Towards Very Early Interrogation of Neurodegenerative Diseases with Diffusion MRI.

by

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ABSTRACT

It is hypothesized that changes in brain tissue microstructure, particularly degradation of neurites (i.e,. axons and dendrites) and synapses, are early drivers of Alzheimer's disease (AD) pathogenesis. Quantitative magnetic resonance imaging (MRI) tools like diffusion tensor imaging (DTI) have long been used to study AD pathogenesis. Using DTI metrics, structural insights of neuro tissue can be inferred but not directly measured. DTI has proven to be an effective tool indicating fractional anisotrophy (FA) differences between groups of varying AD risk factor, but it does not explicitly quantify pathophysiologically-relevant features like neurite density and complexity. This study aims to develop and validate an advanced diffusion MRI acquisition and biophysical modeling platform that can be used to explicitly quantify changes to brain tissue microstructure, specifically neurite density and complexity. Ultimately, this platform will be used to study the pathogenic mechanisms that drive AD in the pre-clinical and clinical setting.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Chapter 1

INTRODUCTION

Nurodegenerative diseases such as Alzheimer's disease (AD) are found in 1 of 9 adults over the age of 65 and account for over 60% of all dementia cases affecting over 6.5 million individuals in the United States (Alzheimer's Association. 2023). This amounts to a heavy burden on the health providers and caregivers. Due to the nuanced symptoms of cognitive decline, diagnosis is a non-trivial task often relying on behavior assessments performed typically by primary care physicians who often feel under-prepared to make such diagnoses. It has been reported that as many as 50% of patients do not receive a clinical diagnosis, leaving millions without treatment options (Bernstein et al., 2019). Because the onset of measurable cognitive symptoms occurs long after changes to neural structure begin (Agosta et al., 2011) there is a distinct need to investigate the early pathogenesis of AD so that strong quantitative diagnostic tests may be established. Furthermore, as new AD drugs enter the market there is a need for tools that enable the study of early treatment efficacy and, eventually, enable earlier intervention. Towards these ends, we seek to develop neuroimaging tools to study early AD-associated changes to the microstructure of neural tissue. These tools will aid in research into the early pathogenic mechanisms thought to drive AD and the development of earlier diagnostic tools and drug discovery.

Neuroimaging has proven invaluable in studying neurodegenerative diseases, owing to its non-invasive nature. High-resolution magnetic resonance imaging (MRI) scans have revealed widespread brain atrophy in AD patients, while positron emission tomography (PET) is adept at tagging tau neurofibrillary tangles and beta-amyloid

plaques, common markers associated with AD (Whitwell et al., 2008; Rowley et al., 2020). However, despite their diagnostic prowess, these techniques have limitations when it comes to unraveling the intricacies of brain microstructure. Specifically, the measurement of structural elements of synapses remains elusive. Parameters such as synapse density, angular complexity, and cell body radius have traditionally only been accessible through histological investigations. Recognizing this gap, we advocate for the development of advanced imaging tools that can quantify brain tissue microstructure, promising a deeper understanding of the neurological underpinnings of diseases like AD. By bridging this gap, these innovative imaging tools hold the potential to deepen our comprehension of neurodegenerative disorders.

Diffusion magnetic resonance imaging (dMRI) is a promising tool for non-invasively and longitudinally investigating the AD-associated earliest microstructural changes to the brain. There is a long history of using dMRI to study the aging brain. Researchers have employed methods such as Diffusion Tensor Imaging (DTI) which provide insights into water molecule diffusion-driven displacement and directionality within a voxel. This approach has been instrumental in suggesting that AD patients have a decrease in white matter (WM) tract integrity (Salminen et al., 2013). WM tracts are bundles of myelinated axons that serve as vital communication pathways within the central nervous system. A notable example is the corpus callosum, responsible for inter-hemispheric signal transmission. A significant advantage of DTI is that it is approved for clinical use; thus, human data are readily acquirable on most clinical MRI scanners or made publicly available via digital repositories. DTI, like other biomarker investigation tools, has a limitation: it does not provide biophysical estimates of tissue structure. Using DTI metrics, structural insights can be inferred but not directly measured. In more recent publications, the use of advanced biophysical modeling

of neural microstructure using dMRI data has been successful in measuring the intricate microstructure of neuronal tissue. Metrics such as neurite density, orientation dispersion/complexity, soma radius, and axon diameter have been estimated in both WM and grey matter (GM) using models. Building upon these advancements, we aim to develop a robust preclinical dMRI protocol that can be used with existing advanced biophysical models such as NODDI (Zhang et al., 2012), SANDI (Palombo et al., 2020), and NEXI (Jelescu et al., 2021) and applied to AD, and ultimately, to longitudinally investigate the pathogenesis of AD using such methods.

Here, we present two studies to illustrate the effectiveness of dMRI in investigating AD. In chapter 2, We utilize DTI to highlight differences in WM tracts between cognitively normal groups possessing a gene associated with increased AD risk. In chapter 3 we demonstrate a powerful dMRI acquisition sequence which can be applied across multiple models and used as the foundation of studies for AD pathogenesis.

Chapter 2

DTI AND A CLINICAL RESEARCH APPLICATION

2.0.1 Introduction

Diffusion Tensor Imaging (DTI) is a subset of MRI imaging that takes advantage of random motion of water molecules and its sensitivity to tissue microstructure (e.g., cell membranes, myelin sheaths, etc.) to determine the apparent diffusivity and microstructure-guided directionality of water diffusion (summarized as in the so-called fractional anisotropy – FA - term in each voxel). A higher FA value indicates stronger "directionality" (anisotropy) of diffusion of the water molecules inside the voxel; conversely, a low FA value indicates that the diffusion has a more spherical (isotropic) shape. DTI is predominantly applied in the identification and characterization of WM tracts in the brain, which are comprised of bundles of myelinated axons. Since the long axis of the axons are oriented in parallel and axonal water is bound by the myelin sheath, water molecules will appear to preferentially diffuse "along" the axis of the tract; i.e., WM tracts can be expected to exhibit high DTI-calculated FA values. However, it is critical to note that the self-diffusivity of water molecules is unchanging and that this appearance of directionality is actually a reflection of the boundary-driven, highly restricted displacement of water molecules in the radial direction of the tract. Other estimates of DTI imaging are Mean Diffusivity (MD), Radial Diffusivity (RD), and Axial Diffusivity (AxD). MD is a measurement of the average diffusion of water in a voxel which we would expect to be higher in WM tracts that are less "intact" due to a loss of structure of the neurons which subsequently

implies that water diffusion is "less restricted." RD represents the diffusion radial (perpendicular) to the axon and is used to suggest the level of myelination (Chang et al., 2017). AxD quantifies diffusion along the axis along the white matter tract. Qualitatively, as RD and AxD diverge, FA increases. By including these measurements, we expect to extract further nuances about the WM tract microstructure.

It is thought that biological change that causes WM tract diffusion to become more isotropic is a decrease in myelination of axons (Aung et al., 2013). This lowers the expected FA values in WM tracts of patients diagnosed with AD. DTI does not tell us anything about why or how the microstructure of the tissue is changing, it only tells that the diffusion of the water molecules is more or less isotropic. Thus inferences must be made as to what are the biological driving forces behind this results. It is expected that neural microstructure begins to change and is detectable using DTI before measurable cognitive impairment (Agosta et al., 2011). We examined a cohort consisting of cognitively normal adults who are carriers of the: (i) APOE-e4 allele, which increases risk of AD development; (ii) APOE-e3 allele, associated with no increased risk; and (iii) the APOE-e2 allele, which reduces the risk of AD development (Reiman et al., 2020). To investigate neural tissue in aging adults we used DTI imaging to determine if APOE-e2, e3, and e4 groups show differences in FA values, possibly indicating that higher-risk groups have lower WM tract integrity prior to the onset of cognitive symptoms.

2.0.2 Methods

We studied scans from a cohort of 281 adults divided into five distinct groups based on the presence of specific APOE alleles 1. These groups included individuals with

e4/4 (n=54), e3/4 (n=66), e2/4 (n=17), e2/3 (n=46), and e3/3 (n=98) genotypes. Mean education was recorded and consistent across all groups. All participants were evaluated and found to be cognitively normal. Homozygous APOE-e2/2 group comparisons were not included due to low numbers of participants n=7. Participants were imaged at the Banner Alzheimer's Institute Phoenix. Images were carried out using a GE Discovery 750 at 3T equipped with a 32-channel head coil. The acquisition protocol consisted of a TR = 9050 ms, TE = 57.2 ms and Resolution = 1.37x1.37x2.7 mm. The DTI sequence parameters included b-values of 0 and 1000 sampling 48 directions.

Preprocessing was performed using DTIPrep (Oguz et al., 2014), a software package designed for the preprocessing and quality assessment of DTI data by detecting and correcting artifacts, thereby improving the reliability and accuracy of subsequent scan analyses. DTI parameters were calculated using weighted-linear fitting with FSL-FDT (Behrens et al., 2003). Voxel wise comparisons of the genetic groups were carried out using Tract-Based Spatial Statistics (TBSS) (Smith et al., 2006) which projects all subjects' FA data onto a mean FA tract skeleton before applying voxel wise cross-subject comparison.

Genotype	**n**	**Mean Age (range)**	**Mean Education (years)**	**%Female**
44	54	64.00 ± 7.76 (47-78)	16.59 ± 1.97	78%
34	66	68.18 ± 8.78 (52-84)	16.32 ± 2.04	76%
24	17	68.71 ± 5.62 (59-78)	16.53 ± 2.87	59%
23	46	71.11 ± 8.40 (51-88)	17.33 ± 1.61	65%
33	98	67.39 ± 8.32 (50-82)	16.46 ± 2.55	77%

Table 1. Summary of Participants' Information.

2.0.3 Results

Shown in Figure 1 and Table 2 the results of the 8 TBSS comparisons of FA are as follows: APOE-e2/3 > APOE-e3/3, APOE-e2/3 > APOE-e3/4, APOE-e2/3 > APOE-e4/4, APOE-e2/4 > APOEe 3/3, APOE-e2/4 > APOE-e3/4, APOE-e2/4 > APOE-e4/4, APOE-e3/3 > APOE-e4/4, and APOE-e3/4 > APOE-e4/4. We noted that widespread lower FA values were found in APOE-e4 carrier comparisons. In addition, the results showed higher FA values in carriers of APOE-e2 allele relative to non APOE-e2 carriers. All group comparisons resulted in a $p < 0.05$ before correcting for multiple comparisons. After correction, APOE-e2/3 (n=48) > APOE-e4/4 (n=54) remained significant.

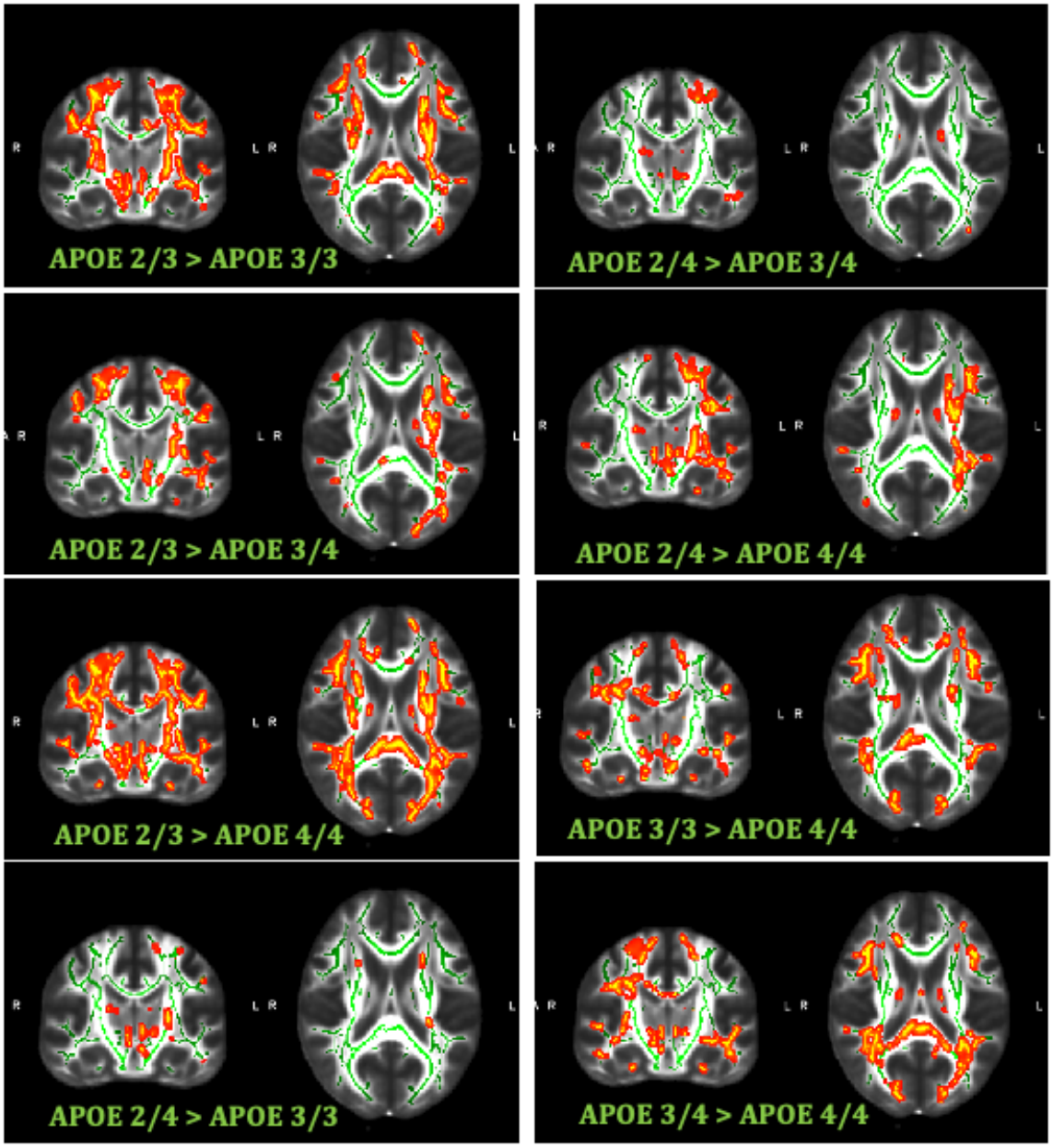


Figure 1. TBSS Comparisons: red/yellow areas indicate voxels where there is a significant FA difference between the compared groups

APOE Group TBSS Comparison of FA Values
APOE e2/3 > APOE e3/3
APOE e2/3 > APOE e3/4
APOE e2/3 > APOE e4/4
APOE e2/4 > APOE e3/3
APOE e2/4 > APOE e3/4
APOE e2/4 > APOE e4/4
APOE e3/3 > APOE e4/4
APOE e3/4 > APOE e4/4

Table 2. Each comparison indicates which APOE group had overall higher FA values on the TBSS tract skeleton. After adjusting for multiple-comparison correction APOE e2/3 > APOE e4/4 remained significant.

2.0.4 Discussion

Our results suggest that the presence of APOE-e4 allele in cognitively normal adults may have a detrimental effect on WM microstructure, which is consistent with previous studies (Salminen et al., 2013). Supported by other studies, our findings also suggest that the presence of the APOE-e2 allele (Reiman et al., 2020; Chiang et al., 2012) may confer a protective effect on tract integrity, as evidenced by higher FA values found in the APOE-e2/3 cohort when compared to both the APOE-E4/4 and e3/3 groups. Previously, this relationship has only been documented using more advanced models such as NODDI (NIR et al., 2021) which require a much more robust acquisition sequence as input, and not with basic DTI analysis. Additionally, future evaluations of DTI measures such as the mean, radial, and axial diffusivity, as well as T1/T2 ratio with an increased number of scan subjects may strengthen the relationship of the effect of APOE on WM tracts of the brain. Additionally, with a larger cohort, whether or not APOE-e2 has a dose-dependent effect may also be established.

We have demonstrated the value of DTI as a clinical research tool, especially in studies of pre-symptomatic AD. However, it is important to note that while DTI provides estimates related to the displacement of water molecules in complex biological structures, it falls short in offering precise structural information. Questions about specific structural changes remain unanswered: Is the myelin sheath thinning? Have the dimensions of axons or soma changed? Is there an increase in diffusion between intra and extracellular spaces? Moreover, does the angular dispersion of neurons exhibit high or low variability? DTI, unfortunately, lacks the direct ability to address these critical questions. To advance our understanding of AD pathology, especially in the pre-symptomatic stage, it is imperative to non-invasively and longitudinally obtain these answers. To achieve this, we propose a pre-clinical AD rat model study and a considerably more advanced dMRI sequence and biophysical modeling scheme (to be discussed in Chapter 3). We hypothesize that these more advanced methods hold the potential to answer the questions mentioned above. Indeed, we aim to shed light on the temporal development of the intricate microstructural changes that are believed to occur in the earliest stages of AD pathogenesis.

Chapter 3

TOWARDS PRECLINICAL VALIDATION OF BIOPHYSICAL MODELS

3.0.1 Introduction

Traditional MRI scans are predominantly utilized for capturing high-resolution images that facilitate visual examinations by radiologists. While these scans offer excellent tissue contrast throughout the body, they represent just one facet of this versatile technology. The field of biophysical modeling powered by dMRI sequences has gained significant traction in the past decade, as articulated by (Novikov et al., 2018). However, more advanced application of dMRI and biophysical modeling in clinical settings has been limited, primarily due to the extended acquisition times required and lack of ground-truth validation of more sophisticated biophysical models of diffusion in tissue. However, innovations such as parallel imaging and compressed sensing has improved the speed of dMRI data acquisition and thus made these methods far more accessible to clinical research and care.

The so-called "Standard Model" of diffusion in brain tissue can be conceptualized as a collection of one-dimensional impermeable sticks, which represent the axons of neurons with no water exchange across the cell membrane. In the context of dMRI, this model is designed to optimize unidirectional diffusion along these sticks, mimicking diffusion in neuronal axons, while also accounting for isotropic diffusion in the extracellular spaces (areas not occupied by the sticks). This means that water molecules diffuse freely in all directions outside of the neurite structures. The following three models are all variations on the standard model: 1. Neurite Orientation

Dispersion and Density Imaging (NODDI) (Zhang et al., 2012). NODDI generates two important estimates: the Orientation Dispersion Index (ODI), which indicates the angular variation of neuronal orientation in each voxel, and the Neurite Density Index (NDI). NODDI's strength lies in its ability to identify WM tracts as you would expect low ODI and high NDI in this tissue type. This is a simple model with only sticks and fixed diffusivity values allowing for it to be driven by smaller data sets thus making it more feasible to acquire in a clinical setting. 2. Soma and Neurite Density Imaging (SANDI) (Palombo et al., 2020). SANDI enhances the standard model by incorporating a spherical ball to account for intracellular diffusion within the soma. This makes SANDI a powerful tool for examining GM tissue where somas are larger and more abundant. From SANDI, empirical biophysical estimates such as Soma Density and Soma Radius are generated, offering valuable insights into GM tissue. 3. Neurite Exchange Imaging (NEXI) (Jelescu et al., 2021). NEXI, like SANDI, is designed to explore GM regions of the brain. It is a departure from the standard model—it does not assume that the sticks (representing axons) are impenetrable and thus requires an estimate of the water exchange rate across cell membranes. To calculate this potentially relevant exchange rate constant between intra- and extracellular compartments, NEXI must consider dMRI data from multiple diffusion times. Consequently, it requires a significantly longer acquisition time, as it must sample multiple directions, b-values, and diffusion times to capture the intricate dynamics of exchange.

Identifying a single "perfect" biophysical model is impossible given that acquired data are finite and noise is prominent in any dMRI data set. Instead of aiming for one model to "rule them all", our efforts focus on a crucial objective: gathering high-quality data compatible with multiple models that might suit different experimental scenarios.

This approach allows us to analyze common model estimates like density and explore potential connections between unique model outputs. Such analyses are vital for informing future model selection and optimization of dMRI acquisition sequences.

Our other aim is to utilize the biophysical parameter estimates from advanced biophysical models to investigate the early pathogenesis of AD through longitudinal preclinical rodent studies. Using AD-predisposed rodents as our scan subjects, we will periodically collect high-quality dMRI data that satisfy the needs of all models described above. This longitudinal approach enables us to study brain density and complexity metrics over time, in both WM and GM, in a model-agnostic manner, and with histology as ground truth verification. By observing these changes as the rodents age, we aim to gain valuable insights into the pathogenesis and progression of AD, utilizing a diverse set of biophysical models and high-quality data to enhance our understanding of this complex neurodegenerative condition.

3.0.2 Methods

A model-agnostic echo-planer imaging (EPI) multi-shot multi-b-value, multi-diffusion-time sequence was developed to accommodate standard DTI calculations as well as fitting of the NODDI, SANDI, and NEXI biophysical models. dMRI data were collected at b-shells of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, and 8 ($ms/\mu m^2$) with 6, 30, 30, 34, 35, 37, 39, 42, 46, 52, and 60 directions for each corresponding b-shell with a total of 412 directions. The number of directions was systematically increased as a function of b-value because: (i) the weighting of the "directionality" of diffusion is expected to increase as b-value increases (i.e., the signal becomes more sensitive to highly restricted protons in structures like WM tracts) and (ii) the signal-to-noise

values at higher b-values is expected to be low. Thus, directions per b-values are increased to strengthen data density at the higher b-values. Low number of directions were sampled at b = 0 and 0.25 since limited sensitivity to directionality of diffusion is expected at this b value range. To accommodate NEXI which, includes parameters intended to quantify exchange time constants across cell membranes, multiple diffusion times must also be sampled. In this sequence we test diffusion times = 20 ms, 30 ms, 40 ms and 50 ms. To maximize the SNR of each scan, different TE values were chosen for each diffusion time: 50 ms, 50 ms, 54 ms, and 64 ms. TR: 1500 ms, small delta: 4 ms. 3 slices with thickness: 0.5mm. The data was collected using MRS DRYMAG 9.4T preclinical scanner.

To ensure consistent sampling across all shells, directions for our sequence were generated using qspace (Caruyer et al., 2012) which generates uniform gradient directions for each shell Figure 2. Total ex-vivo acquisition time was 25 hours, with 6.25 hours for each diffusion time. Signal-to-noise ratio (SNR) was measured by dividing the average signal from a region of interest (ROI) inside the sample by the standard deviation from an ROI outside of the sample as shown in Figure 3. Our scan was performed at room temperature on a fixed rat brain placed in a 15mL falcon tube in a Phosphate-Buffered Saline (PBS.) The sample was removed from cold storage 2 hours before the start of scanning to bring the sample temperature to room temperature (21°C).

The data underwent preprocessing using MRTRIX's dwidenoise (Tournier et al., 2019). Since DTI necessitates a smaller input data set, our scan was truncated to retain only b = 0 and the 34 volumes at 1 $ms/\mu m^2$. The application of both NODDI and SANDI utilized full dMRI dataset the AMICO implementation (Daducci et al., 2015) was used due to its rapid processing capabilities. For NEXI, an extra step was

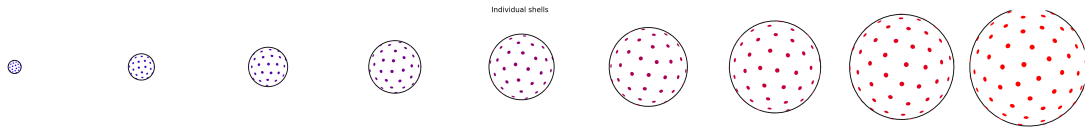


Figure 2. Spherical sampling scheme of directions for 12 shells. Note: Directions associated with b = 0, b = .25 and b = .5 $ms/\mu m^2$
are not included.

taken: a noisemap was generated using dwidenoise from a low b-values scan where b $< 2\ ms/\mu m^2$. To confirm our findings, we compared the estimates from our model with the values reported in existing literature.

3.1 Results

The signal-to-noise ratio (SNR) of the acquired data, characterized by $b = 8\,\mathrm{ms}/\mu\mathrm{m}^2$, exhibited values ranging from 22 to 34, as outlined in Table 3. These results are considered acceptable even under the 'worst case scenario' conditions (i.e., $b = 8\,\mathrm{ms}/\mu\mathrm{m}^2$) Figure 3. In Figure 4, the FA map and orientation dispersion index (ODI) map, generated through the NODDI techniques, effectively delineate white matter (WM) tracts bordering the left and right sides of the corpus callosum. As anticipated, these regions exhibit high FA values, indicative of anisotropic diffusion within WM tracts. Conversely, ODI values in the same tracts were lower, suggesting minimal orientation dispersion within individual voxels. This finding reflects a higher degree of angular coherence among neurons within these regions (Figure 4), aligning with our existing understanding of the brain's microstructure. Within the region of interest (ROI) corresponding to WM, the average ODI intensity was measured at 0.63 ± 0.2, deviating significantly from the expected values of 0.24 ± 0.04. Additionally, the mean ODI signal intensity from the gray matter (GM) ROI was 0.79 ± 0.03, compared to the anticipated 0.59 ± 0.02 (McCunn et al., 2018) (Figure 5). The FA values in these regions were 0.56 ± 0.03.

Table 3. Signal to noise ratio from last volume of b = 8 $ms/\mu m^2$ of each diffusion time sequence.

Diffusion Time	**TE (ms)**	**Inside Signal**	**Outside SD**	**SNR**
20ms	50	406.79	13.54	30.03
30ms	50	317.47	13.88	22.88
40ms	54	458.03	13.33	34.37
50ms	64	397.07	15.94	24.92

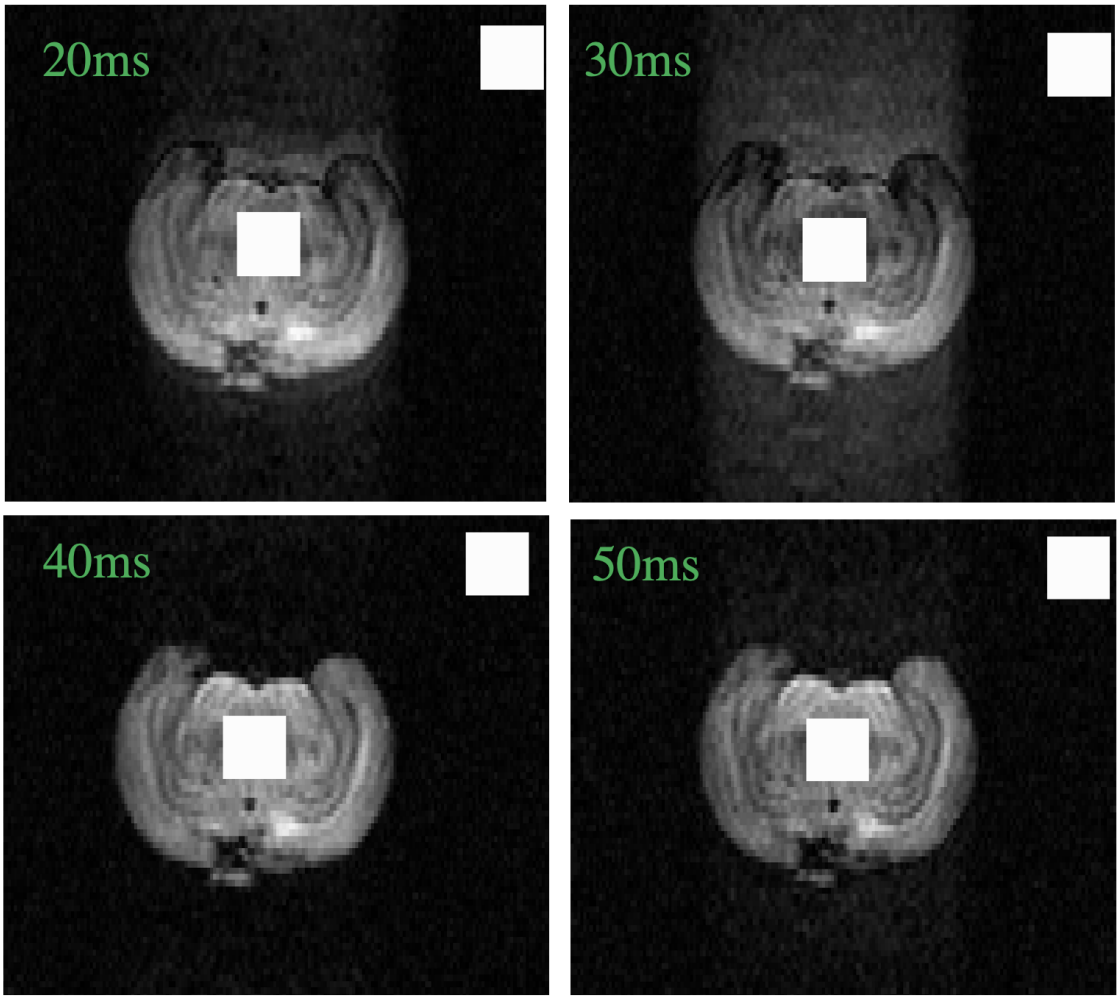


Figure 3. SNR of for each diffusion times measured at b = 8 $ms/\mu m^2$.

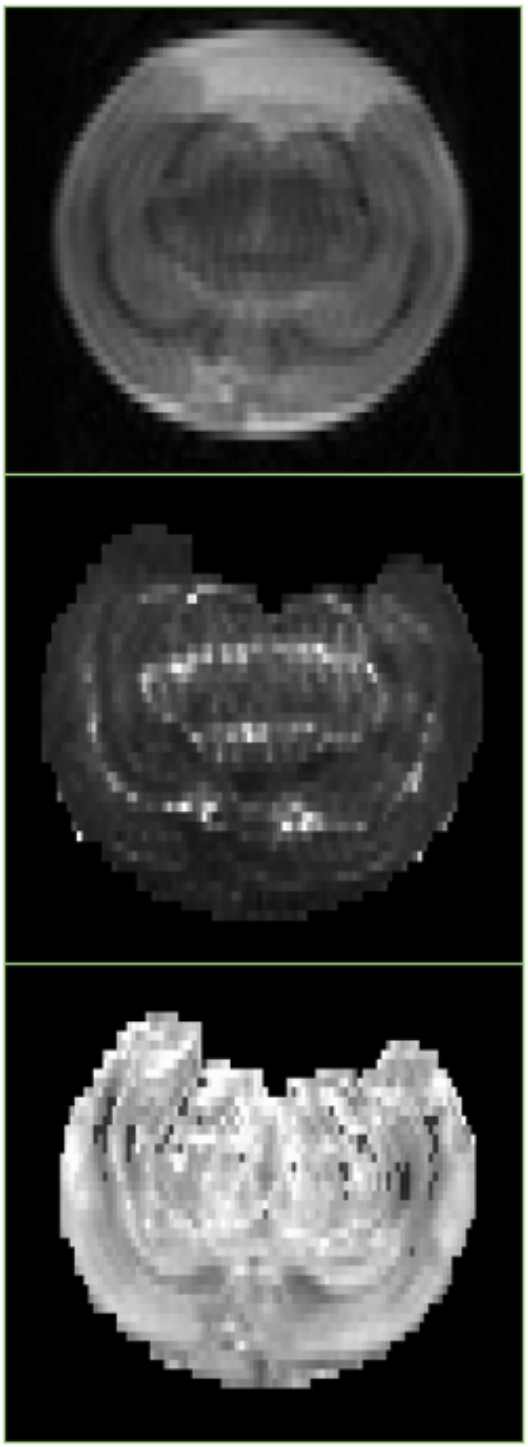
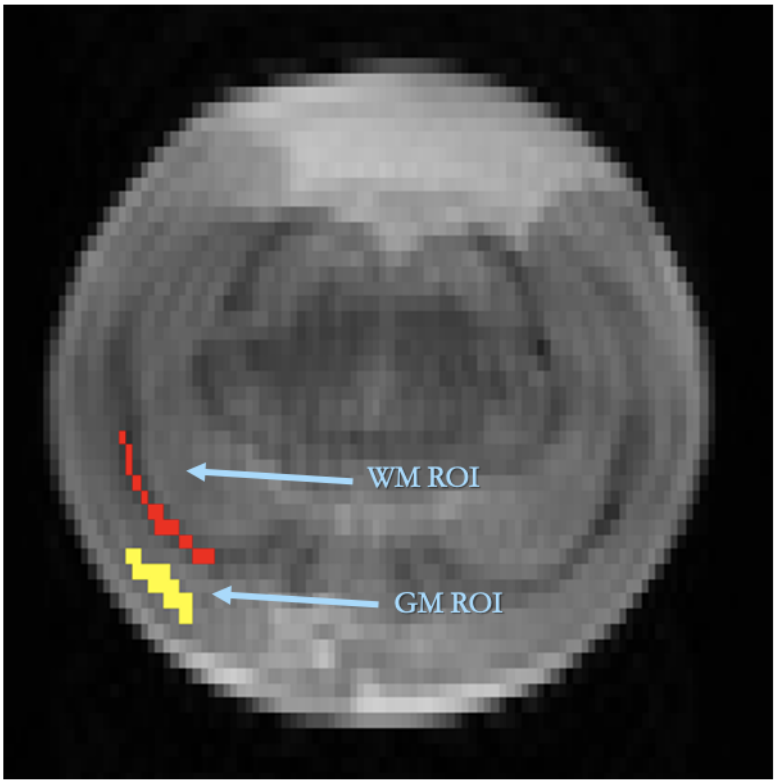


Figure 4. Top: Denoised b0 image; Middle: FA map; Bottom: NODDI ODI map.



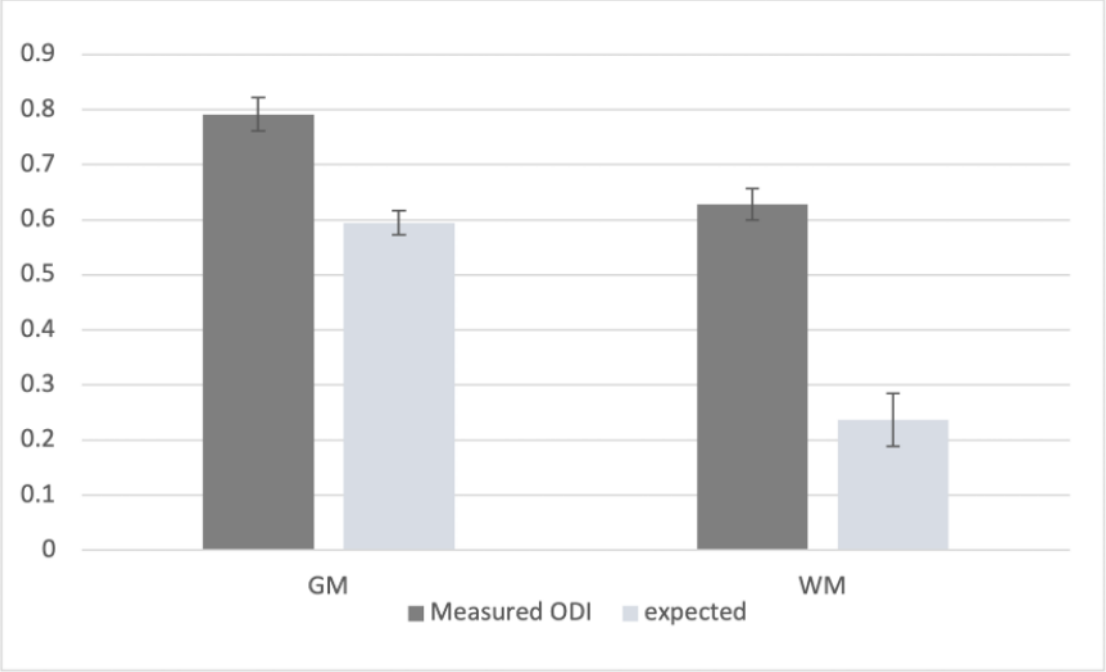


Figure 5. WM ROI we find a average intensity of 0.63 ± 0.2 compared to values of 0.24 ± 0.04. GM ROI was measured at 0.79 ± 0.03 compared to the expected 0.59 ± 0.02 (McCunn et al., 2018).

3.1.1 Discussion

By utilizing our new model-agnostic sequence, we have obtained the necessary data required to perform analysis on the microstructure of both WM and GM regions. We note that SANDI requires specific tuning of model parameters to match input dMRI data. These specific parameters include diffusion time, TE (echo time), and the expected diffusivity of water, which varies significantly due to sample temperature. It's important to highlight that in this study, we did not implement preprocessing corrections for motion. These additional processing steps were deemed unnecessary because our sample was ex vivo, making it immune to movement-related artifacts. Concerning NEXI, there is no consensus on the expected exchange rates in a fixed rodent brain; some studies have indicated pre-exchange lifetimes as low as 2-3 ms or as high as 750 ms (Yang et al., 2018). It important to note that it is theorized that the chemical changes that occur in the preservation process alter the structure in a way that reduce inter-compartment exchange and there's evidence suggesting that variations in preservation protocol can influence measured diffusion times (Shepherd et al., 2009). This is all to say that quantification and relevance of exchange in ex vivo scans is the subject of ongoing and future investigation.

Discrepancies observed between the measured ODI values and the expected values (McCunn et al., 2019) can be attributed to several factors. First, our use of a 64 by 64 matrix size might have induced a partial volume effect where voxels may contain signal from both GM and WM tissue thus increasing the overall average ODI. To explore this phenomenon thoroughly, we plan to replicate the experiment using a higher resolution, specifically a 128 by 128 matrix, as referenced in previous studies. This step will aid in assessing the extent of the partial volume effect. Second, our decision to employ

the AMICO implementation of NODDI was driven by its exceptional computational speed. However, its suitability for analyzing ex-vivo samples remains untested. Our measured FA values were 25% higher than those documented in existing literature. While prior literature indicates that formalin fixation does not substantially impact FA values (Shatil et al., 2018), it is possible that the age and temperature of the sample had an effect. Anticipating further precision as we refine our sequences and extend our analysis to in-vivo scans, we expect our estimates to align more closely with the anticipated values.

We also propose that the logical next step is to refine our sequence such that it can be performed in under 3 hours while maintaining appropriate SNR and sufficient directional sampling. To accomplish this we will examine using smaller data sets i.e reducing b-values and directions as well as other changes to the sequence that will reduce to total scan time such as reducing the number of shots from 16 to 8. We believe that this sequence will be a powerful tool to for pre-clinical rodent studies. By gathering longitudinal estimates from multiple models we expect to reveal changes in neurite density, complexity, and soma radius that occur before the detection of traditional biomarkers. This will allow for insights into understanding the AD pathology genesis and disease progression.

Chapter 4

CONCLUSION

We have demonstrated that dMRI may be a powerful investigative tool for studying changes in neural microstructure associated with pre-symptomatic AD. Specifically, our study showcased the ability of DTI to detect differences in WM tract integrity in cognitively normal adults carrying APOE alleles linked to an increased risk of developing AD. Although DTI is available in clinical settings and can identify changes in generalized water diffusion, it lacks the ability to directly quantify the intricate details of neuronal microstructure and their connections. This limitation highlights the significance of employing advanced biophysical models like NODDI, SANDI, and NEXI. These models offer nuanced metrics that provide powerful insights into neural tissue architecture.

Shown in this study is a robust multi-shell, multi-diffusion time sequence that can be acquired and used to support multiple biophysical models. By applying our sequence and using NEXI, NODDI, and SANDI we believe that we can create a strong tool to non-invasively acquire biophysical information about the microstructure of multiple tissue types in the brain. These efforts will aid future research towards drug discovery and elucidation of pathogenic mechanisms of neural degeneration. Additionally, these tools can be applied non-invasively and longitudinally to any research that concerns changes to neural microstructure.

Important questions pertaining to AD progression can be explored with the tools developed in our study. For instance, in preclinical rodent models of AD we can investigate when microstructural estimates begin to diverge significantly from controls

and whether these metrics exhibit differences from birth. Additionally, these models allow us to analyze whether structural estimates in AD subjects follow linear or non-linear patterns. These inquiries are critical in understanding the disease's development. Furthermore, if these estimates prove accurate and are validated through histology in AD subjects, they can serve as a foundation for exploring the impact of drugs on disease outcomes. For example, recent studies suggest that drugs such as ketamine and psilocybin may influence dendritic spine growth (Shao et al., 2021) and potentially new synapse formation. Exploring the effects of these drugs on the brain using non-invasive and quantitative metrics like neurite density, complexity, and soma radius offers an exciting prospect, potentially shedding light on therapeutic mechanisms in the context of neural microstructure.

In summary, our research has delved deep into the intricate realm of Alzheimer's Disease, leveraging the support of dMRI to shed light on microstructural alterations within the brain. Through analysis and the application of established techniques such as DTI, we have discerned distinctive patterns in the anisotropic diffusion of water within the white matter tracts. Genetic risk factors, as evidenced by our findings, influence these diffusion patterns. Remarkably, our study has not only confirmed decreased anisotropic diffusion in WM tracts among individuals at genetic risk, but has also suggested increased anisotropic diffusion in those possessing genes that mitigate the risk of AD development. These revelations underscore the subtle yet potentially pivotal alterations occurring at the microstructural level and offer insights into the early stages of Alzheimer's Disease.

Central to our contributions is the development of a robust dMRI acquisition sequence, tailored for compatibility with multiple biophysical models. This sequence serves as a gateway to a multitude of biophysical estimates, unraveling the complexities

of both WM and GM microstructure. The development and validation of these tools will mark an important milestone in the capabilities of non-invasive neuroimaging as it relates to the study of neurodegeneration. Looking forward, our focus is on a longitudinal study, where the application of these advanced imaging techniques will illuminate early brain microstructural changes using a transgenic rat model of AD.

In conclusion, our findings underscore the pivotal role of dMRI in unraveling the complexities of Alzheimer's Disease. As we move forward, armed with robust methodologies, we are positioned to make significant strides in deciphering the intricacies of this debilitating condition. With each discovery, we strengthen the foundation upon which future research and therapeutic interventions will be built, ultimately offering tangible progress in the fight against Alzheimer's Disease.

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