ADvanced Diffusion MRI Tractography

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Abstract

The emergence of new neuroimaging techniques combined with sophisticated computational models and algorithms allows to non-invasively probe the structure of the brain in-vivo. Diffusion magnetic resonance imaging (dMRI) is a compelling tool for investigating the structure and geometry of brain tissue based on indirect measurement of the diffusion anisotropy of water. Tractography algorithms are able to reveal global fiber constructs by estimating continuous streamline connections based on the local diffusion information. These techniques contribute to a better understanding of neurological and neuropsychiatric disorders.

However, tractograms are biased by algorithmic parameters, affected by partial voluming of different fiber populations or various tissue types and thus it is difficult to reliably extract biologically meaningful and quantitative measures. Furthermore, the adaptation into clinical research practice is challenging due to time constraints during the acquisition and the difficulty in standardizing diffusion datasets for group comparison.

In this thesis, a flexible and modular software framework is proposed to perform various evaluations and analysis on subject- and group-level, in order to incorporate recent innovations and simplify the processing of structural diffusion MRI datasets. The quality assessment and monitoring is particularly important in patient populations and
multiple tools have been developed to identify various artifacts. A special focus was put on the validation and quantification of tractograms.

The potential of quantitative streamline densities as a meaningful biological marker has been examined in various patient populations. Furthermore, the transfer from high quality acquisition to clinically feasible sequences was studied with respect to the novel diffusion measures. An estimation of axon packing density at the gray to white matter interface was derived, which revealed striking resemblance to myelin estimations from other modalities. An application of fiber density measures in comparison to tensor-based disease measures has been studied in patients suffering from amyotrophic lateral sclerosis, revealing an increased sensitivity to neurological degeneration.
Kurzfassung


In dieser Arbeit wurde ein flexibles und modulares SoftwareFramework entwickelt, welches die Analyse von Diffusions-MRI Daten stark vereinfacht und die Integration und An-

Die Quantifizierung der Faserdichte wurde in verschiedenen Patientengruppen untersucht und hat sich als sinnvoller biologischer Marker erwiesen. Die Durchführbarkeit von zuverlässigen Faserquantifizierungen wurde in Bezug auf verkürzte klinische Diffusions-MRI Sequenzen analysiert und die Berechnung der Faserdichte an der Grenze zwischen der Weissen und Grauen Substanz hergeleitet. Diese Faserdichte zeigt auffällige Ähnlichkeit mit der Schätzung der Myelindichte.

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Chapter 1

Introduction

The complexity of the internal structure makes the brain especially difficult to study and understand. The irregularly folded outer shell, the massive maze of its trillions of wires and the fact that we are most interested in the internal workings and signaling dynamics are only some of the challenges. Histology and tracer studies revealed the anatomy of axon bundles connecting distant cerebral regions. In a network of specialized areas, disconnection leads to dysfunction. This hypothesis is the basis for many neurological disorders. The emergence of new neuroimaging techniques combined with sophisticated computational models and algorithms allows to non-invasively probe the brain in-vivo. Diffusion magnetic resonance imaging (dMRI) is a compelling tool for investigating microscopic tissue properties and has become a popular tool to study the white matter architecture in the brain. The typical voxel dimensions of acquired in-vivo diffusion magnetic resonance imaging data are orders of magnitude larger compared to an axon diameter. Therefore, a single data voxel in the brain white matter is intersected by several thousand different axons. Thus, the measured diffusion signal is a voxel-averaged quantity. Additionally, it is ambitious and challenging to derive quantitative measures. Nevertheless, it is possible to construct a virtual dissection of
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the white matter connections. However, besides the insufficient resolution it is also very difficult to quantify or verify and validate the resulting virtual connections.

1.1 Motivation

While dMRI is widely used to investigate structural changes in psychiatric diseases such as schizophrenia, the recent methodological advances are rarely adopted in clinical studies or translated into clinical practice. Besides hardware limitations and time constraints, standardized tools and procedures have not yet emerged to reliably analyze dMRI datasets in a consistent manner. Moreover, a robust measure to quantify the strength of connectivity between cortical areas might be one of the missing puzzle pieces in order to diagnose and further categorize neurological diseases, especially in early onset stages.

1.2 Outline

Chapter 2 provides a general overview of an extensive pipeline from the diffusion process and its acquisition all the way to a structural connectome. The last part of Chapter 2 is dedicated to the developed framework, which allows to process dMRI datasets on subject and group level using software which was created during the thesis and various external state-of-the-art tools. In Chapter 3, the validation and quality assurance of tractograms are explored by proposing new error measures and a method to improve a given set of streamlines. The transfer of
1.3 Contribution

The analysis of dMRI datasets is a complex and by no means standardized procedure. A lot of expertise and manual monitoring and validation of each processing step is crucial in order to attain meaningful structural biomarkers. To simplify the incorporation of recent innovations into the analysis pipeline for clinical research purpose, a flexible and modular framework was developed to perform various evaluations on subject- and group-level. An important part of the framework are the interfaces between different file formats, conventions, software tools and verification methods. Additionally, many new processing routines for various processing stages have been developed. A special focus was put on the extraction of connectivity measures from biased tractograms by optimizing each fiber contribution in order to fit the streamlines to the measured diffusion signal. The relevance and feasibility in a clinical research environment was examined. New biomarkers based on the optimized fiber tracks were estimated and tested in various state-of-the-art acquisitions into a clinical setting is discussed and evaluated with respect to clinically valuable new biomarkers such as axon packing density, intra-cellular cross-sectional area and fiber density at the gray-to-white matter interface in Chapter 4. Results from a study in patients suffering from amyotrophic lateral sclerosis (ALS) are presented in Chapter 5. Chapter 6 summarizes the thesis and discusses further prospects and perspectives.
1. **Introduction**

populations of healthy volunteers and patients suffering from different neurological diseases.
Diffusion magnetic resonance imaging (dMRI) is a compelling tool for probing microscopic tissue properties and has become a popular neuroimaging method which enables to virtually dissect the human brain.

dMRI acquires one or more $T_2$-weighted reference images and a collection of diffusion-weighted image volumes, in which the $T_2$ signal is attenuate according to the amount of diffusion along prescribed gradient directions. This information can be used to model the directionality of hindering and restricting tissue compartments and the integration of these extracted directions can be incorporated in the construction of spatial trajectories. Virtual streamlines enable the study of axonal connections between functional units of the cortical and subcortical gray matter.

### 2.1 Diffusion

Molecular diffusion is a process that occurs in biological tissue, fluids in particular. Self-diffusion of water molecules can be modeled as a random walk. The dMRI signal measures the history of random displacement of labeled hydrogen spins resolved in the direction of the magnetic field gradients. The cumulative phase change in the spins reflects the changes
in the position-dependent spin frequency induced by the field gradient. Components of the diffusion motion along the direction of the gradient induce such changes. The signal change due to cumulative dephasing is greater when this coincides with a direction that allows greater random displacements, e.g. because of the orientation of a microstructure within which the proton is moving.

In the free diffusion case, the particle motion is spontaneous due to thermal energy. The presence of obstacles other than the diffusion particles is referred to as hindered diffusion. In case of impermeable boundaries, the process is defined as restricted diffusion.

For a homogenous distribution of obstacles or the absence of any boundaries, the diffusion is isotropic and directionally independent. If directed obstacles or barriers are present, the diffusion becomes directionally dependent, and therefore anisotropic, which means that the mean square displacement of the particles is greater along some directions than along others.

2.2 Pipeline Overview

A schematic overview from the diffusion acquisition to the construction of a connectome [1, 2] is given in the following.
Many steps are required in order to get from the acquisition in the scanner to a meaningful connectome. First of all, the image resolution, choice of gradient directions and q-space sampling scheme needs to be addressed for the acquisition. After the data collection, necessary pre-processing steps are performed, e.g. motion and eddy-current correction. A crucial step in every diffusion pipeline is the quality assurance of the diffusion datasets. All datasets get checked for artifacts, excessive head motion, physiological noise et cetera. The next step is the fitting of a diffusion model in each voxel. This step is crucial for the extraction of the directional information which is the basis
for the tractography algorithm. Streamlines can be generated by starting in a seed voxel and proceeding in the most likely direction traversing through the vector field extracted from the diffusion model. The collection of virtual fibers are then clustered or segmented by e.g. intersecting each streamline with regions of interest (ROI) derived from a cortical parcellation performed on an anatomical scan. The final connectome can now be constructed by listing all the connections and applying a desired connectivity measure between distinct cortical areas.

2.3 Acquisition

The most widely used pulse sequence for generating diffusion-weighted images is called Pulsed Gradient Spin Echo (PGSE), also known as the Stejskal-Tanner sequence. It is characterized by a $90^\circ$-$180^\circ$ spin echo pair of radio frequency (RF) pulses with a pair of diffusion sensitizing gradients with strength $G$ around the $180^\circ$-pulse with a gradient duration of $\delta$ and spacing of $\Delta$. The first gradient pulse is introducing a phase shift $\varphi_1$. The spin position $r(t)$ is assumed to be constant during the pulse duration (narrow pulse assumption: $\delta \ll \Delta$)

$$\varphi_1 = \gamma \int_0^\delta G \cdot r(t) dt = \gamma \delta G \cdot r_0$$  \hspace{1cm} (2.1)

where $\gamma$ is the gyromagnetic ratio of the hydrogen atom. After inverting the spins by the $180^\circ$ RF pulse, an identical gradient $G$ is refocusing the spins

$$\varphi_2 = -\gamma \int_{\Delta}^{\Delta+\delta} G \cdot r(t) dt = -\gamma \delta G \cdot r_\Delta$$  \hspace{1cm} (2.2)
2.3. Acquisition

where $r(t)$ is again assumed constant during the second gradient. The induced phase shift after the full sequence is given by \( \varphi = \varphi_1 + \varphi_2 = \gamma \delta G (r_\Delta - r_0) \). A loss of phase coherence due to diffusion leads to an attenuation of the spin-echo signal. The diffusion factor $b$ or $b$-value is defined by the acquisition parameters:

\[
b = (\gamma G \delta)^2 \left( \Delta - \frac{\delta}{3} \right)
\]

with $\gamma$ as the gyromagnetic ratio, $G$ the amplitude of the applied diffusion weighting gradients, $\delta$ the duration of a single gradient pulse and $\Delta$ describing the spacing of the midpoints of the two gradients. However, this formula is only valid for rectangular diffusion gradients with zero rise time and neglecting the effect of other imaging gradients.

### 2.3.1 Q-Space Sampling Schemes

In dMRI, the observed signal is generated from an average over all spins in each voxel. The resulting ensemble average propagator, diffusion propagator or probability density function (PDF) $P(\vec{r}, \tau)$ is the relative spin displacement in the experimental diffusion time $\tau$.

The PDF $P(\vec{r}, \tau)$ is related to the measured signal by the Fourier relationship:

\[
P(\vec{r}, \tau) = \mathcal{F} \{ S(\vec{q}, \tau) \}\]

where $\mathcal{F}$ denotes the Fourier transform with respect to the diffusion wavevector $\vec{q}$. The diffusion wavevector $\vec{q}$ is defined as

\[
\vec{q} = \frac{\gamma}{2\pi} \int_0^\delta \vec{G}(t) dt
\]
As a side note, the q-value ($|\vec{q}|$) can be related to the $b$-value by incorporating the diffusion time $\tau$. Even though, many times only the $b$-value is reported, in order to fully characterize the diffusion experiment, the diffusion time $\tau$ or the timing parameters of the diffusion gradients ($\delta, \Delta$) are also needed ($b = |\vec{q}|^2 \tau$).

Different sampling strategies have been used in order to describe the diffusion propagator (PDF). The q-space sampling scheme is typically also intertwined with the mathematical description or model of the PDF. Common sampling schemes use equidistantly spaced sampling points on a spherical shell with a fixed $b$-value (single-shell), multiple $b$-values (multi-shell) or a full Cartesian sampling of the q-space (diffusion spectrum imaging). Due to the symmetry of the Fourier-space, some acquisition schemes acquire only one half of the q-space.

### 2.3.2 Parallel Imaging

In-plane parallel imaging subsamples the $k$-space by only acquiring every $R$th line reducing the duration of the readout by an acceleration factor of $R$. However, this leads to aliased images. In order to unfold the resulting images, the reconstruction algorithm uses estimates of coil sensitivities to resolve the spatial information in overlapping regions. The widely used techniques are SENSitivity Encoding (SENSE) for fast MRI [3] and Generalized autocalibrating partially parallel acquisitions (GRAPPA) [4]. The application of parallel imaging techniques in diffusion sequences reduces the scan time by shortening the
2.3. Acquisition

readout part, however, for large $b$-values, the total reduction in scan time is only minor. Nevertheless, the shortening of the readout will decrease susceptibility induced distortions. Parallel imaging leads to a reduced signal-to-noise ratio (SNR) due to the acquisition of fewer lines in $k$-space (factor of $\sqrt{R}$ less SNR). Additionally, the SNR is decreased by the condition of the inversion problem. Therefore, the SNR loss depends on the acceleration factor $R$ and the geometry factor ($g$-factor) [3].

2.3.3 Spatial Multiplexing (Simultaneous Multislice)

Extensive q-space sampling schemes increase the total scan time and are often unfeasible, especially in clinical practice. However, recent advancements allow to parallelize the acquisition of multiple slices to decrease the total scan time significantly [5]. Multiple slices are excited simultaneously and the information of multiple coils is used during reconstruction to unfold and separate the different slices. The spatial multiplexing is closely related to the under-sampling of the $k$-space in parallel imaging, however, the under-sampling occurs across slices. This is especially beneficial for sequences with long repetition times (TR), such as diffusion weighted sequences with high $b$-factors, because the total repetition time is reduced by the multislice acceleration factor. Nevertheless, the reduction in repetition time comes with a loss of SNR due to a decrease in the number of acquired samples and an increase in $g$-factor, which also depends on the spacing of the simultaneously excited slices. The $g$-factor penalty can be decreased by introducing a phase-shift between the acquired slices [6].
2. Methods

2.3.4 Temporal Multiplexing

Besides the spatial multiplexing of multiple slices, a temporal parallelization can also be achieved by consecutively exciting multiple slices but separating the k-spaces for each slice in readout direction. A single 180°-pulse is used for all the excited slices. The readout needs to be modified in order to traverse the magnified k-space [7]. Unfortunately, this method is prone to increased susceptibility artifacts due to a prolonged readout and the echo times between different slices will slightly differ due to the excitation pulse separation.

2.4 Pre-Processing

In this section, we refer manipulation of the raw diffusion data performed prior to the fitting of a model. The pre-processing part should aim to minimize motion artifacts between images, eddy-current or susceptibility induced distortions. The importance of an adequate pre-processing pipeline is often underrated. Even though, the possibilities highly depend on the data, the choice of pre-processing steps are almost treated as philosophical choices or individual flavoring. The choice of the applied pre-processing steps may largely influence outcome of diffusion model estimations, tractograms and connectomes.
2.4. Pre-Processing

2.4.1 Quality Assessment

It is very important to inspect the quality of the acquired image volumes. Apart from a visual assessment and the search for various artifacts, some automated checks simplify the quality check enormously. Helpful maps to spot artifacts or outlier volumes are e.g. the residuals of the overdetermined tensor fit. If one of the diffusion volumes is contaminated due to head motion, the residual of this particular diffusion direction will be much larger compared to the other directions. Therefore, the sum of residuals per slice and diffusion direction is a valuable indicator for directionally (or temporally) dependent artifacts. Additionally, it is important to inspect the volumes in all three orthogonal planes, to e.g. identify inter-slice instabilities which might arise from cross-talk or fat-suppression issues. Details about our approach can be found in the quality assessment part of Chapter 5.2.

2.4.2 Denoising

Unfortunately, due to the signal-attenuation induced by diffusion-sensitization and $T_2$-relaxation resulting from the long echo time necessary to accommodate diffusion gradient pulses, the signal-to-noise ratio (SNR) of the dMRI signals is inherently low [8]. The SNR gets even worse for higher $b$-values due to the fact that the gradient strength is limited. Therefore, high $b$-values can only be achieved by increasing the diffusion time $\tau$ and therefore increasing the echo time (TE). Thermal noise that corrupts dMRI measurements propagates to the diffusion parameters of
interest and, as such, influences the quantitative interpretation of the underlying diffusion process. Although attempts have been made to minimize the noise during the acquisition, the limitation of scan time is typically the defining factor in the protocol optimization. Especially in a clinical setting, the strict limitation of the scan time is unavoidable. Therefore, image denoising, i.e. minimizing the variance of the dMRI signals in a post-processing step, can be applied. Fortunately, it has been shown that typical dMRI data exhibit sufficient redundancy due to common practice of oversampling the q-space. However, distinction between signal-carrying features and noise related variance is challenging. Arbitrary thresholding might lead to the loss of signal if the reduction of noise is taken too far. Recently, an elegant, well accepted and already widely employed method was introduced by [9] based on random matrix theory. The proposed denoising technique promises preserving local signal fluctuations of any origin different than thermal noise, including fine anatomical detail.

2.4.3 Motion and Distortion Correction

The correction of distortion and motion generally implicates an image interpolation step at some point. Therefore, it is beneficial to estimate a combined deformation field and apply the resulting correction in a single step for all the corrections. Nevertheless, each step will be described separately in the following.
Eddy Currents

The rapid switching of large gradients will generate eddy currents in nearby electrically conductive elements (also in the gradient coils themselves). These currents will produce time-dependent magnetic fields of typically zero, first and second spatial order. Therefore, the sampled trajectory in $k$-space during the readout will be modified and geometric distortions are introduced. Additionally, the diffusion-sensitizing gradients will also deviate from the nominal shape, which will influence the actual $b$-factor and diffusion direction. Besides multiple correction strategies during the acquisition or image reconstruction, image registration based techniques have been developed, which are able to correct for geometric distortions [10, 11, 12].

Head Motion

The presence of head motion or a general misalignment between different image volumes can be assessed by computing the standard deviation across all the diffusion-weighted images. The outer edges of the brain present a high standard deviation in case of motion. The prevention of head motion in the first place is clearly beneficial to any post-processing strategy. Registration tools can correct for a rigid misalignment of an image volume, however, head motion can also induce signal dropouts. To a certain degree, corrupted diffusion directions can be tolerated, if treated appropriately during the model fit (e.g. [13]). Nevertheless, an image-based registration will also affect the diffusion direction in the gradient table [14]. This
becomes even more problematic, if single slices are reoriented (in contrast to total image volumes). The change in diffusion direction will then vary across slices in the same volume, which results in a spatially varying gradient table.

**Pulsation Artifacts and Physiological Noise**

Cardiac pulsation causes nonlinear motion and local deformations and can corrupt the measured diffusion signal. The resulting signal dropout or residual misalignment between the image volumes will lead to erroneous diffusion models and consequently any subsequently derived measures. Pulsation artifacts are most notably in the brainstem area. To reduce physiological artifacts, the sequence can be triggered to the cardiac cycle. However, the scan time will then depend on the subject’s heart rate and is consequentially prolonged and may become unpredictable.

**Susceptibility-Induced Distortions**

Typical diffusion sequences use single-shot echo-planar imaging (EPI) in order to reduce motion sensitivity. However, these acquisitions suffer from susceptibility-induced distortions. Local magnetic field perturbations are generated at and around boundaries of structures with significantly different susceptibilities (e.g. the air-bone transition of the sinuses). These perturbations lead to an inhomogeneity in the static field and as a consequence, the spatial encoding is corrupted. These artifacts arise mostly in the phase-encoding direction of the EPI
2.4. Pre-Processing

readout. The impact of these distortions becomes obvious if the dMRI data is combined with anatomical image information (e.g. $T_1$-weighted non-EPI images). Therefore, the correction of EPI induced distortions is crucial, especially if e.g. anatomical tissue priors are used during tractography [15] or an anatomical parcellation is used to segment the tractogram.

Most correction methods try to estimate a distortion field from either an acquired field-map or two acquisitions with reversed phase encoding directions [16]. In an ideal case, each diffusion volume would be acquired with both phase-encoding directions, to be able to unwarp and recover the correct image intensities from collapsed regions. However, this prolongs the acquisition by a factor of two. Therefore, a common approach is to only acquire the $b = 0$ image with both phase-encoding directions [12].

2.4.4 Bias-Field Correction

A potential confounder in various image analysis tasks is the presence of a low frequency intensity non-uniformity on the image data. Such bias-fields can be introduced by MRI device related causes including static field inhomogeneity, eddy currents and especially radio frequency (RF) transmission and reception inhomogeneity. On the other hand, bias-fields can also be caused by the imaged object itself, including its shape, position, orientation and its specific magnetic permeability and dielectric properties [17]. The presence of a bias-field becomes especially disruptive in quantitative applications. Numerous methods have been proposed to correct for this artifact either during the
acquisition (e.g. shimming) or during the pre-processing. In dMRI pre-processing, the bias-field is typically estimated on the non-diffusion weighted $b = 0$ image and the inverse of the bias field is multiplied onto each diffusion volume. As long as the correction is multiplicative and not varying across different diffusion direction, the presence of a bias-field can be neglected as long as the diffusion-weighted signal is normalized by the $b = 0$ volume (e.g. for the use of a tensor-model). The applied correction simply cancels out during the normalization process. However, if other diffusion models are applied (e.g. CSD), the diffusion volumes are not normalized by the $b = 0$ reference image and a bias-field will therefore propagate into quantitative measures derived from these models. The state of the art bias-field correction is described in [18].

2.4.5 Gibbs Ringing

The existence of Gibbs ringing artifacts in truncated Fourier representations of non-bandlimited functions has been known for a very long time. However, its occurrence in dMRI has been widely disregarded. Recently, the awareness to this phenomenon and its consequences was raised in the community [19, 20]. Although, ringing is a feature of all MRI images obtained via inverse Fourier transform, it becomes increasingly significant for low resolution and quantitative modalities such as dMRI. The introduced bias of at most 9% of the intensity step can be amplified tremendously in derived parametric maps. A common appearance of this phenomenon is a “negative diffusion” near the ventricle borders, which can manifest as black
2.5. Diffusion Models

voxels in e.g. the fractional anisotropy (FA) map. A suitable method to spot Gibbs ringing is a binary map that flags e.g. negative eigenvalues of the tensor fit, which correspond to "negative diffusion" which is physically impossible.

Besides spatial smoothing, which inherently lowers the spatial resolution of the image and introduces additional partial volume effects, a regularized extrapolation of the \( k \)-space has been proposed [20].

**2.5 Diffusion Models**

The Bloch equations describe the nuclear magnetization as a function of time. The formula was extended by Torrey’s inclusion of relaxation due to diffusion. The Bloch-Torrey equation relates the applied magnetic field vector \( \vec{B} \) to the magnetization vector \( \vec{M} \) in the laboratory frame of reference:

\[
\frac{\partial \vec{M}}{\partial t} = \gamma \vec{M} \times \vec{B} - \frac{M_x \vec{i} + M_y \vec{j}}{T_2} - \frac{(M_z - M_0) \vec{k}}{T_1} - \nabla \cdot (D \nabla \vec{M})
\]

(2.6)

\( \gamma \) is the gyromagnetic ratio, \( T_1 \) and \( T_2 \) refer to the longitudinal and transverse relaxation times and \( M_0 \) is the magnetization at equilibrium in the direction of the z-axis. \( \vec{i}, \vec{j} \) and \( \vec{k} \) are the unit vectors pointing along the x-, y- and z-axis of the laboratory frame. The incorporated diffusion term is derived from Fick’s law, whereby \( D \) stands for the diffusion tensor, which defines the orientation dependence of the diffusion process. The diffusion term can be reduced to \( D \nabla^2 \vec{M} \) when \( D \) is constant (in case of isotropic diffusion). Hall et al. [21] introduced a matrix formulation with a generalized flux term.
The applied magnetic field $B_0$ is assumed to be in the lab frame’s z-axis. The derived generalized equation can be written in the following compact form

$$\frac{d\vec{M}}{dt} = R\vec{M} - \nabla J(\vec{M}) \quad (2.8)$$

with an arbitrary transport process given by $J(\vec{M})$.

### 2.5.1 Diffusion Tensor Imaging

In the diffusion tensor model, the diffusion propagator is assumed to be Gaussian and is therefore given by a 3-dimensional Gaussian distribution and derived from (2.8) with the flux term $J(\vec{M}) = -\vec{D}\nabla \vec{M}$. Besides the relaxation terms from the Bloch equation which are typically collapsed into the non-diffusion weighted pre-factor $S_0$, the solution yields an additional diffusion related attenuation term

$$S(\vec{q}, \tau) = S_0 \cdot e^{-\tau \vec{q}^T \vec{D} \vec{q}} \quad (2.9)$$

or equivalently

$$S(b, \vec{g}) = S_0 \cdot e^{-b \vec{g}^T \vec{D} \vec{g}} \quad (2.10)$$

whereby $\vec{g}$ describes the normalized vector of the applied diffusion gradient. The diffusion coefficient $\vec{g}^T \vec{D} \vec{g}$ is often referred
to as apparent diffusion coefficient (ADC) and is linearly proportional to the logarithm of the signal attenuation:

$$ADC = \frac{\ln\left(\frac{S(b, \vec{g})}{S_0}\right)}{-b}$$ (2.11)

Mathematically, the tensor is defined as a symmetric positive definite $3 \times 3$ matrix $D$:

$$D = \begin{pmatrix} D_{1,1} & D_{1,2} & D_{1,3} \\ D_{2,1} & D_{2,2} & D_{2,3} \\ D_{3,1} & D_{3,2} & D_{3,3} \end{pmatrix}$$ (2.12)

The diffusion tensor consists of six unknown entries, given that the sign of diffusional displacement is not captured, and therefore the minimal requirement of the acquisition is six distinct diffusion directions supplemented by a non-diffusion weighted $b = 0$ volume. By acquiring more diffusion directions, the linear equation system becomes overdetermined and the tensor fit can be solved using a multivariate linear regression or the pseudo-inverse. Decomposing the tensor into its eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and eigenvectors provides the basis for many (still broadly used) tensor metrics. The average ADC, or mean diffusivity

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$ (2.13)

is a valuable marker for ischemia and stroke. The most common parameter however is the quantification of anisotropy, the so-called fractional anisotropy (FA).
\[ FA = \sqrt{\frac{3}{2}} \cdot \sqrt{\frac{(\lambda_1 - \hat{\lambda})^2 + (\lambda_2 - \hat{\lambda})^2 + (\lambda_3 - \hat{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \]  

(2.14)

with \( \hat{\lambda} \) being the mean value of the eigenvalues.

However, the Gaussian assumption will generally be violated in the presence of restricted compartments. Therefore, the diffusion tensor imaging (DTI) model (2.10) was extended in order to estimate excess kurtosis as a metric of non-Gaussianity of the PDF [22]. The flux term is therefore extended with an additional Kurtosis term

\[ J(\vec{M}) = -D \nabla \vec{M} - \frac{1}{3} D \nabla K \cdot D \nabla \vec{M} t \]  

(2.15)

where \( K \) is the fourth order Kurtosis tensor. The solution of (2.15) yields the following

\[ S(b, \vec{g}) = S_0 \cdot e^{-bb_{app}} \cdot e^{-\frac{1}{6}b^2D_{app}^2K_{app}} \cdot \mathcal{O}(b^3) \]  

(2.16)

whereby \( D_{app} \) is the apparent diffusion coefficient and \( K_{app} \) the apparent diffusional kurtosis in the gradient direction \( \vec{g} \).

Nevertheless, the tensor model breaks down as soon as multiple directions of restricted or hindered compartments are present, e.g. in voxels where multiple fiber bundles cross. The estimation of these crossing voxels within the human brain white matter ranges from 30% up to about 90%. As a consequence, there is a considerable demand of being able to resolving more complex fiber geometries in a single voxel.
2.5. Q-Ball Imaging

Q-ball imaging introduced by Tuch et al. [23] was introduced as a fast alternative to reconstruct the fully sampled q-space and the Fourier relation between the PDF and the q-space. The sampling scheme is limited to a single shell and the orientation distribution function (ODF) can be calculated by applying the Funk-Radon transformation.

In comparison to the tensor model, this method is model-free. ODFs are typically decomposed into spherical harmonics (SH) coefficients and the corresponding basis functions. The SH belong to the class of Fourier basis sets and are defined on the surface of the unit sphere $S^2$. The diffusion signal is assumed real and antipodal symmetric, therefore the SH basis is restricted to real symmetric functions.

Many sharpening and regularization techniques have been proposed in order to increase the angular resolution of ODFs. A mathematically elegant solution was proposed in [24]. The Funk-Radon transformation was generalized to a family of transformations, and the Funk-Radon and Cosine Transform (FRACT) in particular yields in improved accuracy and higher angular resolution.

However, it is important to keep in mind that single-shell models can only capture a projection of the PDF and the radial component is sacrificed in favor of a higher angular resolution. In comparison to the tensor model, the diffusion direction(s) cannot be extracted directly. In the tensor case, the eigenvector corresponding to the largest eigenvalue $\lambda_1$ provides a direct estimation of the underlying tissue directionality. In the ODF
2. Methods

In this case, the local maxima of the diffusion distribution need to be extracted first. The so-called peak-finding is an essential prerequisite for most tractography algorithms. A fast and accurate implementation based on a numerical Newton optimization is outlined in [25].

2.5.3 Constrained Spherical Deconvolution

Another prominent diffusion model based on high-angular single-shell acquisitions is the spherical deconvolution [26]. It is assumed that white matter fibers along a single axis have a fixed and identical contribution to the diffusion signal. This contribution or fiber response function is used as kernel. To go from the diffusion signal to a representation of fiber distribution, the signal is deconvolved with the response function. The resulting fiber distribution is called fODF. The response function is typically estimated from the dataset itself by aligning and averaging the signal from voxels with very high anisotropy. The deconvolution is typically constrained with a non-negativity condition, in order to suppress ”negative fibers”. The generation of the response function is a crucial part of this method. Several kernel estimation and iterative calibration methods have been introduced (e.g. [27]) to increase the sensitivity to partial volume effects in the response function and to decrease the occurrence of spurious (false-positive) peaks in the fODF. Recently, the constrained spherical deconvolution (CSD) was extended to a multi-shell, multi-tissue model in order to account for multiple compartments with individual response functions [28].
2.6 Tractography

The aim of fiber tractography is the construction of virtual long-range neuronal pathways supported by the local estimation of the axonal direction. The family of tractography algorithms can roughly be classified into three distinct categories, namely deterministic, probabilistic and global.

2.6.1 Deterministic

First attempts took a streamline approach, whereby starting from a seed-point, the trajectory was integrated along the local diffusion direction. Seeds are typically selected at random or placed in each white matter voxel. Additionally, stopping criteria such as maximum angular change per step size or a minimal FA are deployed. Beyond the tensor model, a choice of direction has to be made. Typically, the ODF peak with the smallest angular deviation with respect to the incoming fiber direction is pursued.

However, deterministic algorithms suffer from a high sensitivity to local noise in the data. Moreover, a single perturbation can propagate along the trajectory and accumulate, resulting in a false positive connection.

2.6.2 Probabilistic

The rationale behind probabilistic tractography algorithms relates to one of the prominent drawbacks of deterministic meth-
methods. Instead of following an exact diffusion direction, a perturbation is introduced on purpose. At each integration step, a random sample is drawn from a probability distribution derived from the diffusion model. The class of probabilistic tractography algorithms improves the sensitivity of deterministic streamline tractography, however the tendency towards false positives is typically even worse.

2.6.3 Global

A third class of algorithms assesses the tracking problem from a global inverse problem perspective. The general mechanism of these methods consists of iteratively minimizing a cost-function, which relates the current fiber configuration to the measured diffusion signal by applying a forward model. Usually, the fiber configuration consists of short fiber segments which are externally forced to follow the given diffusion directions and internally encouraged to form long chains with neighboring segments. Additional priors are needed to e.g. penalize high-curvature [29].

Global methods are more robust to noise and local reconstruction errors. Additionally, these algorithms provide some quantitative correspondence to the diffusion distribution.

2.7 Top-down Streamline Optimization

Inspired by the global tractography algorithms, a top-down approach was introduced by [30, 31]. Instead of constructing a
fetal configuration from small segments, a large set of candidate fibers derived from other algorithms is used as a starting point. A tissue forward model is incorporated in order to compute an estimated diffusion signal. The optimization consists of minimizing the distance between the signal estimator and the measured diffusion data. A similar approach was pursued for the convolutional fiber orientation distribution (FOD) space instead of the signal space [32, 33]. A more elaborated technique has been developed, which enables a flexible choice of forward model and reduced computational load [34]. These methods are promising regarding the quantification of white matter connections. However, the described optimization methods have their own pitfalls. An overview of open challenges is given in [35].

2.8 Statistical Group Analysis

2.8.1 Tract Based Spatial Statistics

Tract based spatial statistics (TBSS) [36] analysis is a widely used voxel-based whole brain analysis for dMRI. The standard processing consists of the following steps. For each subject, an FA image is calculated. All FA images are non-linearly registered to a template space. A mean FA image is created and subsequently skeletonized to build an FA skeleton, representing the main tracts common to all subjects. Finally, the skeleton is projected onto each subject and refined with a local search of the locally maximal FA value in order to correct for registration
errors. Statistical permutation tests are then performed on the FA skeleton. This method can also be applied to other scalar diffusion measures.

2.8.2 Along-tract Quantification

Conventional dMRI group studies often compared bundle averaged diffusion metrics in order to discover changes between healthy and diseased populations. However, the variance of most of the diffusion metrics can be quite large even on a single streamline. Therefore, it was proposed to analyze the course of diffusion parameters along segmented fiber bundles [37, 38]. The profile of the diffusion metric of interest along previously segmented fiber bundles can be directly incorporated into general linear models or other statistical frameworks. For these methods, a registration to a common template space is not required.

2.9 diffPipe Matlab Package

The developed Matlab package is available at https://git.ee.ethz.ch/sommers/diffPipe. Customized processing pipelines can be created on a single subject and group level basis. A brief overview of the available function calls for each processing step is given in the following. Most functions contain many different parameters accessible in the object parameters (e.g. obj.pars.odfCalc).
# diffPipe Matlab Package

## diffPipe

### Preprocessing

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>checkQuality</td>
<td>Diffusion data quality estimation using the residuals from the tensor fit and estimating SNR</td>
</tr>
<tr>
<td>estimSNR</td>
<td>SNR estimation in the Corpus Callosum according to dipy <a href="http://nipy.org/dipy">nipy.org/dipy</a></td>
</tr>
<tr>
<td>estimNoise</td>
<td>Noise-map estimation using dwi2noise (MRtrix) and the residuals from the truncated SH fit</td>
</tr>
<tr>
<td>coregFSL</td>
<td>Eddy- and motion correction implemented in FSL (<a href="http://fsl.fmrib.ox.ac.uk/fsl">fsl.fmrib.ox.ac.uk/fsl</a>, fslwiki: EDDY_CORRECT)</td>
</tr>
<tr>
<td>coregRegnetsch</td>
<td>Motion correction using regnetsch</td>
</tr>
<tr>
<td>coregElastix</td>
<td>Using elastix to correct for motion artifacts (<a href="http://elastix.isi.uu.nl">elastix.isi.uu.nl</a>)</td>
</tr>
<tr>
<td>coregEddy</td>
<td>Motion- and distortion correction by providing multiple volumes with two phase-encoding directions (A-P, P-A).</td>
</tr>
<tr>
<td>coregT1</td>
<td>Rigid registration of the diffusion dataset to the T1 dataset using SPM (<a href="http://www.fil.ion.ucl.ac.uk/spm">www.fil.ion.ucl.ac.uk/spm</a>)</td>
</tr>
<tr>
<td>bdpCorr</td>
<td>Distortion correction for susceptibility induced distortions with the help of a T1-weighted image (<a href="http://brainsuite.org">brainsuite.org</a>)</td>
</tr>
<tr>
<td>denoise</td>
<td>DWI denoising package using overcomplete local PCA (<a href="http://sites.google.com/site/pierrickoupe">sites.google.com/site/pierrickoupe</a>)</td>
</tr>
<tr>
<td>fslBet</td>
<td>skull-stripping and brainMask estimation in FSL</td>
</tr>
<tr>
<td>dynStabCorr</td>
<td>Correction of varying inter-slice intensities caused by the dynamic stabilization option</td>
</tr>
<tr>
<td>averageDyns</td>
<td>Splits and averages multiple dynamics of diffusion scans using MRecon and Regnetsch</td>
</tr>
</tbody>
</table>
## 2. Methods

### Model Fitting

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>fslDTI</td>
<td>Diffusion tensor fit implemented in FSL</td>
</tr>
<tr>
<td>dtiCalc</td>
<td>Diffusion tensor fit (Matlab, mex)</td>
</tr>
<tr>
<td>odfCalc</td>
<td>Matlab implementation of ODF reconstructions for various sharpening parameters including FRT, FRACt and Solid Angle approach</td>
</tr>
<tr>
<td>csdCalc</td>
<td>Constrained Spherical Deconvolution (MRtrix: <a href="http://mrtrix.org">mrtrix.org</a>)</td>
</tr>
</tbody>
</table>

### Tractography

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dtiTrack</td>
<td>A DTI Fact implementation (Matlab, mex) derived from the profTrack algorithm</td>
</tr>
<tr>
<td>dtkTrack</td>
<td>Deterministic, Tensor-based tractography performed in the DiffusionToolkit (<a href="http://trackvis.org/dtk">trackvis.org/dtk</a>)</td>
</tr>
<tr>
<td>dtiMrtrix</td>
<td>DTI based tractography from MRtrix</td>
</tr>
<tr>
<td>factTrack</td>
<td>FACT algorithm adapted to ODFs, modified version of profTrack (Matlab, mex)</td>
</tr>
<tr>
<td>profTrack</td>
<td>profileTracking algorithm based on ODFs, recursive backtracking and neighborhood averaging (Matlab, mex)</td>
</tr>
<tr>
<td>mrtrixTrack</td>
<td>CSD based tractography from MRtrix (multiple algorithms available)</td>
</tr>
<tr>
<td>mrtrixACT</td>
<td>MRtrix tractography using anatomical priors from the T1-weighted image (Anatomically Constrained Tractography), tissue priors are generated in FSL</td>
</tr>
<tr>
<td>spinGlass</td>
<td>Global tractography algorithm adapted from Reisert et al. 2011 (Matlab, mex)</td>
</tr>
</tbody>
</table>

### Segmentation

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>extractRoiFibs</td>
<td>Extract all streamlines which pass through 2 ROIs defined in the roiMap</td>
</tr>
<tr>
<td>filterFibers</td>
<td>Uses the track_vis command line tool to streamline with ROIs defined in the roiMap</td>
</tr>
</tbody>
</table>
2.9. diffPipe Matlab Package

**segmentAFQ**  Segmentation of tractogram into the 20 major fiber bundles, code adapted from [github.com/jyeatman/AFQ](https://github.com/jyeatman/AFQ)

**Optimization**

- **pcaUp**  Up-sampling of clustered streamlines according to Chapter 3
- **pcaOpt**  Fiber optimization with additional up-sampling and a tensor-based diffusion kernel as forward model
- **optLife**  Tractogram optimization using LiFE [github.com/vistalab/life](https://github.com/vistalab/life)
- **optCommit**  Convex Optimization Modeling for Microstructure Informed Tractography (COMMIT) using python (only suited for mac and linux) [github.com/daducci/COMMIT](https://github.com/daducci/COMMIT)
- **optCommit2**  COMMIT adapted for windows including multi-threading

**diffGroup**

- **run**  Batch process to run a given Matlab script on each file or diffPipe object
- **run_par**  Parallelized batch process using Matlab’s parallel pool
- **generateReport**  Generates group-wise overview for various parameters and maps (e.g. quality)
- **tbss**  Runs the complete TBSS pipeline for the given subjects
- **randomise**  Statistics including non-parametric permutation tests including regressors or correlation options
- **afqStats**  Summarizes AFQ statistics for evaluated parameters
Chapter 3

Fiber Up-Sampling and Quality Assessment of Tractograms – Towards Quantitative Brain Connectivity

3.1 Introduction

The performance of tracking algorithms has significantly improved by considering the information contained in orientation distribution functions (ODF) or fiber orientation distribution (FOD), especially in regions with complex fiber configurations [39, 40, 41]. However, tractograms remain biased by algorithmic specific parameters, i.e. stopping criteria, curvature thresholds, seed point distribution and the choice of the tracking algorithm itself, as well as partial volume effects of different fiber populations or various tissue types within the acquired data voxels. This complicates the estimation of reliable tractograms and thus
the extraction of biologically meaningful connectivity measures between brain areas which are a crucial requirement for an accurate, quantitative connectome across different populations [42, 43, 44]. Lastly, besides validation of diffusion pipelines with dedicated phantom data mainly focusing on geometrical metrics of fiber tracts [45], there is currently no objective way to inspect the quality of tractograms in-vivo, especially with respect to accurate quantification of tracking errors.

A first attempt to correct for tractography biases by estimating an actual contribution for each tract was introduced by Sherbondy et al. using a stochastic algorithm on a supercomputer architecture [30, 31]. Another method introduced by Smith et al. is based on a non-linear gradient descent method called spherical-deconvolution informed filtering of tractograms (SIFT). This approach removes fibers of an initially large fiber population to improve the fit between the streamline distribution in each voxel and the fiber ODF [32]. Thereby, a cost function describing the deviation between fiber densities and FOD lobe integrals is minimized by iteratively removing fibers. Fiber densities are calculated by incorporating the length and tangent of reconstructed fibers within a voxel and compared to the corresponding fiber ODF lobes. However, the SIFT approach requires a large amount of initial fibers to determine an
3. Fiber Up-Sampling and Validation

optimized subset of included and excluded fiber tracts.

Its successor, SIFT 2 [33] reduces this requirement, as it determines an effective cross-sectional area for each streamline, represented by a floating-point weighting factor for each fiber, instead of a binary keeping or removing of fibers in comparison to the initial SIFT.

Pestilli et al. introduced a similar method, i.e. linear fascicle evaluation (LiFE), which is based on the diffusion signal, predicted from the connectome, instead of the FOD [46]. The default forward model is a degenerated tensor representing a stick with zero radial diffusivity. To deal with isotropic compartments, the signal mean is subtracted in each voxel prior to the optimization. Daducci et al. pursued a similar approach introducing the Convex Optimization Modeling for Microstructure Informed Tractography (COMMIT) framework [34], though using a more complex forward model by describing both the intracellular stick model, and the extracellular compartment by a tensor. Furthermore, gray matter and cerebrospinal fluid (CSF) are also represented with two distinct isotropic components. It is tempting to interpret the resulting fiber weights as quantitative connectivity measures between brain regions, however, the described optimization methods have their own pitfalls. E.g. in voxels with poor or incorrect fiber representations
due to tracking errors, noise or partial volume contaminations, compartments are typically overcompensated by increasing the weights of the few present fibers, isotropic or extracellular compartments in order to decrease the global fit error. An overview of pitfalls and open challenges is given in [35].

Lastly, the quantification of white-matter properties based on diffusion data also remains challenging. Fiber-specific metrics are quantified by the generally unreliable fiber-count [44] or ROI-based approaches. The evaluation of diffusion metrics along segmented tractography bundles was introduced by [38] and [37]. The Automated Fiber Quantification (AFQ) framework allows the automatic identification and segmentation of major white matter tracts and evaluates scalar diffusion measures such as fractional anisotropy (FA) along these trajectories to quantify changes within the tract diffusion profiles among different subjects or groups [37].

Here, we propose a novel approach, which enables the inspection of the quality and validation of a tractogram optimization such as COMMIT by evaluating FOD characteristics of the error signal along and perpendicular to fiber bundles by utilizing the AFQ framework. The quality metrics proposed allow for a better understanding of the accuracy and error sources of tractograms and help identifying regions with poorly fitted data.
We further show that these metrics, combined with a newly introduced error FA, allow a better interpretation of the directional error distribution. These are important steps towards interpreting fiber weights from a tractogram optimization in a quantitative way to e.g. construct a more meaningful connectivity measure in a connectome. Furthermore, we also present a fiber up-sampling procedure: It allows to increase the number of streamlines of a given fiber bundle, in case of e.g. underrepresentation of a certain structure due to anatomical properties, fiber geometry, seeding pattern or algorithmic constraints. Analyzing the introduced error measures on specific fiber bundles shows the benefit of using up-sampled fiber bundles.

3.2 Materials and Methods

The major steps of a typical connectome generation process is shown in a simplified form in Figure 3.1. It is crucial to perform the optimization after the segmentation and up-sampling steps in order to avoid the partial fiber problematic discussed in [35]. In this work, in contrast to a connectome pipeline, the segmentation step is not based on cortical parcellation, but performed using the AFQ framework. This choice was motivated by the ability of the AFQ framework to reliably quantify measures
3.2. Materials and Methods

Figure 3.1: A schematic connectome pipeline is depicted including the positions for proposed up-sampling and validation steps.

along tracts. The method section is organized as follows. First, the acquisition protocol, preprocessing steps and tractography algorithm is described. However, these parameters can easily be swapped with other protocols or tractography algorithms. Thereafter, the AFQ segmentation, fiber up-sampling, COMMIT optimization and error quantifications including the introduced error measures are described in more detail.

3.2.1 In-vivo Diffusion Data Acquisition

Diffusion MRI data were acquired on a Philips Achieva 3T TX system (Philips Healthcare, Best, the Netherlands), equipped with 80 mT/m gradients and a 32-element receive head coil array, using a diffusion-weighted single-shot spin echo EPI sequence. The study was approved by the local ethics committee and meets the guidelines of the declaration of Helsinki. Written
informed consent was obtained from all subjects. Datasets from 16 healthy volunteers (age: 31.6 ± 8.6, gender: 12 male, 4 female) were acquired with the following diffusion scan parameters: TR: 11.85 s, TE: 66 ms, FOV: 220 × 220 mm², with 40 contiguous slices, slice thickness: 2.3 mm, acquisition and reconstruction matrix: 96 × 96, SENSE factor: 2, partial Fourier encoding: 60%. Diffusion-weighted images were acquired along 64 directions distributed uniformly on a half-sphere with a b-value of 3000 s/mm² in addition to a b = 0 s/mm² scan, resulting in a scan time of approximately 13 min. Additionally, 1 mm isotropic T1-weighted structural images were recorded with a 3D MP-RAGE sequence (FOV: 240 × 240 × 160 mm³, sagittal orientation, 1 × 1 × 1 mm³ voxel size, TR: 8.14 ms, TE: 3.7 ms, flip angle: 8°).

3.2.2 Preprocessing and Tractography

For each dataset, the diffusion data was corrected for eddy-currents and subject motion by FSL (EDDY) [47]. The white matter mask was estimated from the T1 weighted dataset using the tissue segmentation in SPM8 (www.fil.ion.ucl.ac.uk/spm) and transformed back to diffusion space using SPMs coregister function based on normalized mutual information. A Fiber
Assignment by Continuous Tracking (FACT) inspired deterministic algorithm generalized to the Orientation Distribution Function (ODF) was used in the tractography step. The ODF was reconstructed using the FRACT method [Haldar and Leahy, 2013]. The tracking direction was selected according to the local diffusion maximum of the ODF. Ten seeds were started in each white matter voxel, resulting in approximately 700,000 fibers per subject. The estimated white matter mask was only used for seeding purposes and was not utilized as a tractography stopping criterion.

### 3.2.3 Fiber Segmentation and Up-sampling

The segmentation of the tractograms was performed using the AFQ framework [37], which is based on a waypoint ROI procedure as described in [48]. Additionally, a refinement step was applied, which compares each candidate fiber to tract probability maps [49]. To avoid conflicting start and endpoints of fibers running through the two ROIs of the target fiber structure, a flip was performed on all tracts which first passed through the second ROI, resulting in consistent fiber alignment in each bundle. These segmentation steps resulted in the selection of 20 major white matter fiber tracts [37] out of all white matter
fibers contained in the whole brain tractogram (18 bundles as described in [37], and two additional tracts as defined in the online version: https://github.com/jyeatman/AFQ).

Next, to increase the number of fibers of potentially underrepresented fiber populations in the different AFQ segmented bundles, e.g. due to tractography algorithm biases, the following method was applied: The segmented fibers were equidistantly resampled using 80 interpolation points per fiber and principal component analysis (PCA) was applied to all classified and resampled fibers [50]. The space was truncated to the first 80 dimensions (from the 240 point descriptors), whereby more than 99% of the explained variance was still captured. In PCA space, for each bundle separately, new fibers were randomly generated according to the point distribution of the transformed fibers, assuming a bundle specific multivariate Gaussian distribution. The newly generated fibers were transformed back by inverting the linear PCA transformation.

In a further step, potential outliers were identified based on the calculation of a population-mean fiber, i.e. the mean value of all corresponding resampled points of the initial fibers within one fiber bundle. The distance of each randomly generated fiber to the original population-mean fiber was derived by summing up the distances to the nearest points on the mean fiber. New
fibers were only accepted if the distance-threshold to the initial population was met. This threshold was set to the maximum fiber distance of all fibers within the initial population relative to its mean fiber. Newly generated tracts leaving the white-matter mask were also rejected. Based on these fiber population up-sampling steps, additional 10’000 fibers per bundle were generated for each dataset.

Finally, the up-sampled fibers were again segmented using the AFQ framework to apply the same classification criteria to the newly generated fibers as to the initial tractogram. Around 75% of the up-sampled fibers were successfully classified and therefore kept for the further analysis. With the procedure described above, a total of four tractography sets were generated:

<table>
<thead>
<tr>
<th>Set</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFQ</td>
<td>classified AFQ fibers based on the initial tractogram</td>
</tr>
<tr>
<td>AFQUP</td>
<td>AFQ set combined with the up-sampled AFQ fibers</td>
</tr>
<tr>
<td>WB</td>
<td>initial whole brain tractogram</td>
</tr>
<tr>
<td>WBUP</td>
<td>WB combined with the up-sampled AFQ fibers</td>
</tr>
</tbody>
</table>

3.2.4 Fiber Optimization, Optimized Tractogram

The optimization of the different tractogram sets was performed using the COMMIT framework [34] by applying the Stick-
Zeppelin-Ball model [51] for modeling the fiber signal. The intracellular stick model was generated with a longitudinal diffusivity of $d_\parallel = 1.7 \times 10^{-3} \text{mm}^2/\text{s}$. In addition, in each voxel, a hindered contribution was included for every unique FOD peak using the Zeppelin model assuming a perpendicular diffusivity $d_\perp = 0.5 \times 10^{-3} \text{mm}^2/\text{s}$ and longitudinal diffusivity $d_\parallel = 1.7 \times 10^{-3} \text{mm}^2/\text{s}$. Lastly, two isotropic compartments accounting for partial volume with gray matter and cerebrospinal fluid were modeled with diffusivity $d \in \{1.7, 3.0\} \times 10^{-3} \text{mm}^2/\text{s}$. The non-diffusion weighted $b = 0$ image was used to normalize the diffusion data. The convex optimization problem of the following form

$$\arg\min_{x \geq 0} \|Ax - y\|_2^2$$

(3.1)

where $y$ is the vector containing the normalized diffusion signal, $A$ is the linear operator or dictionary and $x$ is the vector of the contributions, was solved using a forward-backward, fast iterative shrinkage-threshold algorithm (https://github.com/daducci/COMMIT), resulting in a solution $\hat{x}$. Stopping criteria for the optimization were either a maximum number of 500 iterations or a minimum relative change of the objective function of $1 \times 10^{-4}$. 

50
3.2.5 Error Quantification

In addition to the normalized root mean square error (NRMSE) of the optimization fit, an actual signal estimator $\hat{s}$ was calculated using $A\hat{x}$, by reverting the $b=0$ normalization. To further examine the differences and similarities between this signal estimator $\hat{s}$ and the acquired diffusion data $s$, a directional error FOD of the signal estimator $\hat{s}$ and the original diffusion data $s$ was calculated. Remaining signal contributions from under- or overrepresented fibers are assumed to remain in the error signal. The FOD for the diffusion signal estimator was reconstructed by applying the constrained spherical deconvolution [26] to the error signal, which is defined by the elementwise difference between the measured and estimated diffusion signals:

$$s_{err}^i = \sqrt{(s^i - \hat{s}^i)^2}$$

In order to use a meaningful deconvolution kernel and to be comparable to the FOD derived from the measured signal $s$, the response function was not re-estimated on the error signal; instead the fiber response from $s$ was used. A maximum spherical harmonics order of $l_{max} = 8$ was used.

Furthermore, a traditional tensor fit of the signal error $s_{err}$ was derived in order to calculate the fractional anisotropy (FA) of $s_{err}$.

To quantify the different error measures along the segmented
and optimized AFQ fiber bundles, we extended the tract profile generation of the AFQ framework. In [37], the locations of the used waypoint ROIs from the segmentation step (2.3) isolate the central trajectories of the fascicles. Next, different scalar diffusion measures (FA, RD, etc.) are evaluated along the central portion of the fiber bundle by clipping and resampling each fiber according to the main segment between the ROIs. Bundle properties are then summarized at each node by taking a weighted average according to the Mahalanobis distance of each fiber tract core as described in [37].

In this work, instead of investigating traditional scalar diffusion quantities as proposed in the AFQ framework, we examined scalar measures such as the fit NRMSE and the introduced error FA along the segmented AFQ tracts. Furthermore, the three dimensional error FOD was also evaluated by calculating longitudinal and perpendicular error FOD amplitudes for each segmented AFQ fiber. These measures depend on the fiber directionality and are not scalar maps. The maximum peak-amplitude along a fiber tract is defined by the maximum FOD amplitude in a cone around the fiber orientation with an opening angle of $\frac{\pi}{6}$. The maximum peak-amplitude perpendicular to the fiber is the maximum of all sampling points outside of this cone (Figure 3.2).
3.2. Materials and Methods

Figure 3.2: Schematics showing the FOD evaluation along a fiber tract: longitudinal maxima are marked by stars (within the cone), perpendicular maxima are marked with circles (outside of the cone).

For every tractogram set \((n = 4)\), following parameters were analyzed along each of the 20 segmented fiber bundles: NRMSE, error FOD along, error FOD perpendicular, and error FA. These measures were tested for statistical significance between the initial and up-sampled tractogram sets and were corrected for multiple comparison using the non-parametric permutation test implemented in FSL [52]. The number of permutations were set to 5000 with a significance level of \(p < .05\).

Furthermore, the up-sampling method was also compared with an increase of seed points during the tractography step. Therefore, the number of seed points was increased incrementally up to a factor of eight in a single subject. The resulting tractogram
sets were segmented using the AFQ framework and either optimized or up-sampled and optimized for the comparison. The up-sampled tractogram sets were also segmented a second time prior to the optimization.

3.3 Results

In Figure 3.3, the mean NRMSE of all four tractogram sets are shown for every subject (N=16) after the optimization with the COMMIT framework. The error in the up-sampled populations (AFQUP and WBUP) is decreased compared to the initial sets (AFQ and WB) for each subject, and comparison at the group level shows a highly significant decrease in the mean NRMSE between AFQ and AFQUP and between WB and WBUP (paired samples, \( p < .001 \)). Furthermore, the whole-brain tractograms (WB and WBUP) also showed lower errors compared to the AFQ and AFQUP.

The different segmented AFQ fiber bundles that are discussed in further detail in the following sections are illustrated in Figure 3.4. Figures 3.5 to 3.8 show the tract profile of the NRMSE, error FA, longitudinal and perpendicular FOD error in selected bundles to illustrate different distributions of the error signal and performance of the up-sampling method.
3.3. Results

Figure 3.3: Optimization results showing the mean NRMSE for each subject between (a) AFQ and AFQUP, and (b) WB and WBUP; (c) group average for the four tractogram sets, the error bars depict one standard error.

Figure 3.5 shows the NRMSE along three major bundles (left and right hemisphere) in the four tractograms sets (AFQ, AFQUP, WB, WBUP). The colored section of the depicted bundles describe the core of the bundle, whereas the x-axis in the subplots shows the 100 parameterized points between ROI 1 and ROI 2. In Figures 3.5-3.8, the ROIs are marked with 1 and 2 to emphasize the start and end region of the parameterization.

The lower error in the up-sampled tractograms (AFQUP, red line, WBUP, black line) compared to AFQ and WB (blue, green...
3. Fiber Up-Sampling and Validation

Figure 3.4: A selection of the discussed segmented fiber bundles of a single representative subject are shown in different colors. In the sagittal view, the right Corticospinal Tract, right Arcuate Fasciculus, right Inferior Longitudinal Fasciculus (ILF), the Callosum Forceps Minor and the right Uncinate Fasciculus are illustrated. The axial slice depicts the left and right Thalamic Radiation, left and right Superior Longitudinal Fasciculus (SLF) and the Callosum Forceps Major.

line) achieved a better fit compared to the initial sets (AFQ, WB). In most parts, the fit error significantly decreased \((p < .05)\) after multiple comparison correction using FSL’s randomise. Regions of statistical significance are highlighted with a transparent overlay in the color of the tractogram set with a higher value (e.g. blue for AFQ).

The FA of the error signal gives further insight into the optimization results. In Figure 3.6, three different types of error...
3.3. Results

Figure 3.5: Mean NRMSE of the optimization over all subjects for the left and right Corticospinal Tract, Thalamic Radiation and Arcuate Fasciculus. Subplots 1-6 shows the mean NRMSE of the AFQ fiber set (blue,) and of the up-sampled tractogram set AFQUP (red). The dashed red and blue lines indicate one standard error. Subplots 7-12 shows the mean NRMSE of the WB fiber set (green), and of the WBUP-tractogram set (black). The dashed green and black lines indicate one standard error. Areas with a significant error reduction (according to FSL’s randomise, $p<.05$) in AFQUP compared to AFQ are overlaid in transparent blue ($AFQ \downarrow AFQUP$) or green ($WB \downarrow WBUP$), respectively.
FA behavior are shown as an example. The Corticospinal Tract showed a statistically significant reduction of the error FA in the up-sampled populations (AFQUP vs. AFQ and WBUP vs. WB), which is desirable in order to reduce a directional bias in the residual diffusion signal. Nevertheless, structural tendencies along the bundle are still visible, especially in the second quarter of the bundle, where the error FA is clearly increased in all of the tractogram sets. The error FA in the Callosum Forceps Major could not be reduced by applying the up-sampling method, and especially in the mid part of the bundle, directional biases in the residual diffusion signal remain clearly visible. In contrast, the Inferior Longitudinal Fasciculus (ILF) revealed a relatively isotropic error signal, expressed by low FA values, and no distinct structure in the error FA, i.e. no directional bias in the residual diffusion signal along the bundle was observed.

In Figure 3.7, the longitudinal FOD error is evaluated along the distinct fiber bundles. The Corticospinal Tract showed a significantly ($p < .05$) reduced longitudinal error in both up-sampled sets compared to the initial tractograms. In the Superior Longitudinal Fasciculus (SLF), the up-sampling reduced the error in the AFQ population (AFQ vs. AFQUP). The longitudinal error was already low in the WB tractogram set for the SLF, and could not be further reduced in a statistically significant
3.3. Results

Figure 3.6: The FA of the error signal is shown along three selected bundles (Corticospinal Tract, Callosum Forceps Major and Inferior Longitudinal Fasciculus). The mean error FA over all subjects derived from the initial optimized, non up-sampled sets are depicted in blue (AFQ) and green (WB), the error FA derived from the up-sampled sets are displayed in red (AFQUP) and black (WBUP). The dashed lines indicate one standard error.

manner by up-sampling the bundle (WBUP). The Arcuate Fasciculus showed a similar behavior, whereas the up-sampling significantly reduced the longitudinal error in the AFQ cases. Additionally, in the WB sets, the up-sampling still significantly reduced the longitudinal error in the temporal part of the bundle (WBUP) but the overall difference is drastically reduced.
3. Fiber Up-Sampling and Validation

Figure 3.7: Longitudinal error FOD peak amplitudes along three representative bundles are shown (Left Corticospinal, Right Superior Longitudinal Fasciculus and Right Arcuate Fasciculus). The mean across all subjects using the initial tractograms are shown in blue (AFQ) and green (WB), the mean longitudinal FOD error in the up-sampled sets are depicted in red (AFQUP) and black (WBUP). The dashed lines indicate one standard error, statistically significant regions ($p < .05$) are highlighted with transparent surfaces (blue: AFQ$\neq$AFQUP, green: WB$\neq$WBUP).

Figure 3.8 depicts the perpendicular FOD error in the segmented fiber bundles of the right Thalamic Radiation, Callosum Forceps Minor and the left Arcuate Fasciculus. For the AFQ case, the up-sampled sets showed a significantly higher error in the Thalamic Radiation and the Arcuate Fasciculus in some parts, even though the overall mean fit error (NRMSE) was...
3.3. Results

Figure 3.8: Perpendicular error FOD peak amplitudes across the selected AFQ bundles (Right Thalamic Radiation, Callosum Forceps Minor, Left Arcuate Fasciculus): the mean over all subjects using the initial tractograms are shown in blue (AFQ) and green (WB). The perpendicular error FOD peak amplitudes in the up-sampled sets are drawn in red (AFQUP) and black (WBUP). The dashed lines indicate one standard error statistically significant regions \( p < .05 \) are highlighted with transparent surfaces (red: AFQUP \( \approx \) AFQ, green: WB \( \approx \) WBUP).

reduced. If all the fibers are taken into account (WB, WBUP), the up-sampled population (WBUP) does not show a significant increase of the perpendicular error anymore.

Figure 3.9 shows a coronal cross section through the Corona Radiata of a single subject. The reconstructed FODs from the measured diffusion signal are depicted in gray, with the colored error FODs derived from the WB set shown on top. Most voxels
exhibit a small error FOD compared to the signal FOD, implicating a good agreement between the signal estimator from the optimization and the measured signal. Nevertheless, in some voxels the error FOD is relatively large compared to the signal FOD. Three of those voxels are highlighted in a, b and c. Figure 3.10 depicts the comparison between increasing the number of seed points during the tractography and up-sampling the segmented fiber bundles in a single subject. Each tractogram set is plotted with the number of fibers on the x-axis in order to compare the same number of fibers. The up-sampling method clearly outperforms the increase in seed points, whereby the largest improvement is achieved by the first up-sampling step.

3.4 Discussion

We have introduced a tool to investigate the quality of a tractogram by further inspecting the directionally dependent error signal between the signal prediction and the measured diffusion signal along reconstructed fiber bundles. Additionally, we presented a method to up-sample a given fiber population in order to achieve better optimization results, i.e. a decreased fit error.
Figure 3.9: A coronal section through the Corona Radiata is shown in a single subject, whereby the signal FODs derived from the measured diffusion signal are depicted in transparent gray, the error FODs derived from the WB set are overlaid in color. Three different voxels are highlighted where the error is significantly larger compared to the other voxels.
3. Fiber Up-Sampling and Validation

Figure 3.10: Different number of seed voxels and the resulting mean NRMSE for the AFQ and AFQUP tractogram sets are shown.

The overall mean fit error averaged over all the white-matter voxels and all subjects showed only small, but nevertheless significant changes comparing the initial (AFQ, WB) with the up-sampled (AFQUP, WBUP) fiber tractograms. These small changes at the group level could be attributed to large inter-subject variability; however, the up-sampled sets achieved a reduced fit error in each single subject (Figure 3.3a and b). The improved signal fit achieved by the up-sampling method was highly statistically significant for both the AFQ vs. AFQUP and WB vs. WBUP tractogram sets. Further inspection of the
NRMSE along the major segmented fiber bundles showed high similarity between the matched left and right structures. However, differences were found between various structures, e.g. the superior part of the Corticospinal Tract was highly improved by the up-sampling method, whereas the frontal part of the Thalamic Radiation was mostly unaffected by the up-sampling procedure. Variable performance of the up-sampling method across structures might be caused by the quality of the initial bundle representation and also by voxels surrounding these bundles. Systemic errors were expected and observed in the AFQ tractogram sets (AFQ, AFQUP) due to the fact that many fibers are not covered by the 20 major bundles and therefore excluded from the optimization. The signal of crossing, non-segmented structures are missing in regions with high NRMSE in the AFQ and the AFQUP tractograms. Whilst the AFQUP set showed a reduced error in almost all structures compared to the AFQ set, a compensation of non-segmented crossing structures remains unachievable by merely up-sampling the segmented bundles without the introduction of missing crossing structures. Segmenting the AFQ bundles introduces an additional source of error due to predefined ROIs and registration steps during the AFQ bundle classification. Fibers, which pass through the distinct bundles but e.g. not through the two ROIs are conse-
3. Fiber Up-Sampling and Validation

sequently unclassified, and therefore missed in the optimization. In the whole-brain sets (WB and WBUP, Figure 3.5) no fiber populations were purposely omitted, and therefore a much more homogeneous NRMSE distribution was found in the brain.

In a next step, we further explored the error distribution across the diffusion directions in each voxel. Therefore, the error FA was calculated and evaluated to reveal potential anisotropy in the error signal (Figure 3.6). In voxels with a good fit, a low anisotropy is expected, i.e. a homogeneous distribution of the error across all diffusion directions. In comparison to the NRMSE, the error FA appears to be a sensitive measure for recognizing badly represented regions, even if all the fibers are taken into account (WB, WBUP). A bad fiber representation or an inaccurate forward model can cause a high error FA as e.g. observed in the middle section of the corpus callosum and parts of the Corticospinal Tract. These bundle segments also have a high signal FA, which might indicate that the chosen forward model underperforms in high FA voxels. Despite the clear distinction of high error FA regions in the graphs, it is rather difficult to define an accurate baseline for the residual error signal in order to distinguish structurally related residuals from pure noise. An accurate signal to noise ratio estimation of the diffusion signal would be needed which is typically also
spatially varying due to multiple acceleration methods. Complex tissue architecture of crossing fiber populations within a single voxel cannot be fully modeled by tensor based metrics, therefore the FOD of the error signal was also evaluated along and perpendicular to the major fiber bundles (Figures 3.7 and 3.8). By disentangling the error signal into a perpendicular and longitudinal error, the exact source of the tractogram error can be observed. Poorly represented structures can therefore be discriminated from over- or underestimated crossing structures. The longitudinal error in the Superior Longitudinal Fasciculi and in the Arcuate Fasciculi differs strongly in the initial populations (AFQ, WB), which is most plausibly caused by segmentation difficulties. However, in case of the Arcuate Fasciculus, applying the up-sampling method in the WB set further significantly reduced the longitudinal error. By further investigating the perpendicular error, a significantly higher error in the AFQUP set was identified for the first time. In the WB sets, this effect diminishes and can therefore be explained by missing fiber populations not embodied in the segmented AFQ bundles. These fiber dependent directional measures combined with the error FA enable to detect and distinguish possible error sources, namely bad fiber bundle representation, missing crossing struc-
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tures or a poor forward-model fit.

Missing crossing structures in the AFQ sets can be found e.g. in the lower part of the Corticospinal Tract. The Cerebellar Peduncles, are passing superior to the first Corticospinal ROI and might be the reason for an increased error FA and NRMSE. Another example is along the middle part of the Arcuate Fasciculus next to the superior region of the Corona Radiata. Besides The Superior Longitudinal Fasciculus, which is also included in the major AFQ bundles, the Posterior Vertical Arcuate and the Vertical Occipital Fasciculus also populate this area and are not segmented using the AFQ framework. The provided tools allow an extensive inspection of tractograms and their optimization by exploring bundle-specific and directionally dependent error measures. To the author’s knowledge, this is the first study that facilitates a deepened insight into the remaining local errors induced by the tractogram or the optimization procedure itself. This step is crucial in order to get a better understanding of the actual goodness of fit of the tractogram.

In Figure 3.9, different cases of error FODs are highlighted. In voxel a), the directionality of the error FOD matches the initial FOD. Most certainly, some of the passing fibers were under or over-estimated in that particular voxel. The error FOD in voxel b) shows a completely different characteristic as the initial
FOD. The geometry of the reconstructed fibers do not match the measured diffusion signal. The third case (voxel c) is a combination of both cases, where the local weighting deviates from the diffusion signal and also the error FOD peaks are slightly tilted. Other voxels show very small error FODs and some spurious peaks do occur, whereby the assumption of an underlying fiber response was violated. Nevertheless, the amplitudes of these error FODs are very small and will not influence the resulting along-tract analysis.

A possibility to mitigate the fiber assumption of the kernel function would be to apply a model-free Funk-Radon-Transformation to the error diffusion signal instead of deconvolving the error signal with a fiber response function. The resulting error orientation distribution function (ODF) would not suffer from spurious peaks.

The ultimate goal of using streamline weights to quantify connectivity strength between cortical regions is still not achievable if the tractogram or the optimization is flawed. The fiber dependent error estimation could also be used to estimate a confidence of the resulting fiber weights aiming to verify the integrity of a quantitative connectivity matrix between cortical regions. Additionally, the difficulty of omitting fibers due to any segmentation and its influence on to the optimization was
shown. In the given framework, the segmentation method can easily be exchanged with a segmentation e.g. based on a cortical parcellation. Thereby, the up-sampling can also be applied to more fiber bundles. However, other segmentation methods e.g. based on a cortical parcellation scheme also suffer from unclassified fibers which cannot be included in the tractogram optimization step.

The introduced fiber up-sampling method improved the tractogram representation, hence led to a significantly superior optimization expressed by a reduced NRMSE. Even though, the introduced approach did not enhance the optimization in every single structure, impairment caused by the fiber up-sampling was not observed. Additionally, the up-sampling was performed per bundle, whereby a bundle segmentation is a necessary prerequisite.

This requirement might be eliminable, if a different sampling strategy is applied to randomly draw samples in the PCA space. The assumption of a multivariate Gaussian distribution is no longer valid in a whole brain fiber population and would lead to many implausible up-sampled fibers.

In Figure 3.10, the fiber up-sampling is compared with increasing the number of seed voxels during tractography. Even an eight-fold increase of fibers did not improve the optimization
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result substantially. However, the up-sampling is not only computationally less intensive, it also introduces new fibers with different features, a larger spatial extent, and therefore novel trajectories and the up-sampled fibers do differ substantially from the initial population. These new fibers contribute significantly to a more optimal tractogram set. Similar effects were reported by [53] while different algorithm parameters were introduced instead of only increasing the number of seed points. The presented framework also enables the comparison of different tractogram sets from various sources and allows a more extensive inspection of each tractogram and its strength and weaknesses without defining an explicit gold standard.

For a reliable fiber quantification, it is crucial to eliminate error sources such as a bad fit due to wrong choice of the forward model, poor tractogram representation caused by the choice of tracking algorithm and tractography parameters or an overcompensation in voxels with a low number of streamlines. As discussed in [33], FOD lobes of voxels containing very little streamlines compared to the actual measured fiber density will result in assigning high weights to those fibers in order to reduce the error in that particular voxel, but introducing biases in all the other voxels traversed by those fibers. Similarly, the COMMIT model will assign higher volume fractions to ex-
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tracellular compartments in order to compensate for missing streamlines representing the intracellular compartment. The presented up-sampling procedure can help to limit voxels containing a low number of streamlines, especially in cases where a higher track density cannot be achieved by simply increasing the number of seed voxels. With respect to global tractography algorithms, an increase of fibers is typically computationally very expensive, whereas the described up-sampling method is very efficient. The introduced error measures such as the error FA or the error FOD itself could also be fed back into the tractography process to influence the placement of new seed voxels or adjust tractography parameters in order to achieve a more representative tractogram. In this study, only single shell diffusion data was used for the optimization, whereas the COMMIT framework clearly benefits from multiple shells e.g. in order to distinguish between different isotropic compartments.
4.1 Introduction

In the human connectome project (HCP), data acquisition techniques and processing protocols have been significantly advanced [54, 55]. Besides progress in post-processing, the HCP provides high quality in-vivo diffusion data acquired with multi-shell diffusion gradient schemes and a high spatial resolution [56]. These datasets have been acquired on a dedicated MR scanner with optimized hardware and less severe acquisition time constraints in comparison to clinical applications. However, not all problems can be addressed by improved hardware and acquisition protocols. One of the major remaining challenges is to reliably extract quantitative measures from tractograms.
4. Fiber Density at the Gray-to-White Matter Interface

across different populations.

Recent developments in global top-down tractography optimizations enable the estimation of fiber contributions and compartment fractions [34, 46, 30, 31, 32, 33]. However, all of these optimization methods have their own pitfalls. An overview of problems and open challenges is given in [35].

Numerous models based on diffusion weighted imaging have been proposed to estimate parameters related to the restricted, intra-axonal compartment, commonly referred to as fiber density [57, 58]. In the work of [34, 33], an optimal weight for each streamline is determined according to a biologically motivated forward model and the measured diffusion signal. By assigning a weight of zero, false positive or implausible connections can be eliminated. The intra-axonal volume (i.e. fiber density) is calculated by multiplying each streamline contribution (fiber weight) by the streamline length. Therefore, the fiber weight is related to the intra-axonal cross-sectional area [57, 59, 58, 33].

During development, the intricate folding of the cortex is formed in order to optimize the wiring and organization of the brain and fit a large cortex in a limited cranial volume [60, 61]. The cortical surface area has been shown to be inversely correlated with gray matter thickness [62, 63] and cortical areas seem to have evolved to optimize inter-areal connections by minimiz-
ing the required axonal volume within the white matter [64]. An increase of cortical thickness allows for more local axonal connections, whereby an increase in sub-cortical surface area might be needed to form more long-range axonal connections traversing the white matter. Due to strict spatial constraints in the brain, we anticipate a homogeneous fiber density at the gray-to-white matter interface (G-WMI).

In this work, we tested this hypothesis by examining the tractography fiber weights from the COMMIT optimization and intersect the streamlines with cortical gray matter regions of interest (ROIs) to estimate fiber density at the G-WMI in 10 healthy subjects from the Human Connectome Project (HCP). Furthermore, the stability and reproducibility of these findings was tested for clinically feasible acquisition schemes by reducing the spatial resolution and utilizing only subsets of the diffusion gradient scheme of the HCP data.

4.2 Materials and Methods

4.2.1 Human Connectome Datasets

MRI datasets of 10 healthy volunteers in the age range of 22 to 35 (6 female, 4 male) were obtained from the HCP Wu-Minn
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database (https://db.humanconnectome.org). Only non-restricted, anonymized open access datasets were used. Therefore, no ethical consent is necessary according to national laws and regulations. Subjects were scanned at a Siemens 3 T scanner equipped with a dedicated, high performance gradient system capable of gradient strengths of 100 mT/m with special gradient amplifiers [55]. Three shells with b-values of 1000, 2000 and 3000 s/mm$^2$ were acquired with 90 diffusion encoding directions on each shell. The spatial resolution was 1.25 mm isotropic. Two phase-encoding direction reversed images for each diffusion direction were acquired. The non-diffusion weighted volumes with $b = 0$ were interleaved with DW volumes such that every sixteenth volume had no diffusion weighting. More details about the acquisition protocol can be found in [56]. The utilized diffusion datasets were already preprocessed by the HCP diffusion pipeline [54]. Briefly, distortions were corrected using a model-based approach that simultaneously takes into account susceptibility and eddy-current induced distortions, as well as head motion.
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4.2.2 Gradient Non-linearity Correction

The diffusion datasets in the HCP suffer from much stronger gradient nonlinearities compared to datasets from conventional scanners. Therefore, the diffusion gradients are not spatially invariant across the field of view. As a consequence, the diffusion weighting can vary up to ±15% [56] and should therefore not be neglected. Unfortunately, many software tools do not allow the use of spatially varying gradient tables within a single volume. In order to examine the severity, we analyzed the effect of the gradient deviations for a simple tensor fit with and without accounting for the spatially varying b-matrix. The direction of the primary eigenvector of the tensor deviated up to 3° from the correctly processed tensor. Scaling of the diffusion weightings due to the varying b-factor emerged as the main effect of the gradient non-linearity. Therefore, we locally modeled the normalized diffusion signal with a mono-exponential signal decay in order to estimate the corrected diffusion signal with a constant b-factor per volume and shell. The mono-exponential signal decay was modeled as follows:

\[
\frac{s(\vec{x})}{s_0} = e^{-b(\vec{x})D} \tag{4.1}
\]
whereby \( s(\vec{x}) \) denotes the local diffusion signal and \( s_0 \) refers to the non-diffusion-weighted \( b = 0 \) image. \( b(\vec{x}) \) is the diffusion b-factor and \( D \) represents the diffusion coefficient. With this approach, we assume a mono-exponential signal behavior for the local regime of \( \pm 15\% \) of the b-value for each shell. With this approximation, the desired diffusion signal \( s_{\text{corr}} \) can be calculated in each voxel with the given formula:

\[
s_{\text{corr}} = s_0 \frac{s_{\text{act}}(\vec{x})}{s_0} \left( \frac{b_{\text{act}}(\vec{x})}{b_{\text{corr}}} \right)
\]

(4.2)

where \( s_{\text{act}} \) represents the measured diffusion signal at a given, spatially varying b-factor \( b_{\text{act}}(\vec{x}) \) and \( b_{\text{corr}} \) represents the desired spatially constant b-factor.

### 4.2.3 Tractography and Global Optimization

Constrained spherical deconvolution with recursive calibration of the response function [27, 26] and fiber tractography was performed in MRtrix3 (www.mrtrix.org) using the default iFOD2 probabilistic tractography algorithm [65] with anatomical tissue priors [15]. For the high-resolution dataset, the maximal fiber length was increased to 312.5\( \text{mm} \) (250 times the voxel size). Additionally, the tractography seed points were determined dynamically using the SIFT model [33]. For all of the experiments,
5 million fibers were generated.

The top-down global tractography optimization was performed using the COMMIT framework [34] by applying the Stick-Zeppelin-Ball model [51]. The forward model consists of an intracellular stick model and an extracellular compartment modeled by a zeppelin to describe white matter (WM) and two distinct isotropic components for gray matter (GM) and cerebrospinal fluid (CSF) compartments.

The intracellular stick model was generated with a longitudinal diffusivity of $d_\parallel = 1.7 \times 10^{-3} \text{mm}^2/\text{s}$. In addition, in each voxel, a hindered contribution was included for every unique FOD peak using the Zeppelin model, assuming a perpendicular diffusivity of $d_\perp = 0.5 \times 10^{-3} \text{mm}^2/\text{s}$ and a longitudinal diffusivity of $d_\parallel = 1.7 \times 10^{-3} \text{mm}^2/\text{s}$. Lastly, two isotropic compartments accounting for partial volume with GM and CSF were modeled with diffusivities of $d \in \{1.7, 3.0\} \times 10^{-3} \text{mm}^2/\text{s}$.

Stopping criteria for the convex optimization solver were set to either a maximum number of 500 iterations or a minimum relative change between two subsequent iterations of the objective function of $1 \times 10^{-4}$. 
4. Fiber Density at the Gray-to-White matter Interface

4.2.4 Fiber Weights vs. Gray Matter Parcellation

Each fiber contribution was projected onto GM regions by intersecting every streamline of the tractogram with cortical ROIs. Thereby, the weight of each streamline was assigned to the intersecting cortical ROIs extracted from the freesurfer parcellation, which is already part of the structural preprocessing of the HCP pipeline, and based on the Destrieux atlas [66]. In the ideal case, each fiber contributes to exactly two ROIs. However, the node assignment is far from perfect [35, 67]. We tried to improve the mapping by allowing the extension of each fiber start-endpoint in the direction of the first / last fiber segment for an additional stretch of 2 mm. This approach is more restrictive compared to the radial search proposed in [67]. Nevertheless, we were still unable to perfectly map the tractogram onto the cortical parcellation. In order to measure the severity of the error emerging from the node assignment, we calculated and report the percentage of unassigned fiber weights to the total sum of fiber weights.

The fiber density at the G-WMI is calculated by dividing the fiber weights by the sub-cortical surface area for each ROI in each subject. The resulting ratio refers to the percentage of sub-cortical surface area which is covered by the intra-axonal
4.2. Materials and Methods

cross-sectional area.

4.2.5 Reproducibility for Clinically Feasible Sequences

We examined the fiber density at the G-WMI in high-quality datasets from the human connectome scanner. In order to test reproducibility in a clinically feasible setting, the high-resolution multi-shell diffusion acquisitions were spatially down-sampled by a factor of two to a voxel resolution of 2.5 mm isotropic using the mrresize function from MRtrix with the sinc-interpolation option. Additionally, the gradient scheme was under-sampled to single-shell acquisition schemes (for $b = 1000$, $b = 2000$ and $b = 3000 \, s/mm^2$) with gradient subsets of 45 and 64 directions (for each shell). The minimum number of 45 directions was chosen to sufficiently characterize the diffusion signal [68]. The gradient subsets for each shell were selected with respect to the minimal energy according to the electrostatic repulsion of the sampling points. However, it is important to mention that a subset of a predefined sampling scheme will not sample the sphere as uniformly as an optimal scheme in a separate acquisition. Additionally, the full gradient scheme was also evaluated and utilized as reference. The complete processing pipeline starting from the constrained spherical deconvolution, tractography
and COMMIT optimization was performed separately for each gradient sampling scheme. Fiber density at the G-WMI was determined for each dataset as described in section 4.2.4. Finally, the root mean square error (RMSE) of the fiber density at the G-WMI between the high and low-resolution datasets and the different gradient subsets was calculated per subject.

4.3 Results

4.3.1 Fiber Weights and Cortical Parcellation

In Figure 4.1, the correlation between the COMMIT weights and the cortical ROI surface area for the high-resolution, full gradient scheme is shown. Each color depicts a separate subject. The mean fiber density resulted in a surface area coverage of $11.01 \pm 2.57\%$ of the subcortical surface area (see also Table 1). The Pearson correlation between COMMIT weights and GM surface is 0.935.

The unassigned fiber weights due to the inability of assigning a streamline to cortical ROIs resulted in $21.41 \pm 1.47\%$. In order to examine the deviation of the regression, we show the mean ratio across the 10 subject for each ROI projected onto the standardized brain parcellation in Figure 4.2, where four different
4.3. Results

Figure 4.1: The correlation between the COMMIT weights and the GM surface area is shown for 10 subjects in 150 ROIs of the Destrieux atlas. The thin colored lines represent the linear regression for each subject, the solid black line depicts the regression across all subjects.

views of the cortical regions are depicted. Besides a very high symmetry between the left and right hemisphere, the notable regions with an increased fiber density are the primary motor, visual and auditory regions. The primary sensory cortex is also slightly elevated. A decrease in fiber density at the G-WMI can be observed in the temporal poles.
4. Fiber Density at the Gray-to-White matter Interface

Figure 4.2: Four different views of the group mean ratio between the COMMIT weights and the ROI areas. Group mean ratios are projected onto the standardized cortical parcellation.

4.3.2 Subsampling of the Gradient Scheme

The RMSE of the ratio for different resolutions and gradient subsets is shown in Figure 4.3. Subplot a) depicts the RMSE of the low-resolution dataset, including all diffusion directions (low res, full set), in comparison to the result of the high-resolution dataset, including all diffusion directions (high res, full set), as a reference. In 4.3 b), the RMSE of the gradient subsampling schemes for the different shells is depicted with the low-resolution full gradient set as reference. The error-bars in both subplots show the standard deviation across the 10 subjects.
The root mean square error of the fiber density at the G-WMI is shown for the down-sampled spatial resolution (a) using the high-resolution as a reference and (b) the subsampled gradient schemes with the low-resolution full set as reference.

The error can be interpreted as the deviation of the surface area which is covered by the cross-sectional intra-axonal surface area in comparison to the respective full set (reference).

Figure 4.4 shows the influence of down-sampling the resolution and of subsampling the gradient set with respect to variations of the fiber density at the G-WMI across the brain. It is important to note that a different scaling of the coloring scheme was applied in order to visualize relative differences and not absolute changes in ratio. The gradient subsets with lower b-values tend to overestimate the fiber density in comparison to the high b-value and multi-shell acquisition.

In Table 4.1, we report fiber density at the G-WMI as percentage
coverage of the intra-axonal cross-sectional area of the total subcortical surface area for the different resolution and gradient subsets (range 11.01 – 19.77%). The standard deviation of the fiber density describes the variance of different ROIs in the same brain averaged over subjects, the deviation of the averaged fiber density across subjects is much smaller. Additionally, we also report the percentage of unassigned fiber weights in order to estimate errors from the node-assignment (range 14.40 – 21.41%).
### 4.4 Discussion

In this work, we tested the hypothesis of a homogeneous fiber density at the G-WMI by correlating tractography fiber weights from the COMMIT optimization with the subcortical surface area. Analysis was performed on the high-resolution datasets of 10 healthy subjects from the HCP using the full multi-shell diffusion gradient sampling scheme. We found a high correlation ($p = 0.935$) between the intra-axonal cross-sectional area (represented by the fiber weights) and the subcortical surface area across different ROIs and subjects. However, we also noted consistent deviations in various structures across all subjects.

The positive correlations indicate that, as expected, a larger ROI might intersect with more streamlines than a smaller ROI. Nevertheless, the magnitude of the correlation and the accordance of the ratio of fiber-weights to ROI area across all 10 subjects is

<table>
<thead>
<tr>
<th>Resolution [mm] isotropic</th>
<th>Gradient set</th>
<th>Mean and std. dev. of fiber density [%]</th>
<th>Unassigned fiber weights [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>Multi-shell, full</td>
<td>11.01 ± 2.57</td>
<td>21.41 ± 1.47</td>
</tr>
<tr>
<td>2.5</td>
<td>Multi-shell, full</td>
<td>13.41 ± 2.91</td>
<td>17.61 ± 1.11</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=1000$, 45 dirs</td>
<td>19.77 ± 3.93</td>
<td>14.40 ± 1.21</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=2000$, 45 dirs</td>
<td>15.28 ± 3.23</td>
<td>16.06 ± 1.26</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=3000$, 45 dirs</td>
<td>13.45 ± 3.01</td>
<td>17.37 ± 1.26</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=1000$, 64 dirs</td>
<td>19.09 ± 3.87</td>
<td>14.67 ± 1.12</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=2000$, 64 dirs</td>
<td>15.04 ± 3.29</td>
<td>16.19 ± 1.21</td>
</tr>
<tr>
<td>2.5</td>
<td>$b=3000$, 64 dirs</td>
<td>13.11 ± 2.96</td>
<td>17.52 ± 1.18</td>
</tr>
</tbody>
</table>

Table 4.1: Mean fiber density and standard deviation at the G-WMI and percentage of unassigned fiber weights are listed for different resolutions and gradient sub-sets.
remarkable. Fiber densities were calculated as the multiplication of fiber-weights and fiber segment length. Conversely, dividing the voxel-wise fiber density by the fiber segment length, results in a cross-sectional area. It is thus tempting to interpret the fiber-weights as a sum of intra-cellular cross-sectional areas of axons, represented by a particular streamline. However, it is important to keep in mind that we assign the MR visible signal to a voxel volume, which is only an approximation (e.g. myelin is ignored completely). Regardless, following this assumption, the ratio of fiber-weights to GM area would represent an axon packing density at the G-WMI. The variance of this ratio among different cortical ROIs is visualized in Figure 4.2 and Figure 4.4, and the standard deviation across ROIs is listed in Table 4.1. Besides a high symmetry of the left and right hemispheres, the primary visual, motor and auditory areas exhibit an increased density at the G-WMI. The communality between these regions is that they all directly process external “inputs” (and also “outputs” in case of the primary motor cortex).

The reproducibility of our findings was also tested for clinically feasible acquisition schemes. We therefore artificially reduced the spatial resolution and subsampled the diffusion gradient scheme to single-shell subsets and different b-factors with different number of gradient directions.
Similar fiber density distributions across the complete G-WMI were found in all subsampled datasets. Nevertheless, especially for lower b-values, the absolute fiber density was over-estimated in comparison to the full diffusion gradient scheme. Fiber density distributions based on high b-value \((b = 3000)\) sets with 45 or 64 directions were almost identical to the full multi-shell dataset. However, the use of larger ROIs may cancel out local variations in the tractogram due to spatial averaging. Hence, a more finely grained parcellation scheme could potentially reveal further differences between the different gradient schemes. Additionally, the high-resolution dataset exhibited a slightly decreased fiber density in comparison to the down-sampled low-resolution dataset, however, this might also be cause by the increased number of unassigned fiber weights during the node-assignment.

We found a conforming fiber density at the G-WMI across different areas and subjects, however there were also marked variations in fiber density in some cortical areas. Whilst this effect is stable across subjects and across multiple gradient schemes with varying b-values and spatial resolutions, it is difficult to determine the cause of these fluctuations. The deviation from the mean fiber density could be caused by biological differences or intrinsic properties of the processing pipeline (e.g. choice of
Furthermore, the node-assignment remains an issue and might also have influenced results. If we compare the percentage of unassigned fiber-weights, a higher spatial resolution and improved angular resolution due to higher b-values or more diffusion directions negatively impact the node-assignment. Unfortunately, the beneficial effects of improved tractograms (at higher spatial and angular resolution) might be mitigated in the applied analysis. Apart from the need of an accurate and reliable assignment of streamlines to GM ROIs, it is also crucial to observe and minimize problems and pitfalls during the optimization as discussed in [35] and [69]. Additionally, it is still unclear if the fully sampled multi-shell acquisition scheme is ideal for the fitting of the utilized microstructure model for the global tractography optimization or if each q-space sample should be weighted according to e.g. the signal-to-noise ratio or the number of sampling points during optimization.

Regardless, a striking resemblance is observed between the axon packing density at the G-WMI and intra-cortical myelin maps derived from $T_1$, $T_2$ and proton-density weighted images [70, 71].

In conclusion, we presented a novel method that allows the indirect quantification of the axonal packing density at the
4.4. Discussion

G-WMI, based on fiber weights derived from tractography optimization. Furthermore, the hypothesis that the intra-axonal cross-sectional area is proportional to the cortical surface area is supported by the presented experiments and can be replicated with clinically feasible spatial resolutions, even with a single shell acquisition scheme.
Chapter 5

Investigation of Neurodegenerative Processes in Amyotrophic Lateral Sclerosis using White Matter Fiber Density

5.1 Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by progressive upper and lower motor signs with a markedly heterogeneous clinical presentation and course. ALS is uniformly fatal with a median survival time of 2 – 4 years from the onset of symptoms [72].

In the last decade, various magnetic resonance imaging (MRI) techniques have been utilized to improve the understanding of the neurobiological mechanisms underlying ALS [73]. Among various MRI techniques, diffusion imaging has proven to be the most reliable method for assessing pathological abnormalities
in ALS \cite{74, 75, 76}. In healthy brain white matter diffusion is anisotropic due to water molecules diffusing more freely along rather than perpendicular to axonal fascicles. A reduction in anisotropy is believed to reflect axonal degeneration and demyelination \cite{77, 78, 79, 80}. Using the so-called fractional anisotropy (FA) value, the degree of diffusion anisotropy can be quantified. Compared to healthy controls, decreasing FA values in the corticospinal tract (CST) of ALS patients were consistently observed using region of interest analysis and spatial profiling \cite{81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93]. Voxel-based morphometry permits assessing the white matter of the whole brain in a single comparison. Using this method, the view of ALS being a multisystem degenerative disorder \cite{74, 94} that extends beyond the motor system could be confirmed \cite{95, 96, 97, 89, 90, 98, 99, 100}. However, results derived using this method may be compromised by imperfect alignment of the DTI data \cite{101} and an inappropriate choice of the smoothing kernel \cite{102}. Tract-based spatial statistics (TBSS) aims to overcome these drawbacks. The native FA images are thereby first registered onto a FA template, followed by the projection onto an alignment-invariant mean FA skeleton \cite{36}. The resulting tract skeletons represent the centers of all tracts common to the study population. The method has been
successfully utilized to evaluate white matter changes in ALS [103, 104, 105, 106, 98, 36].

In recent years, a large number of models has been proposed to infer microstructural features of the neuronal tissue from diffusion-weighted imaging (DWI) data, such as the axonal diameter and the fiber density (FD). Comprehensive overviews have been published by [51] and [107]. In particular the FD may be more sensitive than the FA to the neurodegenerative processes that occur in ALS. However, until recently the techniques suffered either from long acquisition times or computationally intensive fitting procedures. By combining the estimation of local microstructure properties of the tissue with the versatility of fiber tracking, the convex optimization modeling for microstructure informed tractography (COMMIT) framework overcomes these drawbacks [34]. As a result, intracellular compartment maps are generated which are a direct measure of the FD. Using numerical simulations, it was in addition shown that under certain experimental conditions the FD in a given direction is proportional to the diffusion-weighted signal arising from the tissue aligned in that orientation [59]. Thus, the mean diffusion signal (MDS) averaged across a large number of diffusion directions poses a measure of the local FD. The main advantage of this approach is that it is fast and that the MDS
is derived directly from the acquired diffusion signal. Thereby, potentially error-prone post-processing steps such as the fitting of a diffusion model or the reconstruction of streamlines are eliminated.

The present work aims at investigating the potential of the FD and the MDS for evaluating neurodegenerative processes in ALS by comparing ALS patients with healthy age-matched controls and by evaluating longitudinal changes in the parameters. The novel parameters may prove to be more sensitive to the microstructural changes in brain white matter than the more commonly used FA value. The FD and the MDS values are compared in 21 ALS patients (mean age: 62.9, range: 48 – 84) and 12 healthy controls (mean age: 60.4, range: 51 – 69) using TBSS and the results are related to those obtained using the FA value. Beyond that, correlations of the parameters with disease severity are calculated. Finally, changes in the parameters over time are investigated in a subset of 17 ALS patients (mean age: 63.1, range: 48 – 84).
5. Fiber Density and Neurodegeneration in ALS

5.2 Materials and Methods

5.2.1 Patients and Control Subjects

Patients and age-matched healthy controls were recruited at the Neuromuscular Disease Unit / ALS Clinic of the Cantonal Hospital St. Gallen (St. Gallen, Switzerland). Recruitment took place as part of the European project for sampling and biomarker optimization and harmonization in ALS and other motor neuron diseases (SOPHIA). The study was approved by the Cantonal Research Ethics Committee of St. Gallen (St. Gallen, Switzerland). All participants received written and oral descriptions of the study procedures and provided written informed-consent statements in accordance with the declaration of Helsinki before participation in the study.

The initial patient sample consisted of 23 ALS patients (mean age: 62.9, range: 48 – 84, 13 female, 10 male, eight bulbar, 15 limb onset). Two patients had to be secondarily excluded due to insufficient data quality caused by extensive head motion (see section on data quality assessment). Thus, 21 ALS patients (mean age: 64.1, range: 48 – 84, 12 female, nine male, eight bulbar, 13 limb onset) were included in the TBSS analysis. Among these, two had definite, six probable, nine probable laboratory-
5.2. Materials and Methods

supported, and four possible ALS according to the revised El Escorial criteria [108]. Beyond that, longitudinal data were acquired three to six months (5.55 months ± 1.03 months) after the baseline measurement. Four patients were lost to follow up: one patient deceased after the baseline measurement, two patients could not further participate in the study after rapid deterioration of respiratory function, and one patient had to be secondarily excluded due to insufficient data quality (see section on data quality assessment). Thus, 17 ALS patients (mean age: 63.1, range: 48 – 84) were included in the longitudinal analysis of parameter changes over time. For the control group, 13 age-matched healthy controls (mean age: 61.3, range: 51 – 72, eight female, five male) were recruited. One subject had to be secondarily excluded due to insufficient data quality so that the control group during data analysis was comprised of 12 healthy subjects (mean age: 60.4, range: 51 – 69, eight female, four male).

In the ALS patients, disease severity was assessed at each measurement time point using the revised ALS functional rating scale (ALSFRS-R, maximum score: 48) [109]. In the patient group which was compared to healthy controls, the mean ALSFRS-R score was 39.3 (standard deviation: 5.0, range: 22 – 44). In the patient group whose data were analyzed longitu-
5. **Fiber Density and Neurodegeneration in ALS**

Finally, the mean ALSFRS-R score was 39.9 (standard deviation: 3.5, range: 30 – 44) at baseline and 36.4 at follow up (standard deviation: 4.3, range: 27 – 43).

### 5.2.2 Questionnaire and MRI Data Acquisition

Before the scan session, disease severity was assessed using the revised ALS functional rating scale (ALSFRS-R, maximum score: 48), which is a validated measure of motor disability in ALS [109]. Mean ALSFRS score was 38.6 (SD 5.2), ranging from 22 to 44. All MRI data were acquired on a Philips Achieva 3T system (Philips Healthcare, Best, the Netherlands), equipped with 80 mT/m gradients and an 8-element receive head coil array. Diffusion data were acquired using a diffusion-weighted single-shot spin echo EPI sequence with the following parameters: TR: 85.65 s, TE: 49 ms, FOV: 224 × 224 mm², 48 contiguous slices, slice thickness: 2.5 mm, acquisition matrix: 96 × 96, SENSE factor: 2.1, partial Fourier encoding: 60%. Diffusion-weighted images were acquired along 64 directions distributed uniformly on a half-sphere with a b-value of 1000 s/mm² in addition to 6 \( b = 0 \) s/mm² scans, resulting in a scan time of 10 min 8s. Additionally, for structural reference and anatomical priors for the tracking algorithm, 1 mm isotropic T1-weighted images were
recorded with a 3D magnetization prepared rapid gradient-echo (MP-RAGE) sequence (FOV: 240 × 240 × 160 mm\(^3\), sagittal orientation, 1 × 1 × 1 mm\(^3\) voxel size, TR: 8.2 ms, TE: 3.8 ms, flip angle: 8°).

5.2.3 Data Quality Assessment

At first, quality of all DWI data was assessed based on several criteria. To this end, the brain extraction tool [110] from FSL (Analysis Group, FMRIB, Oxford, UK, version 5.0.6) [47] was applied to remove non-brain tissue from the diffusion data and to estimate the inner and outer skull surfaces. Thereafter, the DWI data were corrected for eddy current-induced image distortions and subject motion using the eddy tool in FSL [11]. Diffusion tensor residuals were calculated for every acquired diffusion direction and the nine slices in the whole diffusion dataset with the highest residuals were identified for visual inspection. Furthermore, the MRtrix3 software package (Brain Research Institute, Melbourne, Australia, version 0.3.12) was applied to estimate the voxel-wise noise level using the residuals from a truncated spherical harmonics fit. Plots were generated depicting the twelve slices with the highest noise level, four in sagittal, four in axial, and four in coronal direction, respectively.
In addition, mean signal intensity plots for every diffusion direction and the non-diffusion-weighted image were derived and plotted slice by slice in sagittal, axial, and coronal directions. Peaks in these signal courses often indicate head motion. Based on a subject-wise visual inspection of these signal courses and fitting residuals, a rating was performed on a Likert-type scale by two trained MR physicist. Beyond that, the DWI data were visually inspected for artifacts. As noted above, two patients and one healthy control had to be secondarily excluded in the group comparison and another patient in the longitudinal study based on this quality assessment.

5.2.4 DWI Data Analysis and Parameter Calculation

FD Computation

Constrained spherical deconvolution with recursive calibration of the response function [27, 26] and fiber tractography was performed in MRtrix [111] using the default iFOD2 probabilistic tractography with anatomical tissue priors [15]. The diffusion data was further corrected for susceptibility induced distortions [112], prior to the constrained spherical deconvolution, in order to improve the alignment to the T1 derived tissue priors.
5.2. Materials and Methods

Additionally, the tractography seed points were determined dynamically according to the SIFT model [32]. For all of the experiments, 5 million fibers were generated. The resulting streamlines were optimized using the COMMIT framework [34] with the parameters described in [69] to get a weight for every reconstructed fiber. By modelling and optimizing each compartment contribution accordingly and summing up each fiber weight in every voxel, a FD map (corresponding to the resulting intracellular compartment fraction) can be derived.

**MDS and FA Computation**

FA maps of every dataset were calculated with FSL and the normalized mean diffusion signal (MDS) was derived using the following formula:

\[
MDS = \frac{\sum_{i=1}^{n} \frac{s_i}{s_0}}{n}
\]  

(5.1)

thereby \(S_i\) corresponds to the diffusion-weighted images, \(S_0\) to the non-diffusion-weighted \((b = 0 \text{ s/mm}^2)\) image and \(n\) refers to the total number of directions on the shell. By way of example, Figure 5.1 depicts FD, MDS, and FA maps of a single axial slice in a healthy volunteer for visual comparison.
5. Fiber Density and Neurodegeneration in ALS

Figure 5.1: a) FD, b) MDS, and c) FA maps of a single transversal slice in a healthy volunteer for visual comparison.

5.2.5 TBSS Analysis

TBSS analysis was performed firstly to assess differences between ALS patients and healthy controls and secondly to investigate changes in the diffusion parameters over time in the ALS patients. Thereby, the standard processing steps as described in [36] were used. First, all native FA maps were non-linearly registered to a $1 \times 1 \times 1$ mm MNI152 template. The FA maps were then averaged to create a mean FA map which was thereafter skeletonized to build an FA skeleton that represents the main tracts common to all subjects. Finally, this FA skeleton was thresholded at an FA value of 0.2. The same non-linear registration-, warping-, and skeleton projection operations as derived for the FA maps were subsequently applied to the corresponding FD and MDS maps. To evaluate differences between
ALS patients and healthy controls, voxel-wise analysis based on a general linear model was performed using FSL’s randomize tool [52] with 5000 permutations to correct for multiple comparisons. The TBSS results included threshold-free cluster enhancement [113]. For investigating changes in the diffusion parameters across time, paired statistics was performed again using FSL’s randomize tool with 5000 permutations to correct for multiple comparisons. Two contrasts were computed in each case, testing for positive and negative differences of the parameters in the ALS patients compared to healthy controls and across two points in time, respectively. Furthermore, for the ALS patients, voxel-wise correlations between disease severity (measured by the ALSFRS-R score) and the FD, the MDS, and the FA values were computed by TBSS regression analysis using the ALSFRS-R score as the predictor variable. FSL’s randomize tool was applied with 5000 permutations to correct for multiple comparisons. In the analysis, a p-value < 0.05 was considered statistically significant.
5.3 Results

5.3.1 ALS Patients vs. Healthy Controls and Correlation with Disease Severity

Figure 5.2 depicts the results of the TBSS analysis based on the FD (Figure 5.2 a) and the MDS (Figure 5.2 b) values whereas Figure 5.3 illustrates the results based on the FA values in ALS patients versus age-matched healthy controls. A significant deterioration of the white matter integrity in the patient group, as reflected by reduced FD and MDS values, was found in several white matter regions (see Figures 5.2 a and 5.2 b). A high agreement of the results derived based on the FD and the MDS values was observed. Significant decreases of both parameters were found along the projection fibers, including the right and left parts of the CST and the thalamic radiation. Furthermore, significantly reduced FD and MDS values were detected along the commissural fibers, namely the body of the corpus callosum (CC) as well as the forceps major and minor. Beyond that, TBSS analysis revealed significantly decreased values of both parameters in the association fibers, including the uncinated fasciculus, the superior longitudinal fasciculus (SLF), the inferior longitudinal fasciculus (ILF), and the inferior fronto-
5.3. Results

Figure 5.2: TBSS results of the comparison of a) the FD and b) the MDS values between ALS patients and age-matched healthy controls. Clusters exhibiting statistically significant decreases ($p < 0.05$) in the diffusion parameters are shown in blue on the green TBSS FA skeleton. There is a high anatomical agreement between the clusters derived based on the FD and the MDS values.

occipital fasciculus (IFOF). With respect to the SLF and the ILF, decreased FD values were observed in both hemispheres while reduced MDS values were observed only in the right parts of the fiber tracts. Finally, reduced parameter values were detected in the limbic system tracts, namely the cingulum.

No statistically significant changes in the FA values could be detected but a strong trend towards a statistically significant decrease was observed especially in the CC as illustrated in Figure 5.3 a which shows significant clusters at a p-value of $< 0.10$. With increasing p-values (see Figure 5.3 b for a p-value
Figure 5.3: TBSS results of the comparison of FA values between ALS patients and age-matched healthy controls. Clusters exhibiting a strong trend towards a statistically significant decrease in FA values are shown in blue on the green TBSS FA skeleton: a) clusters at a p-value of $< 0.10$ and b) clusters at p-value of $< 0.20$. There is a high anatomical agreement between the clusters derived based on the FA values and the findings based on the FD and the MDS values.

of 0.20), similar patterns of clusters as derived using TBSS analysis based on the FD and the MDS values showed up.

Finally, TBSS analysis did not reveal significant correlations of the diffusion parameters with disease severity as measured by the ALSFRS-R.

5.3.2 Parameter Changes over Time

TBSS analysis revealed statistically significant changes in the FD and the FA values over time in various anatomical locations.
In contrast, no significant differences of the MDS values were observed between time points ($p > 0.05$).

Figure 5.4 depicts significant changes in the FD values over time. With disease progression, significant decreases in the FD values were observed in the left hemisphere. FD values decreased in parts of the CST, the thalamic radiation, the body of the CC, the arcuate fasciculus, the uncinate fasciculus and in various association fibers, namely the SLF, the ILF, and the IFOF.

Figure 5.5 illustrates significant changes in the FA values over time. Surprisingly, an increase in the FA values was found in several regions in the right hemisphere. These included parts of the CST, the splenium of the CC, the SLF, the ILF, the IFOF, the thalamic radiation, the arcuate, and the uncinate fasciculus.

5.4 Discussion

In the present work, the benefit of the FD and the MDS for evaluating neurodegenerative processes in ALS patients was investigated using TBSS. The findings demonstrate that the MDS and in particular the FD may be more sensitive to white matter changes than the FA value and may permit evaluating pathological processes at an earlier stage of the disease. Data of 21 ALS
Figure 5.4: TBSS results of the changes in the FD values in the ALS patients over time. Clusters exhibiting statistically significant decreases in the FD values are shown in blue on the green TBSS FA skeleton. The two rows a) and b) depict different anatomical locations.
Figure 5.5: TBSS results of the changes in the FA values in the ALS patients over time. Clusters exhibiting statistically significant increases in the FA values are shown in red on the green TBSS FA skeleton. The two rows a) and b) depict different anatomical locations.
patients were compared to those of 12 healthy controls. Beyond that, changes in the parameters over time were evaluated in a subset of 17 ALS patients. The TBSS results were compared to those obtained based on the FA value, a parameter that has been evaluated in numerous clinical studies as a potential indicator for pathological changes in ALS [114, 115, 116]. Compared to healthy controls, the FD and the MDS were significantly reduced in various white matter structures throughout the brain. Although no statistically significant changes in the FA values were observed, a similar trend as for the FD and the MDS values was observed. Furthermore, based on the FD value, continuing deterioration of the previously identified impaired fiber tracts was found in the ALS patients. The results indicate that the FD and the MDS may be more sensitive to the neurodegenerative changes that occur in ALS than the commonly used FA value. TBSS analysis of the FD and the MDS revealed a widespread pattern of white matter impairment affecting both motor and extra-motor fiber tracts which is in agreement with previous results [117, 118, 103, 119, 84, 106, 89, 90, 98, 99, 100] confirming the view of ALS being a multisystem degenerative disorder [74, 94]. Significantly reduced values of the FD and the MDS in ALS patients relative to healthy controls were observed in the projection fibers, the commissural fibers, the association
fibers, and the limbic system tracts. The observed pattern of white matter changes is in close agreement with a previous TBBS analysis based on the FA value [106] but the present findings suggest that the FD and the MDS may be more sensitive parameters for the evaluation of microstructural changes in ALS. The pyramidal cells of the precentral gyrus are also called upper motor neurons. The fibers of the upper motor neurons project out of the precentral gyrus ending in the lower medulla oblongata to form the lateral corticospinal tract on each side of the spinal cord. A lesion or degeneration of these neurons result in a paresis, loss of fine motor skills and spasticity of the upper and lower extremities as typical clinical signs of an upper motor dysfunction or loss [120].

In line with expectations, significantly decreasing values of the FD were observed in various white matter structures throughout the brain. The fiber tracts affected by continuing deterioration agreed with those that had already shown decreased FD values in the group analysis of ALS patients versus healthy controls. No significant changes of the MDS over time were observed. Since DWI data were acquired as part of the European project SOPHIA, DWI was performed with a maximum b-value of 1000 s/mm2. Higher b-values, however, are required to ensure the proportionality of the FD to the diffusion-weighted signal.
5. **Fiber Density and Neurodegeneration in ALS**

[57, 59] and may increase sensitivity of the MDS to pathological processes in ALS. Thus, further studies incorporating higher b-values are needed to corroborate the benefit of the MDS for investigating neurodegenerative processes in ALS. Contrary to expectations, increasing FA values were observed in several white matter structures over time in the right hemisphere. This finding may be explained by the degeneration of minor fiber bundles perpendicular to the dominant fiber bundle in the respective voxel which would artificially increase the FA value despite the occurrence of axonal loss. In musicians, significantly increased FA values were observed in the right arcuate fasciculus after music-cued left-handed motor training [121]. Similarly, the more intensive use of the unaffected hand in ALS patients might lead to increasing FA values in the hemisphere contralateral to the affected hand. So far, relatively few studies have investigated FA changes in white matter structures of ALS patients over time and these studies have yielded inconsistent results. Some observed significantly decreased FA values over time [97, 122, 123] while others found no significant changes in the FA values [124, 125, 90].

Compared to the FD and the FA, it should be stressed that the MDS is derived directly from the acquired diffusion signal and thus does not rely on potentially error-prone post-processing.
steps. Furthermore, the simplicity of its computation facilitates applicability in the clinical setting. The MDS is closely related to the mean diffusivity derived from the diffusion tensor (by a logarithmic transform) but the MDS is not coupled to a tensor model and therefore does not rely on the Gaussian assumption. As noted before, the FD in a given direction is proportional to the diffusion-weighted signal arising from the tissue aligned in that orientation [59]. Thus, the MDS constitutes a measure of the local FD. For this reason, a high correspondence between FD and the MDS findings is expected and was confirmed in the present study.

No significant correlations of the parameters with disease severity have been observed. This may have been caused by the relatively low number of study participants, the composition of the patient group which featured a relatively narrow range of the ALSFRS-R scores or the relative lack of the ALSFRS-R to upper motor neuron involvement. However, inconsistent results have been reported in previous studies with some studies observing correlations of the FA values with the ALSFRS-R [126, 82, 83, 104, 122, 106, 90, 98, 127] while others found that it does not relate to disease severity [118, 128, 86, 105, 129, 92, 130]. When comparing ALS patients to healthy controls, changes in the parameters were predominantly observed in the right hemi-
sphere. Similarly, asymmetry was observed when changes in the parameters over time were evaluated. These findings are in agreement with previous studies in ALS [103, 90, 130] and with the report of asymmetry in motor neuron pathology [131].

A limitation of the present work is the already noted relatively small patient population and in addition, three patients had to be secondarily excluded from data analysis due to severe motion-induced artifacts, two in the group analysis and another one in the longitudinal study. This might be the reason why only a trend towards statistically reduced FA values was observed in ALS patients relative to healthy controls. Due to the limited number of patients, the population was not subdivided into patients with bulbar versus limb onset. Furthermore, the relatively short follow-up period of 3-6 months may be insufficient to reliably capture the full evolution of white matter changes in ALS. However, despite the relatively short interval, three patients were lost to follow up, in one case since the patient deceased prior to the termination of the study and in two cases due to an acute deterioration in their health condition which made further participation in the study impossible. Since a wide range of survival times are observed in ALS ranging from a few months to several decades [72], it is difficult to determine the optimum follow-up period and larger patient
cohorts are required to reliably evaluate white matter changes over time. Nevertheless, despite the relatively short follow up period, changes in various white matter structures throughout the brain were detected using the FD values.

5.4.1 Conclusion

In conclusion, the present work shows that the MDS and in particular the FD show great promise for evaluating white matter integrity and neurodegenerative processes in ALS. The findings suggest that the parameters may be more sensitive to white matter changes than the FA value and may permit evaluating pathological processes at an earlier stage of the disease.
Diffusion MRI tractography allows to non-invasively probe and study the human brain white matter. The incorporation of global top-down optimization techniques enables the derivation of biologically reasonable, quantitative streamline measures. These structural markers show improved sensitivity compared to tensor-based scalar metrics in the investigation of psychiatric diseases (e.g. schizophrenia).

6.1 Discussion

In this thesis, a flexible and modular software framework was developed to perform various evaluations and analyses on subject- and group-level, in order to incorporate recent innovations and to simplify the processing of structural dMRI datasets. The quality assessment and monitoring is particularly important in patient populations and multiple tools were developed to identify various artifacts.
6.2. Outlook

Additionally, the quality assurance of streamline tractograms was explored and new error measures based on global optimization techniques were introduced. Moreover, streamline up-sampling was proposed, which substantially improves the streamline representation of the measured diffusion dataset. The potential of quantitative streamline densities as a meaningful biological marker was examined in various patient populations. Furthermore, the transfer from high quality acquisition to clinically feasible sequences was studied with respect to the novel diffusion measures. An estimation of an axon packing density at the gray-to-white matter interface was derived, which revealed striking resemblance to intra-cortical myelin estimations derived from $T_1$, $T_2$ and proton-density weighted images. An application of fiber density measures in comparison to tensor-based disease measures was studied in patients suffering from amyotrophic lateral sclerosis, revealing an increased sensitivity to neurological degeneration.

6.2 Outlook

In the last decade, a shift towards higher angular resolution and multi-shell acquisition protocols has occurred. Further improvements in reducing scan time will enable the adaptation
6. **Summary**

of more sophisticated scan protocols in a clinical environment. Multislice acquisitions are very promising for various diffusion acquisitions and will reduce the scan time by a factor of $2 - 3$. However, sequences with large b-values exhibit a low SNR due to long echo times caused by the diffusion time and will notably suffer if the reduction factor is pushed too far. In order to increase the SNR, stronger gradients are needed, which enable shorter echo times.

Motion is still a big problem in a clinical setting, especially for longer sequences used in dMRI and post-processing routines will not be able to eliminate severe motion contamination during the acquisition. Therefore, real-time motion monitoring (and correction) systems are needed to improve data quality and to reduce the exclusion rates in clinical studies. Additionally, it is also crucial to adapt more advanced diffusion models and reconstruction techniques in clinical studies where the diffusion tensor is still the method of choice. The two separated fields of diffusion tractography and diffusion microstructure are starting to fuse together due to the ability of measuring multiple $b$-values and high angular resolution simultaneously. Moreover, advancements in the microstructure community experimenting with asymmetric gradient pulse designs claim to be able to measure microscopic anisotropy and orientational coherence.
which might also be beneficial for tractography algorithms.
The limited number of subjects in most clinical studies is another problem that needs to be addressed. Most clinical studies explore statistical differences between a group of patients and healthy controls, however, a single-subject based diagnosis or prediction is desired. Therefore, the large-scale data gathering, sharing and pooling will provide new opportunities despite the emergence of different challenges such as data heterogeneity across different acquisition protocols and scan sites.
A reliable quantification of structural connectivity hinted by the recent improvements in global optimization techniques could drastically improve the significance of dMRI in the investigation and prediction of various neurological diseases. Nevertheless, the validation and a lack of ground truth of global connectivity measures and local microstructure remains an ongoing challenge.
Deep learning and machine learning approaches introduce new possibilities for all processing steps in the diffusion processing pipeline. Valuable improvements are expected in image reconstruction, artifact detection and various pre-processing steps. Tractography algorithms might not be able to directly benefit from machine learning, due to a lack of ground truth. However, machine learning will improve automated diagnosis of various
diseases and predict treatment outcome especially if larger data collections are shared and brought together.


Publications

Journal Publications


Conference Contributions


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