Correlating Tumor Microstructure With Hypoxia Using Magnetic Resonance Imaging

BY

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THESIS

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This thesis is dedicated to my parents who give me the courage and guidance to pursue my goals.
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SM
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Motivation</td>
<td>2</td>
</tr>
<tr>
<td>2. THEORY</td>
<td>5</td>
</tr>
<tr>
<td>2.1 Magnetic Resonance Imaging</td>
<td>5</td>
</tr>
<tr>
<td>2.1.1 Principle of Nuclear Magnetic Moments</td>
<td>5</td>
</tr>
<tr>
<td>2.1.2 Effect of Radiofrequency Pulses</td>
<td>8</td>
</tr>
<tr>
<td>2.1.3 MR Relaxation Processes</td>
<td>9</td>
</tr>
<tr>
<td>2.1.4 MR Signal Reception</td>
<td>11</td>
</tr>
<tr>
<td>2.1.5 MR Image Formation: Gradient Coils</td>
<td>12</td>
</tr>
<tr>
<td>2.1.6 MR Image Formation: The ‘k-space’</td>
<td>14</td>
</tr>
<tr>
<td>2.1.7 MRI Hardware</td>
<td>15</td>
</tr>
<tr>
<td>2.1.8 MRI Sequences</td>
<td>17</td>
</tr>
<tr>
<td>2.2 Diffusion MRI</td>
<td>20</td>
</tr>
<tr>
<td>2.3 Electron Paramagnetic Resonance</td>
<td>24</td>
</tr>
<tr>
<td>2.3.1 Principles of EPR</td>
<td>25</td>
</tr>
<tr>
<td>2.3.2 EPR Oxygen Imaging</td>
<td>26</td>
</tr>
<tr>
<td>2.3.3 EPR Imaging Equipment</td>
<td>27</td>
</tr>
<tr>
<td>3. CORRELATION OF TUMOR VISCOSITY WITH TUMOR HYPOXIA</td>
<td>28</td>
</tr>
<tr>
<td>3.1 Materials, Methods and Results</td>
<td>28</td>
</tr>
<tr>
<td>3.1.1 Diffusion MRI Phantoms</td>
<td>28</td>
</tr>
<tr>
<td>3.1.2 Animal Preparation for ex vivo and in vivo Studies</td>
<td>31</td>
</tr>
<tr>
<td>3.1.3 Ex vivo Experiments</td>
<td>32</td>
</tr>
<tr>
<td>3.1.4 In vivo Experiments</td>
<td>33</td>
</tr>
</tbody>
</table>
4. CORRELATION OF TUMOR MICROSTRUCTURE WITH TUMOR HYPOXIA .......... 38

4.1 Materials, Methods and Results .............................................................................. 38
  4.1.1 Animal Preparation for \textit{ex vivo} and \textit{in vivo} studies ........................................ 38
  4.1.2 \textit{Ex vivo} Experiments .................................................................................. 38
  4.1.3 \textit{In vivo} Experiments .................................................................................. 40

5. CONCLUSIONS AND FUTURE WORK ......................................................................... 43

6. REFERENCES ............................................................................................................ 45

7. VITA .......................................................................................................................... 49
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I: The classification of hypoxia based on the reason for occurrence</td>
<td>1</td>
</tr>
<tr>
<td>Table II: A comparison between MRI and EPRI</td>
<td>27</td>
</tr>
<tr>
<td>Table III: Mean viscosity in tumor and normal region (ex vivo for FSa)</td>
<td>33</td>
</tr>
<tr>
<td>Table IV: In vivo mean viscosity and pO$_2$ in tumor and normal region for MCa4 (n = 1)</td>
<td>36</td>
</tr>
<tr>
<td>Table V: Mean FA in tumor and normal region (ex vivo for FSa)</td>
<td>39</td>
</tr>
<tr>
<td>Table VI: In vivo mean FA and pO$_2$ in tumor and normal region for MCa4 (n = 1)</td>
<td>40</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1: Formation of chronic hypoxic regions in tumor due to limited O₂ supply</td>
<td>2</td>
</tr>
<tr>
<td>Figure 2: A generalized figure based on several radiation therapy studies on the effect of oxygen concentration on radiation dose (Gy)</td>
<td>3</td>
</tr>
<tr>
<td>Figure 3: Alignment of protons in the presence of B₀ giving rise to a net magnetization M</td>
<td>6</td>
</tr>
<tr>
<td>Figure 4: Larmor frequency shown using classical model</td>
<td>7</td>
</tr>
<tr>
<td>Figure 5: Application of RF pulse and the ‘tip angle’.</td>
<td>9</td>
</tr>
<tr>
<td>Figure 6: T₁ relaxation (left) and T₂ relaxation (right).</td>
<td>10</td>
</tr>
<tr>
<td>Figure 7: Free induction decay (FID).</td>
<td>11</td>
</tr>
<tr>
<td>Figure 8: Magnetic field variation due to gradient coil (left) and MRI slice selection (right).</td>
<td>13</td>
</tr>
<tr>
<td>Figure 9: MRI scanner and components.</td>
<td>15</td>
</tr>
<tr>
<td>Figure 10: A simple timing diagram showing the RF pulse and the gradients.</td>
<td>18</td>
</tr>
<tr>
<td>Figure 11: Dephasing of magnetization by T₂* and rephasing it by a 180° pulse during a spin echo sequence.</td>
<td>20</td>
</tr>
<tr>
<td>Figure 12: A diffusion weighted spin-echo pulse sequence.</td>
<td>21</td>
</tr>
<tr>
<td>Figure 13: Variation in diffusion ellipsoid with diffusion type.</td>
<td>23</td>
</tr>
<tr>
<td>Figure 14: Electron spin states in the presence of external magnetic field.</td>
<td>25</td>
</tr>
<tr>
<td>Figure 15: The Bruker 500 AVANCE micro-imaging scanner.</td>
<td>29</td>
</tr>
<tr>
<td>Figure 16: ADC maps of phantoms (left) and their viscosity maps (right).</td>
<td>30</td>
</tr>
<tr>
<td>Figure 17: MRI viscosity vs. expected viscosity (cP).</td>
<td>31</td>
</tr>
<tr>
<td>Figure 18: The 9.4T pre-clinical scanner at UIC.</td>
<td>32</td>
</tr>
<tr>
<td>Figure 19: ADC map of an ex vivo specimen (left) and corresponding viscosity map (right).</td>
<td>33</td>
</tr>
</tbody>
</table>
Figure 20: Schematic for EPR setup used in the study. ............................................................... 35
Figure 21: Comparison of tissue viscosity and partial oxygen pressure in vivo. ......................... 36
Figure 22: The change in viscosity as a function of pO₂. ............................................................ 37
Figure 23: Ex vivo mean diffusivity (A) and fractional anisotropy (B) maps .......................... 39
Figure 24: Comparison of tissue fractional anisotropy and pO₂ in vivo ................................. 41
Figure 25: Correlation between mean pO₂ and FA values within the in vivo tumor volume ....... 42
Figure 26: A micro computed tomography (micro-CT) system (left) that can be used to target hypoxic tumors. EPR information (right) can be used for targeted radiation delivery .......... 43
Figure 27: EPR and MRI conjunction in radiation therapy. ......................................................... 44
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pO₂</td>
<td>Partial pressure of oxygen</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>IMRT</td>
<td>Intensity modulated radiation therapy</td>
</tr>
<tr>
<td>EPROI</td>
<td>Electron paramagnetic resonance oxygen imaging</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<td>DWI</td>
<td>Diffusion weighted imaging</td>
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<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<tr>
<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>FE</td>
<td>Frequency encoding</td>
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<tr>
<td>PE</td>
<td>Phase encoding</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
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<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>MD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>IDL</td>
<td>Interface description language</td>
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<tr>
<td>SEM</td>
<td>Standard error of measurement</td>
</tr>
<tr>
<td>MAS</td>
<td>MRI analysis software</td>
</tr>
</tbody>
</table>
A series of diffusion weighted magnetic resonance imaging experiments were carried out in order to establish a relationship between tumor viscosity and fractional anisotropy, obtained using diffusion weighted magnetic resonance imaging (DWI and DTI), with the corresponding partial pressure of oxygen (pO$_2$) existing inside the tumor volume. The oxygen concentration was derived through a technique similar to magnetic resonance imaging, called the electron paramagnetic resonance oxygen imaging (EPROI).

A series of *ex vivo* and preliminary *in vivo* measurements were performed on murine models with either FSa or MCa4 solid tumor models in their hind limbs. Results through these measurements have shown a high positive correlation (R = 0.85) between the fractional anisotropy and the partial pressure of oxygen in tumor regions. Tissue viscosity has also been demonstrated to be associated with different levels of oxygen concentration in tumors.

As hypoxic or oxygen deficient regions inside a tumor are known to exhibit resistance to radiation therapy, the results from these experiments show that there is a possibility to use both imaging techniques in conjunction to develop efficient ways to target specific areas inside a tumor with dose painting. This may lead to better post-treatment healing and minimize dysfunction by sparing healthy structure with optimized radiation doses. The long-term goal of this study is to help create efficient intensity modulated radiation therapy treatment plan for solid tumors.
1. INTRODUCTION

1.1 Background

Oxygen concentration is an important physiological parameter for assessing tissue health because oxygen molecules form a vital part of energy cycle of the cell. Without enough supply of oxygen, the energy requirements for cells are not met and it eventually perishes. Hypoxia is a broad term which is defined as the below normal supply of oxygen in tissues that can occur due to various reasons. Adequate supply of oxygen depends on many factors such as amount of oxygen in the inhaled air, proper exchange of respiratory gases in lungs, hemoglobin being in sufficient numbers to transport oxygen, and a normal oxidation process. In general, hypoxia can be caused by many factors as defined below:

<table>
<thead>
<tr>
<th>Hypoxemic Hypoxia</th>
<th>Can occur due to decrease in oxygen partial pressure (pO₂) in arterial blood because of respiratory disturbances or disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemic Hypoxia</td>
<td>Occurs when there is a reduction in ability of blood to carry O₂ due to anemia.</td>
</tr>
<tr>
<td>Circulatory or Ischemic Hypoxia</td>
<td>Occurs because of insufficient supply of O₂ to tissue in spite blood being well oxygenated, mainly due to reduced tissue perfusion or the inability of O₂ to diffuse through to the cells.</td>
</tr>
<tr>
<td>Cytotoxic Hypoxia</td>
<td>The cell’s inability to use O₂ due to intoxication as in poisoning.</td>
</tr>
</tbody>
</table>

Table I: The classification of hypoxia based on the reason for occurrence [1].
Hypoxia can also be classified based on the duration of hypoxic conditions: chronic hypoxia and acute hypoxia. In tumors, for example, chronic hypoxia occurs when the distance between cells and the vasculature becomes too large for oxygen molecules to diffuse through to the tissue, thus creating regions of reduced oxygen supply. In such cases, the cells usually remain deoxygenated and die unless they are able to get blood supply. Acute hypoxia, on the other hand, is sort of perfusion-limited event due to fluctuating blood flow in tumor vessels. For example, the random and temporary closing or blockage of a particular blood vessel in tumors [2]. The image below gives a simple illustration of how chronic hypoxia can occur in tumors.

![Formation of chronic hypoxic regions in tumor due to limited O_2 supply.](image)

Figure 1: Formation of chronic hypoxic regions in tumor due to limited O_2 supply.

1.2 Motivation

Approximately, 50% - 60% of human tumors have hypoxic regions [3]. It has been documented from radiation therapy studies that the occurrence of hypoxia make tissue more resistant to radiation and therefore, require a higher dose when it comes to radiation treatment
Efficient radiation treatment requires the presence of adequate oxygen in tissue as they form deoxyribonucleic acid (DNA) damaging free radicals under ionization. Therefore, hypoxic tissues are resistant to radiation doses. Current practice in radiation therapy is to give a uniform distribution of high radiation, to ensure the destruction of oxygen deficient areas. This causes unnecessary damage to healthy tissues and may deteriorate patient life quality after treatment. The knowledge of partial oxygen pressure (pO₂), along with intensity modulated radiation therapy (IMRT) can be useful to target the radiation resistant hypoxic areas, thereby creating a more efficient treatment plan.

Figure 2: A generalized figure based on several radiation therapy studies on the effect of oxygen concentration on radiation dose (Gy). Figure inspired by [8][9][10].

In tumors, oxygen delivery is frequently reduced because of cell proliferation, severe structural changes in tissue microvessels, and abnormal microcirculation. The consequent higher
cellularity and tissue disorganization leads to lower apparent diffusion coefficients for water molecules in malignant tumors when compared against normal tissue [11]. This also creates a heterogeneous environment in tumors where there are areas of low or zero pO$_2$ in adjacent to regions with normal pO$_2$ (normoxic) [1].

Electron paramagnetic resonance oxygen imaging (EPROI) is an established technique to image oxygen concentration with high precision (< 1 torr) [3][12]. It is a technique where the choice of particles for studying magnetic resonance is unpaired electrons. Recent work has shown that using information about pO$_2$ from EPROI, it is possible to predict tumor radiation treatment outcome based on radiation sensitivity of tissue inside a tumor [7]. Magnetic resonance imaging (MRI), on the other hand, is a well-known method of studying tissue microstructure. Water molecules are the primary source of nuclear magnetic resonance (NMR) or MRI signal in tissue. As they are mobile, the random motion of the water molecule in tissue can be characterized by a constant called apparent diffusion coefficient (ADC) [11]. As tissue morphology changes due to disease and degeneration, this gets reflected in the ADC value. In MRI, special diffusion-weighted pulse sequences are used to measure these diffusion coefficients of water molecules and is called diffusion-weighted MRI (DWI). Another imaging technique, known as diffusion tensor imaging (DTI), has been extensively used to get information about tissue orientation and microstructure inside a tumor [13].

This study demonstrates how pO$_2$ concentration is related to tissue viscosity and tissue microstructure in tumors. The focus of this project is to demonstrate and establish a correlation between spatial partial oxygen concentration information from EPROI and corresponding viscosity and fractional anisotropy of tissue inside tumors, using both DWI and DTI respectively.
2. THEORY

2.1 Magnetic Resonance Imaging

MRI today is one of the most powerful tools in the world of clinical and analytical imaging. Although NMR spectroscopy has been in use in research since the 1950s, it was not until 1973 when the first MRI image was acquired by Paul Lauterbur, who shared the Nobel Prize for Medicine in 2003 with Peter Mansfield for their contribution to the invention and development of MRI [14]. MRI is non-invasive, non-ionizing, and can be used to create highly detailed cross-sectional images in three or more planes. MRI also provides the best soft tissue contrast among all current imaging modalities.

2.1.1 Principle of Nuclear Magnetic Moments

The basic underlying physics of MRI is same as that of NMR. At the atomic level, nuclei exhibit a property known as nuclear spin, an intrinsic angular momentum. Certain isotopes of elements have an odd number of protons and/or neutrons such as $^1$H, $^{13}$C, and $^{15}$N, and have an overall positive charge. As a result of having both electrical charge and nuclear spin, the nuclei will possess an intrinsic magnetic moment $\mu$ and the relation can be represented as follows, where $I$ is the angular momentum and $\gamma$ is the gyromagnetic ratio that has a value of 267.54 MHz/T for protons (hydrogen nuclei) [15].

$$|\vec{\mu}| = \gamma |\vec{I}| \ldots (2.1)$$

The human body is 70% - 80% water and thus, MRI observes primarily water protons as small magnets. In the absence of a magnetic field, the orientations of these protons are random and therefore, the net magnetization is zero. However, this situation changes when these protons come under the effect of an external magnetic field $B_0$. The protons then start orienting themselves at an
angle to the external magnetic field, in either the same or in the opposite direction. The magnetic field from these protons cancels out but there is a slight excess in the number of protons aligned parallel to $B_0$. Thus, there is a net-magnetization $\overrightarrow{M}$ in the direction of the external magnetic field as shown in figure 3 [16].

![Figure 3](image)

**Figure 3**: Alignment of protons in the presence of $B_0$ giving rise to a net magnetization $M$.

Under the effect of the external magnetic field, nuclei with nuclear spins have a characteristic resonant frequency. This can be explained via two models: classical mechanics and quantum mechanics [17]. As per the classical model, we can consider the nucleus as a top that spins about its axis. The combined effect of the gravitational force and its spinning movement, causes the top to precess. Similarly, the torque exerted by the external magnetic field on the spinning nucleus is the reason for its circular motion called precession. The rate of precession $\omega_0$ is called the Larmor frequency and it is directly proportional to the strength of the magnetic field as seen below in figure 4 where $\gamma$ is the gyromagnetic ratio.
As per quantum mechanics, the magnetic spins along the magnetic field and opposite of the magnetic field will have different energy states. In case of protons, as there are just two spin states \( m = \pm 1/2 \), there will be two energy levels. In the absence of the external magnetic field, the energies of the random orientations of the magnetic moments are the same. However, when an external magnetic field is applied, the nuclei align themselves in parallel or anti-parallel configurations. The energy difference between the two states is directly proportional to the external field \( B_0 \) and is given by equation (2.2), where \( h \) is the Planck’s constant. Equation (2.3) shows the difference in the number of parallel and anti-parallel nuclei, where \( k_B \) is the Boltzmann’s constant and \( T \) is the temperature in Kelvin.

\[
\Delta E = E_{\text{anti-parallel}} - E_{\text{parallel}}
\]

\[
\Rightarrow \Delta E = \frac{\gamma h B_0}{4 \pi} - \frac{-\gamma h B_0}{4 \pi} = \frac{\gamma h B_0}{2 \pi} = h \omega_0\ldots(2.2)
\]

\[
N_{\text{parallel}} - N_{\text{anti-parallel}} = N_{\text{total}} \frac{\gamma h B_0}{4 \pi k_B T}\ldots(2.3)
\]
2.1.2 Effect of Radiofrequency Pulses

In order to obtain the magnetic resonance signal, radiofrequency pulses must be applied. Magnetic resonance will occur only when an electromagnetic field (in the radiofrequency region of the electromagnetic spectrum) is applied with a frequency equal to the Larmor precession rate, in order to match the energy difference $\Delta E$ between the nuclear spin-levels under a constant field $B_0$. As the radiofrequency (RF) energy is transmitted by an RF transmit coil and for a short duration of time, it is called an RF pulse. At Larmor frequency, resonance occurs and there is efficient transfer of energy from the RF coil to the protons [16]. So, the energy transmitted can be represented as follow:

$$h f = \Delta E = \frac{\gamma h B_0}{2\pi}$$

$$\Rightarrow f = \frac{\gamma B_0}{2\pi} \text{ or, } \omega = \gamma B_0 \ldots (2.4)$$

The magnetic component of the RF pulse $B_1$ produces a torque when it acts in cohesion with the net magnetization $M$. The net magnetization of the protons can be expressed as follows, $\vec{M} = \hat{i}M_x + \hat{j}M_y + \hat{k}M_z$. When no RF pulse is applied, we can say $M$ is equal to $M_z$ (along the direction of the primary magnetic field $B_0$ as shown in figure 3), as there will be no torque acting due to $B_1$. After the application of the RF pulse however, $\vec{M}$ can be tipped to an angle $\alpha$, which is proportional to both the strength of the RF field and the time $\tau_{B1}$. If the RF pulse rotates the net magnetization $\vec{M}$ onto the transverse (x-y) plane, then it is termed as a 90° RF pulse. Similarly, a 180° RF pulse would rotate net magnetization towards the $-z$ direction [16]. The figure 5 below shows how RF pulse $B_1$ affects magnetization $\vec{M}$ and also shows the expression for the ‘tip’ angle $\alpha$. 


2.1.3 MR Relaxation Processes

When there is no RF pulse acting on the net magnetization $\overline{M}$ it is aligned with the longitudinal direction $M_z$ such that $M = M_z$. A 90° RF pulse rotates the longitudinal magnetization onto the transverse plane. Thus $M_z$ becomes zero and $M$ becomes equal to the transverse magnetization. The magnetization then starts to grow back into the longitudinal direction. This is known as longitudinal relaxation or $T_1$ relaxation (spin-lattice relaxation) [16]. The rate at which the protons relax is different for different tissues and is the main source of contrast in $T_1$ weighted images. The spin-lattice relaxation time, $T_1$ is defined as the time it takes the longitudinal magnetization to reach 63% of its final value, assuming it is a 90° RF pulse. The longitudinal magnetization $M_z$ is given by the following equation [14].

$$M_Z = M_0 \left(1 - e^{-t/T_1}\right)...(2.5)$$

We can recall that the protons begin to precess together or, ‘in phase’ under the influence of the RF pulse. This leads to the formation of the transverse magnetization. Immediately after the 90° RF pulse, these protons will rotate about their z-axis and start to ‘dephase’. This occurs as a result of protons precessing at slightly different frequencies due to spin-spin interactions. Due to
dephasing, the transverse magnetization keeps decreasing until the protons are completely dephased, at which the signal becomes zero. This process of relaxation is known as $T_2$ relaxation or spin-spin relaxation [16]. The spin-spin relaxation time, $T_2$ is defined as the time taken by the transverse magnetization to decay to 37% of its original value. Different tissues have different values of $T_2$ which gives rise to $T_2$ contrast. The transverse magnetization $M_{x,y}$ is given by the following equation.

$$M_{xy} = M_0 \left(e^{-t/T_2}\right) \ldots (2.6)$$

In biological tissues, there might be local variations in the $B_0$ field as it may not be perfectly homogenous. This causes the dephasing process to be accelerated. Hence, typically we do not measure a pure $T_2$ relaxation time but a faster relaxation time called $T_2^*$. Additionally, note that equations 2.5 and 2.6 are the solutions to the differential equations put forth by Felix Bloch, that are used to calculate $M$ as a function of $T_1$ and $T_2$ [18].

![Figure 6: $T_1$ relaxation (left) and $T_2$ relaxation (right).](image)
2.1.4 MR Signal Reception

The RF coil is often used both as a transmitter and a receiver. Faraday’s laws of induction states that the voltage $V$ induced in an electrical loop is proportional to the rate of change of magnetic flux $\phi$ [14].

$$V \propto -\frac{d\phi}{dt} \quad \text{(2.7)}$$

The decaying magnetization $M_{xy}$ will induce an electrical current, whereas the recovering $M_z$ will not, as only oscillating signals induce a voltage in the RF coil. The measured signal is often referred to as the free induction decay (FID) as shown in figure 7, because once the RF pulse is switched off, the signal from the protons precessing decays with time. The decay constant is given by the expression $1/T_2^*$, which is the rate with which $M_{xy}$ decays.

![Figure 7: Free induction decay (FID). Figure inspired by [19].](image)

This current can be digitized and stored for subsequent construction of an MR image. To visualize the signal from the nuclei, these decaying signals in the time domain are usually converted into their frequency domain counter-parts through a process known as the Fourier
Transform, invented by the French mathematician Joseph Fourier [20]. Thus, a signal in the time domain $S(t)$ can be represented in the frequency domain by the following equation where $\omega = 2\pi f$.

$$S(\omega) = \int_{-\infty}^{\infty} S(t) e^{i2\pi ft} dt \ldots (2.8)$$

2.1.5 MR Image Formation: Gradient Coils

The NMR signal does not contain any spatial information about the subject. This means that there is no way to differentiate between two points in space. Hence, gradient coils are used in MRI machines to vary the magnetic field in x, y and z directions. Depending on their function they are called the slice-select gradient, the readout or frequency encoding gradient, and the phase encoding gradients. Based on their directions, they can be termed as $G_x$, $G_y$, and $G_z$. Depending whether the slice is axial, coronal, or sagittal, $G_x$, $G_y$, and $G_z$ can be used for slice selection, frequency encoding, and phase encoding [20].

Let us consider the process of slice selection. Suppose $G_z$ represents a linear magnetic field gradient along the z direction with units T/m, where the magnetic field acting on precessing protons is $M_z = M_0 + zG_z$ [14]. Here, at $z = 0$, $M_z$ is equal to $M_0$. The precession frequency of the protons as a function of their position in the z-direction is given by the following equation:

$$\omega_z = \gamma z G_z \ldots (2.9)$$

For slice selection, a selective RF pulse is applied through the transmit coils in order to pick up these varying frequencies arising out of the gradient. For example if the RF pulse has a square shape in the frequency domain, it will only excite those nuclei which are precessing with frequencies in the applied range [20]. Let $\omega_s$ be the frequency of the applied RF pulse applied over an axial slice (which perpendicular to the long axis of the body) with a bandwidth of $\Delta \omega_s$. Then
the nuclei which get excited will have precession frequencies between \( \omega_s + \Delta \omega_s \) and \( \omega_s - \Delta \omega_s \). The thickness of the axial slice selected as shown in figure 8, is given by the following equation where \( T \) is the slice thickness and \( G_{\text{slice}} \) is one of the magnetic field gradients.

\[
T = \frac{2 \Delta \omega_s}{\gamma G_{\text{slice}}} \quad \text{(2.10)}
\]

**Figure 8:** Magnetic field variation due to gradient coil (left) and MRI slice selection (right). Figure inspired by [14].

The slice selection is followed by processes known as frequency encoding and phase encoding. Let us consider a \( 9 \times 9 \) matrix of voxels after an axial slice was selected by \( G_z \) as shown previously. Each voxel will precess with the same frequency \( \omega_0 \) (the Larmor frequency), however they will have different amplitude based on the number of protons in each voxel [20]. If frequency encoding gradient \( G_x \) is applied, we are able to differentiate these voxels based on their spatial frequencies. The central column of the \( 9 \times 9 \) matrix will have a same frequency \( \omega_0 \) as they will not feel the gradient. The right column however will experience a higher magnetic field and thus, the
protons in these voxels will have a higher spatial frequency \((\omega_0^+\))\). On the other hand, the left column will represent a lower magnetic field strength and thus, the protons will precess with a lower frequency \((\omega_0^-)\). Thus, the magnetic field is varied by the application of the gradient that varies in the x-direction and the voxels will start representing protons precessing with different frequencies. As a result, spatial frequency information gets encoded into the MR signal [14].

Similarly, by the application of phase encoding gradient \(G_y\), we are able to allocate different phase angle of spins to different spatial locations. Note that when \(G_x\) is applied, the columns of the \(9 \times 9\) are already arranged as per the different frequencies it represents and are thus out of phase with each other. However, each row still represent the same phase angle of spin. When \(G_y\) is applied, the protons in the top row experience a higher magnetic field than the bottom row as the magnetic field is varied in the y-direction. Hence, they precess faster and attain a different phase angle than the ones in the bottom row. Thus, with the both the gradients \(G_x\) and \(G_y\) acting together, the \(9 \times 9\) matrix of the axial slice could be represented by 9 unique groups of frequencies and phase angles [20].

2.1.6 MR Image Formation: The ‘k-space’

Applying the gradients mean that each voxel is spatially differentiated. All these data points are acquired from the digitized MR signal and stored in a two-dimensional \(m \times n\) data set of ‘k-space’, which is in the spatial frequency domain, by using two-dimensional Fourier transform. Each point in the k-space represents a particular frequency and phase and can be represented as \((k_{FE}, k_{PE})\) where,

\[
k_{FE} = \frac{\gamma}{2\pi} m G_{FE} t\ ...
\]
\[ k_{PE} = \frac{\gamma}{2\pi} n G_{PE} \tau \ldots (2.12) \]

FE refers to frequency encoding and PE refers to phase encoding, t and \( \tau \) are the durations for which the respective gradients are active, and m and n are the sample numbers in the frequency and phase encoding directions respectively [21]. The two-dimensional inverse Fourier transform of k-space data \( S(k_{FE}, k_{PE}) \), which is nothing but the spatial frequency with units cm\(^{-1} \), gives a complex image of \( S(x, y) \), which has both real and imaginary components. MR images are represented as the magnitude of these real and imaging components.

2.1.7 MRI Hardware

![Diagram of MRI scanner and components](image)

**Figure 9:** MRI scanner and components. Figure inspired by [22].
Above is a schematic representation of the basic components of an MRI scanner. They are placed inside a scan room with RF shielding, so that high power RF pulses are prevented from radiating out through the imaging room. The heart of the imaging system is the computer. It controls all the components of the imager. The other primary components are as follows:

a) **Magnet**

It is the most expensive component of the imaging system. It is responsible for producing the static $B_0$ magnetic field. Also known as a superconducting magnet, it is basically an electromagnet made up of a superconducting wire, usually in the form of a solenoid. Such a wire has little to no resistance when it is cooled to temperatures close to absolute zero (4.2 K) by immersing it in liquid helium. The most commonly used material for superconducting wires is an alloy of niobium-titanium [14]. Being flexible, wires made from this alloy can be shaped in the form of a solenoid, which produces a stable uniform magnetic field near the center of the solenoid axes.

In order to maintain liquid helium levels and prevent it from boiling off, the wire and the helium are surrounded by a series of radiation shields and vacuum vessels. These were themselves cooled by an outer container of liquid nitrogen. However, in modern scanners, cryogenic refrigerators are used for the same purpose. They have also diminished the need for replenishing the liquid helium inside the magnet (not before every 2 to 3 years) [22].

As the wire for generating the magnetic field is superconducting, once the magnet is energized by passing current through it, the current circulates through the wire indefinitely without the need of a power source. To improve the homogeneity of the main magnet, shim coils are used
to create a variety of opposing fields, such that it cancels out an inhomogeneity in the $B_0$ magnetic field.

b) **Gradient Coils**

The gradient coils produce the gradients in the x, y and z directions as explained in section 2.1.5 above. These are room temperature coils and based on the configuration, they create the desired gradient. The loud sound that is usually heard during MRI scan is due to the Lorenz forces created by the coils when currents are passed through them. These forces make the gradient coils vibrate and thus, produce the loud sound [23]. As described earlier in section 2.1.5, the gradient coils play a vital role in slice selection and in spatially differentiating the MR signal through frequency and phase encoding.

c) **RF Coils**

The radio frequency coils create the $B_1$ field that rotates the main magnetization vector $M$. They also detect the transverse magnetization as it precesses in the x-y plane. The RF coils can be divided into three categories: 1) transmit and receive coils, 2) transmit only coils, and 3) receive only coils. The RF coils must resonate and efficiently store energy at the Larmor frequency. They can also be divided into the classes of volume coils and surface coils. Volume coils provide RF excitation over a large volume, whereas surface coils are designed for smaller area samples. There are also examples of internal coils which are used for interventional MRI procedures which involve a catheter being inserted into the body with the RF coil being placed on its tip [22] [24].

2.1.8 **MRI Sequences**

An MRI sequence is a combination of RF and gradient pulses which are designed and sequentially arranged to acquire the data to form the desired image. Pulse sequence diagrams are
the schematic representations of these sequences that indicate the relative timing of each of these events. Each one the lines in a pulse sequence diagram represents a certain hardware. Typically, there are minimum four lines in any pulse sequence: one representing the RF transmitter, and the rest three representing each of the three gradient coils [21].

Figure 10: A simple timing diagram showing the RF pulse and the gradients. Figure inspired by [21].

As we see in figure 10 above, first the net magnetization $\vec{M}$ is excited with the application of a RF pulse, while the simultaneous slice select gradient helps select the image slice. It is followed by phase encoding and frequency encoding steps that are responsible for spatially localizing the protons in two dimensions. Gradient activity that does not change with each measurement (slice-select) is represented by a single amplitude or a constant deviation from the baseline. On the other hand, gradient activity which changes with measurement to measurement (phase encoding) are represented by a hashed region, signifying different amplitudes [21].
Additional lines may also be present to demonstrate the activity of other components such as the analog-to-digital converter.

The spin-echo sequence is one of the commonly used sequences in MRI. As we know, a 90° RF pulse is used to tip the net magnetization $\vec{M}$ of the protons onto the transverse plane. As these protons start to dephase (with different precession frequencies) due to $T_2^*$ effect, a 180° pulse is applied to flip the spin vectors, such that the protons precessing slower previously, now precess faster than the previously faster ones and thus, the spins start to rephase. So, in a sense, the decay due to the $T_2^*$ effect is reversed (although the signal will still decay due to spin-spin interactions as seen in figure 11) and what we get is a signal, which can be viewed as an echo of the original decaying signal.

The time taken to reach the peak of the echo after the application of the 90° RF pulse is known as the echo time (TE) [16]. The time after which the 180° refocusing RF pulse is applied is TE/2. Successive 90° and 180° RF pulses are again applied to continue this process of dephasing and rephasing of nuclear spins as shown in figure 11, until the transverse magnetization has completely decayed. The time between successive RF pulses is known as the pulse repetition time (TR).

By varying the TR and the TE time delays in the sequences, we can generate different types of contrast weightings in the MR image. For example, for short TEs ($\leq 20$ ms) and short TRs ($< 700$ ms) we can get $T_1$-weighted images. On the other hand, for long TEs ($\geq 80$ms) and long TRs ($>2000$ ms), $T_2$-weighted images can be acquired [25].
Figure 11: Dephasing of magnetization by $T_2^*$ and rephasing it by a 180° pulse during a spin echo sequence. Figure inspired by [16].

2.2 Diffusion MRI

Diffusion is a process in nature that involves the intermixing of particles without the need of bulk-motion. The physical law that explains this phenomenon is known as the Fick’s law (given below in equation 2.13) that relates the diffusion to the difference in concentration over space. In eq. 2.13, $J$ is the net particle flux, $C$ is the particle concentration and $D$ is the ‘diffusion coefficient’ [26].

$$J = -D \nabla C \ldots (2.13)$$

However, Robert Brown observed that microscopic motions of molecules occur even when there is no concentration gradient. This is known as the ‘random motion’ of particles suspended in fluid, or Brownian motion, resulting out of their collisions with other atoms or molecules. Einstein later was able to quantify this Brownian motion by using $D$ from Fick’s law as shown in eq. 2.14
where \( <x^2> \) is the mean-squared displacement over one dimension, \( D \) is the diffusion coefficient and \( t \) is the diffusion time.

\[
<x^2> = 2Dt \ldots (2.14)
\]

Edwin Hahn, who developed the ‘spin-echo’ experiment, was able to realize the effect of diffusion on the MR signal in 1950. He reported a reduction in the spin echo signal due to the dephasing of spins caused by diffusion [25]. In 1965, Stejskal and Tanner devised a pulsed gradient spin-echo sequence through which, they were able to quantify this attenuation in the MR signal due to the application of a pulsed gradient [27].

**Figure 12:** A diffusion weighted spin-echo pulse sequence.
Figure 12 represents a pulse sequence diagram for the diffusion weighted spin-echo sequence. The time interval between the onset of the diffusion sensitizing gradient and the refocusing pulse is represented by $\Delta$. In the spin echo method of DWI, two diffusion sensitizing gradient pulses are applied, one after the 90° RF pulse and the other after the 180° RF pulse. If there is no change in position of the particles due to Brownian motion (diffusion), the phase changes occurring due to the two pulses cancel out. However, if diffusion happens, the net phase change does not become zero and the overall signal gets attenuated which can be represented as $E(q)$ [26].

In equation 2.15, $E(q)$ is the loss in the MR signal due to diffusion, $b$ or ‘b-value’ depends on the imaging parameters and $D$ is the diffusion coefficient. Here $q = \gamma \delta G$, where $\gamma$ is the gyromagnetic ratio, and $\delta$ and $G$ are the duration and magnitude of the gradient pulse respectively [27].

$$E(q) = e^{-bD}, \text{where } b = q^2 \left(\frac{\Delta - \delta}{3}\right) \ldots (2.15)$$

$$S_i = S_0 e^{-b(\text{ADC})} \ldots (2.16)$$

In diffusion-weighted imaging, the term ‘apparent diffusion coefficient’ (ADC) is used as seen in equation 2.16. It is so because in tissue, the diffusion of water molecules is restricted and the diffusion rates vary based on the local microstructure. Thus, the value of ADC for water will appear to be smaller than that of $D$ (free water) [28]. The term $S_i$ is the diffusion weighted signal intensity of a voxel with the diffusion-sensitizing gradients applied along direction-\textit{i}, while $S_0$ is the signal intensity of the same voxel without diffusion sensitizing gradient. In diffusion weighted imaging (DWI), contrast is generated from these ADC values, arising out of performing the diffusion experiments with at least two b-values, one high and one low (zero for example).
Typically, biological tissue structures are complex and thus, they cannot be represented by a single ADC value, as it will vary depending on the exact orientation of anisotropic anatomic structures. Hence, instead of ADC, diffusion tensors (3-dimensional arrays) are used [29]. In a more advanced diffusion imaging technique, known as diffusion tensor imaging (DTI), instead of just two different b-values, six or more independent diffusion directions are taken into consideration to produce a set of diffusion images [25]. These diffusion weighted images can be converted into a variety of scalar and vector maps that describe a variety of tissue diffusion properties such as mean diffusivity, fractional anisotropy and so on.

![Figure 13](image)

**Figure 13:** Variation in diffusion ellipsoid with diffusion type. Figure inspired by [30].
The diffusion tensor can be conceptualized as an ellipsoid where the eigenvectors that define the three principal axes are the diffusion directions and its eigenvalues $\lambda_1$, $\lambda_2$, and $\lambda_3$, are the radii or the diffusion coefficients along the eigenvectors. The mean diffusivity MD is given by equation 2.17, and equation 2.18 represents the fractional anisotropy FA, which is a measure of the extent to which how anisotropic the diffusion is. Diffusion considered isotropic, that is equal in all directions, has a FA value of zero, which means that the eigenvalues are equal in all directions [28].

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \ldots (2.17)$$

$$FA = \sqrt{\frac{3}{2}} \times \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \ldots (2.18)$$

Thus, using the diffusion tensor model for diffusion in an anisotropic medium, equation 2.16 can be modified as follows [31].

$$S_i = S_0 e^{\hat{b}\hat{D}} \ldots (2.19)$$

where $\hat{b}\hat{D} = \sum_i \sum_j b_{ij} D_{ij}$

**2.3 Electron Paramagnetic Resonance**

Electron paramagnetic resonance (EPR) is a phenomenon that is similar to NMR, the difference being that in EPR, the subjects for the study of magnetic resonance are unpaired electrons. EPR is not as highly used as NMR as most stable molecules do not have unpaired electrons and involves the use of an external spin probe. However, this also means that EPR has great specificity as ordinary molecules do not give rise to EPR spectra.
2.3.1 Principles of EPR

Like nuclei, electrons have spin and hence, due to the combined effect of electrical charge and spin, they possess magnetic moment. Similar to NMR, under the influence of an external magnetic field $B_0$, these electron spins arrange themselves in parallel and anti-parallel configurations. The energy $E$ of a magnetic moment depends on its orientation relative to $B_0$, as shown in equation 2.20, where $g$ in the electron spin $g$-factor (2.0023) and $\mu_B$ is Bohr magneton [12].

$$E = \pm \frac{1}{2} g \mu_B B_0 \ldots (2.20)$$

The difference in energy levels $\Delta E$ of these configurations can be described in terms of the Larmor frequency $\omega_0$, where $\hbar$ is the reduced Planck’s constant and $\gamma_e$ is the electron gyromagnetic ratio.

$$\Delta E = \hbar \omega_0 = g \mu_B B_0$$

$$\Rightarrow \omega_0 = \gamma_e B_0 \ldots (2.21)$$

![Figure 14: Electron spin states in the presence of external magnetic field.](image)
The net magnetization of these electron spins can be perturbed by the application of a B₁ magnetic field, just as it is done in NMR. The solutions to the Bloch equations, which describe the relaxation of spins once the B₁ field is removed are given below:

\[ M_T(t) \propto \exp(i \Omega t) \cdot \exp \left( -\frac{t}{T_2} \right) \] \hspace{1cm} (2.22)

\[ M_Z(t) \propto (1 - M \exp \left( -\frac{t}{T_1} \right)) \] \hspace{1cm} (2.23)

In the above equations, \( \Omega \) is the difference between the Larmor frequency and the operating frequency of the spectrometer, \( M_T(t) \) and \( M_Z(t) \) are the transverse and longitudinal magnetization vectors respectively. Typical EPR T₁ and T₂ relaxation times are of the order of nano to micro seconds. \( M \) represents the initial state of longitudinal magnetization. The captured EPR signal in the time domain is proportional to \( M_T(t) \) [12].

2.3.2 EPR Oxygen Imaging

The oxygen molecule has two unpaired electrons in its triplet state which explains its paramagnetic behavior. Upon interaction with a spin probe, it enhances its relaxation time via the Heisenberg spin exchange. This leads to an oxygen concentration dependent increase in the signal of the probe [32]. The Smoluchowski diffusion equation predicts a linear relationship between pO₂ and the relaxation rates that has been validated for many radicals. Thus, it allows a direct measure of pO₂ with a precision exceeding 1 torr and is a unique feature of EPR oximetry.

\[ R_1 = A \cdot [pO_2] + B \cdot R_x \] \hspace{1cm} (2.24)

In equation 2.24, \( R_1 \) is the spin-lattice relaxation of the injected spin-probe where \( R_1 = 1/T_1 \), \([pO_2]\) is the relaxation due to absolute pO₂ concentration and \( R_x \) is due to factors such as temperature, salinity and viscosity. Both A and B are proportionality constants. As the contribution
from these other factors is negligible under normal conditions, EPRI thus becomes a tool for the
direct measure of oxygen concentration in tissue and hence, in detecting hypoxia [29].

2.3.3 EPR Imaging Equipment

The design of the EPR imager depends on two factors, sensitivity and penetration depth. Sensitivity rises with increasing frequency, whereas the penetration depth of the RF radiation falls with the increasing frequency. Currently, in vivo imagers operate in mainly three frequency ranges: 250–350 MHz, 550–750 MHz, and 1–1.2 GHz or L-band. 250-350 MHz machines are used for larger animals whereas L-band ones are used for small animals or peripheral anatomy [12]. The gradient coils design in EPR is similar to that of MRI. Below is a brief comparison between EPRI and MRI:

<table>
<thead>
<tr>
<th>Category</th>
<th>MRI</th>
<th>EPRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic field at 250 MHz</td>
<td>5.9 T</td>
<td>9 mT</td>
</tr>
<tr>
<td>Radiofrequency pulse width</td>
<td>μsec – msec</td>
<td>10 – 100 nsec</td>
</tr>
<tr>
<td>Relaxation rates</td>
<td>msec – sec</td>
<td>nsec - μsec</td>
</tr>
<tr>
<td>Endogenous probes</td>
<td>Water protons</td>
<td>-</td>
</tr>
<tr>
<td>Exogenous probes</td>
<td>-</td>
<td>Nitroxides, trityls</td>
</tr>
<tr>
<td>Concentration</td>
<td>&gt;60 M</td>
<td>~100 μM</td>
</tr>
<tr>
<td>Stability</td>
<td>Stable</td>
<td>Minutes</td>
</tr>
<tr>
<td>Line width</td>
<td>Hz – kHz</td>
<td>100 kHz - MHz</td>
</tr>
</tbody>
</table>

**Table II:** A comparison between MRI and EPRI.
3. CORRELATION OF TUMOR VISCOSITY WITH TUMOR HYPOXIA

Water protons are the primary source of NMR or MRI signal in tissue. As they are mobile, the random motion of the water molecules in tissue can be characterized by their ADC values [33]. As per the Stokes-Einstein equation, from the self-diffusion coefficient of an isolated Brownian particle, we can calculate the viscosity of the diffusion medium. Equation 2.25 is the Stokes-Einstein expression for self-diffusivity where D is the diffusion coefficient, ‘a’ is the radius of the particle, ‘k_B’ is the Boltzmann constant and η is the viscosity of the diffusion medium.

\[
D = \frac{kT}{6\pi\eta r} \ldots (2.25)
\]

⇒ viscosity (η) = \frac{k_B T}{6 \pi r D} \ldots (2.26)

In hypoxic regions, the additional factors contributing to the relaxation rate of the spin probe in EP ROI (as seen in equation 2.24) cannot be neglected and these factors need to be accounted for. The variations in temperature and salinity are relatively negligible, position independent and their effects on relaxation rates can be accounted for using calibration experiments. Viscosity, however, varies between healthy and cancerous tissue and will affect the oxygen measurement [34]. The purpose of this study was to demonstrate a relationship between tissue viscosity, and its corresponding pO_2. The entire viscosity-MRI study was conducted as follows:

3.1 Materials, Methods and Results

3.1.1 Diffusion MRI Phantoms

The refinement of diffusion MRI imaging techniques as per the needs of the later stages of the study is essential. Water-glycerol phantoms with three different viscosities were used as a part
of the imaging refinement process. They had viscosities of 0.89 cP (distilled water), 3 cP and 10 cP at 25 °C and were prepared by mixing 0%, 38.5% and 62.1% glycerol in water. The phantoms were prepared based on water-glycerol viscosity values from the works of N. E. Dorsey [35]. These phantoms were used to create viscosity maps using the Stokes-Einstein relation (equation 2.26) and obtain the calibration parameter to covert the apparent diffusion maps to viscosity maps. Equation 2.26 could be re-written with an additional factor $\lambda$ in the numerator, where it is the conversion coefficient and was found to be $\sim 0.8$ at 25°C:

The DWI experiments were performed at a 56 mm vertical bore 11.7 T Bruker AVANCE micro-imaging facility equipped with linear triple axis gradients (maximum gradient strength 200G/cm) and a 10 mm RF coil. The experimental parameters were as follows: pulse protocol = diffusion weighted spin echo MRI sequence, slice thickness = 2 mm, number of slices = 7, FOV = 7 mm x 7 mm, TE/TR = 20 ms /1500 ms, $\delta = 5$ ms, $\Delta = 10$ ms, matrix size = 128 x 128 and $b$-values = 0, 100, 200, 300, 500, 750, 1000, and 1500 s/mm$^2$.

![Figure 15: The Bruker 500 AVANCE micro-imaging scanner.](image)
The diffusion maps were calculated using MRI analysis software (MAS), a cross-platform software application written in Interface Description Language (IDL), developed by Mr. William Triplett at the University of Florida. Using the conversion equation given above and known viscosities of water-glycerol phantoms, these maps were converted into the viscosity maps [36]. Below is the figure showing the diffusion map of these phantoms and the corresponding viscosity map derived from the Stokes-Einstein equation and calibration parameter $\lambda$.

![ADC maps of phantoms (left) and their viscosity maps (right).](image)

**Figure 16:** ADC maps of phantoms (left) and their viscosity maps (right).

As seen in figure 16, ADC maps were collected from the water-glycerol phantoms and the corresponding viscosity maps were constructed using a custom-built MATLAB program. The results from the phantoms showed that the used techniques were able to correctly reproduce the standardized viscosity values for the phantoms and thus, the same procedure could be forwarded for *ex vivo* and *in vivo* measurements. The calculated viscosity values from diffusion MRI were
plotted against the expected viscosity values. The relation between them was found to be highly linear with a coefficient of determination value of 0.99 as shown in figure 17.

![Expected Viscosity vs MRI Viscosity (cP)](image)

**Figure 17**: MRI viscosity vs. expected viscosity (cP)

### 3.1.2 Animal Preparation for *ex vivo* and *in vivo* Studies

5 × 10^5 murine tumors cells were injected intramuscularly to the hind limbs of 6 to 8 weeks old C3H mice. The tumors cells were either FSa Fibrosarcoma or Mca4 Carcinomas [7]. These tumors grew to between 1 to 5 cm³ within 1 to 2 weeks. For the *ex vivo* MRI experiments (n = 6), the mice were euthanized using CO₂ (followed by cervical dislocation) and the tumor tissue was fixed using 10% formalin. For the *in vivo* experiments that involved sequential EPROI and MRI experiments, the tumor leg was immobilized using a dental mold of vinyl polysiloxane. Both *in*
*vivo* MRI and EPROI experiments were performed sequentially under isoflurane anesthesia inside facilities at the University of Chicago.

3.1.3 *Ex vivo* Experiments

All *ex vivo* MRI experiments were done using a 9.4 T preclinical Agilent MRI scanner at the Research Resources Center of University of Illinois at Chicago. This machine is equipped with a 38 mm RF coil.

![9.4T pre-clinical scanner at UIC](image)

**Figure 18:** The 9.4T pre-clinical scanner at UIC.

For the *ex vivo* DWI experiments performed on 6 fixed Fsa tumor samples, following parameters were used: pulse protocol = spin echo diffusion tensor MRI, slice thickness = 0.75 mm, number of slices= 20, FOV = 3 cm x 3 cm, TE/TR = 30 ms /4000 ms, number of gradient directions = 6, $\delta = 5$ ms, $\Delta = 20$ ms, matrix size $= 128 \times 128$ and b-values $= 0$ and 1500 s/mm$^2$. The process of obtaining diffusion and viscosity maps was the same as used for phantoms as described in section 3.1.1.
**Ex vivo results:** As seen in figure 19 and table III, the mean viscosity for the tumor region was distinctly higher than that of normal tissue [37]. SEM is the standard error in the measurement.

![ADC map and viscosity map](image)

**Figure 19:** ADC map of an ex vivo specimen (left) and corresponding viscosity map (right).

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Tumor Mean (± SEM) (in cP)</th>
<th>Normal Mean (± SEM) (in cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSa (n = 6)</td>
<td>3.28 (± 0.09)</td>
<td>2.13 (± 0.07)</td>
</tr>
<tr>
<td><em>ex vivo</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III:** Mean viscosity in tumor and normal region (*ex vivo* for FSa).

3.1.4 *In vivo* Experiments

1) **DWI**

*In vivo* experiment was performed upon a single animal with Mca4 tumor in its hind limb. It was done using a 9.4T Bruker MRI Scanner with a 30 mm RF coil at the University of Chicago.
The experimental parameters were as follows: TE/TR = 26 ms / 3500 ms, slice thickness = 0.75 mm, number of slices = 7, δ = 7 ms, Δ = 14 ms, FOV = 2.56 cm x 2.56 cm, matrix size = 128 x 128, and b-values = 0, 50, 250, 500, 750, 1000, and 1500 s/mm², total experimental time ~ 44 min.

2) EPROI

EPR experiments were performed at the Center for EPR Imaging in Vivo Physiology in the Department of Radiation and Cellular Oncology, University of Chicago. It was performed before the in vivo MRI imaging of the same mouse. A pulsed EPR inversion recovery sequence was used for image acquisition. A total of 208 equal solid angle projections were acquired with maximum gradient of 15 mT/m and an isotropic field of view of 4.24 cm. Images were reconstructed using a filtered back projection algorithm.

A triarylmethyl radical OX063, also known as trityl, is a commonly used spin probe for EPR oxygen imaging. It was injected into the mice via the tail vein. It is the spin probe which gives rise to the EPR signal and its signal is enhanced in the presence of oxygen. As the spin-probe has a tissue and tumor half-life lives of just 5 and 30 minutes respectively, additional dose is required after the initial bolus to maintain the signal and image quality [38]. Hence, an infusion pump is used. In this experiment, the rate of infusion was set at 0.78 mmol/kg/hr during imaging time. Image was acquired in 10 minutes and had 1.5 mm spatial and 1 torr pO₂ resolution. Using MATLAB program built by Dr. Boris Epel at the University of Chicago, viscosity images from MRI were registered with EPROI images.
3) *In vivo* Results

The findings from the single *in vivo* specimen with Mca4 tumor, as seen in table IV and figure 21, were also similar to that in the *ex vivo* samples. The data shows that the region of lower oxygen concentration (hypoxic) has a higher viscosity and thus, this indicates that viscosity might have a significant role to play in altering the relaxation rates of the oximetry spin probe. In figure 21, (A) is viscosity (in centipoise or cP) and (B) is the oxygen image (using EPROI) from an axial slice of a murine leg with Mca4 tumor *in vivo*. (C), and (D) are histograms for viscosity and pO$_2$ values from the whole image (blue) and from the tumor volume only (red). Only 7 slices (approximately 25% of tumor volume) were acquired for the viscosity map. Thus, it shows a
smaller amount normal tissue in viscosity histogram. The pO₂ images were acquired for the whole tumor volume.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumor</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cP)</td>
<td>3.49 (± 0.013)</td>
<td>2.30 (± 0.015)</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pO₂ (torr)</td>
<td>11.07 (± 0.16)</td>
<td>27.4 (± 0.30)</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table IV:** In vivo mean viscosity and pO₂ in tumor and normal region for MCa4 (n = 1).

**Figure 21:** Comparison of tissue viscosity and partial oxygen pressure in vivo [37].
Figure 22 below gives the relation between pO$_2$ and viscosity by showing change in viscosity as a function of pO$_2$ in the tumor region \textit{in vivo}. Interestingly, there is a strong negative correlation between tissue oxygenation between pO$_2$ and viscosity in the hypoxic region but the correlation becomes positive for normoxic areas inside the tumor. Further \textit{in vivo} experiments with a larger sample size is required to fully comprehend the reason for this.

\textbf{Figure 22:} The change in viscosity as a function of pO$_2$.
4. CORRELATION OF TUMOR MICROSTRUCTURE WITH TUMOR HYPOXIA

Tumors have been known to initiate drastic changes in tissue microstructure. Unlike normal tissue, tumors lack a sufficient vessel hierarchical structure that is essential for feeding a regularly spaced capillary bed. This disorganization and reduced perfusion leads to higher interstitial pressure and a chaotic vascular tissue structure [39]. DTI based fractional anisotropy (FA) has been often used in studying such changes in tissue microstructure, especially in brain tumors [40].

This study shows how DTI as a technique can also be used to correlate tissue microstructure with hypoxia in tumors and its potential to contribute to targeted radiation therapy. The DTI study was carried out as follows:

4.1 Materials, Methods and Results

4.1.1 Animal Preparation for ex vivo and in vivo studies

Animal preparation was in no way different than in the viscosity studies because the study interest (FA) was a different parameter in the same mouse model. 6 mice samples with FSa tumors in their hind limbs fixed in 10% formalin were used for the ex vivo experiments, whereas a single mouse with MCa4 tumor was used for the in vivo experiment.

4.1.2 Ex vivo Experiments

Deceased mice samples (n = 6) with Fibrosarcomas (FSa) were fixed with 10% formalin and were kept inside a conical for DTI at the 9.4 T RRC Preclinical Facility at UIC. The parameters used were as follows: Pulse protocol = spin echo DTI (semsdw), TE/TR = 30 ms / 4 s, number of slices = 20, slice thickness = 0.75 mm, b-values = 0, 1500 s/mm2, $\delta = 5$ ms, $\Delta = 20$ ms, field of view = 3cm × 3cm, matrix size = 128 × 128, number of different gradient directions = 6.
Figure 23: *Ex vivo* mean diffusivity (A) and fractional anisotropy (B) maps [29].

**Ex vivo results:** The mean diffusivity in tumor region ($MD_{tumor} = 0.61 (\pm 0.1) \times 10^{-3} \text{ mm}^2/\text{s}$) was found to be lower than that of normal tissue ($MD_{normal} = 0.93 (\pm 0.2) \times 10^{-3} \text{ mm}^2/\text{s}$). This was very much in concurrence with already established work and our result in chapter 3 [41]. The mean FA value for tumor was also lower, 0.34 ($\pm 0.01$) as against 0.36 ($\pm 0.01$) for normal regions. Thus, the difference in FA was only marginally lower. This could be the result of fixing of the tumor sample because fixing introduces cross linking of tissue and the loss in original properties [42].

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Tumor Mean (± SEM)</th>
<th>Normal Mean (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSa (n = 6)</td>
<td>0.34 (± 0.014)</td>
<td>0.36 (± 0.013)</td>
</tr>
</tbody>
</table>

**Table V:** Mean FA in tumor and normal region (*ex vivo* for FSa).
4.1.3 *In vivo* Experiments

1) **DTI**

An *in vivo* (with MCa4 tumor) DTI experiment was performed on a mouse using a preclinical 9.4 T Bruker MRI scanner with a custom-built 30 mm RF coil. The experimental parameters were: TE/TR = 19 ms / 2500 ms, slice thickness = 0.75 mm, number of slices = 3, δ = 7 ms, Δ = 14 ms, FOV = 2.56 cm x 2.56 cm, matrix size = 128 x 128, and b-values = 0, 2500 s/mm², total experimental time ~ 39 min, number of gradient directions = 6.

2) **EPROI**

The EPR experiment performed again was the same as the one performed in case of viscosity mapping of MRI. The anesthetized mouse following the DTI experiment was taken to the EPR imager, following which it was euthanized.

3) **In vivo** Results

As seen in table VI, the difference in FA in this case is far more substantial than in the case of fixed tissue. The mean FA in tumor 0.53 (± 0.01) is lower when compared with mean FA in normal region 0.66 (± 0.01).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumor</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA Mean (± SEM)</td>
<td>0.53 (± 0.01)</td>
<td>0.66 (± 0.01)</td>
</tr>
<tr>
<td>pO₂ (in torr) Mean (± SEM)</td>
<td>11.07 (± 0.16)</td>
<td>27.4 (± 0.30)</td>
</tr>
</tbody>
</table>

*Table VI: In vivo* mean FA and pO₂ in tumor and normal region for MCa4 (n = 1) [29]
Figure 24 shows the coregistered pO\(_2\) and FA for MCa4 tumor. Here, (A) is Fractional anisotropy (FA) and (B) is a pO\(_2\) image from an axial slice of murine leg with MCa4 tumor in vivo. (C), and (D) show histograms for FA and pO\(_2\) values respectively, for the whole image (blue) and for the tumor volume only (red). The cyan contour on both FA and pO\(_2\) images represents the digitized tumor contour. On the FA image, slice contours of 10 and 5 torr pO\(_2\) from EPROI are shown.

![Figure 24: Comparison of tissue fractional anisotropy and pO\(_2\) in vivo [29].](image)

When the pO\(_2\) and the FA inside the tumor volume, were plotted against each other, we get a Pearson’s correlation coefficient of R = 0.85, which shows a strong correlation between FA and pO\(_2\). However, similar to viscosity MRI, more in vivo samples are required to establish a concrete relationship between the two.
Figure 25: Correlation between mean pO$_2$ and FA values within the *in vivo* tumor volume [29].
5. CONCLUSIONS AND FUTURE WORK

This study shows the preliminary results of using MRI to compare tissue viscosity and FA with pO$_2$ concentration from EPROI. As we have seen in chapters 3 and 4, hypoxic regions in the FSa and MCa4 solid tumors show higher viscosity, and lower FA in tumors. The FA values in tumor shows a positive correlation with pO$_2$ concentration. Further in vivo experiments with large sample size are required to validate the findings that have come out in these early results.

The long-term goal of this project is to improve the efficiency of radiation therapy by taking into account the O$_2$ concentration and tissue microstructure information. Since EPROI is a functional imaging technique that provides pO$_2$ maps, it along with tissue fractional anisotropy maps from MRI can be applied for efficient treatment plans for the tumor. Thus EPROI, in conjunction with MRI, will be important for future radiation treatment.

Figure 26: A micro computed tomography (micro-CT) system (left) that can be used to target hypoxic tumors. EPR information (right) can be used for targeted radiation delivery.
A radiation therapy machine like an x-ray micro-CT system as shown in figure 26 can be used to target hypoxic areas inside a tumor. If $pO_2$ maps from EPROI and tissue information from DWI and DTI can be successfully registered with x-ray micro-CT (as conceptualized in figure 27), it promises to give radiologists the capability of creating target volumes inside a tumor where more radiation needs to be given to hypoxic area, and apply a lesser dose in normoxic areas. This would go a long way in minimizing the overall radiation dose and ensuring a healthier quality of life for the patient.

**Figure 27**: EPR and MRI conjunction in radiation therapy.
6. REFERENCES


7. VITA

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Education

Master of Science in Bioengineering
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Research Experience

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  Conducted diffusion weighted magnetic resonance imaging experiments on *ex vivo* and *in vivo* tumor samples for MS thesis project titled, “Correlating Tumor Microstructure With Hypoxia Using Magnetic Resonance Imaging”.

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Teaching Experience

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Conference Abstracts


Conference Publications