Morphological characterisation of neural tissue microstructure using the orientationally-averaged diffusion MRI signal

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A B S T R A C T

Diffusion-weighted magnetic resonance imaging (dMRI) is a powerful tool for the characterisation of neural tissue microstructural features. The role of neural projection curvature on the diffusion signal was recently studied for three temporal regimes of the diffusion pulse sequence in search for a description of the different decay trends in the orientationally-averaged diffusion signal reported in in vivo human studies. This work experimentally investigates the effects of neural projection curvedness in one of these regimes, namely the short diffusion time regime. Multi-shell diffusion MRI acquisitions on fixed rat spinal cord were performed using a custom number of diffusion gradient directions on a vertical bore pre-clinical MRI scanner capable of generating 3000 mT/m. Diffusion was probed in three different $q$-values ranges $[450, 970]$, $[600, 1400]$ and $[1500, 1750]$ mm$^{-1}$ using diffusion pulse durations of 1.4, 2 and 2.5ms, respectively. Noise correction was performed on the diffusion data and the orientationally-averaged signal was computed for each shell using a weighted mean. The signal from selected regions in the sample was then fitted to a power law. Results show that gray matter areas exhibit a signal reduction with variable decay trends in the range of diffusion sensitivity values used here. This suggests that gray matter microstructure features are pictured by the orientationally-averaged signal in the high diffusion sensitivity regime and, as theoretically suggested, neurite curvature might play a role in characterizing the signal decay. These preliminary results may prove useful in the development of models for the interpretation of the diffusion signal and the design of acquisition strategies that aim to study the high diffusion sensitivity regime.
To Arianna, that with unconditional love supports me every day...
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Introduction

The introduction of diffusion-weighted magnetic resonance imaging (dMRI) in the early 90s as a tool for non-invasive, in-vivo tissue microstructure investigations opened a new era in the exploration of brain neuroanatomy [1]. Using motion sensitiser gradients, dMRI probes water diffusion occurring at a cellular level and encodes in the MR signal the information about the microenvironment water is moving in [2]. Water motion is influenced by a plethora of microstructural features of the tissue (cell size, shape, density and organization, membrane permeability, components of intra and extracellular environment) [2] which altogether manifest in the acquired signal. This is then used to generate image contrast that helps researchers and clinicians to understand normal brain tissue characteristics and how these change in disease conditions.

Understanding how individual microstructural features of the tissue influence the diffusion signal is challenging. Researchers use a combination of signal acquisition schemes and modelling to tackle the problem [1]. Most signal acquisitions are based on the pulsed gradient spin echo sequence introduced by Stejskal and Tanner [3], where two diffusion gradients are used one before and one after the refocusing pulse. The diffusion signal is then fitted to a model where parameters are related to properties of tissue microenvironment. These models try, using histological observations and/or physical and mathematical description of diffusion, to correlate signal properties to microstructural features of the neural tissue [2]. Tensor based models, such as diffusion tensor imaging (DTI) [2], generate brain maps based on the directionality of water diffusion. More relevant to this study are biophysical models [1], that rely on a compartmental representation of the brain tissue to describe the non-mono-exponential decay of the diffusion signal, thought to reflect hindered and restricted water diffusion [1].

The stick and ball model proposed by [5] uses two compartments to represent the complexity of the brain tissue: straight cylinders for anisotropic structures as dendrites and axons (neural projections), and spheres for the remaining structures and extracellular space. The model provides information, among others, about diffusion anisotropy and volume fraction of dendrites and axons. The usefulness of the model is, however, limited by its simplistic description of the microenvironment. In fact, white and gray matter neural projections are considered to be equivalent. However, as observed by [6], the orientationally-averaged signal from gray matter regions decays faster compared to the one in the white matter regions. To explain these discrepancies, [6] suggested a difference in the membrane permeability between neurons in white and gray matter, while [7] proposed that the faster decay seen in gray matter is to be attributed to the abundance of cell bodies in these regions. A different approach is taken by [8], that described the difference in signal decay in terms of neurite projection curvature.

The focus of this study is to experimentally investigate the theoretical findings of [8] using multi-shell diffusion acquisition. In particular, the short pulse separation
regime is studied, where the distance water molecules travel during the application of the diffusion pulse is smaller or equal to the radius of curvature of the neurite projection [8]. Initially, computer simulations are used to understand the characteristics of the diffusion signal in this regime and optimize the acquisition parameters. Then, using the results from the first stage, diffusion acquisitions on fixed rat spinal cord are performed using a preclinical MRI scanner. This analysis may improve our understanding of the complexity of brain microstructure and its influences on the diffusion MR signal.

In the following section, the principles of magnetic resonance imaging are presented, giving the reader an understanding on the origin of the MR signal and its acquisition. An overview of the neural tissue is then provided, to better grasp how complex the microenvironment diffusion MRI tries to capture is. Afterwards, diffusion is described and, by introducing the Stejskal and Tanner pulse sequence, how the MR signal is made sensitive to it. Some of the models that interpret the diffusion signal are then presented, and how [8] studied the influence of neural projection curvature on the powder-average signal. Finally, a small description of the noise characteristic in the diffusion signal is provided for a better understanding of the noise reduction method used in the data analysis.

\footnote{Fixation is a conservation method used to block the decay of biological tissues.}
Theory

A theoretical background of the MR technique, with focus on signal source, relaxation processes, signal localisation and image reconstruction is provided. Then, a brief description of the water diffusion phenomena is given, and how the MR signal is made sensitive to it. Next, how the diffusion MR signal can be used to study brain tissue microstructure, with particular attention on the curvature of neural processes studied looking at the orientationally-averaged diffusion signal. Before looking at diffusion MRI and its application, an overview of the nervous tissue environment and its complexity is given, since the primary purpose of this work is to study one of its geometric characteristics.

2.1 MRI - how does it work?

Magnetic resonance imaging (MRI) is a non-invasive technique that uses intrinsic properties of tissue to create contrast and generate an image. MRI detects radio-frequency signals coming from induced magnetisation in the tissue after the application of an electromagnetic excitation pulse [9]. A detectable magnetisation in the tissue is promoted by the application of a static magnetic field $\mathbf{B}_0$. Excitation is caused by a secondary oscillating magnetic field, $\mathbf{B}_1$, perpendicular to $\mathbf{B}_0$ and generated by a radio-frequency (RF) pulse using a transmitting coil. Signal is detected using a RF receiving coil that measures the electric potential variations induced by the oscillations of the tissue magnetisation. By timing the application of the RF pulse (or multiple pulses) and the signal acquisition, the intrinsic tissue properties are exploited to generate image contrast. Furthermore, signal localisation is obtained using a set of magnetic field gradients that change the oscillation frequency of the tissue magnetisation and encode its position in the collected signal. Fourier analysis is then used to decode the spatial information and generate an image.

2.1.1 MR signal source

Atoms, whose nucleus has spin as a result of the odd number of protons or neutrons, are magnetic dipoles and can be treated as magnetic moments [10]. The most abundant magnetic dipole in the biological tissue is the hydrogen atom, whose nucleus is made of a single proton. Other atoms, such as isotopes of oxygen and carbon, are magnetic dipoles; however, their percentage fraction in the human body is very small compared to hydrogen, which makes the latter to be the most exploited MR-active atom to generate image contrast [10].

The magnetic moment of MR-active nuclei (spins, protons and MR-active nuclei are used interchangeably and in this work refer to hydrogen atoms) can be represented by a vector with direction and magnitude. Normally, the orientation of the magnetic moments is random in the tissue, with a net magnetisation equal to zero. When an external magnetic field $\mathbf{B}_0$ is applied, spins orient themselves along its direction [9], as is visually described in Figure 2.1. The most important aspect of
2.1. MRI - HOW DOES IT WORK?

Figure 2.1: Spin alignment and precession. By applying an external magnetic field $\vec{B}_0$, spins orientation is forced in the direction of the external field. When this happens, protons start to precess around $B_0$ direction with Larmor frequency $\omega_0$.

This alignment is that it is not static. Spins precess around $\vec{B}_0$ with a characteristic angular frequency $\omega_0$ called Larmor frequency. Precession frequency is defined by the Larmor equation

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

where $\gamma$ is the gyromagnetic ratio, specific for a nucleus, and $B_0$ is the strength of the applied magnetic field. Spins in water have $\gamma = 2.68 \times 10^8 \text{ rad/s/tesla}$ that is commonly converted to $\frac{\gamma}{2\pi} = 42.58 \text{ MHz/T}$.

From the definition of the Larmor frequency it is clear that one can change the precession frequency of the spins by manipulating the magnetic field strength. For example, today’s 3T clinical scanners induce protons to precess at a frequency of $\sim 128$ MHz, that is in the radio-frequency range of the electromagnetic spectrum. The ability to manipulate the precession frequency of spins is a pivotal aspect exploited in signal localisation when performing MR imaging, and is further explained later.

Spin alignment with the external magnetic field occurs in two configurations: parallel or anti-parallel. These represent two quantized energy levels hydrogen atoms acquire experiencing an external magnetic field. Nucleus properties define the number of possible energy states, while the difference between them, $\Delta E$, is proportional to the strength of the external magnetic field. In parallel alignment, the magnetic moment direction of the proton is parallel to $\vec{B}_0$ and the spin is in a lower energy state. In the second scenario, the directions of spin’s magnetic moment and external field are opposed, positioning the spin at a higher energy level. As it can be seen in Figure 2.2, there is a small difference between the population of spins in the two energetic states, and the ratio between anti-parallel $N\downarrow$ and parallel $N\uparrow$ spins is described by

$$\frac{N\downarrow}{N\uparrow} = e^{-\frac{\Delta E}{kT}} \tag{2.2}$$

with $k$ the Boltzmann’s constant and $T$ the absolute temperature in Kelvin. It is important to note that the thermal energy of the system ($kT$) is $10^5$ times bigger than $\Delta E$, leading to a very small difference between the number of spins in the

1T identifies the quantity tesla that measures the flux of magnetic fields. One T is around 20,000 times bigger than the strength of Earth’s magnetic field on surface.
higher and lower energy states. Furthermore, as depicted in Figure 2.2, spins have a slightly higher probability to be in the parallel alignment that results in a so called spin excess \[^9\]. At body temperature, spin excess is millions of times smaller compared to the total number of protons in the sample \[^9\]. Nonetheless, the fact that in a few grams of biological tissue there are Avogadro’s number of protons, results in a spin excess in the order of $10^{17}$ \[^10\].

In thermal equilibrium, when averaging the magnetic moments of a population of protons, the presence of the spin excess results in a longitudinal magnetisation vector $M_0$, parallel to the direction of the main magnetic field. This is the source of the MR signal and its magnitude is defined in terms of spin excess, density of protons in the tissue $\rho_0$ and Planck’s quantum constant $\hbar$ \[^9\]

$$M_0 = \rho_0 \gamma^2 \hbar^2 \frac{1}{4kT} B_0.$$  \hspace{1cm} (2.3)

Moreover, as pictured in Figure 2.2, $M_0$ has only components parallel to $\vec{B}_0$, conventionally the $\hat{z}$ direction. This is explained by the random phase distribution of the spin precessing at $\omega_0$ that results in the mutual elision of the components in the $x−y$ plane, referred to the transverse plane.

To summarize this section, the presence of the constant magnetic field $\vec{B}_0$ induces the generation of a net longitudinal magnetisation vector $M_0$ with no components in the transverse plane. $M_0$ is the source of the MR signal and its magnitude can be manipulated by changing the external magnetic field gradient.

\[^9\]Avogadro number: $6.2 \times 10^{23}$ atoms per mole of substance.
2.1.2 RF excitation pulse and signal generation

The longitudinal magnetisation vector cannot be detected as it is, since it is shadowed by the much larger external field. To obtain a detectable signal, $M_0$ needs to be tilted away from the $\hat{z}$ direction towards the transverse plane [9]. This is accomplished by a secondary magnetic field $\vec{B}_1$, applied at 90° with respect to $\vec{B}_0$ and oscillating in resonance with the Larmor frequency of the spin. $\vec{B}_1$ is generated by a RF transmitting coil and is characterized by an oscillation frequency and a magnitude. Compared to the main field, $\vec{B}_1$ is much smaller in strength [9, 10]; nonetheless, by matching its oscillation frequency with the precession frequency of the spins, resonance occurs. Resonance allows energy transfer from the RF pulse to the spins, that are tipped away from the $\hat{z}$ direction into the transverse plane. This can be visualized as a force that follows the spin precession, pushing constantly the spin magnetisation moment towards the transverse plane [9]. Resonance phenomena, even if easily described above, is the result of energy conservation and quantisation depending on the properties of the hydrogen nucleus and $B_0$ [12].

Another effect of the application of $\vec{B}_1$ is spins gaining phase coherence [10]. As represented in Figure 2.2, spins are uniformly distributed along the precession path, causing no magnetisation components in the transverse plane. The RF pulse induces spins to lose this uniform distribution causing the increase in magnitude of the magnetisation vector component in the $x - y$ plane [10].

To better visualize the results of the application of $\vec{B}_1$, here we introduce the rotating reference frame. The difference between this and the laboratory frame, as

![Figure 2.3: Comparison between laboratory and rotating reference frames. In the laboratory frame (left), the magnetisation vector $\vec{M}$ follows a spiral-like trajectory towards the transverse plane, resulting from the combined precession of the spins around $\vec{B}_0$ and $\vec{B}_1$. In the rotating reference frame (right), the effect of the precession around $\vec{B}_0$ is cancelled by the rotation of the transverse plane around $\hat{z}$ with angular frequency $\omega_0$. Therefore, only the effect of spins precessing around $\vec{B}_1$ is visible which causes the magnetisation vector to be tilted towards the $x - y$ plane by FA (flip angle). In addition, in the laboratory frame, $\vec{B}_1$ rotates round $\vec{B}_0$ with frequency $\omega_{RF}$ matching $\omega_0$. On the other hand, in the rotating reference frame, $\vec{B}_1$ is fixed since it oscillates with the same angular frequency the transversal plane is rotating. The advantage of using the rotating reference frame is that it provides a better visualisation of the effects the RF pulse has on the magnetisation vector.](image-url)
Figure 2.4: Precession of the magnetisation vector in the transverse plane and FID signal. After the application of the RF pulse, \( M_{xy} \) starts precessing around \( \hat{z} \) with angular frequency \( \gamma B_0 \). This produces an oscillating magnetic field that induces a voltage change in the receiving coil. The acquired signal has a particular shape where the decay trend reflects the relaxation processes of the spins in the tissue.

Presented in Figure 2.3 is that the transverse plane rotates around \( \hat{z} \) with angular frequency matching the Larmor precession of the spins. Thus, in the rotating reference frame, the effects of the precession around \( B_0 \) are elided and it is easier to visualize the manipulation of \( \vec{M} \) \[9, 12\]. This becomes clear when looking at the trajectory of \( \vec{M} \) for the two reference frames. As schematically drawn in Figure 2.3, in the laboratory frame \( \vec{M} \) follows a spiral-like rotation towards the transverse plane. This trajectory, called *nutation*, is the result of the combined precession of the spins around \( \vec{B}_0 \) and \( \vec{B}_1 \) \[10\]. On the other hand, in the rotating reference frame, only the precession around \( \vec{B}_1 \) is visible that describes the falling of \( \vec{M} \) towards the transverse plane \[10\]. Moreover, in this reference system, \( \vec{B}_1 \) resembles a static magnetic field perpendicular to \( \vec{B}_0 \).

The combination of spins gaining phase coherence and the resonance phenomena is a magnetisation vector \( \vec{M} \) having components along the main magnetic field direction, \( M_z \), and in the transverse plane, \( M_{xy} \). The magnitude of these two components depends on the how much \( \vec{M} \) is tipped away from the \( \hat{z} \) direction. This is controlled by the amplitude of \( \vec{B}_1 \) and the duration of its application, \( \tau \), as described by \[9, 10\]

\[
\Delta \theta = \gamma B_1 \tau \tag{2.4}
\]

where \( \Delta \theta \) is called *flip angle* (FA). Manipulation of the flip angle is pivotal in all MR application and is used, not only to generate a detectable signal, but also to obtain different types of contrast \[9\].

After the application of the RF pulse, \( M_{xy} \) starts to precess around \( \vec{B}_0 \) with angular frequency defined by Eq. (2.1). This causes a time varying magnetic flux in the \( x - y \) plane that is measured through a RF receiving coil by the induction of an oscillating voltage potential \[9\]. The measured signal is the so called *free induction decay* (FID) signal, whose characteristic shape is pictured in Figure 2.4. FID decay, reflecting the decrease in magnitude of \( M_{xy} \) over time, is the result of relaxation.
processes that depend on tissue characteristics, and are used to generate contrast in MR images.

2.1.3 Relaxation processes
During the RF pulse application, spins absorb energy as a result of resonance phenomena resulting in the net magnetisation vector to be tipped in the transverse plane. After excitation, protons return to the original state by releasing the acquired energy in the environment [9, 13]. This is a spontaneous process influenced by spin mobility, type of molecule protons are bounded to and atoms/molecules spins are close to. All these intrinsic tissue properties are encoded in the decay of $M_{xy}$ and build-up of $M_z$, and this is one of the reasons MR is such a versatile imaging technique. It is important to stress that MRI is not measuring the energy release of individual hydrogen atoms, but the average relaxation processes of a population of spins, identified by the magnetisation vector and representing a region in the tissue.

There are two phenomena inducing the decay of the transverse magnetisation: spin-lattice and spin-spin relaxation [9]. Spin-lattice relaxation, or longitudinal relaxation, describes the build up of $M_z$ after the application of the RF pulse. Spins releasing the excitation energy in the surrounding environment defines the time needed to return to a thermal equilibrium state where $M_z$ is equal to $M_0$ [10]. In particular, the regain in longitudinal magnetisation follows an exponential trend with time constant $T_1$ dependent on tissue properties (Figure 2.5) [10].

$$M_z(\tau) = M_0(1 - e^{-\tau/T_1})\quad (2.5)$$

where $\tau$ is the time after the application of the RF excitation pulse. In regions where spin mobility is limited, $T_1$ is found to be short, while where free water is present, $T_1$ is much longer [10, 12]. This difference finds explanation when looking at the tumbling rate of the molecules spins are bounded to [12]. In particular, spin relaxation occurs more rapidly when the vibration rate of the molecules containing the spins is close to the precessional frequency $\omega_0$ [12]. Medium size molecules, such as lipids, have vibration rates in the MHz region which leads to a more efficient energy transfer between spins and surround environment [12]. On the other hand, free water molecules in body fluids have a much higher mobility and tumbling rate, increasing the time needed by the spins to return to a thermal equilibrium state.

To collect the data needed for the image generation, during the MRI acquisition multiple 90° excitation pulses are used, spaced out by the repetition time TR [9, 12]. In the time interval between each RF pulse, longitudinal relaxation occurs. However, if TR is too short compared to $T_1$, $M_z$ would not have completely recovered before the next excitation pulse. This follows a decrease in signal intensity since the longitudinal magnetisation available to flip into the transverse plane is reduced [12]. Thus, by changing TR, one can weight the image based on the $T_1$ relaxation properties of the different tissues. For example, short TR will create image contrast between tissue regions rich in fat and free water molecules; due to the short $T_1$ relaxation of lipids, fat regions will appear bright in the MRI image because the longitudinal magnetisation is well recovered. By contrast, short $T_1$ regions will appear dark as a result of the partial build up of $M_z$ [12].
The second type of relaxation, spin-spin or transverse relaxation, describes the magnitude decay of $M_{xy}$ due to loss in spin phase coherence \[13\]. The application of the RF pulse flips the net magnetisation vector in the transverse plane while induces the spins to gain phase coherence. Thus, after the application of $B_1$, spins are precessing with the same phase in the $x - y$ plane. Magnetic field variations in the macro and micro environments induce a change in precession frequency among the spins in the same population that results in the loss of phase coherence \[12\, 13\]. This, in turn, leads to the decay in $M_{xy}$ and signal magnitude. It is important to note that, while transverse relaxation occurs, spins also return to their thermal equilibrium state through $T_1$ relaxation.

Spin-spin relaxation is characterized by two time constants, $T_2$ and $T'_2$, describing irreversible and reversible relaxation processes respectively. $T_2'$ characterizes relaxation phenomena due to macroscopic variations in the magnetic field \[12\]. These inhomogeneities are caused by two factors: external magnetic field non-uniformity and variations in tissue magnetic susceptibility\[^{3}\] \[12\]. The former is easily described by imperfections in the main \[13\]. The latter, on the other hand, reflects a more complicated variation of the field uniformity due to tissue properties. Briefly, in tissue there are regions, such as air cavities or highly vascularized areas, that respond differently to the external magnetic field depending on their magnetic susceptibility \[12\]. This causes spins at the boundary between these regions to experience magnetic field variations and thus dephase faster or slower. In general, macroscopic field inhomogeneity are considered constant in time; thus their effect can be reversed using a so called refocusing pulse \[12\]. This allows the MR signal to be characterized only by $T_2$ relaxation phenomena.

$T_2$ relaxation accounts for the loss in phase coherence due to interactions between spins and the magnetic field of close nuclei \[12\, 13\]. In fact, as described at the beginning of this section, each hydrogen atom has its own small magnetic field. This alters, at a micro scale, the magnetic field experienced by near spins and their precession frequency. Moving spins are affected less by $T_2$ since they experience multiple local magnetic fields whose effects even out \[12\]. This is not true for spins fixed

\[^{3}\text{Magnetic susceptibility: tendency of a material to become magnetized. This is influenced by the population of electrons characterising the molecules and atoms in the material}\] 

\[12\]
on large molecules, such as DNA, where they will experience the same variations in local magnetic field resulting in a fast loss in phase coherence. $T_2$ relaxation is an irreversible process that will always affect the signal decay. Thus, spin-spin relaxation is largely used to generate image contrast in MR, since it reflects tissue micro-environment properties.

Collectively, $T_2$ and $T_2'$ are represented in the FID signal by time constant $T_2^*$, that is defined as

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'}.$$  

(2.6)

How the microscopic and macroscopic magnetic field variations influence the decay in longitudinal magnetisation can be described by

$$M_{xy}(\tau) = M_{xy}(0)e^{-\tau/T_2^*}.$$  

(2.7)

where $M_{xy}(0)$ is the magnetisation vector magnitude immediately after the application of the RF pulse (Figure 2.6). Separation between $T_2'$ and $T_2$ effects from Eq. (2.7) can be difficult, especially when $T_2'$ is predominant. However, considering magnetic field inhomogeneity described by $T_2'$ constant, signal recovery can be performed and $T_2$ identified. This is obtained using a second RF pulse, called refocusing pulse, with flip angle of 180° that reverses the accumulated phase caused by the constant magnetic field inhomogeneities. In the time interval $TE/2$ between the 90° and the 180° RF pulses, where $TE$ is the echo time, spins lose phase coherence due to a combination of $T_2'$ and $T_2$. Following the application of the refocusing pulse, spins regain phase coherence (i.e. rephase) and, after $TE/2$ time, the phase lost due to constant field inhomogeneities ($T_2'$) is recovered. This results in the so called echo signal whose magnitude is reduced compared to the FID signal reflecting the still present un-recovered spin dephase due to irreversible $T_2$ relaxation. Thus, looking at the decay trend of the echo signal, $T_2$ effects on the transverse relaxation are identified. A visual representation of the process is pictured in Figure 2.7.

By changing repetition and echo times, MR image contrast changes based on the tissue properties. A detailed description of the methods to generate contrast in
CHAPTER 2. THEORY

Figure 2.7: Spin Echo sequence. After the $90^\circ$ excitation pulse spins precess in the transverse plane with angular frequency $\omega_0$. Due to magnetic field inhomogeneity, spins in the same population precess at different frequencies. In particular here, spins $a$ and $e$ experience a higher local magnetic field than $c$, $b$ and $d$, which makes $a$ and $e$ to precess faster than the other spins. In time, this results in an increase in phase incoherence. After $\text{TE}/2$ time, a second RF pulse is applied causing spins to flip $180^\circ$ around the $x$ axis in the transverse plane. Thus, spins precessing slower ($c$, $b$ and $d$) are brought in front of the spins precessing faster ($a$ and $e$). By continuing precessing, protons rephase and the echo signal is generated after $\text{TE}$ time the application of the $90^\circ$ RF pulse. The loss in magnitude between the FID and the echo signal is the result of irreversible $T_2$ relaxation.

MR is not the purpose of this work. Briefly, short TR (e.g. 500ms) and short TE (e.g. 20ms) generate a so called $T_1$-weighted image. Here, longitudinal relaxation differences between tissue regions are emphasized, while transversal relaxation is restrained by the short TE. Tissue regions rich in fat are brighter compared with areas where free water molecules are present. Long TR time (e.g. 3000ms) and long TE time (e.g. 100ms) generate a so called $T_2$-weighted image. By timing the RF pulses in this way, spin-spin relaxation differences in the tissue are remarked, with regions where spins are free to move appearing brighter compared to areas where hydrogen atoms are bounded to large, less dynamic, molecules. The last common type of tissue contrast in MR is the proton density weighting. In this modality, TR is long and TE is short and image contrast is given by the differences in proton densities between tissues. A visual comparison between the mentioned modalities is picture in Figure 2.8. It is important to clarify that the MR signal is always the result of a combination between $T_1$-weighting, $T_2$-weighting and proton density weighting. By adjusting TR and TE, one selects which of these has a major impact on the image contrast.

It is worth mentioning here that the MR signal can be made sensitive to tissue features not only by timing the RF pulses. Additional gradients can be employed in
2.1. MRI - HOW DOES IT WORK?

Figure 2.8: Comparison between proton density, $T_1$ and $T_2$ weighted MR images. From http://cchen156.web.engr.illinois.edu/SSMRI.html

the pulse sequence to weight the signal to diffusion for example. This opens a new way of investigating tissue properties and will be the topic of upcoming sections.

Space encoding and image reconstruction

The signal acquired by the receiving coil after the application of the RF pulse is the sum of the magnetisation of all the excited spins. Thus, a method is necessary to discriminate the signal from each tissue region. This is achieved by the application of magnetic field gradients that linearly alter $B_0$ making the precession frequency of the spins dependent on their position [9]. The relation between the angular frequency and the magnetic field gradients is described by

$$\omega(x,t) = \gamma (B_0 + xG(t))$$ (2.8)

where $\omega(x,t)$ is the angular frequency of the spins at position $x$ and time $t$ when affected by the sum of $B_0$ and the gradient $G(x)$. Here the direction $x$ is used, however, any direction can be exploited. A visual representation of Eq. (2.8) is pictured in Figure 2.9. Three coordinates are needed to localise the source of the signal in the tissue. This is the reason spatial encoding is performed using three gradient fields: the slice selection, the frequency encoding (or readout) and the phase encoding gradients.

The slice selection gradient, here identified by $G_z$ and along the $z$ direction, is used in concomitance with the RF pulse as a first stage of the MRI signal localisation. As pictured in Figure 2.9 precession frequency changes along the direction of the applied gradient. Thus, by applying a RF pulse with central frequency matching the desired coordinate along the spatial axes, one can selectively excite the spins [12]. Furthermore, the thickness of the slice can be changed by manipulating the frequency band of the RF pulse or the gradient slope [12]. For example, a broad band RF pulse excites a thicker slice because matches the angular frequency of a wide range of positions along the gradient.

After the application of the slice selection gradient, the magnetic moments of the spins selected by the RF pulse precess with angular frequency $\omega_0$ in the transverse plane. To identify the location of the signal in the slice, two more gradients are
needed. It is useful at this moment to introduce the \( k \)-space. This is a spatial frequency space where each point \((k_x, k_y)\) identifies a particular overall magnetic field gradient and time point. In general \( k \) is defined by \[ k(t) = \frac{\gamma}{2\pi} \int_0^t G(t')dt' \] (2.9) and groups the information about the gradient strength and the duration of its application. The acquired signal is directly mapped in the \( k \)-space and, if enough samples of the space are taken, an image can be reconstructed [9]. Continuous and infinite sampling is not achievable due to the limitation in the hardware, and spin relaxation phenomena that kill the signal shortly after the application of the RF pulse.

The readout gradient, identified here by \( G_x \) in the \( x \) direction, is used during the signal acquisition. It induces a linear change in the angular frequency of the spins in the selected slice. By keeping constant the magnitude of \( G_x \) in time, the collected data is mapped in horizontal lines in \( k \)-space. This can be seen by looking at Eq. (2.9), where the value of \( k_x \) increases in time as \( G_x \) is active. For simple acquisition schemes, every TR a horizontal line in \( k \)-space is filled with the recorded data [10].

The phase encoding gradient, here identified by \( G_y \) in the \( y \) direction, is applied between the slice selection and readout gradients. The working principle of \( G_y \) is similar to the frequency encoding gradient, with the difference being that the time interval \( G_y \) is applied is constant and its magnitude changes every TR. This, looking at the \( k \)-space mapping, shifts the \( k_x \) sampling line along \( k_y \). Again, considering Eq. (2.9) by increasing the value of \( G_y \) and keeping constant its application time, \( k_y \) increases. Negative values of \( k_y \) are obtained for \( G_y < 0 \). Figure 2.10 shows a
2.1. MRI - HOW DOES IT WORK?

Figure 2.10: $k$-space representation. The abscissa maps variations in spin precession frequency caused by the frequency encoding gradient. The vertical axes reflects the application of the phase encoding gradient. By performing a 2D inverse Fourier transform of the $k$-space, the MR image is obtained.

representation of the $k$-space described by $(k_x, k_y)$.

Until now we omitted the analysis of the influence the space encoding gradients have on the magnetisation vectors. This, however, is a pivotal aspect of the signal localisation since it is in the phase differences, caused by the linear change in spin precession frequency, that the location of the signal is encoded. It is important to note that here the discussion is focused on the phase difference between magnetisation vectors, the source of the signal, and not on spin dephasing due to relaxation phenomena. The phase difference is calculated compared to the phase the magnetisation vectors have when no encoding gradients are applied. This phase is denoted by $\phi_0$. The phase accumulated due to the application of a gradient field is defined by [9]

$$\phi_G(x, t) = -\int_0^t \omega_G(x, t')dt' = -\gamma x \int_0^t G(t')dt'$$ (2.10)

where the negative sign is to indicate the clockwise precession of $M_{xy}$ around $B_0$ [9]. From the above equation, the phase acquired by the magnetisation vector depends on the time length it is precessing at a certain frequency. Thus, by manipulating the precession frequency using the spatial encoding gradients, the accumulated phase changes. Looking at the slice selection gradient, its shape and duration is designed to produce zero phase difference. This is because there is no need to include information about the $z$ direction in the spin phase since the RF pulse already selected those in the desired $z$ coordinate. The phase accumulated using the frequency and phase encoding gradients is described by Eq. (2.10) and, if the frequency space variable $k$ is used, this results in

$$\Delta \phi = -2\pi x k_x - 2\pi y k_y = -2\pi (x k_x + y k_y).$$ (2.11)

It is clear that there is a direct relation between the $(x, y)$ position of $M$ in the excited slice and the signal value mapped in $k$-space. In fact, the signal acquired by
the receiving coil and mapped in $k$-space is described by

$$s(k_x, k_y) = \int \int \rho(x, y)e^{-i2\pi(xk_x+yk_y)}dxdy$$  \hspace{1cm} (2.12)

where $\rho(x, y)$ is the signal coming from the tissue in position $(x, y)$. The integration represents the fact that each point in $k$-space contains information about all the spins in the slice. As extensively described in [9], the $k$-space signal representation and $\rho(x, y)$, are Fourier transform pairs. Therefore, one can decode the information about the signal location by applying a 2D inverse Fourier transform on the $k$-space

$$\rho(x, y) = \int \int s(k_x, k_y)e^{i2\pi(xk_x+yk_y)}dk_xdk_y.$$  \hspace{1cm} (2.13)

The time series of RF pulses, spatial encoding gradients and data acquisition is known as pulse sequence [10]. Pulse sequences are designed to exploit different tissue properties by timing the RF pulses, as already described previously when discussing $T_1$ and $T_2$ weighted images. An example of pulse sequence is pictured in Figure 2.11, where the influence of the frequency and phase encoding gradients on the $k$-space variables is described.

As it will be described later, the MR signal can be sensitized to water diffusion

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**Figure 2.11:** MRI pulse sequence components and $k$ space mapping. The slice selection gradient is applied along with the RF pulse. A refocusing pulse is present at the end of the slice selection to remove the accumulated phase. After the slice selection, the phase encoding gradient is turned on for a constant period of time. The magnitude of $G_y$ changes every TR time, identifying a vertical position in $k$-space. The readout gradient is constant in magnitude for all TR times and is applied while the MR signal is sampled (black dots). Three samples, $p1$, $p2$ and $p3$ for two different $G_y$ magnitudes, are selected and mapped in the $k$-space. As can be seen, by increasing the time $G_x$ is on, the value of $k_x$ increases, mapping the sampled points on a longitudinal line from left to right. By changing $G_y$ magnitude, $k_y$ value changes the vertical positioning of the mapping line. Thus, using multiple TR times with changing $G_y$ magnitude, the entire $k$-space can be filled with the MR sampled values.
by including in the pulse sequence additional diffusion gradients. This gives the possibility to study microstructural properties of biological tissues, that today finds the major application in brain imaging studies. Before looking at how diffusion MR works, is useful to understand the complexity of the brain microenvironment this technique tries to investigate. The next section provides a brief overview of the brain microstructure, that will help the reader to better understand the models used to interpret the diffusion MR data.
2.2 Brain microstructure complexity

Neurons are specialized cells of the nervous system that are able to generate and propagate electrical signals used to coordinate body’s functions. Communication between neurons occurs through functional contacts called synapses [14]. Here neurotransmitter molecules are secreted in the synaptic cleft by the pre-synaptic neuron and trigger a response in the post-synaptic neuron. The signal is then integrated and eventually transmitted forward. The anatomy of a neuron, visible in Figure 2.12 characterizes their predisposition to intracellular communication. The cell body contains all the organelles needed by the cell to perform metabolic processes, such as mitochondria for energy production and Golgi apparatus for secretion and packing of proteins. From the cell body, two families of cytoplasmic processes generate: the dendrites and the axon.

Dendrites are arboreal ramifications that collect input signals from neighbouring nerve cells and propagate it to the cell body after integration. A single neuron can have multiple main dendritic ramifications that can branch to receive signals from one to as many as 100,000 synapses [14]. The axon is a single specialized process that propagates the signal from the cell body to the synapse and can extend from a few hundreds of micrometers (µm) to even a meter [14, 15]. Axon diameters can range between less than 0.2µm to 10µm. Functionality of the axon is partially dependent on its characteristic cytoskeleton that provides structure and means for material transport along this elongated processes [14, 16].

Axons are classified according to whether they are myelinated or unmyelinated. Myelin is found in sheaths that surround portions of an axon and are the reason of the white appearance of axon-predominant regions in the central nervous system (CNS) [14]. This membranous structure is rich in proteins and lipids, and is generated in the CNS by the envelope of oligodendrocyte cells around the axon.

![Figure 2.12: Schematic representation of neuron anatomy. Dendritic processes extend radially from the cell body. Here, the signal from nearby neurons is collected and transmitted to the cell body. This contains the nucleus and a range of cellular organelles. The single axon extends from the cell body and propagates the integrated signal to the synaptic terminals. From https://commons.wikimedia.org/wiki/File:Blausen_0657_MultipolarNeuron.png under Creative Commons Attribution 3.0 Unported license.](https://commons.wikimedia.org/wiki/File:Blausen_0657_MultipolarNeuron.png)
2.2. BRAIN MICROSTRUCTURE COMPLEXITY

Myelin is pivotal in allowing fast propagation of the signal along the axon [17] [14]. In fact, the function of myelin sheaths is to increase the speed of signal propagation by insulating portions of the axon membrane, allowing for a so called saltatory propagation. Thanks to myelination, propagation velocity increases from 0.5 – 10 m/s up to 150 m/s, and is consequence of biological evolution that would otherwise only be accomplished by increasing axon diameter [14].

In the CNS, neurons constitute only half of the entire cell population. The remaining ~50% is composed by neuroglia cells, or simply glia [18]. This cell family is not able to propagate electrical signals. However, their function of support is pivotal to the activity of neurons [14]. In fact, glia-glia and neuron-glia communications through chemical signalling is shown to influence neural excitation, moderate synaptic strength, and even take part in the activity of neuron sub-networks by mediating their activity [19].

There are four types of glial cells in the CNS: oligodendrocytes, astrocytes, NG2-positive cells, and microglia [16]. These cells have multiple functions and morphologies. Some show cytoplasmic processes that are less prominent and do not have the same function as in neurons [14] [19]. Oligodendrocytes are the protagonists of axon myelination. One oligodendrocyte, as pictured in Figure 2.13, can myelinate more than one axon thanks to its multiple cytoplasmic processes [16]. Astrocytes, named by their morphology that resembles a star, spread throughout the entire CNS thanks to their fine processes [16] [19]. Functions of astrocytes are multiple: maintenance and regulation of extracellular environment ionic concentration [16], bridge substance transfer between blood and neurons [19] and participate during disease response [15]. NG2-positive cells are the precursors of oligodendrocytes and astrocytes. They are uniformly present in all parts of the CNS with thin and long processes that radiate in all directions [16]. The last category, the microglia, represents the immune cells of the CNS. Their presence in the CNS is heterogeneous depending on the needs of different regions [16]. Using their long processes, microglia cells sense the extracellular environment identifying and removing waste products.

![Figure 2.13: Oligodendrocyte envelops multiple axons. Marked in A are the axons that the oligodendrocyte is depositing myelin. A2–A5 axons are completely myelinated, whereas in A1 myelination is in progress. Scale bar 2µm. Figure from [16] with permission.](image-url)
The result of such cellular diversity, in both type and morphology, is a very complex tissue microstructure (Figure 2.14). It is remarkable that in 1 gram of brain tissue around 57 million cells are present [18]. Cellular organisation of such a complex environment varies between CNS regions; the most notorious difference is between the myelin-rich white matter and gray matter. White matter regions are rich in myelinated axons that extend from the cell bodies in gray matter and connect different regions in the CNS [14] [16]. Gray matter regions, on the other hand, are where the majority of neuron cell bodies and dendritic arborisations are present [14]. In additions, glia cells and their processes also crowd gray matter areas [14] making these CNS regions to be extremely intricate from a microstructural point of view.

Changes in the macrostructure of gray and white matter have been actively studied [21] with insights on multiple causes related to such changes. Aging, dementia and multiple sclerosis are some of the causes that induce quantifiable changes in the volumes and activity of gray and white matter [21]. One important aspect of such investigations is the possibility of conducting them on subjects in vivo. Imaging techniques such as computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI) are suited for such investigations and have been used in the last decades to study brain anatomy and understand the plasticity of the CNS [21]. In vivo microstructure investigations, instead, still face big challenges due to the extreme complexity and variability of the CNS micro-environment. MR diffusion weighted imaging (DWI) is one of the predominant tools used today to probe microstructure [16] [22] [1]. The insight provided by DWI is, however, still confined to regions where white matter is predominant.

To conclude, CNS cellular diversity, organisation and interconnections are the origin of its complexity. Understanding the tissue microstructure and its changes as a result of pathological conditions can reveal fascinating aspects on the plasticity of CNS. Magnetic resonance diffusion weighted imaging is one of the tools able to probe tissue microstructure. The working principles and the applications of this technique are the subject of the next sections.

**Figure 2.14:** Staining of CNS cells. (a) H&E staining while (b) Gold & hematoxylin staining. In both, N are neurons, G are glia cells and Np describes regions of cytoplasmatic processes network of neurons and glia cells. It is clear from these stainings that the CNS microstructure is extremely heterogeneous. Being able to study and picture in vivo such environment is a not trivial. Magnification X200 From [20] with permission.
2.3 Diffusion MRI

Diffusion is a mass-transport process that does not require bulk motion. At a molecular level, it is described by the collision of atoms or molecules that are in liquid or gas state [2]. Diffusion was firstly observed and reported by Robert Brown in 1828, when he investigated the random motion of pollen particles suspended in water. Later, he understood that the observed phenomenon was related to the random motion of the water molecules the pollen was immersed into. In fact, above the absolute zero temperature all molecules, in gas or liquid phase, undergo a random thermal motion [23]. A classical description of diffusion was given by Fick’s first law in 1855, where the net flux of particles is related to the diffusion coefficient \( D \) of the medium and a concentration gradient \( \nabla C \) [23].

\[
\mathbf{J} = -D \nabla C
\]  

(2.14)

This implies that a gradient is needed for atoms or molecules to move. Nowadays, however, atoms and molecules are known to always be subjected to thermal motion, which randomness is the cause of zero net flux when no gradient is present [15]. Half a century later, Albert Einstein described the displacement of particles under diffusion using a statistical approach. He considered a large population of particles able to move without restrictions in a 3D space and related their mean displacement to the diffusion coefficient of the medium and the time interval diffusion was observed, hereafter diffusion time. This relation, known as Einstein’s equation, describes the free diffusion process and is [15]

\[
\langle r^2 \rangle = 2nDt
\]  

(2.15)

where \( \langle r^2 \rangle \) is the mean-squared displacement of the particles, \( t \) is the diffusion time, \( D \) is the diffusion coefficient and \( n \) scales the equation based on the dimensionality of the measurement.

If we look at the one dimensional case of Eq. (2.15) (i.e. \( n = 1 \)), the displacement follows a Gaussian distribution with mean zero and standard deviation dependent on the diffusion coefficient [15] (Figure 2.15). The diffusion coefficient reflects intrinsic physical characteristics of the medium and its value is determined by molecule/atom size, temperature and the microstructure of the environment where diffusion is taking place [23]. For example, free water at body temperature has a diffusion coefficient of roughly \( 3 \times 10^{-3} \) mm\(^2\)/s [23]. If the random movement of water is hindered or restricted by cellular or extracellular structures and molecules, the displacement distribution and the diffusion coefficient change. Thus, if one can measure these changes and relate them to variability in the environment diffusion is taking place, then one can infer microstructure properties of such environment [15].

It is important to stress at this point that MR is not able to measure directly the diffusion coefficient of the water molecules in the tissue. In fact, the diffusion coefficient is calculated based on displacement of the water molecules observed in a period of time. In addition, the complexity of the diffusion processes that occur at cellular level are summarized in a voxel scale representation in the MR signal. Thus, what MR measures is an apparent diffusion coefficient (ADC) that is the result of an indirect and average measurement [15, 24]. Nevertheless, ADC groups information
Figure 2.15: Water diffusion. (left) The displacement of a free diffusion process is described by a Gaussian distribution, with standard deviation depending on the diffusion coefficient of the medium. (right) Water diffusion in the tissue is hindered and restricted by the geometrical features of the micro-environment. The blue lines (A) represent hindered diffusion, where water molecules move around cells and other molecules in the extracellular space. Hindered diffusion is considered to be Gaussian, however the diffusion coefficient is lower compared with the one of a free diffusion process. Red lines (C) represent restricted diffusion, where water movement is limited by the cellular membrane (B). Diffusion here is non-Gaussian since the molecules hitting the boundary are forced to change path and thus the displacement distribution changes its properties [15].

about the features of the tissue micro-environment, breaking the spatial resolution of traditional MRI by condensing cellular-level information in one pixel/voxel. This is what makes diffusion MRI (dMRI) a powerful tool for the investigation of microscopic characteristics of in vivo tissues.

The next sections will describe how MR pulse sequences are modified to obtain diffusion-weighted images and how the diffusion information is exploited to describe brain tissue microstructure.

2.3.1 Diffusion pulse-sequence

Considering a standard spin-echo sequence, the MR signal is made sensitive to water diffusion by introducing two additional gradient pulses. This was proposed for the first time by Stejskal and Tanner in 1965, that modified the concept and the theoretical framework introduced by Carr and Purcell in 1954, where a single diffusion gradient pulse was used to probe water motion [15]. In the pulsed-gradient spin-echo sequence designed by Stejskal and Tanner [3], visible in Figure 2.16, the diffusion gradients are applied one before and one after the 180° RF refocusing pulse. The pulse duration, $\delta$, identifies the diffusion encoding time. During this time interval, spins accumulate phase difference, as described by Eq. (2.10), depending on their position [15]. The pulse separation, $\Delta$ or diffusion time, is the time length during which diffusion is probed [15]. These two timing parameters have a pivotal role in the definition of how sensitive the MR signal is to diffusion and what microstructural features of the environment are probed.

A particular case of the Stejskal and Tanner pulse sequence is useful to under-
2.3. DIFFUSION MRI

Figure 2.16: Stejskal and Tanner spin-echo-pulse sequence and its effects on moving water molecules. Two gradient pulses, with duration $\delta$ and separated by $\Delta$, are applied one before and one after the 180° RF pulse. The effect of the application of the diffusion gradient $G_d$ is pictured for two different spins. Both spins acquire a certain amount of phase after the application of the first pulse. In the static spin, this amount will be cancelled out by the second diffusion pulse. However, this does not happen for the moving spin, since its position along the gradient will be different when the second pulse is applied. Thus, a phase difference is gained by the moving spin, resulting in a faster decay of the MR signal. How fast depends on the dephase degree, that is conditioned by the mobility of the water molecules.

Understand how the MR signal is sensitized to diffusion. This is the narrow pulse regime, where the pulse duration is short and no diffusion is considered to take place during the diffusion gradient application [15]. Under this assumption, the phase change introduced by the first pulse gradient is

$$\phi_1 = -\gamma G \delta x_1 \quad (2.16)$$

where $x_1$ is the position of the water molecule when the first diffusion gradient pulse is applied. It is useful at this point to introduce $q$ with units 1/length, that groups the information about the diffusion gradient and the diffusion encoding time:

$$q = \gamma G \delta. \quad (2.17)$$

Thus $\phi_1$ can be written as

$$\phi_1 = -qx_1 \quad (2.18)$$

Similarly, the second pulse introduces a phase change given by

$$\phi_2 = -\gamma G \delta x_2 = -qx_2 \quad (2.19)$$

depending on the spins’ position when the second diffusion pulse is applied. The intermediate 180° refocusing pulse removes spin dephasing caused by reversible processes. Thus, the total phase change induced by the diffusion gradients is

$$\phi_2 - \phi_1 = -q(x_2 - x_1) \quad (2.20)$$

$^4$Here the diffusion gradient direction is omitted from the definition of $q$. However, in a more general case $\vec{q} = \gamma \vec{G} \delta$
dependent on the position $x_2$ of the water molecule when the diffusion gradients are applied. If protons do not move during the diffusion time, $\phi_2 = \phi_1$ and no net dephasing due to motion is present. However, when spins move, the phase changes acquired before and after the refocusing pulse do not cancel out, increasing the dephasing of the spins. Thus, the signal resulting from the sum of the magnetisation vectors is reduced, with reduction depending on the mobility of water in the microenvironment \[15\].

The MR signal solely due to diffusion, $E(q)$, is obtained by taking the ratio between the diffusion attenuated signal and the one with diffusion gradients equal to zero \[15\]. Under unrestricted diffusion conditions, i.e. free diffusion regime, $E(q)$ is described by a mono-exponential decay function

$$E(q) = e^{-q^2D(\Delta - \delta/3)} = e^{-bD} \quad (2.21)$$

where $b$, with typical units s/mm$^2$, describes the sensibility of the MR signal to diffusion and is defined as \[15\]

$$b = \gamma^2G^2\delta^2(\Delta - \delta/3) = q^2(\Delta - \delta/3). \quad (2.22)$$

For high $b$ values, effects of water diffusion are accentuated in the MR signal. From the above definition, high $b$ values can be obtained by increasing diffusion gradient strength or diffusion time. Both methods result in a higher degree of spin phase difference caused by motion; however the information encoded in the diffusion signal is different. Strong diffusion gradient induces a sharp change in phase depending on the spin position. Thus, even low diffusion rates are measurable. High diffusion time, on the other hand, allows diffusion motion to be more susceptible to the environment features due to the increase in the particles mean-displacement.

Recalling that diffusion is a 3D process, water molecules are able to move in all directions with degree of freedom depending on the obstacles and restrictions present in the environment. To capture the tridimensionality of the phenomenon, diffusion is probed in different directions, by changing the orientation of the diffusion gradients in the acquisition pulse sequence. The measurements from the acquisitions in different directions are grouped in a tensor representation of ADC \[23\], from which different metrics are extracted describing characteristics of the environment diffusion is taking place.

### 2.3.2 Modelling the diffusion MR signal

The ability of diffusion MR acquisition to probe microstructural features of the brain tissue became clear at the beginning of 1990, when multiple works \[25\, 26\, 27\] directly related changes and features of the brain tissue to variations in the diffusion signal. It became also evident that not only tissue organisation, such as axons packing in white matter tracts, but also the components, as organelles, cellular membrane and other structural molecules, influence water mobility \[13\]. Different models are proposed to connect the plethora of tissue microstructural features with the dMR signal, and generate contrast in the MR images \[1\]. The major challenge faced by
all models is relating model parameters to single and unique tissue morphology and characteristics. Not being able to do so increases the uncertainty in the interpretation of the diffusion data [24].

One of the applications of dMRI is diffusion tensor imaging, or DTI. This methodology provides a framework for data acquisition, analysis and quantification of diffusion anisotropy in the brain tissue particularly suited to study white matter [28]. Here, the tensor representation of ADC is exploited to compute different metrics reflecting the preferential direction of water diffusion. One of such parameters is the fractional anisotropy (FA) that describes the anisotropy of diffusion at a voxel level, based on the eigenvectors and eigenvalues of the ADC tensor [28]. 2D images using FA as contrast (Figure 2.17) are useful to identify white matter in the brain. In addition, FA is used as a biomarker for the identification of diseases related to white matter fibre degeneration. A reduction in FA is seen in brain regions where white matter fibres are losing myelin, in disease conditions such as multiple-sclerosis [28]. Even if a direct relation between the loss in myelin and the decrease in FA seems natural, many studies have shown that diffusion anisotropy is not only dependent on axon myelination [28]. In fact, FA is reported to be similar in both myelinated and un-myelinated neurons in normal brain tissue. Nevertheless, even if the precise cause is still unknown, changes in diffusion anisotropy pictured by FA are useful in the identification of neurodegenerative disease.

DTI is also used for 3D fibre tractography, where the information about the preferential direction of water diffusion from consecutive voxels is used to track white matter fibre bundles in brain (Figure 2.17) [15, 28]. Using tractography, the connectivity between different brain regions is mapped and visualized. In clinics, for example, this technique proves useful in the definition of the surgical procedure for brain tumour removal. By knowing how white matter bundles are dislocated by the tumour, damage of important fibre tracts can be avoided, reducing post-operative trauma and recovery time. In research applications, 3D tractography is used to map brain connectivity and understand, in combination with other MR methodologies (e.g. functional MRI) how brain circuits work [29].

Applications of fibre tractography and FA are limited by the assumptions of the DTI model. In diffusion tensor imaging water diffusion is assumed to be Gaussian; however the fact that water motion is somehow restricted by features of the tissue microenvironment makes the displacement distribution of water motion non-Gaussian [28]. In addition to this, DTI assumes that the diffusion properties of a single voxel can be summarized by one diffusion tensor. Yet, as described in the previous section, each small volume of brain tissue contains a very large number of neurites and glial cells. Thus, such variation in cell organisation, dimension and shape is implausible to be sufficiently described by a single tensor representation [28]. These limitations introduce errors in the interpretation of the data. For example, when two crossing fibres are present in the same voxel volume, DTI returns low diffusion anisotropy as a result of different fibre orientation [28]. This problem, identified as partial volume effect, reduces the performances of fibre tracking and makes the values of FA to be less reliable. Many research efforts are still ongoing trying to deal with the partial volume problem and the Gaussian diffusion assumption in the DTI model.
Figure 2.17: Fractional anisotropy and fibre tractography using DTI. (left) MR image using FA as contrast. Bright areas (e.g. white matter tracts) indicate brain regions where water motion has a distinct preferential direction. In regions where diffusion is more isotropic, FA values are lower, such as in cortical regions of the brain. Note that, due to the assumptions of the DTI model, low FA can also be found in regions where crossing fibres are present. (right) 3D fibre tracking using DTI.

Another class of models that try to describe the diffusion MR data are biophysical models. These are based on a compartment representation of the tissue microstructure, and describe variations in the diffusion signal in terms of compartment shape, dimension and orientation [1]. Biophysical models use multiple b values and diffusion gradient directions to probe diffusion; this way of collecting diffusion data, allows the identification of decay trends in the signal that are thought to describe hindered and restricted diffusion in the compartmental description of the tissue microstructure [1]. Moreover, changes in the decay trends are used to characterize tissue organisation features, such as fibre orientation and diameter distribution. A wide variety of representations for the intra and extra axonal spaces are proposed [1], and some are pictured in Figure 2.18.

Relevant to this study is the "ball and stick" model suggested by [30], where

Figure 2.18: Biophysical models picture tissue microstructure features using a compartmental description. The intra-axonal space is described using elongated shapes, such as sticks and cylinders, with different density packing and orientations. The extracellular environment and glia cells are commonly described using isotropic-like geometries, to reflect the hindered Gaussian diffusion in these regions. Figure from [1] with permission.

restricted and hindered water motion is described using a two compartment representation of the tissue microstructure. [5] attributes restricted diffusion to the population of neural processes (axons and dendrites), while hindered diffusion to the extracellular space and glia cells. Their modelling is based on three experimental observations, namely (1) the diffusion signal acquired in white and gray matter
regions is not mono-exponential, suggesting a compartment structure of the neural tissue; (2) the ADC is time independent when observing diffusion for a time interval larger than 20ms, indicating that compartment dimension is too big to be mapped by water motion in the $\Delta$ time interval, or too small to contribute to the measured diffusion coefficient; (3) water diffusion across the cellular membrane is highly dependent on the presence of aquaporin proteins, and in particular on aquaporin-4 in the CNS. The expression of this transport protein is exclusive in glia cells, suggesting limited water diffusion across axonal membrane.

Neural processes are modelled as long cylindrical projections with diameter much smaller compared to their length (Figure 2.19). Diffusion in these anisotropic structures is represented by two coefficients, $D_{\parallel} > D_{\perp}$, describing water motion along the length of the projection and across it respectively. The signal arising from a population of such cylinders, with specific density and orientation distribution, is the restricted component of the diffusion signal. The hindered part, on the other hand, is associated with the extracellular environment and glia cells. Here, water molecules can move freely and diffusion is considered to be Gaussian. Moreover, assuming that during the time diffusion is probed, water molecules are able to move between and across glia cells, the diffusion properties of the non-cylindrical regions can be described by isotropic structures, characterized by one diffusion coefficient, $D_{\text{eff}}$. Using this two compartment description, the diffusion signal is modelled as the combination of a hindered component and a restricted component, weighted by their volume fraction in the neural tissue. By fitting the model to the diffusion data, were able to estimate $D_{\text{eff}}$, $D_{\parallel}$, $D_{\perp}$ and $v$, the volume fraction of neural projections.

The power of such model is the ability to directly relate model parameters to important features of tissue microstructure. It is, yet, limited by its simplistic description of the microenvironment. For example, white matter regions are purely represented since the extracellular space around white matter fibres resembles more a cylinder rather than an isotropic sphere, in violation of one of the assumptions. Moreover, the signal originated from white and gray matter projections is considered to be the same, since originating from similar cylindrical structures. However, as later observed by [6], white and gray matter regions express different decay trends. Thus, there are microstructural differences between white and gray matter projections that the stick and ball model does not capture.

Figure 2.19: Stick model representation of a stained rat neurite. Axons and dendrites are replaced by impermeable cylinders in the model. Neurite Figure from http://cellimagelibrary.org/images/807 DOI 10.7295/W9CIL807
The purpose of acquiring diffusion data using different gradient directions is to remove the influence of the ensemble organisation from the observation, allowing the observer to gain information about the elements of the ensemble, without being biased by the overall organisation. To accomplish this, one can disaggregate the organisation of the ensemble by creating a powder of the sample. This is a common practice in solid-state nuclear magnetic resonance (NMR), where powders are used to study the structural properties of crystals, for example. The problem with biological tissues, and especially in vivo studies, is that the sample cannot be made powder. Thus, to obtain a similar result, in dMRI the sample is probed from different directions by changing the orientation of the diffusion gradient. Then, the observations are averaged, resulting in a diffusion signal that resembles the one coming from a powder of the specimen.

The purpose of this work is to experimentally validate a possible improvement of the above model, that accounts for neurite curvature in order to describe the differences between white and gray matter neural projections. This work exploits the orientationally-averaged diffusion signal obtained from multi-shell diffusion acquisition to study how the curvature of neural projections influences the decay trends in the signal. The orientational or powder average signal, is obtained by averaging the signal acquired at one \( b \)-value and with multiple diffusion gradient directions. As extensively described by [31], the direction of the diffusion gradient has a considerable impact on the acquired signal. One way of removing the directional dependence is to collect data from a multitude of directions and average them. The results of such acquisition method resemble an isotropic distribution of all the structures (i.e. compartments) the specimen under investigation is made of (Figure 2.20) [32].

### 2.3.3 Neurite curvature

The stick and ball model proposed by [5] suggests that the powder-averaged diffusion signal from white and gray matter regions exhibits a power law decay in the form of \( \propto q^c \) with exponent equal to -1. This has been experimentally observed in white matter. However, as reported by [6], in gray-matter regions a faster decay is noticed, with \( c \) being \( \approx -1.8 \). This discrepancy could be caused by many factors, that the model does not consider. For example, changes in membrane water permeability between white and gray matter projections [6], and the higher number of cell bodies present in gray matter [7]. Another explanation is proposed by [8], where the role of neural projections curvature on the diffusion signal is used to describe
2.3. DIFFUSION MRI

the differences in signal decay.

In particular, [8] looks at the powder average signal rising from a population of curved neural projections, that are represented by axisymmetric cylindrical compartments where diffusion is taking place. The reason the powder average, \( \bar{E}(q) \), is used in [8] is that here the interest is in the features of the cylinders and not on their spatial organisation.

Under the assumption of \( q \equiv |\vec{q}| \) small enough to consider water diffusion in the cylinder quasi-Gaussian, the diffusion signal for a single cylindrical compartment is approximated by [8]

\[
E(\vec{q}) \approx e^{-TV(B)D} = e^{-(\vec{q})^2(\Delta-\delta/3)D} = e^{-\vec{q}^T V \vec{q}}
\]  
(2.23)

where \( V \), referred to in [8] as the signal decay tensor, captures all the time dependencies. The analytical solution of the orientationally-averaged signal for a population of axisymmetric projections each described using Eq. (2.23) is given by [8]

\[
\bar{E}(q) = \sqrt{\pi} e^{-q^2V_\perp} \text{erf}(q\sqrt{V_\parallel - V_\perp})
\]

where \( V_\parallel \) and \( V_\perp \) are similar to the apparent diffusion component \( D_\parallel \) and \( D_\perp \). If diffusion is limited to occur only along the projection (i.e. \( V_\perp = 0 \), Eq. (2.24) predicts a power law decay, in the form \( \propto q^c \) with exponent -1. The above analytical solution is, however, limited to the case where diffusion is considered quasi-Gaussian in the restricted cylinder compartment. When this is not true because diffusion is observed differently (i.e. \( q \equiv |\vec{q}| \) is large) Eq. (2.24) is not valid and the definition of the powder average signal is not trivial. However, [8] considered large \( q \)-values and provided an analytical solution for the case.

To study the influence of curvature, [8] parametrized the geometrical features of the neural projection using four variables: projection length \( l \), radius of the curvature \( R_c \), radius of gyration \( R_g \) and radius of the projection. A schematic representation of the cylinder parametrisation is presented in Figure 2.21a. The influence of neurite geometry is studied in three different diffusion regimes, correlating the timing parameters of the Stejskal and Tanner pulse sequence with the parametrisation of the projections. For this work, the short diffusion time regime is most significant, where the mean-diffusion length \( \sqrt{D\Delta} \) is smaller or equal to \( R_c \), while the pulse duration is studied for two conditions, short and long \( \delta \).

In the short pulse duration case, \( \sqrt{D\delta} \ll R_c \), water molecules while experiencing the diffusion gradient, are not influenced by the curvature of the neural projection. Under these conditions, [8] theoretically proved that the powder average signal decays with exponent close to -1 in the case of straight and curvy projection, if the \( q \)-value is large. On the other hand, when longer pulse durations are used (i.e. \( \sqrt{D\delta} \approx R_c \), curvature effects influence the powder average signal and a faster decay is visible. In Figure 2.21b, the influence of \( \delta \) on the orientationally-averaged signal is presented. Moreover, Figure 2.22 tries to visually describe how the short pulse duration condition influences what is probed with diffusion.
CHAPTER 2. THEORY

Figure 2.21: (a) Parametrisation of the neurite curvature. The length $l$, the radius of the cylindrical projection $R_0$ and the radius of gyration $R_g$ are global parameters. The radius of gyration gives a description of how disperse the projection is. The radius of curvature $R_c$, on the other hand, is a local parameter, describing how curvy the projection is in each point. (b) By increasing the pulse duration, more and more curvature effects are introduced in the powder signal. This reflects in a faster decay of the orientationally-averaged signal. Figures form [8].

From the above, it is clear that by changing the pulse duration, one can introduce curvature effects in the diffusion signal. In conventional MR scanners, the smallest achievable pulse duration does not fulfill $\sqrt{D\delta} \ll R_c$ and thus, the powder signal from regions with curved neural projections present a faster signal decay. This is coherent with the results of [6], where a power law with exponent $\approx -1.8$ was observed in gray matter while in white matter regions, where neural projects are fairly straight at a voxel level, an exponent close to -1 was visible.

The purpose of this work is to experimentally investigate the influence of neurite curvature on the powder average signal in gray matter regions in the short diffusion time regime ($\sqrt{D\Delta} \leq R_c$) and for small pulse duration ($\sqrt{D\delta} \ll R_c$). Moreover, the large $q$-value regime is also considered to investigate the behaviour of the powder-averaged signal for a wide spectra of diffusion sensitivity values.
2.4. NOISE CHARACTERISTICS

Figure 2.22: Short diffusion time regime for the short (right) and long (left) diffusion pulse duration. By setting $\sqrt{D\Delta} \leq R_c$, diffusion is probed while water molecules are moving in a portion of the curvy neurite projection (light gray boxes). Choosing the diffusion pulse duration (dark gray boxes), the distance water molecules travel while experiencing the diffusion gradient changes. For short $\delta$ (i.e. $\sqrt{D\delta} \ll R_c$), the neural projection is seen as straight. However, when the pulse duration is increased, water can move along the curved projection making the diffusion signal sensitive to the 3D features of the projection, that are represented by a faster decay of the powder average signal.

2.4 Noise characteristics

The MRI signal is corrupted by noise which depends on the experimental setup; higher the spatial or temporal resolution one wants to achieve, higher the level of noise in the acquisition [33]. In diffusion-weighted MR applications, when high diffusion sensitivities are used, the signal to noise ratio (SNR) can be very small, limiting the usability of the acquired data. For this reason, methods for noise handling are a pivotal part of data analysis. This work uses the implementation for the noise correction provided by [34] based on the studies of [35, 33]. In the following sections, a brief description of the noise characteristics in magnitude MRI signal is provided, and how [35, 33] use this information to estimate noise characteristics and correct for them.

The MRI signal is a complex number, where real and imaginary parts are collected separately using quadrature detection. Most MRI applications use the magnitude value of the complex number, which is less sensitive to experimental conditions [33]. The imaginary and real parts of the MR signal are Gaussian distributed, since each in-quadrature RF detector is corrupted by Gaussian distributed noise [33]. The combination of the independent Gaussian distributions in the magnitude signal results in a nonCentral Chi distribution [33]. When the number of channels used to collect the data is one (i.e. two in quadrature RF coils), the nonCentral Chi distribution reduces to a Rician distribution. This distribution can be defined in terms of the standard deviation, $\sigma_g$, of the independent Gaussian distributions describing the real and imaginary signal parts, and the signal intensity, $\eta$ (hereafter underlying signal intensity), that is shadowed by the noise.

In [35] an iterative process is presented for the estimation of $\sigma_g$, where the structure of the diffusion signal and knowledge about the statistics of the Rician distribu-
tion are exploited. Briefly, an initial discrimination between noise-only and noisy pixels is done using the probability distribution function (PDF) of the Rician distribution when the underlying signal is set to zero. From the identified noise-only pixels, the standard deviation of the Gaussian distribution is estimated [35], and used to improve the classification between pixels containing noisy signal and only noise. By iterating the process, the probabilistic identification and estimation of noise (PIESNO) framework proposed by [35] converges to a value of $\sigma_g$ that describes the Gaussian distributions the real and imaginary parts of the MR signal are drawn from.

Using the Gaussian standard deviation, [33] describes a signal transformation framework (hereafter noise-break framework) that converts the magnitude signal from a Rician distribution to a Gaussian distribution. The method is, once again, based on the PDF of the Rician distribution. However, here the underlying signal intensity is not set to zero, but estimated through spline estimation. This provides a representation of how the signal values should look like based on the acquisition parameters. The signal values are then mapped from a Rician to a Gaussian distribution using the estimated $\sigma_g$ and $\eta$.

It is important to note that this methodology is not removing noise. In fact, the noisy signal is only converted from one distribution to another; this is very important because helps in reducing the Rician bias that is a critical aspect in this work. Moreover, having the noisy signal Gaussian distributed allows for simple noise reduction methods, such as averaging. This method for noise reduction was successfully employed in [36], where the Rician bias was removed from the diffusion signal acquired on rat hippocampus to obtain usable data even when the signal intensity was below the noise floor.

---

5 The details of the procedure are far beyond the scope of this work.
## Methods

### 3.1 Data acquisition

Diffusion weighted images using multi-shell acquisitions were acquired on fixed rat spinal cord stored in 4% formaldehyde and, before acquisition, washed with buffered saline solution for 24 hours. The sample was then moved into a 5mm NMR glass tube filled with fluorine solution. Diffusion acquisitions were performed on a 17.6T vertical-bore Bruker Avance III HD pre-clinical scanner (Bruker BioSpin, Billerica, MA, USA) using a RF coil with 5mm in diameter. Temperature was monitored during the acquisition.

Multi-shell diffusion acquisitions were obtained using a spin-echo diffusion-weighted pulse sequence. Four acquisitions were performed probing three different q-value ranges: $[450, 970]\text{mm}^{-1}$, $[600, 1400]\text{mm}^{-1}$ and $[1500, 1750]\text{mm}^{-1}$. The choice of the first q-value interval was based on simulation results, whereas the last two ranges were chosen after having processed the data from the first acquisition. The imaging parameters remaining constant for all acquisitions were: $TR = 2000\text{ms}$, $\Delta = 17\text{ms}$, bandwidth = 100kHz, matrix size = 64 x 64, voxel size = 100 x 100 x 1500 $\mu\text{m}^3$, number of slices = 2 and number of averages = 4. A description of the shell configuration, TE, δ and q-range for each of the four acquisitions is presented in Table 3.1.

The diffusion gradient directions used for the acquisition of the diffusion data and

<table>
<thead>
<tr>
<th>Acquisition</th>
<th>q-range [mm$^{-1}$]</th>
<th>TE [ms]</th>
<th>δ [ms]</th>
<th>n. of shells</th>
<th>shell configuration [directions]</th>
<th>acquisition time [h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>[450, 970]</td>
<td>23</td>
<td>1.4</td>
<td>10</td>
<td>30, 30, 46, 46, 46, 61, 61, 61, 61, 102</td>
<td>74.5</td>
</tr>
<tr>
<td>D2</td>
<td>[600, 1400]</td>
<td>23</td>
<td>2</td>
<td>12</td>
<td>10 each shell</td>
<td>17</td>
</tr>
<tr>
<td>D3</td>
<td>[450, 970]</td>
<td>23</td>
<td>1.4</td>
<td>10</td>
<td>10 each shell</td>
<td>14</td>
</tr>
<tr>
<td>D4</td>
<td>[1500, 1750]</td>
<td>23.5</td>
<td>2.5</td>
<td>5</td>
<td>10 each shell</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 3.1:** Variable acquisition settings for the four experiments. The q-range in all the acquisitions is uniformly sampled based on the number of shells used in the particular acquisition.

The simulations probe half of the q-space identified by a specific q-value. Moreover, the direction sets using 10, 16, 21, 30, 46, 61 and 102 direction are provided by [37]. These sampling directions, along with the weights computed during the orientational average procedure described later, provide a quasi-isotropic sampling of q-space. The directions sets containing 11 to 200 directions (with the exclusion of the one provided by [37]) were obtained using an electromagnetic repulsion algorithm. This minimizes the repulsion energy of a system of charged particles bounded to move on a half surface sphere. The number of charged particles used in the minimisation problem matches the number of directions one requires.

$^1$The sample used for this investigation was obtained from a different study, thus no animal was sacrificed solely for this investigation. The IACUC Protocol associated with the fixed cord is #201803934.
3.2 Data analysis pipeline

For a better understanding of the data analysis pipeline, it is worth describing the structure of the acquired data (Figure 3.1b). Each acquired slice is represented by a 3D dataset where the \((x,y)\) coordinates describe the position in the slice, while the \(z\) coordinate indexes \(\vec{q}\) used during the acquisition, defined by a unique combination of \(q\)-value and gradient direction.

The reconstructed noisy data returned from the magnet software was processed in the Matlab and Java environments. The data analysis pipeline, schematically described in Figure 3.1a, is a six step process that (1) organises the raw data and acquisition parameters in a data structure (\texttt{strData}), (2) controls data reconstruction from \(k\)-space to the spatial domain by computing the number of acquisition channels (\(nCh\)), (3) estimates the signal Gaussian standard deviation (\texttt{estSD}), (4) performs noise correction, (5) computes the weighted orientational average and (6) fits the processed data to a power-law. The following sections describe each processing step providing what the inputs and the resulting outputs are. Note that the data processing is described for a single slice, with multiple slices analyses through an iterative process.

Data organization

The analysis starts with the organization of the diffusion data and the acquisition parameters in a data structure (hereafter \texttt{strData}). In particular, in \texttt{strData} are saved the shell configuration (number of shells and number of directions for each shell), the \(q\)-values, the diffusion gradient directions, the diffusion timing parameters (\(\delta\) and \(\Delta\)) and the raw data of the acquisition. Additional fields for \(nCh\), \texttt{estSD} and \text{SNR} are also allocated in \texttt{strData} and updated through the analysis. The choice of grouping all the data and the information about it in one single data structure was

![Processing pipeline and structure of the diffusion data.](image)

Figure 3.1: Processing pipeline and structure of the diffusion data. (a) The collected data is initially saved in a data structure along with the acquisition settings. Then, \(nCh\) is estimated and used in the third step for the computation of \texttt{estSD}. Subsequently, the noise-break framework converts the Rician distributed noisy signal in a Gaussian distributed one. The orientationally-averaged signal is then computed and the obtained points are fitted to a power law in the form \(q^c\). (b) Using multi-shell acquisitions, each sampled slice is described by a 3D structure where the \(z\) coordinate identifies the data obtained with a combination of \(q\)-value and diffusion gradient direction. All the acquisitions for a specific shell (i.e. \(q\)-value) are grouped together and are identified by a range of \(z\) coordinates.
3.2. DATA ANALYSIS PIPELINE

inspired by the way MRI data is stored. Moreover, having all the information in one place is convenient when feeding the data to the processing functions.

**Computation of nCh to control signal reconstruction**

The input for the estimation of nCh is `strData`. Initially, a diffusion image from the raw data is presented to the user that iteratively selects an only-noise region in the image. The $(x,y)$ coordinates of the selected region are then used to select the only-noise regions in all the diffusion images acquired at different $\vec{q}$. The mean and standard deviation are computed for the collective noise-only region in the 3D raw data. Then, the ratio between the mean and the standard deviation values is compared with the metric in [38] and nCh is identified and saved in `strData`.

This initial processing step is pivotal since the estimation of nCh checks if the reconstruction performed by the scanner software altered the characteristics of the noise while processing the raw $k$-space data. Reconstruction did not change the signal noise characteristics if the estimated number of channels is coherent with the one really used during the acquisition (1 in this work).

**Estimation of estSD**

Using the updated `strData`, the Gaussian standard deviation of the signal is computed using the Java package provided by [34] that implements the PIESNO framework described in [35]. The PIESNO processing steps performed by the Java package are schematically described in Figure 3.2, where the diffusion data from each slice is used to discriminate between noise-only and noisy pixels, and compute estSD. A Matlab function was coded to initialize the required inputs and call the PIESNO

![Figure 3.2](image)

**Figure 3.2:** PIESNO (Probabilistic Identification and Estimation of Noise) estimates the standard deviation (estSD) of the Gaussian distribution that describes the Gaussian noise corrupting the receiving RF coils [35]. The method identifies the pixels only containing noise by looking at the signal distribution, for each pixel independently, obtained at different experimental settings (i.e. $q$-values and directions). The input parameter alpha allows to define a threshold that controls how strict the classification between noise-only and noisy pixels is; lower the alpha value, the easier for a pixel to be classified as containing only noise. estSD is then computed using the noise-only identified pixels. The algorithm is iterative, where the estimation of estSD in one iteration is used in the following to improve pixel classification.
Java functions. The inputs for the PIESNO framework are the raw diffusion data, nCh and the alpha parameter. alpha allows to control how strict is the classification between noise-only and noisy pixels, with higher values making more difficult for a noise-only pixel to be classified as noisy one [35]. The default value for alpha is $10^{-4}$. Here, alpha was set to $10^{-7}$ since this value allowed for a visually better identification of the noisy pixels containing signal.

The output of the PIESNO framework is estSD along with a binary mask that selects the pixels containing signal (i.e. noisy pixels). The mask was used to define the region in the diffusion data to process during the noise correction step.

**Noise correction**

The noise-break framework uses strData and processes each pixel and shell independently, returning the corrected version of the diffusion data. It uses the Java functions provided by [34] that implement the work of [33]. A diagram of the steps performed by the noise correction is pictured in Figure 3.3. The inputs for the Java functions are the raw data for each pixel, the gradient directions used during the acquisition, nCh, estSD, lmax and alpha. The first four inputs are saved in strData, whereas lmax and alpha are computed separately. lmax identifies the order of the spherical harmonics used in the spline interpolation for the estimation of the underlying signal intensity, and its value depends on the number of diffusion gradient directions used in each shell. [34] provided a formula for that calculation of lmax; however, the obtained lmax values were not accepted by the Java functions. Thus,
a modified version of the proposed formula was instead adopted

$$l_{\text{max}} = 2 \text{round}\left(\frac{3 + \sqrt{1 + 8n}}{4}\right) - 4$$ (3.1)

where $n$ is the number of gradient directions used to probe the shell and round identifies the rounding to the nearest integer.

The alpha parameter, similarly to the one in the previous processing step, is a threshold value used to reject signal points identified as not belonging to the Rician distribution described by $\text{estSD}$ and the estimated underlying signal intensity. Lower the alpha parameter, the harder for a data value to be rejected. The Java functions reject a data point by setting its value to zero. Is important to minimize the number of rejected data points because the goodness of the orientationally-averaged signal, computed in the following step, depends on the number of usable (i.e. not rejected) corrected data. The default value for alpha is $10^{-4}$; however in this work $10^{-8}$ was used, since the number of rejected points was reduced while the noise correction was still well performed. In addition to this, and thanks to the possibility of calling the Java functions for the noise correction in the Matlab environment, a second correction was performed on the rejected points. In particular, the natural logarithm of the raw data was used as input for the estimation of the underlying signal intensity instead of the raw data. This further reduced the number of rejected values while keeping a good signal correction$^2$.

To facilitate the use of the Java package for the noise correction, a Matlab function was coded that initialises all the required inputs, calls the Java functions for the noise correction, handles the second correction step on the rejected data points and updates $\text{strData}$ with the corrected diffusion values.

**Weighted orientational average**

Using the updated $\text{strData}$, the weighted orientational average procedure condenses the data from each shell in one single value. The processing steps are described in Figure 3.4 where the data from one pixel is used to outline the procedure. Initially, the rejected values from the noise correction (i.e. zero values) are removed from the shell data, along with the diffusion gradient directions, saved in $\text{strData}$, used to acquire the rejected data points. The remaining directions are used to compute the weights for the shell data values using the code provided by [37]. The purpose of the weights is to correct for the impossibility of collecting infinite points for each shell, allowing for a better approximation of the powder signal. The weighted average is then computed using

$$\bar{E}(q) = \frac{\sum_i s_i(\vec{q})w_i(\vec{q})}{\sum_i w_i(\vec{q})}$$ (3.2)

where $\bar{E}(q)$ is the orientational-average value for that particular shell (i.e. $q$-value), $w_i(\vec{q})$ is the weight value corresponding to the direction the shell value $s_i(\vec{q})$ was collected at. Thus, the output of the weighted orientational average procedure, for

$^2$The goodness of the noise correction was visually tested using synthetic data where noise was added in a controlled manner.
CHAPTER. 3. METHODS

Figure 3.4: Orientational average and power fitting procedure. The powder average signal is computed by taking the weighted mean of all the values belonging to a single shell (i.e. q-value). The weights are calculated to compensate for the non-uniformity of the q-space sampling and improve the directional invariant representation of the sample. The obtained values are then fitted to a power law in the form $aq^c$. The fitting procedure iteratively includes points from the tail of the orientational average (i.e. from the higher q-value to the lowest) and calculates the goodness of fit for each subset. The trend of the goodness of fit is then used to identify the largest subset belonging to the power law decay.

Each pixel, is a one-dimensional signal that condenses the three-dimensional q-space diffusion acquisition.

Power fitting

The last step of the data analysis is the weighted power fitting that fits the orientational average points from the previous step to a power-law in the form $aq^c$. The processing steps are described in Figure 3.4, where the data from one pixel is used to outline the procedure. The data is fitted in Matlab using a non-linear least square method implementing the Levenberg-Marquardt algorithm [39]. Here, the fitting procedure is designed to find the optimal fit parameters $a$ and $c$ by looking at subsets of all orientational-average points. Iteratively, points from the tail of the powder signal (high q-values) are considered during the estimation of the fit parameters. Looking at the trend of the goodness of fit when the subset of points changes, the best subset of orientational-average points is identified, and the corresponding fit parameters $a$ and $c$ are chosen to describe the power law interpolating the data.

To improve the quality of the fit, weights for each orientational average point are computed using

$$w = \sqrt{n \text{estSD}} \quad (3.3)$$

where $n$ is the number of diffusion directions used to sample the shell the orientational average point is representing. This gives more importance to points coming from shells sampled with a higher angular resolution.

The outputs of the power fitting procedure are both $a$ and $c$. However, only $c$ is relevant to this work, since it describes how fast the powder signal decays. By fitting the orientational-average points for all the $(x,y)$ coordinates in the diffusion data, the decay trend for the different tissues types in the sample is identified.
3.3 Simulations and Acquisition parameters optimisation

The performance of the data analysis pipeline was tested and the acquisition parameters were optimized through a series of simulations. In particular, three main simulations were performed with the purpose of (1) evaluating the performance of the noise correction at different SNR values, (2) understanding the influence of the number of diffusion gradient directions on the approximation of the analytical solution in Eq. (2.24) and (3) testing different shell configurations. Additional simulations were also performed to investigate the suitable range of \(q\)-values where the power-law decay trend is visible in the analytical solution of the orientationally-averaged signal and the influence of neural projection orientation variability on the estimation of \(c\). The findings for the additional simulations are reported in the appendix section.

To perform the simulations listed above synthetic datasets were used. These are computed implementing the signal representation in Eq. (2.23) provided by [8] in the Matlab environment. The synthetic data generator, which is schematically described in Figure 3.5, provides an acquisition-like dataset receiving in input the shell configuration and diffusion gradient directions, the range of \(q\)-values, the diffusion timing parameters (\(\delta\) and \(\Delta\) in ms), the diffusion tensor (\(D\) in mm\(^2\)/s) and the noise level one wants to test (SNR).

An important aspect considered during the design of the synthetic data generator is how to produce synthetic data that captures the differences in axon organization between white and gray matter. As described in the brain complexity section, white and gray matter axons are organized differently in space. White matter axons are packed in bundles with all neural projections having a similar orientation, whereas gray matter axons and dendrites spread in all directions without an apparent organization. This difference between white and gray matter is interpreted in this work in terms of axon orientation variability or dispersion. Thus, white matter neural projections can be described by a low orientation variability, whereas gray matter by a high one. To provide a good approximation of the brain tissue in terms of neural projection orientation variability, synthetic data is computed using random rotated

![Figure 3.5: Diagram of the synthetic data generator.](image-url)
versions of the diffusion tensor that represent random orientations of the cylinders in which diffusion is taking place. The number of rotations is set depending on the orientation variability one wants to simulate: high for gray matter and low for white matter. Each combination of \( q \)-value and direction \((\vec{q})\) results, through Eq. (2.23), in a series of \( E(\vec{q}) \) values computed using the different diffusion tensor orientations. Then, for a fixed \( \vec{q} \), the mean of the series of \( E(\vec{q}) \) is taken, representing the synthetic diffusion signal from a randomly oriented population of straight neural projections.

Rician distributed noise is also added to the synthetic signal to simulate the noise characteristics obtained from a MR acquisition using a single channel receiving coil. Two independent Gaussian distributions \((x_{nd} \text{ and } y_{nd})\), with zero mean and standard deviation based on the input SNR, are used to compute the noisy signal as

\[
E(\vec{q})_{\text{noisy}} = \sqrt{(E(\vec{q}) + x_{nd})^2 + y_{nd}^2}
\]

(3.4)

The two normal distributions represent the two independent channels, affected by white noise, used during the quadrature detection of the MR signal.

**Noise correction performance**

To investigate the performance of the noise correction framework with respect to different levels of noise, synthetic data was generated using four SNR values: 5, 25, 100 and 500. The analytical solution in Eq. (2.24) was compared with the noisy and corrected orientational average synthetic signals for the four noise levels. Unchanged synthetic data parameters for the simulation were: \( q \)-value range=\([456, 1100]\)mm\(^{-1}\), 15 shells with 46 sampling directions each, \( \delta=2\)ms, \( \Delta=17\)ms, \( D=3 \cdot 10^{-3}\)mm\(^2\)/s, 100 random orientation of \( D \). The analytical solution was computed using the same diffusion timing parameters, \( q \)-value and diffusion tensor.

A second set of simulation was performed to understand how the number of diffusion gradient direction used to probe the \( q \)-space influence the noise correction. Synthetic data was generated using four different direction sets: 10, 30, 61 and 102. The analytical solution was then compared with the noisy and corrected orientational average signals for the four directions sets. Unchanged synthetic data parameters for the simulation were: \( q \)-value range=\([456, 1100]\)mm\(^{-1}\), 15 shells, \( \delta=2\)ms, \( \Delta=17\)ms, \( D=3 \cdot 10^{-3}\)mm\(^2\)/s, 100 random orientation of \( D \) and SNR=5. The analytical solution was computed using the same diffusion timing parameters, \( q \)-value and diffusion tensor.

**Number of diffusion gradient directions**

The goodness of the approximation of the orientationally-averaged analytical solution with respect to the number of diffusion gradient directions sampling \( q \)-space, was investigated for \( q \)-values in the range between 456 and 1570mm\(^{-1}\). For a fixed \( q \)-value, the analytical solution is compared with the synthetic signal computed using different direction sets, ranging from 10 to 200 directions. The orientational average synthetic signal was considered to well approximate the analytical solution when the mean square error (MSE) between the two values was lower than 0.02 (i.e. the synthetic data is in the \( \pm 2\% \) range of the analytical solution). The lowest number of sampling direction that fulfilled the above criteria was identified as the smallest direction set to approximate the analytical solution. Unchanged synthetic
data parameters for the different simulations were: number of shells=1, δ=2ms, \( \Delta=17\text{ms} \), \( D=3\cdot10^{-3}\text{mm}^2/\text{s} \), 100 random orientation of \( D \). The analytical solution was computed using the same diffusion timing parameters, \( q \)-value and diffusion tensor.

**Shell configuration and data analysis performance**

The performance of different shell configurations with respect to a range of SNR values was tested to identify the optimal combination of diffusion gradient directions for each shell that, with the smallest number of total directions, allowed for a good estimation of the power-law exponent. Moreover, using a variety of shell configurations and SNR values, the data analysis framework was tested, from the data packing to the estimation of \( c \). For a fixed shell configuration (i.e. 15 shells and 20 directions per shell), synthetic data was generated with SNR in the range between 5 and 100. For each SNR value, 15 different synthetic datasets are computed, each with a different realisation of Rician noise\(^3\). Each of the 15 synthetic datasets was independently processed, and the mean \( c \) value of the realisations was computed, along with the standard deviation for the 15 power-law exponent estimations. The unchanged synthetic data parameters for the different simulations were: \( q \)-value range=[456, 1100]mm\(^{-1} \), δ=2ms, \( \Delta=17\text{ms} \), \( D=3\cdot10^{-3}\text{mm}^2/\text{s} \), 100 random orientation of \( D \).

**Note on acquisition parameter optimization**

The parameters to be optimized before the acquisition of the diffusion data were the number of directions for each shell, the diffusion timing parameters (δ and \( \Delta \)), the number of samples in the frequency and phase encoding direction (i.e. pixels of the diffusion image, also called matrix size), the voxel size and the number of acquisitions averaged by the scanner. The identification of the optimal number of directions for a specific \( q \)-value (i.e. shell), was performed by evaluating the results of the simulations described above, whereas the optimization of the remaining parameters was done right before the acquisition. Remembering the need for pulse duration and separation to fulfil \( \sqrt{\delta D} \ll R_c \) and \( \sqrt{\Delta D} \leq R_c \), an initial small value for \( \delta \) and \( \Delta \) was set and adjusted to obtain a \( b \)-value higher than 15000s/mm\(^2\). The voxel size was chosen to have signal in gray matter regions when the highest \( b \)-value was used. The acquisition matrix dimension and the number of averages were specified based on the time available for the entire acquisition.

\(^3\)At each realisation, the random number generator providing the normal Gaussian distributions for the addition of Rician noise was shuffled.
Results

Initially, the simulation results used for the optimization of the acquisition parameters are presented, with focus on (1) the performance of the noise correction at different SNR values and for data generated with different gradient direction sets, (2) the influence of the number of diffusion gradient directions on the approximation of the analytical solution of the orientationally-averaged signal and (3) performance of different shell configurations. The results of the analysis of the diffusion data are then presented for gray and white matter independently.

4.1 Simulations

Noise correction performance

Figure 4.1 shows the performance of the noise-break framework at four different noise levels. The graphs display the analytical solution along with the synthetic noisy and corrected versions of the orientational average signal. The noise correction algorithm worked very well in low SNR conditions (a and b), removing the Rician bias in the SNR = 5 case and not performing substantial corrections at SNR = 25. However, when the signal level is high (c and d), the noise-break fails, altering significantly the signal values.

It is interesting to note that the noise correction framework performs better when the SNR level is rather low. This is not surprising since the noise correction algorithm was designed and tested to operate in the low signal regimes. The fact that at high SNR values the correction is poor suggests a possible numerical instability in the Java package provided by [34]. However, due to the nature of the diffusion signal, one does not expect SNR values higher than 100, in which case no noise correction is needed. In fact, in low noise conditions, the signal distribution resembles more a Gaussian distribution than a Rician one; thus no noise bias is present that needs to be corrected.

It is important to note that the noise correction algorithm performs differently across the range of $q$-values. Consider Figure 4.1a, where synthetic data with SNR=5 was

![Figure 4.1: Performance of the noise-break framework for increasing SNR level. In all graphs the analytical solution (green stars), the noisy signal (red triangles) and the corrected signal (blue stars) are plotted.](image)
processed. The noisy signal agrees quite well with the analytical solution at low \(q\)-values, whereas a visible discrepancy is present for high diffusion sensitivities. This behaviour reflects one of the challenges faced by [33] in developing the noise correction framework, namely the signal is affected differently by the noise based on the acquisition settings (i.e., changes in \(q\)-value). As is can be seen, the noise correction framework is able to handle this phenomenon with signal correction becoming more prominent as \(q\)-value increases, where the Rician bias alters the signal more.

Another result regarding the noise correction is presented in Figure 4.2. Here the performance of the noise correction framework is tested on synthetic data generated with the same SNR=5 but with different sets of diffusion gradient directions. It can be seen that at high angular resolutions (c and d) the correction is better. A possible explanation for this observation can be found in the estimation of the underlying signal intensity \(\eta\). The noise correction uses the noisy signal values from each shell independently to estimate \(\eta\). Thus, when a small angular resolution is used, \(\eta\) is poorly estimated since the number of data points for each shell is limited. This subsequently influences the conversion of the signal from a Rician to a Gaussian distribution and the overall results of the noise correction.

Figure 4.3 shows the estimation of the minimum number of directions required to well approximate the powder average signal when the diffusion sensitivity changes between 456 and 1570mm\(^{-1}\). With increasing \(q\)-value, a higher number of directions is required to approximate the analytical solution of the orientationally-averaged signal.

The above observation, and indirectly the noise correction framework performance, suggest that a higher number of probing directions is required to obtain a good approximation of the powder signal for large diffusion sensitivity values. However, due to time limitations, one needs to optimize the number of directions used for the acquisition. Figure 4.3 provides a starting point for the choice of the number of sampling directions: higher the \(q\)-value, higher the angular resolution required to obtain a good approximation of the powder signal. The reason for this can be found
looking at the physical meaning of the $q$-value, which has units of $\text{mm}^{-1}$. Consider the $q$-value as a magnifying lens which magnification power can change. At low magnification (i.e. small $q$-value) one can skim a written line quite fast, because many letters are visible at the same time. However, increasing magnification (i.e. increasing $q$-value), one can only see a single letter at the time, where more steps are required to cover the same written line. Thus, higher the value of $q$, finer the tool is used to probe diffusion and higher number of directions needed to capture the characteristics of the sample. It is important to note that, as for the magnifier lens example, at high $q$-values more details of the diffusion process are visible, since one looks at "individual" aspects altering diffusion rather than the bulk result of all causes.

**Shell configuration**

Using the results from the above simulations, the number of directions for each shell was identified, and the shell configuration was tested for different noise conditions. Figure 4.4 displays the performance of two shell configurations, optimized (a) and not-optimized (b). The optimized configuration performs well throughout the SNR range tested here, with 100% of the estimated power-law exponents in the $-1\pm10\%$ and $-1\pm5\%$ range. By using a non-optimal number of directions, the estimations of the power-law exponent deviates from the expected value of -1, with 15% and 5% of the values in the 10% and 5%, respectively.

The optimized configuration contains a combination of the direction sets provided by [37]. These sets, in combination with the weights computed during the orientational average processing step, try to obtain a uniform sampling of $q$-space without using a large number of directions. The results in Figure 4.4 clearly describe the importance of the right choice of directions for each shell. The low angular resolution in the non-optimized configuration fails in describing the analytical solution of the orientational-average signal at high $q$-values and does not provide a sufficient number of shell points to the noise-break framework to estimate the underlying signal intensity. Thus, the estimation of the power-law exponent is poor.
Figure 4.4: Shell configuration performance for different SNR values. (a) 10 shells linearly spread between 456 and 1100mm$^{-1}$, with 30, 30, 46, 46, 46, 61, 61, 61, 61, and 102 directions from the smallest to the largest $q$-value. (b) 10 shells, linearly spread between the same $q$-value interval, with each shell having 10 directions. The error bars in both graphs are obtained from the standard deviation of $c$, estimated from 15 realisation of noise for each SNR.
4.2 Diffusion data

Transverse grayscale diffusion images of the fixed spinal cord obtained at different $q$-values are shown in Figure 4.5. The signal to noise ratio level decreases with increasing $q$-value, from a value of $\approx 110$ to $\approx 17$. The sampling direction is $\hat{z}$, leading to the rapid loss of white matter signal when increasing $q$-value (outer region) and persistent signal in the gray matter areas (central butterfly shape).

![Figure 4.5: Transverse section of the cervical portion of fixed rat spinal cord at 4 increasing diffusion weightings. The SNR level, from the lowest to the highest $q$-value, are 110, 62, 34 and 17.](image)

The four regions of interest (ROI) used for the analysis of the diffusion data are shown in Figure 4.6. The smaller ROI (a and c) identifies pixels that belong to gray or white matter in both acquired slices. The broader ROI (b and d) identifies, for each slice independently, a wider region of gray and white matter, which is obtained by a threshold segmentation and is not perfect in terms of only selecting pixels belonging to a specific region.

![Figure 4.6: Regions of interest used for the analysis of the diffusion data. The large ROIs are pictured only for the second slice.](image)

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1SNR is computed as the ratio between the max signal value in the first acquisition (D1) and the $\text{estSD}$ of each acquisition estimated in the third processing step. Using the max signal value of the first acquisition allows for the comparison between the noise levels in the different acquisitions.
4.2. DIFFUSION DATA

4.2.1 Gray matter

Figure 4.7 shows the mean orientational-average signal across the gray matter pixels identified by the small ROI. The graph displays $q\bar{E}(q)$, a scaled version of the powder signal with respect to the $q^{-1}$ behaviour. In this representation, constant signal identifies regions in the data $\propto q^{-1}$ decay, while increasing or decreasing trends highlight a slower or faster decay respectively. Data are presented from the four acquisitions, for the raw as well as the corrected signal. Overall, the difference between the raw and the corrected data is small, with a more noticeable deviation in the fourth acquisition (D4).

![Graph showing $q\bar{E}(q)$](image)

**Figure 4.7:** Scaled mean orientational average signal ($q\bar{E}(q)$) of the gray matter region identified by the red pixels for the second slice of the acquired diffusion data. The four acquisitions are identified by different symbols, which raw and corrected data are presented in different colours. D1 was collected using the optimized shell configuration tested during the simulation, while D2, D3 and D4 used 10 directions for each shell.

Three different decay rates, relative to $q^{-1}$, are visible in the $q\bar{E}(q)$ graph. For small $q$-values ($< 800\text{mm}^{-1}$) a faster decay is present, with both D1, D3 and initial values of D2 describing this trend. Moving towards intermediate $q$-values (between 800 and 1000\text{mm}^{-1}), the orientational average signal decreases following $q^{-1}$. This is mainly captured by the centre points of D2 and the tail of both D1 and D3. At higher $q$-values, D4 and final points of D2 describe a steady increase, whose decay rate is smaller compare to $q^{-1}$.

The variation in the decay trend at different $q$-values is also visible when looking at the histogram of the estimated power-law exponents. In Figure 4.8 the distribution of $c$ values for the small ROI (first and third column) and the large one (second and forth column) are displayed for the four acquisitions and both slices. Note that the results are ordered from the one probing the small $q$-value range (D1 and D3 in first and second row respectively), the intermediate (D2 in third row) and high $q$-value range (D4 in fourth row). In both the small and the large ROI, the centre of the distributions of $c$ values shifts from smaller values to higher ones, describing a reduction in the decay rate when moving from low to high $q$-values. In particular, the powder signal in D1 and D3 decreases following a power-law with mean exponent equal to -1.3 and -1.6, respectively. In D2, the average $c$ value is -0.95 whereas the distribution of D4 is too spread to allow a reasonable description of the decay trend for the high $q$-values it probes.
CHAPTER 4. RESULTS

4.2.2 White matter

As for the gray matter, the scaled version of the orientational average signal, \( q\bar{E}(q) \), is shown in Figure 4.9, for the corrected as well as the raw data. Here the difference between the corrected and the raw results is significant, with D2 and D3 showing the largest discrepancies. D1 does not present any difference between the two versions of the data, and D4 being slightly affected by the correction. Both raw and corrected data points describe a decreasing trend. However, the interpretation of the decay rate, compared to \( q^{-1} \), is not immediate due to the differences in the raw and corrected versions of the signal. In fact, the corrected data shows a fast decay for \( q \)-values smaller than 1000mm\(^{-1}\), captured by D1, D3 and initial points of D2, followed by a slower trend for 1000 < \( q < 1500 \)mm\(^{-1}\) and a \( q^{-1} \) behaviour for higher \( q \)-values. The raw data starts as well with a fast decay for low \( q \)-value; however, above \( q > 800 \)mm\(^{-1}\) the powder average signal shows a constant trend in the \( q\bar{E}(q) \) plot.

Figure 4.8: Distribution of the estimated power-law exponents for D1 (first row), D3 (second row), D2 (third row) and D4 (fourth row). The first two columns describe the results for the first slice, whereas the last two columns for the second slice. Only the corrected data is here presented, with the raw signal showing similar distributions.
4.2. DIFFUSION DATA

Figure 4.9: Scaled mean orientational average signal \( q\overline{E}(q) \) of the white matter region identified by the red pixels for the second slice of the acquired diffusion data. The four acquisitions are identified by different symbols, which raw and corrected data are presented in different colours. D1 was collected using the optimized shell configuration tested during the simulation, while D2, D3 and D4 used 10 directions for each shell.

Figure 4.10 instead of plotting the comparison between the small and large ROI, pictures the differences in the distribution of \( c \) between the corrected (columns one and three) and raw data (columns two and four). The corrected versions of D2 and D3, which are the two acquisitions mostly affected by the noise correction, have a wider \( c \) distribution with a more negative mean when compared to the results from the raw data analysis. On the other hand, the distribution of power-law exponent for the first acquisition is very similar in both corrected and raw version, and also across the slices. Once again, the distribution resulted from the analysis of D4 is too spread to provide significant information.

Considering the change in the mean of the distributions across the four acquisitions we can see that, as for the gray matter regions, there is a shift towards \(-1\) going from low to high \( q \)-values. This is more accentuated for the corrected version of the signal, where the mean changes from \(-1.5\) in D3 to \(-1\) in D2. The variation is less evident in the raw data, where the difference between the mean of D1-D3 and D2 is less than 0.25.
Figure 4.10: Distribution of the estimated power-law exponents for D1 (first row), D3 (second row), D2 (third row) and D4 (fourth row). The first two columns describe the results for the first slice, whereas the last two columns the second slice. Here are pictured the results of the analysis on the large ROI, in both the corrected (first and third columns) and raw version of the data (second and fourth columns)
Discussion

5.1 Simulations

The performance of the noise correction results for variable number of probing directions finds an additional explanation when considering the observation regarding the minimum number of diffusion gradient directions required to well approximate the analytical solution of the orientationally-averaged signal. The unsatisfactory correction results obtained at high $q$-values when a small number of direction was used, may also be caused by the poor approximation of the analytical solution. The synthetic signal did not resemble the analytical solution even before the addition of Rician noise; thus, one can not expect the corrected signal to recover it.

Once again, the importance of the number of directions sampling $q$-space is evident since it plays an important role in the usability of acquired diffusion data.

5.2 Diffusion data

The SNR level throughout the acquisitions is high and well above the minimum value tested during the simulations. It is surprising how much signal is still present in gray matter even at $q = 1600$mm$^{-1}$ ($b=41400$mm$^2$/s with $\Delta=17$ms and $\delta=2.5$ms). The reason for this can be found in the 1.5mm slice thickness and in the well tuning of the MRI scanner before the acquisitions. With a fatter slice, the number of protons in each voxel contributing to the signal is high, which results in a high signal value. One would be tempted to use even bigger slice thicknesses to gain signal and reach even higher $q$-values. The main problem in doing this is the increasing of partial volume effects, where gray matter voxels are contaminated by white matter tissue and vice versa. This reduces the number of usable voxels and, therefore, the reliability of the results. When using spinal cord, the partial volume effects can be addressed by positioning the sample as straight as possible in the NMR tube and by adjusting the transverse acquisition plane in the scanner software making sure it is orthogonal to the length of the sample. In addition, in this work the small regions of interest for white and gray matter were defined looking at pixels belonging to one specific tissue type in both slices.

The second reason for the good SNR level seen in the diffusion data are the pre-acquisition adjustments made at the scanner. Reduction in field inhomogeneities (shimming) and RF coil tune/match are some of the procedures that, if not well performed, affect the signal amplitude and noise level. The shimming procedure requires the adjustment of the magnetic field strength in the $x$, $y$ and $z$ directions to compensate for the field distortions introduced by the sample [10]. Automatic shimming procedures are available in the scanner software; however, manual tuning was required to obtain better results. Tune and match of the RF coil is performed by changing the impedance of the coil electronics to match the resonance frequency of the sample and maximize the energy transfer between the sample and the RF coil.

The results of an un-matched coil can be seen by comparing D1 and D3, where
the values of the first acquisition are 15% smaller compared to the third one. At the end of the first acquisition, and before starting D2, RF coil tuning was controlled and resulted to be off-resonance with the sample. This caused a loss in receiving coil gain during D1 which shows as an overall depletion of signal intensity. The effect is visible in both white and gray matter regions, with similar degree of reduction. To avoid this problem in the subsequent acquisitions, the RF coil tune and match was checked at the beginning of every acquisition, assuring an optimal energy transfer between the sample and the coil.

Even if biased by the reduced signal intensity, acquisition D1 provides useful information regarding the shell optimization procedure and the reliability of the acquired data. In both white and gray matter, the raw and the corrected version of D1 match quite well even if the SNR value is high (≈110). The fact that this happens in both gray and white matter suggests that the angular resolution used for this acquisition is appropriate to probe both high and low neural projection orientation variabilities. By contrast, raw and corrected data for D2, D3 and D4 match only for gray matter, whereas big discrepancies are visible in white matter. In search for an explanation to this difference, the orientation variability between the two tissue types was considered and additional simulations on the performance of the noise correction were carried out. The results of these trials are summarized in the appendix section. Briefly, when a sample is characterized by a low orientation variability (i.e., white matter), a higher number of diffusion gradient directions are needed to obtain a good approximation of the orientational-average signal. Moreover, if the number of probing directions is low, the noise correction is not able to properly correct the signal as shown by previous simulations.

Unexpectedly, the results regarding the noise correction obtained by the simulations and the acquisitions suggest a method for controlling if the angular resolution used to probe $q$-space is high enough to obtain a good approximation of the powder representation of the sample. In particular, if the corrected signal diverges from the raw data at high SNR values, the number of diffusion gradient directions is too small; thus the resulting orientationally-averaged signal is not reliable.

From the above discussion, the results from D2, D3, and D4 in the white matter regions are not reliable, because the orientationally-averaged signal obtained from a 10 directions per shell acquisition does not resemble the powder signal. Thus, no conclusion can be drawn in this study regarding microstructure of the white matter for $q$-values higher than 970mm$^{-1}$. On the other hand, gray matter results are less affected by the small number of probing directions and can be used to discuss the influence of the tissue microstructure on the powder signal. It is important to note that a stronger conclusion can be obtained for gray matter if a larger number of probing directions is used.
5.3 Gray matter

The change in the power-law exponent in the orientational average signal indicates that, at different \( q \)-values, diffusion is influenced by a variety of tissue microstructure features. The rapid signal decay seen for the small \( q \)-value range supports the results obtained by [6] with an average power-law exponent among D1 and D2 of \(-1.86 \pm 0.08\) for \( b \)-values ranging from 3400 to 7500s/mm\(^2\) (i.e. [450, 673]mm\(^{-1}\)). The diffusion signal, as suggested by the analysis of [8], bears the influence of neural projections curvature because the \( q \)-value is not large enough. Exchange phenomena, that were considered to be a possible cause of the fast decay [6], can be excluded here since the diffusion time used during the acquisition (17ms) is too short to allow water to move between the intra and extra-cellular space [41].

It is important to note that the comparison between the power-law decay observed by [6] and the one computed here has a marginal relevance in this work. The differences in experimental conditions and test sample between this study and the one performed by [6] make the data comparison less informative. Nevertheless, the fact that both works agree on the power-law exponent may suggest that gray matter in \textit{in vivo} human brain and fixed rat spinal cord share some tissue microstructure features that are reflected in the orientationally-averaged diffusion signal.

Support to the hypothesis of neural projection curvature influence on the diffusion signal is found in the milder signal decay when moving towards larger \( q \)-values. Simulations performed by [8] suggested the appearance of the \( q^{-1} \) behaviour in the short pulse regime if large enough \( q \)-value is used to probe the sample and the pulse duration is small enough to fulfill \( \sqrt{D\delta} \ll R_c \). The diffusion data identifies 800mm\(^{-1}\) as the minimum \( q \)-value for \( q^{-1} \) to be visible. Moreover, Figure 4.7 shows a constant trend in the range from 800 to 1000mm\(^{-1}\): the orientational average points in this regime (last three points of D1 and D3, and central points of D2) fit a power-law with average exponent between the three measurements of \(-1.064 \pm 0.08\). In addition, the acquisition parameters indicate that \( R_c \) is greater than \( \sqrt{D\delta} = 2.5 \mu m \), using the diffusion coefficient of free water at temperature [2], radius of curvature that is reasonable considering that the diameter of most of the neurons is in the range between 0.5 to 2\( \mu m \). Note that this is a very rough approximation since the diffusion coefficient that should be used is the one describing water diffusion inside the neural projections, which is not yet reported.

An important aspect the analysis of [8] shows, is that \( q^{-1} \) is visible under the conditions of one dimensional diffusion process taking place inside cylindrical compartments. The results presented here suggest that, in the short pulse duration regime, diffusion can be considered one dimensional for \( q \)-values above 800mm\(^{-1}\). Moreover, the orientationally-averaged signal in this high \( q \)-value regime, bears information about diffusion taking place inside the neural projection \textit{i.e.}, in the intracellular space. This, if confirmed by further analysis, will provide a starting point for the design of experimental settings that aim to answer fundamental questions about intra-cellular microenvironment features.

The orientationally-averaged signal in gray matter for \( q \)-values higher than 1000mm\(^{-1}\) shows a slower decay compared to \( q^{-1} \). If confirmed by acquisitions with higher angu-
lar resolution, this slow decay would capture the diffusion signal from the population of almost immobile water molecules restricted in very small compartments. The signal from such regions is very small and has a very slow decay since the diffusion distance allowed by the microstructure constraints is too small to induce a significant change in spin phase. The observation of fully restricted water in gray matter was not yet reported, since it is expected to appear at very high diffusion sensitivities as for the case of white matter studied by [41]. Immobile water can provide important insights of the neural tissue microstructure because related to water molecules trapped in cellular organelles, in glia cells and between myelin sheets.

5.4 White matter

The reliability of the diffusion data at high $q$-values was compromised by the low angular resolution used to probe the sample; thus only the data from D1 can be discussed. The power-law exponent for the small ROI averaged on both the acquired slices for $b$-values ranging between 3400 and 7500 s/mm$^2$ is $-1.22 \pm 0.04$. This value does not agree with the results obtained by [6], where a $q^{-1}$ behaviour was observed for low diffusion sensitivities. A possible explanation for this discrepancy can be found in the difference between the two experiments. Here excised and fixed rat spinal cord was used as sample for the acquisitions, whereas [6] results are based on in vivo human measurements. Tissue fixation undoubtedly plays a role in the observed difference, as the fact that brain and spinal cord neural tissue of humans and rats may share microstructure differences.
Conclusion

Multi-shell diffusion acquisitions of rat spinal cord were used to study the influence of neural projection curvature on the orientationally-averaged signal. Simulations were performed to optimize the number of diffusion gradient directions needed to sample \( q \)-space and obtain a powder representation of the sample, whereas diffusion timing parameters were tuned to investigate the short diffusion time regime. The analysis of the gray matter showed the orientationally-averaged signal decaying following different trends in the range of diffusion sensitivity values tested here, suggesting that the diffusion signal is characterized by gray matter microstructure features. According to the analysis of [8], neurite curvature influences water diffusion, and thus might play a role in the definition of the signal decay trends. Moreover, the appearance of the \( q^{-1} \) decay indicates that water diffusion can be considered one dimensional in the high diffusion sensitivity regime, with the orientationally-averaged diffusion signal capturing properties of the intra-cellular microenvironment. Unexpectedly, the powder signal decay for values above 1000mm\(^{-1}\) is slower compared to \( q^{-1} \); this, if confirmed, will capture the diffusion signal originating from intra-cellular water molecules that are very restricted or immobile.

Further theoretical analysis is necessary to understand the relation between the features of the neural tissue microstructure and the diffusion signal. Alongside the need for comprehensive modelling, advancements in both signal acquisition and processing are required to provide data that can be interpreted. This work shows that gray matter microstructure features are pictured by the diffusion signal and provides a data processing framework for the computation of the orientationally-averaged signal from multi-shell diffusion acquisitions.


[34] Regina Bailey. Highly specific but edgily effective data-processing (hi-speed).


Additional simulations

Min $q$-value to see $q^{-1}$

The minimum $q$-value at which the $q^{-1}$ behaviour starts to appear in the analytical solution in Eq. (2.24) is 456mm$^{-1}$. Figure A.1 shows the trend of the power-law exponent estimated using a moving window that selected regions of the analytical solution. For low $q$-value (e.g. smaller that 200mm$^{-1}$) the analytical solution has a slower decay, which is described by a less negative $c$. Moving towards higher $q$-values, the analytical solution decays faster, with constant rate after 456mm$^{-1}$. With this result, the range of diffusion sensitivities values used for the simulations and the acquisition was set as higher than 456mm$^{-1}$.

It is important to note that Eq. (2.24) not only depends on the diffusion timing parameters and the $q$-value, but also on the diffusion coefficient. This greatly influences the shape of the analytical solution, stretching it going from higher to lower diffusion rates. Thus, the minimum $q$-value at which the $q^{-1}$ behaviour starts also changes, with higher values for lower diffusion rates. Here, the diffusion coefficient of free water was used, which is larger compared to the commonly used coefficients for white and gray matter (0.7s/mm$^2$ WM and 0.83s/mm$^2$ GM [42, 43], but allows the identification of the minimum $q$-value at which, if present, the $q^{-1}$ behaviour should appear.

Orientation variability vs angular resolution

Synthetic data was generated using an increasing number of random orientations of $D$ in the range between 1 and 200. In addition, the analysis was performed for three directions sets, namely 16, 46 and 102. The synthetic data was processed by the data analysis pipeline and the power-law exponent estimated. Unchanged synthetic data parameters for the different simulations were: number of shells=15, $\delta=2$ms, $\Delta=17$ms, $q$-range [456, 1100]mm$^{-1}$, $D=3\cdot10^{-3}$mm$^2$/s, SNR=50.
The result for the above simulations are shown in Figure A.2. It can be seen that higher the orientation variability, the better the estimation of the power-law exponent. Furthermore, higher the number of directions used to sample $q$-space (i.e. 102 directions), smaller the error in the estimation of $c$ at low orientation variability. The minimum number of randomly oriented cylinders that provides a good estimation of $c$ in all three direction sets is 100.

As described by these simulations, neural projection orientation variability influences the number of directions required to probe the sample. At higher orientation dispersion, the simulated sample resembles an isotropic distribution of projections which can be well probed even with a small number of directions. This is because the sample already resembles a powder average of cylindrical compartments from wherever direction one looks at it. On the other hand, a small number of random orientations describes an anisotropic projections distribution. In this case, a higher number of directions are required to probe the specimen because, due to its anisotropy, the features captured by each direction are different. Thus, to remove the dependency on the direction of measurement, one needs to capture all the features of the sample before averaging them.

Figure A.2: Orientation variability influence on the power-law exponent estimation. Simulations were performed using 15 shells, linearly spaced in the [456, 1100]mm$^{-1}$, with 16 (red squares), 46 (blue stars) and 102 (black circles) directions for each shells. The noise level was set to SNR = 50.

Noise correction performance vs orientation variability at high SNR
Synthetic data were generated using four different orientation variability values: 10, 100, 250 and 500. The analytical solution was compared with the noisy and corrected synthetic versions of the orientationally-averaged signal. The number of directions for each of the 15 shells used to generate the synthetic data is 10; this resembles the shell configuration used for diffusion acquisition of D2, D3 and D4. Unchanged synthetic data parameters for the different simulations were: number of shells=15,

\footnote{Recently \cite{44} studied why the computation of the orientational average signal is complicate when $B$ and $D$ tensors are anisotropic and very similar to each other i.e. the rotation of $B$ are similar to $D$. The simulation results described in this work may agree, to some extent, with the findings of \cite{44} (here we use $\vec{q}$ instead of $B$).}
δ=2ms, Δ=17ms, \( D=3\cdot10^{-3}\text{mm}^2/\text{s} \), SNR=70. The analytical solution was computed using the same diffusion timing parameters, \( q \)-value and diffusion tensor.

As displayed in Figure A.3 the signal corrections is well performed when the orientation variability of the sample is high. This result pictures the behaviour seen between the raw and corrected data in white and gray matter for D2, D3 and D4. At high orientation variability (i.e. gray matter), the corrected signal matches the noisy one, as expected due to the high SNR value. On the other hand, at low orientation variability (i.e. white matter), the correction is poor with most of the corrected values much smaller than the noisy data.
What neurons can do is not limited by nature...but by your imagination

Iulian Emil Tampu