

# **UNIVERSITY OF TWENTE.**

Faculty of Science and Technology Biomedical Engineering

## Comparison of 2D and 3D realistic models of white matter microstructure

Christian Licht (s2024993) Master of Science Thesis September 2019

Assignment Committee: Prof. Dr. ir. B. ten Haken<sup>1</sup> Dr. ir. F. F. J. Simonis<sup>1</sup>

<sup>1</sup>Magnetic Detection and Imaging University of Twente P.O. Box 217 7500 AE Enschede The Netherlands Dr. J.P. Marques<sup>2</sup> Dr. R. Hedouin<sup>2</sup>

<sup>2</sup>MR Techniques in Brain Function Radboud University Nijmegen P.O. Box 9101 6500 HB Nijmegen The Netherlands

## Preface

Neurodegenerative diseases, for instance mutliple sclerosis (MS) or amyotrophic lateral sclerosis (ALS), are characterized by a progressive degradation of the myelin sheath. Myelin is an insulation around an axon and enhances signal transmission speed. The loss of myelin hinders the signal propagation and is therefore associated with many cognitive and physical impairments. Specific magnetic resonance imaging (MRI) techniques enable to image myelin in-vivo. By fitting a biophysical model to the measured signal, it is possible to relate properties of white matter to the observed MR signal.

2D white matter models have already been developed to investigate the GRE signal behaviour with respect to myelin's microstructure. In the research group MR Techniques in Brain Function, at the Donders Centre for Cognitive Neuroimaging, Netherlands, 2D realistic white matter models based on electron microscopy data were developed. The assignment was performed in the research group MR Techniques in Brain function.

During this project, a 3D model based on 3D electron microscopy data of white matter was developed. With the developed 3D model, a comparison between the 2D and 3D model was conducted in order to investigate how accurate the 2D models simulate the signal. Thus, the resulting research question for this assignment is: How accurate are 2D white matter models when compared to a more realistic 3D model based on real 3D electron microscopy data?

In order to answer this question the report is structured the following. First, Chapter 1 provides an introduction to the theoretical background and the clinical aspect of this project. Then, in Chapter 2, the realistic 2D white matter model is presented. Afterwards, Chapter 3 shows the development and testing of the 3D model. Chapter 4 demonstrates the signal comparison between a realistic 3D model based on electron microscopy data and the realistic 2D models. Furthermore, the influence of fibers' orientation dispersion on the GRE signal simulation is studied and how 2D models could account for it. The overall discussion of this project is given in Chapter 5. Finally, Chapter 6 concludes the demonstrated research of this project.

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## Abstract

Neurodegenerative diseases, for instance multiple sclerosis (MS) or amyotrophic lateral sclerosis (ALS), are characterized by a progressive degradation of the myelin sheath. Myelin is an insulation around an axon and enhances signal transmission speed. The loss of myelin hinders the signal propagation and is therefore associated with many cognitive and physical impairments. Specific magnetic resonance imaging (MRI) techniques enable to image myelin in-vivo. By fitting a biophysical model to the measured signal, it is possible to relate properties of white matter to the observed MR signal.

The magnetic susceptibility of myelin affects both the magnitude and phase of the Gradient Recalled Echo (GRE) signal. Therefore, studying the signal using realistic models of white matter (WM) microstructure could provide insights into some of its properties and eventually, support to study diseases that are characterized by degradation of myelin sheath. Multi-compartment (intra-axonal, extra-axonal, myelin sheath) 2D models of white matter based on electron microscopy (EM) data have been used in the past to, combined with magnetic susceptibility of the myelin and its orientation in respect to the static magnetic field, simulate the MR signal. Unfortunately, 2D models could have several limitations: they lack the ability to incorporate fibers' orientation dispersion and the shape of axons might be unrealistically elongated and therefore, could not depict the real 3D case accurately. This thesis investigated how accurately the realistic 2D WM models, based on real axon shapes, simulate the GRE signal when compared to a more realistic 3D model. Therefore, a 3D model based on 3D electron microscopy data of white matter was developed. This model enables to simulate the GRE signal with respect to myelin's microstructure in 3D. The comparison between the developed 3D model and the already available 2D model revealed that the 2D model accurately simulates the GRE signal. Furthermore, it was demonstrated that fibers' orientation dispersion influence the simulated signal.

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## List of acronyms

MRI Magnetic Resonance Imaging AVF Axon Volume Fraction MVF Myelin Volume Fraction FVF Fiber Volume Fraction ALS Amyotrophic lateral sclerosis MS Multiple Sclerosis RF Radiofrequency GRE Gradient Recalled Echo FID Free Induction Decay CNS Central nervous System FFT Fast Fourier Transform EM Electron microscopy RM Realistic 2D model vMF von Mises Fisher distribution

## Chapter 1

## Introduction

The aim of this chapter is to provide an introduction to the topics and theory of the subject of this thesis. First, Section 1.1 states the medical aspect of this project. Afterwards, Section 1.2 explains the biological structure and its important characteristics. Then, Section 1.3 provides an introduction into the principle of Magnetic Resonance Imaging (MRI) and its signal generation. Section 1.4 states the concept of magnetic susceptibility with respect to the structure of myelin. Furthermore, the theory of the computation of the field perturbations and the signal are demonstrated. Finally, Section 1.5 combines the previous sections 1 to 4 by introducing biophysical 2D modeling of the Gradient Recalled Echo (GRE) signal with respect to white matter's microstructure.

### 1.1 Relationship of neurological diseases and myelin

White matter in the human brain mainly consists of myelinated axons. Axons are used to transmit signals between certain regions [1]. Myelin is a lipid membrane that is wrapped around axons and works as an insulation [2]. This membrane prevents the axon from interacting with the extra-cellular matrix and therefore, increases signal transmission speed. The microstructure of myelin in white matter is important for healthy brain functions and abnormal myelin conditions are associated with many forms of neuropathology [2].

Neurological diseases such as multiple sclerosis (MS) or amyotrophic lateral sclerosis (ALS) are characterized by demyelination [3]. Whereas MS is characterized by cognitive impairments [4], ALS affects the motor neurons in the brain [5] and therefore, causes physical impairments [1]. Defective myelination or demyelination is a process where axons are damaged [3] or the cells that form the myelin [6]. As a consequence, the myelin sheath degrades or is lost. These losses may be caused by inflammatory processes, mechanical injuries or genetic abnormalities [2]. There-

fore, patients experience problems in regards to communication between the brain and the rest of their body, leading to restricted movements. They suffer from fatigue, weakness, visual loss and cognitive impairments [4].

With 2.3 million cases in 2013, MS is one of the world's most common neurologic disorders [7]. The majority of ALS patients die within 2-5 years of receiving a diagnosis [8]. In 2015,  $\approx$  223,000 ALS cases were reported [9].

Diagnosing MS or ALS is still a challenge and is mostly restricted to testing transmission of nerve signals. [1] Therefore, biomarkers which are sensitive and specific to the neuro degenerative diseases are highly requested to further investigate the cause, progression and treatment of these diseases. A potential biomarker for diagnosing demyelinating diseases is the g-ratio. This parameter describes the ratio of the inner to the outer axon diameter [10].

MRI is an imaging modality, which provides high contrast images in soft tissues in-vivo and is thus, a well-known technique for imaging the central nervous system. MRI has been proven to be a valuable tool to assess myelin during development and demyelinating disease processes [11] for instance via myelin water imaging [12], [13] or quantitative magnetization transfer imaging [14]. However, Myelin Water Fraction (MWF) mapping can also be performed with GRE MR techniques with multicomponent analysis of the signal decay curve [15]. In regards to the clinical setting, Gradient Recalled Echo (GRE) techniques might be advantageous due to the fact that they offer faster acquisitions, low absorption rate and short echo spacing [16].

Different tissues in the human body respond differently to an external applied magnetic field. This response is quantified by the magnetic susceptibility and can spatially vary, for instance due to the myelin content in white matter (WM) brain tissue. [17] Myelin exhibits a complex magnetic susceptibility and therefore, spatially alters the magnetic field. The MR signal, obtained via a GRE technique, is sensitive to the non-uniform magnetic field [18]. As a result, the observed MR signal is altered with respect to the field inhomogeneities caused by the myelin. Thus, microstructural properties of white matter (e.g. g-ratio) are related to the complex MR signal, for instance in MS [19], and could be possibly derived from it [17], [20]. Therefore, analysing the  $T_2^*$  decay curve by combining the GRE signal with biophysical modeling, the measured MR signal can be related to microstructural features of white matter. *Conclusively, combining MRI with biophysical modeling of white matter microstructure could be used to provide more insights into white matter's properties as well as into demyelinating diseases.* 

### 1.2 Central Nervous System

The central nervous system (CNS) comprises the brain (grey and white matter) and the spinal cord. Its function is to monitor and control the entire body by its peripheral divisions, which are distributed to all the muscles, organs, and tissues. Neurons are the basic conducting elements in the nervous system and consist of three major components: (1) cell body, (2) dendrite, and (3) axon. [21]



Figure 1.1: Simplified overview of neuron's components.

The *cell body* contains many of the organelles vital to maintain the cell's structure and function, including the nucleus and nucleolus. Another structuring element are the *dendrites* extending from the cell body and picking up impulses carried toward it. The *axon* is a fiber, which connects to other cells and functions as an electric signal transmitter. The membrane around the axons is called *myelin* and prevents interactions with the fluids in the CNS and therefore, improves signal transmission speed along the axon. The myelin sheath is spirally wrapped around the axon, as demonstrated in figure 1.2b, and is interrupted by gaps, which are referred to as nodes of Ranvier. The site of contact between the axon of one nerve cell and the dendrites and cell body of another neuron is the *synapse*. In addition, nerve cells are supported by glia cells, which form myelin and maintain homeostasis. [21] A schematic overview of a neuron and its signaling pathway is shown in figure 1.1. An abundant amount of axons are usually present in the human body and are thus, tightly bundled (see figure 1.2a).



**Figure 1.2:** (a) Electron micrograph of mouse optic nerve. Myelin and intra-axonal compartment are colorized. Source: [1]

(b) Axon cross section with spirally wrapped myelin sheath.  $R_i$  and  $R_o$  defining the inner and outer fiber radius, respectively.

#### 1.2.1 Axon and myelin

The transmission of an electrical impulse is performed along an axon, which enables sending information. This transmission has to be faster, the further the signal has to travel, in order to ensure an appropriate neurological response. In the simplest way, this can be solved by an increase in axon diameter [1], due to the relationship of the resistance of a cylinder R and its size

$$R = \frac{\rho l}{A} \tag{1.1}$$

with  $\rho$  being the resistivity, l the length and A the cross sectional area of the cylinder.

However, increasing the axonal diameter is limited by two factors (1) high energy demand to maintain ion gradients and (2) space constraints [1]. A way to circumvent this issue and to enable fast signal transmission, is to partly insulate the axon with a sheath. This myelin sheath consists of multiple layers of lipid membranes and is wrapped around the axon [3], see figure 1.2b. However, the sheath is not continuous, in fact, it is interrupted by gaps (nodes of Ranvier), in which the axon is exposed to the extra-cellular space. These gaps are densely packed with  $Na^+/K^+$  pumps, which maintain the ion gradient. In reason of these modifications, the signal velocity in relatively small axons is increased significantly (100 -  $120\frac{m}{s}$ ) [1], when compared to signal transmission speed of relatively small fibers without myelination at about 0.5 to  $10\frac{m}{s}$  [22]. In general, axons with a diameter of >2 $\mu$ m are myelinated,

which represents a threshold at which myelinated axons conduct more rapidly than non-myelinated axons of the same size. [1]

An important structural and functional parameter for circular axons is the g-ratio. It describes the ratio between the inner radius ( $R_i$ ) of the axon to the outer fiber radius ( $R_o$ ), which can be defined as: [10]

$$g = \frac{R_i}{R_o} = \sqrt{\frac{1}{1 - \frac{\pi R_o^2 - \pi R_i^2}{\pi R_i^2}}} = \sqrt{1 - \frac{MVF}{FVF}} = \sqrt{\frac{1}{1 + \frac{MVF}{AVF}}}$$
(1.2)

The g-ratio can be defined as a function of the myelin volume fraction MVF and the axon volume fraction AVF [23], with FVF = MVF + AVF being the fiber volume fraction. A g-ratio of  $g \approx \{0.6...0.8\}$  is mostly observed in healthy white matter [24], because it represents an optimization in regards to speed of signal conduction, cellular energetics and spatial restrictions [1]. Changes in microstructural properties of white matter correlate with neuropathological states [25] and thus, the g-ratio could represent an objective measure to study neurodegenerative diseases [1].

It is important to note that the shape of axons in white matter highly differ [26]. Perge et al [27] reported axon diameters varying from .1 to  $10\mu$ m. Therefore, it is essential to model realistic axons with a variety of different shapes and sizes. Furthermore, the fiber configurations in 3D can vary a lot, as it is demonstrated in figure 1.3.



Figure 1.3: Simplified overview of fiber configurations in white matter.

### 1.3 Magnetic Resonance Imaging

MRI is an imaging modality which enables to image myelin in-vivo. It uses the signal from the nuclei of hydrogen atoms <sup>1</sup>H, present in the water molecule, in order to generate a signal. The nucleus of this atom is a proton, which is orbited by an electron. [28]

The proton can be described by its spin j = 1/2 and its mass m, which means that the proton has a certain angular momentum and due to its electrical charge, a magnetic moment. Thus, protons behave like small magnets and are therefore affected by external magnetic fields and electromagnetic waves. [28]

In an MR scanner, a strong magnetic field  $B_0$  is applied in a defined orientation, z, which causes a small fraction of the the spins to statistically align with the field's orientation. Due to the force of the field acting upon the protons, they undergo precession. This precession is described by the Larmor frequency,  $\omega_0$ , with

$$\omega_0 = \gamma \times B_0 \tag{1.3}$$

with  $\gamma = 42.6 \times 10^6$ Hz/T being the gyromagnetic ratio for <sup>1</sup>H and  $B_0$  the magnetic field strength. [28]

Due to the applied field, more spins tend to be aligned to the magnetic field. The differences in the population of spins produce the induced net magnetization  $M_z$  in a stable spin system [1], [28], resulting in a bulk magnetization of

$$M(t) = \begin{bmatrix} 0\\0\\M_z \end{bmatrix}$$
(1.4)

The magnetization  $M(t) = (M_x(t), M_y(t), M_z(t))$  in a dynamic system, in the presence of an external magnetic field, is given by the Bloch equations

$$\frac{dM_x(t)}{dt} = \gamma M_x(t) \times B_x(t)$$

$$\frac{dM_y(t)}{dt} = \gamma M_y(t) \times B_y(t)$$

$$\frac{dM_z(t)}{dt} = \gamma M_z(t) \times B_z(t)$$
(1.5)

#### 1.3.1 Radiofrequency pulse

By adding energy in the form of an electromagnetic wave with frequency  $\omega_0$  into the stable spin system, applied perpendicular to  $B_0$ , transitions between energy states can be induced. [1] This results in an local excitation of the spin system.

As a result, the net magnetization vector gets deflected towards the transverse (x,y) plane. [28] The flipping of the net magnetization vector is done by an RF pulse  $(B_1)$ , which rotates the magnetization vector about an angle  $\theta(t)$  into the transverse plane, as it is shown in figure 1.4a. The angle  $\theta(t)$  is given by

$$\theta(t) = \gamma B_1 t \tag{1.6}$$

Thus, the resulting magnetization is now denoted by  $M_{xy}$  for  $\theta = 90$  with the  $B_1$  field applied. In addition, it precesses about the *z*-axis, which induces an alternating voltage, picked up by a receiving antenna, which results in an MR signal. By adding gradient fields  $G_x$ ,  $G_y$  and  $G_z$ , the magnetic field can be varied,  $\Delta B_0$ , resulting in a spatially dependent spin frequency alteration. Thus, a relationship between frequency and space is induced and allows to relate the MR signal to a certain spatial location. [28], [29]



**Figure 1.4:** (a) Illustration of tipping of the magnetization vector M. M(t = 0) denotes the magnetization without an RF pulse, initial magnetization. M(t) represents the magnetization after an RF pulse of  $\theta \approx 90^{\circ}$  into the transverse plane.

(b) Illustration of dephasing process. 1) In phase (opaque arrow), 2)Dephasing (transparent arrows)

#### 1.3.2 Relaxation times

After the excitation via the RF pulse, the magnetization begins to return to its equilibrium 1.4). This process is known as relaxation [1] and varies between different tissue types. It is caused by spin-spin and spin-lattice interaction. [28] One differentiates between  $T_1$  (longitudinal relaxation time - spin-lattice relaxation) and  $T_2/T_2^*$  (transverse relaxation time - spin-spin relaxation). When taking relaxation into account, the solution to equations 1.5 are given by

$$M_{x}(t) = (M_{x}(0)cos\omega_{0}t - M_{y}(0)sin\omega_{0}t)e^{-\frac{t}{T_{2}}}$$

$$M_{y}(t) = (M_{y}(0)cos\omega_{0}t - M_{x}(0)sin\omega_{0}t)e^{-\frac{t}{T_{2}}}$$

$$M_{z}(t) = M_{z}(0)e^{-\frac{t}{T_{1}}} + M_{0}(1 - e^{-\frac{t}{T_{1}}})$$
(1.7)

with  $M_0$  being the initial magnetization at equilibrium along z.

Transverse relaxation  $T_2$  is observed when spins lose coherence (dephase). What happens is that the spins do not exchange their energy with their surroundings in fact, they exchange energy with each other (spin-spin interaction). In addition, time independent inhomogeneities of the magnetic field  $B_0$ , caused by different magnetic susceptibilities of the tissue for instance, contribute to the dephasing process. [28] Due to the spatially varying magnetic susceptibility, the field is locally increased or decreased, resulting in spatially varying resonant frequencies, which contribute to the signal decay.

Thus, the decay of the signal is denoted as  $T_2^*$  with  $T_2^* < T_2$  and is called free induction decay (FID). [1] The relationship between  $T_2$  and  $T_2^*$  is approximated by the sum of the relaxation rate and the observed field perturbations

$$\frac{1}{T_2^*} \simeq \frac{1}{T_2} + \gamma \Delta B_0 \tag{1.8}$$

with  $\Delta B_0$  representing the magnitude of the magnetic field changes (field perturbations) across a voxel. [18] This relation between  $T_2^*$  and  $\Delta B_0$  enables modeling of the complex MR signal with respect to the field perturbations and the  $T_2$  relaxation time.

GRE sequences are susceptible to static field inhomogeneities ( $\Delta B_0$ ), caused by the various constituents of the tissue and its compartments such as myelin. [29] The complex GRE signal can thus be expressed as an integral over a certain volume Vaccording to

$$S(t) = e^{-t/T_2} \int_V e^{-2\pi i t \Delta B_0} dV$$
 (1.9)

with  $T_2$  being the corresponding relaxation rate of the volume V.

#### 1.4 Magnetic Susceptibility and Field Perturbations

Magnetic susceptibility,  $\chi$ , denotes the magnetic response of a substance when placed inside a magnetic field. Thus, how easily and in which direction a substance becomes magnetized to an external applied magnetic field. [1] It is defined as

$$\chi = \frac{M}{H} = \frac{M\mu}{B_0} \tag{1.10}$$

with *M* being the macroscopic magnetization of the substance and *H* the magnetic field strength. For diamagnetic materials, e.g. human brain tissues such as white matter [30],  $\chi < 0$  and for paramagnetic/ ferromagnetic substances  $\chi > 0$ .

If a substance with a certain magnetic susceptibility,  $\chi$ , is placed inside a magnetic field,  $B_0$ , the polarization opposes or aligns the field lines inside the substance (see figure 1.5). As a consequence, it reduces the observed signal due to intravoxel dephasing [31].



**Figure 1.5:** Field perturbations caused by different magnetic susceptibilities  $\chi$ .

Myelin is a diamagnetic substance [32] and therefore, causes field inhomogeneities, when exhibited to an external applied magnetic field. The magnetic susceptibility of myelin is described by a second order tensor. [33], [34] Due to its structure, this tensor comprises isotropic,  $\chi_{iso}$ , and anisotropic,  $\chi_{aniso}$ , components [1], see equation 1.11. The anisotropic tensor is graphically demonstrated in figure 1.6a.

The induced spatial magnetization within myelin, M(r), depends on the orientation of the axon to the applied magnetic field,  $B_0$ . If  $B_0$  is perpendicular to the axon, myelin becomes strongly diamagnetic. Axons that are parallel oriented to the applied magnetic field are thus weaker diamagnetic. [34], [35]



Figure 1.6: (a) Graphical illustration of anisotropic magnetic susceptibility tensor.
(b) Graphical demonstration of radial orientation of phospholipids inside myelin. φ denotes the azimuth angle as a function of r, which describes the orientation of phospholipids in the myelin sheath in 2D.

As mentioned in section 1.2, myelin sheath is composed of phospholipids. Due to the dense packing of phospholipids in the myelin sheath, the susceptibility is further described by an anisotropic compartment. As shown in figure 1.6b, phospholipids are radially oriented within the myelin sheath. The anisotropic magnetic susceptibility tensor is thus computed with respect to the phospholipids' orientation inside the myelin, see figure 1.7b.

The total magnetic susceptibility of myelin,  $\chi_{Myelin}$ , is therefore the summation of both susceptibility components,  $\chi_{iso}$  and  $\chi_{aniso}$ , according to:

$$\chi_{Myelin} = \chi_{iso} \cdot \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} + \chi_{aniso} \cdot \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1/2 & 0 \\ 0 & 0 & -1/2 \end{bmatrix}$$
(1.11)

2D models always assume a constant angle between the axon and  $B_0$ , which is not necessarily the case in 3D. Therefore, a more complex 3D shape and orientation of the axon has to be considered, for instance figure 1.7a. Conclusively, the azimuth angle  $\phi$ , with  $\phi \in [0 - 2\pi]$  and the elevation  $\theta$ , with  $\theta \in [0 - pi]$ , denote the orientations of the phospholipids in 3D. This can be taken into account by applying the corresponding rotation matrix to the anisotropic susceptibility tensor, see figure 1.7b.

Thus,  $\chi_{aniso}(r)$  for the 2D model in the reference frame is given by

$$\chi_{aniso,2D}(\phi(r)) = R_z(\phi(r))\chi_{Myelin}R_z^T(\phi(r))$$
(1.12)

with R representing the corresponding rotation matrix. The anisotropic magnetic



**Figure 1.7:** (a) 3D representation of axon in a world coordinate system with orientation  $\theta = 15^{\circ}$ .

(b) Graphical illustration of rotations, defined by phospholipid's orientation ( $\phi$  and  $\theta$ ), applied to anisotropic susceptibility tensor,  $\chi_{aniso}$ .

susceptibility tensor for the 3D model in the reference frame is defined as:

$$\chi_{ansio,3D}(\phi(r),\theta(r)) = R_{y,z}(\phi(r),\theta(r))\chi_{Myelin}R_{y,z}^{T}(\phi(r),\theta(r))$$
(1.13)

Afterwards, the magnetic field perturbations are calculated via a Fourier-based method [36], which uses the varying spatial susceptibility distribution  $\chi_R(\phi(r), \theta(r))$  as input, with  $\chi_R$  being the summation of the isotropic and anisotropic susceptibility tensors.

Each element of a magnetization distribution, M(r), produces a dipolar magnetic field. The total magnetic field, generated at position r, can be described as a function of the sum of each field. By only taking the z component of the magnetization,  $M_z(r)$ , into account, it has been shown that the z component of the generated dipolar field,  $B_d z$ , can be expressed in the Fourier space as

$$B_{dz}(k) = -\mu_0 M_z(k) \left(\frac{k_z^2}{k_x^2 + k_y^2 + k_z^2} - \frac{1}{3}\right)$$
(1.14)

with  $k = [k_x, k_y, k_z]$  being the coordinate position in k space. [36]

The induced magnetization distribution,  $M_z(k)$ , can be calculated with respect to  $\chi(k)$  by applying a 3D FFT of equation 1.10 and substituting it into equation 1.14.  $M_z(k)$  is then further used to calculate the dipolar magnetic field,  $B_{dz}(k)$ , in the frequency domain according to equation 1.14. Afterwards, an inverse 3D FFT is applied to obtain the field perturbations,  $\Delta B_0(r)$ , in the spatial domain.

By using the spatially varying susceptibility distribution,  $\chi_R(\phi(r), \theta(r))$ , as input, one can thus calculate the off-resonance frequency,  $\Delta B_0(\chi_R)$ , according to

$$\Delta B_0(\chi_R) = FT^{-1} \left\{ \frac{1}{3} \hat{H}^T FT\{\chi_R(\phi(r), \theta(r))\} \hat{H} - \hat{H}^T k \frac{k^T FT\{\chi_R(\phi(r), \theta(r))\} \hat{H}}{k^2} \right\} \gamma B_0$$
(1.15)

with  $\hat{H} = [sin(\theta_H) * cos(\phi_H), sin(\theta_H) * sin(\phi_H), cos(\theta_H)]$  being the direction of the applied magnetic field,  $B_0$  being the field strength and the superscript T representing the transpose operation. A derivation of this equation can be found here [36], [33]. Finally, the signal S(t), with respect to  $\Delta B_0$ , can be calculated as it is demonstrated in equation 1.9.

### 1.5 Biophysical modeling of the GRE signal

Biophysical modeling can be combined with MRI in order to decode microstructural features of brain white matter from acquired MRI data. Multi-compartment 2D models of white matter have been developed in the past [1], [20], [34], [37], [38].

These structural models comprise three compartments: (1) intra-axonal, which corresponds to the axon itself, (2) the myelin sheath and (3) extra-axonal compartment (such as water and unmyelinated axons), see figure 1.2. They can be combined with magnetic susceptibility of the myelin and its interaction with the static magnetic field in order to compute the field perturbations in its various compartments. Finally, this model can be used to simulate the produced multi-echo GRE signal, with respect to myelin's microstructure.

According to equation 1.15, the field perturbations can be computed for a given spatial distribution of magnetic susceptibility. Myelin's magnetic susceptibility is the summation of both susceptibility tensors  $\chi_{iso}$  and  $\chi_{aniso}$ , which is demonstrated in figure 1.8. It can be seen that the isotropic tensor creates a dipole pattern, whereas the anisotropic tensor creates high field perturbations in the intra-axonal compartment.

Since a three-compartment model is used, the signal is computed as the sum of the signals of each of the three compartments according to

$$S(t) = \sum_{n=1}^{3} (\rho_n e^{-t/T_{2,n}} \sum_r e^{-2\pi i t \Delta B_n(\chi(r))})$$
(1.16)

with  $\rho_n$  being a relative weight reflecting the water signal received (T1 effect, proton water density, etc.),  $T_{2,n}$  the corresponding transverse relaxation rate, t being the echo time and  $\Delta B_n(\chi(r))$  the corresponding field perturbations for each compartment.

Figure 1.9 demonstrates that field perturbations, caused by myelin's microstructure, affect the multi-echo GRE signal in magnitude and phase. The spatially varying susceptibility of myelin causes the magnitude of the signal to decay faster when compared to the signal evolution in a homogeneous magnetic field. Furthermore, the phase of the signal accumulates over time and behaves non-linear whereas the phase for the homogeneous field is 0.



**Figure 1.8:** Demonstration of the effect the the individual susceptibility tensors. Field perturbations computed for a given axon shape with a magnetic field orientation of  $\Theta = 0,90^{\circ}$  and  $B_0 = 3$ T. First row shows the computed field perturbations for  $\Theta = 0^{\circ}$  and the second row for  $\Theta = 90^{\circ}$ .





For the following simulations  $\phi$  denotes the azimuth angle,  $\theta$  represents the elevation and  $\Theta$  denotes the angle of the applied magnetic field.

#### 1.5.1 Current research status of 2D WM models

Most of the 2D models are based on cylindrical shapes and elliptical spheres. Wharton et al [34] used a hollow cylinder fiber model with isotropic and anisotropic susceptiblity. It was demonstrated that field perturbations, caused by the magnetic susceptibility of myelin, are influenced by the orientation of the fiber with respect to the orientation of the applied magnetic field, as it was experimentally demonstrated by Lee et al [39]. Furthermore, it was proven that these models could be used to compute the orientation of white matter fibers. Nevertheless, it was mentioned that the hollow fiber cylinder model they used is a simplistic representation of the myelin sheath, which enhances the need to develop white matter models based on realistic data. Chen et al [20] developed a multi-compartmental 2D geometric model that models the microstructural arrangement of multiple WM fibers using only isotropic susceptibility and diffusion. It has been shown that the effect of diffusion is negligibly small and therefore, will not be taken into account. Furthermore, Xu et al [40] have demonstrated that the axon's shape influences the computed field perturbations and thus, the simulated signal. They used models with different axon shapes (circular, warped and realistic axon shapes based on electron microscopy data) to investigate the shape's influence on the signal.

It was demonstrated that the frequency distribution of the field perturbations, between a model consisting of circular (unrealistic) axon shapes, differed when compared to a model based on electron microscopy data. Furthermore, it was mentioned that a diversity of shapes and myelin geometries exist in white matter, which has not been studied. Chen et al [20] already suggested to use 3D models in order to investigate more sophisticated geometries and also dispersion. Another potential shortcoming of 2D models could be that they might assume unrealistically elongated fibers.

Conclusively, developing a 3D model based on realistic 3D electron microscopy data could reveal further insights. This model would enable to investigate more so-phisticated axon shapes. Furthermore, the influence of fibers' orientation dispersion could be studied. Dispersion is a parameter that quantifies how much the fibers' individual orientation differ from the average orientation within the data, which is graphically illustrated in figure 1.10.



**Figure 1.10:** Illustration of two fiber bundles with the same average orientation,  $\vec{\mu}$ , but with different dispersion values,  $\sigma_1 < \sigma_2$ .

Therefore, this Masters assignment focuses on the development of a robust 3D white matter model based on 3D electron microscopy data to simulate the GRE signal. This model is further used to conduct a comparison between an already existing realistic 2D model [37], which is based on 2D electron microscopy data. This enables to study the accuracy of the 2D WM models when compared to a more realistic 3D WM model. In addition, the 3D model is used to investigate the influence of dispersion on the simulation of the GRE signal. The research questions for this assignment are defined as:

#### **Research question**

- 1. How accurate are 2D white matter models when compared to a more realistic 3D model based on real 3D electron microscopy data?
- 2. Does dispersion influence the GRE signal simulations?

## **Chapter 2**

## Biophysical 2D Modeling of white matter

The second chapter of this thesis introduces the 2D realistic white matter model. In order to do this, each step to simulate the GRE signal will be visualized.

### 2.1 Realistic 2D Model

The idea is to simulate the GRE signal for several realistic 2D models, which then enables to create a dictionary with parameters such as the FVF and the g-ratio. Thus, the simulated signal will be related to the characteristics of the model. By using a deep-learning algorithm, the neural network will be trained and could learn to select the best suiting 2D model that corresponds to the measured signal. Thus, the biophysical 2D model will be fitted to the measured GRE signal and the best fitting model could then reveal the underlying microstructural properties.

The currently used 2D realistic model is based on a dictionary of realistic axon shapes, which allows the manipulation of the FVF, g-ratio and the amount of axons as described in [37]. The axon shapes were segmented from electron microscopy data of a dog's spinal cord, with an in-plane resolution of 0.26µm.

The simulations in this chapter were performed for the shown axon shape in figure 2.1 and with the parameters stated in table 2.1, which are based on literature values see [1] and [34].  $\rho$  is a relative weight between myelin and the extra-/intraaxonal compartment. Extra/intra axonal compartment are considered to exhibit similar T1 and proton density. *E* representing chemical exchange, which is set to 'off' to simplify the model and  $t_{sim}$  is the simulated echo time.

In order to structure the steps that need to be taken to simulate the GRE signal, the corresponding algorithm 1 for the 2D model is demonstrated.

Table 2.1: Parameters	used for sin	nulation of	complex	GRE	signal	for 2	2D (	and
3D models.								

Parameter	Value	Parameter	Value	
$B_0$	3 T	$\chi_{iso}$ , $\chi_{aniso}$	-0.1e-6	
$T_{2,intra\&extra}$	50 ms	$T_{2,myelin}$	15 ms	
$t_{sim}$	50 ms	ρ	2	
$\gamma$	42.6e6 Hz/T	E	0	

#### Algorithm 1: Simulation of GRE signal - 2D model

- 1: Set variables:  $B_0, H, \chi_{iso}, \chi_{aniso}, \rho, T_2$
- 2: Load 2D axon data (dictionary)
- 3: Create model based on 2D axon data with expected microstructural parameters (FVF, g-ratio) and myelin=1, intra-axon=0.5, extra-axon=0
- 4: Calculate magnetic susceptibility tensor,  $\chi_R(\phi(r))$
- 5: k=1:NumberOfAxons
- 6: Obtain orientation of phospholipids,  $\phi(r)$ , for axon(k) by computing directional gradient
- 7: Create rotation matrix  $R_z(\phi(r))$  with corresponding  $\phi(r)$  values for axon(k)
- Compute magnetic susceptibility tensor for axon(k) according to equation 1.12 and total magnetic susceptibility tensor map for axons(k:NumberOfAxons)
- 9: end
- 10: Compute field perturbations,  $\Delta B_0$ , based on  $\chi_R(\phi(r))$  according to equation 1.15
- 11: Create spatial frequency matrices  $k_{x,y,z}$
- 12: Calculate FFT of  $\chi_R(\phi(r))$
- 13: Compute total field perturbations  $\Delta B_0(k)$
- 14: Inverse FFT to obtain  $\Delta B(r)$
- 15: Compute signal,  $S_{total}$ , with respect to  $\Delta B_0(r)$  according to equation 1.16
- 16: Summation of field perturbations for each compartment
- 17: Compute signal for each compartment:  $S_{intra-axonal}$ ,  $S_{extra-axonal}$ ,  $S_{myelin}$ and total signal,  $S_{total}$ , by summing all signals and applying weighting

In order to simulate the GRE signal, 4 major steps need to be performed. First, the model with a specified FVF and g-ratio is created, see figure 2.1. Secondly, the magnetic susceptibility tensor map has to be calculated with respect to the phospholipids' orientation inside the myelin sheath, shown in figure 2.2. Afterwards, the magnetic susceptibility tensor map is used to compute the field perturbations, see figure 2.3. Finally, the GRE signal is calculated with respect to the field perturbations and the relaxation time of the correpsonding compartment, shown in figure 2.4.

With the axon, shown in figure 2.1a, the magnetic susceptibility tensor  $\chi_R(\phi(r))$  is computed. This calculation is performed with respect to the orientation of the phospholipids radially oriented inside the myelin sheath, see equation 1.12. The lipid orientation (phimap) is shown in figure 2.2.



Figure 2.1: (a) Single axon from the dictionary. Yellow represents the myelin sheath and turquoise intra-axonal compartment. White represents extra-axonal compartment.

(b) Model with several realistic axons from the dictionary.



Figure 2.2: Corresponding lipid orientation of the axon shown in figure 2.1a.

#### 2.1.1 Field perturbations

The field perturbations,  $\Delta B_0$ , are computed with respect to the spatially varying susceptibility,  $\chi_R(\phi(r))$ , according to equation 1.15. Figure 2.3 depicts the calculated field perturbations with respect to the orientation of the applied magnetic field.

Subplot 2.3a shows the computed field perturbations for the case of  $B_0$  being parallel to the axon and incorporating the isotropic as well as the anisotropic susceptibility of myelin. If  $B_0$  is parallel to the axon, field perturbations of  $\approx$  -2Hz are observed inside the myelin sheath. Intra- and extra-axonal compartments exhibit frequencies of  $\approx$  0Hz.



Figure 2.3: (a) Field perturbations for magnetic field orientation parallel to the axon and the corresponding frequency histogram (c). (b) Magnetic field perturbations with a magnetic field applied perpendicular to the axon and (d) showing the corresponding frequency histogram.

Figure 2.3b shows the magnetic field perturbations for  $B_0$  being perpendicular to the axon. In this case, the susceptibility of myelin creates larger and more complex field perturbations. The frequency histograms, shown in figure 2.3c-d, provide more insights into the field perturbations caused by myelin's magnetic susceptibility.

Depending on the orientation of  $B_0$ , myelin, intra- and extra-axonal compartment contribute differently to the field perturbations. The extend and magnitude of the induced magnetization, besides the field strength  $B_0$ , depends on the orientation of the axon and the orientation of the phospholipids inside the myelin sheath, with respect to  $\Theta$ .  $\Theta$  denotes the angle of the applied magnetic field.

For the first case, when  $B_0$  is parallel to the axon, the field perturbations only arise from the myelin. A frequency of  $\approx$  -2Hz is observed, see figure 2.3c.

The second case, if  $B_0$  is perpendicular to the axon, the contribution to the field perturbations becomes more complex. In this case, all of the three compartments exhibit a spectrum of frequencies, which vary mostly between -10 to 10Hz, see figure 2.3d. This is in contrast to the parallel case in which only myelin is the source of perturbations. Frequencies observed inside of myelin range from -10 to  $\approx$ 15Hz. The intra-axonal compartment's frequency is mainly negative ranging from -15 to  $\approx$ 0Hz, which arises from the anisotropic susceptibility.

It is important to note that the extra-axonal contribution would be less if unmyelinated axons were not be regarded as extra-axonal space. In fact, intra-axonal would be the dominant compartment in this case.

#### 2.1.2 GRE Signal

It has already been shown in Section 1.5 that spatially varying magnetic susceptibility of myelin influences the GRE signal. Furthermore, the caused field inhomogeneities strongly depend on the orientation of the external applied magnetic field (see figure 2.3). Therefore, the simulated GRE signal also depends on the orientation of  $B_0$ , because  $\Delta B_0$  is larger for field orientations of  $\Theta \neq 0$ . According to equation 1.9, the exponential decay of the signal's magnitude is thus faster if  $\Delta B_0$ increases.

Figure 2.4a shows the magnitude of the signal and it can be seen that the signal decays faster for larger angles of  $B_0$ , in respect to the axon's orientation. The signal decay is the strongest when  $\Theta = 90^{\circ}$ .

Figure 2.4b demonstrates the corresponding phase of the signal. It is shown that the phase evolution gets steeper when  $\Theta$  increases. If  $B_0$  is parallel to the axon, the phase accumulation is mainly driven by myelin. Since myelin has a short  $T_2$  value, the phase accumulation ends at  $\approx$ t=15ms= $T_{2,myelin}$  and goes back to zero.



**Figure 2.4:** Simulated signal magnitude and phase with different magnetic field orientations. (a) Magnitude of simulated GRE signal (b) Phase of simulated GRE signal.

This arises from the fact that intra- and extra-axonal compartment will dominate the signal at some point, with both exhibiting a longer  $T_2$  value. Furthermore, both compartments have a null phase. This arises from the fact that in the case of  $\Theta = 0^{\circ}$ , intra- and extra-axonal compartment do not exhibit field perturbations and thus, the phase is 0. As  $\Theta$  increases, other compartments, with different  $T_2$  values, contribute to the signal. In this case, intra- and extra-axonal compartment also exhibit field perturbations and as a result, the phase accumulates faster. Furthermore, the phase will not go back to zero. It is demonstrated that phase accumulation is the strongest when  $\Theta = 90^{\circ}$ .

## **Chapter 3**

## Biophysical 3D Modeling of White Matter - From 2D to 3D

The third chapter of this thesis demonstrates the development of the 3D model to simulate the multi-echo GRE signal. Furthermore, its accuracy is compared to the existing 2D model introduced in Chapter 2.

#### 3.1 Methods

The programming was performed in MATLAB (MathWorks, MATLAB R2018b, Natick, Massachusetts, USA) whereby all the simulations of the 3D model were performed with the same parameters as stated in table 2.1, but with a different axon shape. The 3D model was tested on a single axon first.

The first step was to extend the 2D realistic model into the third dimension. Therefore, an axon from the dictionary was replicated along z, yielding a 3D model of size  $150 \times 150 \times 150$ , see figure 3.2.

The next step was to compute the orientations of the phospholipids inside the myelin. As figure 1.6 demonstrates, it is assumed that the phosphpolipids are radially oriented inside the myelin sheath. Thus, it can be further assumed that the orientation of the lipids can be approximated by computing the gradient direction between the inner and outer myelin boundaries. In order to do this, the axon was smoothed by applying a 3D Gaussian filter to the model. Afterwards, the 3D gradient magnitude and direction were computed. In this case, the gradient direction consists of the elevation ( $\theta$ ) and the azimuth angle ( $\phi$ ). Due to varying myelin thickness in real data, a method to check whether the smoothing was sufficient had to be developed. It was found that the smoothing was sufficient, when the gradient magnitude did not exhibit 0 inside the myelin sheath anymore. By extracting  $\phi(r)$  and  $\theta(r)$ , within the myelin sheath, the corresponding phi- and thetamaps were created. The process in

order to compute the phospholipids' orientation is demonstrated in figure 3.3.

Afterwards, the magnetic susceptibility tensor,  $\chi_R(\phi(r), \theta(r))$ , was calculated according to equation 1.13. The anisotropic tensor was computed with respect to the orientation of the phospholipids, denoted by phi- and thetamaps as shown in figure 3.3.

Equation 1.15 was used to compute the field perturbations for the initial matrix size. In order to validate the calculations of the 3D model, the field perturbations for the same axon shape were modeled in 2D. Then, the frequency histograms of the 2D and 3D model were compared, see figure 3.5a and b. Afterwards, the 3D calculations were further verified by applying rotations of about  $\theta = 30^{\circ}, 45^{\circ}, 60^{\circ}$  to the 3D axon. The magnetic field's orientation in 3D was adjusted with respect to the rotations according to  $\Theta_{3D,rot} = \Theta + \theta$ , with  $\theta$  representing the rotation angle of the axon. Thus, the field orientation in 3D was the same as in the 2D model, which enabled to compare both models. This concept is graphically illustrated in figure 3.1. Applying different rotations to the 3D model enabled to investigate, whether the equations 1.13 and 1.15 were correctly implemented in 3D. The field perturbations for the 2D model, the 3D model without rotation and the 3D model with rotation for  $\Theta = 0, 90^{\circ}$  are shown in figure 3.4. The corresponding frequency histogram comparison is shown in figure 3.5.

By applying a mask, a region of interest (ROI) of 40x40x40 was defined, which yielded an FVF and g-ratio of 0.34 and 0.60, respectively. Finally, the GRE signals, originating from the ROI, were computed according to equation 1.16, see fig 3.6. In order to compare the signals, each signal was normalized by dividing each signal by the magnitude of the first echo. Afterwards, by computing the dot product of the differences of two complex signals, the similarity between the signals could be investigated according to:

$$RMSE = \sqrt{\frac{\langle (S_1 - S_2), (S_1 - S_2) \rangle}{length(t_{sim})}}$$
(3.1)



**Figure 3.1:** Demonstration of applying the correct main magnetic field orientation in 3D. For instance, a signal simulated for a field being parallel in 2D  $(\Theta_{2D} = 0^{\circ})$ , would correspond to a field orientation of  $\Theta_{3D} = \vec{\mu}$  in 3D.

### 3.2 Results



**Figure 3.2:** 2D model replicated along z yielding 3D model. Arrows indicate  $B_0$  orientation.

Figure 3.2 shows the replicated 2D model with intra-axonal, myelin and extraaxonal compartment.

Figure 3.3 demonstrates the process for the phospholipids' orientation calculation. The phimap 3D demonstrates that the phospholipids are radially perpendicular orientated within the myelin sheath. Furthermore, thetamap 3D reveals that the elevation of the phospholipids changes with respect to the position inside the myelin sheath. Values at the upper and lower boundary of the axon (z-x plane) are the highest and correspond to the rotation of  $45^{\circ}$ . It is further observed that the elevation of the phospholipids decreases when moving towards the midline of the axon.



**Figure 3.3:** Demonstration of phospholipids' orientation calculation. The shown theta- and phimaps correspond to an axon rotated by  $45^{\circ}$  about the y axis. Dotted line represents midline of axon.

Figures 3.4a-c demonstrate the computed field perturbations for  $\Theta = 0^{\circ}$  for the 2D model (a), the 3D model not rotated (b) and the 3D model rotated (c). It is shown that myelin exhibited field perturbations of  $\approx$ -2Hz. These observations are consistent throughout the slices. Figure 3.5a depicts the frequency histogram comparison between the 2D model and the 3D model without rotation with  $B_0$  being parallel to the axon. The histogram exhibits that myelin is the only compartment that contributes to the field perturbations with  $\approx$ -2Hz. No differences for the 3D model without rotation are observed when compared to the 2D model, see figure 3.5a. Figure 3.5c shows the frequency histogram comparison between the 2D model and the rotated 3D model for  $\Theta = 0^{\circ}$ . It is demonstrated that myelin is the only compartment that exhibits field perturbations. However, the frequency spread of myelin is slightly enlarged for the rotated 3D model, when compared to the 2D model. Furthermore, intra- end extra-axonal compartment exhibit small differences.



Figure 3.4: Computed field perturbations between 2D and 3D model, with and without rotation. (a-c) Demonstrate the field perturbations when the magnetic field was applied parallel to the axon. (d-e) Demonstrate the field perturbations when the magnetic field was applied perpendicular to the axon.

Figures 3.4d-f show the computed field perturbations for  $\Theta = 90^{\circ}$  for the 2D model (d), the 3D model without rotation (e) and the 3D model with rotation (f). It is shown that the intra-axonal compartment exhibited highly negative frequencies and inside the myelin, positive field perturbations are observed. Furthermore, the extra-axonal compartment exhibited positive and negative field perturbations. Fig-

ure 3.5b shows the frequency histogram comparison between the 2D model and the 3D model without rotation for  $B_0$  being applied perpendicular to the axon. It is shown that the observed frequencies for this case are largely spread and each compartment exhibits field perturbations. The intra-axonal compartment exhibits frequencies between -5 to 0 Hz and the myelin compartment -4 to 15 Hz. The extra-axonal compartment's frequencies are mostly observed between -5 to 5 Hz. However, the comparison with the histogram exhibits small differences in the myelin compartment, see figure 3.5b. Figure 3.5d depicts the frequency histogram comparison between the 2D model and the rotated 3D model for  $\Theta = 90^{\circ}$ . It is observed that all three compartments exhibit small differences. The relative largest difference is observed between the myelin compartment and the relative smallest difference for the intra-axonal compartment.



**Figure 3.5:** Frequency histogram comparison for  $\Theta = 0,90^{\circ}$  between 2D and 3D model not rotated (a and b). Images c and d depict the frequency histogram comparison between the 2D and 3D model rotated.

Figure 3.6 demonstrates the signal evolution of the 2D and 3D model (not rotated) with respect to the magnetic field's orientation. It is shown that both models exhibited similar magnitude decay, see figure 3.6a. Furthermore, the behaviour of the phase is also similar between the 2D and 3D model, see figure 3.6b, with  $\Theta = 0^{\circ}$  exhibiting the highest relative difference. The average RMSE between the signals of the 2D and 3D model non-rotated, for  $\Theta = 0, 15, 30, 45, 60, 75, 90^{\circ}$ , was  $0.05\% \pm 0.04\%$ . For  $\Theta = 0^{\circ}$ , the highest RMSE was observed of 0.1% and the lowest RMSE was found to be 0.01% for  $\Theta = 90^{\circ}$ . In regards to the comparison between the 2D and the 3D model rotated, the RMSE was found to be 0.03% for all values of  $\Theta$  and all rotations.



**Figure 3.6:** Signal comparison of 2D (dashed line) to 3D (solid line) model (no rotation 2D) with respect to  $B_0$  orientation. (a) Magnitude for simulated signals. (b) Phase of simulated signals.

### 3.3 Discussion

Figure 3.3 shows the smoothing process used to obtain the phospholipids' orientation inside the myelin sheath. The computed theta values in the upper and lower region of the axon correspond to the rotation of  $45^{\circ}$  applied. However, it is observed that the elevation of the phospholipids decreases when approaching the midline of the axon. This arises from the fact that the elevation of the phospholipids at the middle line of the axon is 0, because they are oriented parallel to the reference axis x. Afterwards, the elevation increases again until  $45^{\circ}$ . The phimap 3D exhibits the typical radial orientation of the phospholipids inside the myelin sheath, when compared to figure 2.2. As a conclusion, the computation of the phospholipids' orientation in 3D is seen to be correct.

The comparison of the field perturbations between the 2D and 3D models were used in order to investigate whether equations 1.13 and 1.15 were correctly implemented in 3D. The field perturbations, shown in figure 3.4, revealed the same frequency pattern as it was seen in the 2D model. If  $\Theta = 0^{\circ}$ , myelin is the only compartment that contributes to the field perturbations, which is in line with what the 2D model exhibits. If  $\Theta = 90^{\circ}$ , the frequency spectrum is wider, since the other compartments such as intra- and extra-axonal compartment exhibit field perturbations. As the comparison between the frequency histograms revealed, shown in figure 3.5, only small differences between the 2D and 3D models were observed. The highest relative differences exhibited the comparison between the 2D and the rotated 3D model, which are attributed to interpolation errors when the rotation is applied. However, these differences were small and therefore, negligible. Conclusively, equations 1.13 and 1.15 were correctly implemented in 3D. In addition, the signal simulation, shown in figure 3.6, demonstrated that the relative differences between the 2D and 3D model were negligibly small.

### 3.4 Conclusion

The above results have shown that the development of a 3D model to compute the field perturbations of 3D axon shapes, with respect to the spatially varying magnetic susceptibility of myelin, was successfully implemented. The comparison of the field perturbations and signals between the already available ground-truth 2D model and the 3D model revealed that the 3D model yields accurate results.

## **Chapter 4**

## Biophysical 3D Modeling - From 3D Electron Microscopy Data

The previous chapter demonstrated the development of the 3D model. This chapter describes the steps that were taken in order to create the 3D model based on 3D white matter electron microscopy data. In addition, the comparison between the 2D realistic and the 3D model based on 3D EM data is given. Furthermore, the influence of fibers' orientation dispersion is investigated.

### 4.1 Methods

The 3D EM data set, shown in figure 4.2a, from the genu of the corpus callosum of a mouse brain, was already segmented by using a 3D electron microscopy segmentation pipeline [41]. Therefore, the myelin mask, see figure 4.2b, and the intra-axonal mask was available. The data size is 1311x1255x430 with a resolution of 7x7x50nm and in-plane down sampling factor of 7, which yielded a nearly isotropic resolution. A sub matrix of size 400x400x400 of the initial EM data was used to create the model and to conduct the comparison.

First, an automated pre-processing algorithm was developed to correct for partly incorrect segmentation. This algorithm enabled to automatically remove falsely labeled structures within the intra-axonal compartment and ensured closed myelin sheaths. First, the intra-axonal mask was filled to remove holes within the intra-axonal compartment. Afterwards, the intra-axonal mask was dilated by one pixel. The third step consisted of combining the myelin mask with the dilated intra-axonal mask to a total map. In order to remove holes in the myelin sheath, the total map was filled and finally, the filled but non-dilated intra-axonal mask was subtracted from the total map. As a result, falsely labeled structures within the intra-axonal compartment could be removed and in addition, all myelin sheaths were closed due to the

dilation of the intra-axonal mask. The results of the pre-processing steps are shown in figure 4.1b.

In order to quantify fibers' orientation dispersion in the data set, the direction of each individual axon, shown in figure 4.2c and d, was computed. The directions,  $\vec{x_i}$ , were found by extracting the eigenvector of the covariance matrix of the pixels' locations of each axon. In order to obtain the resulting orientation of an axon, each direction  $\vec{x_i}$  was used to calculate the corresponding orientation tensor. Afterwards, the average tensor was computed and the resulting average orientation could be found by extracting the eigenvector with the largest eigenvalue, see figure 4.2e.

With  $\vec{x_i}$  and  $\vec{\mu}$ , dispersion,  $\sigma$ , was computed according to equation 4.1. Dispersion is defined as the deviations of orientations of the various axons,  $\vec{x_i}$ , to the average orientation of the axons,  $\vec{\mu}$ . A graphical illustration of dispersion is shown in figure 1.10. In the context of diffusion weighted imaging (FSL [42]), dispersion is calculated according to

$$\sigma = 1 - \frac{1}{n} \sum_{i=1}^{n} \langle \vec{x_i}, \vec{\mu} \rangle^2$$
(4.1)

with  $\sigma$  ranging from 0 to 1, with 0 indicating no dispersion.

The magnetic susceptibility tensor,  $\chi_R(\phi(r), \theta(r))$ , was calculated with respect to the phospholipids' orientation according to equation 1.13. Therefore, each axon was shifted into a smaller matrix to speed up calculations and smoothed individually to compute the gradient directions between the myelin boundaries, as demonstrated in figure 3.3. By calculating the gradient directions, the corresponding  $\phi$  and  $\theta$  values were found for each axon individually, see Figure 4.3. In order to avoid problems with the FFT and the finiteness of the model,  $\chi_R(\phi(r), \theta(r))$  was sufficiently enlarged by zero padding. Afterwards, the field perturbations,  $\Delta B_0(\chi_R)$ , were computed according to equation 1.15. The computed field perturbations for the mentioned model are shown in figure 4.4. Finally, the GRE signal was reconstructed according to equation 1.16. In order to ensure that the GRE signal corresponds to the initial matrix size, a mask was used which enabled to sample the field perturbations of the initial region of interest of 400x400x400. Table 2.1 states the used simulation parameters. First, the simulated GRE signal was compared between the 3D model based on EM data and the realistic 2D models.

#### Signal comparison 2D realistic models and 3D EM model

The GRE signal comparison for the initial 3D EM model was conducted for  $\Theta = 0, 15, 30, 45, 60, 75, 90^{\circ}$ . In order to investigate the accuracy of the 2D models, two different 2D models were used:

- 1. 10 realistic 2D models (see figure 4.5)
- 2. 2D model based on 3D electron microscopy data (see figure 4.6)

Each realistic 2D model contained  $267\pm11$  axons and was created with the same FVF and g-ratio as it was found in the 3D EM data. The signal was computed for each model individually and normalized by dividing each signal by the magnitude of the first echo. Afterwards, the RMSE for each signal of each model was compared to the 3D model. Equation 3.1 was used to determine the similarity between the signals. The comparisons are demonstrated in figures 4.5 and 4.6. Afterwards, the 3D EM model was manipulated to investigate the influence of dispersion on the signal simulation, which is described in the following.

#### Influence of dispersion - Signal comparison 3D EM with artificial dispersion

Dispersion could be a parameter that influences the signal simulation due to the fact that for high dispersion, many fibers differ from the average orientation. As a result, the angle between the applied magnetic field and the fibers vary and therefore, could cause different field perturbations, which in result affect the simulated GRE signal. In order to investigate the influence of dispersion on the simulated GRE signal, higher dispersion values were needed as the one exhibited from the 3D EM data. Therefore, the orientations of all axons and the resulting average orientation,  $\vec{\mu}$ , were computed. By applying a similarity measurement between each orientation of the axons with the average orientation, the axons that differed the most from the average orientation were found. This enabled to create a 3D EM dispersion model with a dispersion value of 0.4. In order to compensate for the loss in regards to the FVF, a 3D mask was used to sample the field perturbations of the axons within a specified region around each axon, shown in figure 4.7, which yielded an FVF of 0.53.

It was tested whether the signal, originating from a model with high dispersion, could be approximated by a 2D model with one magnetic field orientation. However, it was found that the frequency spectrum of the dispersion model was too complex to be approximated by one single magnetic field orientation. Therefore, orientations computed according to the von Mises-Fisher distribution (vMF) were used to introduce dispersion in 2D models. This distribution is used in directional statistics, which describes a probability distribution on a sphere,  $S^{D-1}$ , in *D* dimensions [43]. Its parameters include a concentration coefficient,  $\kappa$ , and a mean direction,  $\vec{\mu}$ . If  $\kappa \to 0$ , the values of the probability density function are spread in the sphere. However, if  $\kappa \to \inf$ , the von Mises-Fisher distribution approaches a dirac function at the mean orientation,  $\vec{\mu}$ . [44] By relating  $\kappa$  to the dispersion value,  $\sigma$ , calculated via FSL definition 4.1, orientations around  $\vec{\mu}$  could be simulated, see figure 4.7c. 50 orientations around  $\vec{\mu}$  were calculated, which corresponded to the same dispersion value as ob-

tained within the manipulated 3D EM data. The generated orientations were then used as the main magnetic field orientation in 2D to approximate fibers' orientation dispersion in the 2D models. Thus, the signal was simulated for each orientation and afterwards, the signals from all orientations were averaged.

Finally, the signal comparison between the 3D model based on 3D EM data with artificial dispersion, five 2D realistic models without dispersion and with dispersion was conducted for  $\Theta = 0, 15, 30, 45, 60, 75, 90^{\circ}$ , shown in figure 4.8. In order to obtain a more intuitive depiction of the observed differences, the RMSE, computed according to equation 3.1, was related to the observed differences between the signals computed for  $\Theta = 0^{\circ}$  and  $\Theta = 90^{\circ}$  for the 2D model. The RMSE, with respect to  $\Theta$ , is shown in table 4.1.

Table 4.1: RMSE values between GRE signals for different  $\Theta$ . Signal of reference is the signal for  $\Theta = 0^{\circ}$ .

Θ[°]	RMSE [%]
0	0
15	0.5
30	2
45	4
60	7
75	9
90	10

### 4.2 Results

In figure 4.1 it can be seen that the pre-processing steps enabled to remove falsely labeled structures within the intra-axonal compartment. It is demonstrated that intra-axonal compartment sometimes exhibited a structure falsely labeled as myelin (yel-low dot in intra-axonal compartment). Furthermore, non-closed myelin sheaths were automatically closed. The successful corrections are indicated by the green arrows. However, some segmentation errors still persisted as indicated by the red arrow.



Figure 4.1: Results of automated pre-processing steps. (a) Shows the 3D model based on non-preprocessed axons and image (b) demonstrates the 3D model with corrected axons. Green arrows indicate resolved segmentation problems and the red arrow indicates persisting segmentation error.

A 3D model containing 365 axons with a FVF and g-ratio of 0.49 and 0.67, respectively, was created based on the myelin mask shown in image 4.2b. Figure 4.2c and d show the individual orientations of each axon within the used EM data, demonstrated in figure 4.2b. It is shown that most axons are mainly oriented along the z-direction. Thus, the average orientation,  $\vec{\mu}$ , of all axons was found to be mainly along z, see figure 4.2e with an angle of  $\approx 6.5^{\circ}$  with respect to the z axis. Furthermore, most of the orientations do not deviate much from the average orientation, see figure 4.2c. For the shown data set, dispersion was found to be  $\sigma = 0.04$ .



Figure 4.2: Raw 3D EM data (a) and myelin segmentation (b) of size 400x400x400.
(c) Frequency histogram of the computed orientations present in the EM model. (d) Depicts the individual orientations of each axon within the sub matrix and image (e) the average orientation, *µ*, of all axons.

Figure 4.3 shows the computed phimap and thetamap for the given axon of the 3D EM data. It is demonstrated that the phospholipids are radially oriented within the myelin sheath, which can be consistently observed throughout the slices. Furthermore, the elevation of the lipids is demonstrated and it is shown that  $\theta$  varies along the z direction of the axon. The orientation of the axon, with respect to the z axis, was found to be  $\theta \approx 9^{\circ}$ .



**Figure 4.3:** The orientation of the phospholipids inside the myelin sheath is given by the azimuth angle,  $\phi$ , and the elevation,  $\theta$ . (a) Computed  $\phi$  values for one axon in 3D. (b) Computed  $\theta$  values for one axon in 3D. Figure 4.4 demonstrates the computed field perturbations for  $\Theta = 0,90^{\circ}$  for the given 3D EM model. The frequency histogram for the case when the magnetic field is applied parallel to the axons is shown in (c). It can be seen that myelin is the dominant compartment which exhibits field perturbations of  $\approx$  -2Hz. However, it is demonstrated that myelin exhibits a slightly larger spread of different frequencies within a range of  $\approx$ -4 to 1Hz. Furthermore, the intra- and extra-axonal compartment are not consistently  $\approx$  0 instead, they exhibit a broader frequency profile of  $\approx$  -1.5 to 1.5Hz.

The frequency histogram for  $\Theta = 90^{\circ}$  is given by figure 4.4d. It is observed that each compartment contributes to the field perturbations by exhibiting a broad frequency spectrum. The intra-axonal compartment's frequency spectrum is mainly negative and ranges from  $\approx$  -10 to 1 Hz. Myelin is observed to exhibit a frequency range of  $\approx$  -5 to 15Hz. The extra-axonal compartment's field perturbations cover a range of  $\approx$  -5 to 5Hz.



**Figure 4.4:** Field perturbations computed with 3D EM model for different magnetic field orientations. (a) Computed field perturbations for  $\Theta = 0^{\circ}$  and the corresponding frequency histogram is shown in (c). (b) Computed field perturbations for  $\Theta = 90^{\circ}$  and the corresponding frequency histogram is shown in (d).

The signal comparison between the 3D model based on 3D electron microscopy data and multiple 2D realistic models, is shown in figure 4.5. It is demonstrated that the differences between the signal from the 2D compared to the 3D model vary with respect to  $\Theta$ . For  $\Theta = 0, 15, 30^{\circ}$ , the differences are about 1.5% to 2% and it is shown that almost all 2D realistic models exhibit the same differences. The highest difference between the 2D models and the 3D EM model is seen for  $\Theta = 30^{\circ}$ . However, for larger  $\Theta$  values it is shown that the differences between the 2D models are larger and are the highest for  $\Theta = 75, 90^{\circ}$ . An average RMSE of  $\approx 1.6\%$  over all models and all values for  $\Theta$  was computed.



Figure 4.5: RMSE comparison between realistic 2D models and 3D model based on 3D electron microscopy data for different main magnetic field orientations.

Figure 4.6 depicts the signal comparison between the 3D EM model and various slices of the 3D EM data (2D EM model). For  $\Theta = 0^{\circ}$ , the RMSE is 1.4% and decreases to 0.35% for  $\Theta = 45^{\circ}$ . Afterwards, the RMSE increases to 2.5% for  $\Theta = 90^{\circ}$ . It is shown that the various slices exhibit small differences for  $\Theta = 0, 15, 30, 45^{\circ}$ , but larger differences for  $\Theta = 60, 75, 90^{\circ}$ . An average RMSE of  $\approx$  1.3% over all slices and all values for  $\Theta$  was calculated.



Figure 4.6: RMSE comparison between various 2D slices of 3D EM data and 3D model based on 3D electron microscopy data.

Figure 4.7 represents the used strategy to create and to sample a manipulated 3D model based on 3D electron microscopy data with artificially induced dispersion. Image (a) shows the 60 selected axons that differed the most from the average orientation and thus, corresponded to a dispersion value of 0.4. Afterwards, a 3D mask was created, which was dilated around each axon, shown in 4.7b. Image 4.7c represents the orientations simulated from the vMF distribution that correspond to a dispersion value of 0.4.



Figure 4.7: Strategy of sampling artificial 3D EM dispersion model. (a) Selected axons that corresponded to a dispersion of 0.4. (b) Dilated 3D mask.(c) Shows the histogram for 200 orientations that correspond to a dispersion value of 0.4 simulated according to the vMF distribution.



**Figure 4.8:** RMSE comparison between the 3D EM model with 0.4 dispersion and multiple 2D realistic WM models (RM) with and without dispersion.

Figure 4.8 depicts the RMSE comparison between the 3D EM model with artificial dispersion of 0.4, and several realistic 2D models (2D RM) with and without taking dispersion into account. It is shown that the realistic 2D models without dispersion exhibit an RMSE of 4.2% for  $\Theta = 0^{\circ}$ . By introducing dispersion into the 2D models it is demonstrated that the differences between the 3D model and the 2D models with dispersion, decrease consistently. The 2D models with dispersion of 0.4 exhibit the lowest RMSE for all  $\Theta$  values. However, for  $\Theta = 60^{\circ}$ , the differences between the 2D models with and without dispersion are the smallest. Nevertheless, it is demonstrated that dispersion has a high impact on the signal simulations for  $\Theta = 0, 15, 30, 75, 90^{\circ}$ .

### 4.3 Discussion

#### Pre-processing

The performed pre-processing steps enabled to improve the quality of the model. It can be seen that most marked areas in figure 4.1 were fixed after the pre-processing steps. However, some errors persisted, which could not be fixed (see red arrow), which negatively influenced the comparison.

#### Fibers' orientation and dispersion

The method to compute the orientation of each fiber was tested for the rotated 3D axon demonstrated in Chapter 3 and was seen to be sufficient. However, this axon was replicated along z and thus, its direction is accurately approximated by a line. It was found that the axons in the 3D EM data set exhibited very complex geometries, as it can be seen in figure 4.9.



Figure 4.9: Complex axon shape of 3D EM data with its computed orientation,  $\vec{x_i}$ .

It can be seen that the orientation,  $x_i$ , approximates the orientation of the axon sufficiently. However, the bump in the middle part of the axon is not taken into account, which might indicate that the representation of very complex axon orientations might not be accurately enough. Therefore, it could be suggested to divide the axon into several sections along the z direction and to compute the axon's orientation section-wise. This would enable to obtain a more detailed depiction of the axon's orientation.

Another suggestion in order to obtain a more in-depth representation of each axon's orientation would be to use an internal dispersion coefficient,  $\sigma_{int}$ . This parameter would represent the internal dispersion of a single axon. For instance, one

could consider to compute the slope of the axon in each slice and therefore, obtain the directional gradient. By relating the slope to the average orientation,  $\vec{\mu}$ , one could quantify the amount of internal dispersion for each axon individually.

For the used 3D EM model, dispersion was found to be low, because most fibers run along z, and thus, the deviation between the main orientation and each axon's orientation is small, see figure 4.2d. The low dispersion arises from the fact that the sample only depicts a very small portion of white matter. Furthermore, the sample was taken from a region which is characterized by low dispersion, as reported by Lee et al [45].

#### Phospholipids' orientation

Due to the complex geometry of the axon, the elevation of the phospholipids inside the myelin sheath varies throughout the slices. However, an average orientation of  $\theta = 8.8^{\circ}$  for the axon was found, which is in line with the corresponding thetamap. Nevertheless, slightly higher values are observed in the shown thetamap, which is attributed to the complex shape of the axon in 3D.

#### Field Perturbations

Due to the fact that the average orientation,  $\vec{\mu}$ , only exhibited a small deviation from the z axis, the applied field for the 3D model corresponded to  $\Theta_{3D} = \Theta_{2D}$ and thus, was not adjusted with respect to  $\vec{\mu}$ . According to table 4.1, the difference between the signal for  $\Theta = 0^{\circ}$  and  $\Theta = 15^{\circ}$  is really small. Thus, it was seen to be valid to use the the same field's orientation as for the 2D model.

The field perturbations for the 3D EM data exhibit a slightly larger spread than the ideal 3D case shown in Chapter 3. As already mentioned in the discussion part about the pre-processing, some segmentation errors still persisted. These errors influenced the computed field perturbations. As it is shown in figure 4.10, falsely labeled structures that were supposed to be intra-axonal compartment exhibited high field perturbations of up to  $\approx$ 10 Hz, for a magnetic field's orientation being parallel to the z direction. In addition, high peaks of field perturbations were also observed due to the finite extent of some fibers, see 4.10 red arrows.

Furthermore, complex axon shapes in 3D, see figure 4.9, influence the calculation of the field perturbations, which result in inconsistent field perturbations. Due to the complex shape, which is not purely along z, the angle between the fiber and the applied magnetic field varies and therefore, creates different field perturbations throughout the slices.



**Figure 4.10:** One slice of the 3D model with the corresponding slice of the field perturbations calculated for  $\Theta = 0^{\circ}$ . Black arrows indicate segmentation error and red errors indicate finite fibers, or fibers that vanished.

#### RMSE 2D RM to 3D EM

It is observed that the differences between the signals are almost constant and vary between 1 to 2%. As table 4.1 states, this difference corresponds to a  $\Theta$  of 15 to 30%, which is seen to be low and therefore, acceptable. Furthermore, it is seen that some signals exhibited lower differences compared to the 3D signal. This could arise from the fact that some models performed better than others, due to the use of axon shapes which were more similar to the ones observed in the 3D EM data. Furthermore, the field perturbations computed for  $\Theta = 0, 15^{\circ}$  mainly arise from the myelin sheath and therefore, the differences between the 2D models is small, as each model exhibited the same FVF.

#### RMSE 2D EM to 3D EM

It is shown that 2D EM slices exhibit a lower RMSE when compared to the 2D realistic model for  $\Theta = 15, 30, 45, 60^{\circ}$ . This could be attributed to the fact that the 2D EM model is based on the same axon shapes as observed in the 3D model and therefore, approximates the 3D signal more accurately. As Xu et al [40] mentioned, a high diversity of axon shapes is present in white matter and therefore, influences the signal simulation. In addition, several slices throughout the 3D EM data were taken, which results in a small change of the FVF within each model, which could yield higher differences between the 2D models. However, the signals of the 2D realistic models and the 2D EM models for  $\Theta = 0^{\circ}$  exhibited almost the same RMSE owed to the fact that the field perturbations for the case  $\Theta = 0^{\circ}$  only arise from the myelin compartment. Since the FVF is almost consistent within the 2D models, no large differences between the 2D RM and 2D EM models are observed.

#### RMSE - Dispersion 2D RM to 3D EM

If  $\Theta$  is parallel to the axon in 2D, the field perturbations only arise from the myelin. However, if high dispersion is present in 3D, it means that many fibers deviate from the average orientation and therefore, are not parallel to the applied field anymore. As a result, higher field perturbations are observed due to the fact that the angle between the applied magnetic field and the fibers is not 0. Thus, the 2D realistic model without including dispersion exhibits the highest difference for  $\Theta = 0^{\circ}$ .

On the other hand a main magnetic field with an orientation of  $\Theta = 90^{\circ}$ , in respect to the fibers' orientation, causes the highest field perturbations. Since high dispersion is observed in the 3D model, many fibers will also be parallel or almost parallel to the orientation of the applied magnetic field and therefore, exhibit smaller field perturbations. Conclusively, the realistic 2D model without dispersion exhibits higher differences than the one with taking dispersion into account.

It can be seen that for  $\Theta = 45,60^{\circ}$ , the RMSE is low for every model. This could arise from the fact that  $\Theta = 45^{\circ}$  represents a combination of both cases of  $\Theta = 0^{\circ}$  and  $\Theta = 90^{\circ}$ . Thus, a combination of parallel as well as perpendicular oriented fibers, with respect to the orientation of the applied magnetic field, are present within the dispersion model. Therefore, the field perturbations for the 2D model with  $\Theta = 45^{\circ}$  and the 3D EM model with dispersion are more similar and therefore, the simulated signal.

However, figure 4.8 reveals that high dispersion indeed influences the signal simulations. Furthermore, the approach to compute the orientations according to the vMF distribution and to take them as the orientations for the applied magnetic field, improves the accuracy of the realistic 2D WM models.

It is important to note that the way of creating dispersion artificially does not represent the real 3D case. Since a mask is used and the field perturbations are only sampled within a defined region around each axon, the influence of field perturbations exhibited by adjacent axons is not considered.

### 4.4 Conclusion

The results of this chapter revealed that the developed realistic 2D WM model accurately simulates the GRE signal when compared to a more realistic 3D model based on 3D electron microscopy data of white matter. Furthermore, it was demonstrated that high dispersion influences the GRE signal simulation and therefore, should be taken into account. The proposed technique to incorporate dispersion in 2D models performed superior to the 2D models with taking dispersion into account.

## **Chapter 5**

## Discussion

This thesis compared 2D and 3D realistic models of white matter in regards to the accuracy of 2D models. A comparison between 2D and 3D models is summarized in table 5.1.

2D Models				
Pros	Cons			
Faster calculations	Less complex axon shapes			
Straightforward change of parameters	Does not intrinsically account for fiber dispersion			
Less memory required	Infinitely long fibers			
Less prone to calculation errors				
Enables modeling of abundant amount				
of axons				
2D EM segmentations common				
3D Models				
Complex/ realistic axon shapes	Longer calculation time			
Accounts for fiber dispersion	3D is more complex to model			
Discretized fiber length	More memory required			
	Prone to calculation errors			
	3D EM segmentations less common			

#### Table 5.1: Pros and cons of 2D and 3D WM models to simulate the GRE signal.

Even though 3D models depict the real case more accurately, they are more prone to error and computationally inefficient. Furthermore, 3D segmentations of WM are not common yet. In addition, Chapter 4 demonstrated that 2D models accurately simulate the GRE signal when compared to a more realistic 3D model based on 3D EM data and therefore it can be concluded that 2D models of white matter accurately approximate the GRE signal.

It was found that the initial segmentation of the 3D EM data was partly incorrect, which caused problems when the field perturbations were calculated. However, the proposed algorithm fixed most of these issues, but some errors still persisted which influenced the signal comparison, especially for the case in which the main magnetic field was applied parallel to the axons. Thus, it is suggested to implement a more sophisticated algorithm to process the 3D EM data, which could improve the comparison. Unfortunately, not many 3D segmentations of white matter are available at the moment. Abdollahzadeh et al [46] have recently proposed a method to segment axons of white matter in 3D, which could be used to re-segment the available EM data. Furthermore, investigating more 3D EM data to study dispersion in more detail could be useful. This would enable to investigate the influence of dispersion on the simulated GRE signal in a more realistic setting.

For the conducted comparison, 10 realistic 2D white matter models were used. It was demonstrated that some models performed better than others. Conclusively, repeating the comparison but with many more realistic 2D WM models, for instance 1000, could exhibit an even better fitting model. Therefore, it is suggested to perform the comparison again in order to find the best fitting model. Thus, it would be possible to determine to which extent the realistic 2D WM models can approximate the GRE signal simulated with the 3D model based on EM data. However, this would require a perfect 3D segmentation of axons.

Another important aspect to mention is that dispersion was artificially introduced in the 3D EM model. By applying the mask, the effect of adjacent axons was neglected, which is not very accurate. However, the observed dispersion in the available 3D EM data was low and thus, an alternative solution with the artificial dispersion was found. As Mollink et al [47] have demonstrated, fiber's orientation dispersion can be higher, especially in the medial region of the corpus callosum. Therefore, it is suggested to investigate more 3D segmentations of white matter with the use of the developed model presented in Chapter 3. This would enable to further study the influence of dispersion in a more realistic model.

During this project a three-compartment 3D model to compute the GRE signal based on myelin's microstructure in 3D was developed. However, the 3D model could be further extended by including other cells besides neurons such as glial cells, e.g. oligodendrocytes in 3D. In addition, incorporating chemical exchange in

the 3D model could be useful to make it more realistic.

Furthermore, developing a 3D model that artificially creates dispersion by rotation of fibers could be useful to correctly create dispersion in 3D data. It could be implemented in such a way that one fiber is placed in the center and each next fiber is rotated according to an orientation computed via the vMF distribution and placed next to the first fiber. Then, the repulsive force is calculated between them and if it is in an acceptable range, the fiber is placed at that spot. This way would also enable to manipulate the FVF in a complex 3D dispersion model. Furthermore, checking for overlapping axons should be performed. 

## **Chapter 6**

## Conclusion

During this thesis, a 3D model to simulate the multi-echo GRE signal, altered by white matter microstructure, was successfully developed. It has been demonstrated that a more realistic 3D model is useful to verify and to improve 2D white matter models.

Chapter 3 described the successful development of the 3D model. It has been proven that the 3D model yields reliable results and is therefore valid to be used to simulate the GRE signal based on 3D white matter segmentations.

In Chapter 4, the 3D model based on electron microscopy data was used to investigate how accurate 2D white matter models can simulate the signal when compared to a more realistic 3D model. It has been demonstrated that 2D models approximate the investigated 3D case accurately. Furthermore, a solution to account for dispersion in 2D models was proposed and it was shown that this approach improves the signal simulation of 2D models, by approximating the 3D signal more accurately. Therefore, it could be useful to extend the current dictionary by a new parameter: dispersion. Simulating the GRE signal with respect to different dispersion values could enable a more accurate encoding of the underlying microstructural features of WM.

Due to the results of the conducted comparison with a more realistic 3D WM model, it can be concluded that it is sufficient to use 2D WM models in order to encode microstructural features of WM from the  $T_2^*$  decay curve. However, future work is needed to investigate more 3D EM data sets to make a robuster conclusion about the extent of the effect of dispersion. Nevertheless, the developed 3D model offers an important basis for realistic WM modeling in the future.

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