QUANTITATIVE ASSESSMENT OF GLIOMA THERAPY EFFICACY USING MAGNETIC RESONANCE DIFFUSION TENSOR IMAGING

by

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A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham, in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering

BIRMINGHAM, ALABAMA

2008
Glioma, a type of malignant brain tumor, is highly aggressive and has poor clinical outcomes, despite aggressive therapeutic strategies like surgical resection, chemotherapy and radiation therapy. The inherent heterogeneous and fast-changing morphology of this tumor is not well understood. Although a number of novel therapeutic drugs are undergoing clinical trials, the quantitative assessment of glioma therapy response remains challenging due to limited in-vivo biomarkers that can differentiate infiltrative tumor from healthy brain tissue. Diffusion Tensor Imaging (DTI) is a Magnetic Resonance Imaging (MRI) technique that can non-invasively assess structural changes in tissue by local measurement of the diffusion of water molecules. It has begun to find utility in imaging the early and subtle changes in brain parenchyma in the presence of pathological changes. The isotropic and anisotropic properties of diffusion obtained by tensor decomposition are shown to be useful in studying the spatial and temporal variations of tissue.

A DTI-based quantitative model was developed for the spatio-temporal analyses of the composition of glioma and surrounding tissue, to permit the longitudinal investigation of the efficacy of therapy using two different treatment strategies. Five patients undergoing an oncolytic virus therapy and five patients undergoing conventional chemo/radiation therapy were evaluated using the method developed. The diffusion based isotropic and anisotropic characteristics were investigated to demarcate the extent of
tumor infiltration into the surrounding brain tissue. We show that DTI is sensitive to rapidly changing white matter pathology. Longitudinally evaluating both isotropic and anisotropic components of diffusion is shown to permit characterization of tumor and tissue quantitatively. Further, data obtained using this spatio-temporal approach is segmented for tissue classification using three techniques.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Graduate Program at UAB for giving me the opportunity to pursue higher studies in Biomedical Engineering – Imaging track, which has been my long cherished dream. I am extremely grateful to my advisor Dr. N. Shastry Akella, for giving me the chance to conduct research in the field of Cancer Imaging. Dr. Akella’s constant support and competent guidance throughout my graduate studies and research work has helped me accomplish my goals.

I would also like to express my sincere gratitude to my committee members – Dr. Donald Twieg, Dr. James Markert and Dr. Burt Nabors. Thanks are also due to Dr. Matthias Karrasch, Dr. Asim Bag, Kathy, faculty and staff of the Department of Biomedical Engineering, and staff of Departments of Neurology and Neurosurgery. I also appreciate the encouragement and suggestions provided by the committee and members of the MR of Cancer Study Group, International Society for Magnetic Resonance in Medicine.

I truly acknowledge the support received from my friends Meenakshi, Mandar, Rajiv, Girish, Nitin, Partha and Rick, throughout my graduate life at UAB.

Finally, I am deeply indebted to my parents and my sister, for their endless love and inspiration.

Research funding for this project was provided by HSF-GEF and P50CA 097247 grants.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>II</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>IV</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>VI</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>X</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>XI</td>
</tr>
<tr>
<td>CHAPTERS</td>
<td></td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2  BACKGROUND AND SIGNIFICANCE</td>
<td>3</td>
</tr>
<tr>
<td>Gliomas</td>
<td>3</td>
</tr>
<tr>
<td>Diffusion Tensor Magnetic Resonance Imaging</td>
<td>7</td>
</tr>
<tr>
<td>Medical Image Segmentation</td>
<td>17</td>
</tr>
<tr>
<td>3  MATERIALS AND METHODS</td>
<td>25</td>
</tr>
<tr>
<td>Overview</td>
<td>25</td>
</tr>
<tr>
<td>MR Diffusion Tensor Imaging</td>
<td>27</td>
</tr>
<tr>
<td>DT Image Analysis</td>
<td>31</td>
</tr>
<tr>
<td>4  RESULTS</td>
<td>37</td>
</tr>
<tr>
<td>5  DISCUSSION</td>
<td>68</td>
</tr>
<tr>
<td>6  CONCLUSION</td>
<td>72</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>73</td>
</tr>
<tr>
<td>APPENDIX: IRB Approval form</td>
<td>80</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

### Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Post contrast enhanced T1- w image in coronal, sagital and axial views showing glioma with enhancing ring</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Diffusion tensor fitted to ellipsoid</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Tracking spatial variations of 2 different tissues in the p:q space</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Tracking temporal variations of same tissue in the p:q space</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Illustration of tissue microstructure in healthy white matter organized in a linear fashion and tumor showing densely packed cells with a necrotic core</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>Patterns of WM fiber tract alteration by gliomas, showing changes in p and q</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>Illustrating crisp clustering of a 2-D feature space</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Illustrating Fuzzy c-means clustering of a 2-D feature space</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>Illustrating Gustafson-Kessel clustering of a 2-D feature space</td>
<td>23</td>
</tr>
<tr>
<td>10</td>
<td>Flowchart summarizing the research methods</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>Diffusion weighted images for a typical slice containing a glioma</td>
<td>29</td>
</tr>
<tr>
<td>12</td>
<td>Schematic of crisp segmentation of p:q space</td>
<td>33</td>
</tr>
<tr>
<td>13</td>
<td>Eigenvalue maps $\lambda_1$, $\lambda_2$ and $\lambda_3$</td>
<td>38</td>
</tr>
<tr>
<td>14</td>
<td>FA map and MD map in grayscale</td>
<td>38</td>
</tr>
<tr>
<td>15</td>
<td>Isotropy p map and Anisotropy q map</td>
<td>39</td>
</tr>
<tr>
<td>16</td>
<td>The p:q space for 4 ROIs selected as appearing Healthy WM, Edema,</td>
<td></td>
</tr>
</tbody>
</table>
Possible Tumor Infiltration and Tumor showing spatial variation ..................40
17 The p:q space for the 4 ROIs for 3 time points showing longitudinal variation ..................................................................................................................41
18 Mean ± SD of p values for different tissue compartments, LP1 ........41
19 Mean ± SD of q values for different tissue compartments, LP1 ........42
20 The p:q space for slice 14, patient LP1 ..............................................42
21 Crisp segmentation of the p:q space .........................................................43
22 Crisp segmentation of a tumor region outlined on anatomic T1 ........43
23 Crisp segmentation showing longitudinal variation in LP1 ..................44
24 Manual ROI selection to encompass tumor, and surrounding tissue and Injection site region on T1 post gad slice ..............................................44
25 Crisp segmentation: Tissue Percent Distribution in ROI .......................45
26 Crisp segmentation: Tissue Percent Distribution at Injection Site ..........45
27 FCM clustering of p:q space for slice 14, patient LP1 .........................46
28 FCM cluster partitioned data: tumor, GM, WM, background, CSF/necrotic and edema ..........................................................47
29 FCM based Segmentation showing longitudinal variation in LP1 ........47
30 FCM based Segmentation: Tissue Percent Distribution in ROI .............48
31 FCM based Segmentation: Tissue Percent Distribution at Injection Site ..........................................................................................48
32 GK clustering of p:q space for slice 14, patient LP1 .............................49
33 GK cluster partitioned data: tumor, gm, wm, background, CSF/necrotic, and edema for LP1 – Study1 ...............................................49
34 GK based segmentation showing longitudinal variation in LP1 ............50
35 GK based segmentation: Tissue Percent Distribution in ROI ................50

VII
36 GK based segmentation: Injection Site Tissue Percent Distribution
37 Manual ROI selection to encompass tumor and surrounding tissue on a T1 post contrast slice of GP5
38 The p:q space for the 4 ROIs for 3 time points showing longitudinal variation for patient GP5
39 Mean ± SD of p values for different tissue compartments, GP5
40 Mean ± SD of q values for different tissue compartments, GP5
41 Crisp segmentation of a region shown outlined on anatomic slice encompassing tumor, edema and necrosis (GP5)
42 Crisp segmentation of 3 time points showing longitudinal variations in GP5
43 Crisp segmentation - ROI Tissue Percent Distribution (GP5)
44 FCM clustering of p:q space of slice #10 in GP5
45 FCM based segmentation of 3 time points from p:q data showing longitudinal variations in GP5
46 FCM based Segmentation - ROI Tissue Percent Distribution (GP5)
47 GK clustering of p:q space of slice #10 in GP5
48 GK based Segmentation of 3 time points from p:q data (GP5)
49 GK based Segmentation - ROI Tissue Percent Distribution (GP5)
50 GK cluster partitioned data for edema across studies for patient GP5
51 GK cluster partitioned data for WM across studies for patient GP5
52 Crisp segmentation - ROI Tissue Percent Distribution (LP2)
53 Crisp segmentation - ROI Tissue Percent Distribution (LP3)
54 Crisp segmentation - ROI Tissue Percent Distribution (LP4)
55 Crisp segmentation - ROI Tissue Percent Distribution (LP5)
Crisp segmentation - ROI Tissue Percent Distribution (GP1)...............................61
Crisp segmentation - ROI Tissue Percent Distribution (GP2)...............................61
Crisp segmentation - ROI Tissue Percent Distribution (GP3)...............................62
Crisp segmentation - ROI Tissue Percent Distribution (GP4)...............................62
Anatomic MR image and GK clustering based tissue classification of diffusion data (GP5)........................................................................70
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crisp segmentation – Percent Tumor Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Crisp segmentation – Percent Edema Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Crisp segmentation – Percent Healthy Tissue Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>Crisp segmentation – Percent Tumor Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>Crisp segmentation – Percent Edema Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>Crisp segmentation – Percent Healthy Tissue Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td>GK based segmentation – Percent Tumor Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>GK based segmentation – Percent Edema Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>GK based segmentation – Percent Healthy Tissue Distribution in Injection Site region for local therapy group patients (LP1-LP5)</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>GK based segmentation – Percent Tumor Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>GK based segmentation – Percent Edema Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>66</td>
</tr>
<tr>
<td>12</td>
<td>GK based segmentation – Percent Healthy Tissue Distribution in manually drawn region for global therapy group patients (GP1-GP5)</td>
<td>66</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>Anaplastic Astrocytoma</td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent Diffusion coefficient</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Diffusion Tensor</td>
<td></td>
</tr>
<tr>
<td>DICOM</td>
<td>Digital Imaging and Communications in Medicine</td>
<td></td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>Echo Planar Imaging</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
<td></td>
</tr>
<tr>
<td>FCM</td>
<td>Fuzzy c-means</td>
<td></td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid Attenuation Inversion Recovery</td>
<td></td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
<td></td>
</tr>
<tr>
<td>GBM</td>
<td>Glioblastoma Multiforme</td>
<td></td>
</tr>
<tr>
<td>GK</td>
<td>Gustafson-Kessel</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>Global therapy Patient</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>Gray Matter</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>Herpes simplex virus type 1</td>
<td></td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
<td></td>
</tr>
<tr>
<td>IVIM</td>
<td>Intra-voxel incoherent motion</td>
<td></td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board for human use</td>
<td></td>
</tr>
<tr>
<td>ISMRM</td>
<td>International Society for Magnetic Resonance in Medicine</td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>Local therapy Patient</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>Mean Diffusivity</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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<td></td>
</tr>
<tr>
<td>p</td>
<td>Isotropic component of diffusion</td>
<td></td>
</tr>
<tr>
<td>pfu</td>
<td>plaque forming units</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>Proton Density</td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
<td></td>
</tr>
<tr>
<td>q</td>
<td>Anisotropic component of diffusion</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>Relative Anisotropy</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
<td></td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
<td></td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

Diffusion Tensor Imaging (DTI) is a new Magnetic Resonance Imaging (MRI) technique that has found utility in imaging a number of neurological diseases and brain pathologies. Since the technique is based on measuring the molecular diffusion of water molecules, it is one of the few imaging techniques that can non-invasively evaluate structural changes of brain tissue at a cellular level. An important application of DTI is imaging malignant brain cancer. Since high grade brain tumors have a poor prognosis, non-invasive monitoring of therapy response is critical to developing an effective treatment strategy. Assessment of therapy response remains challenging, as these tumors have a very heterogeneous morphology that is often difficult to probe using the conventional in-vivo imaging techniques.

In this study, the diffusion properties of tumor and surrounding tissue were studied using DTI. Isotropy and Anisotropy, two diffusion based characteristics were analyzed simultaneously as a 2D feature space. This space was further modeled using an analytical spatio-temporal approach. Clustering techniques were used to differentiate and segment tissues. The segmented tumor was then evaluated for longitudinal variations as a function of therapy. The segmentation approach allows clinicians to evaluate changes to the pathology as a function of time and the type of therapy that was administered.
The significant contributions of this work include presentation of a technique for delineating tumor boundaries from healthy tissue and detecting white matter tumor infiltration, which is not otherwise discernable using conventional MR methods and other imaging modalities. This segmentation approach can be utilized for surgical resection planning and radiation therapy planning. It also finds potential applications in the diagnosis and therapy assessment of other white matter demyelinating and degenerative disorders.

This thesis is divided into six chapters. Following this introductory chapter, chapter 2 provides some background on Gliomas, Magnetic Resonance Diffusion Tensor Imaging, Therapy Assessment of Gliomas, and Medical Image Segmentation. Chapter 3 describes the Research Design and Methods used in this project for Therapy Assessment of Gliomas using DTI, chapter 4 summarizes the results obtained, chapter 5 discusses the results, limitations, future directions, following which conclusions of this work are documented in chapter 6.
CHAPTER 2
BACKGROUND AND SIGNIFICANCE

Gliomas

Brain tumors that develop from the glial cells i.e. the supporting cells of the nervous system are called gliomas. They are the most common type of primary brain tumors, accounting for 60% of the malignancies originating in the central nervous system. Tumors are caused by genetic mutations - either loss of tumor suppressor genes that encode proteins which inhibit cell proliferation and tumor development, or, over-expression of oncogenes, which encode proteins that stimulate tumor proliferation (1). Gliomas may develop from the unsuppressed growth of astrocytes, resulting in initial clinical symptoms such as memory loss, visual impairment, or seizures. High grade gliomas are highly vascular tumors with increased angiogenesis causing breakdown of the blood brain barrier. They typically have a number of pathological features such as necrosis, hemorrhage, increased tumor cell density, infiltrative and vasogenic edema, and hypoxia.

Gliomas are graded according to the World Health Organization (WHO) scheme, by their degree of malignancy as judged by their histology (2). Grade I Pilocytic Astrocytoma is benign and Grade II Astrocytoma is a low grade malignancy. The Grade III Anaplastic Astrocytoma (AA) is malignant. Tumor recurrence is often associated with
malignant progression. Grade IV Glioblastoma Multiforme (GBM) is neurologically
destructive in nature, and is generally accepted as being one of the deadliest forms of
human cancer. GBM usually occurs in older patients, with no prior clinical history of
benign brain tumors. However, 70% of younger patients having prior history of low
grade astrocytomas are diagnosed with GBM despite treatment. They grow aggressively,
invading the healthy white matter tracts surrounding them. The tumor is usually
recalcitrant to chemotherapy with a median survival rate of 10-12 months. GBMs occur
most often in the subcortical white matter of the cerebral hemispheres. However, they
have a high propensity to infiltrate throughout the brain and are hence termed “diffuse”
gliomas.

_Article_ 

_Brain Tumor Therapy_

Cancer cells have unstable genetic structures that are very susceptible to
mutations. Unless a treatment is immediately effective, the cancer may evolve to become
more resistant to treatment. Hence, a number of different treatment strategies are
employed simultaneously, to reduce the chance of a successful mutation. Conventional
therapeutic options currently available for malignant glioma include surgery,
radiotherapy and chemotherapy, and these are not consistently effective across patient
populations. Maximal surgical resection is not a curative procedure, but provides a longer
survival and improved quality of life. Radiation therapy also increases survival, but is not
a curative intervention (3). Benefits of using Temozolomide (Temodal, Temodar;
Schering-Plough, Kenilworth, NJ), a novel alkylating agent that has demonstrated
activity in recurrent gliomas has been reported in literature (4). This chemotherapeutic
agent produces a modest increase in survival, and is being widely accepted in the oncological circles. The diffusively infiltrative nature and increased malignant progression of gliomas renders them resistant to conventional treatment. Understanding the biology of malignant gliomas and developing targeted curative therapies is of significant interest to the glioma research community. As a result, there are new anti-angiogenic drugs, and gene and virus based therapeutic options which are undergoing testing for efficacy in patients with these tumors. Imaging-based evaluations of therapy are gaining popularity as evidenced by the growing number of recent publications utilizing various types of MRI for evaluating brain cancer drugs.

Virus therapy of gliomas can induce tumor cell death. It utilizes the inherent destructive effects resulting from viral gene expression by cytolytic viruses. The host immune response to infection may also contribute to this process. The G207 virus is a genetically modified herpes virus (HSV-1) that replicates only in dividing cells of the tumor, while not affecting the normal non-dividing cells of the brain (5). G207 has been shown to be efficacious in the treatment of malignant brain tumors in murine models (6). Safety has been established by a Phase I study (7) The G207 formulation developed by Medigene AG, Germany, is being tested as a therapeutic alternative for the treatment of gliomas that are inoperable or have developed a resistance to chemotherapy and/or radiotherapy. Combination treatment i.e. administration of oncolytic HSV along with standard therapies like radiation are also being investigated for synergistic effects (8).

The work presented in this thesis is a step toward permitting non-invasive assessment of these modes of therapy.
Clinical imaging of glioma

The chief goals of imaging patients with brain tumors include localization of these neoplasms, determination of extent, type, and malignancy of the tumor. Imaging not only aids in primary diagnosis, but also in planning of treatment including placement of biopsy, resection, radiation, and guided application of experimental therapeutics. Gliomas are clinically imaged using contrast-based Magnetic Resonance Imaging (MRI) and a variety of anatomic techniques. Figure 1 shows a T1 post-contrast (Gadolinium-DTPA) image of a patient with GBM, with arrows indicating the tumor region.

![T1 post-contrast (Gadolinium-DTPA) image of a patient with GBM showing glioma with an enhancing ring](image)

Figure 1. Post contrast enhanced T1 weighted image in Coronal, Sagittal, and Axial views showing glioma with an enhancing ring

Therapy Assessment

Evaluation of new treatments in clinical trials is required for treatment planning, monitoring response, and predicting treatment outcomes. As gliomas are biologically heterogeneous, it is difficult to assess their progression with qualitative imaging methods. An open or needle biopsy of glioma typically provides tissue for histological characterization. After the initial treatment, neuro-imaging plays a critical role in distinguishing recurrent disease from treatment-related changes such as radiation necrosis.
A variety of complementary imaging methods are currently being used to obtain all the information necessary to achieve the aforementioned goals. Computed Tomography (CT) and MRI reveal mostly anatomical information about the tumor (10), whereas Magnetic Resonance Spectroscopy (MRS) and Positron Emission Tomography (PET) provide information regarding the metabolic state and molecular events within the tumor (11). Other therapy assessment measures include histological metrics, qualitative and semi-quantitative clinical indices such as the Karnofsky Performance Score (KPS) that ranges from 100 to 0, with 100 indicating perfect health (12).

**Diffusion Tensor Imaging**

Magnetic Resonance Imaging is a well accepted non-invasive means of probing the human brain, the most complex structure in the body. It is based on measuring signals from $^1\text{H}$ (proton) nuclei present in water that is abundant in the brain. To generate MR contrast based on the physical properties of water molecules, proton density (PD), T1 and T2 relaxation times, and the diffusion coefficient (D) are widely used. The proton density represents water concentration. T1 and T2 are signal relaxation (decay) times after excitation, which are related to the tissue environmental factors, such as viscosity and the presence of nearby macromolecules. Diffusion (D) represents the thermal (or Brownian) motion of water molecules.

In this work, we focus on a diffusion-based approach to studying brain tumor pathology. A simplified equation for MR signal (S) in a Spin Echo (SE) image is:

$$S = PD(1-e^{-TR/T1})e^{TE/T2}e^{-bD}$$

(1)
where the parameters TR and TE are related to the timing of excitation (called repetition time) and the preparation period (echo time) of the MR signal respectively, and, $b$ is the diffusion weighting factor. By modifying these imaging parameters, a number of different image contrasts like Proton Density, T1, T2, and Diffusion can be generated.

Molecular diffusion or Brownian motion refers to the motion of any molecule in a fluid randomly displaced by thermal energy, and was first formally described by Einstein (13). The mean square of the distance covered is proportional to time and diffusion coefficient $D$, and is a function of the diffusing molecule type and the fluid’s viscosity and temperature (14). During their random diffusion-driven displacements, molecules probe tissue structure at a microscopic scale well beyond the typical image resolution, bouncing, crossing, or interacting with many tissue components such as cell membranes, fibers, or macromolecules. The observation of this intra voxel incoherent motion (IVIM) may thus provide unique clues to the structure and geometric organization of tissue (15).

Diffusion can be evaluated by measuring the signal intensity ($S$) attenuation as a function of the so-called diffusion-sensitizing ‘gradient factor’, i.e. “$b$” value. These are related as:

$$S = S_0 e^{-bD}$$

(2)

The $b$ value is a function of the gyromagnetic ratio of the nucleus of interest as well as the gradient strength ($G$) and timings of the diffusion-sensitizing gradients. Stejskal and Tanner proposed a modified SE sequence with intense short duration magnetic field gradients to measure diffusion $D$ in an image (16). By performing two experiments with different $b$ values ($b_1$ and $b_2$) and recording the two corresponding signal intensities ($S_1$
and $S_2$), the signal attenuation, and hence the diffusion coefficient $D$, also known as Apparent Diffusion Coefficient (ADC) can be calculated as shown below:

Experiment 1: $S_1 = S_0 e^{-b_1D}$

Experiment 2: $S_2 = S_0 e^{-b_2D}$

$=> S_2/S_1 = e^{-(b_2-b_1)D}$

$=> D = \frac{-\ln (S_2/S_1)}{(b_2-b_1)}$  

To encode for the amount and rate of movement of water molecules along a certain direction, a pair of equal magnitude and oppositely directed diffusion gradients are applied in a SE T2 weighted pulse sequence, where the strength of the diffusion gradients increases gradually along that direction. A voxel of tissue containing water that has no net movement will result in a T2 weighted signal. However, if there is movement, the faster moving protons undergo larger dephasing, and hence the signal is decreased in that voxel.

Chenevert et. al., investigated the diffusion anisotropy characteristics in neural tissue microstructure using diffusion based spectroscopy and imaging (17). Neural tissue consists of tightly packed and coherently aligned axons that are surrounded by glial cells and are often organized in bundles. As a result, the intracellular and extracellular movements of water molecules are restricted to a greater extent in a direction perpendicular to the axonal orientation than parallel to it. ADC is largest when the diffusion sensitizing gradient is parallel to the neural fiber tract and lowest when it is perpendicular. This anisotropic diffusion can be attributed to the degree of myelination, density of cellular packing, cell membrane (17), or to the local susceptibility-difference-induced gradients (18).
Diffusion Tensor Imaging is an extension of Diffusion Weighted Imaging, where the diffusion directionality is encoded along three orthotropic axes. It was developed by Basser et al., while trying to image the mean particle displacements and the orientation of the white matter fiber tracts (19, 20). The technique works by applying a number of diffusion gradients in different directions, and parameters including extent and orientation of diffusion in the tissue can be measured. The measurements along different axes are then fitted to a 3D tensor model to describe an ellipsoid shape as shown in Figure 2, for representing the average diffusion distance in each direction.

![Figure 2. Diffusion tensor fitted to ellipsoid](image)

The properties of the 3D ellipsoid, namely, the length of the longest, middle, and shortest axes (called eigenvalues, $\lambda_1$, $\lambda_2$, and $\lambda_3$) and their orientations (called eigenvectors, $V1$, $V2$, and $V3$) can be obtained by the diagonalization of the diffusion tensor $D$ that is symmetric along the diagonal.

\[
D_{ij} = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{bmatrix}
\]

(7)

In addition to directional vectors, the tensor in each voxel contains more information than individual scalars such as ADC, T1, T2 and PD. The directional patterns
of white matter tracts can be visualized using the color maps that represent the major eigenvector direction. These may be weighted with the major eigenvalue, or by white matter connection patterns in 3D using white matter tractography (21).

Echo Planar imaging (EPI) is an MR sequence that is often used to obtain Diffusion Tensor Images (22). The single shot sequence lasts only a few milliseconds, capturing signal sensitive to the intravoxel incoherent motion of the microscopic molecules, while not being sensitive to the macroscopic motion like flow. The sequence is thus relatively unaffected by macroscopic motion artefacts and cardiac related brain pulsation (23). A Spin echo or Gradient Echo EPI is modulated with additional bipolar gradients for rephasing and dephasing the spins, applied along several directions. If there is diffusion along the direction of the diffusion gradient, the dephased spins are not perfectly refocused and hence there is signal loss.

Diffusion Tensor Imaging has found applications in imaging a number of white matter demyelinating, degenerative processes, and other pathological conditions, and also in providing clues to brain development and ageing (24). It is also gaining acceptance in imaging psychiatric disorders which may not offer straightforward anatomical variations (25).

**Diffusion Quantitation**

Quantitative scalar indices that are insensitive to patient orientation and used to characterize diffusion anisotropy are made of combinations of terms from the diagonalized diffusion tensor, i.e., the eigenvalues $\lambda_1$, $\lambda_2$, and $\lambda_3$. The most commonly used scalar indices are the Fractional anisotropy and the Mean Diffusivity. Volume Ratio
(VR) and Relative Anisotropy (RA) are two other invariant anisotropy indices that can also be obtained from diffusion datasets (26).

Mean Diffusivity (MD) of the diffusion tensor is simply defined as the average of the sum (trace) of the eigenvalues:

$$D = \frac{1}{3} \text{tr}(D_{ij}) = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$  \hspace{1cm} (8)

MD provides a measure of the total diffusion within a pixel and the overall presence of biological obstacles to diffusion in that pixel, and has units of mm$^2$/s.

Fractional Anisotropy (FA) measures the fraction of the “magnitude” of the diffusion coefficient that can be ascribed to anisotropic diffusion, and is given by,

$$FA = \sqrt{\frac{3}{2} \sqrt{(\lambda_1 - D)^2 + (\lambda_2 - D)^2 + (\lambda_3 - D)^2}} \sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}$$  \hspace{1cm} (9)

FA is a very convenient index because it is scaled from 0 (isotropic) to 1 (anisotropic).

Another novel method of characterizing the intrinsic properties of water diffusion is by decomposing the diffusion tensor into its “isotropic” part and “anisotropic” or “deviatoric” part i.e. how much the tensor deviates from being isotropic (27, 28). Subsequently, the isotropic component “p” and the anisotropic component “q” were reported by Peña, et al. (29). p is a scaled measure of the mean diffusion in the tensor, while q is a measure of the variance or deviation of the eigen-values with respect to the mean diffusion of the tensor, both measurable in mm$^2$/s. Thus,

$$p = \sqrt{3}D$$  \hspace{1cm} (10)
The p:q space is a useful means of spatial and temporal visualization of both the isotropic and anisotropic components of diffusion simultaneously in a 2-D space as shown in Figures 3 and 4. This formalism is utilized extensively for our analyses of brain tumor diffusion characteristics.

\[ q = \sqrt{(\lambda_1 - D)^2 + (\lambda_2 - D)^2 + (\lambda_3 - D)^2} \]

Figure 3. Tracking spatial variations of 2 different tissues in the p:q space

Figure 4. Tracking temporal variations of same tissue in the p:q space

**Diffusion MR Applications in Glioma Imaging**

Diffusion Weighted Imaging has found a number of applications, especially in imaging cytotoxic edema as in stroke (30, 31). Brunberg et al. suggested utilizing ADC maps for differentiating between cystic necrotic and solid lesions in brain tumors,
something that is not possible with T1 and T2 contrasts (32). Decreased ADC is observed in solid lesions, suggesting increased tumor cellularity, whereas necrotic areas demonstrate high ADC. However, Baehring et al., suggested that ADC maps should be used with caution to track treatment response at the early stages of malignant gliomas, as diffusion changes occur spontaneously with minimal change in tumor size and appearance in other MR sequences (33).

Diffusion Tensor Imaging offers a propitious framework to non-invasively explore the organization of white matter in the living human brain, and track its disruption and degeneration. At a macroscopic scale, diffusion of water molecules is anisotropic within white matter, oriented by the axonal fiber directions, and isotropic within gray matter (34). The anisotropic diffusion in white matter could be attributed to a number of reasons such as degree of myelination and cell membrane (35). A number of hypotheses have been developed to explain decreased diffusion in diseases tissues including decrease in cellular change in energy metabolism resulting in cytotoxic edema (36), decrease in extracellular to intracellular spaces and increase in the restriction of intracellular diffusion due to changes in the membrane permeability (37), and increased tortuosity in the extracellular space due to cell swelling (38).

Grade IV glioma is often a rapidly growing tumor, infiltrating and destroying the surrounding healthy white matter (WM) tracts. The tumor tissue is a heterogeneous mass, with areas of necrosis, edema, hemorrhaging, and infiltrated edema along with tumor regions. Conventional MR imaging does not provide good discrimination between tumor and healthy tissue, especially tumor infiltration, since it can be radiologically masked by the edematous regions. DTI-based methods were suggested as one such solution to permit
discrimination of various tumor compartments (39). Tumor infiltration has been shown to cause a reduction in FA in the edematous area surrounding the tumor (40); FA can be a good predictor of tumor cellularity and hence helps estimate proliferation activity (41). ADC was reported to have an inverse correlation with tumor cellularity (42). Inspite of these approaches, a reliable differentiation between infiltration and vasogenic edema based on DTI is currently unavailable.

It is therefore necessary to understand the microstructure of healthy brain parenchyma and tumor affected areas. Figure 5 shows an illustration of these two tissue types.

![Figure 5. Illustration of tissue microstructure in healthy white matter (left) organized in a linear fashion and tumor (right) showing densely packed cells with a necrotic core.](image)

WM fiber tracts appear to be highly affected by infiltrating gliomas, whereas they seem to be relatively preserved in regions of edema (43). Figure 6 shows the possible patterns of WM fiber tract alteration caused by gliomas. These were earlier proposed by Jellison and Field (44) and are as follows:

Pattern 1: Normal high anisotropy in healthy brain parenchyma.

Pattern 2: Substantially decreased anisotropy and increased isotropy suggesting tumor infiltration around tumor margins.
Pattern 3: Slightly decreased anisotropy and increased isotropy suggesting edema with compressed or deviated WM tracts.

Pattern 4: Isotropic diffusion in tumor suggesting complete disruption of WM fibers.

Figure 6. Patterns of WM fiber tract alteration by gliomas, showing changes in p and q.

Glioma Therapy Assessment using DTI

Assessment of glioma treatment currently relies on evaluating changes in tumor morphology provided by conventional anatomic MR images. However, it is believed that changes in the microscopic tumor environment in gliomas soon after treatment occur before tumor regression. Given the inherent dependence on the water molecules, DTI can provide microscopic tissue information giving better insight into the tumor cellularity (45), tumor malignancy (46), degree of white matter tumor infiltration (47), and radiation induced necrosis (48, 49, 50), and thus, aid in therapy assessment. It has been
hypothesized that successful treatment of a tumor with cytotoxic agents would result in
tumor damage and apoptosis, thus altering membrane permeability and cellularity, which
can be detected by diffusion based MR techniques. The technique would allow
interrogation of the spatial heterogeneity of the drug response within the tumor, and help
track changes over time. In this work, we have attempted to provide a method to evaluate
pixel-by-pixel changes in the isotropic and anisotropic components of diffusion, thereby
enabling a prediction of tissue response to therapeutic intervention.

Medical Image Segmentation

Tissue classification is an important process in medical image processing and
involves feature extraction and labeling. Delineation of anatomical structures and other
features of interest can be facilitated by manual, semi-automated, or fully automated
image segmentation techniques. Formally, segmentation is defined as the partitioning of
an image into compartments that do not overlap, and are consistent in image intensity,
texture or other characteristics. Brain MR Images are normally segmented into three
classes of interest: gray matter, white matter and cerebrospinal fluid. Image segmentation
algorithms play an important role in a number of medical image analysis applications like
quantitation of tissue volume, diagnosis, localization of pathology, study of anatomical
structure, treatment planning and computer-integrated surgery (51).

Objective determination of tumor volume is of interest not only in assessing
therapy response, but also in radiation treatment and surgical resection planning (47).
Magnetic resonance imaging can generate a number of contrasts to label the different
tissue structures. There is no widely accepted method today to quantitate tumor volume
An optimal selection of these multi-spectral contrasts can aid labeling of tissues of interest. Knowledge based techniques have been shown to aid in successful labeling of healthy tissues (53).

In DTI dataset based analyses of the brain, the primary knowledge available is in the form of two parameters, namely isotropy and anisotropy characteristics of tissue (54). For example, healthy highly ordered white matter tracts have relatively high anisotropy and CSF has relatively high isotropy. In patients with pathological changes to the brain parenchyma, especially in conditions such as GBM, there is very little a priori information about the underlying pathology. Datasets contain inherent “uncertainties”, making the task of mathematically classifying a tissue a particularly challenging one. In GBM, it is impossible to manually define the extent of tumor based on gadolinium enhanced MR Images, as there is tumor microinfiltration well beyond these boundaries (55, 51). It is difficult to validate this without invasive histopathological studies. Generally, brain tumors vary in size and shape. Malignant gliomas have ragged boundaries, initially in white matter, but later spreading into other tissue, and usually accompanied by edema. They also have significant mass effect, displacing and infiltrating into surrounding tissue. A variety of segmentation methods are tested for tissue-discrimination in this work. These include hard and soft clustering techniques. The theoretical background on these techniques is provided in the ensuing sections of this thesis.
Cluster Analysis

Clustering is a method to partition a given data set into smaller subsets, grouping them together when they share a common trait or feature. It is commonly employed as a statistical analyses technique in many fields, including image analyses and pattern recognition. Cluster analysis is a novel scheme to probe an image feature space. Given a dataset, the clustering algorithm finds several cluster centers that can characterize tissue classes. These classes form partition of the dataset such that the degree of association or relationship is strong for data within partitioned space and weak outside this space. One of the methods by which the data points are clustered is the proximity as defined by a distance measure. Euclidean distance is the commonly used distance metric.

Clustering techniques do not use prior class identifiers, and are thus regarded as “unsupervised”. Clusters on the 2-D feature space can assume different geometrical shapes like squares, circles, or ellipsoids. The performance of the clusters is influenced by spatial relations and distances among the clusters, and can be overlapping or separated. The p:q plot is one such 2-D feature, where data points can be clustered based on certain similarity measures.

Unlike “hard” or “crisp” clustering, where the dataset is grouped into a number of mutually exclusive subsets (See Figure 7), the “soft” or “fuzzy” clustering methods group objects such that they could belong to several clusters with different degrees of membership or association (See Figure 8). K-means clustering algorithm is a hard clustering method that partitions the image into different tissue classes based on intensity value (56). However, it does not account for overlapping signal intensities, and can lead to misclassifications (55). In fuzzy clustering, each point has a degree of belonging to
clusters, rather than belonging completely in just one cluster. The fuzzy membership of each pixel thus accounts for partial volume effects and mixed tissues in datasets such as DTI that are prone to such setbacks (57).

Figure 7. Illustrating crisp clustering of a 2-D feature space

Clustering Algorithms

A fuzzy connectedness between a pair of pixel elements is computed based on their spatial proximity and contrast similarity (58). In cluster analysis, for a given dataset having “N” points given by \(x\), the \(n\) observations are grouped into \(N \times n\) vector \(X\):

\[
X = \begin{bmatrix}
x_{11} & x_{12} & \cdots & x_{1n} \\
x_{21} & x_{22} & \cdots & x_{2n} \\
\vdots & \vdots & \ddots & \vdots \\
x_{N1} & x_{N2} & \cdots & x_{Nn}
\end{bmatrix}
\]

The dataset for this \(n\)-tuple is arranged in the feature space. The mathematical similarity is defined by a distance norm. This distance is measured from a data vector, to a prototypical object in the cluster called the cluster center, which is sought simultaneously with the partitioning of the data. The \(N \times c\) matrix \(U = [\mu_{ij}]\) represents the
fuzzy partitions. The distance matrix defines the membership function $M_{fc}$, i.e. the affinity based on average intensity:

$$M_{fc} = \{ U \in \mathbb{R}^{N \times c} | \mu_{ik} \in [0, 1], \forall i, k; \sum_{k=1}^{c} \mu_{ik} = 1, \forall i; 0 < \sum_{i=1}^{N} \mu_{ik} < N, \forall k \}$$

(12)

for the given conditions:

$$\mu_{ij} \in [0, 1], 1 \leq i \leq N, 1 \leq k \leq c, \quad (13)$$

$$\sum_{k=1}^{c} \mu_{ik} = 1, 1 \leq i \leq N, \quad (14)$$

$$0 < \sum_{i=1}^{N} \mu_{ik} < N, 1 \leq k \leq c. \quad (15)$$

The $i$th column of $U$ represents the membership function of the $i$th subset of $X$. The total membership of each $x_k$ in $X$ equals one.

Prior to any clustering, the given dataset is normalized, using the range method, such that the data varies between 0 and 1:

$$X = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad (16)$$

The Fuzzy c-means and Gustafson-Kessel algorithm are the two fuzzy clustering methods discussed here in this thesis that have the potential of clustering the $p:q$ space to help classify tissue in a brain tumor DTI dataset.

**Fuzzy C-Means Clustering**

The Fuzzy c-means (FCM) algorithm optimizes the minima of the objective functional $J$ i.e. the overall dissimilarity within clusters, iteratively (59):

$$J(X; U, V) = \sum_{i=1}^{c} \sum_{k=1}^{N} (\mu_{ik})^m \| x_k - v_i \|^2_A$$
Where,

\[ \mathbf{V} = [\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_c], \quad \mathbf{v}_i \in \mathbb{R}^n \]  

(18)

is a vector of cluster prototypes (centers), which have to be determined, and

\[ D^2_{ikA} = \| x_k - \mathbf{v}_i \|^2_A = (x_k - \mathbf{v}_i)^T A(x_k - \mathbf{v}_i) \]  

(19)

is the squared inner-product distance norm. The functional J gives a measure of the total variance of each data point \( x_k \) from the cluster center \( \mathbf{v}_i \). The elements in \( \mathbf{V} \) gives the weighted mean of the data items that belong to the cluster, and weights are the membership degrees. The standard Euclidean distance norm is employed in FCM algorithm, and hence the clusters detected have the same shape and orientation i.e. circular, as shown in Figure 8.

\[ D^2_{ikA} = (x_k - \mathbf{v}_i)^T A_i(x_k - \mathbf{v}_i), \quad 1 \leq i \leq c, \quad 1 \leq k \leq N \]  

(20)

Figure 8. Illustrating Fuzzy c-means clustering of a 2-D feature space
**Gustafson-Kessel Soft Clustering**

This algorithm is an extension of the FCM (60). It utilizes the adaptive distance norm for detecting cluster partitions, which may assume any geometrical shape. Figure 9 showing elliptical clusters illustrate this method.

![Figure 9. Illustrating Gustafson-Kessel clustering of a 2-D feature space](image)

Each cluster has its own norm-inducing matrix $A_i$ which denotes the c-tuple of the norm-inducing matrices $[A_1, A_2, \ldots, A_c]$, so they are allowed to adapt the distance norm to the local topological structure of the data points. This yields the inner-product norm:

$$D^2_{ik \cdot A} = (\mathbf{x}_k - \mathbf{v}_i)^T A_i (\mathbf{x}_k - \mathbf{v}_i), \quad 1 \leq i \leq c, \quad 1 \leq k \leq N \quad (21)$$

The objective functional $J$ is given by,

$$J(\mathbf{X}; \mathbf{U}, \mathbf{V}, \mathbf{A}) = \sum_{i=1}^{c} \sum_{k=1}^{N} (\mu_{ik})^m D^2_{ik \cdot A_i} \quad (22)$$

allowing the matrix $A_i$ to vary with its determinant fixed corresponds to optimizing the cluster's shape while its volume remains constant:

$$\|A_i\| = \rho_i, \quad \rho > 0 \quad (23)$$
where

$$A_i = [\rho_i \text{det}(F_i)]^{1/n} F_i^{-1}$$

(24)

and $F_i$ is the fuzzy co-variance matrix of the $i^{th}$ cluster (61):

$$F_i = \frac{\sum_{k=1}^{N} (\mu_{ik})^m (x_k - v_i)(x_k - v_i)^T}{\sum_{k=1}^{N} (\mu_{ik})^m}$$

(25)

For both FCM and GK clustering algorithms, validation measures such as partition coefficient can be calculated as given in Equation 26.

$$PC(c) = \frac{1}{N} \sum_{i=1}^{c} \sum_{j=1}^{N} (\mu_{ij})^2$$

(26)

This provides a measure of the amount of overlapping between clusters, and is critical to the segmentation process that follows clustering.

**Clustering of DTI dataset**

Clustering schemes for diffusion tensor images have been investigated for medical image segmentation (57, 62). However, there are no reports on the utility of clustering schemes of DTI images for brain tumor segmentation.

To employ the fuzzy clustering scheme on DTI data, the p:q plot would provide the 2-D feature space (pattern matrix), where the size of the image or ROI (number of pixels) gives $N$, and $p$ and $q$ are the two observations or features (or attributes).
CHAPTER 3
MATERIALS AND METHODS

Overview

We evaluate two therapy regimens being studied at the Brain Tumor Treatment and Research program at UAB for our DTI-based quantitative model. These are the oncolytic virus G207 therapy and the conventional chemo/radiation therapy. It is felt that the differences in mechanism of action of these two therapies will allow highlighting our DTI-based approach and tissue segmentation.

The key steps in our approach were as follows:

1. A quantitative spatio-temporal model for glioma assessment was developed and evaluated. This was based on computing the isotropic and anisotropic components of diffusion and includes:
   b. Compartmentalization of tumor and surrounding tissue.
   c. Diffusion-based segmentation for the spatial evaluation of glioma.

2. The above spatio-temporal model was evaluated for therapy assessment of:
   a. “Local therapy” (G207 therapy).
   b. “Global” therapy (conventional chemotherapy + radiation therapy).
3. The above spatio-temporal model was used to compare the local and global therapy response.

A summary of the methods is presented in Figure 10.

Figure 10. Flowchart summarizing the research methods.
MR Diffusion Tensor Imaging

Patient population

Five patients with residual/recurrent glioma having failed surgery/radiation therapy no more than 4 weeks and no chemotherapy 6 weeks before enrollment, were inoculated with the G207 virus at sites chosen to ensure effective virus delivery: $3 \times 10^9$ plaque forming units (pfu) G207 was administered by five stereotactic injections of 0.2 ml each into regions of tumor defined enhancement (suggestive of actively growing tumor) in the post contrast T1 weighted images. This was followed by radiation therapy (single fraction of 15, 18 or 24 Gy based on maximum enhancing T1 post contrast tumor diameter), 24 hours post injection. These patients constitute the “Local Therapy” patients (LP1, LP2, LP3, LP4, and LP5). The G207 patients were imaged prior to G207 inoculation (baseline), at 4, and 8 weeks post-inoculation.

Five GBM patients undergoing conventional chemo/radiation therapy were imaged prior to commencement of therapy (baseline), and at 8, and 16 weeks following initiation of therapy. These patients were grouped as “Global Therapy” patients (GP1, GP2, GP3, GP4, and GP5). Both therapies were approved by the University of Alabama at Birmingham Institutional Review Board. A separate secondary analyses protocol was also approved by the UAB-IRB for this thesis project (see Appendix).

Kanofsky’s Performance score (KPS) logs were recorded as a measure of clinical condition at the evaluation following each imaging study.
**Imaging Protocol**

MR Diffusion Tensor Imaging was performed on a 3T MRI scanner (Intera, Philips Medical Systems, Cleveland, OH) using a SENSE head coil. The diffusion single-shot EPI sequence was run with diffusion gradients applied in 15 directions (TR/TE = 3250ms/88ms, FOV 230 mm², slice thickness/gap = 4mm/1mm, 24 slices to cover the tumor, surrounding edema, and regions of possible infiltration, b-value = 1000s/mm², matrix size 256x256. Pre-contrast FLAIR and post-contrast T1 weighted images were also acquired for anatomic reference and visual compartmentalization of the tumor pathology.

All images were stored and retrieved using the DICOM format (Digital Imaging and Communications in Medicine). Patient information was anonymized prior to any image processing to ensure privacy and confidentiality in accordance with the HIPAA regulations.

**DTI Post Processing**

408 DICOM files for each patient study (17 Diffusion image sets for each of the 24 transverse slices) were obtained. These included each of the 15 gradient directions, the B0 image, i.e. the baseline T2-weighted image with no diffusion gradient application, and a trace image, i.e. the average of all the diffusion gradients.

The files were anonymized and renumbered using custom written MATLAB (The Mathworks Inc., Natick, MA) code. The files were then converted to NIfTI format (Neuroimaging Informatics Technology Initiative) using MRIconvert 2.0 software (Lewis
Center for Neuroimaging, University of Oregon, Eugene, OR). Figure 11 shows the 16 diffusion weighted images for a slice (B0 followed by Gradient 1 through 15).

Figure 11. Diffusion weighted images for a typical slice containing a glioma

The files were rearranged and renamed and then imported into FSL (Analysis Group, FMRIB, Oxford, UK) to perform the following post processing steps.
1. Merging for FSL NIfTI Input: The 16 diffusion gradient datasets, each having the 3-D volume of 24 slices were further merged into one 4-D dataset.

2. Eddy Current Correction: Eddy currents produced by the rapidly switching high intensity diffusion gradients in Echo-planar Imaging cause significant shear and stretch artefacts in the image (63). These were corrected by affine registration of all the diffusion gradients images with respect to the baseline volume (64).

3. Brain Extraction: The intracranial tissues were retained by masking out the skull, fat, muscles, air and background noise for all diffusion datasets (65).

4. Generation of eigenvalues: The diffusion tensor consisting of 3 eigenvalues $\lambda_1$, $\lambda_2$ and $\lambda_3$, and their corresponding eigenvectors V1, V2, and V3 were obtained for each pixel in the dataset. The eigenvalues are of interest, and these are saved in ANALYZE format (Biomedical Resource Core, Mayo Foundation).

5. Correction of negative eigenvalues: Negative eigenvalues can generate anomalous anisotropic indices (FA>1), and correction is necessary (66). These were set to zero in MATLAB.

6. Longitudinal Registration: The 3 eigenvalues for the 2nd and the 3rd time point were registered with respect to the first baseline study using 7 degrees of freedom (3 degrees each for translation and rotation, and 1 for global rescale) and Least-squares cost function as the images are from the same patient, same modality, but different session (67, 64).

7. Generation of parametric isotropic and anisotropic maps: FA, MD, p and q maps were generated in MATLAB (26, 29).
DTI Image Analysis

The FA, MD, p and q maps were created as MATLAB files for the entire study volume of each patient data set. The files were then saved for quantitative modeling and segmentation.

*ROI analysis for 4 different tissues of interest*

Four square regions 10x10 pixels in size were selected on the anatomic slice of interest: contralateral healthy white matter, enhancing tumor, dark vasogenic edema, and possible tumor infiltration into normal appearing white matter/edema around the enhancing the tumor margin.

The p and q values for the same 4 regions outlined, for each patient’s study was plotted on the X and Y axes respectively as a 2-D scatter plot. This p:q plot was used for spatial and temporal differentiation of tumor. The mean and SD values for each of the 4 regions for all 3 time points for each patient study was calculated.

*Clustering based Tissue Segmentation*

The high resolution anatomic dataset was used as reference for selecting the slice that contained the maximum tumor volume, and edematic regions, and had at least one injection site coordinate. The corresponding p and q slices were selected (consisting of greatest tumor volume). The anatomic slice was registered using 3 degrees of freedom for 2-D slice to slice registration with Least-squares cost function in FSL (64).
Five types of tissue were classified from the p:q feature space (29): healthy gray matter (gm), healthy white matter (wm), tumor, edema, and necrosis/CSF using the three classification schemes:

1. Crisp segmentation,
2. Fuzzy c-means clustering based segmentation, and,

**Crisp clustering based Segmentation**

The five tissues of interest were investigated for the region space they occupied in the p:q plot, based on the *a priori* information obtained from observing the anatomic images. The p and q behavior was studied for spatial and temporal variations for each of the 5 tissues. The p and q values based on the regions they occupied in the p:q space were compared for agreement with the white matter fiber tract alteration patterns proposed by Jellison, et al, 2005, and discussed earlier (Figure 6) in this report.

The tissues thus classified were then segmented using custom written MATLAB code for normal/abnormal p and q conditions (both p and q in mm$^2$/s) based on the normal and abnormal p:q thresholds as shown in Figure 12.
The segmentation maps were created for all the slices in each patient study. The segmentation maps for the slice of interest were compared for longitudinal variations across studies.

**Fuzzy c-means clustering based segmentation**

The FCM based clustering was performed in MATLAB using the Fuzzy Clustering and Analysis toolbox (Engineering Applications of Soft Computing, Hungary) (68). Custom written MATLAB programs were used to perform segmentation for tissue classification and quantitative analysis.

The p and q slice consisting maximum tumor volume and enhancing ring as observed on the corresponding T1 post contrast weighted was selected. The primary tissues of interest are WM, GM, tumor, edema, and CSF/necrosis. The following steps were performed:

1. Load the p and q maps for the patient’s baseline study, and arrange the dataset as a pattern matrix of 65536 objects each having the 2 features: p and q values
2. Normalize this 65536x2 pattern matrix
3. Compute cluster prototypes (i.e. centers)
4. Compute distances (distance of each pixel to each cluster center)
5. Update the Partition matrix
6. Repeat steps 3-5 until optimality condition is satisfied
7. Extract the distance matrix
8. Map the distance metric i.e. proximity of each data point to cluster center as function of intensity to obtain tissue masks
9. Repeat the steps 1, 3-7 for 2nd and 3rd time point, using the baseline cluster centers, to allow controlled mapping
10. Extract the distance matrices for each data point. This gives a measure of how likely the pixel can be classified as a particular tissue type
11. Segment 6 tissue types: Background, Healthy WM, healthy GM, Necrotic core/CSF, Vasogenic Edema and Tumor for all 3 studies based on maximum likelihood of the data point to belong to that tissue cluster.

**Gustafson-Kessel clustering based segmentation**

The GK based clustering was performed in MATLAB using the Fuzzy Clustering and Analysis toolbox. Custom written MATLAB programs were used to perform segmentation for tissue classification and quantitative analysis.

The p and q slice consisting maximum tumor volume and enhancing ring as observed on the corresponding T1 post contrast weighted was selected. The primary tissues of interest are WM, GM, tumor, edema, and CSF/necrosis. The following steps were performed:
1. Load the p and q maps for the patient’s baseline study, and arrange as pattern matrix of 65536 objects each having 2 features or attributes: p and q values
2. Normalize this 65536x2 pattern matrix
3. Compute cluster prototypes (i.e. centers)
4. Compute distances (distance of each pixel to each cluster center)
5. Update the Partition matrix
6. Repeat steps 3-5 until optimality condition is satisfied
7. Extract the distance matrix
8. Map the distance metric i.e. proximity of each data point to cluster center as function of intensity to obtain tissue masks
9. Repeat the steps 1, 3-7 for 2nd and 3rd time point, using the baseline cluster centers, to allow controlled mapping
10. Extract the distance matrices for each data point. This gives a measure of how likely the pixel can be classified as a particular tissue type
11. Segment 6 tissue types: Background, Healthy WM, healthy GM, Necrotic core/CSF, Vasogenic Edema and Tumor for all 3 studies based on maximum likelihood of the data point to belong to that tissue cluster.

Quantitative Analyses: Manual ROI and Injection Sites

The segmentation maps obtained from each of the three segmentation routines – Crisp, FCM and GK based clustering were saved for further analyses. Code was developed to allow interactive selection of manual regions of interest to be drawn on the anatomic image, and the quantitative analyses results were returned. The results included
percent tissue distribution (tumor, edema, white matter, gray matter, and necrosis/CSF) in each ROI for 3 time points plotted as bar graphs for the segmented data sets.

Additionally, the percent tissue numbers were generated for 5 pixel radii around one injection site for patients receiving the local therapy (LP1-LP5). The injection sites were selected along the tumor regions with the guidance of the STEALTH stereotactic surgery unit (Medtronic Navigation, Colorado, BO). The site co-ordinates were obtained from this system and carefully overlaid onto the segmented data.
A total of 28 DTI datasets (Patients LP3 and LP4 had two time points; remaining 8 patients had 3 time points) were evaluated in our analyses. Due to space constraints, we present select datasets that typify our results. All the results of a representative from the local therapy patient group: patient ‘LP1’ are discussed in detail. The slice encompassing maximum tumor area, necrotic core and edema) was chosen. Results from longitudinal assessment for the three imaging time points: baseline, 4 weeks post therapy, and 8 weeks post therapy are discussed in detail. Significant results representative of global therapy patient group (GP5) are discussed later. This is followed by summary statistics that illustrate the utility of our DTI-based technique for evaluating tumor progression and therapy efficacy.

The diffusion tensor computation from DTI datasets yields the three eigenvalues of interest. Figure 13 shows the three eigenvalues of the chosen image slice. The first map corresponds to $\lambda_1$. The pixels with high intensity in this map imply increased diffusion along the longest axis of 3-D diffusion ellipsoid model.
FA and MD are the two parametric maps obtained from the mathematical calculation of eigenvalues. Figure 14 shows the FA and MD maps. Regions of high intensity on the FA map correspond to the white matter fiber bundles. Regions of high intensity on the MD maps imply edematous regions or cerebrospinal fluid content.

The isotropy $p$ and anisotropy $q$ maps are shown in color in Figure 15, and these are similar to the MD and FA maps respectively. Unlike FA in which intensity is a ratio, and ranges from 0 to 1, the $q$ map is more convenient for use as it has the same units as $p$ map, i.e. mm$^2$/s. $p$ values range from $0 – 6 \times 10^{-3}$, and $q$ values range from $0 – 1.6 \times 10^{-3}$ for the given dataset.
Figure 15. Isotropy p map (left) and Anisotropy q map (right)

To further assess the p and q characteristics in different brain tissues, four regions were selected on the anatomic slice as reference: tumor, possible tumor infiltration along the tumor margins, edema, and normal appearing contralateral white matter. The p and q values were computed from these four regions and were plotted in the 2-D p:q plane, as shown in Figure 16. It can be observed from their spatial tissue distribution in the p:q space, that the healthy white matter has low p value, and high q value with high variance. Tumor varies in its p value, and has very low q value. Edema compartment has slightly lower q than white matter, and high p. Tumor margins having possible tumor infiltration region also demonstrate high p and low q. The findings agree with the proposed white matter fiber alteration patterns, illustrated earlier in Figure 6.
Longitudinal variations in the $p$:$q$ space for each tissue type: tumor, edema, tumor infiltration, and healthy white matter are illustrated in Figure 17. The $p$:$q$ values for the four tissue compartments were plotted individually for the 3 time points: baseline, 4 weeks post therapy, and 8 weeks post therapy. It was observed that in all patients, there was very little variation in the $p$ value for the white matter regions across time. Whereas, the tumor, edema, and tumor infiltration regions showed variation in both $p$ and $q$ values. This is also seen in Figures 18 and 19 that display the mean ± SD values for the same tissues longitudinally.
Figure 17. The p:q space for the 4 ROIs for 3 time points showing longitudinal variation (LP1)

Figure 18. Mean ± SD of p values for different tissue compartments, LP1
The p:q plots presented in Figure 17 indicate certain regions in the image slice. The p:q space for all pixels in this image slice was plotted (Figure 20). Pixels having very high isotropic diffusion ($p > 4 \times 10^{-3}$) suggest cerebrospinal fluid in the ventricles and necrotic regions. The typical $p$ and $q$ range for other tissues were obtained from previous ROI based analyses. Based on these findings, the Crisp Segmentation was performed, compartmentalizing tissues in the p:q space as shown in Figure 21.

Figure 19. Mean ± SD of $q$ values for different tissue compartments, LP1

Figure 20. The p:q space for slice 14, patient LP1
Figure 21. Crisp Segmentation of the p:q space

The image slice was segmented based on crisp clustering. Figure 22 shows the results of crisp segmentation for the tumor region outlined on the corresponding anatomic image. The spatial distribution of white matter (WM), gray matter (GM), edema, tumor, necrosis/CSF, tumor, and image background can be visualized.

Figure 22. Crisp Segmentation of a tumor region outlined on anatomic T1 (LP1)
Figure 23 further shows the longitudinal variation of the tissue in the segmented maps using crisp segmentation. The spatio-temporal results thus obtained were presented earlier this year (69).

![Figure 23. Crisp Segmentation showing longitudinal variation in LP1](image)

For quantitative analyses, two regions: manually drawn and an injection site were overlaid on the segmented maps as shown in Figure 24 and investigated. The percent tissue distribution was computed (Figures 25 and 26). Both regions show gradually increasing edema. Significant variation in all tissue volumes can be noticed, perhaps indicating changes resulting from therapy.

![Figure 24. Manual ROI selection (dotted line) to encompass tumor, and surrounding tissue and Injection site region (red circle) on T1 post gad slice (LP1)](image)
The next set of results presented here are from the FCM technique. Figure 27 shows the p:q space clustered using the FCM clustering algorithm. Six circular shaped clusters were created for the six types of tissue: WM, GM, edema, tumor, CSF/necrosis, and background image.
Each pixel’s distance from each of the cluster centers was computed to yield its degree of membership. The data obtained was then partitioned into 6 groups, containing likelihood measure of a pixel belonging to a certain cluster. Thus, any pixel is presumed to be one of tumor, edema, white matter, gray matter, or background. The closer the data point is to a certain cluster center, the more likely it is to being classified as that tissue. Figure 28 shows the mapping of the proximity of the pixel to each tissue cluster center as intensity level. The tissue masks thus generated permit individual tissue compartmentalization and allow assessment of tissue longitudinally. Segmentation performed using this scheme is illustrated in Figure 28.
Each pixel is then assigned to a tissue compartment based on its proximity to that tissue’s cluster center. For the second and third studies, the pixel’s distance to the cluster center of the first time point is computed and similar cluster partitioned masks are obtained. Figure 29 shows the segmentation results for all three time points.
The percent tissue distribution obtained from FCM based segmentation for manually drawn ROI and injection sites are shown in Figures 30 and 31 respectively.

Figure 30. FCM based Segmentation: Tissue Percent Distribution in ROI (LP1)

Figure 31. FCM based Segmentation: Tissue Percent Distribution at Injection Site (LP1)
Figure 32 shows the clustering results obtained by using Gustafson-Kessel’s clustering algorithm. The adaptive distance norm method in GK clustering allows non-rigid shapes of clusters. The resulting partitioned data maps are displayed in Figure 33.

Figure 32. GK clustering of p,q space for slice 14, patient LP1

Figure 33. GK cluster partitioned data: tumor, GM, WM, background, CSF/necrotic, and edema for LP1 – Study1
The longitudinal evaluation of GK clustering based segmentation shows a slight increase in the edema content (Figures 34, 35, and 36).

Figure 34. GK based segmentation showing longitudinal variation in LP1

Figure 35. GK based Segmentation: Tissue Percent Distribution in ROI (LP1)
The results presented above were representative of the local therapy group. The following results were obtained from Patient GP5 – representative of the Global Therapy patient group. The Figure 37 shows an anatomic slice of this patient.

Figure 37. Manual ROI selection (dotted line) to encompass tumor and surrounding tissue on a T1 post contrast slice of GP5

The longitudinal variations of four ROIs: WM, edema, tumor, and possible tumor infiltration are shown in Figures 38, 39, and 40. The p:q space for WM, edema, and tumor regions for this patient closely matches the pattern for the local therapy patient
LP1. However, the tumor margins displayed increased p and deceased q, suggesting presence of tumor.

Figure 38. The p:q space for the 4 ROIs for 3 time points showing longitudinal variation for patient GP5

Figure 39. Mean ± SD of p values for different tissue compartments, GP5
Figure 40. Mean ± SD of q values for different tissue compartments, GP5

Figure 41 shows the segmented tissues of interest obtained using crisp segmentation for GP5 baseline study. Longitudinal variations can be observed for this slice as shown in Figure 42.

Figure 41. Crisp segmentation of a region shown outlined on anatomic slice encompassing tumor, edema, and necrosis (GP5)
The percent tissue distribution for the manually drawn region of interest is shown in Figure 43. The findings demonstrate a twofold increase in the tumor volume.

The second segmentation method based on FCM clustering yielded slightly similar results. Figure 44 shows the p:q space clustered using FCM clustering algorithm for partitioning the 6 types of tissue: white matter, gray matter, edema, tumor, CSF/necrosis, and background pixel. The resulting segmentation results are shown in
Figure 45 and the percent tissue distribution in the manually drawn ROI are shown in Figure 46. The results demonstrate about 50% tumor involvement in the first study and a very slight increase over studies.

Figure 44. FCM clustering of p:q space of slice #10 in GP5

Figure 45. FCM based segmentation of 3 time points from p:q data showing longitudinal variations in GP5
Figure 46. FCM based segmentation - ROI Tissue Percent Distribution (GP5)

The results obtained by clustering the p:q space for GP5 using the GK clustering algorithm is shown in Figure 47. It can be observed that the edema cluster center in the region (i.e. high p and high q) is similar to that obtained using the crisp segmentation method.

Figure 47. GK clustering of p:q space of slice #10 in GP5
The resulting GK based segmentation and ROI analyses show significant loss in anisotropy in the tumor and surrounding tissue (Figures 48 and 49) for patient GP5. Additionally, an increase in edema and decrease in the WM anisotropy was observed in the second time point for this patient. The individual tissue maps obtained from the partitioned matrix show both isotropy and anisotropy changes clearly (Figures 50 and 51).

![Figure 48. GK based segmentation of 3 time points from p,q data (GP5)](image)

![Figure 49. GK based segmentation - ROI Tissue Percent Distribution (GP5)](image)
Figure 50. GK cluster partitioned data for edema across studies for patient GP5

Figure 51. GK cluster partitioned data for WM across studies for patient GP5

The findings of manually drawn ROI of tumor and surrounding tissue using crisp segmentation method for the remaining patients (LP2, LP3, LP4, LP5, GP2, GP3, GP4 and GP5) are presented in Figures 52 to 59.
Figure 52. Crisp segmentation - ROI Tissue Percent Distribution (LP2)

Figure 53. Crisp segmentation - ROI Tissue Percent Distribution (LP3)
Figure 54. Crisp segmentation - ROI Tissue Percent Distribution (LP4)

Figure 55. Crisp segmentation - ROI Tissue Percent Distribution (LP5)
Figure 56. Crisp segmentation - ROI Tissue Percent Distribution (GP1)

Figure 57. Crisp segmentation - ROI Tissue Percent Distribution (GP2)
Tables 1 - 6 summarize the percent tumor, edema, and healthy brain tissue (white matter and gray matter) obtained by performing crisp segmentation for all patients.
Table 1. Crisp segmentation – Percent Tumor Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>LP1</th>
<th>LP2</th>
<th>LP3</th>
<th>LP4</th>
<th>LP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>39.47</td>
<td>19.74</td>
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</tr>
<tr>
<td>Study 2</td>
<td>14.47</td>
<td>38.16</td>
<td>17.11</td>
<td>21.05</td>
<td>3.947</td>
</tr>
<tr>
<td>Study 3</td>
<td>2.632</td>
<td>28.95</td>
<td>X</td>
<td>X</td>
<td>6.579</td>
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</table>

Table 2. Crisp segmentation – Percent Edema Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>EDEMA</th>
<th>LP1</th>
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<th>LP4</th>
<th>LP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>1.316</td>
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<tr>
<td>Study 2</td>
<td>53.95</td>
<td>26.32</td>
<td>64.47</td>
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<td>Study 3</td>
<td>93.42</td>
<td>53.95</td>
<td>X</td>
<td>X</td>
<td>63.16</td>
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</table>

Table 3. Crisp segmentation – Percent Healthy Tissue Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>HEALTHY</th>
<th>LP1</th>
<th>LP2</th>
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<th>LP4</th>
<th>LP5</th>
</tr>
</thead>
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<tr>
<td>Study 1</td>
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<td>Study 2</td>
<td>31.58</td>
<td>35.53</td>
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<td>73.68</td>
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<tr>
<td>Study 3</td>
<td>3.947</td>
<td>17.11</td>
<td>X</td>
<td>X</td>
<td>30.26</td>
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</table>
Table 4. Crisp segmentation – Percent Tumor Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
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<tr>
<th>TUMOR</th>
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<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>16.05</td>
<td>16.34</td>
<td>29.38</td>
<td>17.41</td>
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</tr>
<tr>
<td>Study 2</td>
<td>6.933</td>
<td>21.97</td>
<td>33.35</td>
<td>13.16</td>
<td>41.82</td>
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<tr>
<td>Study 3</td>
<td>10.87</td>
<td>15.33</td>
<td>29.11</td>
<td>18.56</td>
<td>48.9</td>
</tr>
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</table>

Table 5. Crisp segmentation – Percent Edema Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
<thead>
<tr>
<th>EDEMA</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
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<td>19.01</td>
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<td>Study 2</td>
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<td>36.07</td>
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<td>20.33</td>
<td>21.35</td>
</tr>
<tr>
<td>Study 3</td>
<td>15.72</td>
<td>53.71</td>
<td>40.82</td>
<td>20.29</td>
<td>25.73</td>
</tr>
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</table>

Table 6. Crisp segmentation – Percent Healthy Tissue Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
<thead>
<tr>
<th>HEALTHY</th>
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<th>GP4</th>
<th>GP5</th>
</tr>
</thead>
<tbody>
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<td>Study 1</td>
<td>43.68</td>
<td>45.09</td>
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<td>50.9</td>
<td>32.6</td>
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<tr>
<td>Study 2</td>
<td>54.89</td>
<td>24.94</td>
<td>36.14</td>
<td>48.45</td>
<td>36.43</td>
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<tr>
<td>Study 3</td>
<td>45.3</td>
<td>14.69</td>
<td>28.41</td>
<td>44.12</td>
<td>24.96</td>
</tr>
</tbody>
</table>

The GK clustering algorithm did not converge for data from patients LP3, GP1 and GP3. Tables 7 - 12 summarizes the percent tumor, edema, and healthy brain tissue (white matter and gray matter) obtained by performing GK based segmentation for all patients.
Table 7. GK based segmentation – Percent Tumor Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>LP1</th>
<th>LP2</th>
<th>LP3</th>
<th>LP4</th>
<th>LP5</th>
</tr>
</thead>
<tbody>
<tr>
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<td>37.3333</td>
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<td>22.2222</td>
</tr>
<tr>
<td>Study 2</td>
<td>10.6667</td>
<td>53.5211</td>
<td>X</td>
<td>28.9474</td>
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<td>Study 3</td>
<td>21.3333</td>
<td>78.8732</td>
<td>X</td>
<td>X</td>
<td>23.6111</td>
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</tbody>
</table>

Table 8. GK based segmentation – Percent Edema Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>EDEMA</th>
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<th>LP2</th>
<th>LP3</th>
<th>LP4</th>
<th>LP5</th>
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<tr>
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<tr>
<td>Study 2</td>
<td>20</td>
<td>2.8169</td>
<td>X</td>
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<tr>
<td>Study 3</td>
<td>30.6667</td>
<td>2.8169</td>
<td>X</td>
<td>X</td>
<td>20.8333</td>
</tr>
</tbody>
</table>

Table 9. GK based segmentation – Percent Healthy Tissue Distribution in Injection Site region for local therapy group patients (LP1-LP5)

<table>
<thead>
<tr>
<th>HEALTHY</th>
<th>LP1</th>
<th>LP2</th>
<th>LP3</th>
<th>LP4</th>
<th>LP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>62.6667</td>
<td>46.4789</td>
<td>X</td>
<td>94.7369</td>
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<td>Study 2</td>
<td>70.6667</td>
<td>50.7042</td>
<td>X</td>
<td>61.8421</td>
<td>94.4444</td>
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<tr>
<td>Study 3</td>
<td>49.3333</td>
<td>25.3521</td>
<td>X</td>
<td>X</td>
<td>61.1111</td>
</tr>
</tbody>
</table>
Table 10. GK based segmentation – Percent Tumor Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
<thead>
<tr>
<th>Study</th>
<th>TUMOR</th>
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<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
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<td>20.8624</td>
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<tr>
<td>Study 2</td>
<td>X</td>
<td>53.6411</td>
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<td>13.5516</td>
<td>2.6123</td>
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<tr>
<td>Study 3</td>
<td>X</td>
<td>54.2136</td>
<td>X</td>
<td>17.2177</td>
<td>9.5903</td>
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</tr>
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</table>

Table 11. GK based segmentation – Percent Edema Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
<thead>
<tr>
<th>Study</th>
<th>EDEMA</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>X</td>
<td>14.1528</td>
<td>X</td>
<td>10.0164</td>
<td>18.8871</td>
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<tr>
<td>Study 2</td>
<td>X</td>
<td>13.455</td>
<td>X</td>
<td>9.1653</td>
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<td></td>
</tr>
<tr>
<td>Study 3</td>
<td>X</td>
<td>9.8765</td>
<td>X</td>
<td>11.653</td>
<td>29.82</td>
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</tr>
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</table>

Table 12. GK based segmentation – Percent Healthy Tissue Distribution in manually drawn region for global therapy group patients (GP1-GP5)

<table>
<thead>
<tr>
<th>Study</th>
<th>HEALTHY</th>
<th>GP1</th>
<th>GP2</th>
<th>GP3</th>
<th>GP4</th>
<th>GP5</th>
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<tbody>
<tr>
<td>Study 1</td>
<td>X</td>
<td>48.8459</td>
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<td>44.2571</td>
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</tr>
<tr>
<td>Study 3</td>
<td>X</td>
<td>19.3774</td>
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<td>24.9094</td>
<td>58.604</td>
<td></td>
</tr>
</tbody>
</table>
The results obtained from all three segmentation routines: crisp segmentation, FCM clustering based segmentation and GK clustering based segmentation show variations in tumor and surrounding tissue percentage as a function of time and therapy response. The significant findings from Crisp Segmentation technique are listed below:

1. 4 out of 5 local therapy patients and 2 out of 5 global therapy patients showed significant increase in edematous tissue in the second and third studies.
2. The results from all 5 global therapy patients show decreased WM anisotropy.
3. 3 out of 5 local therapy patients (including 2 therapy responders) show decrease in tumor volume at the injection sites.

The results from FCM and GK clustering segmentation routines are not always in agreement. The GK clustering results show an increase in edema volume for 3 out of 5 patients in the local therapy group.
CHAPTER 5
DISCUSSION

In this work, the diffusion properties of tumor and surrounding tissue were investigated. Various diffusion indices were analyzed. It was found that even though individual inspection of FA and MD images may show abnormal values suggesting tumor affected areas, these maps are not useful for labeling tumor, tumor infiltration into the white matter, edematous, and necrotic regions. The isotropic component $p$ and anisotropic component $q$ for a tissue also do not provide much information when assessed individually. Since glioma is a heterogeneous aggregate of several tissue types, each having characteristic diffusion isotropy and anisotropy, it is very critical to assess both $p$ and $q$ characteristics simultaneously. Our approach would allow assessment of the rapidly changing morphology of gliomas under therapy, and make quantification of the tumor and related biology feasible. To the best of our knowledge, this work represents the first attempt to quantitate tissue characteristics using MR-DTI.

The $p$:$q$ space was helpful in gaining knowledge about the underlying tissue based on its diffusion properties. Healthy white matter, CSF, and abnormal tissue were the three compartments that could be broadly classified using the $p$:$q$ space. Crisp segmentation method was used to compartmentalize 5 tissues: WM, GM, CSF/necrosis, tumor and edema, by incorporating prior knowledge (Figures 6 and 12). The methodology yielded
fair segmentation results that correlated well with anatomic findings (FLAIR and post-contrast T1). The quantitative analyses performed for regions of interest can be compared with existing clinical findings such as histology specimens and KPS for further validation.

Since the abnormal region is a mixture of several tissues: tumor, vasogenic edema, tumor infiltration into WM and necrosis, a more reliable method is required to label these tissues. Fuzzy logic was incorporated in our segmentation methodology. Since a given pixel may belong to two or more tissue types that may have overlapping contrasts, it was felt that fuzzy clustering was appropriate to segment glioma tissue. In the context of MR imaging, this would also account for partial volume averaging of tissue.

Two different fuzzy clustering methods were tested. The clustering of the p:q space using the FCM algorithm showed variation over patient datasets. The cluster partitioning of certain tissues such as WM and CSF/necrosis did not prove to be consistently reliable. This is because both these tissues had high range of either p or q (i.e. high variance in one dimension only), and fitting a circular shaped cluster would exclude some pixels or include other tissue compartments.

The GK clustering algorithm is based on covariance matrix estimation, and performed better than FCM. It is similar to the more complex GG (Gath and Geva) algorithm that is based on maximum likelihood estimation (70). The adaptive distance norm measure allows elliptical shaped clustering, which is appropriate for segmenting white matter, CSF/necrosis and even edematous regions that have fluid around white matter tracts. Figure 60 shows the anatomic and GK clustering based individual tissue images. The baseline study confirms the presence of tumor (indicated by green arrow).
Although the anatomic image of the second study at 8 weeks does not show pathology on the contralateral side, the segmented image obtained from GK clustering confirms loss of anisotropy and increase in isotropy along the white matter region (indicated by red arrow). The region was labeled edema, as it was seen well beyond the tumor area, and along the white matter tracts. These results provide evidence of the change in the integrity of the white matter fibers in the presence of fluid. It also shows that the same regions do have some tumor involvement; as otherwise, the anisotropy in the region would have been significantly compromised. The mild loss in anisotropy could be a radiation induced effect (71, 72).

Figure 60. Anatomic MR image and GK clustering based tissue classification of diffusion data
The Crisp, GK, and FCM clustering approaches for segmentation performed poorly in differentiating gray matter and tumor pixels. Gray matter has low anisotropy and low isotropy. Tumor cells also have low anisotropy, but varying isotropy. The low isotropic diffusion in some tumor regions would explain increased cellularity, as this would restrict isotropic diffusion in both intracellular and extracellular spaces.

Preprocessing steps that included eddy current correction, skull stripping and correction of negative eigenvalues, improved the image quality of the datasets to a certain extent. However, retrospective image quality control measures could not correct for all artefacts produced during DTI image acquisition. Patient motion and variation in patient position and orientation during multiple scans posed significant problems. Although diffusion post processing steps included image registration schemes, the slices were not accurately registered across patient studies. Additionally, patient motion correction and registration could have led to misclassification of pixels during segmentation, especially since we rely on longitudinal quantitation.

MR–DTI imaging of GBM in animal models could help to establish the ground truth for p and q values of healthy versus tumor affected tissue. Additionally, DTI data from an untreated GBM patient, would further help establish the cluster centers for tumor in the p:q feature space, and help reliable longitudinal assessment of therapy response. These are directions that would aid in validating such a quantitative model.
CHAPTER 6
CONCLUSIONS

MR Diffusion Tensor Imaging can be used to generate quantitative surrogate markers of diseased brain parenchyma in the study of complex diseases such as glioma. However, sophisticated medical image analyses techniques are required to extract quantitative information from DTI datasets. A p:q based analysis provides valuable insight into the neuronal tissue microstructure. Cluster analysis of the p:q space aids in tissue segmentation. Three medical image segmentation methods based on the clustering of p:q space were tested for their utility in classifying glioma and surrounding tissue. Differentiation of necrosis, edema, tumor and healthy white matter compartments was shown to be feasible. We were also able to detect subtle changes in the integrity of white matter fibers that is not possible with other non-invasive imaging techniques. This finding may help better understand tumor progression, the effect of various therapies, and provide clues to other pathology and therapy induced effects.

The quantitative approach developed herein is useful for therapy assessment, a critical step for advancing novel treatments for brain cancer. Our model developed could be further refined; however, a priori knowledge about the tumor and surrounding tissue will be necessary. Histological validation would confirm the utility of the p:q approach and the segmentation models for assessing glioma therapy response.
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APPENDIX

IRB APPROVAL FORM

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on November 24, 2003 and expires on October 26, 2010. The Assurance number is FWA00005960.

Principal Investigator: GOEL, PRIYA
Co-Investigator(s): AKELLA, N SHASTRY
Protocol Number: N080311002
Protocol Title: Quantitative Assessment of Glioma Therapy Efficacy using Magnetic Resonance Diffusion Tensor Imaging

The IRB reviewed and approved the above named project on 3/20/08. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.
IRB Approval Date: 3-20-08
Date IRB Approval Issued: 3/26/08

Marilyn Doss, M.A.
Vice Chair of the Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.