Ultra Short MR Relaxometry and Histological Image Processing for Validation of Diffusion MRI

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Ultra Short MR Relaxometry and Histological Image Processing for

Validation of Diffusion MRI

Amin Nazaran

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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Magnetic Resonance Imaging (MRI) is an imaging Modality that acquires an image with little to no damage to the tissue. MRI does not introduce foreign particles or high energy radiation into the body, making it one of the least invasive medical imaging modalities. MRI can achieve excellent soft tissue contrast and is therefore useful for diagnosis of a wide variety of diseases.

While there are a wide variety of available techniques for generating contrast in MRI, there are still many open areas for research. For example, many tissues in the human body exhibit such rapid signal decay that they are difficult to image with MRI: they are “MRI invisible”. Furthermore, some of the newer MRI imaging techniques have not been fully validated to ensure that they are truly revealing accurate information about the underlying anatomical microstructure that they purport to image.

This dissertation focuses on the development of new techniques in two distinct areas. First, a novel method for accurately assessing the MRI signal decay properties of tissues that are normally MRI invisible, such as tendons, ligaments, and certain pathological chemical deposits in the brain, is presented. This is termed “ultrashort MRI relaxometry”. Second, two new image processing algorithms that operate on high resolution images of stained histological slices of the ex vivo brain are presented.

The first of these image processing algorithms allows the semi-automated extraction of nerve fiber directionality from the histological slice images, a process that is normally done manually, is incredibly time consuming, and is prone to human error. This new technique represents one significant step in the complicated problem of attempting to validate a popular MRI technique, Diffusion Tensor Imaging (DTI), by ensuring that DTI results correlate with the true underlying physiology revealed by histological slicing and staining.

The second of these image processing algorithms attempts to extract and segment regions of different “cytoarchitectonic characteristics” from stained histological slices of ex vivo brain. Again, traditional cytoarchitectonic segmentation relies on manual segmentation by an expert neuroanatomist, which is slow and sometimes inconsistent. The new technique is a first step towards automated this process, potentially providing greater accuracy and repeatability of the segmentations in a much shorter time.

Together, these contributions represent a significant contribution to the body of MR imaging techniques, and associated image processing techniques for validation of newer MR neuroimaging techniques against the gold standard of stained histological slices of ex vivo brain.

Keywords: Quantitative MRI, Relaxometry, Histology, Cytoarchitectonic, DTI, Ultra short Echo Time imaging, UTE, 3D cones, Tendon, Brain, Nerve fibers
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DEDICATION

I would like to dedicate this dissertation to my parents, Hamid and Shirin Nazaran, and my brother, Milad.
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6.9 This figure represents different shapes of traverse samples in straight and folded cortical regions. The white area is the region between inner and outer cortical borders. (a) In the subregions that inner and outer cortical borderlines are parallel, the traverse samples are parallel with each other, and are perpendicular to the cortical borderlines. (b) In sub-regions that cortex is folded, the traverse samples are like the slices of a watermelon.

6.10 (a) Part of cortex is shown. (b) A monkey brain sample ROI is shown with red and green lines, representing inner and outer cortical borderlines, respectively. The traverse lines are shown with blue colors in the ROI. The area between two neighboring traverse lines is a traverse sample.

6.11 This figure illustrates how EucDist is measured. The dashed arrow represents EucDist, which is the shortest distance from the centroid of the neurons to the outer cortical border. The shadowed circle represents neurons and the black circles inside the shadow circles represents the centroid of neurons.
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6.13 (a) Labeled reference histological image used for analysis from BrainMaps.org. (b) Cytoarchitectonic parcellation by our expert neuroanatomist based on the calculated laminar map overlaid on the unlabeled reference image. (c) Laminar map calculated cytoarchitectonic parcellation overlaid on the reference image, with magnified view (inset).

B.1 One channel $^{23}$Na and one channel $^1$H coil. (B) Seven channel sodium receive array, surrounded by a large butterfly sodium transmit coil. (C) The new phased array (top view); (D) The new phased array (bottom view).

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NOMENCLATURE

$\vec{B}_0$ External static magnetic field
$\vec{M}_0$ Net Magnetization Vector (NMV)
$\gamma$ Gyromagnetic ratio
$\vec{M}_{xy}$ The traverse component of the magnetization vector
$\vec{M}_z$ The longitudinal component of the magnetization vector
$G_x$ Gradient field in x direction (frequency encoding gradient)
$G_y$ Gradient field in y direction (1st phase encoding gradient)
$G_z$ Gradient field in z direction (2nd phase encoding gradient)
$K_x$ Spatial frequency in x direction
$K_y$ Spatial frequency in y direction
$K_z$ Spatial frequency in z direction
TR Repetition time
TE Echo time
GRE Gradient Recalled Echo
UTE Ultra short Echo time imaging
dMRI Diffusion Magnetic Resonance Imaging
ROI Regions Of Interest
$\alpha$ The angular window width
$\Psi$ The angle of a radial line passing through the center of an angular window
$\Delta \Psi$ The angular spacing between sliding-angular-window bins
$r_c$ The cut-off spatial frequency of the high-pass filter
$E$ Energy
$k_n$ The neuron centroid (n) in the segmented binary image
$A[k_n]$ The area of the neurons with a centroid inside the radial distance from $k_n$
$Num[k_n]$ The number of the neurons with a centroid inside the radial distance from $k_n$
$D[k_n]$ Mean distance from each neuron centroid to the current neuron centroid from $k_n$
$A_c$ Average of all $A[k_n]$ in the segmented binary image
$Num_c$ Average of all $Num[k_n]$ in the segmented binary image
$D_c$ Average of all $D[k_n]$ in the segmented binary image
$L_m$ Maximum number of quantized levels
$\Delta d_{sr}$ Search range to make a traverse line
$\Delta d_{trav}$ The length of the perpendicular line from the middle of the current traverse
$\text{trav}[k]$ Traverse sample k
$Th_d$ Sliding window size in ClusterNum matrix
CHAPTER 1. INTRODUCTION

Magnetic Resonance Imaging (MRI) was invented in 1972 by Paul Lauterbur [4, 5] in an effort to find a non-invasive imaging modality for human diagnosis. MRI is an imaging modality that acquires an image of internal structure without causing damage to the tissue. MRI acquires images using a strong static magnetic field (typically produced with a super-conducting magnet), several less powerful gradient electromagnets, and a radio frequency (RF) field. It does not introduce foreign particles or high energy radiation into the body, making it one of the least invasive medical imaging procedures.

Unlike x-ray based imaging modalities such as computed tomography (CT), MRI can achieve excellent soft tissue contrast. It is therefore useful for the detection of abnormalities in a wide variety of tissues and structures. MRI can be used to see the soft tissues in and surrounding joints. It can be used to differentiate between soft tissues in the brain, and to identify tumors or other lesions. It can even be used to provide less obvious contrasts, such as temperature maps, diffusion maps, or measures of blood oxygenation. The MRI researcher searches for ways to manipulate the nuclear magnetic resonance signal from tissues, using the gradient magnets and the radio-frequency fields, to produce contrasts that are useful for the diagnosis and study of a broad array of diseases.

In this dissertation, I outline work that I have performed that relates to MRI’s capabilities to image both the human brain and other specialized tissues in the body (such as tendon) that exhibit extremely rapid decay of the MR signal and are traditionally “MR invisible”.

Chapters 2 and 3 give background information useful for understanding the rest of the dissertation. The work presented in Chapter 4 describes a novel MRI technique for quantitatively mapping the relaxation characteristics of tissues that normally are MR invisible. Chapters 5 and 6 present some sophisticated image processing algorithms I developed that operate on images of stained histological slices of the human brain. While not directly an application or development in
the field of MRI, this work is motivated by the need to validate several neurological MRI techniques that are in widespread usage.

Finally, Chapter 7 provides a brief summary and conclusion of my work. Additional work applying my techniques to various real-world problems is provided in the appendices.

1.1 Motivation

My first project (Chapter 4) was motivated by the need to develop a technique to quantitatively map the signal decay characteristics of tissues that have traditionally been MRI invisible (as previously mentioned). Many of these tissues, such as tendon, are of great interest in disease diagnosis and progression, and having an accurate method of quantitatively mapping their relaxation properties could provide additional information for the study of these diseases (information that cannot currently be non-invasively obtained using imaging). Many neurological disorders, such as Alzheimer’s Disease, also cause shortening of the MR signal decay time. These techniques can be applied to relaxometry mapping of these tissues as well.

My second and third projects (Chapters 5 and 6) focus primarily on new image processing techniques for analyzing histological slices of the human brain. As previously mentioned, a major motivation for much of my research was to make a tool to validate several MRI techniques that are in widespread usage for neuroimaging. These include diffusion Tensor Imaging (DTI), T1 weighted imaging, and T2 weighted imaging. To validate these techniques, the results achieved from MRI scans need to be correlated with results observed directly through slicing and histological staining of the ex vivo brain, which is considered the gold standard.

Very little work to date has been done to perform this validation. This is largely due to the difficulties associated with extracting the needed data from stained histological slices of the brain ex vivo. The brain is sliced into very thin slices, and each is then placed on a glass slide and stained to allow better visualization of the neurons and other structures in the brain tissue. These stained histological slices are then photographed at a very high resolution, producing a large quantity of data.

Chapter 5 focuses on the automated extraction of nerve fiber directionality from these histologically stained slice images. This is an incredibly time-consuming process to do manually;
nevertheless, it is needed in order to validate the images we are getting using Diffusion Tensor Imaging (DTI), which also purport to show directionality of nerve fibers in brain tissue.

Finally, the work described in Chapter 6 focuses on the automated extraction of certain cytoarchitectonic characteristics of brain tissue from stained histological slices. These are also needed for validation of MRI techniques that attempt to extract the same information non-invasively.

Each of these motivations are described in more detail in the following subsections.

1.1.1 T2* Mapping Using Ultra Short Echo Time MRI

In MRI, every tissue in the human body has an associated time constant, T2, that characterizes the exponential decay of the NMR signal in that tissue after excitation. In some tissues, there are components of the tissue that exhibit very short T2 signal decay, often on the order of hundreds of microseconds. These tissue components do not appear on traditional MRI, as the NMR signal has already gone through multiple time constants of decay before any signal can be acquired.

To overcome this problem, it is necessary to sample the signal as quickly as possible. Ultra short Echo Time (UTE) imaging allows the gap between the time that the NMR signal appears and signal sampling begins to be significantly reduced. The advantage of this rapid signal acquisition is that we can measure and can observe tissues that are not visible using traditional MRI sequences. A T2* map can be reconstructed to evaluate the condition of tissues that have very rapid signal decay (e.g., Achilles tendon and meniscus).

1.1.2 Quantitative MRI Through Histological Analysis

MRI diagnosis can be done qualitatively or quantitatively [6]. Qualitative MRI is defined as visual observation of MRI images for diagnostic purposes by a radiologist. While qualitative MRI diagnosis is an important part of medicine, the practice is subjective. Quantitative MRI (qMRI), on the other hand, deals with specific techniques that are sensitive to changes in the underlying tissue microstructure [7]. qMRI can be used to understand the pathophysiological processes \textit{in vivo}, and can be used for diagnosis, prognosis, and clinical trials [7].

Despite all the potential applications of qMRI, quantitative measurements are often neglected by the majority of the MRI community because its methods are more complicated to im-
Nerve fiber histological analysis for validating DTI results with respect to Alzheimer’s disease

Neurodegenerative diseases such as Alzheimer’s disease (AD) deteriorate the white matter in the brain. Diffusion Tensor Imaging (DTI) provides a visual assessment of white matter integrity by determining the nerve fiber orientation. However, validation of DTI is crucial in assessing the reliability and accuracy of results derived from the technique. Unfortunately, the reliability of the results from DTI when compared to histology, the gold standard, is unknown. Comparing results from DTI to histology is thus particularly important for algorithms that estimate the underlying white matter integrity or architecture. Such mappings are a crucial step in validating non-invasive techniques for assessing nerve fiber orientation in the human brain. For AD studies, there is no reliable way to validate the data derived from diffusion tensor MRI with data derived from stained histological slices of ex vivo brains.

Histological cytoarchitectonic analysis for validating MRI techniques with respect to Autism

Cytoarchitectonics deals with the pattern and distribution of neurons in the cortex. The Cerebral cortex is visible in MRI imaging techniques. Therefore, monitoring the changes in the cerebral cortex can be done in vivo using MRI. However, no reliable method exists to interpret any changes in the cytoarchitecture of the brain detected using MRI with respect to the underlying physiological anatomy since current cytoarchitectonic maps are themselves subjective [9].

1.2 Previous Work

1.2.1 UTE T2* Mapping

Using UTE for disease diagnosis is a relatively new concept. At BYU, a special kind of UTE sequence, called a “3D cones UTE sequence”, has been previously used to perform sodium
MRI. The same sequence lends itself to being used for UTE applications, for reasons that will become clear later in the thesis. T2* maps of tissues that exhibit very rapid signal decay, such as the meniscus, have been done using other UTE techniques. I have modified our custom 3D cones sodium pulse sequence and created a new, rapid, highly SNR efficient UTE T2* mapping sequence, and demonstrated its ability to map short T2* values in both ex vivo brains and in the Achilles tendon.

1.2.2 Nerve Fiber Histological Analysis

In studies of human white matter integrity using DTI, references to anatomical correlations have been qualitative, referring only to already established histological anatomy. This qualitative method for comparing the estimated DTI results to the corresponding underlying histological slice is done by drawing hundreds of thousand of lines, and then measuring the length and directionality of them. These lines will then be used to calculate the directionality of the nerve fibers. Qualitative comparison of DTI results to known anatomical connectivity by a skilled neuroanatomist is another method of comparison. No tool currently exists that directly compares DTI data to the underlying white matter architecture [10].

1.2.3 Cytoarchitectonic Histological Analysis

Previous methods of cytoarchitectonic analysis can be divided into two categories: a) expert observation, and b) human-machine interaction. Expert observation is done through visual inspection by using maps made by Broadmann [11] and Knoblauch [12]. Human-machine interactions are semi-automated algorithms that are used as an alternative way to expert observations. Examples for Human-machine interaction are: gray level index (GLI) [13, 14], sliding windowing based GLI [15, 16], central moments [17], excess mass [18], wavelet transforms [19], density profiles of the cytoarchitectonic layers [20], k-means clustering [21], and nearest neighbor [22–24].

1.3 Dissertation Outline and Summary of Contributions

This dissertation is organized as described below. In each section, I summarized my specific contributions.
Chapter 2 provides background information for general MRI physics.

Chapter 3 consists of a description of UTE imaging, DTI, Brain anatomy, and Histological analysis.

Chapter 4 describes T2* mapping using 3D cones UTE images and their applications in the body. My specific contributions are:

- Adapted a 3D cones UTE sequence utilized for sodium MRI to image hydrogen both in vivo and ex vivo.
- Optimized the trajectory such that we obtained the minimum achievable TE with respect to MRI hardware limitations.
- Optimized the pulse sequence to achieve maximum signal.
- Developed a novel T2* mapping method using multiple image acquisitions from the 3D cones pulse sequence.

Chapter 5 describes the new approach to histological analysis of the directionality of the nerve fibers. The presented semi-automated algorithm first creates a binary-segmented mask of the nerve fibers in the histological image, and then extracts an estimate of average directionality of nerve fibers. It also generates an uncertainty level for the estimate of average directionality. My specific contributions are:

- A segmentation method for separating the nerve fibers from the rest of the tissue.
- A Fourier-domain-based methodology for calculating the directionality of the nerve fibers and estimating their uncertainty over this calculated orientation.

Chapter 6 provides a new cytoarchitectonic analysis of neuronal patterns. The offered semi-automatic algorithm first creates a binary masked image of the neurons in the histological image, and then extracts the sub-regions with distinct cytoarchitecture. My specific contributions are:

- A segmentation method for separating the neurons from the rest of the tissue in very high resolution images (i.e., 32000 pixels x 54000 pixels ).
• Developed a method for finding the pattern of the neurons in cortex.

• Developed a method for identifying sub-regions with distinct neuronal pattern.

I also had a few ancillary contributions at the BYU MRI facility:

• Analyzed UTE sodium MRI results in collaboration with the university of Utah for the breast cancer study.

• Trained principal investigators at the BYU MRI facility for post DTI analysis.

• Established the DTI protocol at the MRI facility.
CHAPTER 2. FUNDAMENTALS OF MAGNETIC RESONANCE IMAGING

The emphasis of this section is to review the key concepts required to understand image formation in MRI. While certain mathematical concepts will be reviewed, the purpose is not to develop a rigorous mathematical framework, but rather to establish important details relevant to the research in this dissertation. Further details can be found in a general text about MRI for the interested reader [25, 26].

2.1 Signal Generation in MRI

Can we get MRI signal from every molecule?

Nuclei with *an odd number of total protons and neutrons* have an intrinsic quality called spin angular momentum while nuclei with an even number of both have a total spin of zero. This nonzero spin gives rise to a small net magnetic moment, \( \mu \), which causes the nucleus to act as a tiny magnetic dipole. The interaction of these dipoles with imposed magnetic fields leads to the generation of electromagnetic radiation that can then be detected by equipment in an MRI scanner. Much of the organic tissue and compounds in the body contain a significant amount of water (H\(_2\)O). The hydrogen atoms in water have a nucleus that consists of a single proton, and thus are capable of being detected using magnetic resonance. Other common nuclei used in MRI include \(^{13}\)C, \(^{19}\)F, \(^{23}\)Na, and \(^{31}\)P.

Magnetization

In the absence of an external magnetic field, the orientation of the magnetic moment of each nucleus is random. Macroscopically, this leads to an overall cancellation of the small moments of each nucleus. This is illustrated in Figure 2.1.(a).
Figure 2.1: In the absence of the external static field, the sum of the magnetic moments in (a) is zero, and no net magnetization vector, $\vec{M}$, is created. When, the external magnetic field, $\vec{B}_0$, is applied, the magnetic moments align with each other: most in parallel with the direction of $\vec{B}_0$ and the rest anti-parallel with it. The sum of the magnetic moments in (b) is no longer zero, and a net magnetization vector is created.

However, if a magnetic field, typically labeled $\vec{B}_0$, is applied, the spins will preferentially align either parallel or antiparallel to the field, as shown in Figure 2.1.(b). The parallel orientation is slightly lower in energy than the antiparallel state, causing spins to prefer to maintain the parallel alignment since they wish to occupy the lowest energy state possible. At field strengths typically used in MRI, thermal energy is sufficient to keep the two states nearly evenly populated. Nevertheless, a small surplus tend to align parallel, or longitudinal, to the applied field $\vec{B}_0$, and thus a small net magnetic moment $\vec{M}$ called the Net Magnetization Vector (NMV) is created in the direction of $B0$ as illustrated in Figure 2.1.(b).

**Precession**

Introducing the nuclear spins to $\vec{B}_0$ has another effect. Magnetic moments in the presence of $\vec{B}_0$ start to follow a circular path, called wobble, while spinning about their axis of rotation if they are not aligned with this static field. This is because the dipole moments experience a torque that attempts to align them with the static field. This phenomenon is also called “precession”, and is illustrated graphically in Figure 2.2.

While experiencing the same external field strength, all magnetic moments of the same type of nucleus precesses at the same frequency about the direction of the static field. The precessional
Figure 2.2: (a) In thermal equilibrium, the net magnetization, green arrow, is oriented in the direction of $\vec{B}_0$. (b) If the spin is made to move out of its equilibrium position, it starts to precess about the direction of $\vec{B}_0$ at the Larmor frequency.

The Larmor frequency is unique for each nucleus, and is called the *Larmor frequency*. The Larmor frequency is defined as:

$$\omega_0 = \gamma B_0,$$

where the gyromagnetic ratio, $\gamma$, is an intrinsic property unique to each nucleus. Some common values for $\gamma$ can be found in the Table 2.1.

Table 2.1: Gyromagnetic ratio for a few molecules. The Larmor frequencies of $^{13}$C and $^{31}$P are similar, likewise the Larmor frequencies of $^{19}$F and $^1$H are similar. In addition, the Larmor frequency of $^1$H is nearly four times greater than $^{13}$C.

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Gyromagnetic ratio, $\gamma$ (MHz/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>42.57</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>11.26</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>10.71</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>40.05</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>17.24</td>
</tr>
</tbody>
</table>


**Excitation**

When a radio-frequency (RF) pulse is applied at the exact Larmor frequency of a nucleus, polarized in a plane perpendicular to \( \vec{B}_0 \), “excitation” occurs. During the excitation process, RF energy is transferred to the magnetic moments from an RF transmit coil, causing the NMV to tip away from the static field \( \vec{B}_0 \) into the transverse plane. The amount that the magnetic moments become tipped away from alignment with \( \vec{B}_0 \) is proportional to the amount of absorbed energy and is called the flip angle \( \alpha \). This process is depicted in Figure 2.3.(b).

![Figure 2.3](image.png)

Figure 2.3: (a) Precessing magnetic moment before applying the RF field. (b) The RF field \( \vec{B}_1 \) perpendicular to \( \vec{B}_0 \) has applied. The magnetic moments spiral out of their equilibrium position. (c) After turning off the transmitter, the magnetic moments spiral up to align with \( \vec{B}_0 \), again. The fluctuating electromagnetic field caused by the magnetic moments generate a RF signal that can be detected by the RF receive coil.

After turning the RF transmitter off, the NMV continues to precess at the Larmor frequency. This precessing magnetic moment generates an electromagnetic field that can then be detected using a receive coil as depicted in Figure 2.3.(c). The strength of the generated radio-frequency signal is proportional to the component of NMV in the transverse plane.

### 2.2 Tissue Characteristics in MRI

#### Relaxation: T1 and T2

After excitation, spins gradually return to their state at thermal equilibrium. The tipped magnetization vector relaxes independently in x-y and z plane, called transverse and longitudinal
relaxation. The longitudinal component exponentially recovers to equilibrium, $\vec{M}$, with time constant $T_1$ while the traverse component, $\vec{M}_{xy}$, exponentially decays with time constant $T_2$. Figure 2.4 shows $T_2$ decay and $T_1$ recovery. The $T_1$ and $T_2$ relaxations are intrinsic properties of the tissue/material under study. Table 2.2 shows $T_1$ and $T_2$ relaxation times for some tissues in the body.

The differential equation for the transversal relaxation is

$$\frac{\partial \vec{M}_{xy}}{\partial t} = -\frac{(M_x \hat{x} + M_y \hat{y})}{T_2}.$$  \hspace{1cm} (2.2)

The general solution for 2.2 is:

$$\vec{M}_{xy}(t) = \vec{M}_{xy}(0)e^{\frac{-t}{T_2}},$$  \hspace{1cm} (2.3)

where $\vec{M}_{xy} = M_x \hat{x} + M_y \hat{y}$. The differential equation for the longitudinal relaxation is

$$\frac{dm_z}{dt} = -\frac{(m_z - m_0)}{T_1}.$$  \hspace{1cm} (2.4)

The general solution for 2.4 is:

$$M_z(t) = M_0 + (M_z(0) - M_0)e^{\frac{-t}{T_1}}.$$  \hspace{1cm} (2.5)

After applying a 90 degrees pulse, $M_z(0) = 0$, and equation 2.5 can be simplified as

$$M_z(t) = M_0(1 - e^{\frac{-t}{T_1}}).$$  \hspace{1cm} (2.6)

The primary cause of $T_2$ decay is local magnetic field inhomogeneity within the tissue, caused by the Brownian motion of molecules. Transverse magnetization reaches a maximum amount of signal immediately after excitation. As time goes on, the interaction between magnetic fields of individual nuclei causes variation in their precession rates. This phenomenon, called “dephasing”, happens when the field variations cause the individual magnetic moments in the NMV to begin to cancel each other out.
Table 2.2: T1 and T2 relaxation times for specific tissues in the body [1–3].

<table>
<thead>
<tr>
<th>Tissue</th>
<th>T1(ms)</th>
<th>T2(ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>812</td>
<td>42</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1420</td>
<td>32</td>
</tr>
<tr>
<td>Heart</td>
<td>1470</td>
<td>47</td>
</tr>
<tr>
<td>Kidney</td>
<td>1194</td>
<td>56</td>
</tr>
<tr>
<td>Cartilage</td>
<td>1240</td>
<td>37</td>
</tr>
<tr>
<td>White matter</td>
<td>1110</td>
<td>56</td>
</tr>
<tr>
<td>Gray matter</td>
<td>1470</td>
<td>71</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>993</td>
<td>78</td>
</tr>
</tbody>
</table>

Figure 2.4: (a) represents T2 decay. After tipping off the spins by 90 degrees, all the spins are in phase. However, as time passes, the spins gets more out of phase and the amount of signal decreases. At last, all the spins get more out of phase and, finally, the noise level is reached. (b) shows T1 recovery. At first, all the spins are in xy plane because of 90 degrees RF pulse. As time passes, more T1 is recovered in the z axis. At last, all the spins are recovered on z axis and the maximum amount of signal from T1 recovery achieved.

Bloch equation

The dynamics of spin precession and relaxation can be described based on Felix Bloch equation. Bloch equation describes the behavior of NMV over time after turning off the transmitter.

\[
\frac{\dot{\vec{M}}}{dt} = \gamma \vec{\mu} \times \vec{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{(M_0 - M_z) \hat{z}}{T_1} + D \nabla^2 \vec{M}
\]  

(2.7)
In equation 2.7, \( \vec{B} = B_1(t)e^{-i\omega_0 t} + B_0 \hat{z} \) and \( D \) is the diffusion coefficient. \( \hat{z} \) is chosen to be the axis parallel to \( B_0 \) and xy axis is parallel to \( B_1 \). \( \gamma \) represents the gyromagnetic ratio, \( B_0 \) the magnetic field vector seen by a net magnetic vector, and \( T_1 \) and \( T_2 \) are the longitudinal and transverse relaxation time constants respectively. In equation (2.1), the first part of the equation describes precession, the 2nd part describes T2 relaxation, and the 3rd part describes T1 relaxation.

2.3 Signal Localization

When only a static magnetic field \( \vec{B}_0 \) is applied, all different locations in the body experience the same field strength and therefore the nuclei at each location precess with the same Larmor frequency. However, making variations in the main magnetic field, \( \vec{B}_0 \), allows us to distinguish between signals that originate from different parts of the body. This is done by creating a gradient in the main magnetic field along a particular direction. Signal localization in MRI is accomplished using a collection gradient coils to alter the main magnetic field along a particular direction. For 3D imaging, the spatial encoding in the x (readout) direction is called frequency encoding, the spatial encoding in y direction is called the first phase encode direction, and the spatial encoding in z direction is called second phase encode direction. For 2D imaging, the gradient in the z-direction is called the “slice selective” gradient, and causes only spins within a certain range in z to become excited.

**Frequency encoding in MRI**

Consider a two-dimensional array of excited spins in the polarizing field \( B_0 \) (as is the case when a thin slice-selective excitation has been performed). Now suppose a spatially varying gradient field is applied while the MR signal is sampled, as shown in Figure 2.5. In this example, the excited slice is in the xy plane, and the gradient field (denoted \( G_x \)) induces a linear variation in magnetic field in the x direction. Spins at different x locations now experience different local magnetic fields strengths, and consequently precess at different frequencies. By analyzing the frequency components of the sampled MR signal, the amount of signal arising from spins at different x locations may be ascertained. In other words, spin location in the x direction has been encoded in
Figure 2.5: The gradient coils enable us to encode the location with the frequency. The magnetic moments at different x locations will precess at different frequencies. The range of frequencies in this figure were calculated for the maximum gradient amplitude of 40mT/m.

the signal frequency. Only one spatial dimension can be encoded in frequency, and subsequently resolved from the detected MR signal.

Phase encoding in MRI

Frequency encoding alone cannot uniquely resolve location, therefore a method for resolving location in at least one additional direction is required. Suppose a gradient field $G_y$ induces a linear variation in the magnetic field in the y direction as shown in Figure2.6. Spins at different y locations then precess at different frequencies while the gradient field is present. When the gradient field $G_y$ is turned off, spins at different y locations will have acquired different relative phases. Spin location along y has thus been encoded in their phase.

2.4 k-space in MRI

In MRI, k-space is the Fourier transform of the image domain. The signal received from the coil is sampled and then stored in k-space. The definition of k-space is based on three spatial frequencies $k_x, k_y, k_z$. The units for the spatial frequencies is cycles/cm, or simply MHz.
Figure 2.6: A gradient field $G_y$ played for a fixed period of time prior to readout results in the magnetic moments at different y locations to accrue different relative phase. This encodes y spatial location in the phase of the sampled signal.

**Traversing k-space in MRI: Trajectories**

Traversing k-space in MRI is done with a specific k-space trajectory. A particular k-space trajectory specifies both the pattern with which k-space should be traversed, and the locations from which samples from the excitation are taken and stored. Typically, k-space trajectories traverse and sample on a Cartesian grid.

**2.5 Repetition Time, Echo Time, and Echo**

To store the sample values related to different locations in the body, in the 3D k-space, we need to repeat the signal acquisition (and excitation) after certain time. This time is called the repetition time (TR). Echo time (TE) is the time between the excitation and the signal measurement. An echo is acquired when the center of k-space is sampled.
2.6 From k-space to Image Formation

Repeated application of excitation, phase encoding, and signal sampling concurrent with frequency encoding eventually yields all of the data necessary to reconstruct an image. The number of repetitions required depends on the size of the image desired and the resolution needed.

In order to form a 2D image, repeated excitation/phase-encode/readout steps must be performed, each with a different phase encoding gradient prior to signal readout. If an $M \times N$ image is required, $M$ excitations must be performed, each followed by a readout with at least $N$ sample points. 3D images can be formed by adding even more repetitions. An $L \times M \times N$ image would require $L \times M$ excitation/phase-encode/ readout steps.

2.7 Pulse Sequences in MRI

Pulse sequences in MRI describe the steps used to acquire all the data needed to reconstruct an image. They specify which part of the body should be excited, how k-space should be traversed (trajectory), and how the samples should be stored in k-space. A simple cartesian pulse sequence called 2D gradient recalled echo (GRE) for T2 imaging will be described in more detail as an example in this sub section.

First, an RF pulse designed to excite a specific range of frequencies is applied along with $G_z$. This way, only the spins within a specific range in $z$ become excited. The rest will not be excited because their Larmor frequency is not within the range of frequencies in the RF pulse. This technique is called selective excitation. Next, both a phase encoding gradient, $G_y$, along with a frequency encoding gradient, $G_x$, are applied. They traverse k-space diagonally to begin in the upper left corner of k-space, after which the $G_y$ gradient turns off. Data is then sampled and stored in k-space while traversing along $k_x$ using a $G_x$ gradient. In the figure, negative and positive lobes can be observed for $G_z$ and $G_x$, which is called gradient compensation. The first lobe is called a dephasing gradient, and the second lobe is called rephasing gradient. Incorporating both lobes produces a larger signal in the centra portion along k-space along the $k_x = 0$ axis where the spins tend to be in phase. The main advantage of using gradient compensation for a GRE sequence is that we always have the maximum signal at $k_x = 0$. 

17
Figure 2.7: (a) represents the 2D GRE pulse sequence. (b) represents how K-space is traversed. Four points have chosen to represent the traverse.

2.8 Field of View and Resolution

Suppose adjacent samples in the $k_x$ and $k_y$ directions are separated by a distance of $\Delta k_x$ and $\Delta k_y$, respectively. Then, FOV and resolution in x and y directions can be described as:

$$\text{FOV}_x = \frac{1}{\Delta k_x} \quad \text{FOV}_y = \frac{1}{\Delta k_y}$$ \hspace{1cm} (2.8)

$$\text{res}_x = \frac{1}{2k_{x,\text{max}}} \quad \text{res}_y = \frac{1}{2k_{y,\text{max}}}.$$ \hspace{1cm} (2.9)

2.9 Image Contrast

Differences in tissue $T_1$ and $T_2$ values can be exploited to generate MR image contrast. For example, suppose that a long repetition time TR is used between excitations, sufficient for near-complete longitudinal ($T_1$) recovery between excitations. Suppose further that we sample the signal during a window centered around a delay of time TE after each excitation, as illustrated in
Figure 2.8: T2 relaxation of gray matter and white matter in the brain. The T2 exponential decay for gray matter, which has T2=71ms, and white matter, which has T2=56ms, have shown.

Figure 2.8. Figure 2.8 shows contrast of White Matter and Gray Matter in the brain. By varying the length of TE, different contrasts based on tissue $T_2$ can be generated. If the signal is sampled after a relatively long TE, only spins with relatively long $T_2$ will still have an appreciable signal, and thus have a much stronger weighting in the image than short $T_2$ tissues. Now consider the case where the repetition time TR is shortened to allow only partial longitudinal recovery between excitations. After a series of excitations, one would expect to detect signal only from tissues with relatively rapid longitudinal recovery, or $T_1$. Signal from longer $T_1$ tissues would be attenuated due to the incomplete longitudinal recovery between excitations. This is known as $T_1$-weighted contrast. In this case, TR is generally chosen to be on the order of T1 of the tissue one wishes to highlight, and the echo time TE is typically kept short to avoid $T_2$ weighting.

2.10 Selective versus Non-selective Excitation

In slice selective excitation, the slice selective gradient is applied along with the RF excitation pulse. It causes that the spins in a specific slice of the tissue to be excited. Slice selective pulses are used to acquire 2D MRI images. In non-slice selective excitation, no gradient is applied.
with the RF pulse. The advantage of applying a non-selective excitation pulse is that the entire volume is excited and a 3D MRI image can be acquired.

2.11 Half Pulse Excitation

To reduce the time between excitation and sampling time, a half pulse excitation technique is used. In half pulse excitation, half of a slice selective RF pulse is used. When two half pulses are used, the effect is as the same as full time excitation.

2.12 Complications in MRI

This chapter has thus far ignored any complications in the imaging process that could degrade image quality. There are, in reality, numerous factors that prevent magnetic resonance imaging systems from functioning ideally. These include effects from non-stationary spins, signal decay during the sampling interval, limitations to transmit and receive coil sensitivities, noise in the surrounding electrical systems and in the body, and a host of confounding factors relating to spins that do not precess at the appropriate frequency (broadly termed off-resonance effects).

Noise

Thermal noise is the primary source of noise in MRI and is often generated by the receiver electronics. The spectral density of the thermal noise is independent of frequency in the range used
in MRI and thus equally affects all the frequencies. Therefore, it can be considered as additive white Gaussian noise. The standard deviation of the noise is dependent on the temperature of the resistor, and effective resistance of the coil.

**Main field inhomogeneity**

Limitations in magnet technology make it virtually impossible to design and fabricate a polarizing magnet that is perfectly uniform. In order to get the magnetic field as uniform as possible over an imaging volume, small shim coils are added to help cancel any non-uniformity. However, even after extensive shimming, some field inhomogeneity inevitably remains. These field variations, although often quite small, introduce error into the imaging process by causing spins to precess at different rates without the application of any gradients.

**Susceptibility-induced field variations**

Perhaps more troublesome in many applications than main-field inhomogeneity are resonant frequency variations arising from differences in bulk magnetic susceptibility across the body. These variations are highly dependent on the geometry and composition of what is being imaged, and are therefore much less predictable than main-field variations. While dynamic shimming can be performed after placement of the body in the magnet, these variations may still be several parts per million or more. Particularly problematic are regions with air/tissue boundaries, or boundaries between tissues with very different susceptibilities. Irregular geometry compounds this problem in the feet, for example.

**Chemical shift**

The magnetic field experienced by certain nuclei can be slightly smaller than expected due to electronic shielding of the nucleus. The most relevant of these effects for this dissertation is the electronic shielding experienced by hydrogen nuclei in lipids (or fats). While the amount of shielding varies with different types of lipid, a chemical shift in resonant frequency of approximately 3.5 parts per million is often assumed. At a field strength of 3 Tesla, this corresponds to a reduction
Figure 2.10: The dephasing causes the signal decays more rapidly than T2 decay. The exponential blue curve represents T2 decay, and the red curve represents T2* decay.

in resonant frequency by approximately 230 Hz. In other words, lipid spins precess about 230 Hz slower than water spins exposed to the same external field.

**T2* decay**

As discussed earlier, Larmor frequency is proportional to $B_0$ field strength. Therefore, any inhomogeneity changes the Larmor frequency. The consequence is that all spins do not precess with the same frequency, and this causes the signal to dephase and thus decay faster than T2 decay. This effect is called T2* decay, and is illustrated in comparison to perfect T2 decay in Figure 2.10.

### 2.13 Examples of MRI sequences

**Spin echo sequences**

Spin Echo sequences acquire an echo from the signal by undoing the phase shifts caused by the magnetic field variations from $B_0$ inhomogeneity and chemical shift. After applying 90 degrees excitation pulse, spins start to precess at different rates and starts to fan out over the transverse plane (figure 2.11. (a-c)). This causes that after a a specific amount of time the net magnetization reaches
Figure 2.11: Spin echo sequences use a 180 degrees pancake flipper pulse to refocus the spins after the excitation. (a) Spins in thermal equilibrium. (b) The spins tipped to the traverse plane by 90 degrees RF pulse. (c) The spins start to fan out by precessing at slightly different frequencies. (d) 180 degrees refocusing pulse is applied along the y-axis. (e) Spins rephased after a specific time.

to zero. Spin Echo sequences apply a 180 degrees excitation pulse, along the y-axis, short time after the 90 degrees pulse to refocus the spins again (figure 2.11. (d-e)).

**T1 and T2 weighted imaging**

A T1 weighted image is produced based on T1 differences in the tissues. T1 weighted images are acquired by using an intermediate TR shorter than T1 of the tissue that we are interested in discriminating, and the shortest possible TE. A T2 weighted image is produced based on T2 differences in the tissues. T2 weighted images are acquired by using a long TR and a TE in the same order of the T2 that we interseted in discriminating.

**Fluid Attenuated Inversion Recovery (FLAIR)**

FLAIR is a special MRI technique that surpasses the signal from fluids (e.g. CSF) to be able to see the changes in the regions close to the fluid better. FLAIR is accomplished by applying an inversion pulse which is a 180 degrees RF excitation pulse.
Functional MRI (fMRI)

fMRI is a neuroimaging MRI technique that is designed to measure brain activities. fMRI generates contrast based on blood oxygenation levels in blood volume, called Blood-oxygen-level dependent (BOLD) contrast imaging. Oxygentaed blood has a slight resonance shift versus the deoxygenated blood and we use that to generate contrast based blood oxygenation level.
3.1 Ultra Short Echo Time (UTE) Imaging

In MRI, T2* values less than 0.1ms are considered super short, 0.1-1ms are termed ultra short, and 1-10ms short. In many biological tissues, such as muscle, cartilage, blood, cerebral spinal fluid, gray matter, and white matter, T2* values range from approximately 20ms to 2s and are not considered short. In traditional MRI, we are typically able to begin sampling the signal several milliseconds after excitation without significant decay of the MRI signal for most soft tissues with T2* in this range (greater than 40ms).

However, to sample short or ultra short T2* values such as those found in tendons, we need to develop techniques that are able to start sampling the signal almost immediately after excitation. This method obtains the maximum achievable NMR signal from the tissue by reducing the time delay between excitation and the beginning of sampling from milliseconds to hundreds of microseconds. This class of sequences, which begin sampling as quickly as possible, are collectively referred to as UTE sequences and begin sampling often 10 to 20 times faster than traditional sequences used clinically [27].

UTE MRI is an emerging technique that shows great promise for visualizing different materials that previously have been “MRI invisible” due to their extremely rapidly signal decay. For example myelin, a substance which coats nerve fibers and aids in signal transmission, is one such tissue component that has traditionally been MR invisible. Calcifications and proteins also exhibit very short T2 time constants, and have traditionally been MRI invisible.

Figure 3.1 shows an MRI image of a foot and a knee with traditional MRI sequence, and a foot and a knee with UTE sequence, and MRI of the knee with a traditional MRI sequence and an UTE sequence. As can be seen, Achilles and meniscus are invisible in the traditional MRI image while they are visible in the UTE image.
Figure 3.1: The location of the Achilles tendon in UTE MRI image (a) and traditional MRI image (b) of the feet are marked with white arrows. These structures have very short T2* decay rates and are usually MRI invisible. The location of Meniscus in the UTE MRI image (c) and traditional MRI image (d) are also marked with white arrows. The UTE images were acquired at a very short TE, 250us, while the traditional MRI images were obtained at longer TE, 3500us. Achilles and meniscus, (a) and (c), respectively, in the UTE image are MRI visible since the image was acquired at very short TE before the signal decays completely. Achilles and meniscus in the traditional MRI image (c) and (d), respectively, are MRI invisible since the image was obtained at longer TE after signal decayed completely.

3.2 Diffusion Tensor Imaging (DTI)

DTI is a magnetic resonance imaging technique that measures the directionality of water diffusion. In DTI, measuring the directionality of water diffusion can be done by calculating a diffusion tensor for each imaged voxel. This diffusion tensor is directly related to the anisotropic restriction of water diffusion that is influenced by underlying micro structural anatomy. DTI has revolutionized the study of brain anatomy, by measuring the integrity of the brain. Prior to the
development of DTI, traditional approaches could only be performed post-mortem (i.e., in cadaver brains), while DTI MRI provides the opportunity to study brain structures in vivo.

3.3 Neuroanatomy and Disease

The main goal of this section is to provide background information needed to understand Chapter 5 and Chapter 6 of this dissertation. In the brain anatomy subsection, common terms are defined. In the histology subsection, a brief overview of what histology is and how histological images are generated is provided.

3.3.1 Brain Anatomy

The brain, by the simplest definition, consists of White Matter (WM) and Gray Matter (GM). Figure 3.2 shows these parts of the brain. Gray matter is composed primarily of neurons. White matter is composed primarily of axons, wrapped in a myelin sheath. The myelin sheath is a fatty substance that insulates each axon; collectively, myelinated axons are called nerve fibers. Nerve fibers in the brain are long and thin wire-like structures that facilitate communication between neurons in the brain [28]. This network of neurons connected by nerve fibers allows us to learn, analyze, and memorize information.

The surface of the brain is covered with a folded layer, called the cortex. The cortex consists of grooves and ridges. The grooves are called gyri and the ridges between the grooves are called sulci. The cortex has a thickness of about 2-5mm, and consists mainly of neurons [29], and performs a variety of cognitive functions. Neurons in each region of cortex have a specific pattern and architecture. Figure 3.2. (a) shows white matter and gray matter, and figure 3.2. (b) shows a specific part of Gray matter called cortex that covers the surface of the brain.

3.3.2 Histological Analysis

Histology is the study of the microscopic structure of tissues. Human organs like the brain consist of tissues, which are groups of similar cells. Histological images are produced by first slicing the tissue into very thin slices. After cutting the tissue, the next step is coloring the tissue, called staining. Staining is done to highlight the nerve fiber and neuronal structures under studies.
allowing better visualization of the tissue. A stained histological slice is shown in figure 3.3. After staining, specific cells will appear with a specific color and almost uniform brightness. The final step is capturing an image of the histological slice at a very high resolution (i.e., 36um), to see the microstructure clearly.

Figure 3.3: This figure shows a histological slice under the glass. The tissue was stained with Luxol Fast Blue/cresyl violet, which stains the nerve fibers in white matter dark blue and the neurons in gray matter light blue.
CHAPTER 4. ULTRASHORT T2* MAPPING WITH A 3D CONES TRAJECTORY

4.1 Introduction

For most tissues in the human body, very little signal decay has occurred by the time MR signal is being sampled. This signal decay often arises from intrinsic properties of the tissue (i.e., pure T2 decay) [30]. However, the signal decay can be accelerated by subvoxel variations in the magnetic field and by other non-idealities (i.e., T2* decay) [31]. Spin echo sequences are typically used to remove the additional signal attenuation due to these non-idealities [30], allowing measurement of the signal with almost pure T2 decay. Nevertheless, for tissues with inherently short true T2 or in areas with rapid subvoxel magnetic field variations (e.g., near iron deposits), spin echo sequences are often not able to recover much signal from the tissue by the time signal sampling occurs. Therefore, a different sequence for detecting these rapidly-decaying signal components needs to be used.

For tissues with rapidly-decaying signal components, ultra-short echo time (UTE) imaging sequences are employed. In UTE sequences, the time between excitation and sampling (the echo time, or TE) is typically under 1 ms [32]. This very short echo time allows us to sample the signal before it has had time to decay for tissues that would have otherwise been invisible (black) on MR images. An example is shown in Figure 4.1. In (a), a sagittal image of the human foot and ankle acquired at an echo time of 5ms are shown. The Achilles tendon, denoted by the white arrow, in this scan is almost completely black, which means that very little signal is detected from the tendon. The same is true for the cortical and trabecular bone. In (b), the same sagittal image was acquired with a UTE sequence employing an echo time of 0.25ms. In this figure, some signal is now detectable in both the Achilles tendon, white arrow, and cortical and trabecular bone. Finally, a difference image can be produced from (a) and (b), which shows only the signal components that exhibited detectable decay between 0.25ms and 5ms after excitation.
Figure 4.1: MR image of the human foot at (a) an echo time of 5ms, and (b) an echo time of 0.25ms. Note that the Achilles tendon (denoted by the white arrow) is completely black in the TE = 5ms image (left), but that some signal is detected from the tendon in the TE = 0.25ms image (middle). If these two images are subtracted, the difference image (c) shows us only the tissues that exhibit extremely rapid signal decay.

As illustrated in the figure, UTE sequences provide a means for detecting these rapidly-decaying signal components. In designing UTE pulse sequences for acquiring rapidly-decaying signal components, there are two aspects that must be carefully considered:

One aspect that must be considered is the duration of signal sampling, typically called the “readout duration”. If significant signal decay occurs during signal sampling, that signal decay results in an exponential decay weighting across our k-space sampling trajectories [33]. This results in degradation of the point spread function (PSF) of our imaging sequence. Therefore, we typically try to keep our readout duration very short for UTE sequences (often 1-2 ms). While some signal decay inevitably occurs during this period, we need to trade off degradation of the PSF and signal-to-noise ratio (SNR), since relative SNR is proportional to the square root of total readout duration.

Another aspect that must be considered is the utilized excitation technique. The limitation of UTE sequences that use the “half pulse” excitation technique [34] is their inability to image in 3 dimensions. These techniques are only capable of exciting a single 2D slice [35]. For this reason, a variety of UTE sequences have been proposed that employ centric k-space trajectories, where signal sampling begins at the origin of k-space and moves outward in some fashion. 3D PR [36], 3D twisted projection imaging (TPI) [37], and 3D cones trajectories [38] have all been implemented for UTE imaging.

While UTE sequences as described allow the detection of positive contrast from rapidly decaying signal components in tissues, the UTE images themselves do not provide quantitative
information about the actual exponential decay time constant T2* in these tissues. In other words, a basic UTE sequence does not produce T2* maps, where each voxel value is an estimate of the actual T2* decay constant associated with tissue at that voxel. However, adapting an UTE sequence for acquiring quantitative information from the tissue through T2* map is important and can have many usage.

Many applications could benefit from actual quantification of these short T2* decay values in various tissues. For example, inflammation in tendons and ligaments would be expected to give rise to a slightly longer T2* decay although still short enough to be practically invisible on traditional MRI. Being able to distinguish these subtle differences in rapid T2* decay could allow direct monitoring of progression and/or healing of tendinopathy. There has also been extensive interest in UTE T2* mapping of the meniscus [39] (the stabilizing cartilaginous structure around the tibiofemoral joint in the knee). Finally, UTE T2* mapping of the brain may provide additional subtle information on the presence and quantity of iron compound deposits, proteins, and other substances that tend to shorten T2*.

In this chapter, we present a 3D UTE T2* mapping sequence based on a 3D cones k-space trajectory. We demonstrate the ability of the sequence to quantify the rapidly-decaying component of T2* in a variety of tissues, including tendon, cortical and trabecular bone, and around iron and protein deposits in a human brain with Alzheimer’s disease (AD).

4.2 Materials and Methods

4.2.1 3D Cones Pulse Sequence

The 3D cones pulse sequence employs a k-space trajectory that begins at the origin of k-space and moves outward in a spiral fashion on the surface of a 3D cone [38]. This is illustrated in Figure 4.2. The sampling density in 3D k-space determines the spatial field of view that can be imaged without aliasing occurring. The surface of the cone is critically sampled to achieve the desired field of view (sometimes requiring multiple excitations to allow interleaved passes over the surface of the cone). Once the surface of a single cone has been critically sampled, the azimuthal angle of the sampling cone is changed on subsequent excitations and the process repeated, ultimately reaching a critical sampling density even at the edges of 3D k-space.
Figure 4.2: The 3D cones k-space trajectory employs a rapid (160 microsecond) non-selective hard RF pulse, after which sampling begins at the origin of k-space, allowing a very short effective echo time. The delay between the end of the RF pulse and the beginning of sampling is only limited by the RF settling time of the involved electronics, which is typically less than about 100 microseconds. The trajectory proceeds outwards from the origin of k-space spiraling around on the surface of a cone. The azimuthal angle of the cone is changed on subsequent excitations once the surface of the current cone is critically sampled to achieve the desired field of view.

The 3D cones k-space trajectory makes efficient use of gradient capabilities (maximum gradient amplitude and gradient slew rate) to sample k-space. It is highly SNR efficient, as the center of k-space is vastly oversampled. Since it is centric, it allows very short echo times, which makes it ideal for 3D UTE imaging. However, note that any aliasing that occurs will not be coherent if MR signal arises from outside the prescribed field of view. Aliasing will be spread across much of the image in a noise-like (but still somewhat patterned) artifact. For this reason, it is critical to ensure that the entire signal-yielding object is within the imaging field of view, or at
the very least that the RF receive coil configuration does not pick up strong signal from outside of the field of view.

Parameters for the 3D cones sequence employed in the work shown here were: TR = 12.1ms, TE ranging from 0.25ms to 5ms, flip angle = 10 degrees, readout duration = 2ms, and hard RF pulse of 160 microseconds duration. Cones gradients were completely rewound to return to the center of k-space at the end of signal acquisition, and gradient spoiling was performed prior to subsequent excitations. In other words, the sequence is a gradient recalled echo (or GRE) sequence. The pulse sequence is shown in Figure 4.3.

Figure 4.3: Pulse sequence diagram of the 3D cones sequence. A 160 microsecond non-selective hard RF pulse is used for excitation, followed by signal readout in k-space using the cones trajectory. The x- and y-gradient waveforms produce the spiraling motion on the surface of the cones in k-space, and the z-gradient amplitude controls the azimuthal angle of the cone. All gradients are fully rewound after signal acquisition, and a large spoiler gradient dephases any remaining transverse magnetization prior to subsequent excitations.
4.2.2 UTE T2* Mapping

In vivo UTE T2* mapping of the human foot and ankle

The foot and ankle of two healthy volunteers were scanned using the 3D UTE T2* mapping sequence described above in a Siemens Trio 3 Tesla whole body scanner. A dedicated 8-channel knee and foot radio frequency coil was used for signal reception. Additional scan parameters were: FOV = 22 x 22 x 22cm, matrix = 220 x 220 x 220, resolution = 1mm isotropic, readout duration = 2ms, with images acquired in the sagittal plane (which in this case means the z-gradient was applied perpendicular to the sagittal plane). Total scan time was one hour. Five separate 3D images were acquired with the 3D cones sequence for foot study at TE = 0.25, 0.5, 0.75, 1.0, and 5.0 ms.

The first subject had never experienced serious trauma to the Achilles tendon in the ankle imaged, while the second subject had undergone a synovectomy of both the main and subtalar ankle joints in which the Achilles tendon had been first surgically severed and then surgically reattached. These subjects were chosen to see if differences in the relaxation properties of the Achilles tendon were evident in the surgically repaired tissue (with significant amounts of scar tissues present) compared to normal tendon.

UTE T2* mapping of the ex vivo brain

A fixed ex vivo human brain of a subject who died with advanced Alzheimer's disease was imaged using the 3D UTE T2* mapping sequence, in a Siemens Trio 3 Tesla whole body scanner. A 32-channel head radio frequency coil was used for signal reception. Additional scan parameters were: FOV = 15 x 15 x 15cm, matrix = 150 x 150 x 150, resolution = 1mm isotropic, readout duration = 4ms, averages = 9, and total scan time = 1 hour and 15 min. Seven separate 3D images were acquired with the 3D cones sequence, each at a different echo time. Echo times employed were TE = 0.25, 0.5, 0.8, 1.0, 2.0, 3.0, and 5.0 ms.
Post processing

After acquiring 3D images with 3D cones sequence, each at different TEs, post processing was performed on each of the 3D magnitude images as follows:

1. A set of difference images was formed from the original images by subtracting each of the images from the final TE = 5.0 ms image. Each of these difference images has an associated $\Delta TE$ that represents the difference between the echo times of the two component images. The voxel intensity in these difference images is now a measure of how much the signal decayed for that voxel in the time interval $\Delta TE$.

2. The first difference image (TE = 5ms - TE = 0.25ms) was thresholded to create a binary mask identifying voxels that didn’t exhibit significant decay during the time interval 0.25 to 5ms after excitation (since these voxels would have very low signal intensity on the first difference image).

3. All of the difference image datasets were multiplied by the binary mask from the previous step.

4. For each non-zero voxel (after masking) in the 3D volume, the corresponding signal intensities from the difference images were paired with their corresponding $\Delta TE$ values, and a mono exponential least-squares fit performed to estimate the rapidly-decaying T2* component for each voxel.

5. A final 3D UTE T2* map was formed from these voxel estimates of T2*. All voxels that were zero in the binary mask are assigned a zero value in the final UTE T2* map.

This process is illustrated graphically in Figure 4.4.

4.3 Results

4.3.1 In Vivo UTE T2* Mapping of the Human Foot and Ankle

Figure 4.5 shows the TE = 0.25ms images (top), TE = 5.0ms images (middle), and corresponding UTE T2* maps (bottom) of the subject with a healthy Achilles tendon (left) and the
Figure 4.4: This figure illustrates the process of estimating the short (< 5ms) T2* component of signal decay in each voxel using the 3D cones UTE sequence. First, a set of 5 images at echo times ranging from 0.25 to 5ms is acquired. From this set, 4 difference images are produced by subtracting the first 4 image from the 5th (TE = 5ms) image. The difference between the echo times associated with these difference images is termed $\Delta T_E$. The signal level in each of the difference images is a measure of how much decay occurred during a time interval of length $\Delta T_E$. The first difference image (TE = 5ms - TE = 0.25ms) is thresholded to produce a binary mask that zeros out any voxels for which significant decay did not occur on that interval. This binary mask is applied to all the difference images, after which a mono-exponential fit is produced on a voxel-by-voxel bases using the 4 difference images to produce a final UTE T2* map.

Excellent uniformity in short T2* estimate is seen across the healthy tendon, giving us confidence that the sequence is giving an accurate estimate (were significant heterogeneity observed in the T2* maps across what we assume is relatively homogeneous tendon, we would have less confidence that the sequence is actually able to detect the rapid signal decay given the noise in the signal). Short T2* values in the tendon range from 0.32 to 0.52 ms, which is in line with previ-
ously estimates of tendon T2*. Short T2* values in the tendon of the subject with Achilles tendon injuries was from 0.5ms to 0.74ms.

Figure 4.5: UTE 3D cones images of the foot and ankle of a normal volunteer (left) and a volunteer who had undergone a synovectomy of both the main and subtalar joints of the ankle, which required surgical severing of the Achilles tendon to access the ankle joint, followed by surgical repair of the tendon. The top row shows the TE = 0.25ms images, the middle row the TE = 5ms images, and the bottom row the corresponding short T2* maps. Excellent heterogeneity in T2* values is seen in the healthy Achilles tendon (left), while areas of heightened T2* are clearly evident in the areas of scarring in the images shown on the right (arrow). These subtle tissue differences are difficult to ascertain from the TE = 5ms images.

Areas of scarring in the Achilles tendon of the second subject are very evident by the differences measured in T2* values. These differences are almost entirely absent in the TE = 5ms image, since the signal has all essentially decayed away at that point. Future studies will explore the differences in T2* in tendinopathy and other inflammations of tendon and ligament.

4.3.2 UTE T2* Mapping of the Ex Vivo Brain

Figure 4.6 shows the TE = 0.25ms (left), TE = 5.0ms (middle), and short T2* map (right) of the ex vivo AD brain. Lesions (white arrows) that appear as simple signal voids in the TE =
Figure 4.6: 3D cones images of the ex vivo brain of a subject afflicted with Alzheimer’s disease is shown. The TE = 0.25ms image is shown on the left. Strong signal is observed coming from a variety of areas in the brain, including the lesion marked by the white arrow. The TE = 5ms image is shown in the center. Notice that much of the signal around the lesion has now decayed, leaving a signal void in that area. Post-processing of the difference images produces the short T2* map shown on the right. Variations in T2* around the lesions are clearly evident in the short T2* map.

5ms image (middle) are evident in both the TE = 0.25ms and short T2* map images (left and right, respectively). We believe these to be areas of both heightened hemosiderin and protein deposits. While some residual signal loss is observed in the immediate vicinity of the lesions, significant variations in short T2* are evident when the data is post-processed to produce the short T2* map shown.

4.4 Discussion

Our UTE short T2* mapping sequence appears to provide a sensitive method for quantifying the rapid (< 5ms) decay components in tissues that exhibit very rapid signal decay, and the preliminary results shown are encouraging. However, there are a number of aspects of the technique that need to be explored and validated in more depth.

First, while the measurements are in line with previous estimates of T2* in, for example, tendon and trabecular bone, the accuracy of the short T2* estimates needs to be further explored and validated. The measurements are relatively consistent across large regions of what is expected to be homogeneous tissue in the examples shown, provides evidence that our estimate is not heavily biased by noise. However, the tests that have been conducted to date have not validated the possibility of a systematic bias in the estimates. Furthermore, the 1mm isotropic resolution we
achieved using the particular scan time and readout duration appears to have provided plenty of signal-to-noise ratio to enable consistent short T2* estimates across the tissues of interest to us. Shorter readout durations (for less T2* blurring), shorter total scan times, and higher resolutions will all compromise relative SNR. Future work is needed to both (1) explore the limits on SNR needed for accurate estimates, and (2) validate the T2* estimates against phantoms of known rapid decay constant to determine whether a systematic bias exists.

Second, more statistical analysis is needed to determine whether the use of magnitude data (which transforms bivariate complex Gaussian noise into either Rayleigh or Rician distributed noise) biases the mono-exponential fit. This can be accounted for in simple T2 or T2* mapping experiments with a more complicated fit model that attempts to estimate and include noise effects in the model. However, the problem is more nuanced in the case of fitting to difference images as we have done.

Finally, further inaccuracies are introduced by our implicit assumption that all observed decay in the difference images is coming from the short component of T2*. For many tissues, decay can be modeled by a bi-exponential fit with both a rapid decay component and a short decay component. We assume that all decay in the 0.25ms to 5ms time window is from the short T2* component, and do a simple mono-exponential fit to the difference data. Any expected decay from a longer T2* component (with say T2* of greater than 30ms) would presumably account for very little decay over that period. Nevertheless, this is a potential source of error which future work should address.

Future work should also include the extension and validation of the technique for other tissues and areas of the body. Notable areas that could benefit from the work outlined here include quantification of short T2* values in cortical bone and trabecular bone, meniscus, cartilage, and cancerous tumors of different varieties.

4.5 Conclusion

The rapid, SNR efficient 3D UTE T2* mapping sequence presented appears to provide consistent and robust estimates of short T2* in tissues that exhibit very rapid MR signal decay. The preliminary experiments in tendon show consistency of estimate across the tendon at values consistent with those estimated using other techniques (found in the literature), and demonstrate the
ability of the technique to detect subtle changes in the tendon. The preliminary work on the ex vivo AD brain shows the potential to distinguish between subtle differences in relaxation properties in the vicinity of what we believe to be iron compound and protein deposits in diseased tissue. While further validation of the technique is still needed, it appears to be a viable candidate for rapid three dimensional mapping of the relaxation properties of certain tissues that were previously barely MR visible.
CHAPTER 5. METHODOLOGY FOR COMPUTING WHITE MATTER NERVE FIBER ORIENTATION IN HUMAN HISTOLOGICAL SLICES

5.1 Introduction

Diffusion Magnetic Resonance Imaging (dMRI) is an imaging technique that measures the properties of water diffusion in tissues, and has revolutionized the study of brain anatomy. Prior to the development of dMRI, traditional approaches to study white matter could only be done post-mortem. The impact of diffusion MRI techniques on the study of neurological disease has been widespread.

Diffusion MRI techniques can produce estimates of water diffusivity in multiple directions, allowing the estimation of a “diffusion tensor” for each imaged voxel that is directly related to the potentially anisotropic restriction of water diffusion [40–42]. This is termed Diffusion Tensor Imaging, or DTI. The restriction of water diffusion is influenced by underlying microstructural anatomy. In white matter, fiber bundles contribute the most to anisotropic diffusion [41, 43]. The linearity of successively adjacent diffusion tensors is calculated to reconstruct fiber bundles. Disease-related changes in the fiber bundles can thus be identified, as (for example) demonstrated in recent studies of Alzheimer’s disease [44–50]. Further studies have shown a direct relationship between white matter integrity and AD cognitive impairment [44, 48, 50, 51].

Validation of DTI is crucial for assessing its reliability and accuracy of measuring white matter diffusion. The algorithms that approximate the underlying white matter architecture need to be accurate. For obvious reasons, a true anatomical comparison for DTI validation is not feasible clinically in humans, but has been attempted in phantom [52, 53] and animal studies [54, 55]. Examples include tractography validation with excised rat spinal cords [56] and enhancement of the optic tracts by the injection of manganese ions [53].

In studies of human white matter structure and fiber reconstruction, references to anatomical correlates have been qualitative, referring only to already established histological anatomy
In AD, white matter degeneration measured with DTI is associated with the progression of cognitive impairment [44, 48, 50, 51]. However, validation of white matter segmentation in DTI has been mostly limited to qualitative comparisons of results to known anatomical connectivity by a skilled neuroanatomist. There is no resource that exists to permit a direct comparison of DTI data to the underlying white matter architecture.

The creation of a methodology for the direct comparison of DTI data with actual underlying white matter architecture is an extraordinarily difficult problem. This paper focuses on one aspect of this problem: namely, the detection of two-dimensional vectors representing white matter fiber orientations in stained histological sections of the post-mortem human brain.

Tissue sections stained specifically for white matter fibers provide qualitative directionality of tracts, but the systematic measurement of the orientation of fibers in histological slices such that they can eventually be directly compared to DTI data is challenging. Examples of published techniques seeking to validate diffusion MRI with histology include those using 2D structure tensors [58–60], Fourier analysis [61], wavelet decomposition [62], oriented filtering [63], micrographs [64], and template matching [65]. While the resolutions achievable with DTI are much lower than those in images of histological sections, and DTI inherently provides three-dimensional information, the extraction of two-dimensional directionality of white matter tracts from images of histological slices is nevertheless an important step in a direct comparison of DTI data to the underlying white matter architecture.

This is particularly important as DTI techniques improve and are extended, and the ability to infer white matter structure on a finer scale increases. Identifying fiber directionality in histological slices has typically involved manual assessment of fiber direction by a trained observer, and is tedious, slow, highly labor intensive, and fatiguing. Furthermore, reproducibility may be poor [66, 67].

In this chapter, we present an algorithm for the semi-automated assessment of fiber directionality from high-resolution images of stained histological sections of the post-mortem human brains. Images of histological sections are divided into subsections which we term “panels”. Then, the algorithm extracts both an average two-dimensional directionality for each panel and an estimated uncertainty level for the directionality vector. The algorithm is rapid and computationally efficient, and is based on a Fourier-domain analysis of a color-thresholded image of each panel.
We assessed the performance of the algorithm by having a trained neuroanatomist evaluate the algorithm-determined directionality in six histological slices (each from a different post-mortem brain) divided into a total of 300 sub-regions of varying dimensions. The weighted \textsuperscript{1} RMS difference between the results of the algorithm and the neuroanatomist determination of directionality were within 15.4 degrees of that determined by the neuroanatomist, giving confidence in the certainty of the algorithm. When a clear dominant fiber direction was identified in a sub-region by the neuroanatomist, the algorithm typically identified a similar direction with a high level of confidence. When no clear dominant fiber direction was identified by the neuroanatomist (or multiple conflicting dominant directions were identified), the algorithm also yielded a low level of confidence in its estimate.

5.2 Materials and Methods

**Tissue preparation and imaging:** Formalin fixed hippocampal specimens were blocked in the coronal plane with a thickness of 2.5mm and embedded in paraffin. The tissue was obtained from the Neuropathology Core at the David Geffen School of Medicine at UCLA. Sections were cut in 10 micron sections using a Leica rotary microtome and then stained with Luxol Fast Blue/cresyl violet, which stains white matter fibers dark blue and nuclei deep purple.

After preparation and staining, each histological slice was scanned using a Leica SCN400 slide scanner at 20x magnification and panels were visualized using the Leica Virtual Microscopy.

**Terminology:** In this manuscript, we refer to a high resolution image of a stained slice as a “histological slice”. Histological slices are then subdivided into regions of interest, “ROIs”, that we wish to analyze in more detail. Finally, each ROI is subdivided into “panels”, each of which is analyzed for average directionality of white matter tracts across the panel (please see figure 5.1). In other words, our algorithm operates at the panel level, identifying a single 2D vector representing average directionality for that panel, along with a single estimate for the uncertainty of the direction for that panel.

\textsuperscript{1} weighted by the confidence of the neuroanatomist on his determination of the directionality
Figure 5.1: The big dark red square shows the ROI and the small green squares represent the panels. Three representative panels are highlighted with orange color.

### 5.2.1 Algorithm

A representative panel from a ROI in the white matter of a histological slice is shown in Figure 5.2. Nerve fibers tend to be dark blue or purple on these stained histological images. Our algorithm attempts to (1) isolate or segment the nerve fibers in the panel, (2) construct an angular histogram that represents the dominance of nerve fiber orientation in each direction, and (3) extract an “average” directionality from the angular histogram, along with an estimate for the uncertainty in the directionality.

Figure 5.2: Histological sample of Luxol Fast Blue stained white matter in the medial temporal lobe.
Segmentation of nerve fibers in a panel

Segmentation of the nerve fibers in a panel can be accomplished by a threshold based algorithm applied after the panel image is mapped to an appropriate color space. Human vision is perceptually uniform, meaning that we can distinguish the nerve fibers from other structures because variations in color across nerve fibers are relatively small compared to the color differences between completely different structures. From an algorithmic point of view, we can automate this process more accurately by choosing a color space for the images which minimizes color variations across nerve fibers while preserving a stark difference in color between nerve fibers and other structures [68, 69]. The RGB color space (the default for our histological slices) is not well suited for the proposed segmentation algorithm. However, the LAB color space is perceptually uniform [70–74], and a better choice for our algorithm. The LAB color space consists of 3 channels: a luminosity channel and two color channels. We found that segmentation was most robustly done on the luminosity channel image, although sometimes information from the two color channels can improve segmentation on the luminosity channel. Our segmentation algorithm consists of the following four steps, which are summarized in Figure 5.3:

1. Convert all histological panels from RGB to LAB.

2. Apply a threshold on the luminosity channel of the first panel using maximum and minimum thresholding values manually determined by the user to separate nerve fibers from surrounding structures.

Figure 5.3: Block diagram of the segmentation step.
3. If this simple luminosity-channel thresholding fails to effectively segment nerve fibers, then a threshold is applied on one of the color channels to identify non-nerve fiber structures. This can then be subtracted from the luminosity-channel thresholded image to improve segmentation of nerve fibers.

4. Once the above steps are completed, we are left with a binary image that contains both actual nerve fibers and some undesired structures (i.e., blood vessels and glia). Fortunately, true nerve fibers tend to occupy a much larger contiguous area than the undesired structures introduced in this process. In this final segmentation step, the areas of contiguous structures in the binary image are computed, and those below a minimum threshold (which are unlikely to be nerve fibers) are deleted.

The segmentation step is now done, yielding a binary mask indicating where nerve fibers are in the panel. Figure 5.4 illustrates the steps described above to make a binary mask of nerve fibers from a representative panel.

Construction of orientation-based angular histogram

Once the segmentation step is done, we analyze the angular distribution of all of the nerve fibers identified in a given panel. However, the identification of a single “direction” for each identified nerve fiber is not easily done. Our algorithm employs a Fourier-domain technique for estimating the dominance of different directions in the segmented image, allowing the construction of an orientation-based angular histogram for each panel. This histogram can then be analyzed to identify the average directionality in the panel. The spread of the angular distributions across the panel can be used to derive an estimate of the uncertainty for the average directionality in the panel.

The presence of sharp edges in a given direction in an image manifests as increased energy in the high spatial frequencies of the Fourier representation of the image. This increase is in angular regions perpendicular to the direction of sharp edges in the image domain. This is illustrated in Figure 5.5(a-d). Thus, by analyzing the angular distribution of energy in the high spatial frequencies in the Fourier domain, we can identify directions present in the segmented image of a panel, and estimate how strongly each of those directions is represented.

To produce an angular histogram for a given panel, the following steps are performed:
Figure 5.4: (a) A sample panel in the white matter of a histological slice. (b) Thresholding on L channel. (c) Detected non-nerve fiber structures on a* channel. (d) Subtraction of (c) from (b). The remaining white structures in (d) are expected to correspond with nerve fibers.

1. Take the 2D Discrete Fourier Transform (DFT) of the panel.

2. Mask out the low spatial frequencies in the DFT image of the panel (equivalent to applying a high-pass filter to the original panel image).

3. Divide the remaining portion of the image into “angular windows” as illustrated in Figure 5.6. Once divided, we sum the energy in each window, and use these sums to form an orientation-based histogram where the intensity of the histogram at a given angle is equal to the image energy found within the corresponding angular window.

Variable parameters for this process include the cut-off spatial frequency of the high-pass filtering step \( r_c \) (measured in pixels in the Fourier domain image), the angular window width \( \alpha \), and the angular spacing \( \Delta\Phi \) between sliding-angular-window bins (Figure 5.6). Note that \( \alpha \) maps to the
Figure 5.5: (a) and (b) show two panels with different dominant nerve fiber directions; (c) and (d) show the magnitude of the 2D Fourier Transform of the segmented versions of figures (a) and (b), respectively. Regions of high energy have been illustrated by red rectangles. The fiber directions are perpendicular to the direction of the bright regions in the magnitude of the Fourier Transform. (e) and (f) represent angular orientation histograms extracted from (a) and (b), respectively. The peak in (e) and (f) represent the dominant nerve fiber orientation in (a) and (b), respectively.

angular extent of each bin in the angular histogram. We use $\Psi$ to denote the angle of a radial line passing through the center of an angular window. The algorithm thus analyzes angular windows with $\Psi$ ranging from 0 to 179 degrees in increments of $\Delta \psi$. 
Figure 5.6: Illustration of the calculation of image energy in angular window slices of the masked DFT panel image. The segmented panel is high pass filtered by zeroing out the center of the 2D DFT (the blue circle above). The energy in the 2D DFT image is then measured across different angular windows (3 shown). \( r_c \) is the cut off frequency, \( \alpha \) is the angular window size, and \( \Psi \) is the angle of a radial line passing through the center of an angular window. In this figure, \( \Delta \Psi \), the angular spacing between angular window slices is equal to the angular window size \( \alpha \).

Let \( F(k_x, k_y) = \mathcal{F}\{f(x,y)\} \) represent the 2DFT of the thresholded panel image, where \( k_x \) and \( k_y \) are spatial frequencies in the x and y directions respectively. The energy, \( E \), in an angular slice of angular width \( \alpha \) centered at angle \( \Psi \) is then given by:

\[
E(\psi) = \sum_{\psi=\psi+\alpha/2}^{\psi-\alpha/2} \sum_{r=r_c}^{[M/|2\cos(w)|]} |F[r \cos(w), r \sin(w)]|^2,
\]

(5.1)

where \( M \) and \( N \) are the width and height of the image (in pixels), and \( r_c \) is the cut off radius (also in pixels).

Again, it is important to note that the orientation of the nerve fiber edges in the masked image are perpendicular to the observed orientation of the edges in the Fourier domain. The dominant direction of each of the panels is apparent as a line of increased energy in the Fourier domain at an angle perpendicular to the dominant direction in the panel. This line in the Fourier domain results from the sharp edges that are present in the mask image.

Note that the angular window size \( \alpha \), cut-off frequency \( r_c \), and angular increment \( \Delta \psi \) should be carefully chosen to yield the best results for a given application. For example, increasing win-
dow size reduces angular resolution and smooths the energy histogram distribution. Increasing the cut-off frequency $r_c$ changes the strength of the dominant directions. Changing angular spacing alters the histogram resolution.

**Orientation estimation from orientation-based angular histogram**

Once an orientation-based histogram is generated for each panel, we can extract a simple average orientation and an estimate of the uncertainty for that panel. While it is tempting to simply find a weighted mean of the histogram (similar to finding the statistical mean of a probability mass function), this can produce undesired results if a peak is centered near 0 and/or 180 degrees as in (Figure 5.7(b)). This problem can be avoided by extending the angular histogram to cover a range of 0 to 359 degrees (effectively replicating it twice). We then calculate the mean and standard deviation of a sliding window with a 180 degree width, starting with a window from 0 to 179 degrees and finishing with a window from 180 to 359 degrees. We then choose the window that has the lowest standard deviation, as illustrated in Figure 5.7(c). In most cases, this will concentrate a directional peak towards the center of the angular window, and yield more accurate results when a peak is clustered near the edges of the 0 to 179 degree window.

Once the sliding window that yields the minimum standard deviation is identified, the average orientation for the panel is then simply computed as the weighted mean of the angular histogram over that minimum standard deviation window. We use the standard deviation of the angular histogram over the minimum standard deviation window as our measure of the uncertainty, where a smaller standard deviation indicates a higher level of confidence in our angular estimate. After extracting the average orientation from the angular histogram for a panel, other useful information can be extracted from the histogram based on the degree to which it is uni-modal vs. multi-modal.

### 5.2.2 Manually Marking Fibers

For comparison, we manually marked fiber directions in 50 panels using the following 3-step process. First, vector lines were drawn close to the nerve fibers using the Neurolucida (Microbrightfield Bioscience) such that the length and the direction of the lines represented the
Figure 5.7: (a) A sample panel in the white matter of a histological slice. (b) The histogram from 0 to 179 degrees yields a mean value of 104 degrees and standard deviation of 60 degrees, which is clearly not an accurate estimate. It is visually evident that the peak is centered closer to 165 degrees. Periodic extension of the angular histogram and identification of the sliding 180-degree window that yields the minimum variance across the window provides a much more robust way of identifying the center of the angular peak. This is illustrated in (c), where analysis of the mean on the 180-degree window shown in green (the minimum-variance window) yields a much more plausible mean value of 165 degrees.

respective dimensions of the nerve fibers. We used the operational definition of nerve fibers as being visually discernible from a clear beginning and end within the plane of the tissue section. An angular orientation histogram from 0 to 179 degrees was then created, with bins centered at each degree. The height of each bin was the total sum of the lengths of the lines within the angular range of each bin. We then followed the same procedure as described in section 2.1.3 to estimate the average orientation and find the standard deviation.

5.3 Experimental Setup

The algorithm described above were implemented in MATLAB (The MathWorks, Inc., Massachusetts). We describe other aspects of our experimental set up below.
5.3.1 Selection of Algorithmic Parameters

**Selection of threshold:** Minimum thresholds were manually chosen for six stained batches. Staining variations from batch to batch can be significant, so this step is important for tuning algorithmic performance. Minimum thresholds were chosen such that the thresholded images contain a minimum amount of undesired structures (i.e., blood vessels and glia). The binary masked images of the undesired structures and the nerve fibers were compared visually, one by one, to the original non-masked images of the panels. The thresholding values that matched the location and width of the undesired structures and the nerve fibers in the panel (by visual inspection) were chosen.

**Selection of \( \alpha, r_c, \) and \( \Delta \Psi \):** In our experiments, other algorithmic parameters were determined by evaluating eight panels with one dominant nerve fiber orientation and sixteen panels with at least two dominant nerve fiber orientation across a range of parameter values. The resulting angular orientation histograms of each of the 24 panels were then visually inspected, and a determination made on which sets of parameters best yielded angular histograms with dominant peaks around the expert’s assessment of fiber orientation in the corresponding panels. Parameter values were varied as follows: \( r_c \) was varied from 2 to 25 pixels (in 1 pixel increments) while fixing \( \alpha = 1 \) degree and \( \Delta \Psi = 1 \) degree. A visual inspection was then performed to determine a good value of the cut-off frequency \( r_c \). This value of \( r_c \) was then used going forward, and the value of \( \alpha \) was varied from 1 to 6 degrees (in 1 degree increments) while keeping \( \Delta \Psi = 1 \). A visual inspection was then performed to determine the best value of \( \alpha \). Finally, the process was repeated with \( r_c \) and \( \alpha \) fixed at the previously-determined values, and \( \Delta \Psi \) was varied. For the histological panels examined, the following values were found to yield reasonable results, and were then used in all subsequent experiments: \( \alpha = 2 \) degrees, \( r_c = 15 \) pixels, and \( \Delta \Psi = 1 \) degree.

5.3.2 Qualitative Validation of the Semi-Automated Method

A qualitative evaluation of the semi-automated method was performed by an expert neuroanatomist (Jonathan J. Wisco) in the following manner. Panels were drawn from ROIs in six histological slices (from six different subjects, 1 histological slice per subject). The performance of the algorithm was tested across panels of different physical dimensions rendered to 512 x 512 pixels to ascertain how the algorithm performed across a range of physical panel dimensions. A
sample of 50 panels was analyzed at a variety of physical dimensions summarized in Table 5.1. To facilitate the comparison of dominance of the nerve fibers with the neuroanatomist’s eyes, a polar histogram of the orientation histogram was constructed. The polar histogram was made by showing the strength of the orientation at each degree in a unit circle. The algorithm was executed on each of the histological panels, yielding an average directionality and uncertainty level. These algorithmically-determined directionalities were then manually evaluated by the expert neuroanatomist. Two qualitative experiments were done. First, binary determination (yes or no) on agreement on the directionality and the major directions in the panels for 300 panels in Table I. Second, drawing a line over 130 panels (from data number 6 and 7 of Table 5.1) such that the orientation of the line represented the average direction of the nerve fibers and the length of the line represented the confidence of him over the average directionality.

Table 5.1: Six histological slices (each from a different post-mortem brain) were used to validate the semi-automated algorithm. 300 panels were drawn from ROIs in the 6 histological slices, 50 from each histological slice, and were analyzed at variety of pixel sizes.

<table>
<thead>
<tr>
<th>Data</th>
<th>Panel size (pixels)</th>
<th>Dimension of each panel (mm x mm)</th>
<th>Pixel size (mm x mm)</th>
<th>Number of panels</th>
<th>Maximum thresholding on l channel (Candela/pixel)</th>
<th>Minimum thresholding on l channel (Candela/pixel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>512 x 512</td>
<td>450 x 450</td>
<td>0.88 x 0.88</td>
<td>50</td>
<td>110;141</td>
<td>55;90</td>
</tr>
<tr>
<td>2</td>
<td>512 x 512</td>
<td>500 x 500</td>
<td>0.98 x 0.98</td>
<td>50</td>
<td>110;115;125</td>
<td>74;85</td>
</tr>
<tr>
<td>3</td>
<td>512 x 512</td>
<td>600 x 600</td>
<td>1.17 x 1.17</td>
<td>50</td>
<td>138;166</td>
<td>88;107</td>
</tr>
<tr>
<td>4</td>
<td>512 x 512</td>
<td>648 x 648</td>
<td>1.26 x 1.26</td>
<td>50</td>
<td>80;90</td>
<td>44;54</td>
</tr>
<tr>
<td>5</td>
<td>512 x 512</td>
<td>692 x 692</td>
<td>1.35 x 1.35</td>
<td>50</td>
<td>86;95;102</td>
<td>42;56;65</td>
</tr>
<tr>
<td>6</td>
<td>512 x 512</td>
<td>776 x 776</td>
<td>1.51 x 1.51</td>
<td>50</td>
<td>86;93</td>
<td>30;49</td>
</tr>
</tbody>
</table>

For the first experiment, for each panel, the neuroanatomist made an initial determination of the dominant directionality of nerve fibers based on visual inspection of the panel and made a decision about the strength of the directionality and also the major directions of the nerve fibers. The neuroanatomist was then asked to take a look at the results of the semi-automatic algorithm, and make a binary determination (yes or no) on whether the algorithmic directionality and uncertainty level was in agreement with his expert determination of directionality for a given panel, and his level of confidence in the calculated directionality. He was also asked to determine whether the dominant nerve fiber directions seen in the polar histogram accurately described the dominance.
of the nerve fiber orientations in the panels. For the second experiment, a subset of 130 panels (from the physical dimensions of 692mm x 692mm and 776mm x 776mm) were randomly chosen to compare the performance of the algorithm in term of directionality to the expert’s decision. To facilitate the measurements at each panel four circles for representing 25%, 50%, 75%, 100% confidence of the neuroanatomist on the direction were shown. Then, the neuroanatomist drew a line from the marked center of the panel toward the fourth circle depends on the strength of the dominance of the nerve fiber orientation in the panel from 0% to 100%. Finally, the weighted RMS was calculated based on the difference in the orientation of the semi-automatic algorithm and the neuroanatomist, and weighted based on the expert neuroanatomist’s confidence on the directionality.

5.3.3 Quantitative Comparison of the Semi-Automated Method to Manual Segmentation

A quantitative comparison of our semi-automated method to the manual-segmentation was then performed using a single histological slice from a single subject. Two trained graduate anatomy students manually marked fibers for a ROI in the histological slice. Manually marking the nerve fibers was performed by segmenting fibers using operational definition of dark blue stained curvilinear structures with a distinguishable beginning and end. Then, the ROI was divided into 50 panels, each with a size of 512x512 pixels and panel size 500x500 mm$^2$. For the manual estimation, a histogram was produced containing 180 bins over the range of 0 to 179 degrees. For the semi-automated method, we used the aforementioned parameters in sub-section 3.1. Polar histograms for both manual and semi-automated methods were created. The average directionality and uncertainty level was then extracted from the histograms (both manual and semi-automated) using the method described in 2.1.3.

5.4 Results

5.4.1 Qualitative Validation of the Semi-Automated Method

The qualitative opinion of the expert neuroanatomist for 300 panels (yes or no agreement) was comparable with the results from the semi-automated algorithm (orientation and uncertainty)
for all of the sampled panels. Also, the dominance of nerve fiber directions seen in the polar histogram matched with the qualitative opinion of the neuroanatomist on the dominance of the nerve fiber orientations in the panels. In addition, the weighted RMS difference between the expert neuroanatomist directionality and the semi-automatic one for 130 panels was 18.3 degrees. The average orientation and the polar orientation histogram for four panels are shown in Figure 5.8. A region of interest containing 123 panels is shown in Figure 5.9, with average orientation shown as a line for each panel. The length of the line indicates the uncertainty of the angular estimate. In addition, the polar histograms and the average orientation for four panels are illustrated.

5.4.2 Quantitative Comparison of the Semi-Automated Method to Manual Segmentation

For each panel, the RMS difference in both average directionality and uncertainty level was calculated. The RMS difference in directionality across the 50 sampled panels was 11.2 degrees.

5.5 Discussion

We are encouraged by the performance of the semi-automated approach in the various comparisons conducted, and believe that it could be a valuable additional tool in the ongoing effort to validate the accuracy of DTI techniques using histology. The results of the algorithm are in reasonable agreement with those obtained through visual inspection by the expert anatomist, and are in even better agreement when compared only to panels in which the expert expressed a high degree of confidence in his estimate. Furthermore, the results were in good agreement with the much more labor and time intensive manual segmentation technique. However, it is important to note that validation of an algorithm such as this is inherently limited by the lack of a gold standard with which to compare. The comparison studies shown are a first step in this process, and future work is needed to more fully validate the algorithm against the wide variety of other techniques that can be applied to the same problem. It is beyond the scope of this paper (intended to introduce the algorithm and provide a basic demonstration of its utility) to provide a more exhaustive comparison against the broad range of other competing techniques.

We were further encouraged by the purely qualitative and binary assessment performed by our expert anatomist. The trained human eye is very good at identifying approximate fiber direc-
Figure 5.8: The average orientation and the polar histograms for panels, calculated by the semi-automatic algorithm, shown as a line for each panel, constructed by the semi-automatic algorithm. The length of the line indicates the uncertainty in the angular estimate (lower length indicate a lower level of confidence in the angular estimate).
Figure 5.9: A region of interest containing 123 panels, with the average orientation direction, calculated by the semi-automatic algorithm, shown as a line for each panel, constructed by the semi-automatic algorithm. The length of the line indicates the uncertainty in the angular estimate (lower length indicate a lower level of confidence in the angular estimate). It should be noted that DTI assigns a single directionality to the population of the nerve fibers, no matter if the fibers are parallel with each other, or not. Therefore, here, we keep defining the directionality of the nerve fibers with one direction even though we have crossing fibers in some panels.

We made the further observation during the outlined studies that a low uncertainty level was produced by the algorithm in panels that clearly had a dominant orientation evident through visual inspection. For panels that exhibit more than one dominant nerve fiber orientation (again through visual inspection), the angular histogram exhibited the expected multiple peaks, and the algorithm yielded a higher level of uncertainty in the direction estimate. The qualitative opinion
of the expert neuroanatomist was that visual inspection of the angular histograms in conjunction with the corresponding panel revealed not only the dominant nerve fiber direction(s) of the panel, but also less dominant directions that were not immediately obvious from inspection of the panel itself. While the comparisons indicate reasonably good agreement between expert opinion, manual segmentation, and the proposed semi-automated algorithm, the differences observed could arise from the ability of the semi-automated algorithm to measure subtle directional trends not obviously visible to the human eye.

The differences between the manual segmentation and semi-automated techniques may also be due to the very different ways in which the two algorithms go about creating the angular histograms. Nevertheless, for panels that exhibit a single strong dominant direction, the polar histograms extracted from the manual segmentation technique were visually very similar to those extracted from the semi-automated algorithm. In some cases, however, both algorithms yielded very different looking polar histogram shapes, but a very similar dominant direction. In these cases, the manual segmentation technique is likely simply not marking relatively small or non-dominant nerve structures that appear in the semi-automated technique. In cases where the directionality estimate from the manual segmentation diverged significantly from the estimate using the semi-automated algorithm, a post hoc informal inspection by the expert anatomist of both analyses was performed. In these cases, the anatomist concluded that the estimate of the semi-automated algorithm was more accurate in a significant majority of cases. Irrespective of these slight variations in performance, the presented Fourier-domain technique appears to be a viable substitute for manual segmentation techniques or expert assessment, and has the potential to be very rapid as the only manual step is tuning the thresholding values for each batch of histological slices.

The proposed technique has some clear limitations. As the variability of the nerve fiber orientations increases, the standard deviation for the panel also increases and the average orientation measurement is less likely to be a good representation of the different orientations existing in a panel. An additional limitation of the technique is sensitivity to lighting and variations in staining. A more thorough study of the effects of these variations on algorithmic performance is needed, but was not possible with our limited dataset and is beyond the scope of this paper. Finally, another clear limitation of the algorithm is its inability to accurately assess through-plane fiber directionality, given the 2D nature of the images of these thin histological slices. It also should be noted that
better techniques may exist for locating the dominant direction of the angular histogram (such as through peak detection algorithms), and the technique could be extended in the future to attempt to identify multiple dominant directions in the angular histograms.

Future work should focus on (1) further validation of the algorithm through more thorough comparisons with other techniques, (2) assessment of the sensitivity of the algorithm to lighting and variations in staining (i.e., Luxol Fast Blue/cresyl violet versus Luxol Fast Blue/hematoxylin and eosin), and (3) evaluation of potential improvements in the interpretation of the produced angular histograms.

Once the algorithm has been more thoroughly validated and tuned, we hope to use it to correlate the average directionality of nerve fibers in histological slices with the directionality of fiber bundles revealed using diffusion tensor MRI on the brain prior to histological evaluation. As previously mentioned, this is a challenging problem for a variety of reasons. Voxel sizes in DTI datasets will typically be 1-2mm isotropic; the histological slices are much thinner, and the resolution of histological images after staining is much higher in-plane. Furthermore, the DTI datasets reveal fiber orientation in three dimensions (including the through-plane direction), while the data extracted from the histological slices fails to capture through-plane fiber orientations. Finally, registration of thin, high-resolution histological images to the corresponding much lower resolution DTI data will be challenging due to differences in resolution and orientation, and sample warping during histological preparation. Nevertheless, it should be possible to validate clear in-plane fiber orientations observed in the DTI datasets with the corresponding histological images, and extract some information about through-plane directionality from series of 2D histological slices.

5.6 Conclusion

In this chapter, we have presented a robust and semi-automated technique for extracting nerve fiber orientation from images of stained histological slices, along with an estimate of uncertainty in the extracted direction. Qualitative evaluation of the new technique by an expert neuroanatomist indicates that the semi-automated technique is indeed accurately identifying what the neuroanatomist would consider the dominant nerve fiber orientation based on visual inspection. Furthermore, the algorithm appears to take into consideration nerve fiber microstructure that is
not immediately evident from rapid visual inspection. Quantitative comparison of the new semi-
automated algorithm against a more time and labor intensive manual nerve fiber segmentation
technique suggests that both techniques yield similar results, further bolstering confidence in the
accuracy of the semi-automated approach.
CHAPTER 6. METHODOLOGY FOR COMPUTING CYTOARCHITECTONIC PATTERN IN HUMAN HISTOLOGICAL SLICES

6.1 Introduction

The human cerebral cortex is composed of a heterogeneously layered sheet of neurons and glial cells [75], folded macroscopically into gyri and sulci [76]. Specific regions of folds are cytoarchitecturally parcellated into sub-regions of cortex based on distinguishable spatial cellular patterns [75]. This cellular architecture can be visually discriminated by dividing the area into several cytological layers, each of which are parallel to the cortical surface [9]. Each cytoarchitectonic area has its own input and output connections with the other areas, and works as the basic processing unit for the cerebral cortex [75].

The density of neurons in the cytoarchitectonic areas changes [77, 78] during the progression of neurodevelopmental disorders (e.g., schizophrenia and autism spectrum). Through in vivo MRI, these changes can be observed using different techniques such as FLAIR (Fluid Attenuated Inversion Recovery) [79], fMRI (functional MRI) [80], T1 [81–83], and T2 [81, 82, 84] weighted images. These observed cytoarchitectonic changes are used to interpret the progression of the disease. However, the accuracy and reliability of the changes is not completely known since they have not been validated with respect to histology.

Validation of commonly used MRI techniques for in-vivo cytoarchitectonic analysis with respect to histology is crucial for assessing the reliability and accuracy of cytoarchitectural differences seen with the progression of the disease. While a true anatomical comparison for MRI validation is not clinically feasible in humans, it has been attempted in animal studies [85,86]. Examples include cytoarchitectonic maps of the macaque monkey brain with reference to MRI [86] and cytoarchitectonic study of the baboon parahippocampal gyrus with respect to MRI [87].

In human studies, there is not a completely objective and reliable histological map that can be used to validate MRI cytoarchitectonic changes with respect to histology. The well-known
maps, including those developed by Broadmann [11], Talairach [88], and Knoblauch [12], might not have enough details to be compared with MRI results [89]. The validation of histology-MRI cytoarchitectonic changes requires quantitative cytoarchitectural maps. The importance of a quantitative cytoarchitectural map is that it increases the number of metrics available for the analysis of neurological disorders or diseases. To date, cell counting on post-mortem tissue ([9,87,90–92]) has been the only quantitative measure of neurodevelopmental or neurodegenerative changes. However, neuron loss may not be the only pathological change occurring.

For obvious reasons, a semi-automatic algorithm with minimum human interaction is highly desired for making a quantitative map. However, the previous attempts to make a semi-automatic algorithm that possesses the potential to be applied to MRI validation, or one which outperforms cell counting measures, have had limited success. Published methods include: gray level index (GLI) [93, 94], sliding windowing based GLI [95, 96], central moments [97], excess mass [98], wavelet transforms [99], density profiles of specific layers [9], k-means clustering [100], and nearest neighbor [101–104]. The results of these algorithms are difficult to interpret anatomically [93–98], are very time consuming [87, 92, 105], and some need significant user interaction (e.g., [9]).

In this chapter, we present an algorithm for the semi-automated parcellation of cytoarchitectonic areas from high-resolution histological images of stained cortex slices of the Rhesus monkey (macaca mulatta) brains at Brainmaps.org. The algorithm first creates a binary mask of neurons from histological sections, then measures the density, size, and number of neurons in a specific radial distance in the binary masked image. Then, a map including all the aforementioned features is constructed. The algorithm finally segments the map into cytoarchitectonic areas.

6.2 Materials and Methods

Tissue imaging: The histological images were acquired at the resolution of 0.05mm from brainmaps.org. Formalin fixed hippocampal specimens were blocked in the coronal plane and embedded in paraffin. Sections were stained with Luxol Fast Blue/cresyl violet, which stains nuclei deep purple.

Terminology In this manuscript, we will refer to a high-resolution image of a stained slice as a “histological slice”. The region of the gray matter on the histological slices that is chosen for
Figure 6.1: Pial surface (black arrow) and cortical-white matter (white arrow) for a histological sample of Luxol Fast Blue stained gray matter.

analysis is called region of interest, “ROI”. A border was drawn to separate the pial surface from the rest of the image, and another border was drawn to separate the cortical-white matter border from the rest of the image. Figure 6.1 shows the pial surface and cortical-white matter for a sample histological slice. The ROI is comprised of the neuronal tissue between the pial surface and the cortical-white matter borders. An ROI can be divided into cytoarchitectonic “sub-regions” by using a “traverse”, which is a line drawn through ROI’s, perpendicular to the pial surface and cortical-white matter borders. The sub-region between two traverse lines is called a “traverse sample”. A traverse is orthogonal to the neuronal lamination or to the cortical surface [106, 107].

6.2.1 Segmenting the Neuron Cells

Segmentation of neuron cells can be accomplished by dividing the image into blocks and then applying a threshold-based algorithm on each block after the image is mapped to an appropriate color space. The RGB color space (the default for our histological slices) is not well suited for the proposed segmentation algorithm. Conversely, the gray scale image is a better choice for the algorithm since the neurons are darker than the rest of the surrounding tissue, and, therefore, have lower brightness compared to the surrounding tissue. A representative window from an ROI in the gray matter of a histological slice is shown in Figure 6.2. Neuron cells tend to be dark purple on these stained histological images.
Our thresholding algorithm is based on Otsu ’s thresholding [108]. The algorithm runs on the gray level histogram and seeks to establish a threshold that minimizes the weighted sum of variances of the two clusters, foreground and background. Our proposed segmentation algorithm has two steps: I) finding the optimum window size and the optimum threshold, and II) thresholding all the neurons in the ROI based on the parameters found in (I).

I. Finding the optimum window size and the optimum threshold

The microscopic light variations in the histological slice image can affect the segmentation results. Traditionally, Otsu ’s thresholding can produce an over-segmented binary masked image, where neurons that are close to each other are connected. To prevent the aforementioned issues,
Figure 6.3: This block diagram describes how the optimum window size is determined. The optimum window is the smallest possible window size that can describe represents the location and shape of individual neurons. We try to find the minimum possible window size for the segmentation since it minimizes the effect of light variations. For a small window size, 1 to 5 times of the size of the neuron, the light variations should be minimum and negligible.

we first need to find the optimum window size and the optimum threshold. The processes for these tasks are summarized in Figure 6.3 and Figure 6.4, respectively.

More specifically, we first choose a relatively large window (e.g., window size of 2000 pixels x 2000 pixels) in the ROI such that the window has different sized neurons, usually found in a laminar pattern. Next, we extract the image in the window and then convert it from RGB to grayscale. The rest of this process is as follows:

1. **Find the optimum window size**: To find the optimum window size, we first choose the size of sub-window. The size of sub-windows is bigger than the size of an individual neuron (e.g., 32 pixels x 32 pixels). We then divide the ROI into small sub-windows to do the segmentation analysis on each of them and acquire binary mask of the neurons in the sub-windows. The segmentation is performed based on Otsu ’s paper to find the threshold. After segmenting all the neurons in the
sub-windows, we concatenate the binary masked images of sub-windows to make the binary masked image of the entire window. The final step is comparing the binary mask of the neurons with the ones in the non-segmented image: If the binary masked image represents the location and shape of individual neurons, the optimum window size is found. If not, we should increase the size of the window and repeat the entire process.

2. **Find the optimum threshold**: When the optimum window size is found, the next step is to find the optimum threshold. This is done by choosing a scaling factor less than 1 (e.g., 0.95), and comparing the binary masked image of neurons to see if the neurons in the binary image are connected or not. After choosing the scaling factor, we multiply the Otsu’s threshold by it, and use this to threshold the binary mask of the neurons in each of the sub-windows. Next, we concatenate the sub-windows to make the binary masked image of the entire window, and compare the binary mask of the neurons with the ones in the non-segmented image. If the neurons look disjointed, this step is done. If not, choose a scaling factor less than the one used before, and repeat the entire process.

II. **Thresholding all the neurons in the ROI based on the optimum window size and the optimum threshold**

After finding the optimum window size and the optimum threshold, we can segment the binary masked image of the neurons in the ROI in a histological slice. We first divide the image within the ROI into the windows based on the optimum window size found in the previous section. We then use the optimum threshold to segment the neurons in the sub-windows, and, finally, concatenate the binary masked images of the neurons in the sub-windows.

Once the above steps are completed, we are left with a binary image that contains both actual neurons and some undesired structures (i.e., noisy pixels). Fortunately, true neurons tend to occupy a much larger contiguous area than the undesired structures introduced in this process. In this final segmentation step, the areas of contiguous structures in the binary image are computed,
Figure 6.4: This block diagram describes how the optimum threshold is found. Otsu’s thresholding, depending on the resolution and the quality of the histological image acquisition, might connect the two adjacent neurons. The optimum scaling factor is the one that separates those adjacent neurons in the binary masked image.

and those below a minimum threshold (which are unlikely to be neuron cells) are deleted. The segmentation step is now done, yielding a binary mask indicating where neurons are in the ROI.

### 6.2.2 Constructing Laminar Pattern Map

Once the segmentation step is done, we can identify the pattern of all the neurons identified in a given ROI by making a cytoarchitectonic laminar pattern map. Constructing a laminar pattern map is done by calculating all the neuron centroids, finding the neurons in a radial distance from the query neuron, and then extracting different features that can be used for discriminating laminar patterns. The centroid of each neuron is used to determine whether the neuron is inside the radial distance or not (see Figure 6.5).
Figure 6.5: This figure represents how it is determined whether a neuron is within a radial distance. Neuronal cell bodies within a given region are presented by shaded circles and the dashed circle represents the radial distance. (a) The centroid of neurons are shown with small black circles inside the shaded circles. (b) The neurons that are determined to be within the radial distance are marked with black color. As can be seen, if a neuron centroid is not within the radial distance, although there might be some overlap between part of the neuron body and the region inside the radial distance, we ignore it.

After finding the neurons within a radial distance, we are able to extract specific features to describe the neurons relative to each other. There are three distinct features that we can use to make the map: distance of, number of, and area of the neuron cells. The following steps are performed for each neuron \(k_n\) in the segmented binary image of neurons to extract features:

(a) calculate the area of the neurons with a centroid inside the radial distance, \(A[k_n]\).

(b) calculate the number of the neurons with a centroid inside the radial distance including the current neuron, \(\text{Num}[k_n]\).

(c) calculate mean distance from each neuron centroid to the current neuron centroid, \(D[k_n]\), for all neurons with centroid inside the radial distance. Note that this is an average over \(\text{Num}[k_n] - 1\) distances.

Each individual feature alone is not a good representative of the pattern of the neurons. Figure 6.6 provides some examples to show why the individual features can be misleading.

To produce the laminar pattern map, we first calculate \((A_c, \text{Num}_c, D_c)\) from all the centroids of the binary masked image of neurons, where \(A_c=\text{average (A)}, \text{Num}_c=\text{average (Num)}, \text{and } D_c=\text{average (D)}\). We then calculate the \(L_2\) norm for all the centroids as follows:
Figure 6.6: This figure illustrates how the distance, number, and area of the neuron cells alone can be misleading. Neuronal cell bodies within a given region are presented by shaded circles. (a) Original pattern. (b) The same number of neurons with varied areas. (c) The same area with a different number of neurons. (d) The same size and number of neurons with a different distance between them.

\[
\text{FeatDist}[k_n] = \| (A[k_n], \text{Num}[k_n], D[k_n]) - (A_c, \text{Num}_c, D_c) \|_2.
\]  

(6.1)

After calculating FeatDist, we normalize and then quantize it to L levels by:

\[
\text{FeatDist}[k_n] = \frac{\text{FeatDist}[k_n]}{\max(\text{FeatDist})}
\]

\[
\text{FeatDist Quantized}[k_n] = \lfloor (L_m - 1)\text{FeatDist}[k_n] \rfloor.
\]

(6.2)

Variable parameters for this process include the radial distance and \( L_m \). The user needs to produce the map for several radial distances and determine which one shows different laminar patterns. The user also needs to decide what the appropriate value for \( L_m \) is based on the perfor-
mance of the algorithm to detect cortical sub-regions. Figure 6.7 represents the laminar pattern at different radial distances.

Figure 6.7: (a-d) The laminar pattern maps constructed at the radial distance of 200 pixels, 250 pixels, 300 pixels and 350 pixels, respectively. The features that were used to make the laminar map were distance of, number of, and area of the neuron cells. The colors represent the FeatDistQuantized value of individual neurons, calculated based on eq. (6.2).

Now, for each centroid of neuron \((k_n)\), we have extracted \((A[k_n], \text{Num}[k_n], D[k_n], \text{FeatDistQuantized}[k_n])\).

### 6.2.3 Identifying Sub-Regions from the Laminar Pattern Map

After generating the laminar pattern map, we need to partition the laminar pattern map into homogeneous sub-regions of cortex. This is done in two steps: 1) dividing the laminar pattern map
into traverse samples, and 2) grouping the adjacent traverse samples as sub-regions based on the similarity among them. Each step is described in more detail below.

**Finding the neurons within the traverse samples**

In this step, we first divide the laminar pattern map into traverse samples, and then identify the neuron centroids within each traverse sample. Here, we use the modified version of Schleicher et al. [106] for constructing the traverse samples. To produce the traverse samples, the following steps are performed (see figure 6.8):

(a) Mark the first traverse line by defining the starting points in the inner cortical border line and the outer cortical border line.

(b) Define the search range in both the cortical lines. This search range is where we want to find the traverse line.

(c) Go perpendicular from the middle of the current traverse line for a specific distance.

(d) Detect as many possible traverse lines crossing both the end point of the perpendicular line and the points in the search range.

(e) Choose the traverse line with the minimum length from the pool of all possible traverse lines.

(f) Choose the crossing points in the inner and outer cortical lines of the new traverse line as the new starting point and repeat the process to produce the next traverse line.

It should be noted that if the cortical borderlines are straight, the traverse samples have a rectangular shape, and if that part of the ROI is folded, the traverse samples are like the slices of a watermelon as shown in Figure 6.9.

Variable parameters for this process include search range, $\Delta d_{sr}$, and the length of the perpendicular line from the middle of the current traverse, $\Delta d_{trav}$, measured in pixels. Note that $\Delta d_{sr}$ is calculated as $\Delta d_{sr} \geq 2 \times \Delta d_{trav}$ to make sure the algorithm can find an appropriate traverse line in the searching range. Figure 6.10 shows the traverse samples for a monkey brain sample ROI.
Figure 6.8: This figure illustrates the step that are taken to make the traverse windows. (a) shows an example region, inner and outer cortical border lines. (b) shows the first traverse line drawn by hand. The bold circles on the border (traverse) lines show where the traverse line crosses inner and outer cortical border lines. (c) shows the perpendicular black line from the middle of the traverse, with length of $\Delta d_{trav}$ pixels, and red search range lines, with length of $\Delta d_{sr}$ pixels, on the inner and outer cortical borderlines. (d) represents some lines that cross both search range lines and the end of perpendicular line from the middle of the traverse. (e) The line with the minimum length is chosen as the traverse line and the perpendicular black line from the middle of the current traverse line is drawn. (f) We repeat the same process as (d) for the current traverse line.

Once the process of defining traverse lines is completed, the traverse samples can easily be identified as the area between two contiguous lines. We then can identify all the neuron centroids within a traverse sample ($m$), called $\text{trav}[m]$. Now, for each neuron centroid ($k_n$), we have extracted $(A[k_n], \text{Num}[k_n], \text{D}[k_n], \text{FeatDistQuantized}[k_n], \text{trav}[k_n])$. 

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Figure 6.9: This figure represents different shapes of traverse samples in straight and folded cortical regions. The white area is the region between inner and outer cortical borders. (a) In the subregions that inner and outer cortical borderlines are parallel, the traverse samples are parallel with each other, and are perpendicular to the cortical borderlines. (b) In sub-regions that cortex is folded, the traverse samples are like the slices of a watermelon.

**Grouping the traverse samples**

Once the neuron centroids that belong to each traverse sample are determined, we partition the laminar pattern map into distinct sub-regions based on the similarity of the neuron centroids within the traverse samples. The similarity of laminar pattern can be measured based on the “FeatDistQuantized” value of and the location of each neuron centroid in the traverse samples. The location of each neuron centroid \( k_n \) in a traverse sample can be determined as the shortest Euclidean distance from each neuron centroid \( k_n \) in a traverse sample to the outer cortical border and rounded to the nearest pixel, \( \text{DistEuc}_{\text{border}}[k_n] \), as shown in Figure 6.11. It should be noted that the location of neuron centroids at the width of the traverse sample is ignored. After calculating \( \text{DistEuc}_{\text{border}}[k_n] \) for each neuron centroid, we are able to measure the similarity of the laminar pattern among the traverse samples based on \( (\text{FeatDistQuantized}[k_n], \text{trav}[k_n], \text{DistEuc}_{\text{border}}[k_n]) \) as is described in the rest of this subsection.

In this chapter, we adopt a region growing algorithm to segment the ROI into the sub-regions with similar laminar pattern. Each traverse sample at first is considered as an initial “sub-region”. Then, these smaller sub-regions, sharing the similar laminar pattern, are iteratively merged.
Figure 6.10: (a) Part of cortex is shown. (b) A monkey brain sample ROI is shown with red and green lines, representing inner and outer cortical borderlines, respectively. The traverse lines are shown with blue colors in the ROI. The area between two neighboring traverse lines is a traverse sample.

Together based on a criterion to make larger sub-regions. The similarity criterion of the traverse samples consists of content similarity and texture similarity.

The content of a traverse sample can be statistically described based on the distribution of FeatDistQuantized values within the traverse samples. This distribution of FeatDistQuantized values can effectively be summarized in a histogram (see 6.12. (a-b) ). Therefore, the context similarity of the traverse samples (trav[m] and trav[m']), con(trav[m], trav[m']), can be determined by measuring Chi-Square distance of their histograms:

$$\text{con}(\text{trav}[m], \text{trav}'[m']) = \frac{1}{2} \sum_{r=1}^{l_m} \frac{(I[r] - J[r])^2}{I[r] + J[r]} , \quad (6.3)$$

where I and J are histograms with $l_m$ bins. The histograms count the number of FeatDistQuantized values, associated with neuron centroids in trav[m] and trav[m'], that fall into each bin. The bins, here, are FeatDistQuantized values from 0 to $l_m - 1$. Chi-square distance does not provide any measure for texture similarity since it is not sensitive to spatial dependency of FeatDistQuantized values within the traverse samples.
Figure 6.11: This figure illustrates how EucDist is measured. The dashed arrow represents EucDist, which is the shortest distance from the centroid of the neurons to the outer cortical border. The shadowed circle represents neurons and the black circles inside the shadow circles represents the centroid of neurons.

Texture similarity can be calculated by considering the position of FeatDistQuantized values, in another word spatial pattern, within the traverse samples. If the spatial pattern of FeatDistQuantized values within two neighboring traverse samples are similar, the texture of two traverse samples can also be considered similar. The spatial pattern of FeatDistQuantized values within the traverse samples can be statistically summarized based on average and standard deviation (std) of position of the individual FeatDistQuantized values in them (see 6.12. (c-d) ). To do that, we first calculate the average and standard deviation of $\text{DistEuc}_{\text{border}}$ for all the neuron centroids which belong to a specific FeatDistQuantized value in a traverse sample as:

$$\text{avg}(y, \text{trav}[m]) = \{\text{average}(\text{DistEuc}_{\text{border}}[k_n]) | \forall k_n \in \text{trav}[m] & \text{FeatDistQuantized}[k_n] = y\}$$

$$\text{std}(y, \text{trav}[m]) = \{\text{std}(\text{DistEuc}_{\text{border}}[k_n]) | \forall k_n \in \text{trav}[m] & \text{FeatDistQuantized}[k_n] = y\}.$$  

(6.4)
We then calculate the weighted average and standard deviation as the representative of texture in a traverse sample as follows:

\[
\text{avg}(\text{trav}[m]) = \left\{ \frac{\sum_{r=1}^{l_m} W_r \text{avg}(r,\text{trav}[m])}{\sum_{r=1}^{l_m} W_r} \right\} \quad (6.5)
\]

\[
\text{std}(\text{trav}[m]) = \left\{ \frac{\sum_{r=1}^{l_m} W_r \text{std}(r,\text{trav}[m])}{\sum_{r=1}^{l_m} W_r} \right\}, \quad (6.6)
\]

where \( W_r \) is the number of neuron centroids in \( \text{trav}[m] \) with \( \text{FeatDistQuantized} = r \). The final similarity criterion is calculated as:

\[
\text{Merge}(\text{trav}[m], \text{trav}[m']) = \alpha_1 \ast \text{con}(\text{trav}[m], \text{trav}[m']) + \\
\alpha_2 \ast |\text{avg}(\text{trav}[m]) - \text{avg}(\text{trav}[m'])| + \\
\alpha_3 \ast |\text{std}(\text{trav}[m]) - \text{std}(\text{trav}[m'])|,
\]

where \( \alpha_1 + \alpha_2 + \alpha_3 = 1 \).

Here, we use a merge and split algorithm to partition the laminar pattern map. This is done in two steps: (1) merging the traverse samples by using a large threshold to obtain big regions, and (2) splitting the big regions into smaller regions by using smaller thresholds.

The iterative merging process starts with merging the most similar adjacent sub-regions. As we continue the process, the less similar sub-regions are being merged into the more similar sub-regions until the similarity criterion of the sub-regions has reached a specific threshold. Obviously, when two traverse samples \( \text{trav}[m], \text{trav}[m'] \) are merged to make a larger sub-region, the neuron centroids for each of the traverse samples are shared.

In the splitting step, we assume that the pattern in each individual region is different than the other regions and, therefore, we use different thresholds to split each of the big regions. We first find the regions from the merging step with more than a specified number of traverse samples. Then, for each big region, we find the individual traverse samples within them and use a threshold to divide the region into smaller ones. The threshold to split each region is determined independently.
Figure 6.12: This figure represents how content and texture of a traverse sample is calculated. Maximum number of levels for the traverse samples within the ROI, \( l_m \), is 5. (a) The shadowed colorful circles are the neuron centroids. Each group of neuron centroids with a specific \( \text{FeatDistQuantized} \) value is shown with a distinct color. (b) The histogram, with \( l_m \) bins, which represents the content of the traverse sample based on the number of neuron centroids with a specific \( \text{FeatDistQuantized} \). (c) The double arrow shows where neuron centroids with specific \( \text{FeatDistQuantized} \) are located. (d) Mean and standard deviation of \( \text{DistEuc}_{\text{border}} \) for the neuron centroids with a specific \( \text{FeatDistQuantized} \) value can be used to represent texture information. The weighted average based on eq. (6.5) and the weighted standard deviation based on eq. (6.6) is 500 and 100, respectively.

6.3 Experimental Setup

A high-resolution, coronal histological image of a Nissl stained Rhesus monkey (macaca mulatta) brain slice at the level of the hippocampus, pulvinar and cerebral aqueduct was acquired from Brainmaps.org was for the study. The slice number 0721 from the dataset number RH04 was acquired at the resolution 0.05 mm. An ROI over the right side of the monkey brain was defined. The reference map for this histological slice has also provided at Brainmaps.org.
The algorithm described above was implemented in MATLAB (The MathWorks, Inc., Massachusetts). The parameters for experimental set up were chosen based on visual inspection by an expert neuroanatomist (Jonathan J. Wisco) and determined to give reasonable results. First, The optimum window and threshold were determined by segmenting three different regions with size of 2000 X 2000 pixels in the histological slice. The optimum threshold was 0.8 and the optimum window size was 62 pixels. Next, the laminar pattern at different radial distances were created. The one that was most similar to the laminar pattern of the histological slice, chosen by the expert neuroanatomist, had the radial distance of 250 pixels. For generating the traverse samples, $\Delta d_{trav} = 256$ pixels, and $\Delta d_{sr} = 512$ pixels were chosen. The threshold value used to generate distinct sub-regions was 1120. The summary of the variable parameters are presented in Table 6.1.

Table 6.1: Variable parameters that are used in this chapter, the section or subsection that they were defined, and their values are presented.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Section/subsection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum threshold</td>
<td>0.8</td>
<td>6.2.1: Segmenting neuron cells in a window</td>
</tr>
<tr>
<td>Optimum window size</td>
<td>62 pixels</td>
<td>6.2.1: Segmenting neuron cells in a window</td>
</tr>
<tr>
<td>Radial distance</td>
<td>250 pixels</td>
<td>6.2.2: Constructing the laminar pattern map</td>
</tr>
<tr>
<td>Number of levels ($L_m$)</td>
<td>10</td>
<td>6.2.2: Constructing the laminar pattern map</td>
</tr>
<tr>
<td>$\Delta d_{sr}$</td>
<td>512 pixels</td>
<td>6.2.3: Finding the neurons within the traverse sample</td>
</tr>
<tr>
<td>$\Delta d_{trav}$</td>
<td>256 pixels</td>
<td>6.2.3: Finding the neurons within the traverse sample</td>
</tr>
<tr>
<td>$Th$</td>
<td>1120 pixels</td>
<td>6.2.3: Merging the traverse samples</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.33</td>
<td>6.2.3: Merging the traverse samples</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>0.33</td>
<td>6.2.3: Merging the traverse samples</td>
</tr>
<tr>
<td>$\alpha_3$</td>
<td>0.33</td>
<td>6.2.3: Merging the traverse samples</td>
</tr>
</tbody>
</table>

An expert neuroanatomist (Jonathan J. Wisco) manually evaluated and marked the locations where major changes in the laminar pattern map occurred based on visual inspection, without referring to the detected regions by the algorithm. He also marked the sub-regions in the original histological image based on the location of the sub-regions in the laminar map. The same expert neuroanatomist then evaluated the performance of the semi-automated method based on visual inspection. He compared the regions that he marked with the regions that the algorithm detected. He also compared the regions that are marked at Brainmaps.org by other neuroanatomists with the regions the algorithm detected.
6.4 Results

We compared the cytoarchitectonic parcellations of the reference image (from BrianMaps.org, figure 6.13.(a)) with those done by our expert neuroanatomist and with the calculated laminar map using our algorithm. The expert neuroanatomist manually marked cytoarchitectonic borders on the calculated laminar map and these were overlaid on the reference image (red lines, figure 6.13.(b)). Our automated algorithm parcellations were also overlaid on the reference image (figure 6.13.(c)). In area TEO, neither anatomists from BrainMaps.org, nor our group found changes in the cytoarchitecture. However, our algorithm demarcated TEO into three different areas (figure 6.13.(c)). This demonstrates that the algorithm can differentiate slight changes in the cytoarchitecture.

6.5 Discussion

The algorithm we are proposing here cannot eliminate the effect of tissue artifacts in making the laminar pattern map. However, since the algorithm focuses on spatial properties of neurons, tissue artifact poses no threat to the algorithm any more than manual analysis ignores tissue artifact. Any tissue section with an artifact that clearly disrupts the metrics we are measuring here, of course must be taken into account, and we discourage using this algorithm on sections that have serious tears, knife chatter, freezing artifacts, and anything else that would compromise the integrity of spatial relationships. The algorithm can detect more non-homogeneous sub-regions than the human eye.

Cytoarchitectonic parcellation is typically performed by a neuroanatomist based on variations of laminar patterns observed in Nissl stained histological sections. In this study, the laminar pattern map provides detail that is indistinguishable in a typical Nissl stained image. Our expert neuroanatomist observed more cytoarchitectonic borders than what was observed by experts at Brainmaps.org. Further, our automated algorithm mathematically calculated more borders based on features that our neuroanatomist did not see through visual inspection alone. Therefore, our automated method marked borders based on more granular information and placed more borders.

We acknowledge that the borders are based on cytoarchitecture alone, without information on white matter patterns, or physiological information provided by immunohistochemistry stains. Without these other parameters, it is difficult to determine if the borders calculated by the
algorithm are functionally relevant, even though the anatomical differences are clear. Also, the specific cortico-cortical or cortico-bulbar connectivity may be underestimated in a stain specific to function, and are rather best represented by the cytoarchitectonic variability seen in our maps. That is, the cytoarchitectonic maps may be a better indicator of brain connectivity than functional maps. It is also possible that small variations in cytoarchitecture do not equate to functional differences. Having said that, the results of the algorithm raises the question of whether we should be considering more granular anatomical areas with regard to function.

6.6 Conclusion

The proposed algorithm here uses the number of, area of, and distance of neurons cell bodies simultaneously to make the laminar pattern map. The detection of individual cortical sub-regions is based on the similarity of texture and content information through the laminar pattern map in smaller scale, traverse samples. The evaluation of the algorithm by an expert neuroanatomist, based on visual inspection, indicates that the semi-automated technique is identifying what the neuroanatomist would consider cytoarchitectonic sub-regions.
Figure 6.13: (a) Labeled reference histological image used for analysis from BrainMaps.org. (b) Cytoarchitectonic parcellation by our expert neuroanatomist based on the calculated laminar map overlaid on the unlabeled reference image. (c) Laminar map calculated cytoarchitectonic parcellation overlaid on the reference image, with magnified view (inset).
CHAPTER 7. SUMMARY AND CONCLUSIONS

This dissertation described a novel technique to quantitatively map relaxation characteristics of tissues with extremely rapid signal decay, which are normally MRI invisible. This dissertation has also described new histological techniques with the potential to measure the accuracy of popular and widely used neurological MRI techniques including DTI, T1 weighted imaging, and T2 weighted imaging. The results are summarized as following.

7.0.1 Summary

Chapter 4 presented an approach to measure the relaxometry of tissues that cannot currently be non-invasively obtained using MRI imaging. A rapid and highly SNR efficient 3D UTE sequence, called UTE 3D cones, was adopted to get signal from tissues with very weak MRI signal. Then, a T2* map was constructed by measuring the amount of signal at different TE's by using the 3D cones sequence. Results were demonstrated in both tendon and ex vivo AD brain, which are high interest for disease diagnosis and progression. For the tendon experiment, a healthy volunteer and an unhealthy volunteer with both the main and subtalar joints of the ankle were scanned. In the ex vivo AD brain experiment, the AD brain of the subject with Braak stage VI, the last stage of the disease. The results demonstrate the ability of the technique to detect subtle differences in relaxation properties of tendon, for both the healthy and injured tendon, and in the brain. While further validation of the technique is still needed, it appears to be a viable candidate for rapid three dimensional mapping of the relaxation properties in certain tissues that were previously barely MR visible.

Chapter 5 presented an approach for the automated extraction of nerve fiber directionality from high-resolution images of stained histological slices. The presented semi-automated algorithm first created a binary-segmented mask of the nerve fibers in the histological image, and then extracted an estimate of average directionality of nerve fibers through a Fourier-domain analysis.
of the masked image. It also generated an uncertainty level for its estimate of average directionality. The results of the algorithm were assessed by an expert anatomist (J.J. W) qualitatively and quantitatively. The average orientations of the semi-automatic method were first compared to a qualitative expert opinion based on visual inspection of nerve fibers. A weighted RMS difference between the expert estimate and the algorithmically determined angle (weighted by expert’s confidence in his estimate) was 15.4 degrees, dropping to 9.9 degrees when only cases with an expert confidence level of greater than 50% were included. The algorithmically determined angles were then compared with angles extracted using a manual segmentation technique, yielding an RMS difference of 11.2 degrees. The presented semi-automated method is in good agreement with both qualitative and quantitative manual expert-based approaches for estimating directionality of nerve fibers in white matter from images of stained histological slices of the human brain. The technique appears to be a good candidate for validating DTI from MRI with respect to the gold standard histology.

Chapter 6 presented an approach for differentiating distinct cortical sub-regions of the brain. The algorithm first extracted the neuron bodies in an ROI within the cortex and, then, made a laminar pattern map based on the distance of, size of, and number of neurons. After that, the laminar map was partitioned into traverse samples and, finally, distinct sub-regions based on the content and texture similarity of the traverse samples were reconstructed. The results of the algorithm were assessed by an expert neuroanatomist (J.J. W). The expert neuroanatomist manually marked cytoarchitectonic borders on the calculated laminar map and these were overlaid on the reference image, acquired at BrainMaps.org. Our automated algorithm parcellations were also overlaid on the reference image. The results of the algorithm showed that the algorithm detected more sub-regions than the ones marked at BrainMaps.org or marked by the expert neuroanatomist on the laminar map. The evaluation of the detected sub-regions by the expert neuroanatomist indicates that the algorithm can differentiate slight changes in the cytoarchitecture. It remains to be seen if these anatomical differences have functional implications.

7.0.2 Suggestions for Future Work

There are a number of areas where the current research described could be expanded or related topics could be explored in the future:
• The UTE work presented in this dissertation ran on a limited number of volunteers. In addition, the resolution of the images in this dissertation was 1mm isotropic. Modifying the UTE sequence to get higher resolution images with high SNR is the next step in the UTE sequence development. Aside from the technical improvement, there are a variety of applications for this new technique that can be explored.

• The principle area for future development of the histological process presented in Chapters 5 and 6 is to create a methodology to register the histological images with the images from MRI. Such a methodology, could then be used to validate DTI with respect to histology or be used to evaluate changes in the cortex of the brain acquired from the MRI with respect to histology.
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APPENDIX A. GLOSSARY

AD: Alzheimer’s disease.

B0: main static magnetic.

B1: RF field used for excitation and receiving the signal.

Echo time: please see the definition for TE.

DTI: Diffusion Tensor Imaging: it measures the directionality of water diffusion in the brain.

FLAIR: Fluid Attenuated Inversion Recovery.

Flip angle: the amount of the net magnetic vector is tipped off from the alignment with B0.

fMRI: functional MRI: neuroimaging MRI technique that is designed to measure brain activities.

GRE: Gradient Recalled Echo.

k-space: the space in which the received signal is samples and is stored.

MRI: an imaging modality that acquires an image without causing damage to the tissue.

NMV: Net Magnetization Vector.

qMRI: quantitative MRI: specific techniques that are sensitive to changes in the underlying tissue microstructure.

Repetition time: please see the definition for TR.
T1 Relaxation: exponential recovery time constant.

T2 Relaxation: exponential decay constant.

T2* relaxation: exponential decay constant faster than T2.

TE: time between excitation and sampling of the center of k-space.

TR: repetition time: time between repetitions of the pulse sequence.

UTE: Ultra short Echo time imaging is used to get signal from tissues with rapid T2* decay.
APPENDIX B. AN SNR COMPARISON BETWEEN A SODIUM PHASED ARRAY COIL AND A SINGLE CHANNEL COIL

B.1 Purpose

Sodium imaging has the potential to reduce the false positive rate in breast cancer diagnosis, and may be able to improve the ability to stage tumors [1]. However, the signal-to-noise ratio (SNR) of sodium imaging is significantly limited when compared to hydrogen imaging. This is due to sodium’s lower intrinsic concentration within the body, its lower gyromagnetic ratio, and its faster signal decay rates. Coil design plays a crucial role in the overall SNR of MR imaging. Phased array coils are commonly used to improve the SNR in MRI by combining small surface loops that have higher sensitivity and smaller noise volume when compared to larger coils. Phased array coils have demonstrated their ability to improve sodium MRI at 3T. Sodium breast arrays have only recently begun to be investigated at 3T [2] and 7T [3]. We previously constructed a 3T phased array sodium breast coil [2] which had greatly improved SNR compared to single-loop designs. In order to accommodate a wider range of women being evaluated for breast cancer, we have created a new sodium phased array coil, with a large, open design that can accommodate large breast sizes. SNR is compared to a single channel sodium coil (with proton trap).

B.2 Methods

Single channel coil (Figure B.1.A)

The coil used for comparison has a single transmit/receive 1H loop and a single transmit/receive $^{23}$Na loop. The radius of the coil is 133 mm. The $^{23}$Na loop contains a proton trap to decouple the sodium loop from the proton loop.
Figure B.1: One channel $^{23}\text{Na}$ and one channel $^{1}\text{H}$ coil. (B) Seven channel sodium receive array, surrounded by a large butterfly sodium transmit coil. (C) The new phased array (top view); (D) The new phased array (bottom view)

**New phased array coil (Figure B.1. B-D)**

The new phased array coil consists of 7 sodium loops that are overlapped to minimize coupling to adjacent sodium loops. Each loop has a diameter of 74mm. The loops were mounted on a 3D printed structure that has an overall length of 264mm, width of 179mm, and depth of 73mm at the center. Transmit occurred through a large butterfly coil, with the butterfly coil bent in the center so that it operates in quadrature. A butterfly loop was used to improve the transmit efficiency of the coil through quadrature operation, instead of using a less efficient large square loop that surrounds the entire structure.

**B.3 MRI Experiment**

Experiments were done on a 3T Siemens TIM Trio MRI machine, using a spherical NaCl/CuSO4 phantom. A 3D cone ultra short echo time (UTE) sequence [3] was used with scan parameters: TR=120ms, TE=0.3ms, 2.5mm resolution, FOV=20cm, flip angle = 60 degrees, and 2 averages. A
flip angle map was obtained using the dual-angle method for both coils at 30 and 60 degrees with similar scan parameters.

### B.4 Results

SNR maps from both coils are shown in the Figure B.2. SNR measurements from both coils in two regions of interest are in Table B.1. As can be seen, the new phased array design has higher SNR near the surface loops of the coil (2-3x) and has reduced SNR near the center of the phantom (50%), when compared to the single channel coil.

![Normalized SNR maps of two coils, scaled identically. (A) Resulted image from one channel $^{23}$Na and one channel $^1$H phased array. (B) Resulted image from the new designed coil.](image)

Figure B.2: Normalized SNR maps of two coils, scaled identically. (A) Resulted image from one channel $^{23}$Na and one channel $^1$H phased array. (B) Resulted image from the new designed coil.

<table>
<thead>
<tr>
<th>Coil name</th>
<th>SNR at the center</th>
<th>SNR at the edge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual tune coil</td>
<td>67.3</td>
<td>60.4</td>
</tr>
<tr>
<td>Phase array coil</td>
<td>33.1</td>
<td>167.8</td>
</tr>
</tbody>
</table>

Table B.1: SNR results comparing the two coils.
B.5 Discussion

The overall benefit of this larger array is that the sensitive volume of the coil is much larger than that of single channel coil, due to the much larger volume that can be contained within the coil; the phased array is 264 X 179 mm$^2$ long/wide, while the trap coil is only 133 mm in radius. In addition to the larger volume, near the surface loops the coil obtained higher SNR (2-3x) than the single channel coil. The inhomogeneous transmission of the coil causes the flip angle to decrease near the center of the coil, which resulted in a lower SNR than could be obtained if the transmission had been more homogeneous. Improvements to this coil could include the implementation of a more homogeneous transmit to enable higher SNR from the center of the coil and additional sodium loops for increased coverage.

B.6 Conclusion

This work demonstrated a new phased array sodium breast coil with a design that accommodates a wide range of breast size, suitable for typical breast cancer patients or women at risk for breast cancer. The coil obtained 2-3 times the sodium SNR near the loops when compared to a simple loop of 133mm diameter.
C.1 Abstract

A new method to measure species with very short T2* values is presented. The method is based on 3D cones UTE technique and is done by acquiring difference between images at two different echo times. This is the first try to measure species with very short T2* values like proteins in a clinical MRI machine.

C.2 Introduction

It is very difficult to directly image proteins in the brain with MRI due to a very weak and rapidly decaying (short T2*) MR signal. Normal MRI techniques are not suitable for probing the signal of very short T2* tissues, since the signal from these tissues has typically decayed before it is sampled. We explored the use of a custom 3D UTE technique to detect signal from very short T2* tissue in an ex vivo brain with known Braak VI tauopathy.

C.3 Methods

We implemented a 3D UTE MRI sequence with a 3D cones k-space trajectory, and conducted ex vivo scans on a 3 Tesla scanner of a formalin fixed human temporal lobe from a subject with known Braak VI tauopathy, heaviest in the hippocampus and para hippocampal gyrus. We chose an area of the brain for our study that we expect to have high volume of beta amyloid and tau proteins. We then acquired the UTE images at TEs of 0.4, 0.6, 0.8, 1, 1.2, 1.5, 2, 2.5, 3, and 3.5 ms and TR of 16ms. Resolution was 1mm isotropic and the FOV was 15 cm in all directions. Each of these scans was repeated at flip angles ranging from 5 to 60 degrees. Difference images were then formed by subtracting the TE=3.5ms images from the TE=0.4ms images, effectively
suppressing longer T2* tissues. We defined four regions of interest (ROIs) in areas with visible short T2* signal in the hippocampus and then calculated an average T2* for each ROI by fitting the signal from each echo to a simple mono exponential curve. T1 was then estimated for each ROI by ascertaining which flip angle yielded the largest signal for the corresponding ROI, and applying the Ernst angle formula relating maximum signal to flip angle and T1.

C.4 Results

Figure C.1.(a-c) shows UTE image at TE=0.4ms, TE=3.5ms, and the difference image of TE=0.4 and 3.5 ms, respectively. We measured T2* values in the short T2* tissues at approximately 1.5–2.6ms for the ROIs having about 5-12 pixels. Figure C.2 represents the mean signal intensity of one the bright spots with respect to TE value, and the measured T2* value, 1.6ms. We hypothesize that these regions yielding short T2* MR signal correlate with areas with heavy Tau protein deposits. The T1 estimated in these regions was approximately 300ms. Short T2* signal is detected both in areas around the hippocampus as well as around blood vessels.

Figure C.1: UTE 3D cone image of an ex vivo brain with known Braak VI taopathy; (a) UTE 3D cone image of the brain at TE=0.4ms; (b) UTE 3D cone image of the brain at TE=3.5ms; (c) difference image of TE=0.4 and 3.5 ms.
Figure C.2: The plot shows T2* value for one of the bright spots of the difference images of the brain at different TE values.

C.5 Conclusions

A novel 3D UTE MRI sequence with a 3D cones k-space trajectory was used to image short T2* tissues in the hippocampus. Future work will seek to determine if the short T2* signal observed is water bound to protein deposits as hypothesized.
APPENDIX D. RESOLVING NORMALLY INDISTINGUISHABLE INHOMOGENEOUS TISSUE USING 3D CONES ULTRA-SHORT ECHO TIME MRI

D.1 Background

Determining Alzheimer’s disease (AD) disease stage has been traditionally done through Diffusion Tensor Imaging by examining the structure of the brain. Other MRI imaging techniques like GRE are unable to get microscopic subtlety on the tissue although they get contrast based on relaxation time. Here, we explored the use of a custom 3D UTE technique to differentiate the signal from different regions of the brain, including white and gray matter sub-regions, in an ex vivo brain with known Braak VI taopathy. The technique can sub-differentiate inhomogeneous tissue types, may help to resolve disease states, and can give us physiological contrast.

D.2 Methods

We implemented a 3D UTE MRI sequence with a 3D cones k-space trajectory, and conducted ex vivo scans on a 3 Tesla scanner of a formalin fixed human temporal lobe from a subject with known Braak VI taopathy. We then acquired the UTE images at TEs of 0.4, 0.8, 1, 1.5, 2.5, and 3.5ms and TR of 14ms and flip angle of 15 degrees. FOV was 10 cm and resolution was 1mm isotropic in all directions. We also acquired 2D GRE images with TR of 450ms, TE of 10ms, FOV of 12cm, and resolution 0.6 x 0.6 x 3mm.

D.3 Results

Our preliminary results (please see Figure D.1 and Table D.1) show that the designed 3D UTE cones provides give us contrast at different TEs at different locations in the brain and, therefore, this makes it possible to discriminate different tissue types that cannot be seen in GRE. In Figure D.1, two ROIs in the hippocampus (ROI1 and ROI2), two ROIs in the sub-cortical white
Figure D.1: Six ROIs are defined in each image: (a) GRE image; (b) UTE image at TE=0.4ms; (c) UTE image at TE=1 ms; (d) UTE image at TE=3.5ms. ROI1 and ROI2 are two close regions in hippocampus, ROI3 and ROI4 are two close regions in sub-cortical white matter, and ROI5 and ROI6 are two close regions in cortical gray matter.

In the GRE image in Figure D.1, (a), ROI1 and ROI2 are black (very small amount of SNR). However, in the UTE images shown in Figure D.1. (b-d), ROI1 and ROI2 are not black. In Table D.1, the difference in SNR between close ROI pairs (ROI1-2, ROI3-4, and ROI5-6) can be seen.

D.4 Conclusions

A novel 3D UTE MRI sequence with a 3D cones k-space trajectory was used to distinguish different sub-tissue in the white and gray matter. Future work will seek to determine which TE corresponds to each microscopic constituents of conventionally homogenous tissue.
Table D.1: SNR within different regions shown in Figure D.1. As can be seen, for ROI1-4, the GRE sequence at TE=10ms results in low SNR while the UTE sequence at TE=0.4ms acquires high SNR. Also, the amount of SNR at different TEs is different for close ROI pairs (ROI1-2, ROI3-4, and ROI5-6).

<table>
<thead>
<tr>
<th>Sequence name (TE)</th>
<th>ROI1</th>
<th>ROI2</th>
<th>ROI3</th>
<th>ROI4</th>
<th>ROI5</th>
<th>ROI6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRE (10ms)</td>
<td>3.8</td>
<td>22.1</td>
<td>72.5</td>
<td>70.2</td>
<td>107.1</td>
<td>109.9</td>
</tr>
<tr>
<td>UTE (0.4ms)</td>
<td>182.2</td>
<td>330.1</td>
<td>318</td>
<td>341.1</td>
<td>343.2</td>
<td>338.2</td>
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<tr>
<td>UTE (0.8ms)</td>
<td>151.3</td>
<td>290.4</td>
<td>295.4</td>
<td>311.3</td>
<td>317.8</td>
<td>314.2</td>
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<tr>
<td>UTE (1ms)</td>
<td>137.2</td>
<td>237.7</td>
<td>281.5</td>
<td>295.7</td>
<td>301.8</td>
<td>298.6</td>
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<tr>
<td>UTE (1.5ms)</td>
<td>102.5</td>
<td>225</td>
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<td>253.8</td>
<td>258.9</td>
<td>257.5</td>
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<tr>
<td>UTE (2.5ms)</td>
<td>71.2</td>
<td>193.2</td>
<td>228.9</td>
<td>242.6</td>
<td>243.2</td>
<td>242.2</td>
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<tr>
<td>UTE (3.5ms)</td>
<td>34.8</td>
<td>126.8</td>
<td>166.4</td>
<td>176.8</td>
<td>174.9</td>
<td>175.2</td>
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