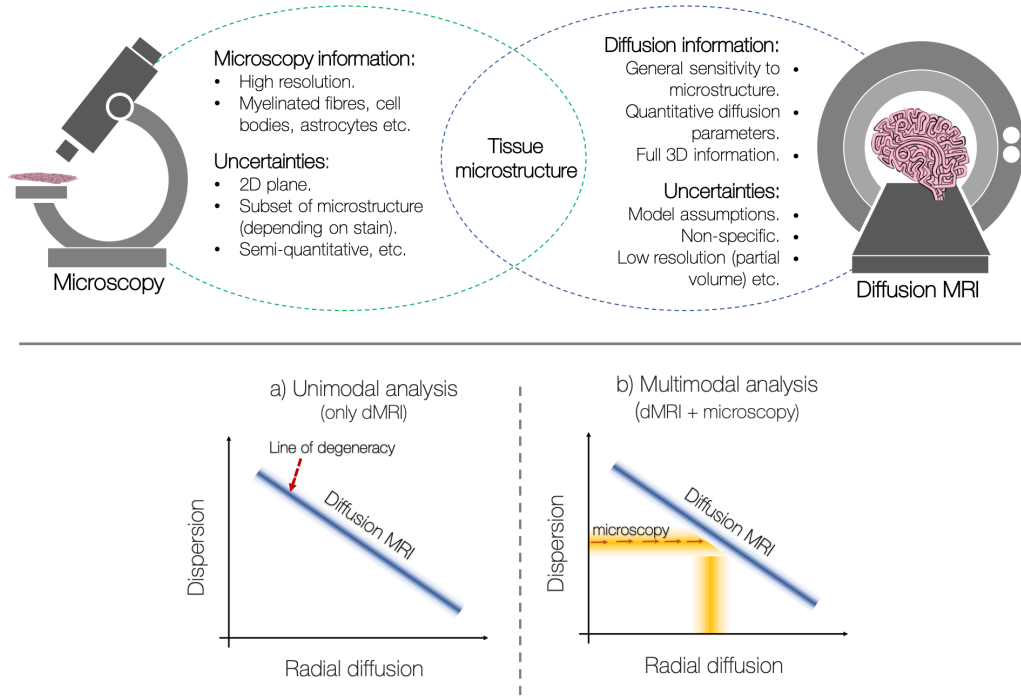


Figure 4.1: Top: Microscopy data can provide highly detailed information about specific microstructural features of the tissue at sub-micrometre resolutions. In comparison, the diffusion MRI signal is an indirect measure of the same microstructure of interest. Due to the complementary information provided by the two modalities, we propose a data-fusion framework which simultaneously analyses diffusion MRI and microscopy data from the same tissue sample to drive a single, joint model. Bottom: (a) A highly dispersed fibre population with low radial diffusivity may produce the same diffusion MRI signal as a single, coherently ordered fibre population with high radial diffusivity. The blue line labelled ‘Diffusion MRI’ represents a simplistic but graphical representation of this degeneracy. (b) Microscopy can estimate and, in the joint analysis, constrain the fibre dispersion in each MRI voxel to overcome the degeneracy; the diffusion MRI data can now provide accurate estimates of radial diffusion. Critically, as the microscopy acts as a soft constraint (rather than fixing the dispersion), both the diffusion MRI and the microscopy data simultaneously inform on the fibre dispersion. Information about the radial diffusion comes from diffusion MRI data alone. The shading represents noise in the data and uncertainty in the parameter estimates.

a single, coherently oriented fibre population which is then used to define a global radial diffusivity. Alternatively, promising developments by Lasič *et al.* [150], Szczepankiewicz *et al.* [151], and more recently by Cottaar *et al.* [152] demonstrate that, by combining linear and spherical diffusion tensor encoding, it is possible to disentangle microscopic diffusion anisotropy from orientation dispersion. Nonetheless, current analyses of conventional diffusion MRI data (based on single diffusion encoding and which do not assume global diffusivities) are unable to distinguish these two distinct fibre configurations.

Microscopy can be used to determine the fibre orientations at a much finer spatial resolution (typically $\sim$ micrometer or sub-micrometer per pixel) and so can estimate the (typically 2D) fibre orientation distribution in each diffusion MRI voxel. However, microscopy alone is typically uninformative of the diffusion properties of the tissue. In a joint diffusion MRI-microscopy model (as illustrated in Figure 4.1, bottom), once the fibre orientations and amount of within-voxel fibre dispersion are constrained by the microscopy data, diffusion MRI can accurately estimate the radial diffusion. In this manner a joint model should be able to overcome the degeneracy and separate out fibre dispersion from radial diffusion. This could provide insights into how these two parameters vary across the brain in both health and disease.

There are many ways to formulate a joint model. In this report we focus on one approach based on constrained spherical deconvolution (CSD, [40]). As a popular data-driven analysis, CSD estimates the underlying fibre orientations from a diffusion MRI dataset. In CSD, the diffusion signal is considered to be the convolution of the fibre orientation distribution (FOD) with a fibre response function (FRF). Here the FRF describes the diffusion signal from a single, coherently oriented fibre population. In the model, the FRF is first estimated empirically (typically from voxels with large fractional anisotropy [40, 53, 52, 51]) and subsequently used as a global deconvolution kernel to determine the underlying FOD. CSD is typically thus based around two main assumptions: that it is possible to identify a region which contains a single, non-dispersed fibre population, and that the FRF

estimated from this region holds globally. In other words, it is possible to estimate a valid ‘brain-wide’ FRF. These assumptions may be challenged if we consider there to be non-zero orientation dispersion in typical MRI voxels, and the diffusion characteristics of white matter to be dependent on microstructural properties such as axonal diameter, packing and myelination that can vary across the brain [153, 7]. Thus, the estimation of a brain-wide fibre response function could be unreliable and has been shown to produce spurious results [154, 53] when poorly estimated. To overcome this limitation, various attempts have been made to assume a more local (rather than global) response function. Anderson *et al.* [155] derive a voxel-wise FRF from the estimated apparent diffusion coefficient, whilst Jeurissen *et al.* [52] and Dhollander *et al.* [51] estimate a tissue specific response function and perform multi-tissue CSD. Nonetheless, alongside the recent and complementary publication by Schilling *et al.* [156], this report constitutes some of the first work to actively demonstrate the extent of FRF variation across the brain.

In this work we combine diffusion MRI and histology data from the same human tissue sample to investigate the diffusion properties of white matter under conditions where multiple modalities are informative of the ‘true’ fibre configurations. To highlight the benefits of the data-fusion framework, this report considers a joint model which is based on non-parametric CSD. We show that by including histology data into the joint model we are able to overcome the degeneracy of fibre dispersion and radial diffusion. As such, we can simultaneously estimate the diffusion profile of a single fibre bundle (the FRF) and the underlying fibre orientation distribution (the FOD) on a voxel-by-voxel basis. In our results we investigate how the FRF changes across several white matter regions, and consider the implications this may have on both the reliability of CSD-based analyses and our understanding of the white matter microstructure.

4.2 Methods

This section will first describe the principles of CSD, and how these were developed into our joint modelling approach (c.f. *Joint modelling*). As a generative model which spans multiple modalities, the joint model error balances three terms (a diffusion MRI-data fidelity term, a microscopy-data fidelity term and a complexity penalty) with two regularisation factors. Thus, *Simulations* describes how simulated data (with a known ground truth) was used to determine appropriate values for the two regularisation factors. Finally, *Postmortem data acquisition* provides details of the co-registered high-resolution diffusion MRI (0.4mm isotropic) and myelin-stained histological data used in this study.

4.2.1 Constrained spherical deconvolution

In constrained spherical deconvolution (CSD) the diffusion-weighted MR signal attenuation, S , measured along an orientation parametrised in spherical coordinates by angles (θ_0, ϕ_0) is considered to be the convolution of the FOD with the single-fibre response function (FRF):

$$S(\theta_0, \phi_0) = \iint_{S^2} FOD(\theta, \phi) FRF(\theta_0 - \theta, \phi_0 - \phi) \sin(\theta) d\theta d\phi \quad (4.1)$$

where both the FOD and FRF are defined on the unit sphere. The FRF describes the signal profile of a single coherently-oriented fibre bundle and both the FRF and the FOD can be expressed in terms of spherical harmonics [40].

After estimating an appropriate FRF, the FODs are reconstructed on a voxel-wise basis by deconvolving the FRF from the diffusion signal S . As the deconvolution is sensitive to noise, the spherical deconvolution is constrained to minimise physically impossible negative peaks in the reconstructed FOD [157]. To constrain the optimisation a modified Tikhonov regularisation method [55] is typically employed to iteratively penalise negative amplitudes on the FOD and update the FOD estimation.

This report will compare results from the standard CSD model (a deconvolution) with those from the novel joint modelling approach (a generative model). In the standard CSD model, processing was performed using the MRtrix3 package (www.mrtrix.org, [158, 54]) and the FRF was determined empirically using Tournier’s approach [159]. Briefly, the Tournier algorithm iterates between response function estimation and CSD to determine the 300 most likely ‘single-fibre’ voxels from which the FRF is estimated.

4.2.2 Joint modelling

The principles of constrained spherical deconvolution were extended to enable joint estimation of the FRF and FOD for datasets with both diffusion MRI and microscopy data. Although the joint modelling approach could be applied to any type of microscopy data from which we can extract fibre orientations, in this work we analyse histological images which have been stained for myelin and co-registered to 0.4 mm isotropic diffusion MRI data (c.f. *2.4 Postmortem data acquisition*). Through structure tensor analysis of histological images [58, 59, 60], the primary fibre orientation was estimated per pixel after which 1400×1400 orientations were combined into a frequency histogram to generate a 2D microscopy-derived FOD ($FOD_{2D,micro}$) for a ‘superpixel’ comparable to the spatial resolution of diffusion MRI. In the model, the microscopy-derived FOD acted as a soft constraint on both the fibre orientation and amount of dispersion in each MRI voxel. This allowed estimation of the diffusivities both parallel (d_{axial}) and perpendicular (d_{radial}) to the fibre.

The generative model is described in Figure 4.2. In the joint model, the FRF and FOD were first estimated across a densely sampled sphere and then combined to predict the diffusion MRI signal, S , along each gradient direction, $k(\theta_0, \phi_0)$, using Equation (4.1). Additionally, the FOD was projected onto the 2D plane ($FOD_{2D,joint}$) and re-normalised to fit the microscopy-derived $FOD_{2D,micro}$; for details see *Section 2.3.1: Histopathology*. To retain simplicity and avoid overfitting, in the joint model the FRF was characterised by an axially-symmetric diffusion

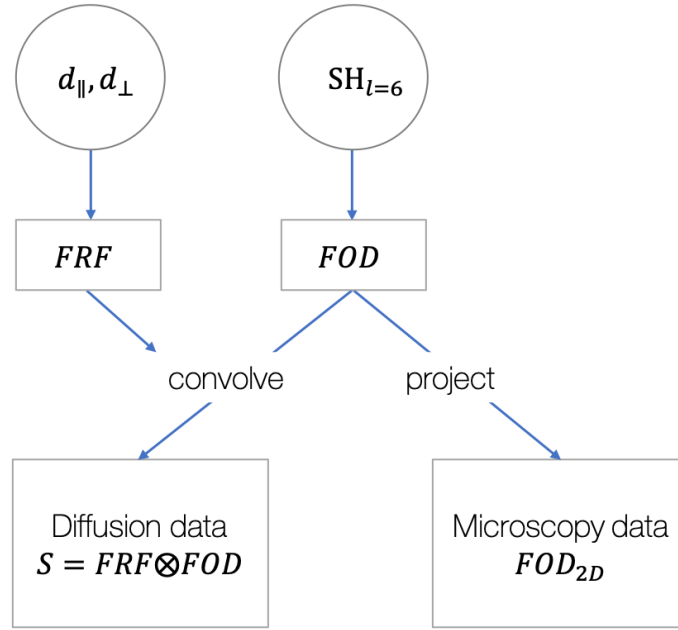


Figure 4.2: The generative joint model. Here the fibre response function (FRF) was considered an axially-symmetric diffusion tensor characterised by diffusivities (d) parallel and perpendicular to the fibre. The fibre orientation distribution (FOD) was defined on a spherical harmonic basis set of order $l = 6$. In the joint model, the FRF and FOD were simultaneously fit to the diffusion data (through convolution) and the FOD projected onto a 2D plane for comparison with the microscopy-derived FOD.

tensor, whilst the FOD was defined on a spherical harmonic basis set of order 6. The model thus contained 30 free parameters: 2 diffusivities (which determined the FRF) and 28 spherical harmonics coefficients (which determined the FOD).

The model parameters were fitted by minimising a cost function E made of three terms,

$$E = E_{diff} + \lambda_{micro} E_{micro} + \lambda_{complex} E_{complex}, \quad (4.2)$$

where E_{diff} describes the diffusion MRI-data fidelity term, E_{micro} the microscopy-data fidelity term, and $E_{complex}$ a complexity penalty. The predicted diffusion MRI signal, S , was compared to the diffusion MRI data, Y , along gradient direction $k \in [1, N]$, using the mean sum of squared residuals,

$$E_{diff} = \frac{1}{N} \sum_{k=1}^N (Y_k - S_k)^2, \quad (4.3)$$

This assumes a zero-mean Gaussian distribution for the diffusion MRI noise, though the true distribution will depend on a various factors including the b-value (where Rician distributed MRI data can be approximated as having Gaussian noise in the moderate-to-high SNR regime), the number of receive coils, and the method for combining signals across multiple channels. The projected FOD ($FOD_{2D,joint}$) was compared to the microscopy-derived $FOD_{2D,micro}$ using the symmetric Kullback-Leibler divergence D_{KL} [160],

$$E_{micro} = D_{KL}(FOD_{2D,micro} || FOD_{2D,joint}) + D_{KL}(FOD_{2D,joint} || FOD_{2D,micro}), \quad (4.4)$$

where the Kullback-Leibler divergence of two discrete probability distributions P and Q is defined as,

$$D_{KL}(P||Q) = - \sum_i P(i) \log \frac{Q(i)}{P(i)}. \quad (4.5)$$

In the model, i indicates the discrete values of θ , the in-plane angle over which $FOD_{2D,micro}$ and $FOD_{2D,joint}$ are defined. As the Kullback-Leibler divergence becomes numerically unstable when $P(i)$ or $Q(i)$ are close to zero, we set a lower bound such that $P(i), Q(i) \geq 2 \times 10^{-16}$. The lowest value in the real histology FODs was found to be 4×10^{-8} .

A third error term, the ‘complexity penalty’, minimised the presence of spurious peaks in the 3D FOD. The penalty consisted of two parts. The first penalised unphysical, negative peaks in the FOD. The second penalised small positive peaks and excessive undulations in the FOD shape, which are unlikely to be biologically meaningful. Firstly, positivity in the FOD was enforced in a manner similar to Tournier *et al.* [40]. The spherical harmonics (which describe the 3D FOD) were projected onto a densely sampled sphere, the magnitude of any negative peaks summed (creating E_{neg}) and then set to 0. Thus, the negative peaks did not contribute to either the predicted diffusion signal S nor $FOD_{2D,micro}$. To discourage the presence of small positive peaks or excessive undulations in the

FOD, the complexity penalty minimised the L1-norm of the spherical harmonics coefficients, $x \in [1, 28]$ where,

$$E_{complex} = E_{neg} + \sum_{n=1}^{28} |x_n|. \quad (4.6)$$

The three error terms were combined into a single cost function (Equation 4.2), with regularisation factors λ_{micro} and $\lambda_{complex}$ respectively, and minimised using MATLAB's non-linear solver *fmincon* [161]. Optimisation constraints ensured that the FOD, d_{axial} and d_{radial} were positive and that $d_{axial} \geq d_{radial}$. The joint model was initialised as follows: in each voxel, the spherical harmonics coefficients output from CSD [40] provided an initial estimation of the FOD and the FRF was initialised to $d_{axial} = 0.25 \mu m^2/ms$, $d_{radial} = 0.05 \mu m^2/ms$. MATLAB's simulated annealing algorithm *simulannealbnd* [161] was used to generate three sets of starting parameters from the above initial conditions. The model was optimised for each set of starting parameters, and the solution with the lowest error chosen. With this procedure, the joint model was able to simultaneously optimise both the FRF and FOD on a voxel-by-voxel basis.

4.2.3 Simulations

This section will describe two sets of simulations which were used to determine appropriate regularisation factors λ_{micro} and $\lambda_{complex}$. Diffusion MRI and histology data was simulated for a fairly simple single-fibre configuration. The first set of simulations was used to determine an appropriate value of λ_{micro} and did not include a complexity penalty ($\lambda_{complex} = 0$). We evaluated whether, through the inclusion of histology data (increasing λ_{micro}), the joint model was able to separate fibre dispersion from radial diffusion by assessing how faithfully the model could quantify the fibre orientation dispersion. Here we included 3 parameters: the azimuth, θ , and inclination, ϕ , of the FOD peak, and the symmetric fibre dispersion around the peak, characterised by the orientation dispersion index (*ODI*, Equation 4.7). In the second set, λ_{micro} was fixed and a cross-validation approach was taken to

optimise $\lambda_{complex}$. Here spherical harmonics were used to describe the FOD and we assessed how the residual error of the model changed with respect to $\lambda_{complex}$.

In both sets of simulations a dispersed FOD was approximated by a Watson distribution with concentration parameter κ [49, 39]. As proposed by Zhang et al. [39], an orientation dispersion index was defined as,

$$ODI = \frac{2}{\pi} \arctan(1/\kappa) \quad (4.7)$$

whereby the ODI varies from 0 (no dispersion) to 1 (isotropic dispersion). As above, the diffusion signal was calculated as the convolution of the FOD with an axially symmetric FRF (Equation 4.1) after which zero-mean Gaussian noise was added. In a similar manner to Sotiropoulos *et al.* [46], assuming $S_0 = 100$, the SNR was defined as $SNR = S_0/\sigma_{noise}$. Simulation parameters were fixed to values of $ODI = 0.25$, $d_{axial} = 0.2 \mu m^2/ms$, $d_{radial} = 0.1 \mu m^2/ms$, $SNR_{MRI} = 15$.

Structure tensor analysis [58, 59, 60] of the histology data produces a single fibre orientation per pixel, where each orientation can be seen to represent the in-plane component of a (myelinated) fibre in the 3D FOD (as defined by MRI, assuming perfect co-registration). To simulate histology data, we emulated this process in reverse: fibre orientations were sampled from the Watson distribution (which describes the 3D FOD) using rejection sampling, projected onto a 2D ‘‘histological’’ plane and combined into a frequency histogram. Rejection sampling is an acceptance-rejection method which allows us to generate observations from the Watson distribution. Briefly, orientations (θ, ϕ) were randomly sampled across the sphere. For each orientation, the value of the normalised Watson distribution $P(\theta, \phi)$ was evaluated and a number was randomly sampled from the uniform distribution $U(0, 1)$. The orientation (θ, ϕ) was accepted if the randomly generated number was less than, or equal to, $P(\theta, \phi)$. In simulation, 1400×1400 (accepted) samples were drawn from the Watson distribution to match the number of fibre orientations in each ‘superpixel’ of the real data. No additional noise was added to the histology data.

We first determined an appropriate value for the weighting of the histology data λ_{micro} , whilst setting $\lambda_{complex} = 0$. As we were primarily interested in how accurately the model could separate fibre dispersion from radial diffusion, a wrapper function was used to simplify the joint model such that the model contained only 3 free parameters: ODI , d_{axial} and d_{radial} . To do this the fibre orientation was fixed to a specific direction, (θ, ϕ) , after which the Watson-like FOD could be fully characterised by the ODI alone (rather than the 28 spherical harmonics of the normal joint model). Diffusion MRI and histology data was simulated for a single-fibre population oriented along (θ, ϕ) and a given ODI . The simplified joint model was fit to the simulated data using a Markov chain Monte Carlo (MCMC) method, Metropolis Hastings [84], to find optimal values of ODI , d_{axial} and d_{radial} . d_{axial} and d_{radial} were initialised using the same parameters as for the real data ($d_{axial} = 0.25 \mu m^2/ms$, $d_{radial} = 0.05 \mu m^2/ms$) whilst the dispersion was initialised to $ODI = 0.5$. The optimisation was constrained such that the ODI , d_{axial} and d_{radial} were positive and $d_{axial} \geq d_{radial}$. Here the error term $E = E_{diff} + \lambda_{micro} E_{micro}$ was interpreted as the log-likelihood and the parameters had uniform priors within their bounds. It was preferable here to use MCMC for optimisation (instead of *fmincon* above) for two reasons. Firstly, the multiple MCMC samples identify combinations of parameters which produce an equally good fit. This allowed us to investigate both the precision and accuracy of the model. Secondly, as there were fewer model parameters to fit (2 diffusivities and the ODI , rather than the 30 free parameters above), the optimisation was better suited to MCMC methods. In these simulations of a single fibre population, the number of model parameters was greatly reduced as we fit a single-fibre FOD of symmetric dispersion, characterised by the ODI . Due to the tight restrictions on the form of the FOD, this simplified model was not used on real data. In real data, we describe the FOD using spherical harmonics coefficients rather than the ODI of a Watson distribution, allowing for greater complexity in the FOD shape (e.g. multiple peaks with complex dispersion). The above procedure was first performed for a fibre population oriented along the histological plane ($\phi = 0$) where the histological data was most informative of the

FOD shape, and then repeated for various inclinations, ϕ . Finally, the simplified model was used to investigate the robustness of the joint model to mis-alignment of the histology-diffusion MRI data. Here the histology FOD was rotated with respect to the diffusion MRI data and the model evaluated for its accuracy and precision in estimating the *ODI* and radial diffusivity.

For the second set of simulations, a cross-validation approach was used to determine an appropriate weighting of the model complexity penalty, $\lambda_{complex}$. The complexity penalty aims to minimise spurious peaks in the FOD by penalising non-zero spherical harmonics which are unsupported by the data. If $\lambda_{complex}$ were too low it would be ineffective, but if set too high, the spherical harmonic coefficients would be unable to accurately describe the FOD. The latter would be identified in a cross-validation study as it would result in a high error of the model with respect to a validation dataset. For the cross-validation study, data was simulated for a single-fibre population (as described above) and divided into training and validation datasets of equal proportions. That is, each set included half of the gradient directions, distributed fairly evenly across the sphere. For various $\lambda_{complex}$, the model parameters d_{axial} , d_{radial} , and the spherical harmonic coefficients were fit to the training data, after which the out-of-sample residual error was calculated.

4.2.4 Postmortem data acquisition

This study analyses formerly obtained diffusion MRI and histology data; for a detailed description of samples, the data acquisition and post processing see [47]. Briefly, postmortem brain tissues, which had been immersion fixed in formalin, were obtained from the Oxford Brain Bank, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK. The postmortem tissues were from three male donors with no known neurological conditions who died of non-brain related disease. From each brain, a 5mm thick coronal section was extracted at the level of the anterior commissure to include various anatomical regions of interest: the corpus callosum, centrum semiovale, corticospinal tract, and cingulum bundle. Prior to

scanning, each section was placed in a syringe and, where necessary, immobilised with additional gauzes. The syringe was filled with Fluorinert (FC-3283, 3 M™, St. Paul, USA), a proton-free, susceptibility-matched fluid which contributes no MR signal and maximises field homogeneity.

Diffusion MRI data was acquired on a 9.4T preclinical MRI scanner equipped with a 100 mm bore gradient insert (Varian Inc, CA, USA) and with a maximum gradient strength of 400 mT/m. At a spatial resolution of 0.4 mm isotropic, a diffusion-weighting of $b = 5000 \text{ s/mm}^2$ was achieved with a single-line spin-echo sequence (TE=29 ms, TR = 2.4s) over a total of 120 gradient directions. An additional eight images were collected with negligible diffusion weighting ($b = 8 \text{ s/mm}^2$). This equated to approximately 8 hrs 45 mins scan time per tissue specimen.

After MRI scanning, the tissue was sectioned in half along the anterior-posterior direction. One half was processed for classic immunohistochemical staining of myelin and the other for polarised light imaging. As previous work [47] determined that the myelin staining provided better predictions of dispersion (those more consistent with diffusion MRI data), this study focused solely on these histological images. For immunohistochemical staining, the tissue was paraffin embedded and sectioned at $6 \mu\text{m}$ along the coronal plane. To visualise the myelin content, three sections of tissue were stained with antibodies against proteolipid protein (MCA839G; Bio-Rad; 1:1000) and imaged at a spatial resolution of $0.28 \mu\text{m/pixel}$. Structure tensor analysis [58, 59, 60] of the histological images estimated the primary fibre orientation per pixel. 1400×1400 fibre orientations were then combined into a frequency histogram to generate a 2D histology-derived FOD, $FOD_{2D,micro}$, at a spatial resolution comparable to the 0.4 mm diffusion MRI data. Due to the symmetric nature of the diffusion MRI-derived FOD, we calculated a symmetric (rather than asymmetric) FOD from the histology data, which was fully described over $\theta \in [-\pi/2, \pi/2]$. The $FOD_{2D,micro}$ was then normalised such that $\sum_{\theta} FOD_{2D,micro}(\theta) = 1$.

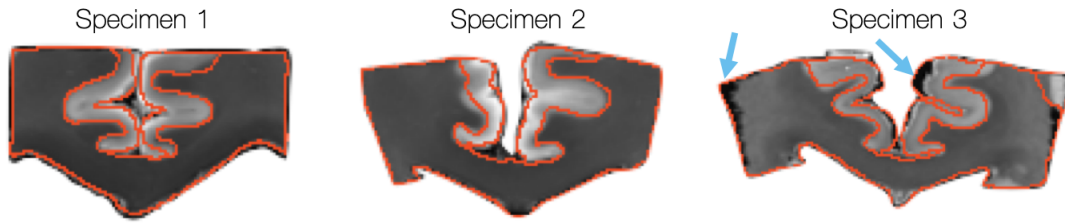


Figure 4.3: Previous evaluation of the diffusion MRI-histology registration (reprinted with permission from [47]). Generally, the co-registered data shows good alignment, with many of the tissue boundaries within one diffusion MRI voxel of each other (i.e 0.4mm). There are also areas of poor alignment, particularly in specimen 3, highlighted by the blue arrows.

To allow for voxel-wise comparison of the multimodal data, the diffusion MRI and histology were co-registered using a 2D registration based on a Modality Independent Neighbourhood Descriptor (MIND) [162] algorithm. The MIND algorithm computes a local modality-independent similarity metric and so was highly applicable to the multimodal data in this study. Co-registration of MRI-microscopy data is typically challenging due to the large number of deformations (e.g. shearing or tearing) that may occur in the histology tissue preparation. Nonetheless, previous evaluation [47] of the registered data found the tissue boundaries to be generally well aligned (within one 0.4mm MRI voxel (Figure 4.3)). All three specimens show especially good registration in the corpus callosum facilitating robust voxel-wise comparisons. However, slight mis-alignment was apparent at some tissue boundaries, particularly in specimen 3.

4.3 Results

4.3.1 Model validation

Using simulated data we first assessed whether the joint model was able to separate fibre dispersion from radial diffusion as intended. To determine an appropriate weighting of the histological data, λ_{micro} , we examined the model specificity and accuracy when estimating d_{axial} , d_{radial} and the ODI . Figure 4.4 considers a FOD

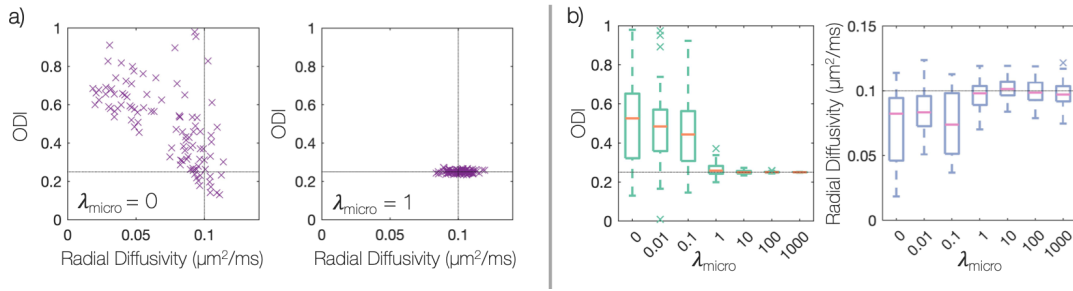


Figure 4.4: Overcoming the degeneracy between fibre dispersion and radial diffusion. Here, simulated data for a single-fibre FOD has been fit using MCMC. Note, the fibre orientation dispersion index (ODI) ranges from 0 (no dispersion) to 1 (isotropic dispersion) and the light grey lines represents the ground truth. Here we simulate data for a single fibre population oriented along the histological plane, where the histological data is most informative. a) Each point shows a sample from MCMC where the cost function of the joint model has been minimised with equally good fit. When the model only considers the diffusion MRI data (left), we see a clear degeneracy as expected. However, the degeneracy may be overcome through the inclusion of histology data to the model (right). Part b) examines the effect of λ_{micro} , the weighting of the histological data. When $\lambda_{micro} = 1$, the joint model was able to estimate both the ODI and radial diffusivity to a high degree of accuracy and precision.

oriented along the histological plane, where the histology is most informative. Figure 4.4a shows samples from fitting a simulated FOD using MCMC where the ODI , d_{axial} and d_{radial} were optimised simultaneously. With only diffusion MRI data included in the model (Figure 4.4a, left), we found a clear degeneracy between fibre dispersion and radial diffusion as expected. A similar degeneracy exists between d_{axial} and the ODI which is not shown. In comparison, Figure 4.4a (right) depicts the same fit but with both histology and diffusion MRI data included in the model. Here the histology data acted to constrain the within-voxel fibre dispersion. Even though the histology data was only informative of the fibre dispersion in 2D, the joint model was able to overcome the degeneracy and estimate both the fibre dispersion and radial diffusion simultaneously.

In the model, the histologically-derived error was weighted by a regularisation factor λ_{micro} ; when $\lambda_{micro} = 0$, the histological data was excluded from the model, corresponding to the left part of 4a. Figure 4.4b shows how the accuracy of estimating d_{radial} and the ODI varies with respect to λ_{micro} . When $\lambda_{micro} = 1$,

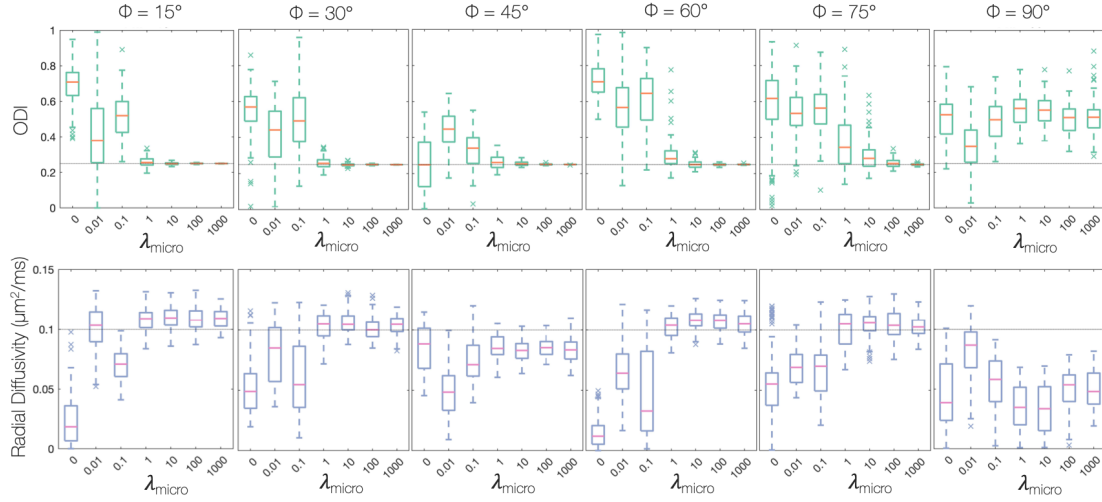


Figure 4.5: How the accuracy of the joint model, for a given λ_{micro} , is affected by the FOD inclination with respect to the histological plane, ϕ . As in Figure 4.3, data was simulated for a single-fibre FOD and fit using MCMC. The light grey line represents ground truth values. When $\lambda_{\text{micro}} = 1$, the joint model was able to faithfully estimate both the ODI and radial diffusivity for fibres of inclinations up to 60° out of the histological plane. For an inclination of $\phi = 45^\circ$ and when $\lambda_{\text{micro}} \geq 1$, the joint model estimated a value of radial diffusivity lower than the ground truth. The estimated value appears consistent across $\lambda_{\text{micro}} \geq 1$. When investigating noiseless data, the model consistently estimated radial diffusivities very similar to the ground truth (data not shown). Therefore we can conclude that such discrepancies between the estimated radial diffusivity and the ground truth (which are consistent for $\lambda_{\text{micro}} \geq 1$) are artefacts due to noise, not the performance of the model.

we observe a break in the degeneracy between d_{radial} and the ODI , demonstrated by the increase in precision and accuracy.

In Figures 4.4a,b the FOD was oriented along the histological plane, such that the 2D histological data was highly informative of the FOD shape. In contrast, the histological data should be minimally informative for FODs oriented out of the histological plane. Figure 4.5 evaluates the performance of the model for fibre FODs with increasing inclination, ϕ , with respect to the histological plane. For $\lambda_{\text{micro}} \geq 1$, both d_{radial} and the ODI were estimated with good accuracy for fibre inclinations of up to 60 degrees. Accurate estimation of d_{radial} and the ODI for fibres of higher inclination required increasing values of λ_{micro} . Finally, for an FOD oriented perpendicular to the histological plane, the degeneracy between the ODI and d_{radial} remained for all λ_{micro} .

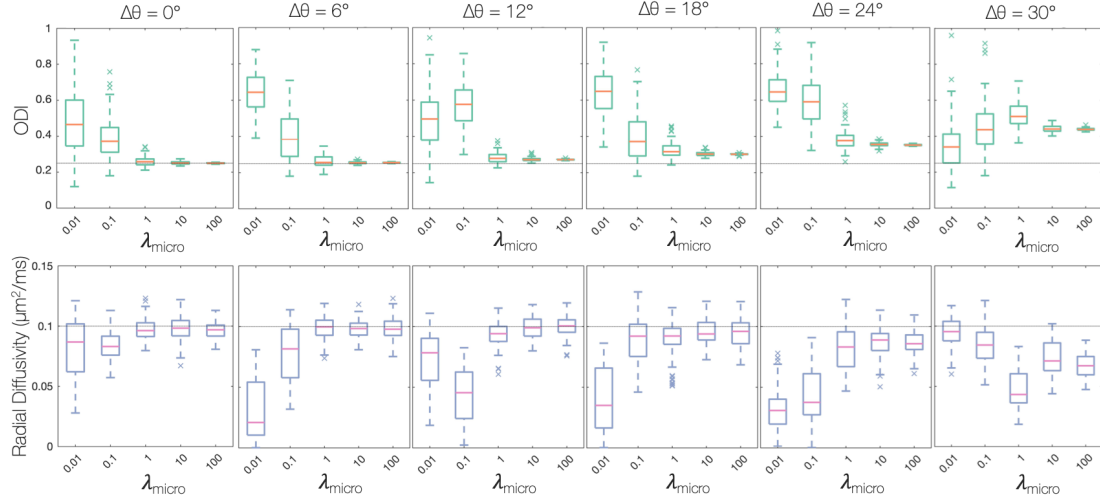


Figure 4.6: Evaluating the robustness of the joint model to mis-alignment (here rotation) of the histology FOD with respect to the diffusion data. Data was simulated for a single fibre population oriented in the histological plane, the histological FOD was rotated $\Delta\theta$ with respect to the diffusion MRI data, and optimised using MCMC. Again, the light grey line represents ground truth values. For $\lambda_{micro} > 1$ and rotations $\Delta\theta \geq 12^\circ$, the joint model estimated the ODI with high precision, but without accuracy: the ground truth no longer lies within the distribution of estimated values. For $\lambda_{micro} = 1$, the model could estimate the ODI and radial diffusivity with both good precision and accuracy for rotations $\Delta\theta \leq 12^\circ$.

On occasion, in Figure 4.5, the joint model appeared to estimate radial diffusivities higher ($\phi = 15^\circ$) or lower ($\phi = 45^\circ$) than the ground truth. Notably, the estimated radial diffusivity is consistent across $\lambda_{micro} \geq 1$. When evaluating noiseless data, the joint model repeatedly recovered the ground truth values of both radial diffusivity and ODI (data not shown). Therefore, we conclude that these deviations are an artefact of the diffusion MRI noise, not the performance of the model.

Figure 4.5 suggests that it is optimal to set λ_{micro} to a very high value (≥ 100). Since the simulated histology FOD is both noiseless and perfectly aligned to the diffusion MRI data, this is to be expected. For a large λ_{micro} , the histological FOD becomes similar to a ‘ground truth’ or a hard constraint. In real data however, there are various sources of noise in the histology images (e.g tissue deformation or tearing) and some degree of mis-registration of the diffusion MRI-histology data. This will result in a lack of spatial overlap and/or a rotation of the histology data with respect to the diffusion MRI data. Figure 4.6 considers the robustness of the

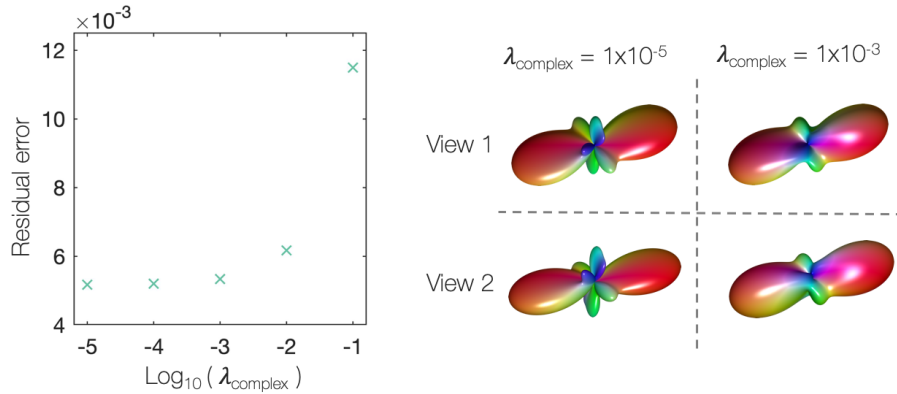


Figure 4.7: Determining $\lambda_{complex}$, the weighting of the complexity penalty. A complexity penalty was added to prevent spurious peaks occurring in the FOD. Left: Having performed cross-validation on simulated data, the residual error of the out-of-sample data was estimated for various $\lambda_{complex}$. $\lambda_{complex} = 1 \times 10^{-3}$ was chosen for all future optimisations due to the unsubstantial increase in the residual error. Right: An example voxel from the postmortem dataset. The voxel was optimised both with a very low complexity penalty ($\lambda_{complex} = 1 \times 10^{-5}$) and with $\lambda_{complex} = 1 \times 10^{-3}$. We see how the complexity penalty successfully minimises the presence of spurious peaks in the FOD which are unlikely to be biologically meaningful.

joint model to rotations, $\Delta\theta$, of the histology FOD with respect to the diffusion data. As in Figure 4.4, Figure 4.6 considers simulated data for a single-fibre population oriented in the histological plane ($\phi = 0$). For large $\lambda_{micro} > 1$ and rotations $\Delta\theta \geq 12^\circ$, the model estimated both the *ODI* and radial diffusivity with increasing precision, but with reduced accuracy. For $\lambda_{micro} = 1$, the model could estimate the *ODI* and radial diffusivity with good precision and accuracy both for rotations $\Delta\theta \leq 12^\circ$ (Figure 4.6) and fibres of inclination $\phi \leq 60^\circ$ (Figure 4.5). Therefore, $\lambda_{micro} = 1$ was chosen for all further optimisations: the histology FOD was considered to be a soft-constraint and the model estimates were less susceptible to mis-registration or histology artefacts.

With $\lambda_{micro} = 1$, a procedure of cross-validation was used to determine the weighting of the complexity penalty, $\lambda_{complex}$. Figure 4.7 plots the out-of-sample residual error with respect to $\lambda_{complex}$. To remove spurious peaks from the FOD requires the highest complexity penalty supported by the data (i.e. one which does not detrimentally increase the residual error). Consequently, $\lambda_{complex} = 1 \times 10^{-3}$

was considered optimal.

4.3.2 Residual error

Figure 4.8 compares the fit of the joint model and CSD to both the diffusion MRI and histological data. For standard CSD, the FRF was estimated empirically from the most likely ‘single-fibre’ voxels using the Tournier algorithm [159]. Once estimated, the FRF was held constant across the sample and used as a deconvolution kernel to estimate, in each voxel, the FOD from the diffusion MRI data. In comparison, the joint model acted as a forward model, fitting to both the diffusion MRI and histology data to estimate the FRF and FOD on a voxel-by-voxel basis. Figure 4.8 shows the mean absolute percentage error between the predicted diffusion signal (convolution of the FOD and FRF, Equation 4.1) and the diffusion MRI data (top), and the Kullback-Leibler divergence (Equations 4.4, 4.5) between the histological data and the model FOD when projected into the histological plane (bottom).

When compared to CSD, the joint model shows a slight increase in the diffusion MRI-associated error which is concurrent with a largely improved fit to the histological data. This is as expected: CSD by definition minimises the error with respect to the diffusion MRI data. In comparison, the joint model considers additional information about the ‘true’ fibre dispersion (from the histology data) to avoid overfitting.

4.3.3 Variations in axial and radial diffusivities

Standard CSD methods define a global FRF and thus assume that the diffusion characteristics of the tissue remain constant across the sample. In contrast, the joint model overcomes the degeneracy between fibre dispersion and radial diffusion to estimate the FRF on a voxel-by-voxel basis. In the joint model the FRF is considered an axially symmetric diffusion tensor described by d_{axial} and d_{radial} . So, the joint model can estimate how the axial and radial diffusivities (and thus the FRF) vary voxel-wise across the sample.

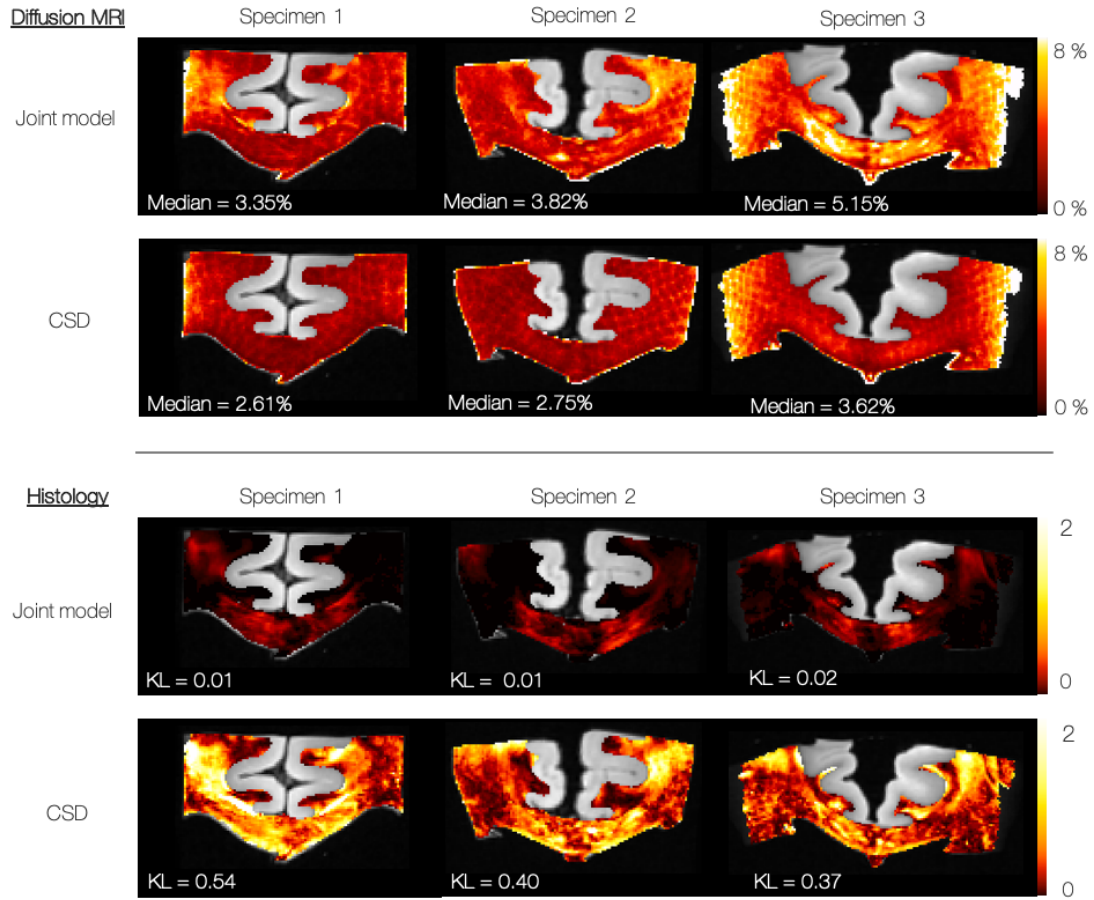


Figure 4.8: Top: The residual error (calculated as the mean absolute percentage error) of the model fit to the diffusion MRI data only. Bottom: The error of the model with respect to the histological data, defined as the symmetric Kullback–Leibler (KL) divergence. When $KL = 0$, the 2D-FODs are identical. Compared to CSD, there is a slight increase in the residual error of the joint model with respect to the diffusion MRI data. This is to accommodate the highly improved fit to the histological data.

Figure 4.9 shows considerable variation in the estimated axial and radial diffusivities across all three specimens. Here we should again note the less accurate registration of diffusion MRI and histology data in specimen 3 (Figure 4.3). Previous work [47] demonstrates the mis-alignment of the tissue around the grey matter tissue boundaries of specimen 3 as well as the more robust registration across the corpus callosum. Values of axial and radial diffusivity outside of the corpus callosum in specimen 3 should therefore be viewed with a sceptical eye. Nonetheless, in Figure 4.9 (top) we generally see a similar pattern of variation in both axial and radial diffusivities, with particularly low values of diffusivity often found

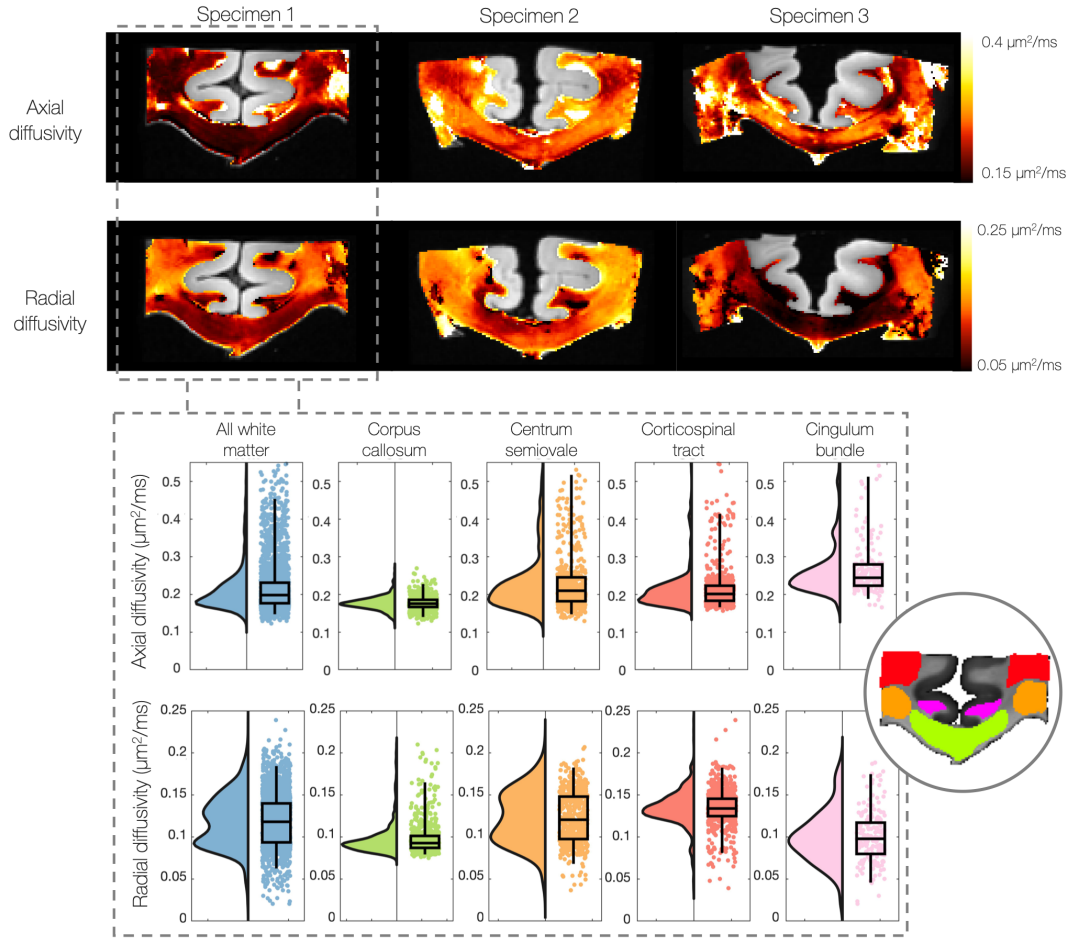


Figure 4.9: Heatmaps (top) and raincloud plots (bottom, specimen 1 only) show how the axial and radial diffusivities (and thus the FRF) vary on a voxel-wise basis across the white matter. We find notably lower radial diffusivities in the corpus callosum compared to other white matter tracts: the centrum semiovale, corticospinal tract and cingulum bundle. Additionally, we find two apparent distributions of radial diffusivities in the white matter. These results challenge standard CSD methods where a global FRF is assumed constant across the sample. Note the change in scale bar between the axial and radial diffusivities, with the radial diffusivity generally lower.

in the corpus callosum.

To assess how the diffusivities vary with anatomical regions of interest, Figure 4.9 (bottom) compares the distribution of axial and radial diffusion in all white matter voxels to those only in the corpus callosum, centrum semiovale, corticospinal tract and cingulum bundle. We see considerably lower diffusivities in the corpus callosum, as well as two distinct distributions of radial diffusivities across all

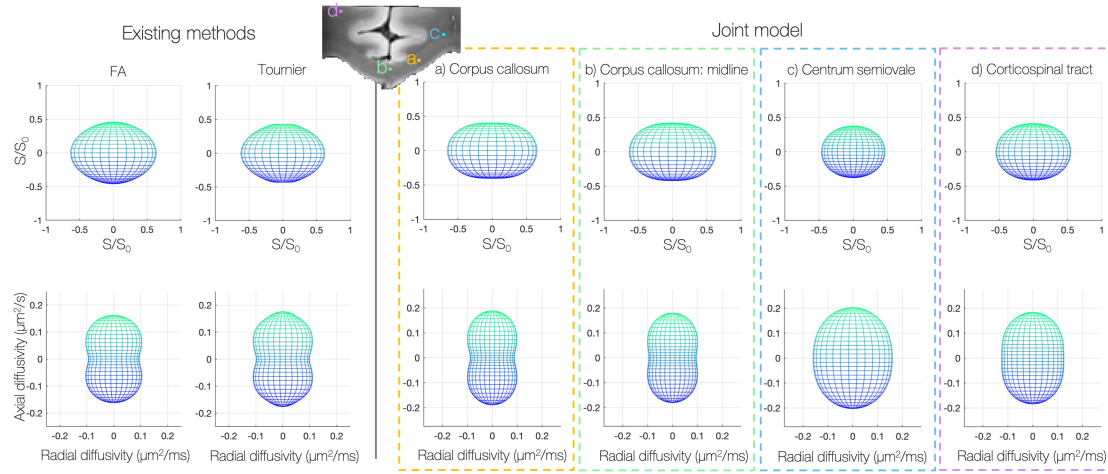


Figure 4.10: Variations in the observed fibre response function (FRF); here the FRF describes the diffusion profile of a single fibre bundle aligned vertically with respect to the page. The axial and radial diffusivities which describe the FRF are plotted below. A global FRF was first estimated from the 300 highest FA voxels and the Tournier algorithm [159] which selects the most likely ‘single-fibre’ voxels (left). This was compared to the FRF estimated by the joint model in the corpus callosum, centrum semiovale and corticospinal tract. The joint model estimates the FRF on a voxel-wise basis. However, here we consider local changes in the FRF; the FRFs shown were characterised by the mean axial and radial diffusivities over a 2×2 neighbourhood in each anatomical region. We see how the joint model predicts in general a more anisotropic (oblate) FRF in the corpus callosum and that the FRF appears to vary considerably with anatomy.

white matter voxels.

4.3.4 Fibre response function

Figure 4.10 compares three ways of estimating the FRF from the diffusion MRI data alone: the two conventional approaches estimate a global FRF, whilst the joint model estimates the FRF on a voxel-by-voxel basis. The FRF was first estimated from the 300 highest FA voxels and from the most-likely ‘single-fibre’ voxels defined by the iterative Tournier algorithm [159, 158]. This was compared to the joint model estimates of the FRF for voxels in the corpus callosum, centrum semiovale and corticospinal tract. To consider local changes in the FRF (rather than the voxel-wise variations of Figure 4.9), Figure 4.10 shows the mean FRF across a 2×2 neighbourhood of voxels in each anatomical region.

Here both conventional methods estimated the FRF from voxels either in the corpus callosum or the cingulum bundle (data not shown): both of which are known to contain coherently oriented, single-fibre voxels. In Figure 4.10, we see how the conventional FRFs most closely resemble those estimated by the joint model in the corpus callosum, as expected. Although the FRFs are similar, there are also notable differences. In the corpus callosum (Figure 4.10a)), the joint model estimated an FRF with lower radial diffusivity and higher diffusion anisotropy ($d_{axial} - d_{radial}$) when compared to the conventional FRFs. This would be consistent with standard methods conflating fibre dispersion with diffusivity in the voxels used to generate the FRF.

In the joint model, the FRF appeared to vary considerably with neuroanatomy. Compared to the corpus callosum, Figure 4.10 depicts a higher radial diffusivity in the corticospinal tract as well as a more spherical FRF in the centrum semiovale.

Notably, Figure 4.10 demonstrates FRF variability across the corpus callosum, which is typically considered a homogenous tract of coherently oriented fibres. Comparing the example FRF's in Figure 4.10, the midline of the corpus callosum is characterised by lower axial diffusivity, higher radial diffusivity and a subsequent decrease in diffusion anisotropy.

4.3.5 FODs

Notable changes to the estimated FODs were also observed. Figure 4.11 (top) shows representative voxels from the corpus callosum of specimen 1 where, when compared to the CSD, the joint model estimated narrower FODs and a lower degree of dispersion in the underlying fibres. This finding was consistent across many (but not all) voxels in the corpus callosum of all three specimens.

Furthermore, in the joint model we sometimes observed the emergence of additional distinct fibre populations in the FOD. Figure 4.11 shows an example from the centrum semiovale; a known region of crossing fibres.

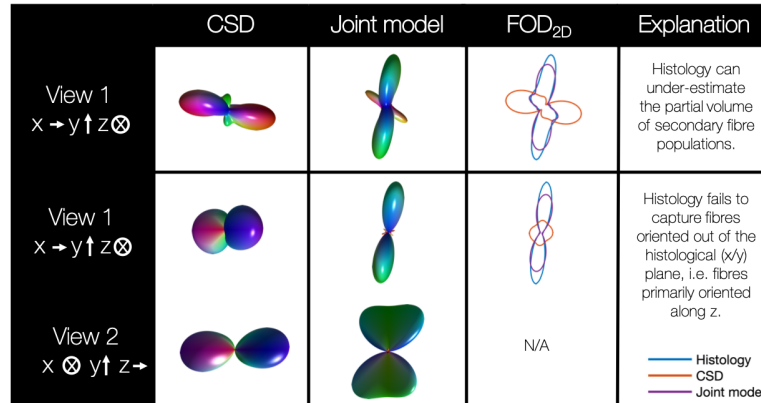


Figure 4.12: Two cases where the histological data might bias the 3D FOD predicted by the joint model. Histology may under-estimate the volume fraction of in-plane secondary fibre populations (top), or omit out-of-plane fibres from the histology-derived 2D FOD (bottom). Here the 3D FOD is seen from two orthogonal views: looking onto (view 1) and out of (view 2) the histological plane (denoted x/y).

Figure 4.11 right (‘FOD_{2D}’) shows the improved fit of the FOD when projected into the histological plane and compared to the histologically derived $FOD_{2D,micro}$. This is quantified by the reduced KL divergence as shown in Figure 4.8. We see here how the histological data acts as a soft constraint on both the fibre orientation and amount of dispersion in the MRI voxel.

4.3.6 Biasing the shape of the FOD: model limitations

Figure 4.12 demonstrates two cases where, due to limitations of the 2D histological data, the histology may bias the joint model prediction of the FOD. Firstly, the histology may underestimate, or totally omit, the volume fraction of a secondary fibre population (Figure 4.12 top). We see how the joint model thus attempts to minimise the presence of a secondary fibre population, the resultant FOD being perhaps biased or biologically improbable.

Secondly, the histological data is most sensitive to fibre bundles in, or close to, the histological plane. This effect was first observed in simulated data (Figure 4.5) where the joint model was able to accurately estimate the fibre dispersion and radial diffusion for fibres of inclination angles $\phi \leq 60^\circ$, but struggled to faithfully

reconstruct highly inclined FODs with good accuracy. As the fibre inclination was increased, the histology FOD became evermore circular and information about the FOD dispersion was lost: for highly-inclined fibres, the model again became degenerate. Figure 4.12 (bottom) shows a single voxel from real data in two orthogonal views: looking down on the histological plane (view 1), and perpendicular to the plane (view 2). In CSD, we see a single fibre population, primarily oriented out of the histological plane; the CSD-derived 2D FOD is consequently fairly circular. The histology-derived FOD however looks substantially different. This may be due to the histology-derived FOD over-emphasising either in-plane components of mostly through-plane fibres or minor fibre populations which lie in the histological plane. Alternatively, there could be mis-registration of the diffusion MRI and histology data such that the two datasets are incompatible. Consequently, as the joint model tries to fit the histological data, it assumes a much less biologically plausible FOD shape. Both examples in Figure 4.12 were taken from the centrum semiovale on the left hand side of specimen one: a known region of complex fibre configurations and, in this specimen, a region of high diffusion MRI-associated error in the joint model.

4.3.7 Orientation of the FOD peak

In the joint model, the histological data constrains both the fibre orientation and amount of dispersion in each diffusion MRI voxel. This is likely to affect the orientation of the estimated FOD peak in two respects. Here we define the peak orientation by the azimuthal angle in, and inclination out of, the histological plane. Firstly, any mis-registration of the diffusion MRI and histology data may cause a rotation in the azimuthal angle. Secondly, as the histological data is most informative of in-plane fibres, the joint model may favour FODs of low inclination. Figure 4.13 investigates these effects by comparing the orientation of the primary peaks of FODs estimated by both CSD and the joint model. Figure 4.13a shows the angular deviation of the two peaks where the three samples have a median difference of 12.8° , 12.9° and 13.5° . Figures 4.13b and c compare the

azimuth and inclination of the primary peaks respectively. We see how all three specimen show very close correlation with the robust regression line close to the line of unity and r-values between $r = 0.93 - 0.98$. Note that here we use robust linear regression to limit our sensitivity to outliers and that the r-values of robust regression (here calculated using MATLABs *fitlm* [161]) are typically inflated when compared to standard linear regression.

In Figure 4.13b specimen 1 we see a prevalence of joint model estimated FOD peaks at azimuthal angles of $\theta \pm 90^\circ$, i.e along the superior-inferior axis. This is due to an almost equal prevalence of histology-defined FODs with peaks at these angles and is probably caused by an artefact in the histology data. This however does not appear to greatly impact either the inclination (Figure 4.13c) or total angular difference (Figure 4.13a) between the CSD and joint model derived FODs of specimen 1. Overall, Figure 4.13 indicates a markedly stable relationship between the FOD peaks derived from CSD (diffusion MRI analysis only) and the joint model (combined diffusion MRI and histology analysis).

4.4 Discussion

In the joint model we utilised a data-fusion framework to combine co-registered diffusion MRI and histology data from the same tissue samples, acquired from three postmortem human brains. This allowed us to test the validity of a brain-wide fibre response function as used in CSD [40]. With the inclusion of histological data, we were able to constrain both the fibre orientation and amount of dispersion within each MRI voxel, albeit in 2D. Consequently, the joint model could overcome the degeneracy between fibre dispersion and radial diffusion to estimate the FRF and FOD simultaneously on a voxel-by-voxel basis.

Using simulated data, we evaluated the model specificity and accuracy. For simulated data of single-fibre populations, the joint model provided particularly robust estimates of both orientation dispersion and radial diffusion for fibre populations in

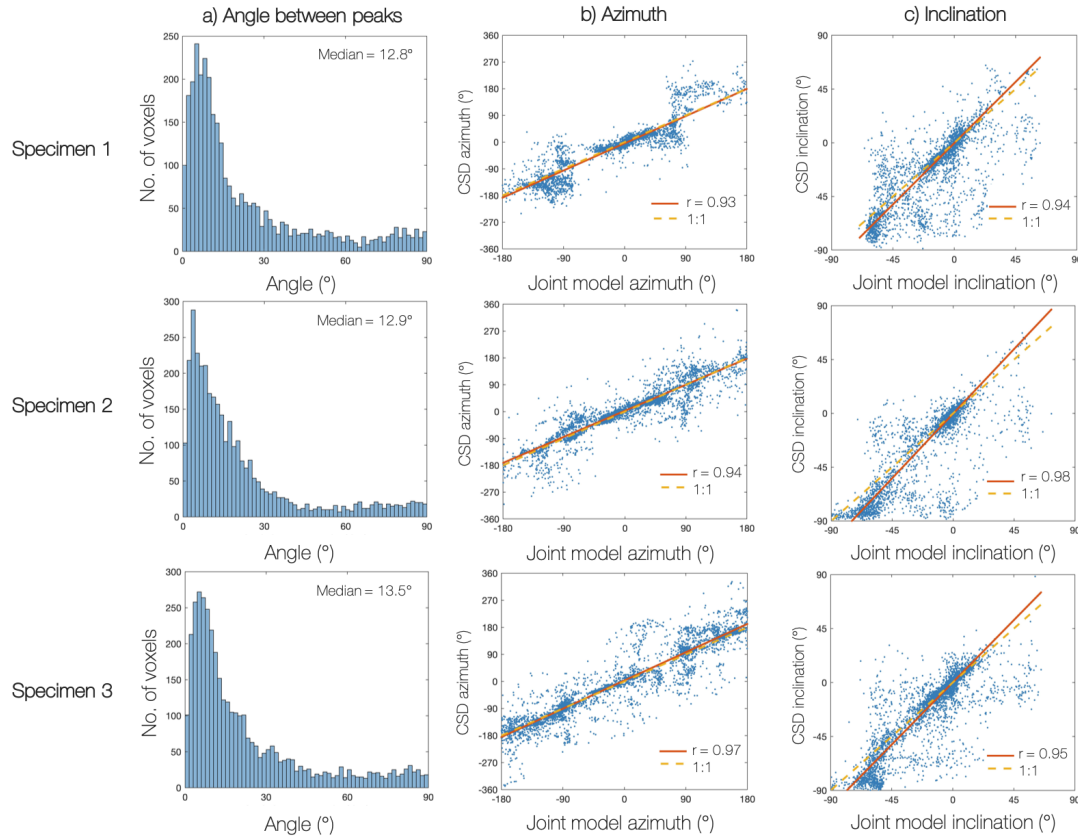


Figure 4.13: Comparing the orientation of the primary FOD peak derived from both CSD and the joint model. The peak orientation is described by an azimuth and an inclination angle which is defined with respect to the histological plane (inclination = 0°). In the joint model, any mis-registration of the diffusion MRI and histology data may result in a rotation of the peak azimuth. In addition, as histology is most informative of fibres lying in or close to the histological plane, the joint model might favour FODs of low inclination angles. In all 3 specimen there is a low angular deviation (a) between the CSD and joint model FOD peaks, with good correlation between the azimuthal (b) and inclination (c) angles: the robust regression lines are close to unity with r -values between 0.93-0.98.

or close to the histology plane (up to inclinations of 60°), where the histological data was most informative. However, in both simulation and post-mortem data the joint model was less effective at reconstructing out-of-plane fibre populations. This was due to an inherent limitation of the microscopy technique, where fibres oriented out of the plane aren't readily detected in the 2D histological data. The bias of the model could be reduced if the microscopy was able to reconstruct the FOD in 3D; 3D polarised light imaging developed by Axer *et al.* [63], or 3D confocal microscopy from Schilling *et al.* [163, 156] being two examples of such

techniques. In addition, future work could take a more heuristic approach where the regularisation factor λ_{micro} could depend on fibre inclination, as a proxy of how informative the microscopy data is of the 3D FOD.

In this joint model, the FRF was characterised by axial and radial diffusivities, which appear to vary considerably across the human brain. Generally, both diffusivities seem to follow a similar pattern of variation, with particularly low values of radial diffusivity found in the corpus callosum. Contrary to the assumption of a brain-wide FRF in CSD, our results demonstrate that the FRF is dependent on the local anatomy. This supports recent findings by Schilling *et al.* who used 3D confocal microscopy to investigate FRF variation across the brain of a squirrel monkey [156]. FRF variation is expected as tissue characteristics such as axonal density, packing and myelination are unlikely to hold across the entire brain [153]. Indeed, characteristically different fibres should each have their own unique FRF, although in practice this would be difficult to model. For example, areas of the corpus callosum have been reported to have only 30% myelination [164] which would significantly impact the FRF; decreased myelination has been shown to be associated with high radial diffusivity [165, 166, 167].

Not only does FRF variation challenge the assumption of the ‘brain-wide FRF’ used in CSD, but also implies that current analyses (which typically estimate the FRF from voxels in the corpus callosum) may be biasing the shape of their reconstructed FODs throughout the brain. Indeed, in known regions of crossing fibres such as the centrum semiovale, the joint model estimated distinct secondary fibre populations which were often recognisable but not well defined in the CSD-derived FODs. The latter effect could have important implications for tractography where secondary and tertiary fibre populations facilitate the determination of less prominent tracts through crossing fibre regions, where the tracts derived from the joint-model FODs may have considerably different paths and/or spatial extent to those from CSD-derived FODs [156]. Furthermore, the joint model often estimated FODs of lower dispersion in single-fibre voxels, particularly across the corpus

callosum. Current algorithms which estimate the FRF directly from the data [53, 159, 159] typically estimate the FRF from voxels in the corpus callosum which exhibit high fractional anisotropy. However, even these voxels are unlikely to contain a perfectly coherent fibre bundle. Indeed, recent studies of both histology [116], polarised light imaging and diffusion MRI data [47] have found significant levels of dispersion in the corpus callosum, particularly at the midline [47]. To obtain accurate estimates of the FOD, both this study and that by Schilling *et al.* [156] support the development of analyses which estimate a local rather than global FRF. Both approaches are however limited as they require co-registered diffusion MRI and microscopy data in postmortem tissue samples. Building on the work by Lasič *et al.* [150] and Szczepankiewicz *et al.* [151], Cottaar *et al.* [152] combine linear and spherical tensor diffusion encoding to overcome the degeneracy between fibre dispersion and diffusion anisotropy to estimate both on a voxel-wise basis, with highly consistent results. Similar techniques could provide exciting new avenues for local FRF estimation in in vivo data and more faithful FOD reconstruction in spherical-deconvolution based analyses. Additionally, future work could focus on developing a technique which iterates between FRF estimation and FOD reconstruction, to determine population specific FRFs.

Multimodal datasets also present a number of challenges: one being that the microscopy data is only representative of a subset of the tissue microstructure. Here the tissue sections were stained to visualise the myelin content of the tissue, yet only pixels above a certain staining intensity were recognised as myelinated fibres. Fibres that failed to meet the staining threshold (perhaps those with less myelin and typically lower diameter), and other cell types (e.g. glia) may have therefore been underrepresented or omitted from the microscopy-derived FOD. Secondly, as the histological stain was only sensitive to myelin, other cell types (e.g. glia) were excluded from the microscopy FOD. If the unmyelinated axons or glia both contributed to the diffusion signal and acted to increase the dispersion of the ‘true’ FOD, the FOD of the joint model would underestimate the amount of dispersion compared to that of the true contributing microstructure. Nonetheless,

as seen in Figure 4.11, the joint model did not always act to fit to the histological data perfectly. Indeed the joint model often estimated a slightly higher degree of dispersion than prescribed by the histology and on occasion assumed a substantially different FOD, if this was both driven and supported by the diffusion MRI data. This is a result of the histology acting as a soft constraint, rather than fixing the fibre orientation and dispersion.

The postmortem diffusion MRI data used here presents a second challenge. Although the data provides high spatial resolution (0.4 mm isotropic), it has relatively low diffusion contrast. This is likely due to the postmortem interval (time between death and fixation) and immersion fixation of the human brains from which our samples were taken. It is most likely that the meninges, specifically the pia mater and the components of the dura, were attached during immersion fixation of the postmortem brains in this study. As the samples originated from the centre of each brain, the uptake of fixative to this area will have been particularly slow as fixative will have had to diffuse from the cortical surface to reach the callosum, increasing the apparent postmortem interval. Prior to fixation, post-mortem tissue may begin to decompose, wherein both the postmortem interval and fixation method have been shown to change the diffusion properties of the tissue [168, 169]. This likely explains both the inter-specimen variation in estimated diffusivities and why the diffusivities reported here are low when compared to in vivo data where, for comparison, the NODDI model assumes $d_{axial} = 1.7 \mu m^2/ms$ [117, 39]. It is important to note that the joint model generalises to any dataset which includes diffusion MRI alongside microscopy data from which we can estimate fibre orientations. This encompasses existing, open access, diffusion MRI and histology datasets [163, 170, 47], as well as data from alternative microscopy techniques such as the mesoscopic fibre orientations obtained from polarised light imaging [63, 145, 47]. As such, future work aims to apply the CSD-based joint model to other datasets, of both human and monkey (perfusion-fixed at death) brain tissue.

Thirdly, the joint model simultaneously analyses co-registered diffusion MRI and microscopy data from the same tissue sample. The accuracy with which the model estimates the FOD, axial and radial diffusivities is therefore dependent on the quality of the registration. Diffusion MRI-histology registration is particularly challenging as histological images suffer from various non-linear tissue deformations (e.g. due to sectioning, tissue shrinkage or tearing) or imaging artefacts such as dirt, air bubbles or un-even immunohistochemical staining. This study analyses previously published, pre-processed data where the diffusion MRI and histology data was registered using a Modality Independent Neighbourhood Descriptor (MIND) algorithm [162]. Previous evaluation [47] of the tissue boundaries (Figure 4.3) showed generally good alignment of the diffusion MRI-histology data with the tissue boundaries mostly within one MRI voxel (0.4mm). Nonetheless, there are areas which show less good alignment where the joint model will estimate the FOD and diffusivities with a higher degree of uncertainty. The development of reliable tools for high-quality co-registration of microscopy and diffusion MRI data [171, 172, 173] will greatly aid future joint-modelling work and the field of diffusion MRI validation in general.

With very high-quality co-registered data, the joint modelling approach could further take advantage of the highly detailed fibre orientations obtained from histology. Here, we combine 1400×1400 histologically-derived fibre orientations into a frequency histogram to create a 2D FOD which is considered symmetric about the origin. The FOD is therefore essentially a summary measure, where ultra-high-resolution information about the spatial distribution of the fibre orientations within the MRI voxel is lost. Instead, the histology data could describe an asymmetric FOD and the joint-modelling approach could be used to either validate or drive the modelling of FOD asymmetry [146]. Alternatively, we could use the joint model to directly predict the diffusion signal (through the convolution of the FRF and FOD, c.f. Equation 1) in each histology pixel, or over a small local neighbourhood, much smaller than the MRI voxel. In this manner, a joint modelling approach may be able to differentiate crossing fibres from those in fanning, bending or kissing configurations which are

known to produce the same diffusion signal [48]. Furthermore, approaches utilising the high-spatial resolution of the microscopy data could be used to quantify the biases or errors associated with inferring microscopic tissue features from macroscopic (on the order of millimetres) and antipodally symmetric diffusion signals.

Finally, this report looks through the lens of a convolution-based joint model to detail both the advantages and challenges of a diffusion MRI and microscopy data-fusion framework. As such, we recognise this model to be only one example of a joint modelling approach. The microstructure model at the centre of the data-fusion framework can itself take a range of forms, or be extended to increase both its complexity and specificity. For example, future work will consider a multi-compartment model of the fibre response function, which distinguishes intra- from extra-axonal space, each characterised by a unique diffusion profile. In this manner, we hope to disentangle which tissue compartment is driving the FRF variation across the brain. Alternatively, the joint-modelling approach could be utilised to obtain high-precision estimates of fibre dispersion from, for example, a NODDI-based model [117]. Lastly, future joint models will benefit from a Bayesian framework where the datasets are combined optimally in the sense of precision-weighting, i.e. the shared parameters get information from the fused datasets while accounting for uncertainties inherent to the data, noise, and model structures.

4.5 Conclusion

The joint model takes a data-fusion approach, combining diffusion MRI and histology data from the same tissue sample to investigate the diffusion properties of white matter under conditions where multiple modalities are informative of the ‘true’ tissue microstructure. In the model, histology acted as a soft constraint on both the fibre orientation and amount of dispersion in each diffusion MRI voxel. This allowed us to overcome the degeneracy between fibre orientation dispersion and radial diffusion, to estimate the FOD and FRF on a voxel-by-voxel basis. Our results demonstrate

how the diffusion characteristics of a single fibre (here characterised by axial and radial diffusivities) vary considerably across the brain. Notably, the diffusivities were found to vary both between and within white-matter tracts; even in the corpus callosum where the microstructure is typically considered fairly homogenous. These findings contradict the assumption of a ‘brain-wide’ FRF, currently used in many deconvolution-based analyses. Finally, our results suggest that current diffusion models may be overestimating dispersion in single-fibre voxels and underestimating the number of distinct fibre populations in known regions of crossing fibres; both of which have important implications for tractography [45].

4.6 Contributions

The data used in this study was published previously by Mollink *et al.* [47]. Jeroen Mollink acquired and preprocessed the data, including structure tensor analysis of the myelin-stained slides and co-registration of the MRI and microscopy data. My personal contributions include: project conceptualisation; design, development and implementation of the joint model; data simulation; analysis and presentation of results; manuscript writing, editing and review.

5

The BigMac dataset

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Foreword

We present a truly unique dataset, the BigMac dataset, that combines ultra-high angular resolution diffusion MRI and multi-contrast microscopy in a single, whole macaque brain. The BigMac dataset includes data that spans four orders of magnitude, from microscopy images with micron or sub-micron resolution, to MRI signals on the order of millimetres. Crucially, the MRI and microscopy images are co-registered together to facilitate quantitative comparisons or joint modelling of the microscopy and MRI-derived tissue parameters. Here we detail the extensive acquisition and careful curation of the data, that together build BigMac into a innovative resource for open dissemination to researchers worldwide.

Publications relating to this work

Conference proceedings:

Howard AFD, Jbabdi S*, Khrapitchev AA, Sallet, J, Daubney G, Mollink J, Scott C, Sibson N, Miller KL*, “The BigMac dataset: ultra-high angular resolution diffusion imaging and multi-contrast microscopy of a whole macaque brain”, Proceedings of the 27th Annual Meeting of the ISMRM, Montreal, Canada, 2019.

5.1 Introduction

Our ability to characterise the human connectome has been greatly advanced by the scientific community's access to big, open data. Big data can be population based to examine both inter- and intra-subject variability in, for example, the 1,200 participants of Human Connectome Project [102] or the $\leq 100,000$ imaging participants of the UK BioBank [174]. Alternatively big data can aim to characterise a single connectome in exquisite detail, a beautiful example being the MyConnectome project [175] where a single subject was scanned 3 times a week for over 50 weeks.

When acquiring big data, there are several advantages to imaging non-human primates or mouse models rather than human data, and postmortem rather than in vivo tissue. To acquire high quality MRI data, smaller brains or tissue sections fit into small-bore preclinical magnets with higher field strengths, while postmortem tissue can be scanned for exceptionally long times. Furthermore, postmortem data avoids signal instabilities from physiological movement that can fundamentally limit the signal-to-noise. For example, Lui *et al.* [176] scanned one hemisphere of a postmortem Marmoset brain for over 2 weeks on a 14 T/8.9 cm vertical bore MRI spectrometer to obtain diffusion MRI data at ultra-high spatial resolution and 64 μm isotropic voxels. Additionally, postmortem data can be sectioned, stained and imaged using invasive microscopy techniques to obtain gold standard estimate of the 'true' tissue structure. One example is the Big Brain histological atlas where a single postmortem brain was sectioned, stained for cell bodies, imaged at high spatial resolution and reconstructed in 3D [177]. However, there remains a lack of resources which combine microscopy with both in vivo and postmortem MRI data in the same tissue, and so the link between the 'true' tissue structure and the MRI signal remains relatively unexplored. Here postmortem diffusion MRI acts as a crucial intermediary between in vivo diffusion MRI and postmortem microscopy data: the postmortem and in vivo MRI data are similar in nature (i.e. contain similar diffusion MRI signals), but image the tissue in a considerably different state

(postmortem, fixed tissue versus in vivo); the postmortem MRI and microscopy data share a similar tissue state, though the signals are different.

Here we aim to curate and explore a unique, multimodal dataset that can inter-connect microstructural features of the tissue (from high resolution microscopy data) with MRI signals throughout the brain. This research requires data that i) combines complementary MRI and microscopy data; ii) relates high-quality postmortem MRI and microscopy to in vivo MRI; iii) facilitates whole brain analysis with densely sampled data throughout; and iv) provides the above in a single specimen. The latter is essential as only when combining data from the same brain can we be sure that we are comparing like-for-like and thus not over generalising, or ignoring important inter-subject variations. Few existing datasets fulfil all these requirements. For example, existing data combining MRI and microscopy in the same tissue sample are often limited to small tissue sections [47], a single microscopy contrast or minimal MRI data [177], or compare data from different tissue samples, overlooking considerable between-brains variability. To address each of these goals, here we present “The BigMac Dataset” - **Big** data in a **M**acaque brain. The BigMac dataset combines in vivo MRI with extensive postmortem (including ultra-high angular resolution diffusion imaging (ultra-HARDI)) and multi-contrast microscopy data in a single macaque brain.

Figure 5.1 shows some example images from the BigMac dataset. Post sacrifice, a comprehensive MRI dataset (~ 270 hours scanning time) was acquired which includes both high resolution (0.3 mm isotropic) structural images and extensive diffusion MRI data at two spatial resolutions (0.6 and 1 mm isotropic), b-values of up to $10 \text{ ms} / \mu\text{m}^2$ and 1000 gradient directions in each of the outer two shells (‘ultra-HARDI’). With high b-value ultra-HARDI data, we obtain unprecedented diffusion contrast and angular resolution to characterise the diffusion signal in great detail, estimate secondary fibre populations with increased consistency and precision, and improve the reconstruction of white matter tracts through crossing

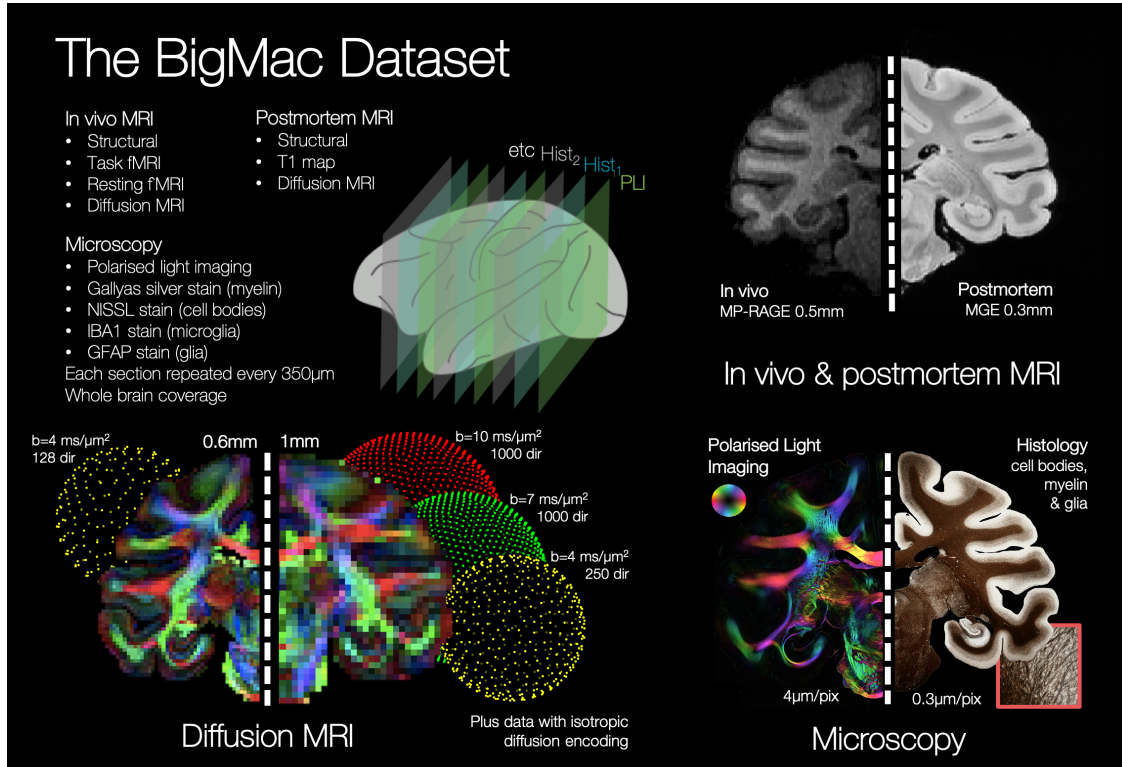


Figure 5.1: A summary of the data in the BigMac dataset. A single macaque monkey was scanned both in vivo and postmortem. The in vivo MRI data includes structural, task functional MRI (fMRI), resting fMRI and diffusion MRI. The postmortem diffusion MRI data contains up to 1,000 gradient directions for b-values of 4, 7 and 10 ms / μm² and two spatial resolutions (0.6 and 1 mm isotropic). T₁ maps and data with spherical tensor encoding were also collected. For comparison, polarised light imaging and histology provide high-resolution information about the white matter microstructure at 4 μm/pix and 0.28 μm/pix respectively. In total, the postmortem MRI acquisition constituted around 270 hours scanning time and the microscopy data took >1000 hours to acquire.

fibre regions. Furthermore, with 1000 gradient directions we retain dense sampling on an arbitrary 2D plane for direct comparison with 2D microscopy data.

After scanning, the entire brain was sectioned along the rostral-caudal axis, with consecutive sections processed for one of five microscopy contrasts (Table 5.1, where the 6th section is still unallocated and stored for longevity). The sections were sequentially allocated to either polarised light imaging (to visualise the orientation of myelinated fibres) [61, 62, 63], or histological staining of myelin, neuronal bodies or microglia. These sections were then imaged at high resolution (0.28-4 μm/pixel) and co-registered to the MRI [173, 178]. Through co-registration we re-create a full

3D volume that is populated with detailed, registered microscopy data.

Importantly, the BigMac dataset also includes in vivo MRI data. This provides an excellent opportunity to link high-quality postmortem MRI and microscopy data (detailed structural connectivity) to typical in vivo data (structural and functional connectivity) within the same brain. Through this extensive, multi-modal, multi-contrast data, the BigMac dataset aims to characterise the structural connectivity of a single, whole macaque brain in exquisite detail.

The BigMac dataset is interesting from both an anatomical and methodological viewpoint. For those interested in microstructural neuroanatomy, the densely sampled multi-contrast microscopy can be used to examine both the myelo- and cyto-architecture in great detail and develop novel white/grey matter atlases or parcellations. For those interested in diffusion modelling, the comprehensively sampled diffusion MRI space - which is co-registered to microscopy data - can be used to drive protocol optimisation, advance biophysical modelling of the tissue microstructure and direct validation of many current and future diffusion MRI models. Finally, for those interested in machine learning approaches to image analysis, the BigMac dataset will support the development of novel algorithms which jointly model MRI and microscopy data to, for example, map quantitative imaging biomarkers to specific features of the tissue microstructure.

This chapter will first walk through the extensive MRI and microscopy data acquisition of the BigMac dataset. Here we acquire data from multiple MRI scanning sessions and two different microscopy techniques through the development of novel protocols and/or processing pipelines. Secondly, we detail the careful processing and curation of several terabytes of collected data, to transform the BigMac dataset into a comprehensive resource for open dissemination online. Finally, we show some example analyses which demonstrate the unique information provided by the ultra-HARDI data included in BigMac.

In vivo MRI	Resolution (mm iso)	b-value (ms/ μm^2)	# gradient directions	# non-DW volumes	Linear / spherical encoding	TE/TR (ms/s)
Structural	0.5	-	-	-	-	4.0 / 2.5 *
Diffusion MRI	1	1	60	28	Linear	102 / 8.3 *
Resting fMRI	2	-	-	-	-	**
Task fMRI	2	-	-	-	-	**

*Most recent in vivo data (monkey at 10.8 years of age) **Various protocols

Postmortem MRI	Resolution (mm iso)	b-value (ms/ μm^2)	# gradient directions	# non-DW volumes	Linear / spherical encoding	TE/TR (ms/s)
Structural	0.3	-	-	-	-	7.8 / 0.097
T ₁ maps	0.6	-	-	-	-	8 / 10
a) Diffusion MRI	0.6	4	128	8	Linear	25.4 / 10
b) Diffusion MRI	1	4	250	10	Linear	42.5 / 3.5
Diffusion MRI	1	7	1000	40	Linear	42.5 / 3.5
Diffusion MRI	1	10	1000	40	Linear	42.5 / 3.5
Diffusion MRI	1	4	(30)	2	Spherical	42.5 / 6.4
Diffusion MRI	1	4	50	3	Linear	42.5 / 6.4
c) Diffusion MRI	1	7	(30)	2	Spherical	42.5 / 6.4
Diffusion MRI	1	7	50	3	Linear	42.5 / 6.4
Diffusion MRI	1	10	(30)	2	Spherical	42.5 / 6.4
Diffusion MRI	1	10	50	3	Linear	42.5 / 6.4

a) High spatial resolution, b) Ultra-high angular resolution (ultra-HARDI), c) Combining linear & spherical tensor encoding

Microscopy	Thickness (μm)	Staining	Visualisation	Imaging resolution
Polarised Light Imaging	50	None	Myelinated fibres	4 $\mu\text{m}/\text{pix}$
Histology	50	Cresyl violet	Neurons	-
Histology	50	IBA-1	Glia	-
Histology	50	Gallyas silver	Myelin	0.28 $\mu\text{m}/\text{pix}$
Histology	100	GFAP	Glia	-
Histology	50	-	-	-

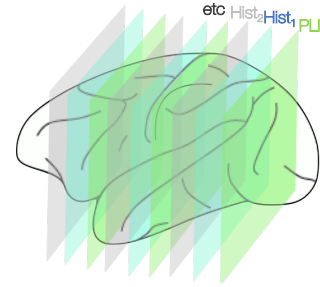


Table 5.1: A summary of the data which constitutes the BigMac dataset. The dataset combines in vivo and postmortem MRI with multi-contrast microscopy. All data were acquired from a single, adult macaque monkey brain. iso=isotropic; #=number; DW=diffusion-weighted; TE=echo time; TR=repetition time. Note, the ‘# gradient directions’ for data with spherical tensor encoding indicates the number images (repeats) acquired.

5.2 Data acquisition

The BigMac data was acquired on a single adult macaque brain. Prior to sacrifice, the macaque was scanned in vivo over multiple scan sessions where structural, task fMRI, resting state fMRI and diffusion MRI data were collected. At 11.8 years of age, the brain was perfusion fixed in formalin after which extensive postmortem MRI data was acquired. After scanning, the entire brain was sectioned along the rostro-caudal axis, with consecutive slices processed for different microscopy contrasts. A summary of the acquired data is presented in Table 5.1.

This thesis includes microscopy data from both polarised light imaging and slides stained with Gallyas Silver which specifically image the myeloarchitecture of the brain - i.e. the myelinated axons. Although this is a subset of the future ‘complete’ BigMac dataset, the raw data alone is approximately 2.5 TB in size.

This section will first briefly describe the animal history and then the in vivo data that will be made publicly available as part of the BigMac online dataset. The animal was cared for, and in vivo data were acquired by, our collaborators Dr Jerome Sallet, Dr Rogier Mars and colleagues from the Department of Experimental Psychology and the Comparative Anatomy group, University of Oxford, to whom we are very grateful. All procedures were performed under licenses from the United Kingdom (UK) Home Office in accordance with the UK Animals (Scientific Procedures) Act 1986 and with European Union guidelines (EU Directive 2010/63/EU). No new or additional in vivo data or procedures were conducted as part of this project.

The section will then detail the extensive postmortem MRI and microscopy data that were acquired as part of this thesis.

5.2.1 Animal history and tissue pathology

At the heart of the BigMac dataset is the brain of a single rhesus monkey (*Macaca mulatta*, male). In vivo data were acquired over multiple years and as part of various studies.

During one study, the BigMac monkey underwent intraoperative bilateral lesioning of the orbitofrontal cortex. This planned intervention was conducted as part of a behavioural study (in preparation). The postmortem MRI data in Figure 5.2 shows the extent of the bilateral lesions approximately one year after surgery.

As lesions often lead to substantial death and degeneration of neuronal cells in the surrounding white and grey matter, these two lesion sites facilitate the study of atypical or abnormal appearing brain tissue in the BigMac dataset. This is

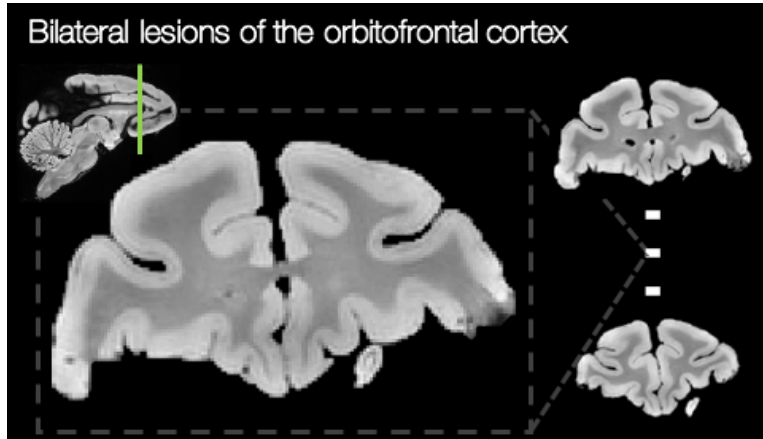


Figure 5.2: Bilateral lesioning of the orbitofrontal cortex where postmortem structural MR images show the extent of the lesion. The top middle image is the most posterior, and the bottom most anterior, of those shown. The lesion occurred approximately 1 year prior to the scans shown.

important as the biophysical interpretation of many diffusion models is based on typical, healthy white matter, and so their performance in abnormal brain tissue is often compromised or poorly understood. For example, many diffusion models assume non-exchanging tissue compartments. However, in damaged tissue, demyelinated fibres may have fast exchange between the intra- and extra-axonal space and therefore violate this modelling assumption. When the modelling assumptions are violated, the estimated model parameters will likely be biased. The BigMac dataset can be used to validate MRI models against microscopy ground truth in both normal and abnormal brain tissue. Here we can both test the sensitivity of the MRI models to tissue abnormalities (e.g. how abnormal does tissue need to be before we detect it as abnormal in our MR model), and understand how their performance relates to biological markers of interest (e.g. does a change in diffusion kurtosis relate to a difference in axonal orientation dispersion or myelination - both of which are quantifiable from either PLI or myelin-stained histology sections).

In addition to the planned lesion, inspection of the postmortem data (Figure 5.3) shows a fairly substantial abnormality in the left hemisphere which extends from the inferior portion of the supramarginal gyrus, up through the post-central sulcus. This abnormality could relate to a cerebral bleed, which perhaps occurred post

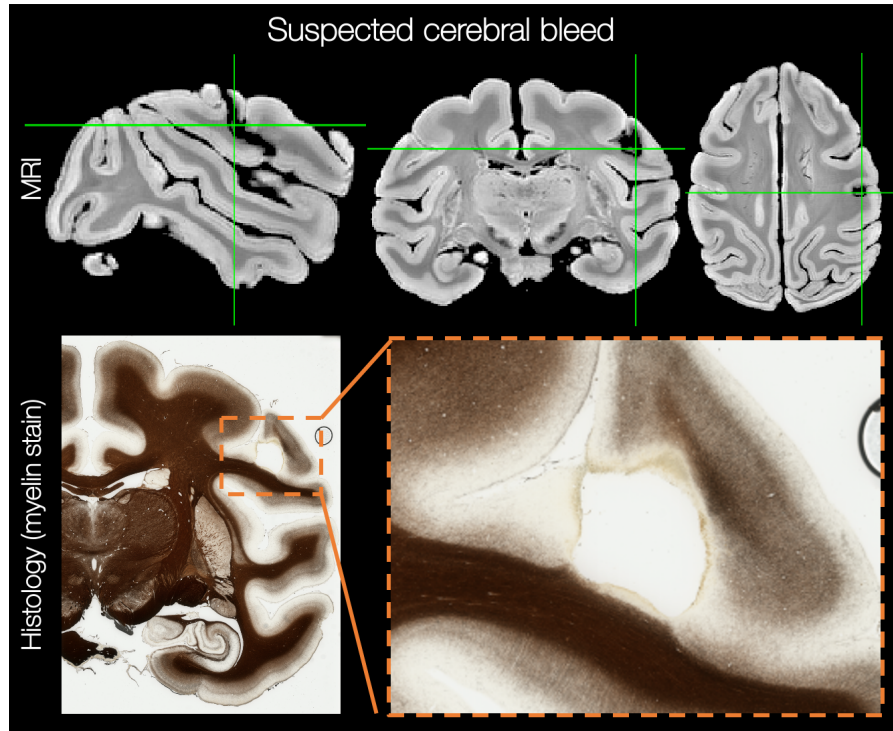


Figure 5.3: Both the postmortem MRI (top) and histology data (bottom) show a region of tissue damage in the BigMac brain. This may be indicative of a postoperative cerebral bleed, though there was no indication of this at the time. This affords the study of both typical and abnormal tissue within the BigMac brain.

operatively; though nothing was suspected at the time. Tissue damage and loss in this region was also seen in the microscopy data.

At 11.8 years of age the animal was anaesthetised and the brain perfusion fixed with 90 % saline and 10 % formalin, extracted and then stored in 30% sucrose formalin.

5.2.2 In vivo MRI

Prior to sacrifice, the animal partook in a number of studies [179, 180] in which behavioural and imaging data were acquired. To give two examples, the BigMac monkey features in a study by i) Papageorgiou *et. al* [179] who combine functional MRI with various decision-making tasks to investigate the role of the ventromedial prefrontal cortex, and ii) Sallet *et. al* [180] who link flexible behaviour to changes in both the MRI-derived structure and function of a fronto-cortical network.

The BigMac in vivo MRI data includes structural images, diffusion MRI, resting-state fMRI and task fMRI over a variety of tasks. The data were acquired at various time points throughout the animals adult life. The in vivo data were acquired on a 3 T whole-body scanner ($G_{\text{max}} = 4 \text{ G/cm}$) with a four-channel phased-array receive coil and a local transmit coil (Windmiller Kolster Scientific). Importantly, the last in vivo dataset was acquired only 1 year before sacrifice, at 10.8 years of age. As such, the age-induced atrophy between the in vivo and postmortem data should be roughly similar.

To compare the quality of the in vivo data and postmortem data, Figure 5.4 (top) shows example unprocessed structural images from the most recent in vivo imaging session. The in vivo image was acquired using a T_1 -weighted Magnetization Prepared - RApid Gradient Echo (MP-RAGE) sequence with 0.5 mm isotropic resolution, $TR/TE=2.5 \text{ s} / 4.01 \text{ ms}$ and 128 slices. This is then compared to postmortem data, as described below.

5.2.3 Postmortem MRI

Postmortem data were acquired on an 7 T small animal scanner (Agilent Specrometer, 400 mT/m), fitted with a $G_{\text{max}} = 40 \text{ G/cm}$ gradient coil (Agilent, 210/120 mm) and a 72 mm Birdcage receive/transmit RF coil (Rapid Biomedical). Prior to scanning the brain was rehydrated in phosphate-buffered saline to remove any lasting fixative and to somewhat restore both the diffusivity and T_2 of the tissue [29, 168]. The brain was then packed into a plastic holder filled with Fluorinert (FC-3283, 3 MTM, St. Paul, USA), a proton-free, susceptibility-matched fluid which is both MR invisible and improves field homogeneity.

As the diffusion properties of brain tissue are highly dependent on the tissue temperature, the temperature was controlled via a constant flow of air through the scanner bore and air conditioning of the scan room. This was previously found to keep the temperature of both the gradient coil and a tissue phantom

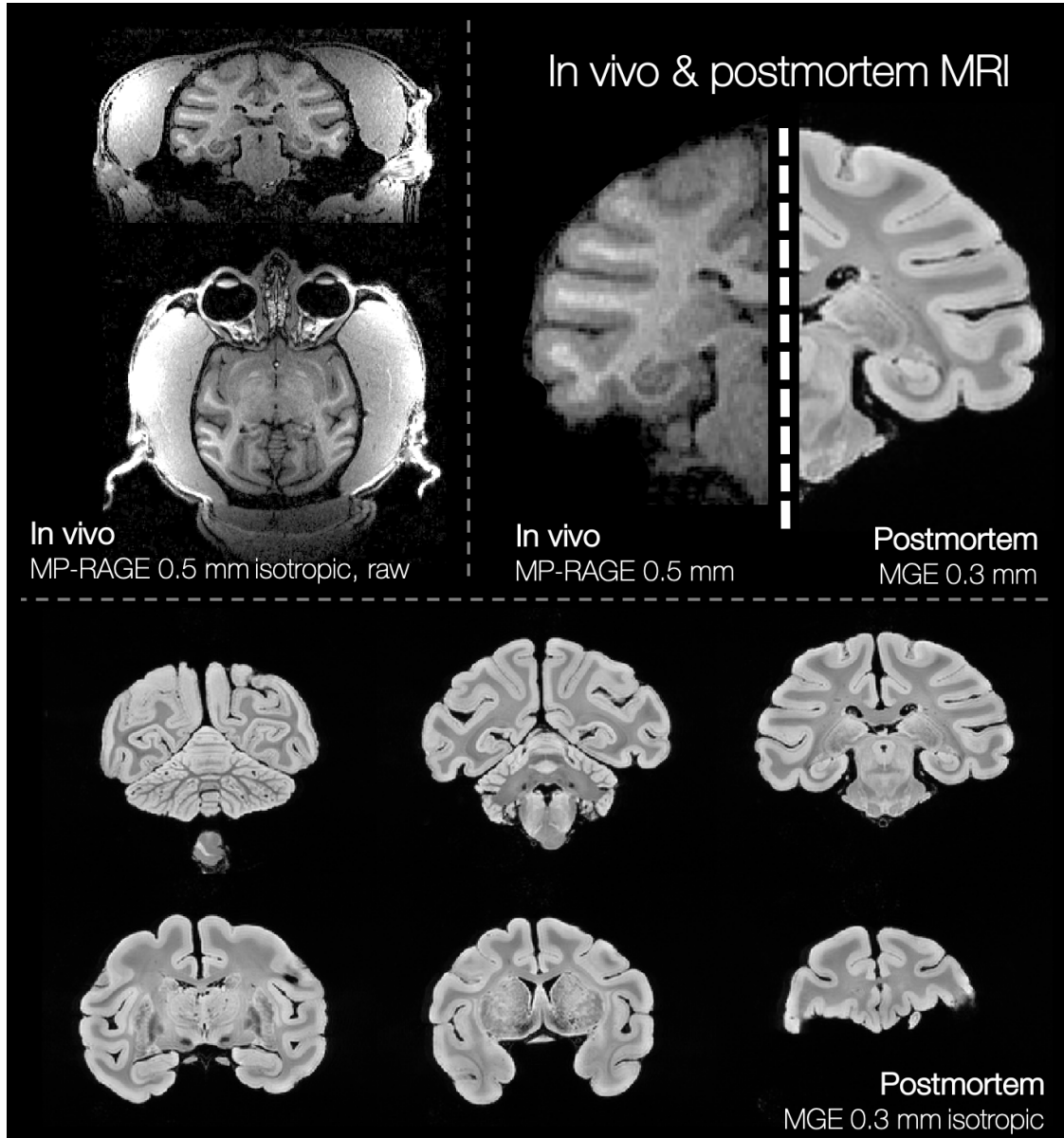


Figure 5.4: The BigMac dataset includes both in vivo (structural, functional and diffusion MRI) and postmortem (structural MRI, diffusion MRI and microscopy) data in the same animal. Here we compare example structural images from both the in vivo and postmortem protocol. Though there is inverted contrast between the in vivo and postmortem structural images, the data is highly complementary. Furthermore, in the high-quality postmortem data (bottom) we can see the anatomy in detail due to the high 0.3 mm isotropic resolution. The top left image is the most posterior and the bottom right the most anterior, where we again see the bilateral lesion of the orbitofrontal cortex.

stable (within 1°C of room temperature) during postmortem scan sessions which can last many tens of hours.

The BigMac postmortem MRI data was acquired over three different scanning sessions:

1. 29th March - 5th April 2018: Acquisition of $b = 7$ and $10\text{ ms}/\mu\text{m}^2$ ultra-HARDI data with 1000 diffusion-weighted gradient directions per shell and 1 mm isotropic resolution.
2. 6th - 9th April 2018: Acquisition of the 0.3 mm structural MRI, T_1 maps and 0.6 mm $b = 4\text{ ms}/\mu\text{m}^2$ diffusion-weighted data.
3. 20th - 23rd April 2018: Acquisition of 1 mm $b = 4\text{ ms}/\mu\text{m}^2$ diffusion-weighted data plus the protocol combining linear and spherical tensor encoding at $b = 4, 7$ and $10\text{ ms}/\mu\text{m}^2$.

The brain was not repacked between sessions, though slight deformations did occur as the tissue relaxed over time. In total, the postmortem MRI data acquisition took ~ 270 hours scanning time.

Note, the scanning protocols for the first and third scanning period were developed specifically for the BigMac dataset. The second scanning session followed a pre-existing protocol. Consequently, the data from the second scan session is similar to other, pre-existing datasets on various animals which were acquired by our collaborators, and which will greatly facilitate future cross subject comparisons and validation.

Structural MRI

Structural images were acquired using a multi gradient echo (MGE 3D) sequence: TE/TR = 7.8/97.7 ms, flip angle 30°, 0.3 mm isotropic resolution, FOV = 76.8 x 76.8 mm. The structural images were corrected for signal inhomogeneities due to a small radio frequency bias field and then segmented into two tissue classes to generate white and grey matter masks (FSL's FAST, [94]). The estimation and correction of the small bias field was required to enable a good white/grey matter segmentation.

Figure 5.4 (bottom) shows example postmortem structural images from the BigMac dataset. Here we see the anatomy in great detail due to the very high spatial resolution with excellent contrast between the cortex and white matter. This data is highly complementary to the in vivo T_1 -weighted data (Figure 5.4).

Due to their high image quality and anatomical detail, the postmortem structural MRI acted as a crucial intermediary in the co-registration of the diffusion MRI and microscopy data (*c.f. Co-registration*).

T_1 maps

T_1 maps were acquired using similar imaging parameters as the 0.6 mm postmortem diffusion MRI data but now with an inversion recovery preparation: TE/TR = 10 s/8 ms, FOV = 76.8 x 76.8 mm, resolution 0.6 mm isotropic and 12 inversion times from 12 to 6000 ms.

Diffusion-weighted MRI: linear tensor encoding

The diffusion MRI protocol has 3 main objectives (Table 5.1): i) high spatial resolution imaging, ii) high angular resolution imaging, and iii) combining linear and spherical tensor encoding. This thesis focuses on data pertaining to the first two objectives, the protocol of which is described in this section. The protocol for

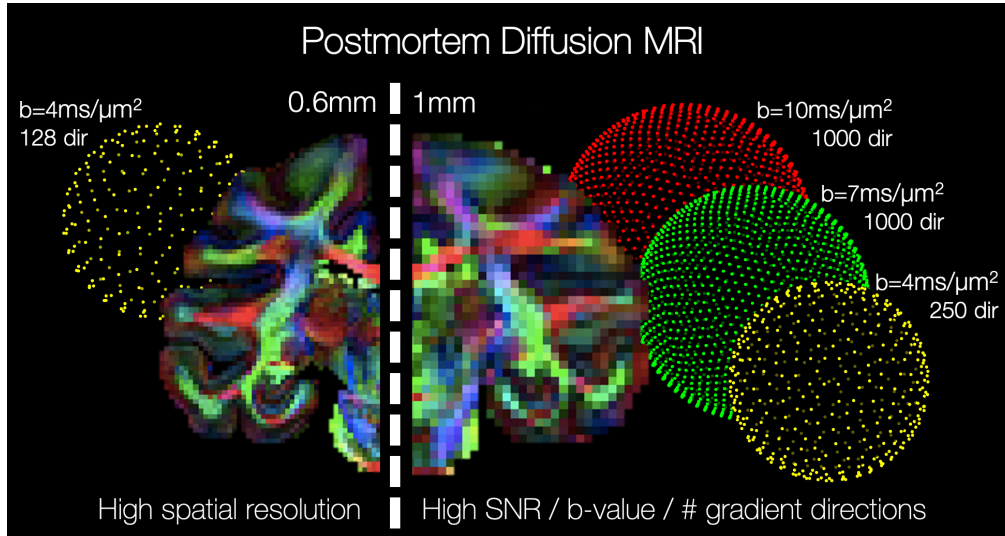


Figure 5.5: A summary of the postmortem diffusion MRI data included in the BigMac dataset. High spatial resolution (0.6 mm isotropic) data were acquired at a b-value of $4 \text{ ms}/\mu\text{m}^2$ with 128 gradient directions. High angular resolution data were also acquired at a spatial resolution of 1 mm isotropic, with multiple b-values (4, 7 and $10 \text{ ms}/\mu\text{m}^2$) and up to 1000 gradient directions per shell (‘ultra-HARDI’ data). At 1 mm, we were able to acquire ultra-HARDI data within a reasonable scan time (≤ 1 week) and push to high b-values whilst retaining good SNR. Data was also acquired with spherical tensor encoding (not shown).

the third objective is described in the following section (*c.f. Diffusion-weighted MRI: spherical diffusion encoding*).

Figure 5.5 summarises the high spatial and angular resolution postmortem diffusion MRI data included in the BigMac dataset. The diffusion MRI data includes two spatial resolutions: high resolution 0.6 mm data at $b = 4 \text{ ms}/\mu\text{m}^2$ as well as 1 mm data at b-values of 4, 7 and $10 \text{ ms}/\mu\text{m}^2$. Here the 0.6 mm data reduces partial volume effects, which facilitates characterisation of complex fibre geometries, while the 1 mm data facilitates the acquisition of high b-value data whilst retaining good signal to noise ratio (SNR). In addition, at 1 mm we collected 1000 gradient directions (‘ultra-HARDI data’) in the outer two shells within an achievable scan time (< 1 week).

The diffusion-weighted data were acquired using a spin echo multi-slice (DW-SEMS) sequence and single-line readout. Here the spin echo sequence was preferable over EPI as, though the sequence is fairly slow, it does not suffer from susceptibility or eddy-current induced distortions associated with EPI that typically lead to

reduced signal-to-noise ratios (SNRs). A summary of the diffusion MRI protocol is presented in Table 5.2. To ensure that data from different shells retain the same diffusion propagator, both the time between the gradients (i.e. the diffusion time, Δ) and gradient duration (δ) were kept constant for all data with 1 mm isotropic resolution. The desired b-value was achieved by modifying the amplitude of magnetic gradient, G .

In the 1 mm data, images were acquired in batches of 26 volumes where one volume with negligible diffusion weighting ($b \sim 0 \text{ ms}/\mu\text{m}^2$) was followed by 25 diffusion-weighted volumes. Two sets of gradient directions were used in the 1 mm diffusion-weighted data: one with 250 gradient directions ($b = 4 \text{ ms}/\mu\text{m}^2$), the other with 1000 gradient directions ($b = 7, 10 \text{ ms}/\mu\text{m}^2$). For both sets, the gradient directions were generated using GPS (an FSL tool, [181]) and were evenly distributed across the sphere. Subsequently, the directions were ordered so that any consecutive subset of gradient directions (e.g the first 100 gradient directions) also gave good coverage across the sphere (orderpoints, Camino [182]). In this case, were the scan interrupted or prematurely stopped, we would retain reasonable angular coverage. Finally, to minimise heating of the magnetic gradients, the diffusion-weighted gradient directions within each batch of 25 were reordered to ensure that highly co-linear directions were not played out in close succession.

The 0.6 mm $b = 4 \text{ ms}/\mu\text{m}^2$ data followed a different protocol. Here 128 diffusion-weighted gradient directions were acquired, followed by 8 volumes with negligible diffusion weighting.

To reduce the effect of signal drift - i.e. slow temporal changes in the signal magnitude due to the transfer of heat from the gradients to the scanner's passive shim materials - the scanner was driven to a level of approximate equilibrium prior to acquiring diffusion-weighted images at a given b-value. Here a set of 'warm-up' diffusion-weighted gradient directions were acquired, but these data were not used in subsequent analysis. The warm-up period was approximately 1.5 hours for the

b-value (ms/ μm^2)	Resolution (mm isotropic)	TE (ms)	TR (s)	FOV (mm)	δ/Δ (ms)	G (G/cm)	t_{dir} (mins)	# gradient directions	# non-DW volumes
4	0.6	25.4	10	76.8x76.8	7/13	32	21.3	128	8
4	1	42.5	3.5	76x76	14/24	12.0	4.1	250	10
7	1	42.5	3.5	76x76	14/24	15.9	4.1	1000	40
10	1	42.5	3.5	76x76	14/24	19.1	4.1	1000	40

Table 5.2: Imaging parameters for the diffusion protocols in the BigMac dataset. TE=echo time; TR=repetition time; FOV=field of view; δ =gradient duration; Δ =diffusion time i.e. time between gradients; G=gradient magnitude; t_{dir} =imaging time per gradient direction; #=number; DW=diffusion-weighted.

$b = 4 \text{ ms}/\mu\text{m}^2$ data at 0.6 mm isotropic resolution, and ~ 4 hours for data with 1 mm resolution. These times were derived from preliminary experiments.

Diffusion-weighted MRI: spherical tensor encoding

Recently, there has been a strong interest from the diffusion MRI community on spherical tensor encoding. Spherical tensor encoding allows for the separation of effects due to the fibre orientation distribution and the diffusion properties, to estimate additional microstructural parameters such as μFA [151, 150].

In BigMac, data were also acquired with spherical tensor encoding at b-values of 4, 7 and $10 \text{ ms}/\mu\text{m}^2$. The gradient waveforms used for isotropic encoding were kindly provided by Michiel Cottaar. Due to additional stress on the magnetic gradients when performing the spherical tensor encoding, the repetition time (TR) was increased (with respect to the ultra-HARDI data) to 6.4 s. For each b-value, 30 images (repeats) were acquired with spherical tensor encoding. Complementary data with linear tensor encoding and the same TR were also acquired: 50 gradient directions per shell + 5 with negligible diffusion weighting. The imaging parameters otherwise matched those from the ultra-HARDI data: TE/TR = 42.5 ms/6.4 s, $\delta/\Delta = 14/24$ ms, FOV = 76x76 mm, 1 mm isotropic resolution. The gradient amplitude G was adjusted to produced the required b-values of $b = 4, 7$ and $10 \text{ ms}/\mu\text{m}^2$.

Note, this data is yet to be processed and is not the focus of this thesis. All

future analyses presented in this thesis are based on data with high spatial or angular resolution (i.e. objectives 1 and 2).

5.2.4 Microscopy

Using the BigMac dataset, we can link the MRI signal to microscopy data which has both ultra-high resolution data and high specificity. This section details the various microscopy contrasts included in BigMac. Note, the brain was sectioned, stained and imaged ('processed') in two batches. The brain was first cut around the level of the caudal tip of the central sulcus to create two tissue blocks, representing the rostral half and caudal half. First, the rostral (i.e. anterior) block was sectioned, stained and imaged, after which the caudal (i.e. posterior) block was processed using a highly similar protocol.

Each tissue block was sectioned along the rostro-caudal axis to produce thin coronal tissue sections. Consecutive sections were allocated, in order, to one of six contrasts, that was then repeated moving rostral to caudal:

1. Polarised light imaging to visualise myelinated fibres
2. Immunohistochemical staining of Nissl bodies in neurons: Cresyl violet
3. Immunohistochemical staining of microglia: ionized calcium-binding adaptor molecule 1 (IBA-1)
4. Gallyas silver staining of myelin
5. Immunohistochemical staining of glia: glial fibrillary acidic protein (GFAP)
6. Unassigned section

All sections were 50 μm thick, except for the GFAP histological sections which were 100 μm . Each stain was repeated every 350 μm throughout the brain. The unassigned section was returned to formalin and stored for longevity.

Digitisation (or imaging) of the tissue sections is very time consuming. Hence, slide digitisation is an ongoing process. When digitising the tissue sections, we do not acquire a single image, but scan across the slide, acquiring 10s or 100s of smaller, overlapping fields of view which are then later stitched together. For each of the five microscopy contrasts (polarised light imaging and the four histology stains) there are approximately 160 tissue sections or slides, where polarised light images took approximately 4 hours to acquire per slide, and histology images took approximately 2 hours per slide.

Polarised Light Imaging

Polarised light imaging utilises the birefringence of myelinated axons to estimate the primary fibre orientation per pixel [61, 62, 63]. Here unstained tissue sections were imaged using a Leica DM4000B microscope (Leica, Germany) adapted for PLI with an LED light source, a polariser, a quarter wave plate with its fast axis at 45 degrees to the transmission axis of the polariser, and a rotatable polariser (the analyser) (*c.f. Chapter 2*).

Due to the large size of the BigMac tissue sections, multiple images or fields of view were acquired across each sample and later stitched together using bespoke software (adapted from [47]) to form a whole slide ‘mosaic’ image. For each field of view, images were taken as the analyser was rotated from 0 to 180 degrees in 9 equidistant steps. A 2.5x magnifying objective produced an imaging resolution of $\sim 4 \mu\text{m}$ per pixel. Background correction was performed [183, 47] to account for light source variations across the image, after which a sinusoid was fitted to the pixelwise image intensity as a function of the analyser rotation. The transmittance, retardance, in-plane and inclination angle were derived from the sinusoid phase and amplitude (*c.f. Chapter 2*).

Figure 5.6 shows example PLI mosaics from the BigMac dataset. The output images are maps of the tissue transmittance, retardance, inclination and in-plane

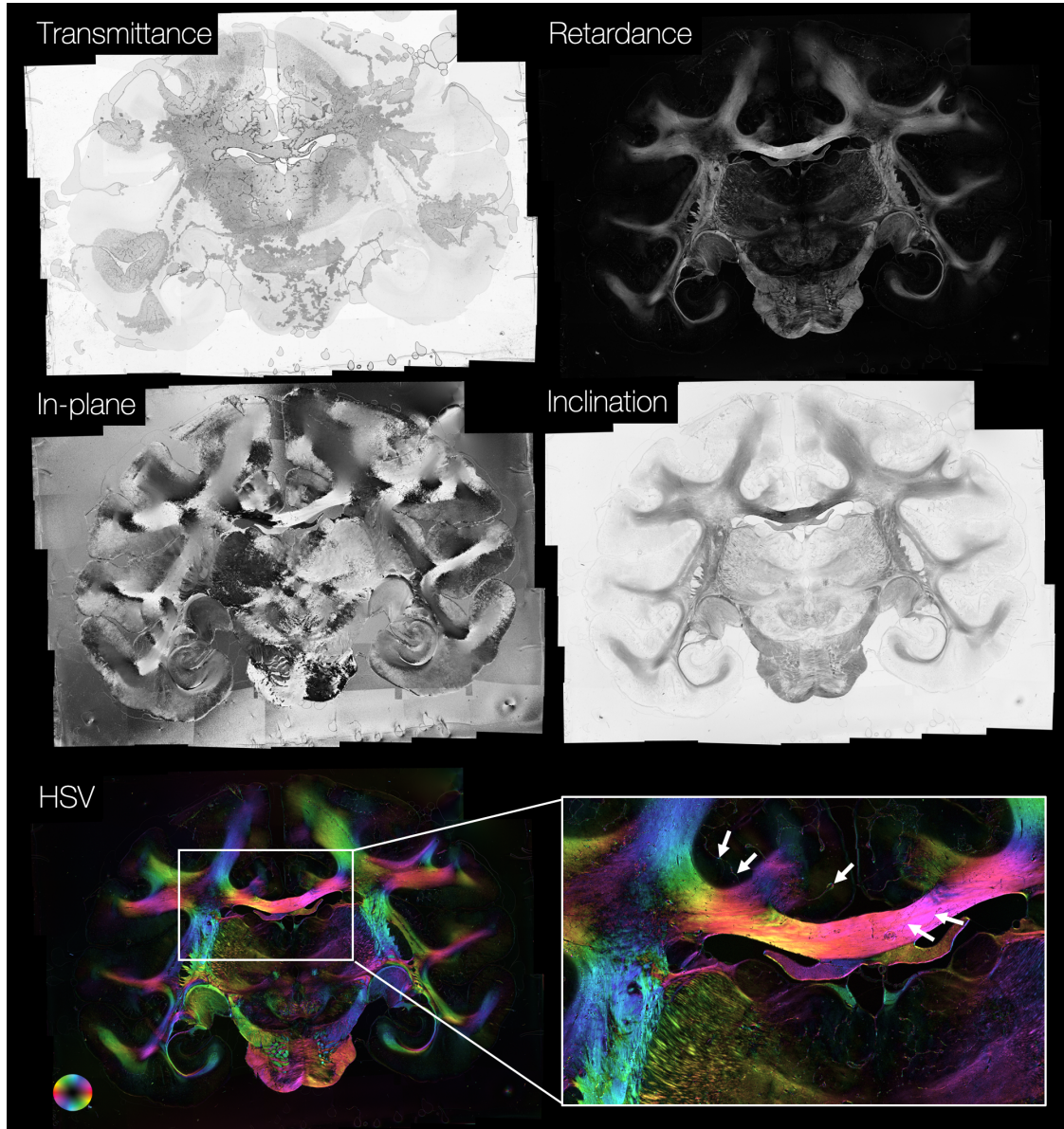


Figure 5.6: Example transmittance, retardance, in-plane and inclination maps from PLI. The in-plane and retardance images are combined in a composite HSV image. Though the slide is badly affected by bubbles and artefacts (see transmittance image), the artefacts appear to have little effect on the other PLI maps (retardance, in-plane and inclination). Consequently, only very minor artefacts are seen in the HSV image (white arrows). The whole slide PL images were acquired for > 160 sections throughout the BigMac brain.

angle, as well as the composite HSV (hue-saturation-value) image. As described in Chapter 2, the retardance map is dependent on both the inclination and amount of birefringent material (i.e. myelinated fibres) within the PLI-pixel [61, 62, 63]. By assuming that the amount of myelin is approximately constant across the white matter, we estimate the inclination angle (Figure 5.6 "Inclination"). In the HSV image, the hue is dependent on the in-plane angle of the myelinated fibres, and the value is given by the tissue retardance.

PLI data were acquired for >160 slides throughout the brain. Figure 5.7 shows example PL images from different locations along the anterior-posterior axis where image 1 is the most anterior and image 8 the most posterior. Here we see the myelinated fibres in high detail at a resolution of 4 μm per pixel. The PLI data can, for example, delineate the organisation of the internal capsule (Figure 5.7a), track white matter bundles projecting between deep grey matter structures such as the globus pallidus and the putamen (Figure 5.7b), as well as pontocerebellar fibres extending from the basilar sulcus of the pons and around the midbrain (Figure 5.7c). In the cerebellum (Figure 5.7d) we see i) beautiful organisation of the cerebellar nuclei and ii) how the PLI data separates the molecular and granular layers of the cerebellar cortex; the molecular layer is the top, outermost layer of the cerebellar cortex (white arrows on inset) and the granular layer (yellow arrows) lies between the molecular layer and the white matter. Notably, in some regions the molecular layer shows fairly strong birefringence (retardance), with in-plane angles parallel to the cortical surface, a possible explanation of which is given below. In comparison, the molecular layer at the gyri crown (blue arrows) tend to have low retardance. Although the retardance in the granular layer is low, we see a low density of more individually identifiable fibres or fibre bundles which generally extend from the white matter and into the molecular layer.

To understand the origin of the apparent birefringence in the molecular layer, we should consider the known architecture of the cerebellar cortex (Figure 5.8). The granular layer (between the white matter and the molecular layer) contains cerebellar

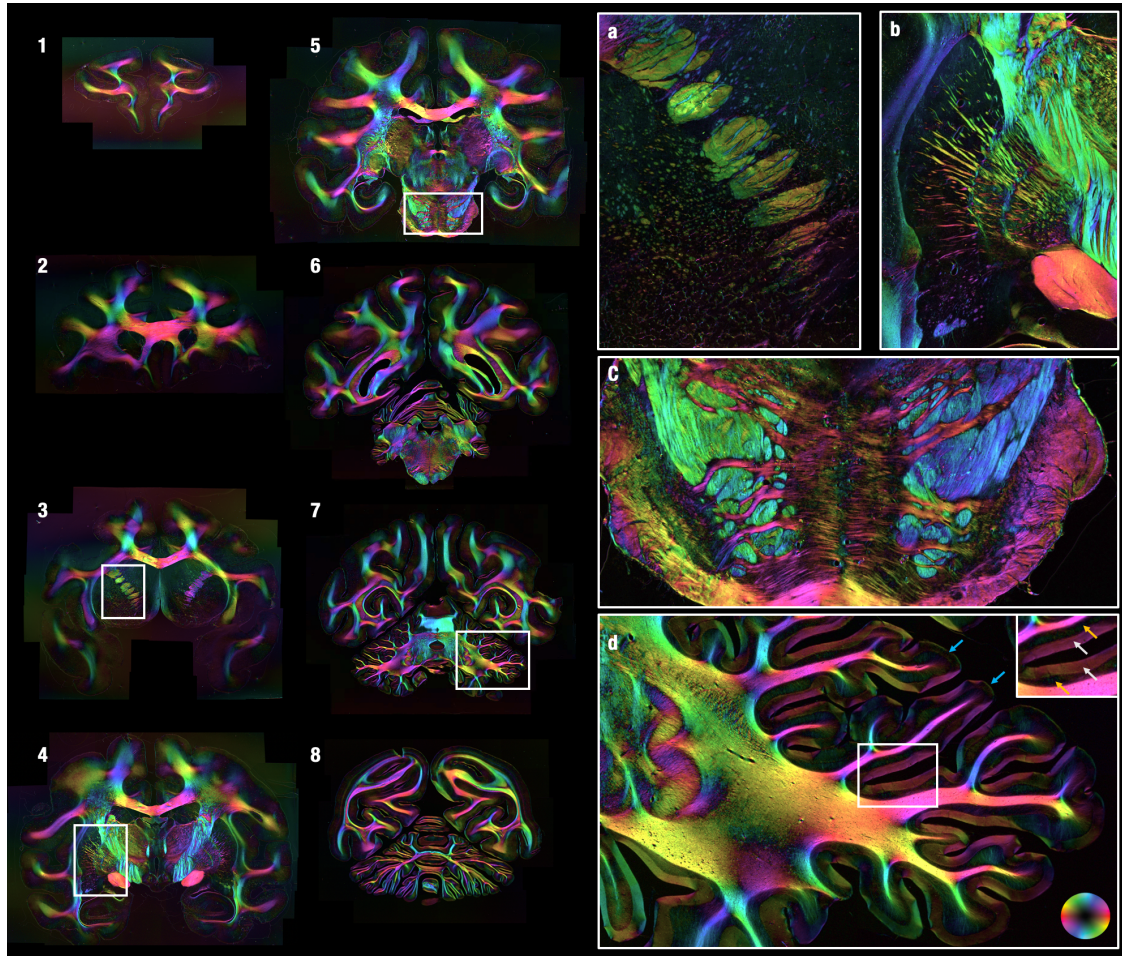


Figure 5.7: Example PL images throughout the brain. Image 1 = most anterior, 8 = most posterior of the images shown. a-d: The myeloarchitecture is viewed in great detail due to the 4 μm PLI resolution. The yellow, white and blue arrows point to (in order) the granular layer, the molecular layer, and the gyri crown (see main text for further information).

granule cells, some of the smallest but most numerous cells in the brain. The axon of the granule cell extends vertically into the molecular layer, where it then splits into two horizontal branches in a ‘T-like’ fashion. These branches are known as ‘parallel fibres’. The Purkinje cells sit with their soma in the ‘Purkinje layer’, at the interface of the granular and molecular layer, and the Purkinje dendritic tree extends into the molecular layer. The parallel fibres of the granule cells run through the Purkinje dendrites, forming synaptic connections with the Purkinje dendritic spine.

The parallel fibres are often assumed to be exclusively unmyelinated [184]. However, using electron microscopy Wyatt *et al.* [185] found myelinated fibres of 0.4-1.1 μm

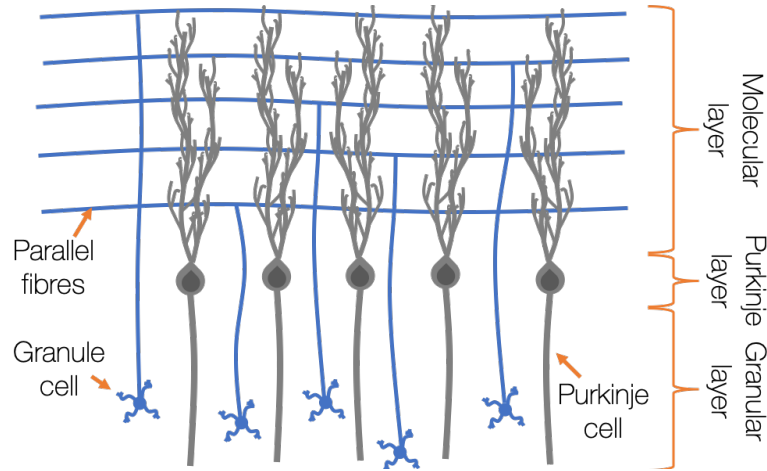


Figure 5.8: The structure of the cerebellar cortex. This highly simplified schematic focuses solely on the granule and Purkinje cells, to illustrate the parallel fibres in the molecular layer. Note, the dendritic tree of the Purkinje cells has highly anisotropic dispersion. Here we see the axis of least dispersion, where the Purkinje dendritic tree fans out most in the through-page orientation.

diameter in the Macaque molecular layer. These myelinated fibres, running parallel to the cortex, could cause the coherently oriented birefringence of the molecular layer (Figure 5.7d). Interestingly, Wyatt *et al.* found a larger number of myelinated fibres in the molecular layer near the Purkinje layer, and fewer towards the cortical surface. Consequently, we might expect to see a gradient in the PLI retardance across the molecular layer, though this is not evident in the data. Finally, although myelinated parallel fibres provide a possible explanation for the coherently oriented birefringence of some regions in the molecular layer of the cerebellar cortex, this interpretation is highly speculative and requires further investigation. Future work will look to see whether the Gallyas myelin-stained slides show evidence of such myelinated parallel fibres in the cerebellar cortex.

PLI artefacts

The rostral PLI sections were mounted using a hard-set mounting medium (Fluor-Save, Merck). Unfortunately, over time we saw artefacts develop on the slides, which are likely bubbles and may be due to shrinkage of the mounting medium as it hardens over time. This artefact is clearly seen on the transmittance image of Figure 5.6. Fortunately, the retardance, in-plane and inclination maps do not

appear to be substantially affected by the artefact, though some edge effects are faintly visible, as highlighted by the white arrows.

In some rostral PLI sections we see background birefringence outside of the tissue which varies slowly across the slide (Figure 5.7 sections 1-5 where 1 and 3 are worst affected). This is due to the slides being coated in gelatine. Gelatine is frequently used to aid the mounting of tissue sections onto glass slides. Unfortunately, gelatine is also highly birefringent [186]. Nonetheless, the PLI orientations within the white matter do not appear greatly affected, where the birefringence of the myelin appears to dominate [65]. In order to avoid these artefacts, the caudal sections (which were processed second) were instead mounted with an aqueous mounting medium (Polyvinylpyrrolidone, PVP) on plain glass slides without gelatine coating.

PLI of silver-stained slides

Finally, Figure 5.9 shows polarised light imaging of a silver-stained section. These images are noisier than the unstained sections used specifically for PLI because the stain reduces the amount of light transmitted through the tissue. Nevertheless, the PL images in Figure 5.9 do capture fibre orientations that are consistent with the known neuroanatomy. Therefore, these images facilitate the direct comparison of fibre orientations derived from both polarised light imaging and myelin-stained histology slides. This is important as although both modalities are thought to provide ‘gold standard’ estimates of fibre orientation, they have been found to produce slightly different results. For example, the estimates of orientation dispersion from PLI were previously found to be lower than that from histology [47]. Furthermore, were PLI acquired on all BigMac silver-stained slides, we would double the density of PLI data in the BigMac brain.

Gallyas silver staining

The Gallyas silver staining method [187] was performed on one set of sections in the BigMac dataset. In this method, myelin can bind to colloidal silver particles, and

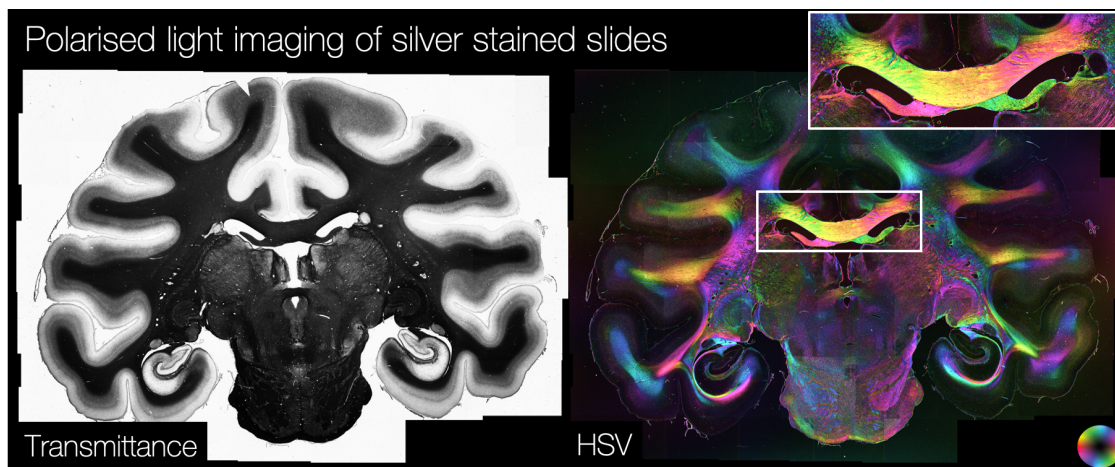


Figure 5.9: Polarised light imaging of a silver stained slide. Though the amount of transmitted light is low when compared to that from unstained sections specifically processed for PLI (Figure 5.6), where this makes the PLI fitting more noisy, the fibre orientations are as we might hope and follow known neuroanatomy.

thus turn a deep brown. Consequently, this staining facilitates easy visualisation of the myeloarchitecture [188]. After staining, the sections were cover-slipped, sealed and digitised using a Leica Aperio Scanscope AT Turbo slidescanner (Leica, Germany) with a x40 magnifying objective. This produced an imaging resolution of $0.28 \mu\text{m}/\text{pix}$, where the histology image resolution is more than 10 times that in PLI. Due to the large slide size, many of the central sections were digitised in two images.

Figure 5.10 shows example images of the digitised slides. Of the sections shown, the left image is the most anterior, and the right image is the most posterior, at the approximate level of the central sulcus. We see interesting detail in both the white and grey matter where, up close, we can visualise single, thin axons in detail due to both the sensitivity of the stain and the sub-micron imaging resolution. In the digitised images we can track complex patterns of fibre projection for example, from the white matter into the cortex (a), between sub-cortical structures (b), within the highly complex geometry of the hippocampus (c) or through the deep white matter (d). Furthermore, in the deep white matter we see different tissue ‘textures’ as well as large scale, ‘wave-like’ undulations of fibres in the corpus callosum (d): a region which is typically considered homogeneously ordered with little fibre dispersion.

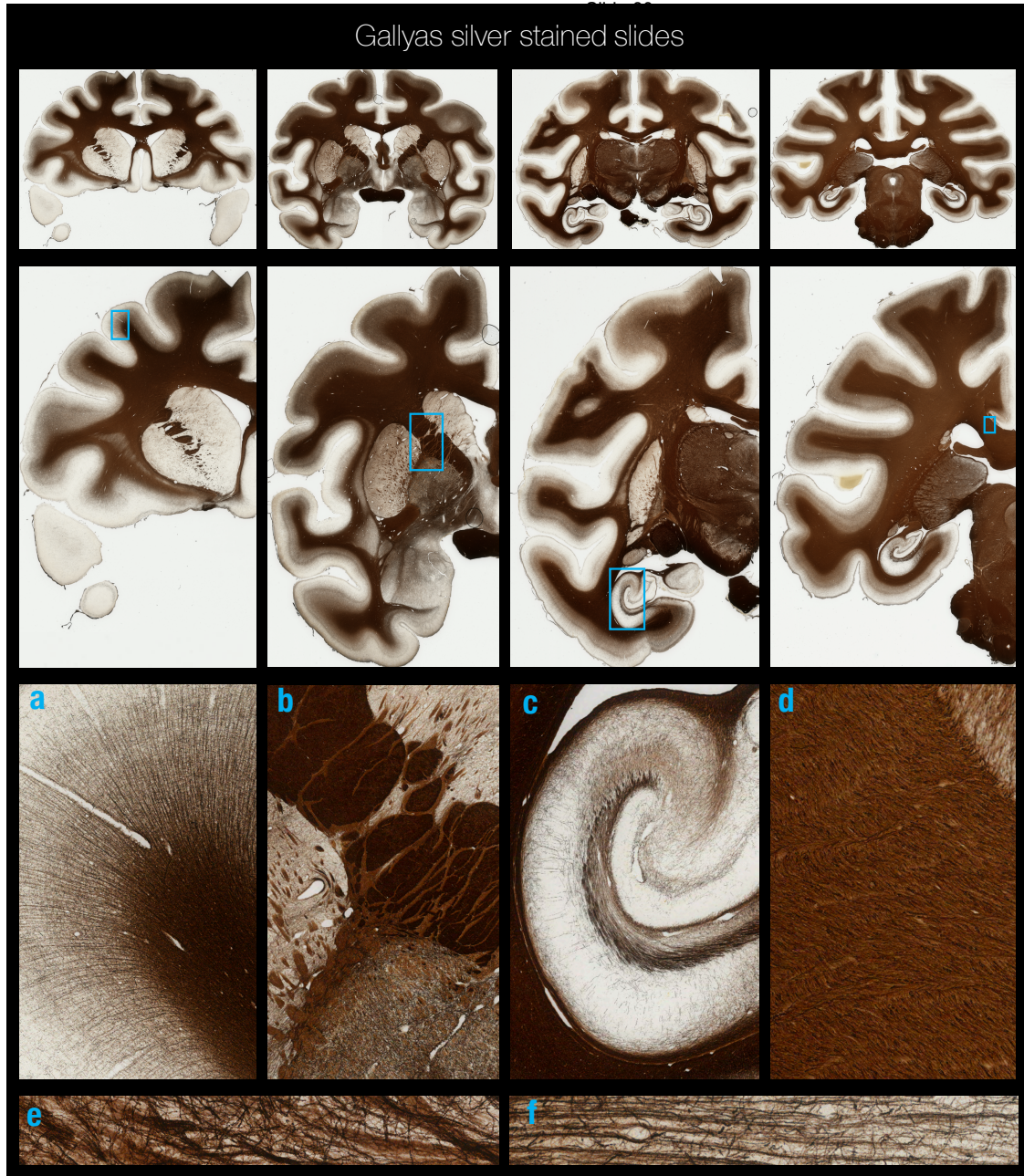


Figure 5.10: Example digitised Gallyas silver stained slides from the BigMac Dataset. With a spatial resolution of $0.28 \mu\text{m}$ / pixel, we see the myelinated fibres in great detail, visualising single axons at the grey/white matter boundary (a,c,e,f) and fibre undulations in the deep white matter (d).

5.3 Resource curation

Once acquired, the MRI and microscopy data were prepared for open dissemination. This included preprocessing of the MRI data, structure tensor analysis of the Gallyas silver stained slides and co-registration of the MRI-microscopy data, as outlined below.

5.3.1 MRI preprocessing

The postmortem MRI data was found to have few distortions, so minimal preprocessing was applied. For example, the data did not need correcting for susceptibility or eddy current distortions, as is typically required. This is largely due to the brain being placed in a susceptibility-matched fluid and the data acquired with a single-line readout instead of the typical echo planar imaging (EPI). The main corrections were a) registration, b) correction of signal drift, and c) signal normalisation. Here the term ‘experiment’ is used to describe images pertaining to the same b-value that were acquired in succession.

Registration

Despite scanning a stationary postmortem brain, there are several reasons as to why the MR images had to be registered together, as outlined below. First, the images from within the same ‘experiment’ (i.e. pertaining to the same b-value) were linearly registered together. Afterwards, data acquired from different experiments or scan sessions were co-registered, as outlined in Figure 5.11. The transforms described in Figure 5.11 (both linear matrices and non-linear warp-fields) are provided in the BigMac resource, enabling researches to move between imaging domains with ease.

The ultra-HARDI data for both $b = 7$, and $10 \text{ ms}/\mu\text{m}^2$ were acquired within the first scanning session. At specific time points throughout the week-long acquisition, the central scanner frequency was recalibrated. This occurred three times during the $b = 7 \text{ ms}/\mu\text{m}^2$ acquisition and 4 times during $b = 10 \text{ ms}/\mu\text{m}^2$. Because of the

recalibration, images acquired with different scanner central frequencies are shifted (translated) with respect to one another. To correct for these translations, the data were rigidly registered to a reference S_0 image (i.e. a volume with negligible diffusion weighting) from the ultra-HARDI dataset. Here the reference image was taken to be the mean S_0 image from the first ‘set’ of images which were all acquired with the same central frequency. The registration was performed using FSL’s FLIRT with spline interpolation of the data [105, 106].

Data from the second scan session includes high spatial resolution (0.4 mm isotropic) $b = 4 \text{ ms}/\mu\text{m}^2$ diffusion data as well as the detailed (0.3 mm isotropic) structural scan. The S_0 images associated with the 0.6 mm diffusion data ($b = 4 \text{ ms}/\mu\text{m}^2$) appeared to slowly drift in position along the readout direction. This was likely caused by a drop in the scanner temperature as the S_0 images were acquired in succession at the end of the scanning session where, as the gradients were no longer in use, the temperature likely decreased. A change in scanner temperature can cause a shift in the central frequency, which in turn results in both a translation of the image and a reduction in the signal magnitude, as seen in the data. To correct for signal drift, the S_0 images were linearly registered and normalised to the first S_0 i.e. that which most likely represents the ‘true’ S_0 of the diffusion-weighted data before the scanner temperature began to fall. The data were corrected using FSL’s FLIRT [105, 106] where the transformation was restricted to only consider translation along a single axis. The $b = 4 \text{ ms}/\mu\text{m}^2$ 0.6 mm diffusion-weighted data were then co-registered to the postmortem structural image using linear registration (FSL FLIRT, [105, 106]).

Upon inspection of the data, the brain shape appeared to change or ‘relax’ slightly between scanning sessions. In particular there was relaxation along the anterior-posterior axis so that, were the images only linearly registered, the most anterior and posterior sections of the brain would not align. To account for these deformations, non-linear transformations were generated both between the $b = 4 \text{ ms}/\mu\text{m}^2$ 1 mm data and the ultra-HARDI data, and between the ultra-HARDI data and the postmortem structural image (FSL’s FNIRT, [189, 181]). The respective

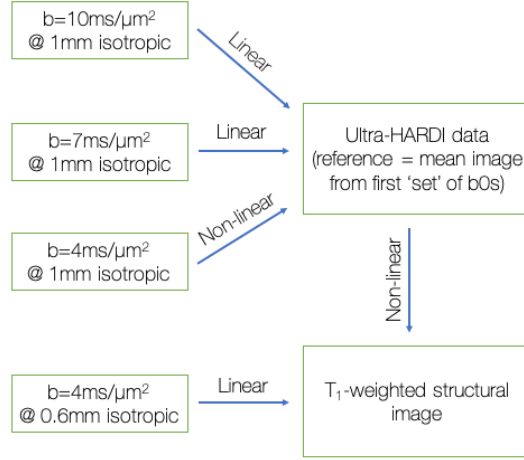


Figure 5.11: Co-registration of the BigMac postmortem MRI data.

transformations were then concatenated so that images are easily transformed between the native space of any two experiments.

Finally, to integrate the BigMac dataset with other datasets, non-linear transformations [189, 181] were computed between the postmortem structural image and the F99 standard template [190].

Signal drift

In all experiments, the signal magnitude, measured as the mean signal across S_0 images, was seen to decrease over time. To correct for signal drift, a linear trend with respect to time was fitted to the S_0 images and subsequently regressed from the data (both the S_0 and diffusion-weighted volumes).

Data normalisation

Most diffusion models approximate the S_0 image by taking the mean S_0 image across all volumes, assuming that the signal magnitude is constant both across time and b-values. In contrast, here we found the S_0 signal to vary between experiments and shells. Were diffusion models naively applied to concatenated data from the BigMac dataset, the results may be biased (e.g. the kurtosis would be misestimated). Therefore, we also provide diffusion-weighted data which is normalised to the mean S_0 of the $b = 10 \text{ ms}/\mu\text{m}^2$ ultra-HARDI data.

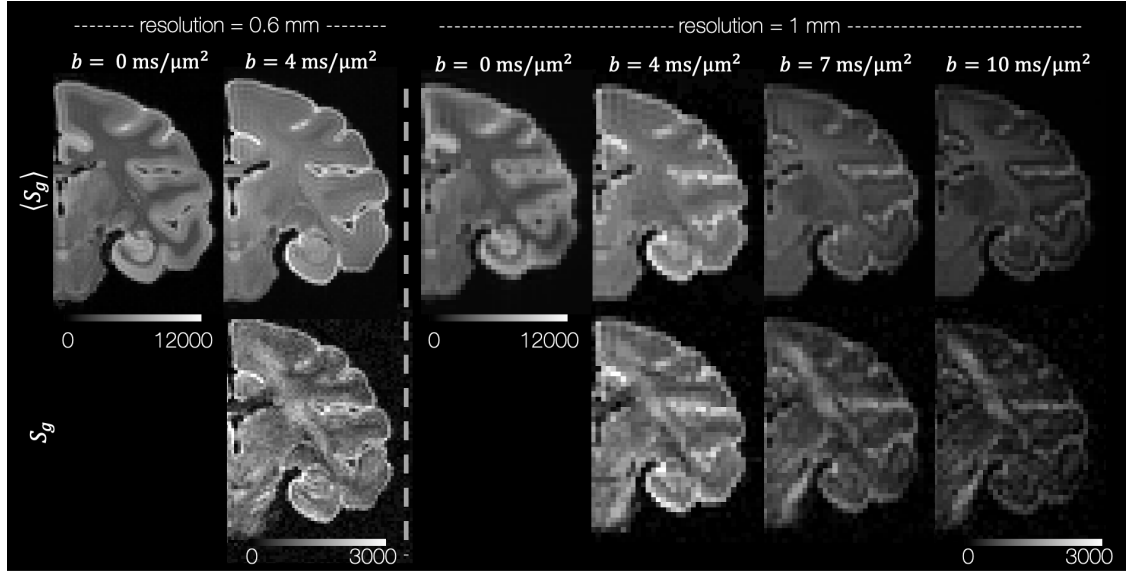


Figure 5.12: Example diffusion MR images from the BigMac dataset. The mean image across all volumes (top) is compared to a single diffusion-weighted image (bottom). The diffusion contrast is seen to increase with b-value, whilst the data retains good SNR even at very high $b = 10 \text{ ms}/\mu\text{m}^2$.

Figures 5.12-15 attest to the quality of the BigMac diffusion data. Example preprocessed images from the diffusion MRI protocol are shown in Figure 5.12. We see how even at very high b-values the data retains good SNR and shows excellent diffusion contrast.

Figure 5.13 examines the diffusion signal profile as we move to higher b-values and angular resolution. Here spherical harmonics of order 12 were fitted to the attenuated diffusion signal. The 3D diffusion profiles at $b = 4 \text{ ms}/\mu\text{m}^2$ (left) with 250 gradient directions are then compared to the ultra-HARDI data at $b = 7 \text{ ms}/\mu\text{m}^2$ (middle) and $b = 10 \text{ ms}/\mu\text{m}^2$ (right). We quantitatively see how the signal magnitude decreases with increasing b-value. Furthermore, the signal profiles at $b = 10 \text{ ms}/\mu\text{m}^2$ are highly detailed, where these details are often either missing or easily misinterpreted as noise in the $b = 4 \text{ ms}/\mu\text{m}^2$ profiles. Reassuringly, many of the details in the $b = 10 \text{ ms}/\mu\text{m}^2$ data are often visible, though to a lesser extent, in the $b = 7 \text{ ms}/\mu\text{m}^2$ data.

Figure 5.14 shows how our ability to characterise the diffusion signal is affected by the angular resolution of the data. Here we compare $b = 10 \text{ ms}/\mu\text{m}^2$ data with

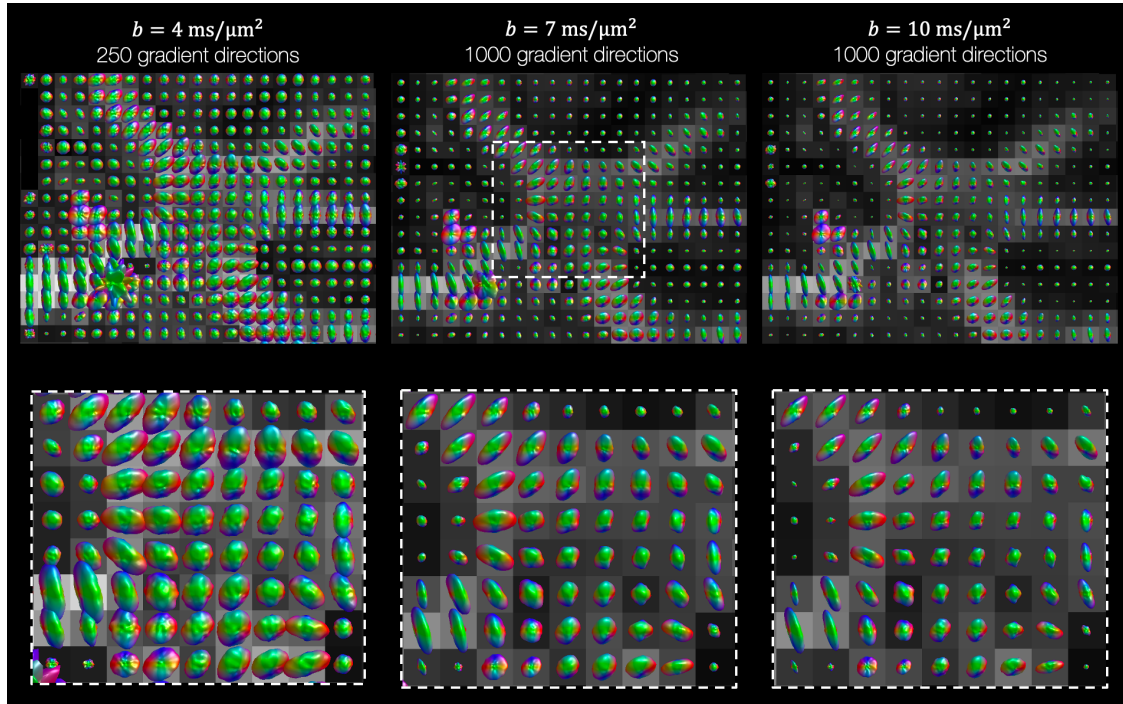


Figure 5.13: A qualitative assessment of the diffusion profile as a function of b -value. Spherical harmonics of order 12 were fitted to the attenuated diffusion signal and the glyphs overlaid on a fractional-anisotropy image. At high b -value, the overall signal magnitude decreases (as expected) and we see more structured shape in the diffusion profile, particularly in crossing fibre regions.

only 32 gradient directions, to that with 64, 250 and the full 1000 directions. Each point represents the diffusion signal along a single gradient direction and we show a single voxel from two orthogonal views. Due to tight time constraints, the majority of in vivo protocols only acquire ~ 60 gradient directions per shell and so only sparsely sample the 3D diffusion signal. In comparison, with the 1000 gradient directions of the ultra-HARDI data we are able to characterise the diffusion signal in great detail and ask questions such as how the number of gradient directions affects our estimation of the ‘true’ diffusion signal.

Finally, Figure 5.15 shows how, with 1000 gradient directions, we retain dense sampling of the diffusion signal on any arbitrary 2D plane for comparison with 2D microscopy data. Focusing on the hippocampus, Figure 5.15 shows data from gradient directions within 3° of the 2D (x-y) plane. At the level of a single, arbitrary voxel, we can compare the BigMac ultra-HARDI data in blue to equivalent points

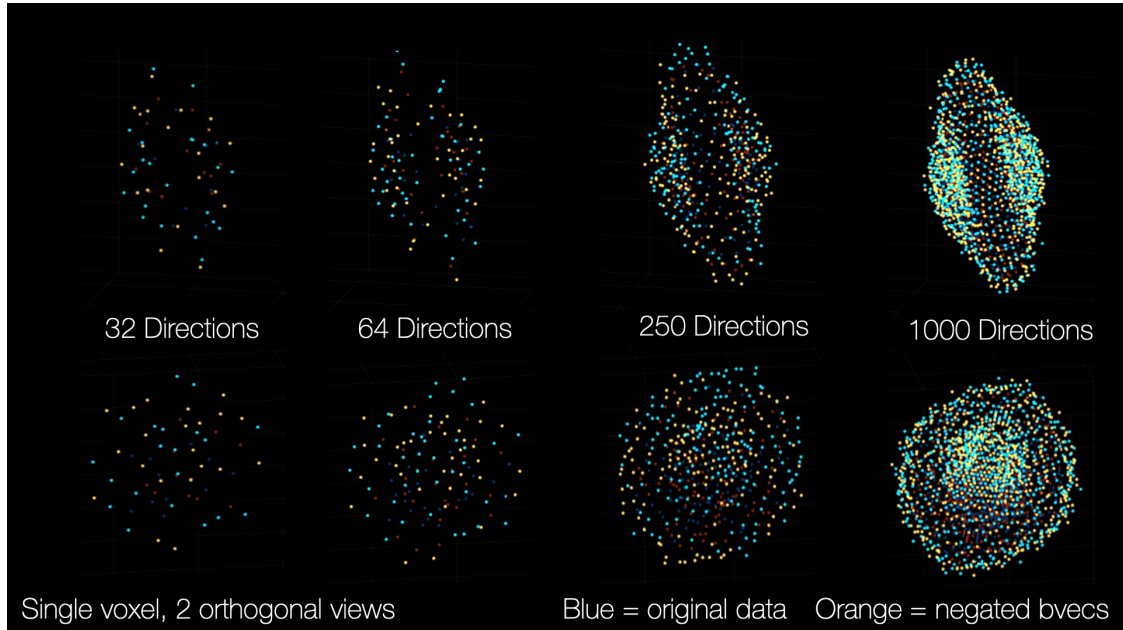


Figure 5.14: Characterisation of the diffusion signal in 3D. With 1000 gradient directions we can estimate the diffusion signal in great detail. Here we see the diffusion profile of a single voxel from two orthogonal views; orange/red indicates the original data-points, whilst blue is the equivalent data for negated b-vectors. We see how low angular resolution data may not faithfully reconstruct the ‘true’ diffusion profile.

from a dataset with 64 gradient directions as shown in orange. We see how with 1000 gradient directions we are now able to characterise the diffusion signal in 2D. This is essential if we want to compare the diffusion signal with information from inherently 2D microscopy data and will allow for the validation of many present and future diffusion MRI models.

5.3.2 Structure tensor analysis of Gallyas silver stained slides

Structure tensor analysis [58, 59, 116, 60] is a type of texture analysis which, when applied to the red channel of the digitised Gallyas images, extracted the primary fibre orientation per microscopy pixel (Figure 5.16). Across a local neighbourhood of 150 x 150 pixels, the fibre orientations were then combined into a frequency histogram to produce a fibre orientation distribution for a $\sim 40 \times 40 \mu\text{m}$ ‘superpixel’. Summary statistics were also extracted at the level of the superpixel and are shown

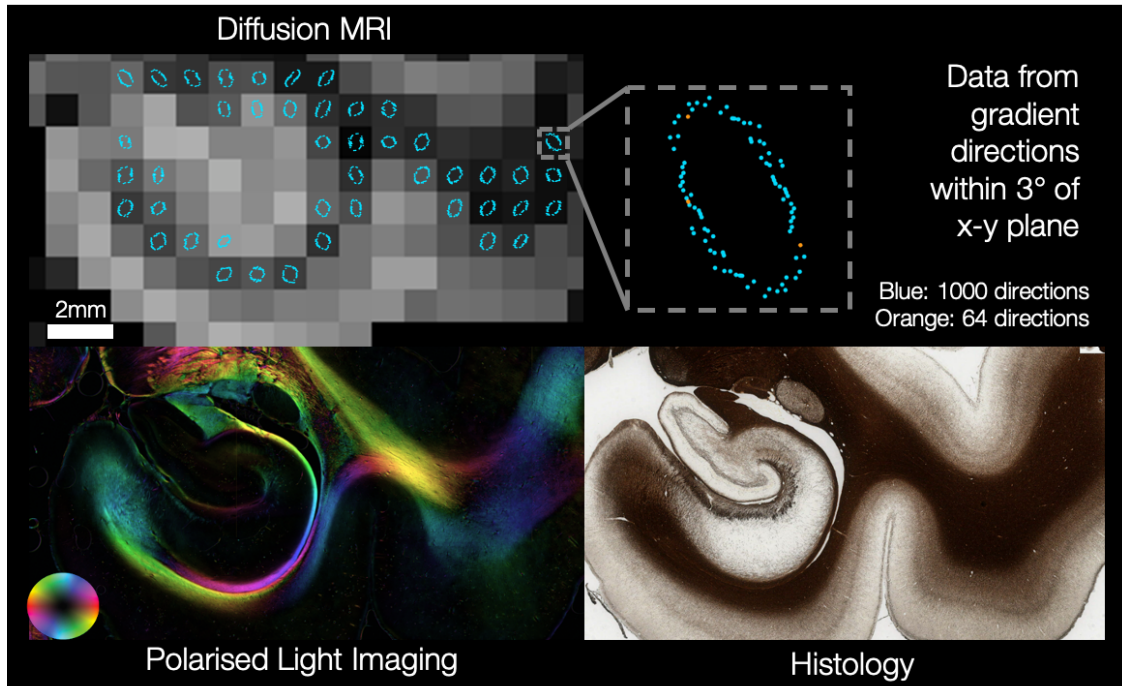


Figure 5.15: The dense sampling of the diffusion signal on the 2D plane facilitates robust comparison of diffusion MRI (top) and microscopy data (bottom). Top (left): A $b = 0 \text{ ms}/\mu\text{m}^2$ image is overlaid with a 2D slice of the ultra-HARDI data; each blue dot shows the signal from a gradient direction within 3° of the 2D-plane. Top (right): For comparison, the equivalent points from a dataset with 64 gradient directions are shown in orange.

in Figure 5.16 e-i. The superpixel parameters include:

1. The fibre orientation distribution

Description: The fibre orientations were combined into a frequency histogram (bin size = 2°).

Use: By providing the fibre orientation distribution per superpixel, we downsample the data whilst retaining detailed information about the high-resolution orientations. That being said, we do lose information about each pixel's location. From these histograms the fibre orientations can be then combined over many superpixels for comparison with the MRI data (as below).

2. The mean fibre orientation

Description: The circular mean of the fibre orientation distribution.

Use: Probing structure tensor-derived fibre orientations at a $\sim 40 \mu\text{m}$

resolution to gain neuroanatomical insight. Because the image is downsampled (from GBs to MBs) and saved in a standard file format (from SVS to TIF) it is much easier to work with. For example, it can be opened by a standard image viewer or loaded into memory in MATLAB or python. This is particularly beneficial for open data dissemination as it allows researchers to get a feel for the data without having to download many gigabytes of the original SVS files.

3. The fibre orientation dispersion index at $\sim 40 \mu\text{m}$

Description: A Bingham distribution was fitted to the fibre orientations within the superpixel. The dispersion parameter κ was then converted to the orientation dispersion index, $ODI = 2/\pi \arctan(1/\kappa)$.

Use: Probe fibre dispersion at a $\sim 40 \mu\text{m}$ resolution to gain neuroanatomical insight.

4. The fibre orientation dispersion index at 1 mm

Description: As above, though here the Bingham distribution was fitted to fibre orientations from a $1 \times 1 \text{ mm}$ neighbourhood, centred on the superpixel (details below).

Use: These dispersion estimates are at the resolution of the ultra-HARDI MRI data and thus can be used to validate MRI-derived estimates of the fibre dispersion index.

5. The mean RGB image

Description: as in name.

Use: Later used for co-registration of the MRI-histology data.

As the amount of orientation dispersion within a local neighbourhood is highly dependent on the spatial extent of the superpixel or voxel, to facilitate fair comparison of the MRI/microscopy-derived dispersion, the microscopy-derived ODI was computed at the resolution of the ultra-HARDI MRI data (1 mm). Here, a sliding window was used to calculate the orientation dispersion index over a $1 \times 1 \text{ mm}$ neighbourhood centred on each superpixel. Similarly, microscopy-derived

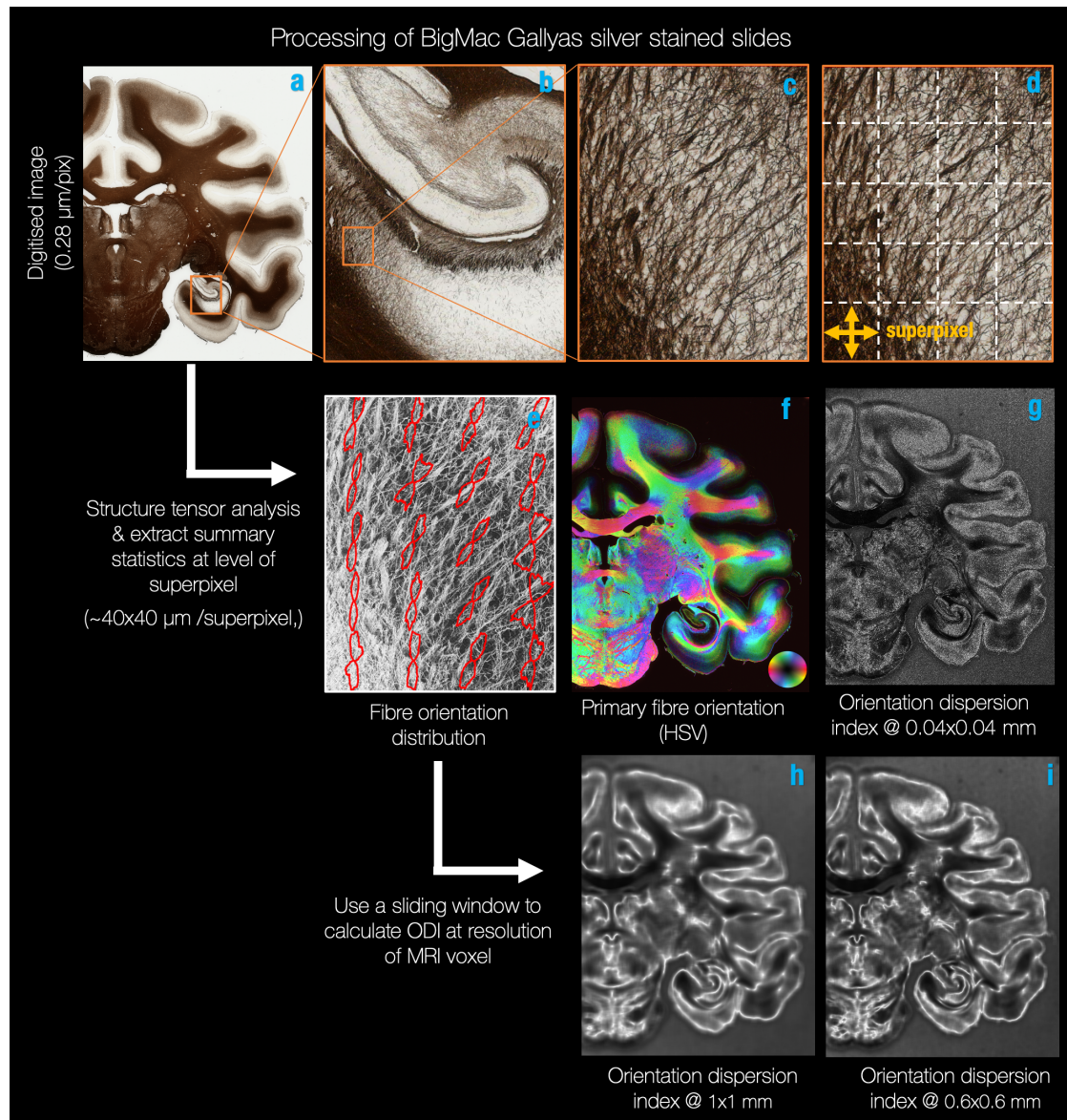


Figure 5.16: Structure tensor analysis was applied to the Gallyas silver stained slides to estimate a fibre orientation per 0.28 μm pixel (a-d). These orientations were then combined into a 2D frequency histogram after which the summary statistics (f-i) were calculated.

estimates of ODI over a 0.6 x 0.6 mm superpixel were generated for comparison with the high resolution MRI data.

Instead of using the sliding window method above, we could instead tile the data here at the size of the MRI voxel (i.e. over a 0.6 or 1 mm superpixel). This tiled image (with 0.6 mm or 1mm resolution) could then be registered to the MRI data for comparison with the MRI-derived estimate of dispersion. However, to obtain MRI and microscopy dispersion estimates in the same space, the microscopy dispersion maps would need to be warped into the MRI space, or the MRI maps warped into the microscopy space (or both warped to the midway point). Here, one (or both) of the maps would need to be interpolated where, at this coarse (0.6 mm or 1 mm) resolution, the interpolation could substantially impact the dispersion estimates (the 0.6 or 1 mm maps of dispersion are not necessarily smooth). By instead using a sliding window, we produce dispersion estimates for a local 0.6 or 1 mm neighbourhood whilst critically retaining a pixel size of 40 μm . The 40 μm maps were then registered into the 3D MRI space. For each voxel, the microscopy-derived estimate of dispersion was calculated as the mean dispersion estimate over all of the pixels that were mapped into that voxel. Consequently, by using the sliding window method we minimise how interpolation (due to registration) affects our dispersion estimates.

Finally, the fibre orientations extracted from structure tensor analysis correspond well with those derived from PLI, giving confidence to the output of both techniques. Figure 5.17 (top) shows the primary fibre orientation derived from the structure tensor analysis per $\sim 40\mu\text{m}$ superpixel. The orientations are colour-coded in HSV (hue-saturation-value) space, where, in a similar manner to the PLI data, the hue is dependent on the fibre orientation and the value is weighted by $(1 - r)$ where r is the pixel intensity in RGB space i.e. pixels in heavily stained white matter are bright compared to those in the lightly stained grey matter. This image is then compared to an adjacent section imaged with PLI (Figure 5.17 bottom). Here the image hue again shows the in-plane fibre orientation, whilst the value

is dependent on the PLI retardance. Neither PLI nor structure tensor analysis can necessarily be considered as a "gold standard" for deriving fibre orientations. However, the similarity of the images, despite the very different manner by which the orientation estimates were derived, provides a mutual corroboration of the two methods. Furthermore, we see how the two modalities provide subtly different information. For example, when a PLI pixel (at $4\text{ }\mu\text{m/pix}$ resolution) contains crossing fibre populations of approximately equal weighting and perpendicular orientations, the PLI-derived in-plane angle becomes uninformative, the retardance is low and the HSV image dark. In Figure 5.17 we see how this results in a darker bands appearing between fibre tracts of different orientations. For example, where the pink and teal fibres intermingle, as indicated by the white arrows in Figure 5.17 inset. These darker bands often give the image a perception of depth and a more '3D look' when compared to the structure tensor output (Figure 5.17 top). In these crossing fibre regions, the original Gallyas image has an order of magnitude finer spatial resolution (at $0.28\text{ }\mu\text{m/pixel}$) and so can likely resolve both fibre populations, the mean of which is shown in Figure 5.17 (top) .

5.3.3 Co-registration of MRI and microscopy data

Co-registration of the BigMac MRI and microscopy data is highly challenging for a number of reasons. Firstly, the spatial resolution of the data spans up to 4 orders of scale, from microscopy images with sub-micron resolution to the $\sim$ millimetre MRI data. Secondly, the contrast between the MRI-microscopy images is substantially different. Because the different modalities capture very different tissue features, there isn't necessarily a one-to-one mapping (e.g. it isn't as simple as matching grey and white matter). For example, Figures 5.6 and 5.10 show how in both the Gallyas and PL images, neither the grey/white matter boundary nor outer tissue edges are consistently well defined. Thirdly, the thin tissue sections may be deformed during sectioning or microscopy preprocessing (e.g. staining and/or mounting). For example, the tissue can shrink or tear, or there may be dirt or bubbles in the

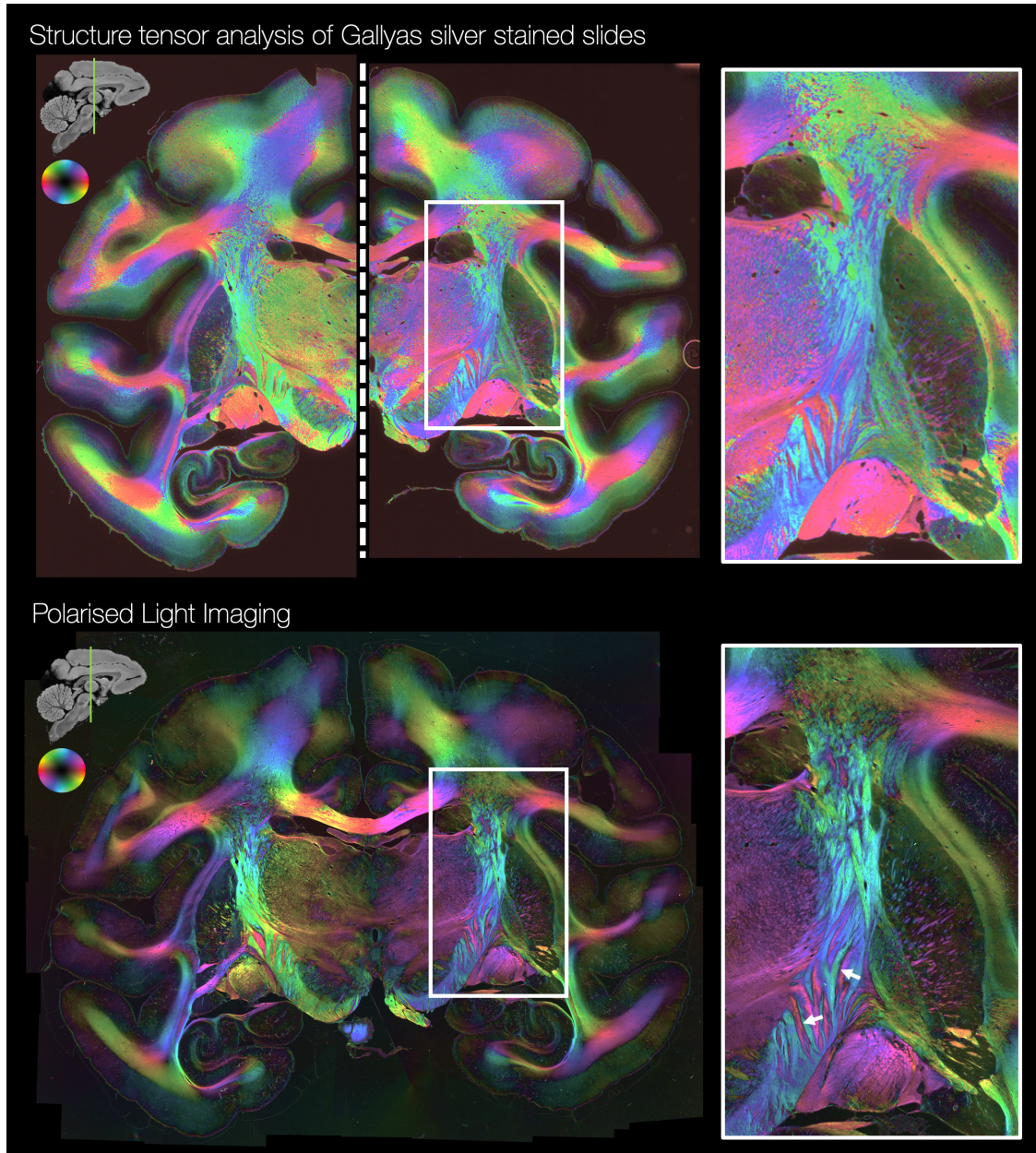


Figure 5.17: The fibre orientations derived from the Gallyas silver stained slide (top) are compared to an adjacent PLI slide. We see remarkable consistency between the images with both modalities capturing the myeloarchitecture in detail. Inset: Both modalities show the detailed organisation of the corticospinal tract. Note here the image contrast and brightness of the PL image has been adjusted to better visualise the fibres extending into the grey matter, for comparison with the histological equivalents.

microscopy slides (e.g. the PLI transmittance maps in Figure 5.6). Finally, the inherently 2D microscopy must be registered into the 3D volume - a particularly difficult optimisation with many degrees of freedom.

If MRI-microscopy co-registration is achievable, the returns can be great. The mapping of parameters between MRI and microscopy space facilitates the validation of MRI-derived tissue parameters with microscopy ground truths. Through co-registration we can obtain *quantitative* rather than just *qualitative* MRI-microscopy comparisons, mapping cells on the order of microns to MRI signals on the order of millimetres. Furthermore, co-registration enables the joint modelling of MRI and microscopy data - a exciting avenue of research. Joint modelling or data-fusion techniques can be achieved through the development of novel biophysical models [56] (*c.f. Chapter 4*), deep learning algorithms or more traditional machine learning approaches. To date, there has been little work in the space of MRI-microscopy joint modelling, largely due to the lack of tools which can accurately co-register MRI and microscopy data.

For the BigMac dataset, we have access to TIRL, a new MRI-microscopy registration tool by Huszar *et al.* [173, 178] that is specifically designed to overcome the above challenges and facilitate accurate MRI-microscopy co-registration. TIRL utilises a sequence of linear and non-linear transforms to register 2D microscopy images into 3D MRI volumes. The primary TIRL pipeline was developed under the assumption that photographic, blockface intermediaries were available to guide the registration. This is because TIRL was initially designed for human sections, where small tissue blocks (a few centimetres cubed) are typically extracted from either the full brain or thick slabs of tissue. These tissue blocks are then sectioned into thin tissue slices for histological staining. Consequently, blockface images act as a crucial intermediary between the thin histology slides and the full 3D brain volume. Because i) the macaque brain (6cm diameter) is considerably smaller than the human brain and ii) we acquired fairly thick sections (50-100 μ m thick), in BigMac we were able to section full coronal slices, mitigating the need for blockface images. Because no

blockface images were acquired during the BigMac sectioning, the TIRL protocol for the BigMac dataset required specific modifications that were achieved through a collaboration with Istvan Huszar, the TIRL developer.

As cross-modality registration within MRI (e.g. co-registering diffusion and structural data) is well established (FSL's FNIRT/FLIRT), it was sensible to identify a single MRI target that worked best for registration with the microscopy images. The microscopy data could then be registered to other MRI contrasts through concatenation of the TIRL and FSL transforms.

In the BigMac dataset, the polarised light images and structure tensor output were registered to the postmortem structural MR image. The resolution of the images were 4, 40 and 300 μm respectively. The structural MR image was chosen as the target image as i) it was the MR data with the highest spatial resolution and ii) it provides good grey/white matter contrast. To drive the registration, we selected the microscopy images with the best grey/white matter contrast (most similar to the structural MR image) and the most well-defined tissue boundaries. Consequently, for the Gallyas slides we used the structure tensor RGB output. For PLI we used the 'inclination' map which, when compared to the transmittance images, are relatively unaffected by the 'bubble' artefact (Figure 5.6). The microscopy images were first resampled to the resolution of the structural MR data and registered into the 3D imaging volume. TIRL outputs a series of transformations which allow the user to transform either images, pixel or voxel coordinates between domains. This is essential as it allows us to map the high-resolution microscopy pixels to precise 3D coordinates in the MRI volume.

In the most rostral and caudal microscopy sections, the tissue from each brain hemisphere is entirely disconnected and distance between the hemispheres is no longer meaningful. Therefore, each hemisphere was registered separately to the MR data with the aid of hand-drawn tissue masks. Similarly, the cerebellum was masked and registered separately to the cerebrum.

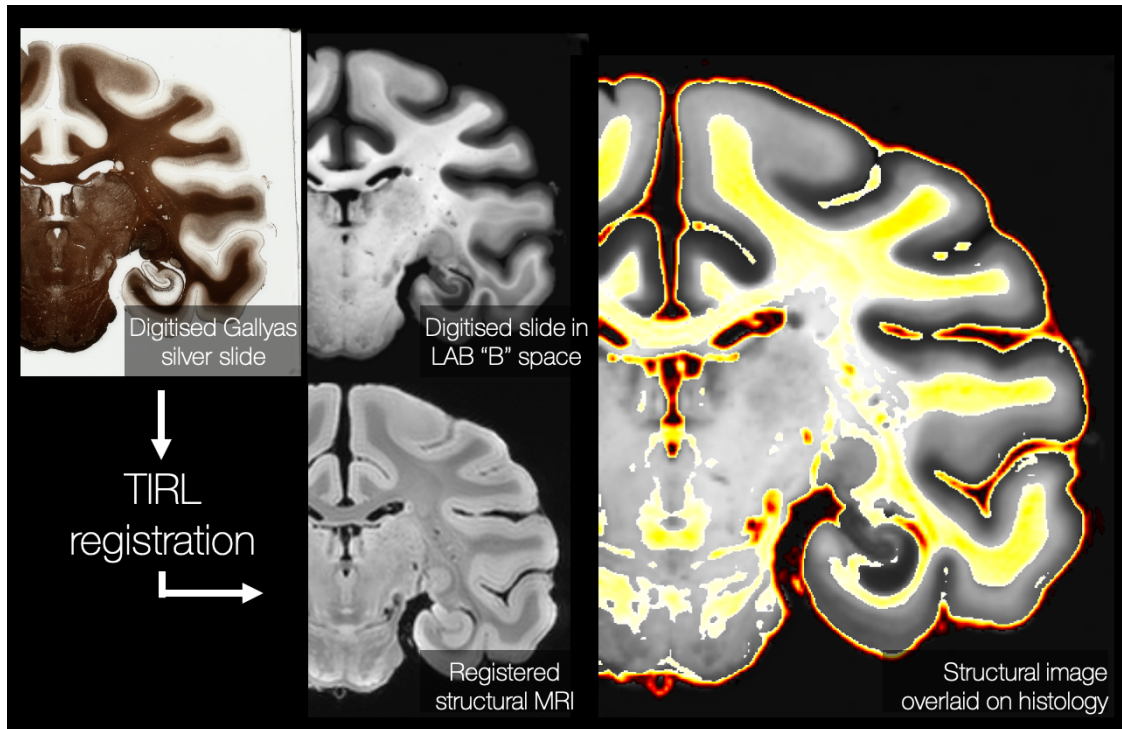


Figure 5.18: An example TIRL registration. The Gallyas silver stained slide was registered to the postmortem structural MRI. The digitised slide was first converted to the LAB space, after which the image was down sampled and non-linearly registered to the 3D MRI volume. The LAB space is based on the opponent colour model of human vision, where any given colour is represented as the combination of lightness ("L"), a position along a red-green axis ("A") and that along a blue-yellow axis ("B"). Because the tissue boundaries were fairly well defined in the LAB "B" image (though difficult to determine in the RGB space), the "B" image was used to drive the MRI-microscopy registration. The registered structural MRI is shown below. Right: The registered structural MRI is overlaid on the LAB microscopy image to assess the high quality registration. We see close agreement in both the tissue edges and the position of the white/grey matter boundary.

Figure 5.18 shows an example output from TIRL where the right hemisphere of a Gallyas silver stained slide has been registered to the postmortem structural MRI data. Figure 5.18 (right) shows the quality of the TIRL transform where the MRI data maps very closely to the microscopy image, both at the white/grey matter boundaries and the tissue edge.

Once the TIRL MRI-microscopy mapping has been generated, the TIRL platform can be used to map the high-resolution microscopy pixels into the 3D MR volume in any MR domain. For example, if the transform between the diffusion MRI and

structural space is known (e.g. though computation with FSL tools), the microscopy pixels can be mapped directly into the diffusion MRI space. In addition, TIRL can handle rotationally dependent metrics such as vectors and tensors, which are appropriately rotated according to the non-linear TIRL warp field. For example, the PLI-derived fibre orientations (consisting of both an in-plane and inclination angle) were mapped from 2D PLI space into the 1 mm diffusion MRI domain for comparison with the ultra-HARDI MRI data.

5.4 Analysis

This section details some of the first analyses of the BigMac data. Here we focus solely on the BigMac MRI data and examine to what effect the ultra-HARDI diffusion MRI data provides unique information when compared to data with lower angular resolution and/or higher spatial resolution.

5.4.1 Ultra-HARDI data: fibre orientations and tractography

We first examined whether the high b-value ultra-HARDI diffusion data provided unique microstructural information (in the form of fibre orientations) when compared to more ‘conventional’ quality data. Here, the ball and stick model [38, 45] was used to estimate the fibre orientations from data acquired at two spatial resolutions (0.6 and 1 mm isotropic), multiple b-values ($b=4, 7$ or $10 \text{ ms}/\mu\text{m}^2$), and varied angular resolution (≤ 1000 gradient directions). In the following results we investigate only voxels within the white matter (see Figure 5.19 for mask).

Figure 5.19 shows how, as the angular resolution of the data was increased, the ball and stick model estimated an increasing number of voxels with 3 or more fibre populations (Figure 5.19 top) and that the fibre orientations were estimated with increased precision (Figure 5.19 bottom). Here the 1 mm diffusion data ($b=4, 7$

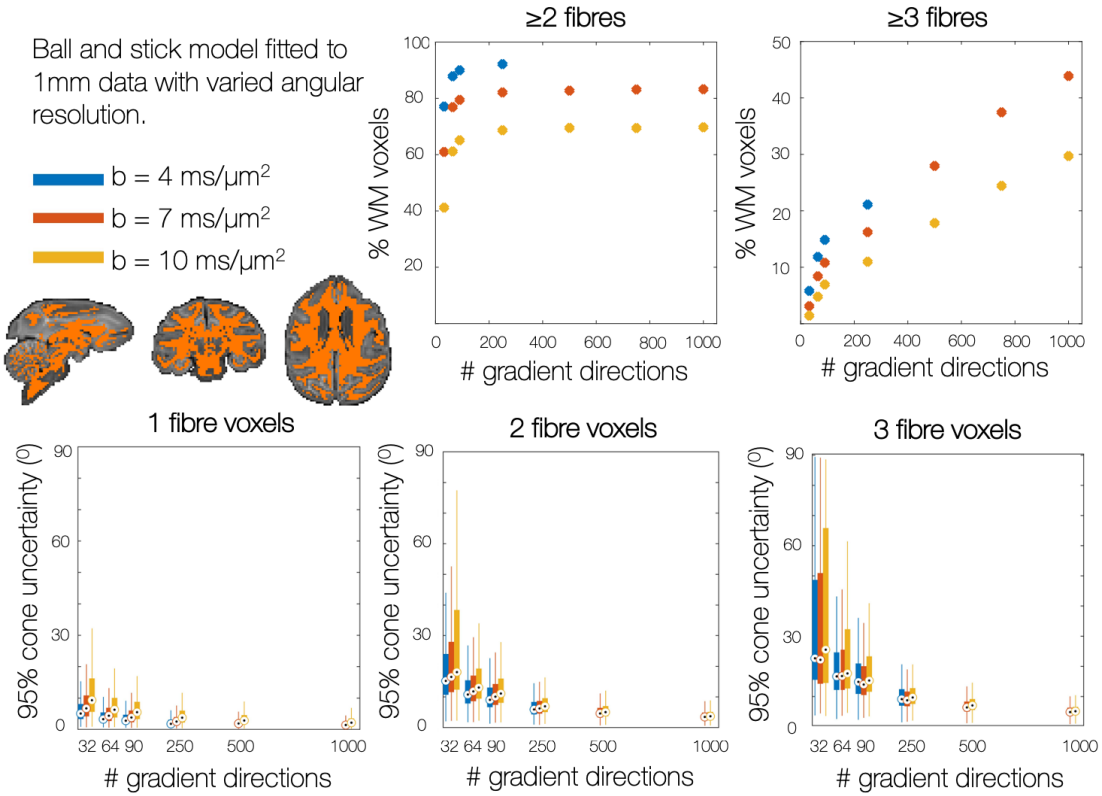


Figure 5.19: The ball and stick model was applied to BigMac diffusion data with varied angular resolution. When applied to data with increased angular contrast (b-value), the ball and stick model estimated fewer voxels as having multiple fibre populations (top); with increased angular resolution (# of gradient directions), the fibre orientations were estimated with increased precision (bottom), and tertiary fibre populations were recovered from previously ‘two-fibre’ voxels (top). The white matter mask is shown in orange.

or $10 \text{ ms}/\mu\text{m}^2$) was split into subsets of gradient directions (32, 64, 90, 250, 500 directions) and the ball and stick model fitted to each subset of data. In Figure 5.19, voxels were classified as having ≥ 2 or 3 fibres if the volume fraction of the secondary or tertiary fibre population was ≥ 0.95 .

After >90 gradient directions, Figure 5.19 shows no substantial increase in the number of voxels with at least secondary fibre populations (i.e. the number of single-fibre voxels is unchanged), but a large increase in the number of tertiary fibre populations. To investigate this effect further, Figure 5.20 compares the 1, 2 and 3-fibre masks for data with different angular resolution. Figure 5.20 top shows how the increased number of tertiary fibres in high angular resolution data

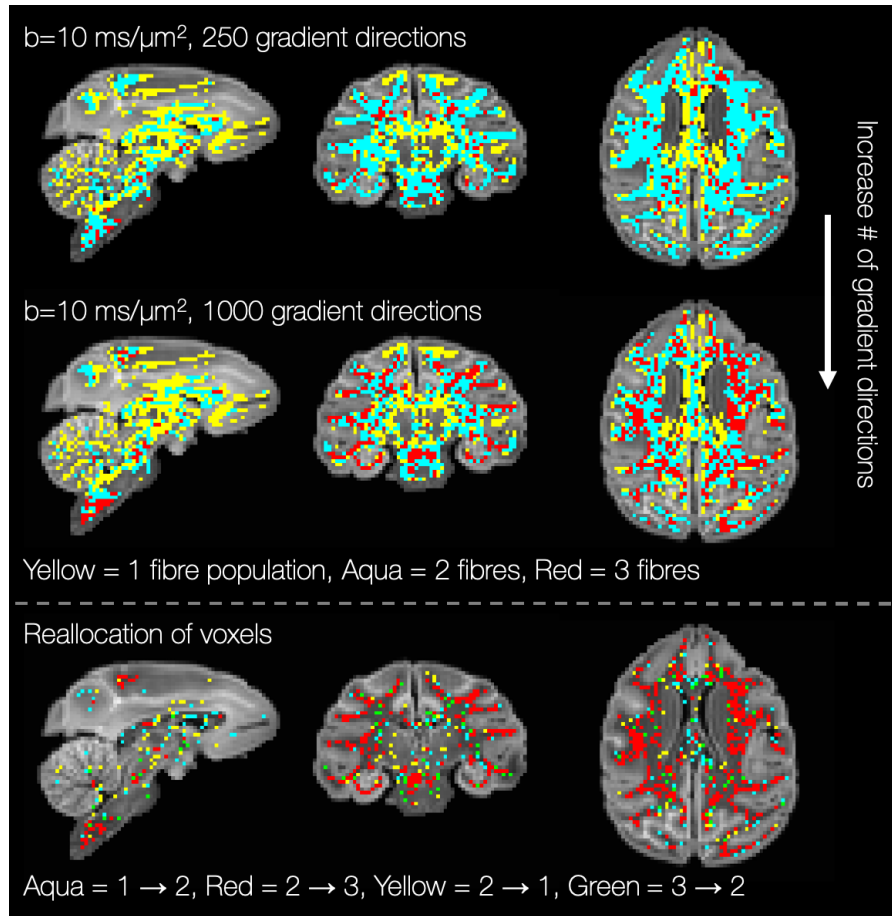


Figure 5.20: Top: Masks showing which voxels are classified by the ball and stick model as having one (yellow), two (aqua) or three (red) fibre populations. Bottom: Here, the colours indicate how voxels are allocated when moving from the top to the middle mask i.e. from data with 250 gradient directions to 1000 directions. For example, the aqua mask shows one-fibre voxels in the 250 direction data but were reclassified as two-fibre voxels in the 1000 gradient direction data.

was largely driven by voxels near the gyri being reclassified from 2-fibre to 3-fibre voxels (Figure 5.20 bottom, red voxels). Concurrently, there were an approximately equal number of voxels ($\sim 5\%$ of voxels across the white matter) reclassified from 1-fibre to 2-fibre voxels (Figure 5.20 bottom, aqua voxels) and from 2-fibre to 1-fibre voxels (yellow). This resulted in the number of single fibre voxels staying approximately constant. These voxels did not follow a clear spatial pattern but were fairly randomly scattered across the brain.

Figure 5.19 bottom shows the precision with which the orientation of the primary, secondary and tertiary fibre populations was estimated. We see how, as angular

resolution data was increased, the ball and stick model shows a decrease in the 95% cone of uncertainty for all fibre populations. This effect is most striking in the tertiary fibres.

As increased precision is not synonymous with increased accuracy, the ball and stick model could be over-fitting to the data. Though a true test of accuracy would be to compare these fibre orientations in an anatomical ground truth provided by the microscopy data (an avenue for future work), in an initial investigation of consistency, Figure 5.21 examines the tertiary fibre orientations for their biological plausibility, where consistent orientations describing known neuroanatomy are more likely accurate. Here we compare the tertiary fibres estimated from the high spatial resolution $b = 4 \text{ ms}/\mu\text{m}^2$ data with 90 gradient directions (left), to those estimated from the $b = 10 \text{ ms}/\mu\text{m}^2$ data with a subset of 250 gradient directions (middle), and the ultra-HARDI data at $b = 10 \text{ ms}/\mu\text{m}^2$ with 1000 gradient directions (right). Here we do not consider any of the diffusion MRI data a ground truth, but instead ask whether the fibre orientations follow our neuroanatomical expectations. Figure 5.21 shows consistent structure in the tertiary fibres of the ultra-HARDI data: in the coronal plane (a) we see the tertiary fibres linking the deeper white matter with the top of the cortex; in the sagittal plane (b), the tertiary fibres follow the sulcus in the pattern of U-fibres; and in the axial plane (c), fibres track along the gyri. None of these biologically plausible fibre populations are seen in detail the low angular resolution data.

Finally, we consider whether the more biologically plausible fibre orientations from ultra-HARDI data lead to improved reconstruction of white matter tracts with tractography. Figure 5.22 compares tractography reconstruction of the superior longitudinal fasciculus (SLF) II from data with either high spatial resolution (left), or high angular resolution and b-value (right). Although this tract is not difficult to reconstruct in human data, our group has struggled with its reconstruction in the macaque. Here probabilistic tractography was performed using FSL's AutoPtx [191]

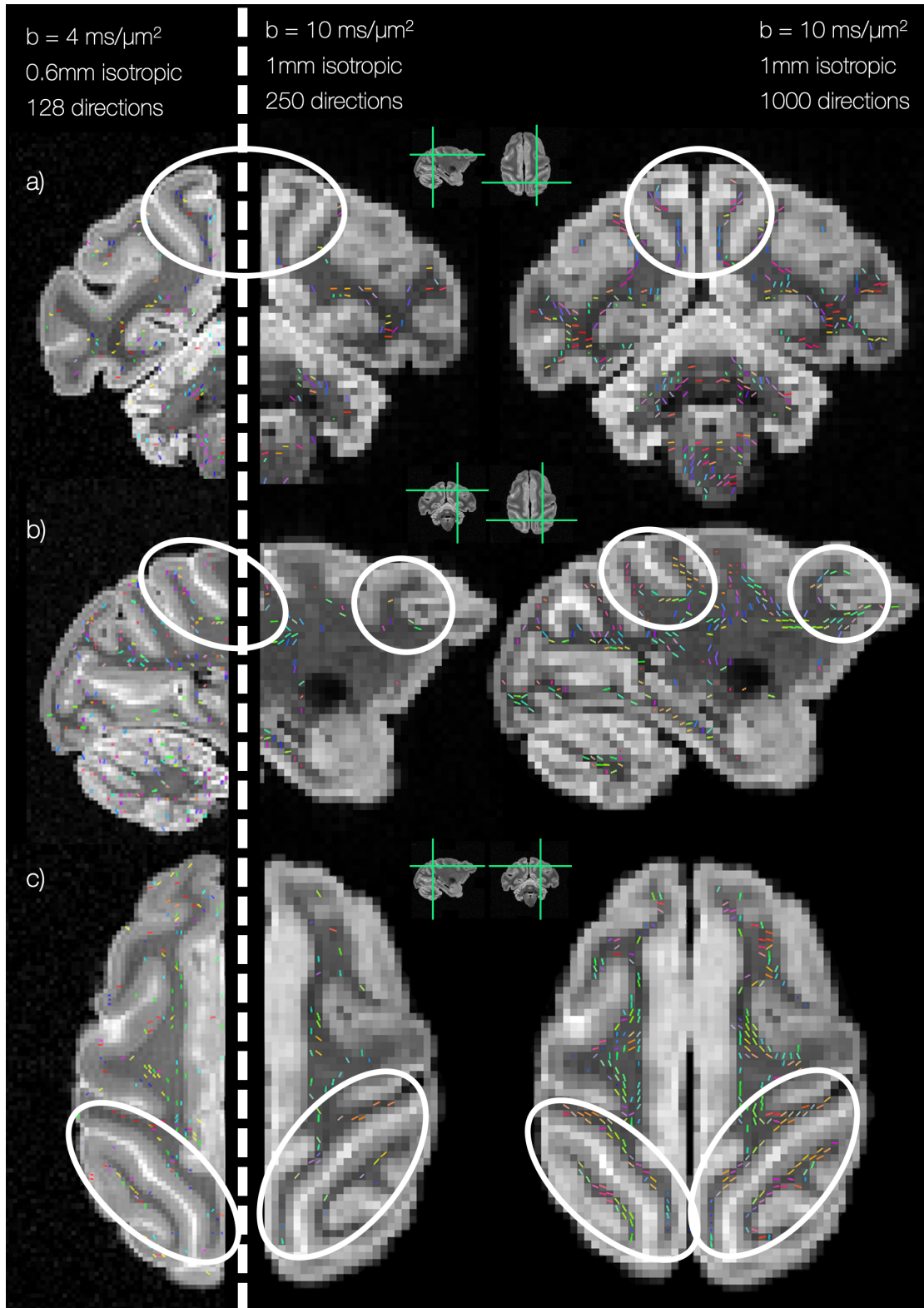


Figure 5.21: The tertiary fibre orientations from the ball and stick model are investigated for their biological plausibility. High spatial resolution (0.6 mm) $b = 4 \text{ ms}/\mu\text{m}^2$ data (left) is compared to 1 mm $b = 10 \text{ ms}/\mu\text{m}^2$ data with 250 gradient directions (middle) and the full 1000 directions (right). We see coherent tertiary fibre orientations in the ultra-HARDI data which are not seen in the more ‘conventional’ quality data.

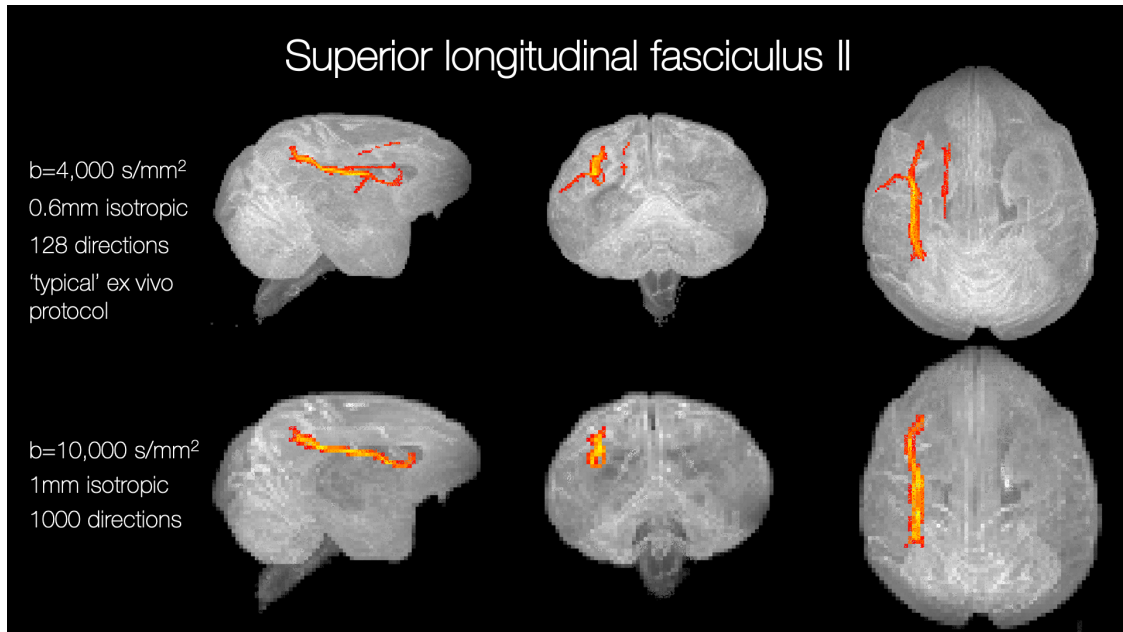


Figure 5.22: The superior longitudinal fasciculus was reconstructed using probabilistic tractography from high spatial but lower angular resolution data (top) and 1 mm ultra-HARDI data (bottom). We see how the ultra-HARDI data produces a more reliable tract which follows our anatomical expectations with fewer noisy offshoots.

with predefined seed, target and exclusion masks (which were kindly provided by Rogier Mars).

Figure 5.22 shows the results from tractography where we use a high threshold to examine the tract centre (i.e. the voxels with the highest density of streamlines). In Figure 5.22, the $b = 4 \text{ ms}/\mu\text{m}^2$ high spatial resolution data produces a tract with several noisy offshoots that do not follow our anatomical expectations: the main body or core of the SLF II is not expected to branch out laterally, and the more medial streamlines are likely associated with the SLF I. In the ultra-HARDI data however, the high angular resolution and contrast allows us to reconstruct a tract with a single, uniform core and a higher density of streamlines extending the main tract body into the frontal regions. This is likely due to the improved tracking of fibres through complex crossing fibre regions. When viewed by a collaborator with expert knowledge of macaque neuroanatomy, the ultra-HARDI tract was deemed to be considerably more consistent with the known spatial extent and connectivity of the SLF II.

Now with co-registered MRI and microscopy data, we are in the position to validate these ball and stick-derived fibre orientations and tracts against microscopy-derived equivalents.

5.5 Conclusion

We present a novel resource, the BigMac dataset, which combines ultra-high angular resolution MRI with multi-contrast microscopy across a whole adult macaque brain. This chapter describes how, through extensive data acquisition and careful curation, the BigMac dataset has been built into a platform for open dissemination to researchers worldwide. The ultra-HARDI data provides unique information which will aid the reliable estimation of structural connectivity. Furthermore, the microscopy data visualises the highly detailed ‘true’ tissue microstructure due to its ultra-high spatial resolution. As a unique, multimodal resource, the BigMac dataset will enable neuroscientists to ask new and fundamental questions. For example, it will enable researchers to i) link brain connectivity across spatial scales spanning four orders of magnitude; ii) understand the relationship between relatively crude but non-invasive MRI signals and cellular properties measured by invasive microscopy; and iii) develop novel approaches to extracting rich information from in vivo MRI data by leveraging the specificity of microscopy-derived gold standards. Thus, once released, the BigMac dataset will provide an open access platform from which we can interconnect microstructural features with MRI signals throughout the brain.

5.6 Contributions

This multi-modal multi-disciplinary project has relied on many collaborators across our Imaging Centre, to whom I am extremely thankful for their valuable contributions. In particular, I would like to thank Jerome Sallet and Rogier Mars for their ongoing advice and for facilitating this ambitious work. As the above chapter

centres on the BigMac data acquisition and curation, here I detail those who have directly contributed to those aspects of the project: the postmortem MRI data was acquired by Alexandre Khrapitchev, who also helped optimise the postmortem MRI protocols; the in vivo MRI aspect of BigMac was led in its entirety by Jerome Sallet; Jerome also developed the MRI protocol for acquisition of the postmortem 0.6 mm diffusion MRI, structural MRI and T_1 maps and did much of the postmortem tissue preparation and handling; the protocol for isotropic diffusion encoding was developed by Michiel Cottaar; the tissue was sectioned, stained and mounted for microscopy by Greg Daubney; much of the microscopy data was acquired and processed by Adele Smart; Adele also hand-drew tissue masks for the microscopy images and ran much of the MRI-microscopy co-registration; the software and protocol for the co-registration of the MRI-microscopy data were developed by Istvan Huszar; the original software for polarised light imaging and structure tensor analysis were previously developed by Jeroen Mollink; the anatomical masks for tractography were provided by Rogier Mars. Furthermore, I would like to thank Connor Scott, Jesper Anderson, Moises Hernandez Fernandez, Paul McCarthy, Michiel Kleinnijenhuis and Menuka Pallegage Gamarallage for their advice at stages throughout this project, and Nicola Sibson for providing access to the MRI scanner. My personal contributions to this project include: conceptualisation and project lead; design and optimisation of the bespoke week-long postmortem diffusion MRI protocol to acquire ultra-HARDI data; MRI data preprocessing; advising on histology sectioning, staining and mounting techniques; digital scanning of the first histology slides; the first PLI data acquisition; supervising Adele, our research assistant, in microscopy data acquisition and processing; visualising and interrogating microscopy data; developing analysis pipelines for PLI fitting and stitching, structure tensor analysis of histology slides, and MRI-microscopy co-registration; MRI-microscopy data curation; data modelling, analysis and presentation of results.

6

Future directions and final remarks

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This thesis considers different ways in which we can overcome known parameter degeneracies and improve the specific microstructural interpretability of the diffusion MRI signal. The data in this thesis spans modalities, species, tissue states and scales: Chapter 3 utilises ultra-strong gradients available on only a few scanners worldwide, Chapter 4 requires postmortem tissue samples, Chapter 5 describes a unique macaque resource that took > 1000 hours to acquire. Consequently, this data can only be obtained on a few subjects and the models cannot be directly applied to typical, clinically achievable data. Nonetheless, the data and results of this thesis provide insights into how certain tissue features influence the observed diffusion signal. This knowledge will aid design of future, more specific, microstructural modelling of diffusion MRI.

6.1 Future work

The work in this thesis demonstrates two main results: i) that the diffusion characteristics vary across the brain, and ii) that there are unique opportunities afforded by MRI-microscopy data fusion analyses. Here we suggest future work in both domains.

6.1.1 Characterising diffusivities across the brain

Though Chapters 3 and 4 differ widely in their methods (high b-value *in vivo* imaging versus joint MRI-microscopy modelling), in both Chapters our results demonstrate how the diffusion characteristics vary considerably across the white matter. Though this is perhaps not surprising - where ideally (and with infinite imaging resolution) the diffusion signal from each fibre or fibre bundle would be modelled individually - it challenges the assumption of global, fixed diffusivities, as used in many current diffusion models. Consequently, future work should aim to both reproduce these results and further understand the biophysics underpinning the observed diffusivity variations across the brain. To this end, some specific projects are suggested below.

Confirmation of results

The results of Chapter 3 and 4 should be confirmed via different techniques and/or methods. In particular, it would be interesting to see whether the high axial diffusivities reported in Chapter 3 are reproducible across studies.

In Chapter 3, because the dispersed stick model relies on the simultaneous estimation of the intra-axonal axial diffusivity and the fibre orientation dispersion, if the dispersion estimate is inaccurate, the estimates diffusivity could be biased. This limitation is particularly relevant in crossing fibre regions where, as the model currently assumes a single fibre population per voxel, the dispersion estimates in multi-fibre voxels may not be very biologically meaningful. Consequently, our results

in crossing fibre regions should be interpreted with caution and we are somewhat restricted in our ability to interpret intra-axonal variations across the white matter.

This limitation can be overcome through the development of a multi-fibre dispersed stick model. Alternatively, we can develop a method that estimates the intra-axonal axial diffusivity without estimating dispersion. The spherical mean technique [124, 192] provides one way of achieving this. In the spherical mean technique, microstructural features are related to the powder-averaged diffusion signal, i.e. the average signal across all gradient directions. Crucially, the powder-averaged signal is independent of the underlying fibre orientation distribution and thus only dependent on $F = f_{in} * S_0$ and $d_{\parallel}$. Thus, if we can find a way to separate the F from $d_{\parallel}$ in high b-value, powder-averaged data, our diffusivity estimation should not be biased by having to also estimate the underlying fibre orientation distribution.

As Markus Nilsson recently suggested, by taking the ratio of the powder-averaged signal from two images, each acquired at high b-value but with different b-tensors [107], both F and $d_{\parallel}$ can be estimated simultaneously from high b-value data. We look forward to collaborating with Markus on this exciting project.

In Chapter 4, using the joint MRI-microscopy model we analysed only a small section of postmortem tissue and so could not comment on how the fibre response function (and thus the diffusion characteristics) varies across the wider brain. In future work, we will apply the joint model to the BigMac data with an aim to test the reproducibility of our results and characterise any canonical patterns of variation in the diffusion characteristics across the brain. Furthermore, by changing how we parametrise the fibre response function and fitting to multiple b-values simultaneously, we can extend the joint model to concurrently estimate separate diffusion characteristics for the intra- and extra-axonal space.

Validation

Where possible our results should be validated against 'gold-standard' estimates obtained from other modalities. Though the diffusion characteristics are themselves

difficult to validate, the orientation dispersion estimates from the dispersed stick model (Chapter 3) can be validated against microscopy-derived ground truths. Because the orientation dispersion and intra-axonal axial diffusivity covary, if the dispersion estimates are found to be realistic, this will increase our confidence in the axial diffusivity estimates. Accounting for the reduced diffusivity in postmortem tissue, this validation study will require data with 3 shells above $\sim 15 \text{ ms}/\mu\text{m}^2$. Unfortunately, the BigMac dataset does not include such high b-value data and so a new dataset will need to be acquired.

Relating diffusion characteristics to specific tissue features

The relationship between the diffusion characteristics and other aspects of the tissue microstructure warrant further investigation. Our results in Chapter 3 suggest an interesting relationship between the intra-axonal axial diffusivity and axon diameter where the two parameters appear to anti-correlate. Currently, this supposed relationship is purely qualitative, where the observed high-low-high pattern of axial diffusivity across the corpus callosum is broadly compared to the low-high-low pattern of axon diameters reported in other literature. In a quantitative comparison study, estimates of both axon diameter and intra-axonal axial diffusivity should be acquired in the same subjects and directly correlated.

Furthermore, these experimental investigations should be backed up by theoretical simulations. Following the work by Lee *et al.* [91, 90] and Callaghan *et al.* [131] (amongst others), the diffusion signal can now be simulated from axons with highly realistic morphology. By utilising this simulation framework, we can extend the simplistic simulations of Chapter 4 to investigate more thoroughly how various microstructural parameters affect the observed intra-axonal axial diffusivity from the dispersed stick model. Particular parameters of interest include the axon diameter, undulation, variations in caliber, as well as the presence of mitochondria.

Global diffusivities in comparative studies

Our results challenge the assumption of a global diffusivity where, in Chapter 3,

we demonstrate how estimated parameters of the NODDI model (which assumes a fixed, predefined axial diffusivity) change as the assumed axial diffusivity is varied. However, these results do not necessarily imply that the NODDI parameters won't show meaningful differences between tissue states or in a comparative study of patients and controls. Consequently, a future simulation study (with known ground truth values) should investigate the validity of the NODDI parameters (and those output from other diffusion models assuming global diffusivities) in comparative studies. This work may provide useful insights into how we should interpret the NODDI model parameters as informative 'indices' of the tissue state, rather than specific microstructural features.

6.1.2 The BigMac dataset & data fusion

The BigMac dataset opens up many, new avenues of research, from basic neuroanatomy and cross-species analysis, to the origins of the MRI signal. Recently we have achieved co-registration of the BigMac MRI and microscopy data with excellent results. Here we suggest a few projects based on the co-registered data that will both demonstrate the power of BigMac in neuroscience research, and further develop it as an open access data platform for dissemination to researchers worldwide.

Development of BigMac histological atlas

Due to the vast time it takes to acquire histological data, we are still in the process of digitising the histology slides. However, once digitised, the slides will be co-registered into the 3D MRI volume. Consequently we will produce a multi-contrast 3D histology atlas that will eventually contain registered data from polarised light imaging and 4 complementary histology contrasts (Gallyas silver, Cresyl violet, IBA1 and GFAP). This atlas will highly complement the BigBrain human histological atlas [177], though with the additional benefit of providing co-registered MRI data (both *in vivo* and postmortem) from the same tissue sample.

Furthermore, to extract quantitative metrics from the histological data, we aim to develop algorithms - utilising both deep learning and more traditional machine learning approaches - to automatically segment the microscopy images to differentiate cell types and extract biologically-meaningful stained area fractions. By automating this process and applying it to all BigMac histology slides, we will be able to map cytoarchitectonic variations across the brain. These maps could then be used to drive microscopy-derived segmentations of the cortex at multiple scales (e.g. using data-driven clustering techniques or dimensionality reduction). Note, both the original high resolution ($0.28\ \mu\text{m}/\text{pixel}$) data, the co registered data and the quantitative maps will be made publicly available via the BigMac online platform for others to use in their research.

Diffusion model validation

The BigMac dataset facilitates the direct comparison of MRI parameters and microscopy data on a voxelwise basis, and so is an excellent resource for validating many current diffusion models. In a proof of principle study, we have begun to develop analysis pipelines that compare MRI-derived estimates of dispersion to those from either polarised light imaging or histology. As our results in Chapter 3 suggest that i) the intra-axonal axial diffusivity is higher than that typically assumed by NODDI, and ii) the orientation dispersion is dependent on the assumed axial diffusivity, it will be interesting to see whether the NODDI estimate of dispersion is better matched to that from microscopy when the assumed axial diffusivity is increased.

3D polarised light imaging

MRI-microscopy joint analysis is difficult for a number of reasons, one being that the microscopy data is typically only 2D and so cannot inform on the full 3D fibre orientation distribution. However, in the BigMac dataset this limitation may be overcome if we can estimate 3D fibre orientations from polarised light imaging. Typically, polarised light imaging estimates an in-plane and inclination angle per pixel [61, 62, 63, 63]. However, the sign of the inclination angle - i.e. whether it is

above or below the plane - is often ambiguous and the estimated inclination is thus ignored. Using a specialist tilting sample stage, Axer *et al.* [63] have shown it is possible to remove the ambiguity and estimate a unique inclination angle.

Alternatively, an opportunity for 3D PLI is also afforded by co-registered diffusion MRI and PLI data: diffusion MRI can inform us about the fibre orientations in 3D and thus can resolve the PLI inclination angle ambiguity to reconstruct the full 3D PLI tensor at the native microscopy resolution. In this manner we hope to reconstruct dense, whole brain, 3D matrix containing 3D PLI fibre orientations at a spatial resolution of $4 \times 4 \times 350 \mu\text{m}$. This can then be used to perform whole brain microscopy-derived tractography with unprecedented spatial resolution.

Not only do we expect the PLI tractography to provide new neuroanatomical insights, but as the microscopy tractography will be performed in MRI space, the microscopy-derived streamlines can be directly compared to those estimated from diffusion MRI. Furthermore, due to the richness of the BigMac MRI data, we can investigate how b-value, spatial and angular resolution (and any combinations thereof) affect our estimates of the structural connectome. This information may then inform future MRI protocol design [193] and the development of advanced tractography algorithms, particularly in studies that aim to determine specific tracts of interest which are difficult to reliably estimate using current approaches.

Characterising patterns of gyral connectivity: a multi-voxel, data-fusion approach

BigMac can enable the development of sophisticated multi-voxel, multi-modal analyses to characterise patterns of connectivity across the brain. In particular, we will first consider where connections enter the cortical folds (gyri) and connect to cortical grey matter, which are poorly characterised by current MRI estimates. Gyri share many common features, implying the existence of a stereotyped, ‘canonical’ architecture. Here we propose the joint analysis of MRI and microscopy data in

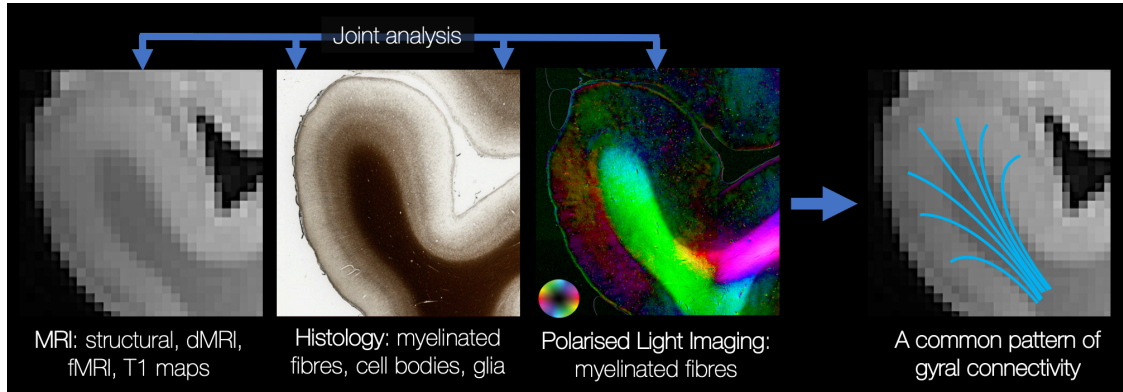


Figure 6.1: Characterising the canonical gyrus.

a model which simultaneously considers both the topography, cyto- and myelo-architecture of the gyrus, to provide enhanced modelling of gyral connectivity (Figure 3). Using a Deep Neural Network (likely a CNN or GAN), we can directly map the MRI signals to the ‘true’ gyral microstructure from microscopy. By analysing all gyri across the BigMac brain, we can first ask whether the concept of a canonical gyrus holds and characterise ‘common’ patterns of gyral connectivity. This model can subsequently be applied to *in vivo* human MRI (in the absence of ground truth microscopy data) to improve connectivity estimates. This project will build on the recent promising work by Yendiki *et al.* [194] who have demonstrated how neural networks can be trained on co-registered MRI-microscopy data to predict patterns of connectivity within the white matter.

Voxel-wise mapping of tissue microstructure to MRI signals in the cortex

An opportunity afforded by the BigMac dataset is to better understand the information content of MRI at the level of individual voxels. Here we propose a bottom-up approach to microstructural modelling, first asking to what extent specific tissue features contribute to voxel-wise changes in the MRI signal, and using this knowledge, develop informed biophysical models of the tissue. Furthermore, if by using the BigMac dataset we can learn how microscopic tissue features map onto MRI signals, this mapping can then be applied in *in vivo* data to predict the cellular structure of brain regions, potentially in *in vivo* human brains.

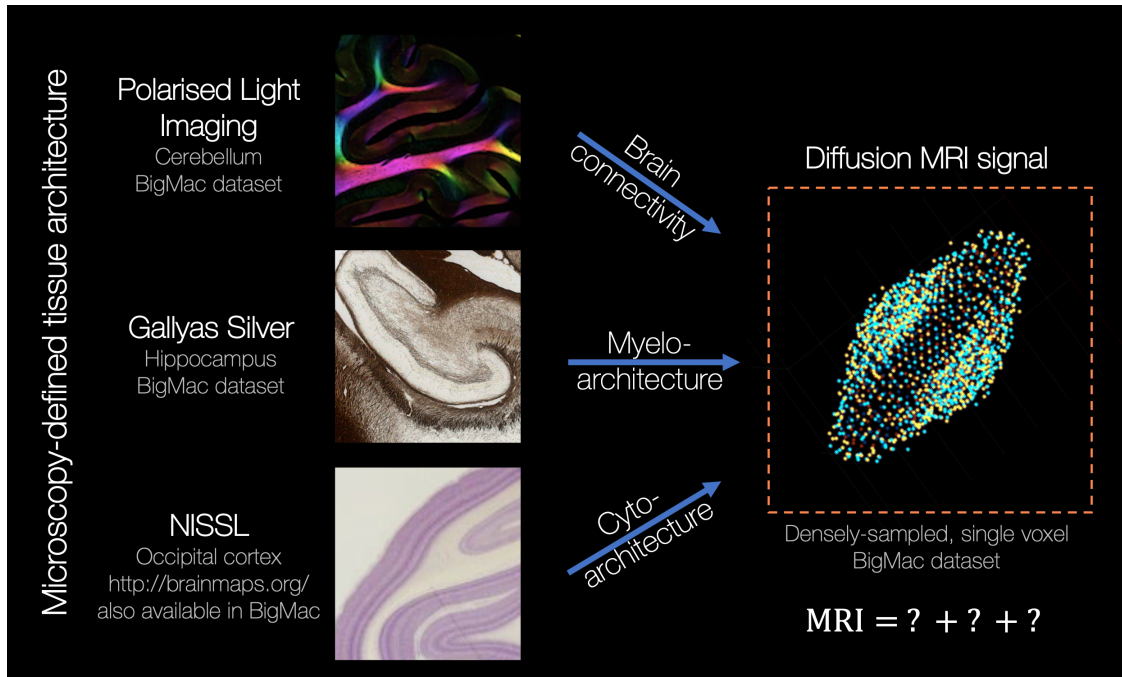


Figure 6.2: Mapping microscopic tissue features to macroscopic MRI signals.

Future work will begin by analysing the cortical grey matter where the biological basis of the diffusion MRI signal is still poorly understood [195]. As appropriate, this analysis will involve both traditional machine learning approaches, such as regression, and more novel deep learning algorithms, e.g. for segmentation of the microscopy images where, in the Cresyl sections, we hope to quantitatively map different cell *types* across the brain.

Development of BigMac online platform

Finally, few institutions have the expertise or resources to acquire a dataset of this nature, but if properly curated, many could benefit from its existence. In future work, we will create an open-access online platform for the BigMac dataset, which will be continually enhanced with data, tools and methods for others to use in similar research. This will include the micro-to-mesoscale full-brain connectivity atlas based on both microscopy (as described above), providing unprecedented anatomical detail, and high-quality MRI.

6.2 Final remarks

The work in this thesis constitutes the beginning of an exciting adventure into the field of neuroscience. With modern machine learning approaches, we are now able to quantitatively analyse data on a scale that, due to time and computing requirements, has previously not been possible. Through multi-disciplinary, collaborative research, our science can cross modalities, scales, species and populations. Because of open access and data sharing initiatives, scientists from across the world are collaborating on Big Data to drive new exciting hypotheses and neuroscientific discoveries. It's a fascinating time to join the neuroscience community and it will be interesting to see what we discover next.

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