Thesis Proposal: A Framework Towards Assessing Brain-Tissue Microstructure Properties from Diffusion Imaging: The Modeling, The Tool and The Evaluation

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Project Summary

I will develop a computational framework for using diffusion MRI to measure and evaluate the association of voxel-based microstructure properties in the brain tissue with white-matter-disease progression *in vivo*. Diffusion MRI measures the diffusion properties of the white matter, and has become one of the most popular techniques in brain research for assessing a number of neurological disorders. Common biomarkers derived from diffusion MRI, such as fractional anisotropy (FA), are useful indicators of major microstructure changes, though they are nonspecific at the microstructure level: the same observation of FA changes could be caused by two distinct microstructure change processes which may include a combination of changes in myelin, axon caliber, cellular packing density and membrane permeability. I hypothesize that measuring and analyzing specific disease-affected microstructure changes *in vivo* will provide insights into and make possible earlier detection of white-matter diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS). My goal is to collaborate with brain scientists to develop an analytical model and a microstructure-property recovery algorithm, and applying these prediction measures to the study of human brain.

Using this framework, I will develop analytical models of water diffusion in the white matter from diffusion MRI. The analytical models will be incorporated into a computational tool that extracts microstructure properties of the white matter that are otherwise not obtainable *in vivo*. The microstructure properties I aim to extract are: axon caliber, glial-cell caliber, the compartmental-volume fraction, axon-caliber distribution profile, and the membrane permeability. I will instantiate the framework using a combination of tools: (1) an interactive graphical interface for selecting region-of-interest (ROI) in diffusion MRI data. (2) an analytical model of water diffusion for estimating microstructure properties through a computational procedure. Tool (1) enables brain scientists to perform interactive analyses of neural anatomy in their desired regions more efficiently. Tool (2) provides a computational method which, using diffusion MRI, allows microstructure properties of the white matter to be extracted from analytical models of water diffusion. The framework will allow brain scientists to explore microstructure properties and assess white-matter diseases non-invasively.

I will validate my framework by gauging the accuracy and consistency of microstructure measurements at several different levels. First, I will validate the estimation results from simulated MRI data with a set of microstructure parameters pre-defined in the simulation. Second, I will validate estimation results from animal data with histological sections of a number of isolated regions from the same subject or the recorded genetically identical subjects.

One of my primary goals is to apply *in-vivo* microstructure measurements to the disease study of human subjects. The framework will be applied to normal human subjects to validate its (1) feasibility in extracting microstructure properties *in vivo*, and (2) reliability in performing segmentation of the corpus callosum (CC). If the study of normal subjects proves to be reliable, the framework will be applied for analyzing specific microstructure changes in white-matter diseases *in vivo*. One of the following three major white-matter diseases will be studied: (1) Alzheimer's Disease (AD), (2) Human Immunodeficiency Virus (HIV), and (3) Multiple Sclerosis (MS). Voxel-based group analysis will be performed between patient and controlled group. The disease study results will be quantitatively validated with existing clinical and histological observations.

A Contributions

Specific contributions of this work include:

A.1 *In-vivo* Estimation of Microstructure Properties to Improve Brain Disease Progression Analysis Studies have shown that changes to microstructure properties (e.g. axon caliber) are detected in an early stage of the brain diseases. Currently, however, these properties can only be measured in the brain using invasive histological methods. The goal of my project is to develop computational methodology for diffusion imaging data modeling in order to measure disease-affected microstructure changes *in vivo*. I anticipate that these measures will provide more specific insights into and make possible earlier detection of brain diseases. Most of the following contributions centers on the evaluation of this main contribution.

The microstructure properties I aim to estimate are: axon caliber, glial-cell caliber, the compartmental-volume fraction, axon-caliber distribution profile, and the membrane permeability. Quantitative validation of the estimation will be done by measuring and comparing the accuracy and consistency of the results with a set of ground truths at two different levels:

- Estimation results from simulated MRI data will be validated with a set of microstructure parameters pre-defined in the simulation.
- Estimation results from animal data will be validated with histological sections of a number of isolated regions from the same subject or a recorded genetically identical subject.

A.2 Four-Compartmental Geometric Model Incorporating Water Exchange to Achieve Higher Accuracy in Microstructure Parameter Estimation The brain-tissue structure is extremely complicated and a geometric model is usually an approximation of that structure. In the scope of this thesis project, I aim to build a more realistic model with four compartments representing the axons, their myelin sheaths, the glial cells and the extracellular compartment. Water exchange is modeled between these compartments. I hypothesize that the model will more closely represent the microstructure of the white matter and thus improve the accuracy of microstructure information extraction. I will evaluate the accuracy improvement of the extracted microstructure parameters with the well-known CHARMED model (a composite hindered and restricted model of diffusion) [8].

A.3 General Guidelines for Optimizing the Diffusion-MRI Experimental Design to Enable *in-vivo* Acquisition and Achieve More Accurate Microstructure-Parameter Estimation The current parameter estimation procedure requires special data acquisition with high gradient strength ($300mT/m$) and may take up to 11 hours [11], circumstances which are not possible to obtain with live subjects. I aim to analytically evaluate and derive the experimental guidelines for optimizing: (1) length of time for the experiments and (2) estimation accuracy of specific microstructure parameters.

This contribution will be demonstrated in the following two ways:

- Demonstrate the feasibility of parameter estimation from experimental protocols that are achievable *in-vivo*.
- Demonstrate the improved accuracy in parameter-estimation results compared with results from protocols that were not yet optimized.

A.4 A Probabilistic Modeling Framework for Recovering Variability in Axon Caliber and Uncertainty in Fiber Orientation The axons in the brain tissue are non-uniform in size and direction. I aim to model the variability in axon caliber and uncertainty in fiber orientation through probabilistic model. I will validate the probabilistic model results in the following two ways:

- axon-caliber distribution will be validated using simulation data [23] modeling different parts of the corpus callosum (CC) matching the distribution found in [2, 27]. The extracted axon-caliber distribution will be quantitatively compared with histological findings in [2, 27].
- fiber orientation recovery will be validated using a fiber phantom configuration. The recovered fiber orientation using the framework will be compared with the pre-defined fiber orientation in the phantom data.

A.5 Human Application Study: Towards Better Assessment of Human-Brain Tissue in Healthy and Diseased States Non-invasively The framework will be applied to normal human subjects for microstructure parameters recovery and anatomical segmentation. If the framework proves to be reliable from the study of the normal subjects, I will use the framework to quantitatively analyze one of the following three diseases: (1) Alzheimer's Disease (AD), (2) Human Immunodeficiency Virus (HIV), and (3) Multiple Sclerosis (MS).

A.5.1 A More Direct *In-vivo* Measurement of Microstructure Properties in the Human-Brain Tissue and the Application to Anatomical Segmentation One of my primary goals is to apply non-invasive microstructure measurements on human studies. The application and validation of the framework in healthy normal subjects is a fundamental step towards disease study. In this study, we aim to:

- Validate the feasibility of recovering the microstructure properties in human subjects using the framework.
- Validate the reliability of performing anatomical segmentation using the extracted microstructure information from the framework.

A.5.2 Towards Better Assessment of Microstructure-Change Correlation in Disease Progression Currently, most *in-vivo* white-matter-disease studies that use diffusion MRI rely on indirect biomarkers such as FA. The fact that FA is a nonspecific summation index of the observed diffusion tensor imaging (DTI) signal over the entire pixel, masking the ability to distinguish between the different pathologies affecting different microstructure components. I hypothesize that studying the specific microstructure changes *in vivo* will: (1) enable earlier detection of brain disease, (2) provide more direct assessment of how disease affect the brain microstructure, and (3) provide a more sensitive measurement of the degree of disease progression.

I aim to test my hypothesis in the following ways:

- Voxel-based group analysis (patient and controlled group) will be conducted to compare the recovered microstructure-parameter changes due to disease with recorded clinical changes findings.
- The revealed microstructure changes will be evaluated for their capability to interpret the observed nonspecific biomarker-changes (FA, MD) due to disease in the literature and validate their inferred microstructure changes.
- The sensitivity of these *in-vivo* microstructure measurements will be compared with the existing nonspecific biomarkers for assessing the progress of white-matter disease.

The framework will be applied to one of the three major white-matter diseases: (1) AD, (2) HIV, and (3) MS. Please refer to the methods section of this proposal for detailed plans of the disease study.

B Background and Significance

The proposed framework in this project could ultimately provide a non-invasive way of obtaining more specific information about microstructure changes in the progress and treatment of brain disease. In this section, I start with a description of the advantages of my proposed method, provide some of the background leading and enabling my approach, describe the significance of my framework and its application for human study in healthy and diseased brain, and explain some of the significant challenges I will face in developing and validating my tools.

B.1 Advantages of My Proposed Methods over Current Methods My framework will have one or more of the following advantages over all current methods of which I know:

- The accuracy of my framework will be validated at two levels: (1) simulation data with predefined parameters as ground truth, and (2) animal data with histological data as ground truth.
- The microstructure modeled with multiple compartments (axons, myelin sheath and glial cells) incorporating water exchange between them. Previous work assumed two compartments with no exchange between them [7, 11].
- Extracts microstructure information with no assumption about the fiber orientation. Previous works assume a known fiber direction [7, 11].
- Probabilistic Modeling framework will be developed to handle uncertainty in axon-caliber variability and fiber orientation.
- Microstructure information will be extracted from human data *in vivo*. The *in-vivo* microstructure analysis will also be applied on the corpus callosum (CC) segmentation. All previous *in-vivo* work on extracting microstructure information has only been applied on simulation or rat data [11, 4].
- If the study of normal subjects is successful, microstructure changes in major brain diseases will be measured and analyzed to provide a more direct diagnosis of the disease progression. All previous non-invasive brain-disease studies have relied on nonspecific biomarkers [39, 16, 19].
- Guidelines for optimizing the experimental design for diffusion MRI to reduce acquisition time and improve estimation accuracy will be established. Previous work only focused on acquisition optimization for a specific axon caliber [4].

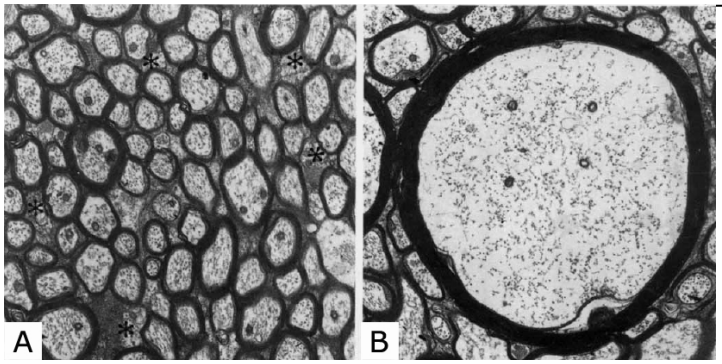

Figure 1: *A: Electron microscopy of axons in anterior region of the corpus callosum (CC) in an adult rhesus monkey. Notice the small unmyelinated axons (asterisks). B: Electron micrography of axons in midbody region of the same CC shown in A. In the center of this micrography is an example of the very large axons encountered only in midbody region of the CC. Both microscopy, 8,500x magnification. Figures from [27].*

B.2 Significance: Assessing Brain-Tissue Microstructure Properties Non-invasively using Diffusion Imaging Diffusion MRI measures the mean displacement of water molecules. The fact that water diffusion is sensitive to the underlying tissue microstructure provides a unique potential to infer properties of the brain tissue from diffusion MRI. Axons are in effect the primary transmission line of the nervous system in the brain, and they form complex neural networks. The structure of such networks in both healthy and diseased states is essential for studying brain diseases [29].

Microscopic analyses (Figures 1) have provided invaluable, high resolution information about tissue structure. However, this is an invasive histological procedure and almost all microscopic imaging of central nervous system (CNS) was carried out on chemically fixed or frozen tissue [10]. My project aim to measure brain-tissue properties *in-vivo*.

B.3 Significance: Reveal Microstructure Properties to Provide More Specific Biomarkers for Brain Study Demyelination in multiple sclerosis (MS) and axonal degeneration in Alzheimer's disease (AD) are two examples of diseases that primarily affect the neuron but cause changes in the white matter structure as well.Current *in-vivo* diffusion MRI brain studies rely on indirect diffusivity-based measures as biomarkers [36, 33, 44, 45]. Fractional anisotropy (FA) became the most widely used index [9]. The major limitation of these indirect biomarkers is that they are nonspecific: FA would show a similar indication of reduction at a number of combined pathological changes at a microstructure level, and we could not distinguish between them.

I hypothesize that direct estimation of these tissue properties (e.g. axon caliber) could provide greater insights into brain diseases and aid in their earlier detection and treatment. I will evaluate the sensitivity of these measures in assessing tissue changes by comparing them with indirect measures such as FA found in the literature [16, 42, 21, 19]. The recently discovered non-Gaussian nature of water diffusion within tissue structure [14] enables us to recover microstructure properties that were not accessible before. A recent approach [8, 7] imposes a geometric model on the signal decay enabling the extraction of specific information on various microstructure compartments. However, this work has three key limitations: it requires (1) high gradient strengths ($300mT/m$ in [11]), (2) long acquisition times (2 hours in [11]), and (3) prior knowledge of the fiber orientation. My project will aim to address these limitations. Details are discussed in the following sections.

B.4 Significance: A Four-compartment Model to Improve Extracted Brain-Tissue Microstructure Properties A model needs to be imposed on the diffusion signal decay in order to derive compartment-specific information about brain-tissue microstructure. Stanisz's model was the first to use the geometric properties of the sample in order to extract the mean size of geometric compartments (ellipsoids and spheres representing axons and cells) from complicated signal decay. This model was very specific to the optic nerve, however, and therefore did not take into account the variability in sizes of the compartments [41]. The Peled model [34] accounted for geometrical variability by modeling axons as impermeable cylinders with size distribution in the fitting procedure for diffusion in the sciatic nerve. While the model introduced tissue heterogeneity, it was not implemented in 3D space and did not take into account other diffusion processes. The CHARMED model [8, 7] uses a similar framework as [41] and [34], but defines the diffusion process in 3D. It decomposes the measured signal to diffusion processes and assumes the assignment of each of the diffusion processes to specific compartments.

The white matter of the CNS contains axons, their myelin sheaths and glial cells. The function, microstructure properties and relationship of these cellular elements changes in response to white-matter diseases. The existing two-compartment models with no exchange do not accurately describe the microstructure properties of the CNS. I aim to better model the white matter tissue structure with a four-compartment model incorporating

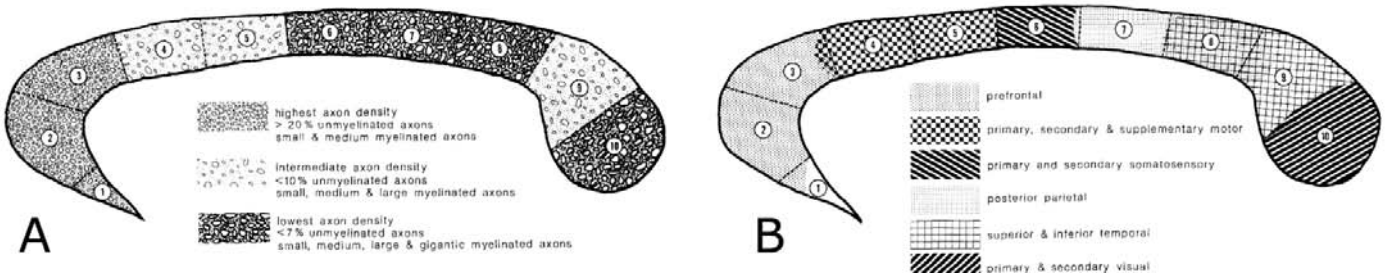

Figure 2: *Studies [2, 27] have shown that the largest myelinated axons and the smallest proportion of unmyelinated axons are found in regions of the corpus callosum (CC) that carry projections from primary sensory cortices. The smallest myelinated axons and largest proportion of unmyelinated axons are found in regions of the CC that carry projection from association cortices. A: A summary diagram of five identifiable sub-regions with different axon composition based on the sector analysis of the corpus callosum. B: Data on the topography of axons within the callosum adapted from the studies of Pandya et. al. [32], drawn in the same format and scaled to the same size as the drawing in A. Figures from [27].*

water exchange. I hypothesize that this model will more closely represent the microstructure of the CNS and thus improve the accuracy of *in-vivo* microstructure information extraction.

B.5 Significance: Axon-caliber Variability Estimation Most previous works [4, 40] has assumed a single axon caliber per voxel and did not account for the variability in axon caliber. Studies [2, 27] have shown that axon caliber is non-uniform even within a voxel (Figures 2). Barazany et al. [12] accounted for the caliber variability by modeling axon caliber distribution using gamma distribution. This work demonstrates the feasibility of extracting axon caliber variability although the accuracy of imposing such a distribution remains a challenge.

B.6 Significance: Unknown Fiber Orientation Recovery One of the fundamental limitations in previous microstructure-estimation work [8, 7, 11] is that they require prior knowledge of the fiber orientation. This could be inhibiting the potential of whole brain parameter mapping. Recently, Alexander et al. [4] demonstrated the feasibility of estimating axon caliber of unknown fiber orientation using an active imaging approach to optimize the orientationally invariant protocol, although the study was only validated on single axon caliber simulation data. I aim to solve this challenge in animal and human data where the fiber orientation is uncertain.

B.7 Significance: Optimized Protocol to Enable *In-vivo* Microstructure-Property Estimation Previous work on microstructure parameter estimation [11] requires high gradient strengths ($300mT/m$) and long acquisition times (2 hours) making it inapplicable to *in-vivo* studies for human subjects. In those cases, the diffusion signal may reach the noise level before enabling full characterization of the decay curve [6]. The protocol optimization is challenging since a wide range of experimental settings (b values and diffusion time) are required to extract different axon populations. Study [4] suggested that optimized acquisition protocols can be derived using a stochastic optimization of the Cramer-Rao lower bound (CRLB). I aim to optimize the protocol to enable *in-vivo* human and animal microstructure estimation.

B.8 Background: Matching Histological Data with the MRI Scans for Histological Validation In [24], the DTI datasets were acquired from the intact specimen, processed for histology and stained for myelin. The histological slices were successfully aligned with corresponding DTI "slices" in order to provide feedback about the fidelity of DTI-based models of fiber tracts. Fig. 3 shows a direct aligned comparison among anatomical MRI, DTI, and histology in the same image plane. The data was acquired in the brain of a larger ($\approx 1kg$) prosimian, the Aye-Aye lemur. This work demonstrates the feasibility of matching histological slices with MRI scans and provides background for the histological validation process of this project.

B.9 Significance: Disease Impact on Microstructure Properties and Application Studies I propose to apply my microstructure-parameter estimation methods to one of the following diseases: AD, HIV, and MS as my validation process for the project. I anticipate that extraction of direct tissue properties including axon caliber, myelin thickness and compartmental volume fraction will help us to better understand disease-related pathology changes.

B.9.1 Alzheimer's Disease (AD) Although AD is generally believed to affect gray matter, previous histological and MRI studies showed pathological changes in the white matter. Rose et. al. [39] found that patients with AD showed a highly significant reduction in the integrity of the associated white matter fiber tracts, while the pyramidal tract integrity was preserved. This confirms the clinical impression that cognitive decline is more prominent than motor disturbance at the presentation of probable AD. Bozzali [16] found higher MD and lower

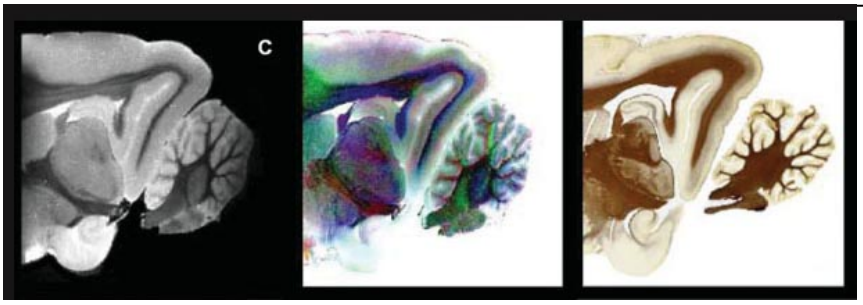

Figure 3: *Aligned comparison of imaging modalities. Left: T2-weighted MRI. Middle: Color-coded diffusion-tensor MRI. Right: Gallyas fiber stain.*

FA in various brain regions of AD patients while intervoxel coherence (C) showed no difference. Since C is a intervoxel coherence measure where as FA is intravoxel coherence, this finding may suggest that the amount of tissue disorganization of the white matter in AD is relatively small. Although these observations support the findings of white-matter-microstructure changes of AD[17], the actual underlying microstructure changes remain unknown in the current in-vivo studies.

B.9.2 Human Immunodeficiency Virus (HIV) One of the biggest health concerns globally and in the United States is HIV. HIV is detected in the CNS within two weeks of the initial infection [25]. HIV may be one of the most challenging diseases to study. The presence of HIV predominately involves the sub- cortical regions, with a particular predilection for the basal ganglia and white-matter pathways [26, 18, 15]. The specific damage to the myelin sheaths includes the white-matter gliosis, loss of white-matter volume, and white- matter pallor [30]. A number of non-invasive studies using DTI have demonstrated abnormalities associated with HIV infection, but the findings have not been universal [42, 21, 35, 46, 38, 37].

B.9.3 Multiple Sclerosis (MS) Multiple sclerosis (MS) is an autoimmune condition in which the immune system attacks the CNS and is the most common cause of neurologic disability in young adults. While demyelination is without doubt an important pathological process that leads to neurological symptoms, histological studies proved that axonal loss plays a major role in contributing to disability [22, 20, 43]. Olga et al. [19] have shown that patients with MS have lower FA and higher MD in the corpus callosum (CC) than controls. Axonal loss, with associated expanding extracellular space [13], may lead to the observed increase in diffusivity and reduction in anisotropy in the CC of patients with MS. Interestingly, the differences in diffusion parameters reached statistical significance only in the splenium of CC though the reason is unclear. It is hypothesized that it could be related to the differences in fiber composition of CC [1]. Thin fibers are most dense in the splenium and smaller axons seem to be preferentially susceptible to injury in MS. The reduction in the density of fibers passing through the CC could reflect Wallerian degeneration of axons transected in remote MS lesions.

C Preliminary Studies

In this section, I demonstrate preliminary studies pertinent to the framework that are in place to strengthen the success of the proposed project.

C.1 Feasibility: Achieving Higher Accuracy in Microstructure-parameter Estimation with a New Geometric Model Incorporating Water Exchange I demonstrate the improved estimation of axon caliber and diffusion coefficient with a new geometric model incorporating water exchange [47]. A model needs to be imposed on the diffusion signal decay in order to derive microstructure parameters. The only geometric model that defines the diffusion process in 3D assumes two compartments with non-abutting impermeable cylinders representing axons [8, 7, 11, 4]. Experiments have shown, however, that exchange does exist and a recent study suggested that exchange should be included if axons are modeled with two compartments [31]. My preliminary work has shown that a new two-compartmental non-abutting cylindrical model with permeable membranes incorporating water exchange has improved the estimation of axon caliber and diffusion coefficients [47]. Previous work [4] had a much lower accuracy in recovering smaller calibers ($\approx 2\mu m$). In [47], the recovered axon caliber had about the same variance for both small and large axons($\approx 1-7\mu m$). Also, the extracted diffusion coefficient was quite close to the true value while previous work had a downward bias in the estimation [4]. This demonstrates the potential of achieving higher accuracy in parameter estimation with a more realistic model for tissue microstructure.

C.2 Feasibility: Interactive Tool for Region-of-Interest (ROI) Analysis Disease-associated microstructure white-matter pathology change is usually not homogeneously distributed and often involves specific brain regions. For example, cognitive decline is more prominent than motor disturbance in AD patients. The

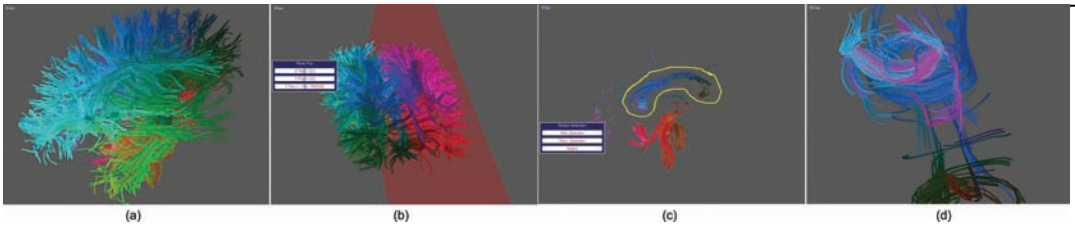

Figure 4: *Our Region-of-interest (ROI) application: sketching selection and interaction with data from the brain of a normal subject.*

main advantages of ROI analysis are that the regions can be chosen on the basis of a priori hypotheses, and can be located in a specific part of the brain. This methodology consists of drawing ROIs in specific areas of the brain, using anatomical knowledge, and then quantifying microstructure parameters within those areas.

Our ROI system [48] allows expert users of the application to select regions interactively with a 2D-based sketching mechanism (Fig. 4). The user selects axis-aligned planes in 3D (Fig. 4b) then views, as a 2D slice image (Fig. 4c), the colors of streamtubes representing the white matter intersecting that slice. Next the user makes a free-form closed curve. The streamtubes that pass through this region are selected as ROI (see Fig. 4d). This axis-aligned view and selection method fits neuroscientists' expertise with sectional anatomy to interpret aligned 2D images of the brain.

C.3 Data Simulation I demonstrate that simulation data has been successfully generated for estimation of a set of important microstructure parameters in my previous work [47]. Our simulation data was based on a Monte-Carlo diffusion simulation of a rectangular arrangement of cylinders with permeable membranes, incorporating water exchange. In the proposed work, I plan to improve the flexibility of our simulation data by using the simulation approach in [23]. In this simulation process, tissue is modeled as parallel cylinders with distributed caliber that are allowed to deform and abut when their edges touch. This simulation model more closely represents the tissue structure in the brain.

C.4 Disease Study: On-going Collaboration I demonstrate that we have established close collaboration with the following groups for each brain disease that I plan to study:

- AD: Prof. Stephen Correia at Psychiatry and Human Behavior Department in Brown University who works on the white matter impacted by normal aging and in disorders that can cause dementia.
- HIV: Prof. Robert Paul and Prof. Ronald Cohen at Brown Medical School who work on cognitive function in HIV infection.
- MS: Prof. Jack Simon at University of Colorado Health Sciences Center who works on MS pathology in the normal and abnormal appearing white matter.

D Research Design and Methods

I propose to develop and refine my framework for brain-tissue microstructure extraction in a spiral approach. Figure. 5 illustrates the general steps of each iteration. I will iteratively validate and refine my model in order to improve its accuracy and efficiency. This spiral approach comprises a number of steps, some of which may run in parallel. First, I will develop a four-compartment geometric model that represents the underlying structure of the brain. Second, I will analytically determine how water diffusion affects the signal decay this geometric model. Third, I will evaluate and acquire guidelines for optimized *in-vivo* protocols. Fourth, I will generate simulated diffusion MRI and collect animal and human (healthy and diseased) scans for validation and application study. Fifth, I will validate the estimation results at several different levels and compare the accuracy with the well-known CHARMED model [8]. Finally, I will perform an application study on human corpus callosum (CC) segmentation as well as a disease study.

D.1 Geometric Model Development The geometric model is usually an approximation of the exceedingly complex underlying structure of the brain tissue. I aim to design a four-compartment geometric model incorporating water exchange to improve the estimation of the microstructure parameters. I will start with our current geometric model [47] with two compartments assuming parallel non-abutting cylindrical axon cells with partially permeable membranes embedded in an extra-cellular medium. The new model will have four compartments modeling the axons, their myelin sheaths, glial cells and extracellular space in the white matter. The axons will be modeled with cylinders. The axon myelin sheaths will model the effect of the water exchange with cell membranes of varying permeability. The glial cell will be modeled with spheres similar to [41]. Finally, the extra-cellular space will be modeled with hindered diffusion.

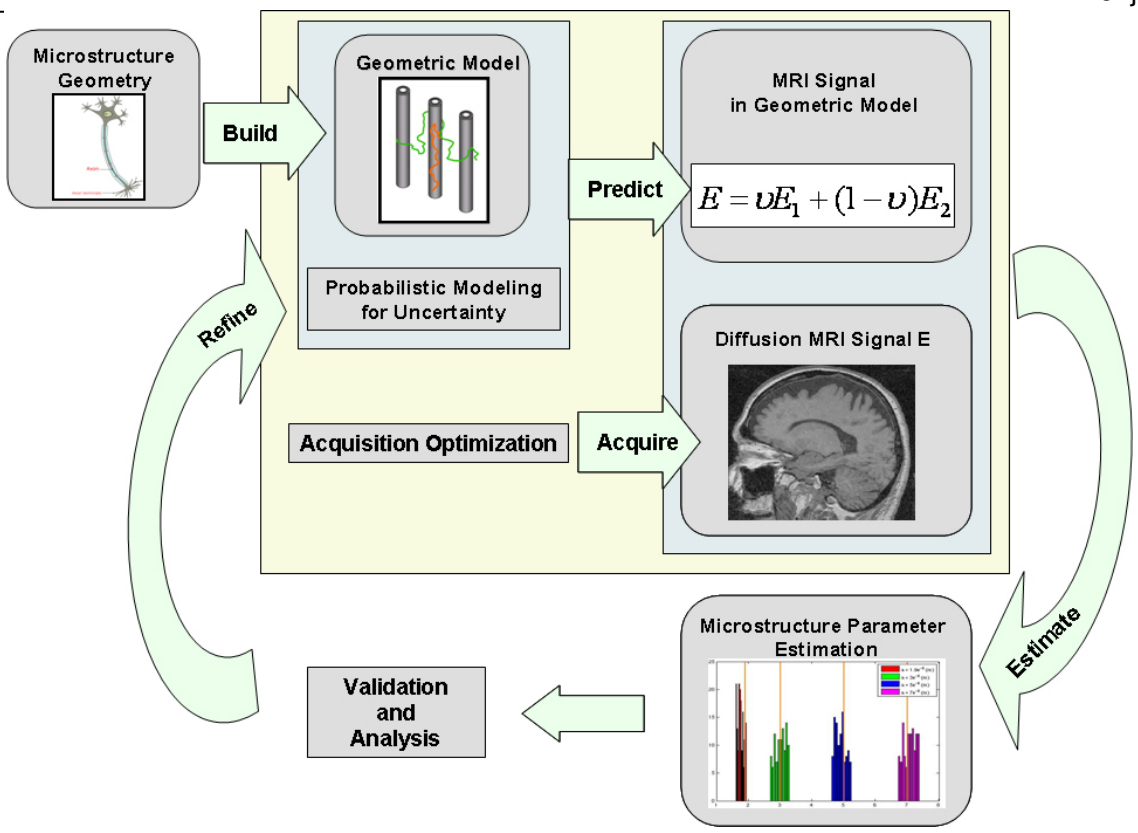

Figure 5: *Illustration of the general steps of the development and validation process of the project.*

D.2 Analytic Model Development The analytic model is a solution or approximate solution of the diffusion equation for water molecules in the pre-defined geometry of the tissue. This equation predicts the corresponding MR signal from the tissue. In order to derive the diffusion equation, the measured signal will be decomposed according to the geometric model. The analytic model will assume the assignment of each of the different processes to specific compartments in the geometric model. I will examine the conditional propagator which describes the motion of water molecules under-going diffusion. The conditional propagator obeys Fick's Law. Given the constraints of my geometric model, I will define my initial condition, boundary condition, and boundary relationship to solve for the conditional propagator. Finally, I will derive the signal attenuation by applying the Fourier Transformation to the conditional propagator that describes the spin displacement.

D.3 Multiple-Parameter Estimation from Analytic Model I will apply Markov Chain Monte Carlo (MCMC) procedure for multiple-parameter estimation from my analytic model of water diffusion. MCMC methods attempt to simulate direct draws from some complex distribution of interest. The general MCMC estimation procedure will be similar to our previous work [47].

D.4 Measuring the Axon Caliber Variability As I have mentioned earlier, the axon caliber may be non-uniform even within a voxel and its distribution is uncertain [27] (Figure. 2). Barazany et al. [11] used gamma distribution to account for axon caliber variability and extracted axon diameter distribution for rat's corpus callosum. Although this work demonstrates the feasibility of extracting axon caliber variation, the accuracy remains a challenge. I aim to start with gamma distribution and derive an optimal distribution or non-parametric approach to extract the axon caliber variation in a given voxel.

D.5 Extracting the Unknown Fiber Orientation Existing work for *in-vivo* microstructure-parameter measurement requires prior knowledge of the fiber orientation [11, 4]. The gradient in these studies needs to be applied perpendicular to the fiber direction. In this project, I will use multi-directional MRI scans combined with acquisition optimization (described below) to demonstrate how I would go beyond previous work and extract the axon parameters without prior knowledge of the fiber orientation in animal and human data. I hypothesize that the directional information could enable us to extract the unknown fiber orientation.

D.6 Voxel-based Analysis Voxel-based analysis will be applied to my validation study. It consists of performing an analysis on a voxel-by-voxel basis in order to localize the changes related to tissue abnormalities [5]. I will combine this approach with region-of-interest (ROI) analysis to be spatially specific, and histogram analysis to be unbiased. It involves co-registration of microstructure-parameter maps into a standard space, and then making comparisons of parameter values between groups or testing for correlations with an external variable, such as disability or age. I will extend our existing interface [48] from the circle-to-select mechanism (Fig. 4) to paint-to-select in order to better handle the study of small voxels.

D.7 Acquisition Optimization I aim to optimize acquisition parameters (gradient strength G, diffusion time Δ, pulse duration δ) in order to improve microstructure estimates and enable *in-vivo*. An objective function will be constructed to reflect the precision of model-parameter estimates for a particular protocol. The stochastic optimization of the Cramer-Rao lower bound (CRLB) based on Alexander et al. [4] will be used to find the protocol that minimizes the objective function. The combination of optimizations to consider are constrained by the requirement of *in-vivo* imaging protocol. Those requirements and constraints include [4]:

- The tolerable acquisition time that limits the number of measurements.
- Both power and safety constraints that limit the maximum gradient strength.
- Model-parameter estimation that must be orientationally invariant.

D.8 Validation and Analysis In this section, I will describe the various validation processes that the framework will be tested on. The following validation guidelines remain the same throughout all the validation studies discussed here:

- The following microstructure parameters will be extracted: axon caliber, glial-cell caliber, the compartmental-volume fraction, axon-caliber distribution profile, and the membrane permeability.
- The estimated mean value will be compared with the ground truth for voxel-based analysis.

D.8.1 Validation for the Accuracy of the Estimated Model Parameter Quantitative validation of microstructure-parameter estimation will be done by measuring and comparing the accuracy and consistency of the estimation results at the following two levels:

- Simulation Data
  - *Data:* Simulation data generated using Monte Carlo procedure based on [23].
  - *Ground Truth:* Pre-defined model parameters for the simulation.
- Animal Data
  - *Data:* Live Animal (macaque or sheep) MRI scan.
  - *Ground Truth:* Histological images from the same animal or published histological information from the recorded genetically identical animal.
  - *Validation Sites:*
    1. Corpus Callosum (CC), where most histological information have been recorded. Studies have shown found smaller axon caliber in anterior and posterior regions and larger axon caliber in midbody of CC.
    2. Cerebellum, where axon caliber uniformity in axon is most apparent.

D.8.2 Validation of Accuracy Improvement for Microstructure-Parameter Estimation The framework's improvements for parameter estimation will be validated using simulation data with the well-known CHARMED model (a composite hindered and restricted model of diffusion) [8].

D.8.3 Validation for Uncertainty Recovery The framework's ability to reveal the variability in axon caliber and uncertainty in fiber orientation will be validated with simulation data:

- Axon caliber variability validation:
  - *Data:* Simulation data [23] for different parts of the corpus callosum (CC) matching the distribution found in [2, 27].
  - *Evaluation:* Quantitatively compare the extracted axon caliber distribution with histological findings in [2, 27].
- Axon fiber orientation uncertainty validation:
  - *Data:* Phantom configuration of single fiber orientation.
  - *Evaluation:* The extracted fiber orientation will be quantitatively compared with the phantom fiber configuration.

D.9 Application Studies To further quantitatively evaluate my microstructure estimation framework, I plan to apply my framework to human subjects. The framework will be applied to the corpus callosum of normal human subjects for microstructure-parameter recovery and segmentation. If the framework proves to be reliable of the normal subject study, I will perform a *in-vivo* application study on one of the major brain diseases.

D.9.1 Application Study of Normal Human Subjects: Corpus Callosum (CC) Measurement and Segmentation I plan to apply the estimation framework to study the human CC. The CC is the largest white matter structure in the brain and it connects and facilitates the communication between the left and right cerebral hemispheres. Segmentation of the CC plays an important role in understanding the connectivity of the brain [2, 3, 28]. Moreover, several detailed histological studies of the CC in the brain have been carried out, allowing us to perform reliable validation studies [2, 27].

In the study I will:

- Measure the microstructure parameters *in-vivo*: axon caliber, the compartmental-volume fraction, axon-caliber distribution profile, and the membrane permeability.
- Quantitatively compare the estimation measurements with recorded histological information.
- Perform CC segmentation based on the derived axon caliber.
- Quantitatively compare the segmentation result with traditional tractography based segmentation.

D.9.2 Disease Study: Microstructure Properties as an *In-vivo* Biomarker for Brain Disease Study The ultimate goal of the project is to computationally determine microstructure changes in brain disease *in-vivo*. If the framework proves to be reliable from the above normal subject study, I will perform a *in-vivo* application study on one of the following diseases: (1) Alzheimer's Disease (AD), (2) Human Immunodeficiency Virus (HIV), and (3) Multiple Sclerosis (MS). The principle aim of this part of the project is to evaluate the specific hypotheses for the disease using direct microstructure properties derived from this framework in three ways:

- Voxel-based group analysis (patient and controlled group) will be conducted to compare the extracted microstructure-parameter changes with clinical changes due to disease.
- The revealed microstructure changes will be evaluated for their capability of interpreting the observed nonspecific biomarker-changes (FA, MD) due to disease in the literature and validate their inferred microstructure changes.
- The sensitivity of these *in-vivo* microstructure measures will be compared with the existing nonspecific biomarkers for assessing the progress of white-matter disease.

I will apply my methods to one of the three major white-matter diseases:

- Alzheimer's Disease (AD):
  - *Validation Sites:* Corpus Callosum (CC) region in the frontal, temporal and parietal lobes of the patients
  - *Current observation:* Increased mean diffusivity and lower FA have been observed [16]
- Human Immunodeficiency Virus (HIV):
  - *Validation Sites:* 10 regions displaying studied in [16].
  - *Current observation:* Significantly increased FA and MD in the SP group compared to the SN group have been displayed in these 10 regions [16].
- Multiple Sclerosis (MS):
  - *Validation Sites:* Body, genu and splenium of the Corpus Callosum (CC) region
  - *Current observation:* Increased mean diffusivity and lower FA have been observed [19].

Timeline

Time	Milestone
Year 1, Month 1	1st version of four-compartmental geometric model
Year 1, Month 2-3	Analytical model predicting water diffusion
Year 1, Month 4	Optimized experimental protocol
Year 1, Month 5	s data ready for first validation process
Year 1, Month 6	First validation process done for parameter estimation
Year 1, Month 7	CHARMED model implemented for validation comparison **Thesis proposal draft** sent to committee members
Year 1, Month 8	First validation process with CHARMED **Thesis proposal**
Year 1, Month 9	Refined geometric model
Year 1, Month 10	Analytical model from the refined geometric model
Year 1, Month 11	Optimized experimental protocol for the new model
Year 1, Month 12	Simulation data ready for second validation process
Year 2, Month 1	Second validation process done for parameter estimation Animal data for validation ready
Year 2, Month 2	Second validation results compared with CHARMED model
Year 2, Month 3-4	Refined model to handle axon caliber variability
Year 2, Month 5	Simulation data ready for axon caliber variability validation
Year 2, Month 6	Axon caliber variability validation done
Year 2, Month 7-8	Refined model to handle fiber orientation uncertainty
Year 2, Month 9	Simulation data ready for fiber orientation uncertainty validation
Year 2, Month 10	Fiber orientation uncertainty validation process done
Year 2, Month 11-12	Refined model for animal studies Third validation process done for parameter estimation
Year 3, Month 1	Human data for application studies ready
Year 3, Month 2-3	Refined model for human application studies
Year 3, Month 4-5	Human study parameter estimation done
Year 3, Month 6	Human study results analyzed
Year 3, Month 7	Corpus callosum segmentation done
Year 3, Month 8	All human study results analyzed and compared with literature
Year 3, Month 9-10	First draft of dissertation
Year 3, Month 11-12	**Dissertation and thesis defense**

Appendix

Terms and Abbreviations	Descriptions
Axon	The core nerve fiber extending from a neuronal cell body. It conveys signal from one nerve cell to another nerve cell or to muscle or gland.
Alzheimer's Disease (**AD**)	Alzheimer's disease (AD) is an irreversible, progressive brain disease that slowly destroys memory and thinking skills, and eventually even the ability to carry out the simplest tasks.
Corpus Callosum (**CC**)	A structure of the mammalian brain in the longitudinal fissure that connects the left and right cerebral hemispheres.
Central Nervous System (**CNS**)	The part of the nervous system that functions to coordinate the activity of all parts of the bodies of multicellular organisms.
Diffusion Magnetic Resonance Imaging (**Diffusion MRI**)	A magnetic resonance imaging (MRI) method that produces *in vivo* images of biological tissues weighted with the local microstructural characteristics of water diffusion. The field of diffusion MRI can best be understood in terms of two distinct classes of application - Diffusion Weighted MRI and Diffusion Tensor MRI.
Fractional Anisotropy (**FA**)	A scalar value between zero and one that describes the degree of anisotropy of a diffusion process.
Grey Matter (**GM**)	The part of the central nervous system, such as cerebral cortex, that contains nerve cell bodies and appears gray.
Multiple Sclerosis (**MS**)	An autoimmune disease in which the body's immune response attacks a person's central nervous system, leading to demyelination.
Myelin	The coating around nerve fibers (axons) that acts to insulate the fibers and promote efficient electrical conduction.
Human Immunodeficiency Virus (**HIV**)	A lentivirus that can lead to *acquired immunodeficiency syndrome* (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections.
Markov Chain Monte Carlo (**MCMC**)	A class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its equilibrium distribution.
ROI	Region-of-interest
White Matter (**WM**)	The part of the central nervous system that contains myelinated nerve fibers and appears white.

Literature Cited

[1] F. Aboitiz, A.B. Scheibel, R.S. Fisher, and E. Zaidel. Fiber composition of the human corpus callosum. *Brain Res*, 598(1-2):143–153, 1992.

[2] F. Aboitiz, A.B. Scheibel, R.S. Fisher, and E. Zaidel. Individual differences in brain asymmetries and fiber composition in the human corpus callosum. *Brain Research*, 598:154–161, 1992.

[3] A.L. Alexander, J.E. Lee, M. Lazar, R. Boudos, M.B. DuBray, T.R. Oakes, J.N. Miller, J. Lu, E.K. Jeong, W.M. McMahon, et al. Diffusion tensor imaging of the corpus callosum in autism. *Neuroimage*, 34(1):61–73, 2007.

[4] DC Alexander. A general framework for experiment design in diffusion MRI and its application in measuring direct tissue-microstructure features. *Magnetic Resonance in Medicine*, 60(2):439–48, 2008.

[5] J. Ashburner and K.J. Friston. Voxel-based morphometry-the methods. *Neuroimage*, 11(6):805–821, 2000.

[6] Y. Assaf, D. Ben-Bashat, J. Chapman, S. Peled, IE Biton, M. Kafri, Y. Segev, T. Hendler, AD Korczyn, M. Graif, et al. High b-value q-space analyzed diffusion-weighted MRI: Application to multiple sclerosis. *Magnetic Resonance in Medicine*, 47(1):115–126, 2002.

[7] Y. Assaf, T. Blumenfeld-Katzir, Y. Yovel, and P.J. Basser. AxCaliber: a method for measuring axon diameter distribution from diffusion MRI. *Magnetic Resonance in Medicine*, 59(6), 2008.

[8] Y. Assaf, R.Z. Freidlin, G.K. Rohde, and P.J. Basser. New modeling and experimental framework to characterize hindered and restricted water diffusion in brain white matter. *Magnetic Resonance in Medicine*, 52(5):965–978, 2004.

[9] Y. Assaf and O. Pasternak. Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. *Journal of Molecular Neuroscience*, 34(1):51–61, 2008.

[10] Yaniv Assaf and Yoram Cohen. *Inferring microstructural information of white matter from diffusion MRI*, chapter 7. Academic Press, first edition, 2009.

[11] D. Barazany, P. J. Basser, and Y. Assaf. In-vivo Measurement of the Axon Diameter Distribution in the Rats Corpus Callosum. *Proceedings of the 16th Scientific Meeting of the International Society for Magnetic Resonance in Medicine, Toronto, Canada*, page 567, 2008.

[12] Daniel Barazany, Peter J. Basser, and Yaniv Assaf. In vivo measurement of axon diameter distribution in the corpus callosum of rat brain. *Brain*, 132:1210–1220, 2009.

[13] D. Barnes, PMG Munro, BD Youl, JW Prineas, and WI McDonald. The longstanding MS lesion: a quantitative MRI and electron microscopic study. *Brain*, 114(3):1271, 1991.

[14] PJ Basser, J. Mattiello, and D. LeBihan. MR diffusion tensor spectroscopy and imaging. *Biophysical Journal*, 66(1):259–267, 1994.

[15] J.R. Berger and A. Nath. HIV dementia and the basal ganglia. *Intervirology*, 40:122–131, 1997.

[16] M. Bozzali, A. Falini, M. Franceschi, M. Cercignani, M. Zuffi, G. Scotti, G. Comi, and M. Filippi. White matter damage in Alzheimer's disease assessed in vivo using diffusion tensor magnetic resonance imaging. *British Medical Journal*, 72(6):742–746, 2002.

[17] A. Brun and Englund. E. A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. *Ann Neurol.*, 19(3):253–262, 1986.

[18] H. Budka. Neuropathology of human immunodeficiency virus infection. *Brain Pathology*, 1(3):163–175, 1991.

[19] O. Ciccarelli, D.J. Werring, G.J. Barker, C.M. Griffin, C.A.M. Wheeler-Kingshott, D.H. Miller, and A.J. Thompson. A study of the mechanisms of normal-appearing white matter damage in multiple sclerosis using diffusion tensor imaging. *Journal of neurology*, 250(3):287–292, 2003.

[20] CA Davie, GJ Barker, AJ Thompson, PS Tofts, WI McDonald, and DH Miller. 1H magnetic resonance spectroscopy of chronic cerebral white matter lesions and normal appearing white matter in multiple sclerosis. *British Medical Journal*, 63(6):736–742, 1997.

[21] C.G. Filippi, A.M. Ulu, E. Ryan, S.J. Ferrando, and W. van Gorp. Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain. *American Journal of Neuroradiology*, 22(2):277–283, 2001.

[22] L. Fu, PM Matthews, N. De Stefano, KJ Worsley, S. Narayanan, GS Francis, JP Antel, C. Wolfson, and DL Arnold. Imaging axonal damage of normal-appearing white matter in multiple sclerosis. *Brain*, 121(1):103, 1998.

[23] MG Hall and DC Alexander. A tissue model of white matter undergoing tissue swelling. *Proc CDMRI workshop at MICCAI, New York, NY, USA*, 2008.

[24] Jason A. Kaufman, Eric T. Ahrens, David H. Laidlaw, Song Zhang, and John M. Allman. Anatomical analysis of an aye-aye brain (daubentonia madagascariensis, primates: Prosimii) combining histology, structural magnetic resonance imaging, and diffusion-tensor imaging. *Anatomical Record*, 287A(1):1026–1037, November 2005.

[25] FC Krebs, H. Ross, J. McAllister, and B. Wigdahl. HIV-1-associated central nervous system dysfunction. *Advances in pharmacology (San Diego, Calif.)*, 49:315, 2000.

[26] K. Kure, KM Weidenheim, WD Lyman, and DW Dickson. Morphology and distribution of HIV-1 gp41-positive microglia in subacute AIDS encephalitis. *Acta Neuropathologica*, 80(4):393–400, 1990.

[27] A.S. LaMantia and P. Rakic. Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. *The Journal of Comparative Neurology*, 291(4), 1990.

[28] A. Lundervold, N. Duta, T. Taxt, and AK Jain. Model-guided segmentation of corpus callosum in MR images. In *Computer Vision and Pattern Recognition, 1999. IEEE Computer Society Conference on.*, volume 1, 1999.

[29] S. Mori and J. Zhang. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*, 51(5):527–539, 2006.

[30] BA Navia. Clinical and biologic features of the AIDS dementia complex. *Neuroimaging Clinics of North America*, 7(3):581, 1997.

[31] M. Nilsson, J. Lätt, E. Nordh, R. Wirestam, F. Stĺhlberg, and S. Brockstedt. On the effects of a varied diffusion time in vivo: is the diffusion in white matter restricted? *Magnetic Resonance Imaging*, 2008.

[32] D. N. Pandya and B. Seltzer. *The topography of Commissural Fibers*, pages 47–73. AR Liss, 1986.

[33] NG Papadakis, D. Xing, GC Houston, JM Smith, MI Smith, MF James, AA Parsons, CL Huang, LD Hall, and TA Carpenter. A study of rotationally invariant and symmetric indices of diffusion anisotropy. *Magnetic resonance imaging*, 17(6):881, 1999.

[34] S. Peled, D.G. Cory, S.A. Raymond, D.A. Kirschner, and F.A. Jolesz. Water diffusion, T2, and compartmentation in frog sciatic nerve. *Magnetic Resonance in Medicine*, 42(5), 1999.

[35] A. Pfefferbaum, M.J. Rosenbloom, E. Adalsteinsson, and E.V. Sullivan. Diffusion tensor imaging with quantitative fibre tracking in HIV infection and alcoholism comorbidity: synergistic white matter damage. *Brain*, 130(1):48, 2007.

[36] C. Pierpaoli and P.J. Basser. Toward a quantitative assessment of diffusion anisotropy. *Magnetic Resonance in Medicine*, 36(6):893–906, 1996.

[37] AB Ragin, P. Storey, BA Cohen, RR Edelman, and LG Epstein. Disease burden in HIV-associated cognitive impairment A study of whole-brain imaging measures. *Neurology*, 63(12):2293–2297, 2004.

[38] A.B. Ragin, P. Storey, B.A. Cohen, L.G. Epstein, and R.R. Edelman. Whole brain diffusion tensor imaging in HIV-associated cognitive impairment. *American Journal of Neuroradiology*, 25(2):195–200, 2004.

[39] S.E. Rose, F. Chen, J.B. Chalk, F.O. Zelaya, W.E. Strugnell, M. Benson, J. Semple, and D.M. Doddrell. Loss of connectivity in Alzheimer's disease: an evaluation of white matter tract integrity with colour coded MR diffusion tensor imaging, 2000.

[40] Rosario Sance, Andres Santos, Maria J. Ledesma-Carbayo, Timothy Behrens, and Saad Jbabdi. Estimation of axonal radius from diffusion MR data in directionally heterogeneous tissues. *11th International Conference on Medical Image Computing and Computer Assisted Intervention Workshop*.

[41] G.J. Stanisz, G.A. Wright, R.M. Henkelman, and A. Szafer. An analytical model of restricted diffusion in bovine optic nerve. *Magnetic Resonance in Medicine*, 37(1), 1997.

[42] M.M. Thurnher, M. Castillo, A. Stadler, A. Rieger, B. Schmid, and P.C. Sundgren. Diffusion-tensor MR imaging of the brain in human immunodeficiency virus-positive patients. *American Journal of Neuroradiology*, 26(9):2275–2281, 2005.

[43] B.D. Trapp, J. Peterson, R.M. Ransohoff, R. Rudick, S. Mork, and L. Bo. Axonal transection in the lesions of multiple sclerosis, 1998.

[44] AM Uluğ and PC Van Zijl. Orientation-independent diffusion imaging without tensor diagonalization: anisotropy definitions based on physical attributes of the diffusion ellipsoid. *Journal of magnetic resonance imaging: JMRI*, 9(6):804, 1999.

[45] C.F. Westin, SE Maier, H. Mamata, A. Nabavi, FA Jolesz, and R. Kikinis. Processing and visualization for diffusion tensor MRI. *Medical Image Analysis*, 6(2):93–108, 2002.

[46] Y. Wu, P. Storey, BA Cohen, LG Epstein, RR Edelman, and AB Ragin. Diffusion alterations in corpus callosum of patients with HIV, 2006.

[47] W. Zhou and D. H. Laidlaw. An Analytical Model of Diffusion and Exchange of Water in White Matter from Diffusion-MRI and its Application in Measuring Axon Radii. *Proceedings of the 17th Scientific Meeting of the International Society for Magnetic Resonance in Medicine, Hawaii, USA*, 2009.

[48] W. Zhou, P.G. Sibley, S. Zhang, D.F. Tate, and D.H. Laidlaw. Perceptual coloring and 2D sketching for segmentation of neural pathways. 2006.