Multi-parametric Magnetic Resonance Imaging (MRI) in Prostate Cancer

by

Deanna Lyn Langer

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy, Graduate Department of the Institute of Medical Science University of Toronto

© Copyright by Deanna Lyn Langer 2010
Multi-parametric Magnetic Resonance Imaging (MRI)

in Prostate Cancer

Deanna Lyn Langer

Doctor of Philosophy 2010

Institute of Medical Science

University of Toronto

Abstract

Prostate cancer is extremely prevalent, with shifting patient demographics leading to an increasing number of men balancing treatment efficacy with associated side-effects. Non-invasive characterization of disease – useful for guiding biopsy, to monitor disease progression during active surveillance, or for treatment planning of focal therapies – could have a significant impact on patient management. Through its excellent anatomic imaging capabilities and its ability to characterize physiologic properties, magnetic resonance imaging (MRI) has the potential to fulfill clinical goals; however, further improvements are necessary to maximize accuracy and impact. Thus, this thesis presents: 1) the development of a multi-parametric model to combine parameters derived from measurement of T2 relaxation, diffusion weighted imaging, and dynamic contrast-enhanced MRI to improve the discrimination between normal and malignant peripheral zone tissue; 2) determination of the impact that the
presence of normal tissue within regions of tumour has on the measurement of apparent diffusion coefficient (ADC) and T2 relaxation in the peripheral zone; and 3) relationships between MRI measurement and underlying prostate tissue composition. A common patient cohort was used for all studies, with prostate cancer patients having \textit{in vivo} MRI prior to prostatectomy followed by whole-mount histologic sectioning of the surgical specimens, facilitating the use of pathology as a gold-standard for all analyses. In the first study, the optimal multi-parametric model combines ADC, T2, and volume transfer constant ($K^{\text{trans}}$) to yield the probability of malignancy for each voxel. Performance of the model is better than each single parameter, but not significantly so compared to ADC. The second study demonstrates that there is no difference in ADC and T2 between tumours containing significant portions of normal tissue and the surrounding normal tissue itself, indicating that full characterization of prostate cancer with MRI may be limited. Finally, by determining relationships between MRI parameters and tissue characteristics, the third study suggests mechanisms driving MR image appearance in the prostate, including the visualization of cancer. Taken together, this thesis presents potential improvements to prostate cancer imaging, and provides further insight into the interplay between the underlying histology and MRI.
Acknowledgements

I would like to acknowledge both the professional and the personal support I have received over the past years. I have been privileged to work for, work with, and be assisted by a strong team of co-workers, friends and family; and although I cannot possibly put quite the right words together to give appropriate thanks, I'll do what I can.

I am fortunate to have been funded through a number of sources, and would like to thank the Prostate Cancer Research Foundation of Canada, Muzzo Fund (Princess Margaret Hospital Foundation), Campbell Family Cancer Research Institute Prostate Cancer Program, the University of Toronto and Institute of Medical Science (Open Fellowships), and the International Society of Magnetic Resonance in Medicine for stipend and conference funding.

I would like to thank my Masters supervisor, John Rowlands, PhD, for laying the groundwork for any time I’ve managed to be successful in conveying my ideas to an audience. I would also like to thank my current supervisory committee: Brian Wilson, PhD, John Trachtenberg, MD, CM, and Greg Stanisz, PhD; my meetings have been an (almost) pleasant experience, and I’ve appreciated your contributions and assistance.

The project itself simply wasn’t feasible without the commitment and hard work of everyone involved, and I’ve been lucky to have a diverse, talented, and friendly team to work within. I would particularly like to thank Andrew Evans, MD, PhD, and Theodorus van der Kwast, MD, PhD, for their dedication and enthusiasm for work that required a considerable amount of increased effort from the usual clinical routine, and for being a tonne of fun to work with in the pathology lab. In addition, I would like to credit Dev Olshansky in pathology at Toronto General Hospital for her extra efforts in preparation, and Laibao Sun, MB, MSc, at Sunnybrook for generating exquisite whole mount sections to serve as the base for all pathology interpretation.
Each chapter seemed to require a new set of statistical tools, and so I would like to acknowledge the valuable advice and consultation received from Gina Lockwood, MMath and Melania Pintilie, MSc in the Department of Biostatistics at the Princess Margaret Hospital, as well as friends and statistical gurus Gabrielle Page, Manolo Romero, and Norman Farb.

I would like to give heartfelt thanks to my supervisor, Masoom Haider, MD, for giving me the opportunity to branch out into a truly translational space – allowing me to join a fantastic project in its early stages, and letting me learn and grow along the way. I’ve appreciated your direction and our discussions, and am grateful that you took the formal leap of faith into academic supervision and all of its administrative hassles. I sincerely hope I haven’t done too much to scare you off doing it again in the future.

I’m lucky to have a strong community of friends and teammates behind me, both at work and at play. Thanks to Jeremy Hoisak for keeping me well-caffeinated over many years, and to Lisa Di Diodato for her open door and her friendship. I would also like to thank the extensive volleyball and ultimate communities for their support and escapism – especially ‘Tula ladies, Tri-campus volleyball, and the entire Tuesday night volleyball crew.

And – finally – to my family: thanks, Adriana and Bob Brown, for keeping me fed at least once a week and for your unwavering advocacy; thank you, Mom and Bob, for an environment that encouraged curiosity from the beginning, and for our family’s unique balance of creativity and logic. And to my partner, Kevin: thank you for challenging me to be my best in sport, science, and all of our other endeavours; for your unconditional support; and for all adventures along the way. Thank you for sharing the journey.
# Table of Contents

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1: Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Prostate Cancer: Diagnosis and Treatment</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Magnetic Resonance Imaging of the Prostate and Prostate Cancer</td>
<td>9</td>
</tr>
<tr>
<td>1.3.1 T2-weighted MR Imaging</td>
<td>9</td>
</tr>
<tr>
<td>1.3.2 Parametric MRI</td>
<td>14</td>
</tr>
<tr>
<td>1.4 Multi-parametric Magnetic Resonance Imaging</td>
<td>25</td>
</tr>
<tr>
<td>1.5 Summary of Aims and Research Contributions</td>
<td>29</td>
</tr>
<tr>
<td><strong>Chapter 2: Methods</strong></td>
<td>32</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>33</td>
</tr>
<tr>
<td>2.2 Patient Population</td>
<td>33</td>
</tr>
<tr>
<td>2.3 Magnetic Resonance Imaging</td>
<td>34</td>
</tr>
<tr>
<td>2.4 Whole-mount Histology</td>
<td>42</td>
</tr>
<tr>
<td><strong>Chapter 3: Prostate Cancer Detection with Multi-parametric Magnetic Resonance Imaging</strong></td>
<td>46</td>
</tr>
<tr>
<td>3.1 Abstract</td>
<td>47</td>
</tr>
<tr>
<td>3.2 Introduction</td>
<td>48</td>
</tr>
<tr>
<td>3.3 Methods</td>
<td>50</td>
</tr>
<tr>
<td>3.4 Results</td>
<td>54</td>
</tr>
<tr>
<td>3.5 Discussion</td>
<td>61</td>
</tr>
<tr>
<td>3.6 Summary</td>
<td>65</td>
</tr>
<tr>
<td><strong>Chapter 4: The Impact of Intermixed Normal Tissue within Prostate Cancer on Measurements of Apparent Diffusion Coefficient and Quantitative T2</strong></td>
<td>67</td>
</tr>
<tr>
<td>4.1 Abstract</td>
<td>68</td>
</tr>
<tr>
<td>4.2 Introduction</td>
<td>69</td>
</tr>
<tr>
<td>4.3 Methods</td>
<td>70</td>
</tr>
</tbody>
</table>
Table of Tables

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1: Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>Table 1.1 – Risk Group Stratification for Clinically Localized Prostate Cancer</td>
<td>6</td>
</tr>
<tr>
<td>Table 1.2 – Tumour Localization Using T2-weighted MRI @1.5-T with endorectal coil</td>
<td>14</td>
</tr>
<tr>
<td>Table 1.3 – Quantitative T2 in Normal and Malignant Prostate</td>
<td>16</td>
</tr>
<tr>
<td>Table 1.4 – Apparent Diffusion Coefficient (ADC) in the Prostate</td>
<td>20</td>
</tr>
<tr>
<td><strong>Chapter 2: Methods</strong></td>
<td></td>
</tr>
<tr>
<td>Table 2.1 – Characteristics of Twenty-nine Prostate Cancer Patients</td>
<td>34</td>
</tr>
<tr>
<td>Table 2.2 – Acquisition Parameters for <em>in vivo</em> MRI</td>
<td>37</td>
</tr>
<tr>
<td><strong>Chapter 3: Prostate Cancer Detection with Multi-parametric Magnetic Resonance Imaging</strong></td>
<td></td>
</tr>
<tr>
<td>Table 3.1 – Summary Parameter Values for Tumour and Normal Prostate in Peripheral Zone (PZ) Tissue</td>
<td>56</td>
</tr>
<tr>
<td><strong>Chapter 4: The Impact of Intermixed Normal Tissue within Prostate Cancer on Measurements of Apparent Diffusion Coefficient and Quantitative T2</strong></td>
<td></td>
</tr>
<tr>
<td>Table 4.1 – Summary of median ADC and T2 values measured in normal peripheral zone tissue, sparse tumour, and dense tumour</td>
<td>79</td>
</tr>
<tr>
<td>Table 4.2 – Summary of tumour-to-normal matched-pair differences and log2−transformed ratios for ADC and T2 in sparse and dense tumours</td>
<td>79</td>
</tr>
<tr>
<td><strong>Chapter 5: Investigative the Relationship between Magnetic Resonance Imaging-Derived Parameter Values and Tissue Composition in the Prostate</strong></td>
<td></td>
</tr>
<tr>
<td>Table 5.1 – Mean Slope ± Standard Error for MRI Parameter <em>versus</em> Proportion of Cellular Component</td>
<td>94</td>
</tr>
<tr>
<td>Table 5.2 – Summary MRI Parameter Values for Tumour and Normal Prostate in Peripheral Zone Tissue</td>
<td>95</td>
</tr>
<tr>
<td>Table 5.3 – Summary Values for the Percentage Area of Cellular Components in Tumour and Normal Peripheral Zone Tissue</td>
<td>95</td>
</tr>
</tbody>
</table>
# Table of Figures

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1: Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 1.1 – Male anatomy through the level of the prostate.</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.2 – Prostate zonal anatomy: sagittal (a) and transverse (b) views</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.3 – Gleason grade.</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.4 – Planes of Human Anatomy.</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.5 – T2-weighted MRI of the Prostate at 1.5-Tesla.</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.6 – T2-weighted MRI (a) and quantitative T2 map (ms) (b) from corresponding slice locations.</td>
<td>16</td>
</tr>
<tr>
<td>Figure 1.7 – T2-weighted MRI (a) and map of apparent diffusion coefficient (ADC) (mm²/s) (b) from corresponding slice locations.</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.8 – T2-weighted MRI (a) and map of the volume transfer constant ($K_{trans}$) (min⁻¹) (b) from corresponding slice locations.</td>
<td>24</td>
</tr>
<tr>
<td><strong>Chapter 2: Methods</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 2.1 – Determination of oblique MRI imaging planes.</td>
<td>36</td>
</tr>
<tr>
<td>Figure 2.2 – T1 estimation of gadolinium-doped vials using inversion recovery (a) and multiple flip angle (b) acquisitions, with comparison of mean values (c).</td>
<td>40</td>
</tr>
<tr>
<td>Figure 2.3 – Contrast agent concentration in tissue ($C_t$) versus time: sample data and curve fit using two-compartment model and assumed arterial input function.</td>
<td>42</td>
</tr>
<tr>
<td>Figure 2.4 – Gel embedding and 3 mm sectioning of fixed surgical specimen.</td>
<td>43</td>
</tr>
<tr>
<td>Figure 2.5 – Whole-mount H&amp;E-stained section (a) with corresponding ex vivo (b) and in vivo (c) T2-weighted MRI.</td>
<td>45</td>
</tr>
<tr>
<td><strong>Chapter 3: Prostate Cancer Detection with Multi-parametric Magnetic Resonance Imaging</strong></td>
<td></td>
</tr>
<tr>
<td>Figure 3.1 – Manual alignment of parameter maps according to outer prostate contour.</td>
<td>51</td>
</tr>
<tr>
<td>Figure 3.2 – Sample distribution of values from a single patient for regions of interest in normal (blue) and malignant (red) tissue for all single parameters.</td>
<td>52</td>
</tr>
<tr>
<td>Figure 3.3 – Median values for all regions of interest in normal and malignant peripheral zone tissue.</td>
<td>55</td>
</tr>
</tbody>
</table>
Figure 3.4 – Correlation between MRI-derived parameters. ..............................57
Figure 3.5 – Distributions of all parameter and model values in tumour (red) and normal (blue) peripheral zone tissue. .................................................................58
Figure 3.6 – Receiver operating characteristic (ROC) curves for all parameters: quantitative T2, ADC, $K^{\text{trans}}$, $v_e$. ...........................................................................59
Figure 3.7 – Receiver operating characteristic (ROC) curves for 3-parameter logistic regression model (LR-3p), quantitative T2, and ADC. .................................60
Figure 3.8 – Whole-mount histologic section (a) and corresponding anatomic T2-weighted MRI (b), input parametric maps (ADC (mm$^2$/s) (c), quantitative T2 (ms) (d), $K^{\text{trans}}$ (min$^{-1}$) (e)), and tumour probability map (LR-3p (no units) (f)): tumour visible in all input parametric maps. ........................................................................62
Figure 3.9 – Whole-mount histologic section (a) and corresponding anatomic T2-weighted MRI (b), input parametric maps (ADC (mm$^2$/s) (c), quantitative T2 (ms) (d), $K^{\text{trans}}$ (min$^{-1}$) (e)), and tumour probability map (LR-3p (no units) (f)): tumour not visible in all modalities. .............................................................................63

Chapter 4: The Impact of Intermixed Normal Tissue within Prostate Cancer on Measurements of Apparent Diffusion Coefficient and Quantitative T2

Figure 4.1 – Histologic examples from normal and malignant regions..............71
Figure 4.2 – Schematic example of regional assessment of whole-mount histologic section .................................................................................................................72
Figure 4.3 – Whole-mount sections, corresponding T2-weighted MR images, and ADC and T2 maps for a dense tumour example .........................................................75
Figure 4.4 – Whole-mount sections, corresponding T2-weighted MR images, and ADC and T2 maps for a sparse tumour example .........................................................76
Figure 4.5 – Distributions of median values from all ROIs, all tissue types.........77
Figure 4.6 – Distributions of log$_2$-transformed tumour-to-normal ratios of matched-pair median values. .........................................................................................78

Chapter 5: Investigating the Relationship between Magnetic Resonance Imaging-Derived Parameter Values and Tissue Composition in the Prostate

Figure 5.1 – Prostate segmentation schema. .........................................................88
Figure 5.2 – Sample mark-up images from image segmentation of histology.....89
Figure 5.3 – ADC $\text{versus}$ %area for each tissue component, z-score normalized with regression result. ..............................................................................................91
Figure 5.4 – T2 relaxation $\text{versus}$ %area for each tissue component, z-score normalized with regression result .................................................................92
Figure 5.5 – Representative histologic sections (a, b) with corresponding ADC (c) and $K^{\text{trans}}$ (d) maps. .........................................................................................94
Chapter 6: Discussion

Figure 6.1 – Sample data from finite element modeling for deformable registration of whole-mount histology to MRI. .................................................................115
### Symbols and Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>3-dimensional</td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
<td></td>
</tr>
<tr>
<td>AIF</td>
<td>arterial input function</td>
<td></td>
</tr>
<tr>
<td>$A_z$</td>
<td>area under the receiver operating characteristic (ROC) curve</td>
<td></td>
</tr>
<tr>
<td>$b$</td>
<td>diffusion weighting</td>
<td></td>
</tr>
<tr>
<td>$C_p$</td>
<td>concentration of contrast agent in blood (plasma)</td>
<td></td>
</tr>
<tr>
<td>$C_t$</td>
<td>concentration of contrast agent in tissue</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>central gland</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
<td></td>
</tr>
<tr>
<td>DCE</td>
<td>dynamic contrast-enhanced</td>
<td></td>
</tr>
<tr>
<td>DWI</td>
<td>diffusion-weighted imaging</td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>echo planar imaging</td>
<td></td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
<td></td>
</tr>
<tr>
<td>FSE</td>
<td>fast spin echo</td>
<td></td>
</tr>
<tr>
<td>FSPGR</td>
<td>fast spoiled gradient echo</td>
<td></td>
</tr>
<tr>
<td>Gd</td>
<td>gadolinium</td>
<td></td>
</tr>
<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin</td>
<td></td>
</tr>
<tr>
<td>$K^{trans}$</td>
<td>volume transfer constant</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>MRSI</td>
<td>magnetic resonance spectroscopic imaging</td>
<td></td>
</tr>
<tr>
<td>MVD</td>
<td>microvessel density</td>
<td></td>
</tr>
<tr>
<td>PCa</td>
<td>prostate cancer</td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
<td></td>
</tr>
<tr>
<td>PSA</td>
<td>prostate-specific antigen</td>
<td></td>
</tr>
<tr>
<td>PZ</td>
<td>peripheral zone</td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>radiofrequency</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
<td></td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>spin-lattice (longitudinal) relaxation time</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>spin-spin (transverse) relaxation time</td>
<td></td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>inversion time</td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
<td></td>
</tr>
<tr>
<td>$v_e$</td>
<td>extravascular extracellular volume fraction</td>
<td></td>
</tr>
<tr>
<td>$v_p$</td>
<td>blood plasma volume fraction</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 1

Introduction
1.1 Introduction

Despite advances in detection and treatment, prostate cancer remains a considerable health concern for men as they age, accounting for almost 10% of all male cancers, worldwide (Quinn et al. 2002). In Canada, an estimated 25,500 cases are expected to be detected in 2009, making prostate cancer the most frequently diagnosed male cancer (Canadian Cancer Society 2009). Although mortality rates are much lower, prostate cancer is still the 3rd-highest cause of male cancer death, behind lung and colorectal cancers. Thus, despite the considerable disparity between incidence and mortality rates, there is still significant room to improve outcomes, which includes balancing patient survival with quality of life for patients living with disease.

The advent of the prostate-specific antigen (PSA) test has provided a simple diagnostic tool, and has likely contributed to the detection of prostate cancer at an earlier, more treatable stage, as well as to a reduction in age of newly diagnosed patients (Mettlin et al. 1998, Bartsch et al. 2001). However, prostate cancer has a slow growth rate (Schmid et al. 1993) and may remain indolent (not leading to the death of the patient) even if treated conservatively (Albertsen et al. 2005). Thus, although PSA testing is useful in the context of early detection, it has also led to increases in lead time and overdiagnosis (i.e., disease that would not have otherwise been detected during the patient’s lifetime) (Draisma et al. 2009). Taken together, and including upward trends in life-expectancy over time, the number of men living with prostate cancer and balancing treatment options with their associated side-effects will likely increase. As a consequence, there is a need for advances that enable accurate identification and localization of malignancy requiring treatment. As will be introduced in this chapter, imaging has the potential to play an increasing role both within the current clinical paradigm, and also in facilitating the use of novel therapies that offer improved side-effect profiles. This thesis addresses the current limitations in prostate cancer localization with magnetic resonance imaging (MRI), presenting methods to improve discrimination between normal...
and malignant tissue in an image, and demonstrating the relationships between characteristics of prostate tissue and MRI parameter values.

1.2 Prostate Cancer: Diagnosis and Treatment

Initial detection of prostate cancer often occurs during a routine physical exam, when results from one or both of a digital rectal exam or PSA test are considered suspicious for cancer. These methods are suitable for initial screening; however, as PSA levels may be elevated as a result of either malignancy or benign processes such as benign prostatic hyperplasia or prostatitis (Stamey et al. 1987, Nadler et al. 1995), a diagnosis of prostate cancer cannot be established without examination of tissue. The prostate is located in the approximate centre of the body (Figure 1.1) and consists of three zones (Figure 1.2): the peripheral zone, and the central and transitional zones, often referred to together as the central gland. The peripheral zone is located along the posterior and apex of the gland, adjacent to the rectum, and is where the majority (~70%) of prostate cancers originate (McNeal et al. 1988, Chen, M. E. et al. 2000). Thus, tissue samples are obtained through the rectum by needle biopsy performed under transrectal ultrasound guidance. The number of cores obtained varies between health care centres, however, eight or more cores distributed through the gland are typical, with additional cores taken in areas suspicious for cancer on transrectal ultrasound (Hricak et al. 2007). A pathologist reviews all biopsy samples for a patient and determines the presence or absence of cancer in each core, the percentage of core length containing malignant glands, and the Gleason score, determined from the Gleason grade(s) present (Gleason et al. 1974, Epstein et al. 2005, Montironi et al. 2005) (Figure 1.3). Extracapsular extension or seminal vesicle invasion, if present, is also noted. This information is consolidated to provide an estimate of whether disease is likely clinically localized, then used to stratify patients into categories reflecting their risk of recurrence after radical treatment (Table 1.1) (Kattan et al. 1998).
Figure 1.1 – Male anatomy through the level of the prostate. The prostate gland is approximately 3 - 5 cm in diameter, located immediately anterior to the rectum, and inferior to the bladder with the urethra running through it. The proximity to the rectum to the posterior of the gland facilitates transrectal ultrasound-guided biopsy. However, the location of the prostate – deep within the body, and adjacent to sensitive structures – leads to challenges for therapy. (© 2005 Terese Winslow, U.S. Govt. has certain rights. Reprinted with permission from T. Winslow.)

Figure 1.2 – Prostate zonal anatomy: sagittal (a) and transverse (b) views. The level of the three transverse sections (A,B,C) are shown in the sagittal view. The prostate is composed of the peripheral, central, and transition zones. The peripheral zone is located along the posterior edge of the gland, as well as the apex. The transition and central zones are located in the centre-to-superior aspect of the prostate, surrounding the urethra (*) and ejaculatory ducts (arrows), and are often described together as central gland tissue. (Reproduced with permission of AMERICAN ROENTGEN RAY SOCIETY: American Journal of Roentgenology (Kundra et al. 2007) (c) 2007)
Figure 1.3 – Gleason grade. A diagram depicting Gleason grades (1-5) in prostate adenocarcinoma, as well as four major architectural patterns (e.g. small gland – I, to solid sheet – IV). Gleason grades reflect the degree of differentiation of prostate cancer, with grades 1 and 2 closely resembling normal prostate. Gleason grade 3 is more poorly differentiated, but still has recognizable glands. Malignant glands begin to fuse in Gleason grade 4, and typically become a solid sheet by Gleason grade 5. The Gleason score is the sum of the two most common grades in the tumour, and thus ranges from 2-10. The cohort studied in this thesis have primarily Gleason score 6-7 tumours. (Reprinted by permission from Macmillan Publishers Ltd: Modern Pathology (Srigley 2004) © 2004)

Accurate clinical assessment of prostate cancer is thus vital to ensure appropriate risk determination. Currently, accuracy is impacted by sampling error of the biopsy. Biopsy samples are typically ~0.8 mm in diameter (18-Gauge) and ~15-20 mm in length, representing only a small portion of the gland (0.04% of a 40 mL gland) (Montironi et al. 2005). This has the consequence that the most extensive or aggressive portion of the tumour may not be characterized and, in fact, both upstaging and an increase in Gleason score from biopsy to evaluation of the surgical specimen post-prostatectomy are frequently reported (Steinberg et al. 1997, Montironi et al. 2005). An accurate, 3-dimensional (3D) description of disease location and extent could be used to guide the placement of the biopsy needle, ensuring a more-representative sample of the tumour is
extracted for assessment. This suggests a role for imaging in improving early clinical assessment; however, potential benefits of prostate cancer characterization may also be realized during therapy.

Table 1.1 – Risk Group Stratification for Clinically Localized Prostate Cancer

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>PSA   (ng/mL)</th>
<th>Gleason score</th>
<th>T Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt; 10</td>
<td>2 – 6</td>
<td>T1 – T2a</td>
</tr>
<tr>
<td>Intermediate</td>
<td>10 – 20</td>
<td>7</td>
<td>T2b – T2c</td>
</tr>
<tr>
<td>High</td>
<td>&gt; 20</td>
<td>8 – 10</td>
<td>T3a Extracapsular extension</td>
</tr>
<tr>
<td>Very High</td>
<td>&gt; 20</td>
<td>8 – 10</td>
<td>T3b – T4 Seminal vesicle invasion to some invasion of adjacent structures</td>
</tr>
</tbody>
</table>

PSA – Prostate-specific antigen
T Stage – Full description of TNM Staging criteria can be found in: (National Comprehensive Cancer Network 2009), ST-1
All Indicated Risk Groups are N0 and M0 (no nodal involvement or metastatic disease)
Data for this table extracted from: (National Comprehensive Cancer Network 2009), PROS-1.
The presence of any of a higher risk factor shifts a patient into the next risk group.

Treatment options vary according to risk group, as well as factors such as life expectancy, patient health, and preference. One option for men with low-risk prostate cancer is to follow an active surveillance protocol (Klotz 2006), deferring treatment until their disease shows signs of progression – potentially indefinitely. This strategy avoids morbidity associated with therapy, but requires the patient to be monitored closely, including multiple repeat biopsies, and may contribute to patient anxiety (Latini et al. 2007). As the presence of any Gleason grade 4 or 5 disease upgrades risk classification to intermediate, and is an indication that active surveillance may no longer be sufficient, accurate sampling of the tumour during initial or follow-up biopsy is imperative. Full characterization and mapping of prostate cancer could also provide added assurance that a patient’s tumour burden is low, and be employed to monitor changes over time.
For men in the intermediate risk group, the primary options for therapy involve treatment of the entire gland, and include surgical removal (radical prostatectomy) or external beam radiation therapy. Although these therapies offer good long-term survival outcomes (Thompson et al. 2007), side effects such as incontinence and erectile dysfunction are common (Kundu et al. 2004, Thompson et al. 2007). Thus, for men in this population, as well as men in the low-risk group for whom active surveillance is not preferred, the treatment of their cancer may have a significant impact on their quality of life. Although prostate cancer is most often multifocal, a proportion of patients (~20% to as much as 50%) will present with a single focus of tumour (Villers et al. 1992, Wise et al. 2002, Mouraviev et al. 2007). For this population, therapies that target a portion of the gland may be appropriate. Focal therapies, including high intensity focused ultrasound, cryotherapy, photodynamic therapy, laser ablation, or conformal radiation therapies (intensity modulated radiation therapy or brachytherapy) seek to restrict therapy to malignant tissue, minimizing treatment to normal structures and potentially reducing the incidence of side effects (Carroll et al. 1997, Barqawi et al. 2007, Eggener et al. 2007). However, in order to be most effective, focal therapies must be guided – the target must be clearly defined.

The majority (75 – 90%) of prostate cancer cases are localized to the prostate at the time of detection (Mettlin et al. 1998, Jemal et al. 2009). Of these, approximately 85% of patients are in the low or intermediate risk categories. Thus, any improvements in care in this patient population will have a large impact. Imaging has the potential to play a vital role when optimizing the treatment of each patient. Identification of the location and extent of disease would facilitate targeted biopsies to sample the most extensive or aggressive location of tumour, and the depiction of the cancer used to provide guidance during active surveillance, or enable optimal delivery of focal therapies.

Imaging modalities used during the assessment of prostate cancer include transrectal ultrasound, MRI, computed tomography (CT), radionuclide bone
scanning, and combined positron emission tomography (PET)/CT (Yu et al. 2000, Hricak et al. 2007, Fuchsjager et al. 2008). However, the use of CT or bone scans is primarily limited to the detection of metastases, and PET/CT has not yet demonstrated adequate sensitivity for use in primary prostate cancer. Due to its low cost and availability, transrectal ultrasound is widely used to guide biopsy and measure prostate volume (Terris et al. 1991, Yu et al. 2000, Hricak et al. 2007, Fuchsjager et al. 2008). Although staging, specifically the presence of extracapsular extension or seminal vesicle invasion, can also be performed with transrectal ultrasound, a prospective multi-institutional trial demonstrated no significant improvements in performance over digital rectal exams (Smith et al. 1997). Transrectal ultrasound is also not currently used for tumour localization (Yu et al. 2000, Hricak et al. 2007), although the use of microbubble contrast agents during contrast-enhanced ultrasound is showing promise to improve tumour detection (Halpern 2006).

The primary clinical role of MRI for prostate cancer is to assist in assessing clinical stage through detecting extracapsular extension or seminal vesicle invasion (Mullerad et al. 2004, Wang, L. et al. 2004, Futterer 2007, Graser et al. 2007), with T2-weighted MRI performed using an endorectal coil shown to have superior performance for staging compared to T2-weighted MRI using a body coil, transrectal ultrasound, or digital rectal exam (Huch Boni et al. 1995). However, the excellent soft-tissue imaging capabilities, as well as the potential to probe physiologic characteristics in the same imaging session, have led to the extensive investigation of MRI for prostate cancer localization (Scheidler et al. 1999, Wefer et al. 2000, deSouza et al. 2007, Graser et al. 2007, Haider et al. 2007, Testa et al. 2007). The focus of studies presented in this thesis has been to 1) investigate methods to combine multiple MRI datasets (i.e., multi-parametric MRI) to improve the detection and characterization of prostate cancer, and 2) determine the underlying pathologic and histologic features of the prostate tissue that impact signal in MRI. Therefore, the next section introduces MRI measurements and techniques, with an emphasis on clinical prostate and prostate cancer imaging.
1.3 Magnetic Resonance Imaging of the Prostate and Prostate Cancer

1.3.1 T2-weighted MR Imaging

Through the manipulation of magnetic fields, and harnessing both the properties of water molecules and their abundance in tissue, MRI is able to perform cross-sectional imaging capable of depicting differences in soft tissues. This is possible because water contains hydrogen, whose nuclei consist of a single, unpaired proton, spinning about their axis. The proton is referred to as a ‘spin’, and has properties related to its environment. Depending on the parameters used to acquire the MR image, various properties of the spins, sensitive to differences in tissue, can be measured. These properties include: the spin-lattice (longitudinal) relaxation time (T1), which depends on the transfer of energy between spins and the surrounding lattice; the spin-spin relaxation time (T2), related to how rapidly the spins dephase; and proton density (N(H)). The differences in T2 between soft tissues have caused T2-weighted MRI to be commonly employed for anatomic imaging. In the prostate, T2-weighted MRI provides the best depiction of zonal anatomy, and is thus the primary MRI technique. Image sets are usually acquired in axial, sagittal and coronal planes (Figure 1.4) in thin-sections (~3 mm slice thickness) and a field of view (FOV) on the order of 14 cm. Reconstructed voxel dimensions in the plane of the image (i.e., in-plane) are typically sub-millimetre (Yu et al. 2000, Hricak et al. 2007, Fuchsjager et al. 2008).

To be useful diagnostically, and well-tolerated by the patient, a clinical T2-weighted protocol must be capable of providing high-quality anatomic images with a minimum of artifact or distortion, within a reasonable time. At 1.5-Tesla (T), the most commonly used field strength in clinical MRI, this is most often achieved through the use of a Fast Spin Echo (FSE) sequence, with combined pelvic phased-array and endorectal surface coils to transmit and receive the radiofrequency pulses that facilitate MR imaging. The endorectal coil is placed immediately adjacent to the prostate to increase the signal received from the
gland, thus improving the signal-to-noise ratio (SNR). However, this comes at a cost of signal uniformity; the coil is more sensitive to immediately-adjacent tissues, and images tend to be brighter near the posterior aspect of the gland. Signal then decreases as the distance from the coil increases, i.e. towards the anterior of the gland. Figure 1.5 shows axial-oblique and coronal sections through the prostate taken with a standard clinical T2-weighted MRI protocol; the signal drop-off can be appreciated in the axial-oblique images.

**Figure 1.4 – Planes of Human Anatomy.** T2-weighted MR images are typically acquired in three planes: sagittal, coronal, and transverse (also referred to as ‘axial’), defined according to their orientation in the human body. Each plane shown here can be thought of as a single slice that depicts the anatomy contained at that level of section. These terms typically refer to orthogonal planes; if a plane is tilted slightly, e.g. the transverse (axial) plane is angled such that one side is slightly closer to the subject’s head, and the other slightly closer to the subject’s feet, it is referred to as an ‘oblique’ plane, keeping the original directional indicator. (e.g., axial-oblique) (created by Yassine Mrabet, permission granted to copy, distribute and/or modify this document under the terms of the [GNU Free Documentation License](https://www.gnu.org/copyleft/fdl.html), Version 1.2 or any later version published by the Free Software Foundation; with no Invariant Sections, no Front-Cover Texts, and no Back-Cover Texts. A copy of the license is included in the section entitled “[GNU Free Documentation License](https://www.gnu.org/copyleft/fdl.html)”.)
The relatively high signal intensity of periprostatic fat in T2-weighted MRI assists in differentiating between various pelvic structures such as the prostate, rectum, muscles, bones, and bladder (Figure 1.5) (Schnall et al. 1990, Futterer 2007, Fuchsjager et al. 2008). Within the prostate, the bright normal peripheral zone can be distinguished from the central gland, which tends to have lower signal intensity (Futterer 2007, Hricak et al. 2007, Fuchsjager et al. 2008). Central gland tissue is often less homogeneous than tissue in the peripheral zone, frequently containing nodules of benign prostatic hyperplasia. Nodular regions of hypo-intensity in the peripheral zone are considered suspicious for prostate cancer on T2-weighted images (Schnall et al. 1990, Futterer 2007, Graser et al. 2007, Fuchsjager et al. 2008). However, benign conditions such as post-biopsy hemorrhage, dense, fibromuscular stroma common in scar tissue, and prostatitis may also lead to a decrease in signal, and have been implicated as potential sources of false-positive findings (Quint et al. 1991, Sommer et al. 1993, Shukla-Dave et al. 2004). Cystic atrophy can also cause an increase in signal intensity in peripheral zone tissue, which can lead to the interpretation of adjacent normal (non-cystic) peripheral zone as malignant (Sommer et al. 1993, Jager et al. 1996). In the central gland, heterogeneity due to the presence of benign prostatic hyperplastic nodules can make tumour detection in this zone challenging. However, homogeneous regions of low signal intensity having poorly defined margins or without a low signal intensity rim can be characteristic of malignancy (Futterer et al. 2006, Hricak et al. 2007).
Figure 1.5 – T2-weighted MRI of the Prostate at 1.5-Tesla. Axial-oblique images at the level of the base (a), mid-gland (c), and apex (e), with the slice location depicted by the dashed line (---) on the coronal view (b, d, f). B – bladder; ERC – endorectal coil; SV – seminal vesicles; CG – central gland; PZ – peripheral zone; PCa – prostate cancer; ED – ejaculatory ducts; U – urethra. The central gland appears heterogeneous, due to the presence of benign prostatic hyperplasia nodules. Relative to central gland, peripheral zone tissue appears bright. Prostate cancer is seen in the peripheral zone as a hypo-intense region. See Figure 1.2 for corresponding schematic of zonal anatomy. Patient orientation and scale-bars (1 cm per division) are shown for all axial-oblique views in e, and all coronal views in f.
The sensitivity (i.e., the percentage of tumours correctly identified) and specificity (i.e., the percentage of non-tumour segments correctly identified) for localization of prostate cancer using T2-weighted 1.5-T MRI plus an endorectal coil ranges from 36 – 82% and 46 – 92%, respectively; individual study results are tabulated in Table 1.2. Factors influencing detection include the size and location of the tumour, as well as the degree of differentiation (Ellis et al. 1994). Detection is also more accurate for peripheral zone tumours than for lesions in the central gland (Jager et al. 1996, Haider et al. 2007). To improve characterization of tumour location and extent, a number of studies have suggested that additional MRI techniques be added to the clinical protocol, including quantitative mapping of T2 relaxation, diffusion-weighted imaging (DWI), dynamic contrast-enhanced (DCE) MRI, and magnetic resonance spectroscopic imaging (MRSI). As signal intensity in T2-weighted MR images is not absolute – i.e., relative differences in signal are visualized, but absolute and inter-patient values are not meaningful, the calculation of parametric maps derived from these additional MRI datasets also provide a means to determine quantitative values. This feature can be used to improve consistency in interpretation, and also facilitates the use of voxel values in quantitative models to describe image features. Additionally, each MRI protocol may be sensitive to different aspects of tissue physiology, and thus may improve biological characterization of the cancer or surrounding normal tissue. The next section, therefore, introduces each technique and its application in prostate and prostate cancer MRI.
Table 1.2 – Tumour Localization Using T2-weighted MRI @1.5-T with endorectal coil

<table>
<thead>
<tr>
<th>Study</th>
<th>nPts</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>A_z</th>
<th>Note(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Scheidler et al. 1999)</td>
<td>53</td>
<td>77</td>
<td>81</td>
<td>0.73</td>
<td>WM as reference 2 readers (κ = 0.43), one line per reader</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PZ tumours only</td>
</tr>
<tr>
<td>(Wefer et al. 2000)</td>
<td>47</td>
<td>^a67</td>
<td>^b69</td>
<td>NR</td>
<td>WM as reference 2 readers, average score used</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^aWhole gland, ^bbase, ^cmidgland, ^dapex</td>
</tr>
<tr>
<td>(Graser et al. 2007)</td>
<td>106</td>
<td>82.3</td>
<td>90.3</td>
<td>0.802</td>
<td>WM as reference 3 readers (κ = 0.53 – 0.57), one line per reader</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78.7</td>
<td>65.6</td>
<td>0.776</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.2</td>
<td>79.5</td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td>(Haider et al. 2007)</td>
<td>49</td>
<td>^a54</td>
<td>^b57</td>
<td>^c36</td>
<td>WM as reference (in quarters) Values for the ^awhole gland, ^bPZ, ^cCG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>^a91</td>
<td>^b91</td>
<td>^c92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^c0.79</td>
<td></td>
</tr>
<tr>
<td>(Testa et al. 2007)</td>
<td>26</td>
<td>54</td>
<td>75</td>
<td>NR</td>
<td>WM as reference Whole gland (5 patients had only CG tumours)</td>
</tr>
</tbody>
</table>

nPts – number of patients; Sens – sensitivity; Spec – specificity; A_z – area under the receiver operator curve (ROC); κ – interobserver agreement; NR – not reported; WM – whole-mount histologic sections; CG – central gland; PZ – peripheral zone

1.3.2 Parametric MRI

1.3.2.1 Quantitative T2

Contrast in T2-weighted MRI is a result of differences in the spin-spin relaxation time (T2) for each tissue, which characterizes the decay rate of the transverse magnetization vector after being flipped 90 degrees from alignment with the main magnetic field (B₀) into the transverse (x – y) plane. However, in T2-weighted imaging, the sequences have been optimized to maximize soft-tissue contrast in a minimum scan time, versus permitting calculation of absolute values of T2. Signal intensity is thus not consistent between scans, and decreases as a function of distance from the endorectal coil. Although techniques to remove signal intensity variation have been suggested (Liney et al. 1998), this strategy does not improve the consistency of inter-patient values. Thus, the use of voxel
values obtained from T2-weighted MRI is limited. By acquiring T2-weighted images at various echo times (TEs) with a long repetition time (TR) to minimize the effect of spin-lattice (longitudinal) relaxation (T1), values of T2 relaxation times for each voxel in the image can be calculated using a mono-exponential decay relationship:

\[ M_{xy} = M_0 e^{-t/T2} \]  

(1.1)

where \( M_{xy} \) and \( M_0 \) are the transverse and main magnetization vectors, respectively, and \( t \) is the time elapsed after the flip to the transverse plane (Hashemi et al. 2003). This assumes that one T2 dominates the signal measured at that spatial location, and is the basis for T2-mapping in the majority of prostate studies (Liney et al. 1997, Gibbs et al. 2001, Chan et al. 2003, de Bazelaire et al. 2004, Kershaw et al. 2009); however, recent work has also demonstrated bi-exponential behaviour in the prostate (Storas et al. 2008).

Despite the advantages of providing consistent values across patients and scans, and removing the signal changes as a function of coil distance, the basis of contrast in quantitative T2 and T2-weighted MRI is the same. Voxel size is typically increased for T2 mapping in order to maintain a reasonable scan time, causing a pixelated appearance of the map, relative to a T2-weighted image (Figure 1.6). Routine measurement of T2 for prostate cancer applications is therefore not often performed, but useful when absolute values are required, providing a quantitative version of the signal information contained in T2-weighted MRI. The transverse relaxation time, T2, of prostate cancer is significantly lower than that of normal peripheral zone tissue, with values ranging from 65 – 103 ms for malignant tissue and 96 – 160 ms for normal peripheral zone reported (Table 1.3). These differences can primarily be related to the increase in cellular density associated with prostate cancer, which increases macromolecular content, and displaces the open spaces in the lumen, thus decreasing free water content (Mitchell et al. 1987). Positive correlation between T2 and citrate concentration has also been demonstrated in prostate tissue.
(Liney et al. 1996), and is likely due the presence of citrate in the extra-cellular, free water in peripheral zone lumen.

Figure 1.6 – T2-weighted MRI (a) and quantitative T2 map (ms) (b) from corresponding slice locations. The contrast is similar between the T2-weighted and T2 map images and, in both images, prostate cancer (two locations, indicated by ‘*’) is hypo-intense compared to the surrounding normal peripheral zone tissue. However, the values for each pixel in the T2 map represent the average T2 relaxation in that location, and the signal drop-off occurring as a function of distance from the endorectal coil is no longer present. In order to maintain a reasonable scan time, the resolution of the T2 map is less than the T2-weighted image.

Table 1.3 – Quantitative T2 in Normal and Malignant Prostate

<table>
<thead>
<tr>
<th>Study</th>
<th>nSub</th>
<th>Normal (ms)</th>
<th>Malignant (ms)</th>
<th>Note(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Liney et al. 1997)</td>
<td>16</td>
<td>96 ± 15</td>
<td>65 ± 6</td>
<td>All PCa patients; values in normal or malignant PZ</td>
</tr>
<tr>
<td>(Gibbs et al. 2001)</td>
<td>a12 b8</td>
<td>136 ± 40</td>
<td>a8 82 ± 18</td>
<td>Values in a patients, and volunteer b1 PZ and b2 CG</td>
</tr>
<tr>
<td>(Chan et al. 2003)</td>
<td>11</td>
<td>128 ± 43</td>
<td>103 ± 28</td>
<td>9 patients</td>
</tr>
<tr>
<td>(Kershaw et al. 2009)</td>
<td>13</td>
<td>160 (143-174)</td>
<td>NA</td>
<td>Values in a normal PZ and b BPH in CG</td>
</tr>
</tbody>
</table>

nSub – number of subjects; TE – echo time; PCa – prostate cancer; PZ – peripheral zone; CG – central gland; NA – not applicable; BPH – benign prostatic hyperplasia
1.3.2.2 Diffusion-Weighted Imaging

Diffusion-weighted imaging (DWI) measures the diffusion of water molecules occurring as a result of Brownian motion, with the measured signal in tissue sensitive to the presence of micro-structures that can impede diffusion or flow (Le Bihan et al. 1986, Koh et al. 2007, Thoeny et al. 2007); thus, DWI can be useful in assessing or comparing pathologies such as stroke or cancer, where structural changes, including changes in cellular density or extracellular space, are expected (Huisman 2003, Koh et al. 2007). Measurement of diffusion is performed by applying two gradients of equal, but opposite, strength. At the end of the second gradient, molecules that have remained stationary will be in phase, and have recovered maximum signal. If, however, water molecules have moved during the application of the first and second gradients, the signal will be reduced. In addition to tissue characteristics, dictating the degree to which movement of the water molecules are restricted, the signal change is also a function of MRI acquisition parameters described by the b-value ($b$), where:

$$ b = \gamma^2 G^2 \delta^2 (\Delta - \frac{\delta}{3}) $$

(1.2)

$\gamma$ is the gyromagnetic ratio, $G$ is the gradient strength, $\delta$ is the time the gradient is applied, and $\Delta$ is the time between the start of each gradient (Stejskal et al. 1965, Bammer 2003, Thoeny et al. 2007). Signal can then be described by:

$$ S_i = S_0 e^{-b_i \cdot \text{ADC}} $$

(1.3)

where $S_0$ is the signal in an image obtained without the diffusion gradient applied ($i.e., b = 0$), $S_i$ is the signal for an image obtained using the $i^{th}$ b-value, $b_i$, and ADC is the apparent diffusion coefficient. All moving water molecules contribute to the overall measured signal, $S_i$. Thus, ADC is sensitive to diffusion in all tissue compartments, e.g. the extracellular extravascular space, intracellular space, and vasculature, the weighting of which is determined in part by the b-values used in acquisition (Thoeny et al. 2007). Additionally, by applying the diffusion gradients
in multiple directions, the ADC for each direction can be measured. However, although non-isotropic results have been determined in the prostate (Gibbs et al. 2001, Manenti et al. 2007), the directionally-averaged ADC or ‘trace’ is more typically reported.

![Figure 1.7 – T2-weighted MRI (a) and map of apparent diffusion coefficient (ADC) (mm²/s) (b) from corresponding slice locations.](image)

The regions of prostate cancer (two locations, indicated by ‘*’ in Figure 1.7) are apparent in ADC as regions of restricted diffusion. Some image distortion can be noted in the ADC map; however, in this example the distortion primarily affects the appearance of the endorectal coil.

In prostate cancer, ADC is significantly lower compared to the value in surrounding normal peripheral zone tissue (sample ADC map shown in Figure 1.7, with literature values summarized in Table 1.4). Concurrent review of ADC maps with T2-weighted endorectal MRI has led to an improvement in tumour localization (Haider et al. 2007, Yoshimitsu et al. 2008). The change in ADC with prostate cancer has been attributed to an increase in cellular density and disruption of ductal architecture in the peripheral zone (Gibbs et al. 2001, Issa 2002, Hosseinzadeh et al. 2004, deSouza et al. 2007). As a measure of water diffusion, ADC measured using similar parameters (i.e., consistent b-values) should relate to the underlying tissue characteristics. However, there is still an appreciable overlap seen in ADC values for prostate cancer versus normal peripheral zone within single studies, despite the fact that the direction of relative (intra-patient) differences is consistent (Issa 2002, Hosseinzadeh et al. 2004,
Mazaheri et al. 2008). This suggests that cellular microstructure likely varies between patients, which could potentially impact the characterization of lesions with DWI. As Gleason grades are related to gland morphology (Figure 1.3), changes in ADC might be expected to relate to Gleason grade or scores. Nevertheless, although some studies report a significant correlation between ADC and Gleason (deSouza et al. 2008, Tamada et al. 2008, Yoshimitsu et al. 2008), these findings have not been consistent (Wang et al. 2009, Zelhof et al. 2009).

Clinical measurement of ADC in the prostate generally assumes a mono-exponential signal decay, and calculation is often performed from images acquired with a low b-value (often $b = 0$), plus one b-value in the range of 600–1000 s/mm$^2$. However, a few studies have demonstrated bi-exponential behaviour (Mulkern et al. 2006, Riches et al. 2009). These refinements in measurement technique may have prognostic implications; for example, de Souza et al. found a correlation between either $\text{ADC}_{\text{fast}}$ or $\text{ADC}_{\text{slow}}$ (corresponding to perfusion and diffusion components, respectively) and patient risk group, whereas there was no relationship between $\text{ADC}_{\text{overall}}$ and risk (deSouza et al. 2008).

Signal changes in DWI are interpreted as being due to the diffusion of water molecules in the tissue, and thus DWI is extremely sensitive to any other sources of displacement, e.g., bulk motion. Fast acquisition protocols such as single-shot echo planar imaging (EPI) are thus required (Turner et al. 1990, Turner et al. 1991). Echo planar imaging is prone to distortion artifacts caused by susceptibility differences (Ludeke et al. 1985, Bammer 2003, Hashemi et al. 2003, Zand et al. 2007). The use of an endorectal coil for prostate MRI can thus be problematic, as air within the coil is immediately adjacent to the tissue of interest – i.e., the prostate gland itself, and, specifically, the peripheral zone. To reduce distortion, the air in the coil can be displaced with material that better matches properties of the tissue, such as a barium sulphate suspension (Rosen et al. 2007).
Table 1.4 – Apparent Diffusion Coefficient (ADC) in the Prostate

<table>
<thead>
<tr>
<th>Study</th>
<th>nSub</th>
<th>Normal</th>
<th>Malignant</th>
<th>Note(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Gibbs <em>et al.</em> 2001)</td>
<td>a12</td>
<td>b3.4 ± 0.7</td>
<td>b1.3 ± 0.2</td>
<td>EPI; 8 b-values: 0 – 720 s/mm²</td>
</tr>
<tr>
<td></td>
<td>b8</td>
<td>b4.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Issa 2002)</td>
<td>19</td>
<td>a1.8 ± 0.5</td>
<td>b1.6 ± 0.4</td>
<td>EPI; b-values: 64, 144, 257, 401, 578, 786 s/mm²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b1.4 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Chan <em>et al.</em> 2003)</td>
<td>11</td>
<td>1.6 ± 0.4</td>
<td>b1.4 ± 0.5</td>
<td>EPI; b-values – 5, 750 s/mm²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b1.4 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Hosseinzadeh <em>et al.</em> 2004)</td>
<td>10</td>
<td>1.6 ± 0.3</td>
<td>b1.3 ± 0.4</td>
<td>EPI; b-values: 0, 1000 s/mm²</td>
</tr>
<tr>
<td>(Kozlowski <em>et al.</em> 2006)</td>
<td>14</td>
<td>a°1.6 ± 0.3</td>
<td>b1.4 ± 0.2</td>
<td>FSE; b-values: 0, 600 s/mm²</td>
</tr>
<tr>
<td>(deSouza <em>et al.</em> 2007)</td>
<td>33</td>
<td>a°1.7 ± 0.2</td>
<td>b1.5 ± 0.1</td>
<td>EPI; b-values – 0, 300, 500, 800 s/mm²</td>
</tr>
<tr>
<td>(Kozlowski <em>et al.</em> 2008)</td>
<td>15</td>
<td>a°2.0 ± 0.2</td>
<td>b1.5 ± 0.1</td>
<td>EPI; b-values: 0, 600 s/mm²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b1.2 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mazaheri <em>et al.</em> 2008)</td>
<td>38</td>
<td>1.7 ± 0.2</td>
<td>b1.4 ± 0.2</td>
<td>EPI; b-values – 0, 800 s/mm²</td>
</tr>
<tr>
<td>(Tamada <em>et al.</em> 2008)</td>
<td>a90</td>
<td>b°1.8 ± 0.3</td>
<td>b°1.3 ± 0.1</td>
<td>EPI; b-values – 0, 800 s/mm²</td>
</tr>
<tr>
<td></td>
<td>b125</td>
<td>b°1.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

nSub – number of subjects; EPI – echo planar imaging; LSDI – line scan diffusion imaging; FSE – fast spin echo; PZ – peripheral zone; CG – central gland; BPH – benign prostatic hyperplasia

1.3.2.3 Dynamic Contrast-Enhanced MRI

Angiogenesis – the formation of new blood vessels – is often associated with malignancy, with the new vessels tending to be chaotic, larger in diameter, and more permeable than established normal vasculature (Carmeliet *et al.* 2000, Padhani *et al.* 2001). A number of studies have measured an increase in microvessel density (MVD) in prostate cancer (Bigler *et al.* 1993, Mydlo *et al.* 1998, Kiessling *et al.* 2004, Schlemmer *et al.* 2004), potentially enabling the
detection or localization of tumours through imaging techniques sensitive to these characteristics. Vascular properties can be probed using T1-weighted dynamic contrast-enhanced MRI (DCE-MRI); a bolus of low molecular weight paramagnetic contrast agent is injected intravenously, and a series of images is acquired. The contrast agent, typically gadolinium based, diffuses from the vascular space into the surrounding extravascular extracellular space and is visible initially as an increase in the T1-weighted signal (Weinmann et al. 1984, Tofts 1997, Barentsz et al. 1999, Choyke et al. 2003, Alonzi et al. 2007). To capture the dynamics in signal intensity, the MRI sequence must be capable of rapid imaging over the desired volume while maintaining a reasonable in-plane spatial resolution (Barentsz et al. 1999, Choyke et al. 2003, Alonzi et al. 2007, Hricak et al. 2007, Fuchsjaeger et al. 2008), e.g. multi-slice fast spoiled gradient echo (FSPGR) imaging. Once a DCE-MRI dataset has been acquired, evaluation can be performed a number of ways including: qualitatively, to locate focal regions of increased uptake (Villers et al. 2006, Girouin et al. 2007); by characterizing properties of the enhancement curve (e.g., area under the contrast-enhancement uptake curve, time to peak enhancement) (Engelbrecht et al. 2003, Kim et al. 2005); or through quantification, using pharmacokinetic models to describe parameters related to vascular properties (Tofts 1997).

Visual assessment of enhancement has been successful in improving tumour localization (Girouin et al. 2007), does not make any assumptions about the mechanism of contrast, and requires a minimum, if any, post-processing – thus providing a rapid tool to assist in prostate cancer evaluation. However, interpretation is reader-dependent, and quantitative evaluation is not possible, limiting utility for inter-patient or inter-site comparisons or correlation with physiologic processes. Curve-characterization methods have successfully demonstrated the ability to improve prostate cancer localization with DCE-MRI (Jager et al. 1997, Engelbrecht et al. 2003, Kim et al. 2005), but are limited in their ability to describe underlying physiologic features in the tissue, although some correlations have been suggested (Tofts 1997, Evelhoch 1999). They may also be more sensitive to image acquisition properties such as gain, sequence
parameters, and scaling factors (Parker et al. 1997, Tofts et al. 1999, Padhani et al. 2001), and thus have reduced reproducibility in inter-site studies or between MRI vendors.

Accurate quantification using pharmacokinetic models is challenging, requiring a number of steps and baseline assumptions. For example, a commonly employed model in oncology assumes two compartments – intravascular and extravascular extracellular – with isodirectional, immediate exchange between them (Tofts 1997). Leakage of the contrast agent into the extravascular extracellular space (described by the volume fraction, $v_e$) is governed by the volume transfer constant, $K_{\text{trans}}$, which is related to physiologic properties such as permeability, flow, surface area, or a combination of these properties depending on whether the system is flow or permeability limited (Tofts et al. 1999). Determination of $K_{\text{trans}}$ and $v_e$ from DCE-MRI requires knowledge of the concentration of contrast agent flowing into the tissue of interest, i.e. the arterial input function (AIF), or $C_p$. As the measurement of the AIF with MRI can be subject to errors due to inflow effects, insufficient temporal resolution to accurately capture temporal variation, and/or partial volume effects (Ivancevic et al. 2003, Yankeelov et al. 2005, Zand et al. 2007), analytic descriptions of population-based measurements have been frequently employed. In particular, the combined model described in Walker-Samuel et al. incorporates two population-based AIFs to describe both initial and long-term contrast agent concentration (Fritz-Hansen et al. 1996, Tofts 1997, Walker-Samuel et al. 2006), and, in the prostate, was demonstrated to improve the reproducibility of $K_{\text{trans}}$ and $v_e$ over the use of a reference tissue-derived patient-specific AIF (Walker-Samuel et al. 2007). Thus, this population-based model AIF was employed in this thesis during pharmacokinetic analysis of DCE-MRI.

In order to convert signal enhancement, sensitive to inter-system/scan variability as mentioned above, to a more consistent measure of contrast agent concentration in tissue ($C_t$), the native T1 – i.e., the T1 of the tissue prior to
injection of the contrast agent – must be known. The tissue concentration of the contrast agent can then be approximated by:

$$C_t \sim \frac{1}{r_1 T_{10}} \left( \frac{S(t) - S(0)}{S(0)} \right)$$  \hspace{1cm} (1.4)

where $r_1$ is the in vivo tissue relaxivity, and $S(0)$ and $S(t)$ are the signal at times = 0 and $t$, respectively (Tofts 1997). For spoiled gradient echo sequences, signal is a function of T1, MRI acquisition parameters such as the repetition time (TR) and flip-angle ($\alpha$), and a factor proportional to the equilibrium longitudinal magnetization, $M_0$:

$$S(\alpha) = M_0 \sin \alpha \frac{1 - e^{-\alpha T/R}}{1 - \cos \alpha e^{-\alpha T/R}}$$  \hspace{1cm} (1.5)

(Bluml et al. 1993, Deoni et al. 2003). Thus, by obtaining data using multiple flip-angles, $T_{10}$ can be obtained through curve fitting techniques. Finally, with $C_t$ and $C_p$ known or approximated, $K^{\text{trans}}$ and $v_e$ can be obtained by fitting:

$$C_t = D K^{\text{trans}} \sum_{i=1}^{2} a_i \frac{e^{-(K^{\text{trans}}/v_e)m_i}}{m_i - K^{\text{trans}}/v_e} + v_p D \sum_{i=1}^{2} a_i e^{-m_i}$$  \hspace{1cm} (1.6)

$D$ is the injected dose of the contrast agent, $t$ is the time from bolus injection, $a_i$ and $m_i$ are constants characterizing the AIF, and $v_p$ is the blood plasma volume fraction (Tofts 1997). Estimates of $v_p$ tend to be small (Buckley et al. 2004) and do not impact determination of $K^{\text{trans}}$ or $v_e$ (Walker-Samuel et al. 2006); thus, the second term is often not included during curve fitting of $C_t$. A sample map of $K^{\text{trans}}$ is shown in Figure 1.8, along with the corresponding T2-weighted image.

Significant increases in $K^{\text{trans}}$ and, to a lesser extent, $v_e$, have been established for malignant compared to normal peripheral zone tissue (Padhani et al. 2000, Buckley et al. 2004, van Dorsten et al. 2004, Kozlowski et al. 2006). However, reports of correlation between DCE-MRI and specific tissue properties such as Gleason score and MVD have been mixed, with both significant (Engelbrecht et al. 2003, Schlemmer et al. 2004, Ren et al. 2008) and
insignificant (Padhani et al. 2000, Kiessling et al. 2004) findings. The relationship between DCE-MRI and histologic features may be affected by transient shutdown of vessels, which would decrease the directly-measured correlation between DCE-MRI and MVD, or changes in vessel permeability that may not manifest solely as changes in MVD. However, relationships between the histologic features themselves are also unclear, with evidence both for (Bono et al. 2002) and against (Brawer et al. 1994, Rubin et al. 1999, Schlemmer et al. 2004) significant correlation between MVD and Gleason score. In summary, DCE-MRI currently provides clinical value for localizing prostate cancer, and the potential to understand underlying physiologic differences between tumour and normal prostate tissue; however, mechanisms governing both qualitative and quantitative changes are not yet fully understood.

Figure 1.8 – T2-weighted MRI (a) and map of the volume transfer constant ($K_{\text{trans}}$) (min$^{-1}$) (b) from corresponding slice locations. Compared to surrounding normal peripheral zone tissue, the regions of prostate cancer (indicated by ‘*’) have higher $K_{\text{trans}}$ values. By viewing the T2-weighted image and the $K_{\text{trans}}$ map in conjunction, the small tumour on the patient’s right may be more apparent than just with review of the T2-weighted image alone. Interpretation of central gland tissue is challenging, as benign prostatic hyperplasia nodule may also exhibit an increase in $K_{\text{trans}}$, as noted in this example.
1.4 Multi-parametric Magnetic Resonance Imaging

The addition of quantitative T2, DWI and DCE-MRI to a clinical protocol ideally provides some insight into the underlying physiology or structure of the tissue, contributing to discrimination of malignant versus normal tissue. As these techniques have all been demonstrated to be useful in localizing prostate cancer, it is likely that there is some overlap as to which changes in tissue properties cause changes in MRI measurements. However, despite some similarities, they are also distinctive: for example, the measurement of water diffusion with DWI versus modeling the leakage of contrast agent from vasculature with DCE-MRI. Thus, a combination of techniques may either reinforce or complement each other, ideally providing improved localization. This multi-parametric approach, i.e., using more than one MRI technique, has been taken to identify ischemic tissue in stroke (Bernarding et al. 2000, Jacobs et al. 2001), classify tissue in brain tumours (Di Costanzo et al. 2006, McMillan et al. 2007, Verma et al. 2008), and characterize breast lesions (Jacobs et al. 2003). In prostate cancer, initial multi-parametric MRI studies are demonstrating improved performance over single-parametric techniques for discriminating between malignant and normal prostate tissue. Multi-parametric MRI schema have included both qualitative (using visual assessment of the image sets) (Kozlowski et al. 2006, Tanimoto et al. 2007, Chen, M. et al. 2008), or quantitative (combining voxel values to develop a classification or probability model for malignant versus normal tissue) (van Dorsten et al. 2004, Reinsberg et al. 2007, Mazaheri et al. 2008) methods.

Qualitative methods require radiologist review of all datasets, assessing each for the suspicion of cancer. This has the advantage of simplicity; it precludes the development of a robust quantitative model and does not require additional processing steps beyond generation of the parametric maps employed, eliminates the need for accurate spatial registration between techniques, and harnesses the knowledge of experts trained at interpretation for prostate cancer characteristics aside from voxel value (e.g., morphology). However, manual interpretation is subjective; results are thus dependent on the
experience and expert opinion of the reviewer, and are a source of inter-observer and potentially inter-site variability. The review of each dataset is also labour intensive, with the time required increasing with the addition of each technique. Finally, strategies to resolve discrepancies between datasets are not straightforward. Despite these limitations, qualitative multi-parametric MRI methods have been successful. For example, Tanimoto et al. compared the sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve ($A_2$) for three protocols (A: T2-weighted MRI; B: T2-weighted MRI + DWI; C: T2-weighted MRI + DWI + DCE-MRI), and found a significant improvement in sensitivity and $A_2$ with protocol C over A (T2-weighted MRI alone), although no significant differences between protocols B and C (Tanimoto et al. 2007). All datasets for a given protocol were reviewed concurrently and an overall score assigned, reflecting the confidence that the tissue being reviewed was normal or malignant. Although this interpretation scheme allows flexibility in interpretation – the reviewer can weight each dataset differently from case to case, depending on appearance of the lesion – the result relies heavily on the decision of each individual observer. Kozlowski et al. also explored the combination of DWI and DCE-MRI, finding an increase in sensitivity when the union of all datasets was employed, e.g., a region was considered positive for cancer if either the DWI- or DCE-MRI-derived parametric maps (ADC and $K^{\text{trans}}$, respectively) had positive findings (Kozlowski et al. 2006). By using the union, sensitivity is maximized, but at the potential cost to specificity, as a false positive in either technique will be a false positive in the multi-parametric assessment. Chen et al. used an averaged score from all techniques employed, T2-weighted, DWI, and magnetic resonance spectroscopic imaging (MRSI) (Chen, M. et al. 2008). This likely reduces the false-positive rate for the multi-parametric method, at a cost of sensitivity, but makes the assumption that each technique should contribute equally to the final assessment; in the case where one individual technique is more accurate, optimal results would likely be obtained by using a weighted combination.

Quantitative methods use the voxel values from each dataset and combine them to provide an indication of the presence of prostate cancer in that
voxel. The output is also quantitative – either a binary value indicating the presence or absence of cancer in a given voxel (van Dorsten et al. 2004, Reinsberg et al. 2007), or the probability that that voxel is malignant (Mazaheri et al. 2008). Input data thus do not require radiologist review and, as the output is numeric, a map identifying regions of suspected prostate cancer can potentially be generated. This greatly simplifies work-flow by reducing the number of datasets for review, and also provides more objective means for combining each parameter to obtain a final value improving consistency, facilitating the use of quantitative multi-parametric MRI in large-scale trials or screening studies, and for inter-site use. However, as voxel values are being combined, registration between parametric maps becomes increasingly important to ensure optimal results. Although all image sets are typically acquired in the same session, some bulk and peristaltic motion can displace the prostate, and some datasets (e.g., DWI) often contain distortion artifacts. The source of these registration errors can be minimized during acquisition by the administration of a drug to reduce peristalsis, or through addition of a barium sulphate suspension in the endorectal coil to reduce susceptibility artifact (Rosen et al. 2007). However, these issues are present during both training and testing of a model, and thus the performance of a developed model is likely to only be improved through further optimization of registration.

Strategies to perform quantitative multi-parametric MRI for prostate cancer vary: van Dorsten et al. used 2x the standard deviation of the mean $K^{\text{trans}}$ or metabolite ratio values in normal peripheral zone as a cut-off to identify voxels likely to be malignant (van Dorsten et al. 2004), extending criteria often used in spectroscopy to be applicable to multi-parametric MRI. Although they performed no formal comparison of single- versus multi-parametric performance, they observed that additional malignant voxels are correctly identified with the addition of $K^{\text{trans}}$ to the use of metabolites only. This method is simple to implement, however determination of the cut-off value(s) employed is somewhat arbitrary. Reinsberg et al. used a linear discrimination approach to optimally separate tumour from normal voxels on a plot of the log of metabolite ratios versus ADC.
The line was determined by iterating over possible slopes until the area under ROC curve, $A_z$, was maximized (Reinsberg et al. 2007). Their combined model achieves a significantly higher specificity at the same sensitivity (~90%) compared to each input parameter alone. Mazaheri et al. also used the combination of ADC and metabolite ratios, however their combined model was derived using logistic regression (Mazaheri et al. 2008). Their final performance for the combined model was higher than either metabolite ratios or ADC, however this improvement was not significant for the latter ($p = 0.09$). Logistic regression differs from the previous methods in that the output is a continuous variable reflecting the probability that a voxel is malignant, versus a binary classification. This distinction may be useful in a clinical setting by facilitating the quantitative, objective combination of each parameter, while maintaining enough information to permit interpretation for regions at higher or lower risk of malignancy; if this functionality is not desired, a strict threshold to define normal versus malignant can be set a posteriori. Although these quantitative multi-parametric methods all have the potential to be used to generate maps of tumour location or probability, none of these studies attempted to do so. This may have been due to their inclusion of spectroscopy data, as voxel sizes are significantly larger in MRSI (~3.4 – 9 mm in-plane resolution; 6.9 – 15 mm through-plane resolution in these studies). This limits utility of these methods as, in addition to the challenges of interpreting a large, pixilated map, partial volume effects in the large voxels greatly reduce the ability of the technique to accurately define tumour boundaries and may also increase the minimal size of a detectable lesion.

These studies all suggest the promise of using multi-parametric MRI to improve the localization of prostate cancer; however, to date, none have been implemented using methods that allow both the potential scope of physiologic information available as well as the spatial resolution and image quality attainable with MRI to be captured in one quantitative result. In moving forward, emphasis on techniques that are feasible in a clinical setting, and ideally provide
one output map for interpretation, are likely to have the maximum impact on patient management.

1.5 Summary of Aims and Research Contributions

Mapping of the location and extent of prostate cancer could facilitate guided biopsy, target definition for focal therapy, or non-invasive monitoring during active surveillance. The overall aim of this thesis was thus to improve the characterization of prostate cancer, including: the use of multi-parametric MRI to improve detection in the peripheral zone (Chapter 3); understanding a potential source of false-negative results in MRI (Chapter 4); and correlating MRI parameter values to the specific histologic composition of the tissue (Chapter 5). Each chapter is summarized below:

Chapter 2: Methods

All analyses were performed on subsets of a common patient cohort, all of whom had biopsy-confirmed prostate cancer, and MRI prior to prostatectomy. Thus, Chapter 2 details the clinical characteristics of the patient cohort, and methods that are common to all chapters.

Chapter 3: Prostate Cancer Detection with Multi-parametric Magnetic Resonance Imaging

Many MRI techniques are employed for prostate cancer imaging, and the ability of combined techniques to improve performance have been explored. However, the mapping of tumour location with techniques suitable for prospective application has yet to be attempted. Chapter 3 tests the hypothesis that, using methods suitable for use in a clinical setting, multi-parametric MRI performs better than single-parameter MRI in identifying malignant voxels in prostate peripheral zone tissue. MRI techniques were chosen based on their potential to provide complementary information in a multi-parametric model and the resolution feasible using clinical protocols, and included T2-mapping, DWI, and DCE-MRI. Feature vectors consisting of sets of parameters derived
from voxels in pathologically defined tumour and normal peripheral zone regions were used in training and testing. A step-wise logistic regression model was developed, providing statistical rationale for inclusion or exclusion of parameters, and defining the optimal weighting of each parameter in the final model. The performance of the final model was compared against each single-parameter. Sample tumour probability maps were generated to demonstrate the feasibility of prospective use of the model in a clinical setting.

Chapter 4: The Impact of Intermixed Normal Tissue within Prostate Cancer on Measurements of Apparent Diffusion Coefficient and Quantitative T2

Multi-parametric MRI is impacted by the strengths and weaknesses of each imaging technique. As single-parameters, ADC and T2 provided good performance for identifying malignant voxels in the peripheral zone. However, in this patient cohort there were examples of tumours that were not visible on maps of ADC or T2. Preliminary assessment revealed the presence of normal peripheral zone tissue, intermixed with malignant glands. Therefore, Chapter 4 tests the hypothesis that tumour heterogeneity – specifically, the presence of normal peripheral zone regions within the tumour boundary – reduce the ability of MRI to differentiate between tumour and normal tissue. Peripheral zone tumours were classified as ‘sparse’ or ‘dense,’ depending on the relative content of normal versus malignant tissue, and the ADC and T2 values for each tumour type were compared against normal peripheral zone controls.

Chapter 5: Investigating the Relationship between Magnetic Resonance Imaging-Derived Parameter Values and Tissue Composition in the Prostate

The utility of various MRI techniques in prostate cancer is based on the sensitivity of MRI to the underlying physiology, including – but not limited to – malignant status. Detailed study of the relationships between MRI parameters and histologic features in prostate tissue has been limited to few parameters in both MRI and histology, and to small regions of interest in histology. Improved
understanding of the histologic basis of MRI could assist in characterizing tumours for characteristics associated with poor patient outcome, and in interpreting false–positive or –negative imaging findings. Chapter 5 tests the hypotheses that 1) there is an underlying relationship between specific histologic properties and MRI parameter values, and 2) these histologic properties are altered in prostate cancer arising in the peripheral zone. Corresponding regions in both parameter maps (ADC, T2, $K_{\text{trans}}$, $v_e$) and H&E-stained whole-mount histology were defined using anatomic divisions of the peripheral zone and central gland; regions of interest for tumours were defined at pathology, as were control regions in normal peripheral zone tissue. Relative areas of cellular components (%area of nuclei, cytoplasm, stroma, and lumen) were calculated using colour-based segmentation of digitized histology. Significant linear relationships between relative areas and mean MRI-parameter values were assessed, including values from normal peripheral zone and central gland regions (i.e., excluding regions containing a mixture of tumour and normal) as well as peripheral zone tumours. Significant differences between specific tissue composition in normal and malignant peripheral zone were also tested.

Chapter 6: Discussion

The final chapter is an overall summary and discussion of the work detailed in the thesis. The contributions of each data chapter to the improved characterization or understanding of MR imaging of prostate cancer are discussed, as are some limitations and suggestions for potential improvements.
Chapter 2

Methods
2.1 Introduction

This thesis consists of analyses performed on magnetic resonance imaging (MRI) datasets and histology acquired as part of a University Health Network Research Ethics Board approved trial (REB #05-0386-CE) to explore methods to improve prostate cancer localization. As such, image acquisition, histology, and calculation of parametric maps was consistent across patients, and analysis for each data chapter performed on a sub-set of the patient population summarized here. Chapter 2, therefore, describes the clinical characteristics of the twenty-nine patients included in analyses, as well as methods that were consistent across the entire thesis; any deviations are subsequently noted in the Methods section of the individual data chapters.

2.2 Patient Population

Patients with biopsy-confirmed, localized prostate cancer who were due to undergo radical prostatectomy were approached for inclusion in the study. Exclusion criteria included any prior treatment for prostate cancer, or contraindications for MR imaging or contrast agent, and all patients were determined to be free of nodal or bone metastases using preoperative imaging. Between June 1, 2006, and February 5, 2008, thirty-one patients gave informed consent. Patients had MRI (described in detail in the next sections), followed by surgery. The surgical specimens were processed to generate whole-mount histologic sections (described in section 2.4). The median time between MRI and surgery was 20 days (range: 1 – 139 days). One patient’s histology was sent to another institution for review, and one was processed incorrectly; hence, these patients were not included in any subsequent analyses. The clinical characteristics of the twenty-nine patients available for study are summarized in Table 2.1.
Table 2.1 – Characteristics of Twenty-nine Prostate Cancer Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Datum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>64 [44 – 72]</td>
</tr>
<tr>
<td>Prostate-specific antigen level</td>
<td>4.6 [1.4 – 11]</td>
</tr>
<tr>
<td>Pathologic stage**</td>
<td></td>
</tr>
<tr>
<td>T2a</td>
<td>5</td>
</tr>
<tr>
<td>T2b</td>
<td>1</td>
</tr>
<tr>
<td>T2c</td>
<td>17</td>
</tr>
<tr>
<td>T3a</td>
<td>5</td>
</tr>
<tr>
<td>T3b</td>
<td>1</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

**Definition of pathologic T Stages given in Chapter 1, Table 1.1
Note – Unless otherwise indicated, data are number of patients

2.3 Magnetic Resonance Imaging

Data (Image) Acquisition

MRI was performed prior to surgery on a 1.5 Tesla MRI system (Excite HD; GE Healthcare, Milwaukee, WI, USA) using a four-channel torso array surface coil coupled to a receive-only endorectal coil (both coils from MEDRAD, Warrendale, PA, USA). To reduce peristalsis, 30 mg of hyoscine butylbromide (Sandoz, Quebec City, PQ, Canada) was delivered intravenously to patients without contraindications for the drug (e.g., glaucoma, irregular heartbeat, or urinary retention). In later patients (13 of 29), the endorectal coil was filled with a 100% w/v barium sulphate suspension (Liquid Polibar; E-Z-EM, Westbury, NY, USA) to reduce susceptibility artifact. Localization images were acquired, followed by image sets to depict prostate anatomy and to permit calculation of the parametric maps used in analyses. All acquisition parameters are contained in Table 2.2. Anatomic imaging was achieved using T2-weighted fast spin echo (FSE) scans acquired in sagittal, and oblique axial and coronal planes. Oblique planes were
prescribed to be perpendicular to the rectal wall/prostate interface, as per Figure 2.1. The T2-weighted image sets were followed by axial-oblique: diffusion weighted imaging (DWI) with a single-shot echo planar imaging protocol (EPI), used to generate maps of apparent diffusion coefficient; T1-weighted fast spoiled gradient echo (FSPGR) images, to assist in identifying regions of hemorrhage; multi-echo FSE for T2-mapping; a multiple flip-angle FSPGR dataset for T1-mapping; a short FSPGR series used to establish timing for contrast agent delivery; and a FSPGR dataset with contrast enhancement (DCE-MRI) to map pharmacokinetic parameters. DCE-MRI included the acquisition of data at two time points prior to injection of a 20 mL bolus of contrast agent (gadopentetate dimeglumine [Magnevist; Bayer Schering Pharma, Berlin, Germany]) at a rate of 4 mL/s, followed by a 20 mL saline flush. Contrast agent injection was performed using a power injector (MEDRAD Spectris MR injection system; MEDRAD, Warrendale, PA, USA). Slice orientation, thickness (3 mm), and locations were prescribed to match the anatomic axial-oblique image set.
Figure 2.1 – Determination of oblique MRI imaging planes. A sagittal T2-weighted MR image through the prostate (blue outline (–)) (a) is used to determine the imaging plane for coronal-oblique (b) and axial-oblique (c) anatomic T2-weighted MRI. The angle of the anterior rectal wall/posterior prostate face (purple line in a (–)) defines the in-plane column axis for coronal-oblique images (b), and the through-plane axis for axial-oblique images (c). In-plane axes for the coronal- and axial-oblique images shown in (b) and (c) are denoted by (---) in the sagittal image (a). sup – superior; inf – inferior; ant – anterior; post – posterior; R – right; L - left. (Anatomic planes of section are defined in Chapter 1, Figure 1.4)
Table 2.2 – Acquisition Parameters for *in vivo* MRI

<table>
<thead>
<tr>
<th></th>
<th>T2-weighted</th>
<th>Diffusion EPI</th>
<th>T1-weighted SPGR</th>
<th>T2 Mapping FSE Multi-echo</th>
<th>T1 Mapping FSPGR Multi-flip</th>
<th>DCE FSPGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sagittal</td>
<td>Axial</td>
<td>Coronal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR (ms)</td>
<td>4750</td>
<td>6500</td>
<td>4600</td>
<td>4000</td>
<td>175</td>
<td>2000</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>90</td>
<td>101.5</td>
<td>96</td>
<td>77</td>
<td>4.2</td>
<td>10 TEs: 9-90ms</td>
</tr>
<tr>
<td>Flip Angle (°)</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>ETL</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>144</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Bandwidth (kHz)</td>
<td>15.65</td>
<td>20.83</td>
<td>20.83</td>
<td>166.7</td>
<td>62.5</td>
<td>31.25</td>
</tr>
<tr>
<td>FOV (cm)</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>14</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Slice Thick. (mm)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>#slices/locations</td>
<td>22</td>
<td>30</td>
<td>22</td>
<td>27</td>
<td>57**</td>
<td>28</td>
</tr>
<tr>
<td>matrix (N_x x N_y)</td>
<td>320x192</td>
<td>320x256</td>
<td>320x256</td>
<td>128x256</td>
<td>320x160</td>
<td>256x128</td>
</tr>
<tr>
<td>NEX</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Scan time (min:sec)</td>
<td>1:30</td>
<td>5:19</td>
<td>3:55</td>
<td>10:40</td>
<td>2:58</td>
<td>9:44</td>
</tr>
</tbody>
</table>

DCE – dynamic contrast-enhanced; FSE – fast spin echo; EPI – echo planar imaging; SPGR – spoiled gradient echo; FSPGR – fast spoiled gradient echo; TR – repetition time; TE – echo time; ETL – echo train length; FOV – field of view; N_x x N_y – number of frequency x phase encoding steps; NEX – number of excitations

**Note – T1-weighted SPGR sequence was prescribed to cover a region greater than the prostate only**
Image Analysis – Parametric Mapping

Acquired images were analysed to provide semi-quantitative parametric maps, determined on a pixel-wise basis using software developed by the author (Deanna L Langer) in Matlab (version 7, The Mathworks, Natick, MA, USA). All data fitting was performed using the unconstrained nonlinear optimization function provided in Matlab (‘fminsearch’), minimizing the sum of the squared error:

\[
sse = \sum_{i=1}^{n} (y_i - f(p_i))^2
\]  

(2.1)

where \( y_i \) is the measured value, and \( f(p_i) \) is the value calculated using the set of parameters, \( p_i \), derived from the fit for \( i = 1:n \) data points.

ADC was calculated directly from the diffusion-weighted data according to:

\[
ADC = -\frac{1}{b} \ln \frac{S}{S_0}
\]  

(2.2)

where the diffusion factor, \( b \), was equal to 600 s/mm\(^2\), and \( S \) and \( S_0 \) are the signal values with and without the diffusion gradient applied, respectively. The T2 relaxation time was determined assuming a mono-exponential decay relationship between the signal acquired from the multi-echo FSE dataset and the echo time (TE) used:

\[
S(TE) \propto Me^{-TE/T2}
\]  

(2.3)

where \( M \) is a factor related to proton density and coil sensitivity profile. Maps of the longitudinal relaxation time, T1, were calculated assuming a single T1 value for the tissue (\( i.e., \) a mono-exponential recovery (Stanisz et al. 2005)), and using a two-step process; the first step determined a preliminary estimate of T1 for each voxel, and the second applied a calibration to correct inaccuracy in the initial estimate, as described below.
To calculate the preliminary estimate of \( T_1 \), the signal obtained from the multi-flip FSPGR dataset was used in conjunction with:

\[
S(\alpha) = M_0 \sin \alpha \frac{1 - e^{-TR/T_1}}{1 - \cos \alpha e^{-TR/T_1}}
\]

where \( \alpha \) is the flip angle, \( S(\alpha) \) is the signal at each flip angle, \( M_0 \) is a factor proportional to the longitudinal magnetization, and \( TR \) is the repetition time (Bluml et al. 1993, Deoni et al. 2003). Inaccuracy in the flip angle, \( \alpha \), due to RF inhomogeneities can have a significant effect on \( T_1 \) estimation (Cheng et al. 2006), and thus additional experiments were performed to evaluate systematic bias in \( \alpha \) evaluation, resulting in the second processing step being included to calibrate the results obtained from the multi-flip FSPGR dataset. A phantom, consisting of vials doped with variable amounts of gadopentate dimeglumine, immersed in a water bath, was scanned using both an inversion recovery sequence with multiple inversion times (TI) (TI = 50, 200, 400, 800, 1000 ms (+2000 ms for one experiment)) and a 3D multi-flip FSPGR protocol with five nominal flip-angles (\( \alpha = 2^\circ, 3^\circ, 7^\circ, 10^\circ, 20^\circ \)). \( T_1 \) was estimated for each pixel in the inversion recovery dataset according to:

\[
S(TI) = M_0 (1 - 2e^{-TI/T_1})
\]

Values were estimated for the FSPGR dataset using the equation for \( S(\alpha) \) above. Both measurements were performed in the same session and repeated on two separate occasions. As shown in Figure 2.2, the two \( T_1 \) protocols yield values that are linearly related, but not 1:1. Using a minimum of two known \( T_1 \) values, the linearity can be used to calibrate the \( T_1 \) estimates from 3D-FSPGR; these reference values were taken as the values for muscle (\( T_1 = 856 \) ms) and fat (\( T_1 = 343 \) ms) reported in de Bazelaire et al. (de Bazelaire et al. 2004), and a calibration curve determined from the linear relationship between the reference values and values calculated from large regions of interest in patient datasets. The regions of interest included left and right internal obturator muscle and periprostatic fat, and were acquired from 6 – 10 slices through the level of the
prostate, then used to scale T1 values. Using this technique, the mean T1 value for the prostate was 1139 +/- 58 ms (range: 1017 – 1226 ms), which is within the range of values reported in the literature (916 – 1670 ms) (Buckley et al. 2004, de Bazelaire et al. 2004, Noworolski et al. 2005, Kershaw et al. 2009).

Figure 2.2 – T1 estimation of gadolinium-doped vials using inversion recovery (a) and multiple flip angle (b) acquisitions, with comparison of mean values (c). Estimated T1 values differ substantially for the two protocols, although a linear relationship between the protocols is apparent. Values in (c) are the mean values for regions of interest in each vial ± the standard deviation, and include values derived from data in (a) and (b), as well as paired datasets from a separate session.

DCE-MRI analysis was performed using a two-compartment model of perfusion (Tofts 1997) and the assumed arterial input function described by Walker-Samuel et al. (combined model, (Walker-Samuel et al. 2006)). Contrast agent concentration in tissue was estimated for each pixel at each time point:

\[
C_t = \frac{1}{r_i T_1} \left( \frac{S(t) - S(0)}{S(0)} \right)
\]

(2.6)
where $r_i$ is the in vivo tissue relaxivity (4.5 L mmol$^{-1}$ s$^{-1}$ (Stanisz et al. 2000)), $T_{10}$ is the calibrated, intrinsic T1 relaxation of the tissue in the absence of Gd-DTPA (determined in the previous step), $S(t)$ is the signal at each time point, $t$, and $S(0)$ is the signal at baseline, i.e., prior to contrast enhancement. All pre-contrast images were averaged to generate $S(0)$. From the two-compartment model presented in Tofts (Tofts 1997), two quantitative measures of tissue perfusion – volume transfer constant, $K_{trans}$, and extravascular extravascular volume fraction, $v_e$ – were derived from:

$$C_i = DK_{trans} \sum_{i=1}^{2} a_i \frac{e^{-(K_{trans}/v_e)t} - e^{-m_it}}{m_i - K_{trans}/v_e}$$  \hspace{1cm} (2.7)\]

The injected dose of contrast agent, $D$, was calculated knowing the injected volume ($V = 20$ mL), concentration of contrast agent ([Gd] = 0.5 mmol/mL), and each patient’s weight; dose was then $V*[Gd]/weight$, and was approximately 0.1 mmol/kg. The arterial input function terms were as reported by Walker-Samuel et al.: $a_1 = 36$ kg L$^{-1}$; $m_1 = 4.9$ min$^{-1}$; $a_2 = 13$ kg L$^{-1}$; $m_2 = 0.08$ min$^{-1}$. Sample contrast agent concentration in tissue ($C_i$) versus time curves, with results of curve-fitting, are shown in Figure 2.3.
2.4 Whole-mount Histology

Immediately after removal from the patient, each prostate was injected with buffered formalin in 20 – 40 locations with a 21-gauge syringe and immersed in formalin for a minimum of 24 hours; this technique ensures adequate fixation of the gland, without affecting tissue morphology (Ruijter et al. 1997). The anterior, left, and right faces of the prostate were marked with different colours of ink, performed to facilitate orientation of each section after histologic processing as the ink remains visible on the outer contour of each section. The prostate specimens were then embedded in an alginate gel (HistOmer; Vibratome, St. Louis, MO, USA) using the following procedure: a layer of gel (~1–2 cm) in the liquid-phase was placed in a flat-bottomed mould, levelled, and allowed to set; the flat, posterior face of the prostate was placed on the layer to approximate the rectal wall/prostate interface in vivo, and the midline of the gland centered along the long axis; the mould was filled with liquid-phase gel, and thin rods were used
to hold the gland in place until the gel solidified. Photos of the fixed, painted gland and gel-embedding procedure are shown in Figure 2.4.

**Figure 2.4 – Gel embedding and 3 mm sectioning of fixed surgical specimen.** The fixed specimen is painted (a) to identify left (green), right (red), and anterior (yellow) aspects of the gland. An alginate gel powder (b) transitions from a liquid phase, when mixed with water, to solid gel, and is used to embed the specimen (c). After *ex vivo* MRI of the gel + tissue is performed, the block is sliced into 3 mm sections (d), with multiple sections measured prior to reaching tissue to confirm slice thickness (e). The resulting sections, at the level of the tissue, are shown in (f). The front face is identified with ink, the gel is removed, and the sample is sent for H&E-stained whole-mount histologic processing.

To confirm orientation of the gland in the gel and potentially improve reproduction of the axial-oblique plane of *in vivo* MRI, *ex vivo* MRI was performed on the embedded specimen. *Ex vivo* MRI was also useful as an intermediary between *in vivo* T2-weighted MRI and whole-mount histology. The mould was placed square within an 8-channel knee coil on the 1.5 Tesla MRI, and axial-oblique T2-weighted MR images acquired at 5° intervals around true-axial orientation. T2-weighted *ex vivo* MRI acquisition parameters were: fast spin echo (FSE); TR/TE, 4000/102.5; matrix 256 x 224; echo train length, 16; bandwidth, 15.6 kHz; number of excitations, 2; anteroposterior phase encoding; 3
mm slice thickness; field of view, 14 cm; time ~ 2:45 mins. The ex vivo images were then inspected concurrently with in vivo, and the angle resulting in the best visual correspondence determined. A higher-resolution T2-weighted ex vivo dataset was then obtained at this angle (reconstructed voxel dimensions: 0.27 x 0.27 x 1.0 mm\(^3\)) with a three-dimensional fast recovery FSE sequence; TR/TE, 2200/103.1; matrix 320 x 224; echo train length, 39; bandwidth, 41.7 kHz; number of signals acquired, one; anteroposterior phase encoding; 1 mm slice thickness; field of view, 14 cm; time ~ 13 mins. After ex vivo MRI, the gel-embedded sample was sliced into 3 mm sections using a rotary blade; when necessary, a wedge was used to duplicate the angle determined during ex vivo imaging. Slice thickness was verified prior to reaching tissue by measuring 6-12 slices of gel with a ruler (Figure 2.4e). The gel was removed, and hematoxylin and eosin (H&E) stained whole-mount histology sections generated from the 3 mm slices. All gel embedding and sectioning was performed in collaboration between genitourinary pathologists (Theodorus H van der Kwast, PhD, MD or Andrew J Evans, PhD, MD) and the author (Deanna L Langer). Whole-mount H&E-stained sectioning was performed by Laibao Sun, MB, MSc, in the laboratory of Martin J. Yaffe, PhD (Sunnybrook Health Science Centre, Toronto, Ontario). A sample whole-mount H&E-stained section is shown in Figure 2.5, with corresponding ex vivo and in vivo MRI.

Whole-mount sections were reviewed by Dr. van der Kwast, and all regions of prostate cancer delineated. Inclusion criteria for tumours in subsequent analyses required lesions be a minimum dimension of 3 x 3 mm\(^2\) and have a Gleason score \(\geq 6\). Malignant regions closer than 3 mm in the same section or in the same location on adjacent slices were considered parts of the same tumour, as per criteria defining multifocality (Villers et al. 1992, Ruijter et al. 1996). For each tumour, the histologic section containing the largest surface area of malignancy was identified and used in further analyses. On these sections, Dr. van der Kwast also delineated contiguous regions of normal, typically-appearing peripheral zone tissue, i.e., containing a mixture of benign
glands intermixed with loose stroma and connective tissue. The corresponding slice location on MRI was determined for each whole-mount section identified for analysis by comparing the location and appearance of a combination of anatomic features on *in vivo* MRI, *ex vivo* MRI, and histology; features included the urethra, ejaculatory ducts, benign prostatic hyperplasia nodules, and number of slices from the apex and base of the gland. Slice locations were confirmed by a radiologist (Masoom A Haider, MD), who transferred both tumour and normal outlines from histology to MRI. Additional anatomic landmarks, such as the radial location or distance to the outer contour or surgical capsule, were used as references while translating the outlines from histology to imaging.

Subsets of these MRI anatomic images or parametric maps, whole-mount H&E-stained histology sections, and malignant and normal peripheral zone tissue outlines were used as the basis of all analyses.

Figure 2.5 – Whole-mount H&E-stained section (a) with corresponding *ex vivo* (b) and *in vivo* (c) T2-weighted MRI. Common features such as benign prostatic hyperplastic nodules can be identified in all images. The *ex vivo* MRI serves as a useful visual intermediary between histology and *in vivo* MRI, having similar contrast characteristics to *in vivo* MRI, but more closely resembling the structure seen on histology.
Chapter 3

Prostate Cancer Detection with Multi-parametric Magnetic Resonance Imaging

This chapter is adapted with permission from the following publication:

Deanna L. Langer, MSc, Theodorus H. van der Kwast, PhD, MD, Andrew J. Evans, PhD, MD, John Trachtenberg, MD, CM, Brian C. Wilson, PhD, and Masoom A. Haider, MD. (2009) “Prostate Cancer Detection with Multi-parametric MRI: Logistic Regression Analysis of Quantitative T2, Diffusion-Weighted Imaging, and Dynamic Contrast-Enhanced MRI.” Journal of Magnetic Resonance Imaging 30(2): 327-34.
3.1 Abstract

To identify malignant voxels in the peripheral zone of the prostate, a multi-parametric model was developed that combines parameters derived from magnetic resonance imaging (MRI) (apparent diffusion coefficient (ADC), quantitative T2, volume transfer constant ($K_{\text{trans}}$), and extravascular extracellular volume ($v_e$)). All voxels used in development and testing of the model originated from regions of interest (ROIs) defined as ‘tumour’ or ‘normal’ on whole-mount histologic sections. Receiver operating characteristic (ROC) curves were generated for each parameter, and the area under the ROC curves ($A_z$) calculated. Step-wise logistic-regression modeling was performed to determine the optimal combination of parameters for identifying malignant voxels, and $A_z$ for each parameter and the final model compared. The best-performing single-parameter was ADC, where $A_{z,\text{ADC}}$ was significantly greater than that of $K_{\text{trans}}$ and $v_e$ ($p < 0.002$), and greater (but not significantly) than $A_z$ for T2 ($p = 0.026$) (mean $A_z$ [95% confidence interval]: $A_{z,\text{ADC}}$: 0.689 [0.675, 0.702]; $A_{z,T2}$: 0.673 [0.659, 0.687]; $A_{z,K_{\text{trans}}}$: 0.592 [0.578, 0.606]; $A_{z,ve}$: 0.543 [0.528, 0.557]). The optimal multi-parametric model, LR-3p, consisted of combining ADC, T2, and $K_{\text{trans}}$ (mean [95% confidence interval]: $A_{z,LR-3p}$: 0.706 [0.692, 0.719]). Compared to $A_z$ of each single parameter, LR-3p performed significantly better than T2, $K_{\text{trans}}$, and $v_e$ ($p < 0.002$), and better than ADC ($p = 0.090$), but not significantly so. Using LR-3p, maps of tumour probability were generated, illustrating the suitability of the model for prospective use in identifying suspicious regions in the prostate.
3.2 Introduction

T2-weighted MRI provides excellent visualization of prostate anatomy, and is capable of localizing prostate cancer with some degree of accuracy. However, further improvements are necessary to be useful in applications such as guiding biopsy, characterizing disease properties or monitoring changes during active surveillance, or facilitating accurate treatment planning for focal therapy delivery. By adding MRI studies that provide some insight into the underlying physiology, such as diffusion weighted imaging (DWI) or dynamic contrast-enhanced (DCE) MRI, some of these improvements are being realized. Methods that combine multiple techniques, i.e., multi-parametric MRI, have the potential to exploit differences in tissue properties and provide a better description of tumour location and extent. In the prostate, multi-parametric MRI is showing promise to outperform single-parameter MRI in prostate cancer applications. Multi-parametric techniques can be divided into qualitative (Kozlowski et al. 2006, Tanimoto et al. 2007, Chen, M. et al. 2008) and quantitative (van Dorsten et al. 2004, Reinsberg et al. 2007, Mazaheri et al. 2008) methods; however, to achieve the clinical goals stated above, optimal output should include a map or image indicating either the presence or absence of cancer, or the risk of malignancy for each voxel. This precludes the use of qualitative methods, and thus this chapter focuses on the development of a quantitative multi-parametric model suitable for prospective mapping of prostate cancer.

The type of values contained in the output generated from multi-parametric MRI – binary or continuous – is determined by the choice of model or method used to combine datasets. The methods developed by both van Dorsten et al. and Reinsberg et al. generate binary output, with the former choosing a threshold based on the distribution of normal values, and the latter employing a linear discrimination approach (van Dorsten et al. 2004, Reinsberg et al. 2007). This differs from Mazaheri et al., who developed a logistic regression model to determine the probability of malignancy for each set of input data (Mazaheri et al. 2008). Although binary classification provides a simplified result (tumour versus
normal), inclusion of intermediate values, such as the continuous probability from a logistic regression model, may assist during lesion assessment, and provides the reviewing physicians with additional information to be used at their discretion. For example, treatment boundaries may be increased during treatment planning for focal therapies to include marginal regions if they are located at a distance from sensitive structures or, conversely, decreased if near tissue implicated in the incidence of side-effects. Maps of continuous values could also potentially play a role during active surveillance if early changes in tissue can be visualized. As the output from a logistic regression model is continuous, and also well-suited for determining the significance and weight of each parameter within a multi-parametric model, this method was used to develop the model in this study.

To obtain an optimal result – *i.e.*, a model that best describes future input – training and test data should reflect the desired task. The use of summary measures derived from regions of interest (ROIs) reduces the impact of noise on the model. However, partial volume averaging errors are likely (*i.e.*, the inclusion of tissues with differing characteristics), and the model may not accurately reflect the inherent heterogeneity of the underlying tissue. Thus, in other MRI/modeling applications (*e.g.*, DCE-MRI), voxel-wise analyses have been suggested as being more appropriate (Padhani *et al.* 2001). In addition to concerns over averaging between tissue types, the use of ROIs may also impact the spatial coherence of each parameter value; *e.g.*, the voxel location corresponding to the median value of one parameter may be distant from the voxel location for the median value of another. This possibility is emphasized by the observation of Kozlowski *et al.*, who noted that the regions of low ADC did not entirely overlap with regions of rapid-enhancement in the majority of their cases (Kozlowski *et al.* 2006). In fact, this discrepancy is the basis of the strength of multi-parametric methods; if all parameters were suspicious for malignancy in the same location, it is likely that there would be no improvement possible from their combination except potentially to reduce noise.
The purpose of the work presented in this chapter was to develop a multi-parametric MRI model, suitable for prospective tumour mapping in the peripheral zone of the prostate, and having improved performance over single-parameter methods. Established, clinical MRI techniques, capable of reasonable (<1 millimetre) resolution, were used and included DWI, quantitative T2, and DCE-MRI. Benign and malignant regions were defined on whole-mount pathology, independent of imaging results; benign regions included ‘normal’ tissue, free of non-malignant pathology such as atrophy or cystic changes. Voxels extracted from these normal and malignant regions were used in the development and testing of a multi-parametric logistic regression model. Finally, the performance of the final model was compared against each single-parameter.

3.3 Methods

Imaging and pathology data from twenty-five of the twenty-nine patients summarized in Chapter 2 were included for analyses in this chapter. The remaining four patients were excluded due to having: tumours originating solely in central gland tissue (n = 2); significant motion during DCE-MRI acquisition (n = 1); and prostate cancer consisting only of micro-foci (having a diameter <3 mm) (n = 1). All imaging and parametric mapping was performed as described in Chapter 2; however, as the original in-plane voxel size differed between the DWI acquisition and the T2 and DCE-MRI data (0.55 mm per side for DWI, compared to 0.78 mm per side for T2 or DCE-MRI), the ADC maps were re-sampled for this chapter only to permit spatial co-registration of voxel values.

The histologic section containing the largest cross-sectional area of each peripheral zone tumour was used, with MRI data from the corresponding slice location. The MRI parameter maps were manually aligned, if needed, to account for between-scan motion of the patient (Figure 3.1): the outer contour of the prostate was delineated on the map of T2 and the outline transferred directly to the ADC and $K_{\text{trans}}$ maps; the maps were shifted individually in voxel increments, such that the contour delineated the prostate for all parameter maps; any shifts determined for $K_{\text{trans}}$ were applied to $\nu_e$. Analyses were performed using voxels
extracted from the ROIs delineating each tumour on the T2 map, as well as a region in normal peripheral zone tissue for use as a control. Voxels were excluded from analyses if curve fitting had failed during DCE-MRI parameter determination, i.e., if the fit failed to converge, or \( v_e \) was outside of the range [0, 1]. Feature vectors used for multi-parametric model development and verification corresponded to the set of parameter values at each spatial location within each ROI; i.e., a subset of \{ADC(\textit{i,} \textit{j}), T2(\textit{i,} \textit{j}), K^{\text{trans}}(\textit{i,} \textit{j}), v_e(\textit{i,} \textit{j})\}, where \((\textit{i,} \textit{j})\) is the voxel located at the \textit{i}th row and \textit{j}th column of the images.

Figure 3.1 – Manual alignment of parameter maps according to outer prostate contour. The outer contour of the prostate was delineated on the map of T2 (green, a), and transferred directly to the re-scaled ADC map (cyan, b), and original resolution \( K^{\text{trans}} \) map (white, c). In this example, the ADC map was shifted two voxels (1.56 mm) to the right in order to match the original (cyan) contour (simulated here by re-drawing the contour in green, two voxels to the left), and \( K^{\text{trans}} \) required no shift. Any transformation performed on \( K^{\text{trans}} \) maps was also performed on \( v_e \) (d). The regions of interest in tumour (red outline) and normal (blue outline), transferred from pathology to the T2 map, were then directly applied to the ADC, \( K^{\text{trans}} \), and \( v_e \) maps.
Parameter values for tumour versus normal tissue were compared using the median values calculated for each patient; for patients having more than one tumour, all tumour voxels were binned prior to determination of the median, as were all normal voxels. Median values are more robust to the presence of outliers, and the generation of one tumour and one normal value for each patient ensured equal representation of each patient during testing; sample distributions of parameter values are shown for one patient in Figure 3.2. Both paired and unpaired non-parametric tests were performed on the tumour and normal median values – signed rank tests for paired median data, and Wilcoxon rank sum tests on the unpaired. The paired tests reflect whether relative (intra-patient) differences between tumour and normal parameter values are significant, whereas the unpaired tests reflect whether differences in values are significant over the population.

Figure 3.2 – Sample distribution of values from a single patient for regions of interest in normal (blue) and malignant (red) tissue for all single parameters. Although the distributions for each parameter are approximately normal in some cases, e.g., ADC, the use of the median value as a summary measure is more robust than the mean in the presence of skewed distribution or outliers.

The full feature vector set, consisting of all voxel data, was used to: determine the Pearson Correlation coefficient (r) between all parameters (Sokal et al. 1995); generate receiver operating characteristic (ROC) curves; and as
input during logistic regression modeling. Re-sampling of the feature vector set was performed using bootstrapping methods (1000 iterations). Bootstrapping facilitates model development and validation with the same dataset, and yields a non-parametric estimate of the confidence interval around each mean \( A_z \) (Sokal et al. 1995). To generate each bootstrap sample, the full dataset is randomly sampled with replacement to produce a distribution with the same number of elements as the original. Each new distribution will thus contain repeats of some voxel values, and be missing others. For large samples, this property results in bootstrap estimates of the mean and confidence limits that are good approximations of the overall population statistics (Sokal et al. 1995).

For each parameter, the mean area under the ROC curve (\( A_z \)) and 95% confidence-interval was calculated from the bootstrapped data, where each individual \( A_z \) for each bootstrap sample was estimated using non-parametric methods (Obuchowski 1997). Differences between the areas were determined, as were the bootstrap p-values. The significance level, \( \alpha \), was adjusted using a Bonferroni correction (Sokal et al. 1995), and \( A_z \) for the four potential input parameters (ADC, T2, \( K_{trans} \), \( v_e \)) compared; significant p-values for comparing \( A_z \) were thus < \( \alpha \), where \( \alpha = 0.05/6 = 0.008 \) for the six possible comparisons. The logistic regression model was developed by adding parameters step-wise based on decreasing area (starting from the highest \( A_z \) and working down). After each parameter addition, the significance of the change in deviance was tested (\( \Delta \text{deviance} = \chi^2 \) with one degree of freedom; the p-value then reflects the significance of the improvement of fit achieved through the addition of the parameter to the model). Generalized estimating equations with an independence working correlation matrix were used to account for within-patient correlations, and correct standard errors and p-values of the logistic regression coefficients. The final logistic regression model was compared with each parameter, with \( \alpha \) again adjusted using a Bonferroni correction (significant p-values < \( \alpha \), where \( \alpha = 0.05/4 = 0.0125 \) for the four possible comparisons). All statistical analyses were performed in Matlab (version 7, The Mathworks, Natick,
MA, USA), with the exception of the use of generalized estimating equations which was performed in the R statistical environment (v.2.6.2, R Foundation for Statistical Computing, Vienna, Austria) using geepack (v.1.0.13).

3.4 Results

Most patients had a single focus of prostate cancer (16 of 25). However, six patients had two peripheral zone tumours, two had three, and one had four separate tumours; thus, thirty-eight tumour and thirty-eight normal ROIs were included in analyses. Twenty-three of the thirty-eight tumours had a Gleason score of 6, with the remainder (15 of 38) having a Gleason score of 7. The median cross-sectional area for the tumour ROIs was 37 mm$^2$ [range: 9.5 – 311 mm$^2$]. Median values for all ROIs are presented in Figure 3.3 and summarized in Table 3.1. ADC and T2 values were significantly lower in tumour (1.28 x10$^{-3}$ mm$^2$/s and 89 ms, respectively) than in normal tissue (1.47 x10$^{-3}$ mm$^2$/s and 112 ms, respectively) for both non-parametric matched-pair (p < 0.001) and overall (p < 0.005) tests. In matched-pair samples (i.e., intra-patient comparisons), the median volume transfer constant, $K_{\text{trans}}$, was significantly higher in tumour than normal (0.30 versus 0.25 min$^{-1}$, respectively, p = 0.013), and extravascular extracellular volume, $v_e$, showed a trend towards being lower for tumour versus normal (0.28 versus 0.29, respectively, p = 0.067); neither $K_{\text{trans}}$ nor $v_e$ were significantly different in unpaired tests of tumour versus normal values ($K_{\text{trans}}$ – p = 0.17; $v_e$ – p = 0.67).
Figure 3.3 – Median values for all regions of interest in normal and malignant peripheral zone tissue. Distributions are shown for all parameters, ADC (a), T2 (b), $K_{\text{trans}}$ (c) and $v_e$ (d), with each 'x' representing the median value for a region of interest, and lines connecting normal and tumour values for the same patient. The red □'s in each distribution represents overall medians (reported in Table 3.1). Although there is considerable overlap between normal and tumour distributions, tumour values are typically lower than normal for ADC and T2, and higher than normal for $K_{\text{trans}}$ and $v_e$. This difference was significant using matched-paired tests for ADC, T2, and $K_{\text{trans}}$, and was significant for unpaired tests for ADC and T2 ($p < 0.05$ for all significant results). (PCa – prostate cancer)
Table 3.1 – Summary Parameter Values for Tumour and Normal Prostate in Peripheral Zone (PZ) Tissue

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal PZ mean ± sem**</th>
<th>Tumour mean ± sem**</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 (ms)</td>
<td>112 [71, 225]</td>
<td>89 [71, 137] a,b</td>
</tr>
<tr>
<td></td>
<td>119 ± 8</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>ADC (x10^{-3} mm^2/s)</td>
<td>1.47 [1.01, 2.07]</td>
<td>1.27 [0.94, 1.64] a,b</td>
</tr>
<tr>
<td></td>
<td>1.51 ± 0.05</td>
<td>1.27 ± 0.03</td>
</tr>
<tr>
<td>K_{trans} (min^{-1})</td>
<td>0.25 [0.09, 0.77]</td>
<td>0.30 [0.14, 1.25] b</td>
</tr>
<tr>
<td></td>
<td>0.29 ± 0.04</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>ν_e (no units)</td>
<td>0.29 [0.06, 0.44]</td>
<td>0.28 [0.11, 0.46]</td>
</tr>
<tr>
<td></td>
<td>0.29 ± 0.02</td>
<td>0.31 ± 0.02</td>
</tr>
</tbody>
</table>

*a normal PZ and tumour significantly different using Wilcoxon rank sum testing (p < 0.05)  
*b normal PZ and tumour significantly different with match-pair signed rank tests (p < 0.05)  
**Note – due to the non-normal distribution of values, valid comparisons are between medians. However, means and standard error of the mean (sem) are provided for reference.

The total number of feature vectors derived from all ROIs was 6862 (2416 normal, 4446 malignant), which was reduced to 6460 (2308 normal, 4152 malignant) after voxels with failed DCE-MRI fitting were excluded, i.e., the fitting procedure failed to converge; these 6460 voxels were used to look at correlation between parameters, to construct all ROC curves, and in logistic regression modeling. Correlation between parameters was, in general, weak, with the highest correlation seen between T2 and ADC (r = 0.520); scatter plots are presented in Figure 3.4. Histograms of all tumour and normal feature vector data, generated for each parameter, are shown in Figure 3.5; receiver operating characteristic (ROC) curves for the individual parameters are shown in Figure 3.6. ADC had the highest performance (mean A_{z,ADC}, [95% confidence interval]: 0.69, [0.68, 0.70]), and was significantly greater than A_{z,Ktrans} (0.59, [0.58, 0.61], p < 0.002) and A_{z,ve} (0.54, [0.53, 0.56], p < 0.002). A_{z,ADC} was also larger than A_{z,T2} (0.67, [0.66, 0.69], p = 0.026) although this difference was not significant according to the Bonferroni-corrected threshold (i.e., α = 0.008).
Figure 3.4 – Correlation between MRI-derived parameters. Each point represents the corresponding parameter values (labelled on the x and y axes) for the same voxel. (blue markers represent voxels in normal peripheral zone, red markers represent voxels in malignant tissue)
Figure 3.5 – Distributions of all parameter and model values in tumour (red) and normal (blue) peripheral zone tissue. Histograms are shown for each single parameter ((a) ADC, (b) T2, (c) $K_{\text{trans}}$, (d) $v_e$), as well as tumour probability calculated from the multi-parametric model ((e) LR-3p). Ideally (i.e., in the case of perfect separation between tumour and normal values), red and blue distributions would be distinct. Data were derived from the filtered, final feature vector set (4152 malignant and 2308 normal voxels), and histograms were generated such that there are 200 bins across the range shown. Corresponding receiver operating characteristic (ROC) curves are shown in Figures 3.6 and 3.7.
Figure 3.6 – Receiver operating characteristic (ROC) curves for all parameters: quantitative T2, ADC, $K^{\text{trans}}$, $v_e$. Mean $A_z$ and 95%-confidence interval (95%CI) values were calculated from bootstrapped samples. $A_z,\text{ADC}$ was significantly greater than $A_z,K^{\text{trans}}$ or $A_z,v_e$ ($p < 0.008$), but not $A_z,T2$ ($p = 0.026$).

Reductions in deviance were significant during the addition of ADC ($\Delta\text{deviance} = 612$), then T2 ($\Delta\text{deviance} = 117$), then $K^{\text{trans}}$ ($\Delta\text{deviance} = 45$) ($p < 0.001$ for each addition); $v_e$ did not account for a significant improvement in the model ($\Delta\text{deviance} = 0.76$, $p = 0.39$), thus the final logistic regression model (LR-3p) consisted of ADC, T2 and $K^{\text{trans}}$. The probability of a voxel being malignant was:

$$
\Pr = \frac{e^{3.176-1378ADC-0.0089T2+0.715K^{\text{trans}}}}{1+e^{3.176-1378ADC-0.0089T2+0.715K^{\text{trans}}}}
$$

(3.1)
The p-value for each coefficient in LR-3p was not always significant (p-value for the constant, ADC, T2, and $K^{\text{trans}}$: <0.001, 0.001, 0.016, 0.28 respectively). Histograms of malignant probability, calculated using LR-3p for the tumour and normal feature vectors, are shown in Figure 3.5e. The ROC curve for LR-3p is shown in Figure 3.7, along with ADC and T2 for comparison. The area under the ROC curve for LR-3p (mean $A_z,_{\text{LR-3p}}$ [95% confidence interval]: 0.71, [0.69, 0.72]) was significantly higher than $A_z$ for T2, $K^{\text{trans}}$, and $v_e$ ($p < 0.002$ for all). Additionally, although $A_z,_{\text{LR-3p}}$ was also greater than $A_z,_{\text{ADC}}$, this difference was not significant ($p = 0.09$). Finally, the multi-parametric model was developed to be capable of prospective mapping of regions suspicious for cancer in the peripheral zone of the prostate. Thus, two examples of input data and LR-3p output maps are shown in Figures 3.8 and 3.9.

![Figure 3.7](image_url)

**Figure 3.7** – Receiver operating characteristic (ROC) curves for 3-parameter logistic regression model (LR-3p), quantitative T2, and ADC. (T2 and ADC curves are repeated from Figure 3.6 for comparison.) LR-3p tended to follow the regionally ‘highest’ ROC curve, with behaviour similar to T2 for high-sensitivity, and similar to ADC for high-specificity. $A_z,_{\text{LR-3p}}$ was significantly greater than $A_z,_{\text{T2}}$ ($p < 0.002$), and tended to be greater than $A_z,_{\text{ADC}}$ ($p = 0.09$, not significant).
3.5 Discussion

This chapter demonstrates that the overall performance of MRI for detecting cancer in the peripheral zone of the prostate may potentially be improved with multi-parametric methods. The developed model combines information from multiple datasets to generate one comprehensive value to assess the risk of malignancy, precluding review of each MRI scan separately. Identification of regions in malignant or normal peripheral zone tissue was determined on whole-mount histologic sections by a pathologist, blinded to imaging appearance; these regions were transferred to MRI based on anatomic location, versus the presence or absence of MRI features suggestive of malignant status. Thus, potential bias in the determination of the training regions on imaging was reduced. All voxels within these regions contributed to evaluation of each parameter and training/testing of the model, more accurately representing the wide range of values and spatial correspondence encountered in a multi-parametric image set, compared with the use of summary values. The multi-parametric model, LR-3p, was better-able to distinguish between voxels in normal and malignant peripheral zone tissue, as determined quantitatively through the calculation of the mean area under the ROC curve, $A_z$. Additionally, Figures 3.8 and 3.9 demonstrate that the model is suitable for generating maps of malignant probability from input parameter datasets, and could thus be applied prospectively to assist in tumour localization. Although the improvements in $A_z$ for LR-3p over single parameters were not statistically significant in all cases – specifically when compared to the apparent diffusion coefficient – Figure 3.9 provides an example of where the inclusion of parameters in addition to ADC improves visibility of the peripheral zone tumour. Additionally, in the future, the methods described in this chapter can be used as a template to test the impact that the inclusion of novel or improved parametric maps may have on the performance of multi-parametric MRI.
Figure 3.8 – Whole-mount histologic section (a) and corresponding anatomic T2-weighted MRI (b), input parametric maps (ADC (mm$^2$/s) (c), quantitative T2 (ms) (d), $K^\text{trans}$ (min$^{-1}$) (e)), and tumour probability map (LR-3p (no units) (f)): tumour visible in all input parametric maps. Tumour and normal peripheral zone regions were identified on histology, and are delineated in black and blue, respectively, in (a), and with solid and dotted lines, respectively, in (c-f). LR-3p values are only valid for peripheral zone tissue. Hypo-intense regions can be seen in ADC and T2 corresponding to the tumour delineated in a, with slightly hypo-intense ADC and T2 values also seen on the contra-lateral side of the prostate. $K^\text{trans}$ is increased from surrounding tissues in the area of tumour, with fewer regions of higher intensity contra-laterally. The lesion is clearly visible in LR-3p, and is more distinct from the background and contra-lateral side, compared to the single parameter maps. (patient age, 49 y; prostate-specific antigen level, 4.18 ng/mL; pathologic stage, T2c; Gleason score, 6)

The performance of all single- and multi-parametric methods studied, as quantified by $A_z$, tends to be below, but within the range encompassed by, previous studies ($A_z$ range: [0.52, 0.90]) (Scheidler et al. 1999, Chan et al. 2003, Engelbrecht et al. 2003, Haider et al. 2007, Reinsberg et al. 2007, Mazaheri et al. 2008); however, is important to note that every voxel within an ROI was included in this work, as opposed to the use of summary values or overall lesion detection. This produces more realistic training data, takes into account variability present as a result of true physiologic variation within an ROI (e.g., Gleason patterns in prostate cancer), and maintains spatial correspondence between parameter values. Multi-parametric models based on summary values do not capture this
physiologic heterogeneity, potentially limiting their utility for tumour mapping; however, the increased noise inherent to voxel-wise data may decrease apparent performance, as there is likely to be increased overlap between tumour and normal values.

Figure 3.9 – Whole-mount histologic section (a) and corresponding anatomic T2-weighted MRI (b), input parametric maps (ADC (mm$^2$/s) (c), quantitative T2 (ms) (d), $K_{\text{trans}}$ (min$^{-1}$) (e)), and tumour probability map (LR-3p (no units) (f)): tumour not visible in all modalities. Tumour and normal peripheral zone regions were identified on histology, and are delineated in black and blue, respectively, in (a), and with solid and dotted lines, respectively, in (c-f). LR-3p values are only valid for peripheral zone tissue. The tumour was not visible by T2-weighted MRI, and is not clearly defined as a hypo-intense lesion by ADC or T2. Increased $K_{\text{trans}}$ is seen, corresponding well to tumour location and extent. In the LR-3p probability map, the tumour can be appreciated against the background. Although in this case the entire lesion is not described in LR-3p, the multi-parametric map represents improvement over ADC and T2, and is reduced in noise compared to $K_{\text{trans}}$. (patient age, 68 y; prostate-specific antigen level, 4.62 ng/mL; pathologic stage, T2a; Gleason score, 6).

The relatively poor performance of the DCE-MRI-derived parameters, the volume transfer constant, $K_{\text{trans}}$, and the extravascular extracellular volume, $v_e$, was likely influenced by a number of factors, including noise in the voxel-wise data, as well as choices made during the pharmacokinetic modeling such as the use of an assumed arterial input function. However, the significance of the reduction in deviance resulting from its addition indicates that $K_{\text{trans}}$ is contributing
to the overall goodness of fit of the model. Additionally, correlation between either pharmacokinetic parameter ($K_{\text{trans}}$ or $v_e$) and ADC or T2 was <0.5, suggesting that DCE-MRI provides complementary information in a multi-parametric protocol. This is demonstrated in Figure 3.9, as the lesion is primarily visible in the $K_{\text{trans}}$ map (Figure 3.9e), versus the other two input parameters (ADC and T2, Figures 3.9c and 3.9d), ultimately translating to the increase in tumour probability seen in the LR-3p map (Figure 3.9f). Any improvements to DCE-MRI acquisition or analysis may, therefore, further improve the overall performance the multi-parametric model, although the model should be re-trained and tested prior to implementation.

Logistic regression was selected to develop the multi-parametric model, since optimal weighting of each parameter is determined objectively, the output is a continuous variable reflecting the probability that a voxel is malignant, and, once developed, the model can be applied to prospective datasets without requiring radiologist review at each stage. This differs from earlier works that require review and risk assessment of each dataset (Kozlowski et al. 2006, Tanimoto et al. 2007, Chen, M. et al. 2008), or studies that use binary discrimination methods (van Dorsten et al. 2004, Reinsberg et al. 2007). Mazaheri et al. also pursued logistic regression analysis (Mazaheri et al. 2008), developing their model using mean values from ROIs for ADC and metabolite ratios. Their results are comparable, namely that $A_z$ for multi-parametric MRI is greater than that of the single parameters input to the model, with the improvement significant compared to metabolite ratios and, as in this work, tending towards significance versus $A_{z,\text{ADC}}$ ($p = 0.09$ in both studies). They did not extend their results to attempt tumour probability mapping, but were limited by the resolution of the MRSI scan. This limitation is in fact common to all quantitative multi-parametric MRI studies reported to date, and greatly reduces the utility of these models; accurate target delineation for focal therapy is restricted by the large voxel size (dimensions approximately 3.4 – 9 mm in-plane and 7 – 15 mm through-plane), and results may be impacted by mixtures of normal and malignant tissues within the voxels. Additionally, compared to a
higher resolution technique, fewer voxels will contain tumour. Thus, a false-negative result in a single voxel may have significant repercussions, as it could potentially lead to the entire lesion being missed. In comparison, the MRI methods included in the development of the multi-parametric model presented in this chapter are much higher in resolution; the in-plane voxel size is less than a millimetre (0.78 mm) per side, and the slice thickness is 3 mm. Thus, the clinical utility for applications such as monitoring prostate cancer progression in active surveillance, guiding biopsy, or target delineation for focal therapy is potentially greater with the methods described here.

Training and test data were limited to normal and tumour voxels in peripheral zone tissue in the prostate. This was necessary as, because the parameter values measured in the central gland differ from those measured in the peripheral zone (Padhani et al. 2000, Gibbs et al. 2001, Issa 2002, Buckley et al. 2004, Sato et al. 2005, de Souza et al. 2007), each zone requires separate datasets for model development. The limited number of central gland tumours in the entire patient cohort (5 in 29 patients) restricted their study at this time; however, the same methods used for the peripheral zone could be applied in the future to develop and test a multi-parametric model specific to the central gland. Additionally, ROIs for ‘normal’ data were restricted to regions in healthy peripheral zone tissue; although all non-malignant peripheral zone tissue could be included, pathologies such as fibrosis, inflammation or atrophy would likely have been present, potentially confounding results. Future work should include testing the multi-parametric model performance in non-normal – but non-malignant – tissue. However, a suitable cohort that includes representation of each non-malignant pathology for a number of patients is required, and is thus beyond the scope of the work described in this chapter.

3.6 Summary

This chapter details the development of a multi-parametric MRI model to identify malignant voxels in peripheral zone prostate tissue. Single-parameters were derived from DWI, quantitative T2, and DCE-MRI imaging studies, with the final
model including ADC, T2, and $K^{\text{trans}}$. The multi-parametric model thus incorporates information from different aspects of prostate physiology (water diffusion, chemical environment, vascular properties) into a single value. The model has inherent limitations in performance, as demonstrated in this study, but has the advantage of not relying on reader interpretation for diagnosis. Hence, multi-parametric MRI analysis capable of objectively mapping tumour probability is feasible, and may offer better detection of prostate cancer in the peripheral zone over single-modality techniques.
Chapter 4
The Impact of Intermixed Normal Tissue within Prostate Cancer on Measurements of Apparent Diffusion Coefficient and Quantitative T2

This chapter is adapted with permission from the following publication:

4.1 Abstract

The effect of intermixed normal and malignant peripheral zone tissue on quantitative magnetic resonance imaging (MRI) was studied by comparing normal tissue with tumours having substantial normal tissue contributions (‘sparse’) versus those comprised mainly of malignant glands (‘dense’). Prior to radical prostatectomy, maps of apparent diffusion coefficient (ADC) and T2 relaxation time were obtained using a 1.5-T clinical MRI system with endorectal coil. Thirty-nine peripheral zone tumours were reviewed on whole-mount histology, and regions assessed to contain primarily (>60%) normal peripheral zone tissue were delineated. Tumours were categorized as ‘sparse’ if more than 50% of their cross-sectional area was primarily normal peripheral zone regions, and were considered ‘dense’ otherwise. Normal peripheral zone tissue was outlined separately on the same sections. Tumour and normal tissue outlines were transferred to corresponding ADC and T2 maps, and median values calculated. Values were compared using multiple regression analysis. Matched-pair tumour-to-normal tissue differences and log2-transformed ratios were assessed using nonparametric tests. Thirty-one percent (12 of 39) of tumours were sparse; sixty-nine percent (27 of 39) were dense. ADC and T2 was significantly lower for dense tumours than for normal tissue using both paired and unpaired testing (p < 0.017), but no significant differences were determined between sparse tumours and normal peripheral zone. Log2-transformed tumour-to-normal tissue ratios were significantly less than zero for dense tumours for both ADC and T2 (p < 0.001), however no difference was determined for sparse tumours; comparison of the ratios calculated for dense and sparse tumours also ascertained that ratio values for dense tumours were significantly lower than sparse (p < 0.05). In conclusion, sparse prostate tumours have similar ADC and T2 values to those of normal peripheral zone tissues. This may limit detection and volume assessment of some prostate cancers using MRI.
4.2 Introduction

Knowledge of tumour location and extent in prostate cancer is essential to facilitate accurate targeting during focal therapies, and may also directly affect patient management decisions during active surveillance. To facilitate improved localization and characterization of prostate cancer, multi-parametric magnetic resonance imaging (MRI) strategies are being pursued (van Dorsten et al. 2004, Kozlowski et al. 2006, Reinsberg et al. 2007, Tanimoto et al. 2007, Chen, M. et al. 2008, Mazaheri et al. 2008). Chapter 3 presented a multi-parametric MRI model to combine diffusion weighted imaging, T2 relaxation, and dynamic contrast-enhanced MRI to improve discrimination between malignant and benign voxels in peripheral zone tissue. The model is the first example of a quantitative method to combine these MRI techniques, and the input datasets and modeling methods were chosen based on their suitability for prospective application to patient data to generate maps of malignant probability. However, the overall performance of any multi-parametric technique is influenced by the performance of each individual parameter. Thus, Chapter 4 identifies a potential source of false-negative imaging results, based on tissue characteristics of peripheral zone tumours.

Tissue composition in prostate cancer is heterogeneous in nature; tumours are typically composed of densely packed malignant glands, but may also consist of few malignant glands scattered within normal tissues. The density of the stromal components within tumours also varies and may have prognostic implications, with dense, reactive stroma shown to be related to disease recurrence (Yanagisawa et al. 2007). Large, moderately- or poorly-differentiated tumours in the posterior half of the prostate are easier to detect using MRI (Ellis et al. 1994), however little has been written about how the specific tumour composition influences MRI signal characteristics. The presence of normal peripheral zone tissue interdigitated with malignant glands may reduce contrast between tumour and normal tissue, and has been noted as a source of volume discrepancy between pathologic and MRI findings by Jager et al. and Quint et al.,
or suggested as a factor leading to a false-negative MRI result by Schiebler et al. (Schiebler et al. 1989, Quint et al. 1991, Jager et al. 1996). Preliminary visual inspection of the cohort described in Chapter 2 suggested that the presence of normal tissue within the tumour may also be influencing the detection of peripheral zone cancers with ADC and T2. Chapter 4 therefore presents a study to quantitatively determine the impact the presence of normal peripheral zone tissue in a tumour has on measurements of ADC and T2 in prostate cancer. Using semi-quantitative criteria developed in conjunction with genitor-urinary pathologists, tumours were divided into two categories: ‘dense’ tumours, consisting primarily of malignant glands and reactive stroma; and ‘sparse’ tumours, comprised of a mixture of scattered malignant glands and normal peripheral zone tissue. The median ADC and T2 values calculated for these two tumour types were compared against values obtained in control regions of normal peripheral zone tissue, as well as against each other.

4.3 Methods

From the twenty-nine patients summarized in Chapter 2, twenty-six were included in this study. Three patients were excluded, as their cancers originated solely in central gland tissue (n = 2), or had tumours that consisted of micro-foci only (<3 mm) (n = 1). All imaging, parametric mapping, and whole-mount histologic sectioning was performed as per Chapter 2. After delineation of peripheral zone tumours on whole-mount histology, a pathologist (Theodorus H. van der Kwast) further inspected each section containing the largest cross-sectional area of prostate cancer for each tumour focus. Normal peripheral zone tissue was defined as a combination of benign glands and loose stroma. All regions within the tumour, >1.5 mm x 1.5 mm, consisting of >60% normal peripheral zone were identified in a semi-quantitative review and were considered ‘sparse’ malignant tissue. The 60% threshold reflected a cut-off that was felt to be reproducible by Dr. van der Kwast; in practice, these regions tended to be >80% normal peripheral zone tissue. Remaining tumour regions were considered ‘dense’ malignant tissue, and consisted of tissue with a high
malignant gland to stroma ratio or a high proportion of smooth muscle, desmoplastic, or collagenous stroma. Examples of each tissue type are given in Figure 4.1.

Figure 4.1 – Histologic examples from normal and malignant regions. Samples of loose stroma are indicated with ‘+’s; benign glands with ‘∗’; and malignant glands with arrows. (a) Normal peripheral zone tissue is characterized by a mixture of loose stroma and benign glands. Sparse regions in tumours consist of normal peripheral zone tissue infiltrated by scattered malignant glands; (b) malignant glands are intermixed with benign glands and loose stroma; (c) a line of malignant glands traverses through otherwise normal loose stroma. (d-f) In contrast, dense regions in tumours consist of (d) a high proportion of malignant glands, (e) dense smooth muscle tissues, uncharacteristic of normal peripheral zone tissue and visible as solid staining, (f) or malignant glands mixed with desmoplastic stroma. (Hematoxylin-eosin stain; magnification, x100)

Sections were digitized at a resolution of 300 dots per inch and saved using Adobe ImageReady CS2 9.0 (Adobe Systems Incorporated, San Jose, CA, USA). Regions were traced on the images in Matlab version 7 (The Mathworks, Natick, MA, USA), and cross-sectional areas calculated for all sparse and dense tissue components in each tumour. A primary classification of ‘sparse’ or ‘dense’ was given to each tumour, based on the dominant tissue component (>50% cross-sectional area) in the lesion. A secondary classification of ‘homogeneous’ was given to the tumour if >70% of the entire cross-sectional area was one tissue type; tumours were considered ‘heterogeneous’ otherwise. As a control, a region
of interest (ROI) of normal peripheral zone was delineated. Minimum dimensions for this region were 3 mm x 3 mm and had to contain exclusively normal peripheral zone tissue. A schematic example of an annotated whole-mount section is shown in Figure 4.2.

![Schematic example of regional assessment of whole-mount histologic section](image)

Figure 4.2 – Schematic example of regional assessment of whole-mount histologic section. The boundary between the peripheral zone and central gland is indicated by the dotted line. Sparse malignant regions greater than 1.5 x 1.5 mm were identified within each tumour at pathologic review. Tumours were given an overall classification of ‘sparse’ if >50% of the cross-sectional area consisted of sparse malignant regions, and ‘dense’ if >50% of the cross-sectional area consisted of dense malignant regions. A secondary classification of homogeneous was given if >70% of the tumour was sparse or dense; otherwise, tumours were considered heterogeneous. On the same section, a region of normal peripheral zone tissue was also identified. This schematic represents a tumour that would be classified as dense and homogeneous. PZ – peripheral zone.

Tumour and normal ROIs were transferred from histology to ADC and T2 maps by a radiologist (Masoom A. Haider), and the median value of each ROI calculated. To investigate differences between all normal peripheral zone, sparse tumour, and dense tumour values – while accounting for variations in the number of tumours per patient – two-stage multiple regression analysis was performed for each tissue type pair (e.g., sparse tumour versus normal peripheral zone tissue): the first stage modeled inter-patient variability; the second stage added tissue type, controlling for inter-patient effects. The p-value associated with the $R^2$ change was assessed for significance after the significance level, $\alpha$, was corrected for the multiple comparisons using a Bonferroni adjustment (significant p-value $< \alpha$, where $\alpha = 0.05/3 = 0.017$ for the three possible comparisons). To evaluate within-patient differences, matched-pair tumour and
normal values were compared using Wilcoxon signed rank tests, as were the log₂-transformed tumour-to-normal ratios against ‘0.0’ (corresponding to no difference between tumour and normal values). These two tests correspond to evaluating image ‘contrast’ through assessment of both differences in parameter values, and the ratio of values from within the same image. Finally, Mann-Whitney U-tests were used to determine significant differences between within-patient differences or log₂-transformed ratios in sparse versus dense tumours. SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) was used for multiple regression analyses, and all other statistical analyses (Wilcoxon signed rank and Mann-Whitney U-tests) were performed in Matlab 7 (The Mathworks, Natick, MA, USA).

4.4 Results

Thirty-nine tumours from twenty-six patients were reviewed. Twelve tumours were classified as sparse (size: median 25 mm², range 10-72 mm²), and twenty-seven classified as dense (size: median 51 mm², range 14-148 mm²). Eighteen patients had a single focus of cancer, six patients had two foci, two patients had three foci, and one patient had four foci. The majority of all tumours were ‘homogeneous’ (90% (35/39)), as defined using the criteria described in the methods; however, many tumours had a small component of the other tumour type (51% (20/39)). Twenty-two tumours were Gleason grade 6, and seventeen were Gleason 7. All sparse tumours were Gleason 6. Sample ROIs from pathology, T2-weighted MRI, ADC and T2 maps are shown for dense and sparse cancers in Figures 4.3 and 4.4.

For both ADC and T2 measurements, normal peripheral zone was significantly higher than dense tumour (p < 0.001 for both ADC and T2), but was not different from sparse tumours (p = 0.99 and p = 0.10 for ADC and T2, respectively). ADC and T2 values were higher in sparse tumours than in dense. These differences were not significant, however sparse versus dense tumours for ADC approached significance (p = 0.02 and p = 0.44 for ADC and T2, respectively). Median values for normal peripheral zone, sparse tumours, and dense tumours are summarized in Table 4.1, and plotted in Figure 4.5.
There was no significant difference in matched-pair sparse tumour and normal peripheral zone values (p = 0.58 and p = 0.62 for ADC and T2, respectively); however, normal peripheral zone values were significantly higher than those in dense tumour for both ADC and T2 (p < 0.0001 and p = 0.0002 for ADC and T2, respectively). All difference values calculated from matched-pair tumour and normal values are summarized in Table 4.2. Log$_2$-transformed tumour-to-normal ratios, summarized in Table 4.2 and shown in Figure 4.6, were significantly lower than zero in dense tumours (p < 0.0001 and p = 0.0001 for ratios in ADC and T2, respectively), but not sparse (p = 0.57 and p = 0.73 for ratios in ADC and T2, respectively). For both ADC and T2, sparse and dense tumour values were significantly different from each other using log$_2$-transformed ratios (p = 0.004 and p = 0.03 for ADC and T2, respectively). When the relative differences between sparse and dense tumour values were compared, the tumour types were significantly different for ADC (p = 0.01), and tended towards significance for T2 (p = 0.08).
Figure 4.3 – Whole-mount sections, corresponding T2-weighted MR images, and ADC and T2 maps for a dense tumour example. (a) A region in normal peripheral zone tissue (blue outline) and the outer extent of the dense tumour (red outline) were outlined on the H&E-stained section. The corresponding T2-weighted MRI (b), ADC map (mm$^2$/s) (c), and T2 map (ms) (d) are shown. Compared to the normal region, the tumour is visible as an area of hypo-intensity. The crossed-out lesion in (a) did not meet the size criteria for evaluation. (patient age, 64 y; prostate-specific antigen level, 3.47 ng/mL; pathologic stage, T2a; Gleason score, 6)
Figure 4.4 – Whole-mount sections, corresponding T2-weighted MR images, and ADC and T2 maps for a sparse tumour example. (a) A region in normal peripheral zone tissue (blue outline) and the outer extent of the sparse tumour (red outline) were outlined on the H&E-stained section. The corresponding T2-weighted MRI (b), ADC map (mm²/s) (c), and T2 map (ms) (d) are shown. This tumour is not visible on any of the MR images. (patient age, 44 y; prostate-specific antigen level, 9.14 ng/mL; pathologic stage, T2a; Gleason score, 6)
Figure 4.5 – Distributions of median values from all ROIs, all tissue types. For both ADC (a) and T2 (b), dense tumours were significantly lower than normal peripheral zone tissue (p < 0.017) and there was no statistical difference between normal peripheral zone tissue and sparse tumours. Median values from each ROI are shown by each ‘x,’ and the medians of the distributions are plotted as ‘□’.
Figure 4.6 – Distributions of log₂-transformed tumour-to-normal ratios of matched-pair median values. For both ADC (a) and T2 (b) measurements, dense tumour log₂-transformed ratios are significantly lower than zero (p < 0.0001 and p = 0.0001, respectively), while log₂-transformed ratios for sparse tumour values were not significantly different from zero (p = 0.57 and 0.73, respectively). With both measurements, there was a significant difference between sparse and dense tumour populations (p = 0.004 and p = 0.03, respectively). Each ratio is shown as an ‘x,’ and the medians of the distributions are plotted as ‘□’. A value of -1.0 corresponds to a twofold difference in the matched tumour value from normal value. Tumour and normal values used to determine each ratio have been obtained from the same slice location.
Table 4.1 – Summary of median ADC and T2 values measured in normal peripheral zone tissue, sparse tumour, and dense tumour

<table>
<thead>
<tr>
<th>Normal</th>
<th>Sparse</th>
<th>Dense</th>
</tr>
</thead>
<tbody>
<tr>
<td>median [range]</td>
<td>median [range]</td>
<td>median [range]</td>
</tr>
<tr>
<td>ADC (x10^{-3}) mm(^2)/s</td>
<td>1.51 [1.03 – 2.20]</td>
<td>1.40 [1.15 – 2.11]</td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>111 [64 – 227]</td>
<td>102 [79 – 154]</td>
</tr>
</tbody>
</table>

\(^a\)Significantly different by two-stage regression, cut-off for significance (\(\alpha\)) = 0.017

Table 4.2 – Summary of tumour-to-normal matched-pair differences and \(\log_2\)-transformed ratios for ADC and T2 in sparse and dense tumours.

<table>
<thead>
<tr>
<th>Difference (Tumour – Normal)</th>
<th>Sparse</th>
<th>Dense</th>
</tr>
</thead>
<tbody>
<tr>
<td>median [range]</td>
<td>median [range]</td>
<td></td>
</tr>
<tr>
<td>ADC (x10^{-3}) mm(^2)/s</td>
<td>-0.04 [-0.38 – 0.23](^c)</td>
<td>-0.26 [-1.1 – 0.02](^a)</td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>-2 [-83 – 37]</td>
<td>-16 [-139 – 25](^a)</td>
</tr>
</tbody>
</table>

\(\log_2\)(Tumour/Normal)

<table>
<thead>
<tr>
<th>Sparse</th>
<th>Dense</th>
</tr>
</thead>
<tbody>
<tr>
<td>median [range]</td>
<td>median [range]</td>
</tr>
<tr>
<td>ADC (no units)</td>
<td>-0.02 [-0.40 – 0.26](^c)</td>
</tr>
<tr>
<td>T2 (no units)</td>
<td>-0.04 [-0.84 – 0.45](^c)</td>
</tr>
</tbody>
</table>

\(^a\)Significantly lower than matched normal value, \(p \leq 0.0002\)

\(^b\)\(\log_2\)-transformed ratio significantly lower than zero, \(p \leq 0.0001\). ‘Zero’ corresponds to equal values for the matched pair.

\(^c\)Sparse and dense tumours significantly different, \(p < 0.05\)

Note – Tumour and normal values used for each difference or ratio were obtained from the same slice location.
4.5 Discussion

This chapter demonstrates that sparse tumours, which consist of predominantly normal peripheral zone tissue, have ADC and T2 values that are statistically indistinguishable from those of normal peripheral zone tissue. In comparison, dense tumours composed of higher malignant gland and stromal tissue content had ADC and T2 values significantly lower than normal peripheral zone. The accuracy of tumour detection and localization were not directly assessed; however, as image assessment is based on relative contrast between lesions suspected of being malignant and the surrounding normal tissue, these results suggest a mechanism that may reduce the correspondence between MRI and pathology.

Within-patient contrast was assessed using matched-pair tests as well as comparing log$_2$-transformed tumour-to-normal ratios against 0 (i.e., representing a non-transformed ratio of 1:1). Additionally, the overall distributions for each tumour type were compared against the distribution of values obtained in normal tissue, which is significant in the context of using the absolute ADC or T2 values in applications such as quantitative multi-parametric modeling. In all cases, dense tumour values were significantly different from normal peripheral zone tissue, whereas there were no significant differences determined for any of the sparse tumour comparisons. Although it is logical that tumours containing a high proportion of normal tissues will be less distinct than those consisting of malignant glands and dense stroma, this study provides quantitative verification that some tumour types or regions within tumours may be intrinsically undetectable by ADC and T2. The results may, in principle, be used to understand contributing factors to false-negative findings in other studies, or poor radiologic-pathologic correlation.

Statistical conclusions for ADC and T2 were the same, which was unsurprising given the underlying mechanisms governing ADC and T2. Increases in cellular density and disruption of ductal architecture have been suggested to result in the differences in ADC measured between tumour and
normal peripheral zone tissue (Gibbs et al. 2001, Issa 2002, Hosseinzadeh et al. 2004, deSouza et al. 2007), whereas T2 is primarily affected by the abundance of free water and local macromolecular environment (Mitchell et al. 1987). The higher percentage of malignant glands and dense stromal components in dense tumours are more likely to displace free water within the lumen, compared to the less-compact distribution of malignant glands in sparse tumours. Despite the quantitative nature of ADC and T2, it is apparent that there is a wide distribution of normal and malignant peripheral zone values (Figure 4.5). For ADC, the overlap in values has also been documented in the literature (Issa 2002, Hosseinzadeh et al. 2004, Mazaheri et al. 2008), which makes establishment of a strict threshold to differentiate tumour versus normal tissue challenging. The differences in ADC and T2 measured between sparse and dense tumours demonstrate one mechanism of how MRI signal is dependent on the features of the tissue being imaged, in addition to simply overall malignant status (i.e., tumour versus normal). However, although these results indicate that the presence of normal tissue within prostate cancer impacts signal in MRI, more detailed analyses of the relationships between parameter values and histology is necessary to develop general relationships.

To reduce inaccuracies that may be incurred during sub-tumour registration between pathology and MRI, each tumour was given an overall classification of ‘sparse’ or ‘dense,’ based on the proportion of sparse or dense regions within the tumour. The secondary classification of homogeneous or heterogeneous facilitates preliminary exploration into the extent to which tumour margins or detectability may be affected. In the patient cohort, all dense (27) and eight of the twelve sparse tumours were homogeneous (i.e., >70% one type). However, 51% (20/39) of all tumours had both sparse and dense components. These results have clear clinical implications: although lesion extent for active surveillance or target delineation for focal treatment of dense tumours may be well-characterized using ADC and T2, there may still be regions of the tumour that are intrinsically undetectable. Additionally, some sparse tumours may lack any dense portion and thus may be missed entirely. An example is shown in
Figure 4.4, where a large tumour focus, indicated in core biopsies, was not visible on T2-weighted MRI, ADC, or T2 mapping. Under-treatment of the true tumour volume could negatively bias patient outcome and reduce the perceived efficacy of therapy. Thus, this work provides a note of caution for prescribing tight treatment margins based on MRI. However, it is noteworthy that all sparse tumours were Gleason 6; further study of the prognostic implications of sparse versus dense Gleason 6 tumours will be of value if MRI is used to guide therapy.

4.6 Summary

This chapter investigates the impact the presence of normal peripheral zone tissue within the tumour boundary has on ADC and T2 values. Descriptions of sparse and dense tumours were introduced, where sparse tumours contain high proportions of normal peripheral zone tissues, and dense tumours consist predominantly of malignant glands and dense, desmoplastic stroma. Median ADC and T2 values for sparse tumours and dense tumours were compared to those of normal peripheral zone using both overall and matched-pair tests, thus testing both overall as well as relative (within-patient) differences. The results indicate that although ADC and T2 distinguish dense tumours from normal, sparse tumour values are not significantly different from normal peripheral zone tissue. Thus, in conclusion, the presence of normal peripheral zone components in prostate cancer places inherent limitations on the ability of ADC and T2 to fully characterize tumours in the peripheral zone of the prostate.
Chapter 5

Investigating the Relationship between Magnetic Resonance Imaging-Derived Parameter Values and Tissue Composition in the Prostate

This chapter is adapted from the following manuscript, *in press* in Radiology:

Deanna L. Langer, MSc, Theodorus H. van der Kwast, PhD, MD, Andrew J. Evans, PhD, MD, Anna Plotkin, MD, John Trachtenberg, MD, CM, Brian C. Wilson, PhD, and Masoom A. Haider, MD. “Prostate Tissue Composition and MR Measurements: Investigating the Relationships Between ADC, T2, $K_{\text{trans}}$, $v_e$, and Corresponding Histologic Features.” *Radiology, in press.*
5.1 Abstract

The relationship between magnetic resonance imaging (MRI)–derived parameters and the underlying tissue composition was studied for normal and malignant prostate. Prior to prostatectomy, T2-weighted, diffusion-weighted imaging, T2-mapping, and dynamic contrast-enhanced MRI was acquired at 1.5-T with an endorectal coil. Maps of apparent diffusion coefficient (ADC), T2, volume transfer constant ($K_{\text{trans}}$) and extravascular extracellular space ($v_e$) were calculated. Whole-mount hematoxylin and eosin–stained sections were generated and digitized at histologic resolution. The percentage area (%area) of tissue components (nuclei, cytoplasm, stroma, luminal space) was measured using image segmentation. Corresponding regions in MRI and histology were defined using anatomically-defined segments in peripheral zone and central gland tissue. Tumour and normal peripheral zone regions were identified at pathology. Each parameter:component pair was assessed using linear mixed-effect models, and tumour versus normal peripheral zone values compared using non-parametric tests. ADC and T2 were inversely related to %area$_{\text{nuclei}}$ or %area$_{\text{cytoplasm}}$, and positively related to %area$_{\text{lumen}}$ (p ≤ 0.01); these trends were reversed for $K_{\text{trans}}$ (p < 0.001). $K_{\text{trans}}$ had a significantly negative (p = 0.01) and $v_e$ positive (p = 0.008) slope versus %area$_{\text{stroma}}$. $v_e$ was inversely proportional to %area$_{\text{nuclei}}$ (p = 0.05). All MRI parameters were significantly different between tumour and normal peripheral zone (p ≤ 0.05), as were all %areas of tissue components (p ≤ 0.001) except for stroma (p = 0.48). In conclusion, MRI-derived parameters measured in the prostate are significantly related to the proportion of specific histological components that differ between normal and malignant peripheral zone tissue.
5.2 Introduction

Currently, the best predictors for the long-term outcome of prostate cancer patients include the biopsy Gleason score, prostate-specific antigen levels, and stage (Kattan et al. 1998), and thus influence the choice of therapy. Morphologic features such as nuclear or lumen size have been shown quantitatively to relate to primary Gleason pattern (Venkataraman et al. 2009) and disease progression (Donovan et al. 2008), and have the potential to contribute to management strategies. However, due to sampling error, tissue properties from biopsy may not accurately reflect those determined post-radical prostatectomy (Steinberg et al. 1997, Montironi et al. 2005, Van As et al. 2008). To assist in tumour localization and potentially improve the characterization of disease, imaging protocols such as diffusion weighted imaging (DWI) and dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) have been added to standard T2-weighted imaging (Jager et al. 1997, Engelbrecht et al. 2003, Kim et al. 2005, Haider et al. 2007, Yoshimitsu et al. 2008). Previous strategies to combine multiple parameters were discussed in Chapter 3 (van Dorsten et al. 2004, Kozlowski et al. 2006, Reinsberg et al. 2007, Tanimoto et al. 2007, Chen, M. et al. 2008, Mazaheri et al. 2008), and a logistic regression model to quantitatively combine DWI, T2 relaxation and DCE-MRI was developed to differentiate between malignant and normal peripheral zone tissue. However, ultimately it is not the malignant status of the tissue (‘tumour’ versus ‘normal’) that is the source of contrast in the image, but rather the underlying histologic or physiologic features.

In Chapter 4, it was demonstrated that the apparent diffusion coefficient (ADC) and T2 relaxation values of tumours consisting of significant portions of normal tissue (defined as ‘sparse’ tumours) are not significantly different from those of surrounding normal peripheral zone. These results suggest a pathologic property that may influence the detection of a tumour by MRI; however, the identification of ‘sparse’ regions within prostate cancer was subjective, with visual assessment and delineation performed on regions of tumours by a pathologist.
Histopathologic correlates to T2-weighted signal in the prostate have also been established previously (Schiebler et al. 1989, Quint et al. 1991), and, more recently, an inverse relationship between apparent diffusion coefficient (ADC) or quantitative T2 and cell density determined (Gibbs et al. 2009, Wang et al. 2009, Zelhof et al. 2009). A correlation between MRI and immunohistochemical markers of proliferation has also been identified (Shukla-Dave et al. 2009, Wang et al. 2009), suggesting a role for MRI in evaluating the malignant potential of individual prostate cancers. Further elucidation of the relationship between MRI and corresponding histologic features could have a tremendous impact on patient care, facilitating guided biopsy to the most-aggressive location of cancer or providing a surrogate for properties related to outcome. However, the study of properties beyond a measure of cell density or nuclear area has been limited, as has the range of MRI parameters investigated. Additionally, histologic analysis has been performed on selected regions in the available tissue, potentially introducing bias.

Thus, the purpose of the study detailed in this chapter was to determine the relationship between various tissue components (proportions of nuclei, epithelial cytoplasm, stroma, luminal spaces) and in vivo MRI parameters (ADC, T2, volume transfer constant ($K^{trans}$), and extravascular extracellular volume fraction ($v_e$)). Datasets included in vivo MRI obtained from prostatectomy patients, prior to their surgery, and corresponding whole-mount histologic sections, generated from the surgical specimen. For each patient, the entire whole-mount histologic section for the location included in the study was digitized at histologic resolution, which facilitated the use of image segmentation methods to extract the proportions of each tissue component. Anatomic divisions were used to define regions of interest (ROIs) for study, providing an objective registration method for all datasets. Additionally, pathologically homogeneous tumour and normal ROIs were defined in the peripheral zone on histology and transferred to imaging. Finally, linear mixed-effect methods were used to assess the relationships between the proportions of tissue components and the MRI-derived parameters. These relationships were compared to differences in both
tissue components and MRI-derived parameters in tumour versus normal peripheral zone tissue.

5.3 Methods

From the twenty-nine patients summarized in Chapter 2, twenty-four were included in this study; the five patients of the twenty-nine were excluded because their slides could not be digitized. Additionally, DCE-MRI data from one of the twenty-four patients was excluded due to significant motion during acquisition. All imaging, parametric mapping, and whole-mount histologic sectioning was performed as per Chapter 2. The section containing the largest cross-sectional area of the index lesion for each patient was digitized at histologic resolution (20x objective magnification) with an Aperio ScanScope XT brightfield scanner (Aperio Technologies, Vista, CA, USA). The whole-mount section and corresponding MRI slice were divided to generate ROIs using the following method: the entire gland was radially-sectioned into eighths, and peripheral zone within the posterior-four sections delineated to generate ROIs. Central gland tissue was then radially-sectioned into quarters and the posterior-two central gland sections included; excluding anterior central gland limited the influence of signal drop-off. ROIs were drawn on histology to avoid non-luminal spaces (e.g., tears) and were excluded if significant portions of tissue were fragmented. For MRI, regional division and contouring was performed on T2-weighted images and transferred directly, with appropriate scaling, to T2, $K^{\text{trans}}$, and $v_e$ maps. The ROIs were also transferred to ADC maps, and the outer edges adjusted to reflect any distortion. The pathologist-identified peripheral zone tumours and normal tissue control regions were also transferred to MRI, as per Chapter 2. To limit averaging between normal and malignant tissue, peripheral zone and central gland ROIs were excluded from further analyses if they contained tumour. A schematic of the anatomic segmentation is shown in Figure 5.1.
To objectively register MRI and histology, the entire prostate was divided radially into eighths, and peripheral zone (PZ) tissue present in the posterior segments delineated (PZ-divisions: ---). Similarly, the central gland (CG) was divided into quarters and the posterior segments outlined (CG-divisions: ...). Peripheral zone tumours were identified on histology, and outlines transferred to MRI. To minimize partial-voluming effects, sections were removed from regression analysis if they contained a mixture of normal and malignant tissue, and the tumour ROI included. In this example, five sections would have been included (2-PZ, 2-CG, 1-tumour).

Cellular components were identified using colour-based segmentation (Positive Pixel Count algorithm in ImageScope v8.0, Aperio Technologies, Vista, CA, USA). Pixels are identified as positive or negative according to user-defined hue/window settings, and each area is reported. Calculation of the relative area (%area) of each component was accomplished using two [hue, window] settings (Setting 1 – [0.1, 0.5]; Setting 2 – [0.7, 0.35]), optimized for each slide by adjusting the window on a test region to minimize false positive and negative pixels. This adjustment was slight (median absolute adjustment, 0.02; maximum, 0.07), and compensated for variations in stain intensity resulting from differences in section thickness or processing. Representative H&E segmentations are shown in Figure 5.2. Settings were determined by the author; however, accuracy and inter-observer variation was verified by having a pathologist (Andrew J. Evans) independently process a subset of cases (5 of 24) to compare to original values. The %area of each component was calculated as the ratio of the

Figure 5.1 –Prostate segmentation schema. To objectively register MRI and histology, the entire prostate was divided radially into eighths, and peripheral zone (PZ) tissue present in the posterior segments delineated (PZ-divisions: ---). Similarly, the central gland (CG) was divided into quarters and the posterior segments outlined (CG-divisions: ...). Peripheral zone tumours were identified on histology, and outlines transferred to MRI. To minimize partial-voluming effects, sections were removed from regression analysis if they contained a mixture of normal and malignant tissue, and the tumour ROI included. In this example, five sections would have been included (2-PZ, 2-CG, 1-tumour).
segmented area to the total ROI area. For nuclei, the segmented area corresponded to pixels identified in the entire ROI as negative using setting 1 (Figure 5.2b); cytoplasm+nuclei corresponded to all positive pixels in setting 2 (Figure 5.2c), and thus cytoplasm was the subtraction of the two segmentation results; stroma was identified as the area of negative pixels in setting 2 (Figure 5.2c); finally, luminal spaces corresponded to the difference between the total area of each ROI and the sum of all positive and negative pixels.

![Sample mark-up images from image segmentation of histology.](image)

The mean value was determined for each ROI segment, and each MRI parameter. Although median values are a more accurate summary value for non-normal distributions or in the presence of outliers, in this case, mean values better-reflect the %area value computed for each tissue component and thus were used in regression analysis. All distributions, i.e., the population of all ROI values for each MRI parameter or %area, were tested for normality using
Lilliefors testing. As the data were not normally distributed, all distributions were \( \log_2 \)-transformed and re-tested. Z-score normalization was then performed. Two parameters (ADC and T2) were not normal after \( \log_2 \)-transformation, due to the presence of outliers. For these cases, a 95%-trimmed distribution was generated and confirmed to be normal. The trimmed mean and standard deviation was then used to calculate z-scores for those distributions. The relationship between each component:parameter pair was determined using linear mixed-effect models, testing the slope for significance against zero; the patient number was included as the random effect variable, to account for within-patient correlation of the data. Mixed-effect modeling was performed using the Nonlinear Mixed-Effects Models (nlme) library in the R statistical environment (v.2.6.2).

Comparisons between values in malignant versus normal peripheral zone tissue were performed using non-parametric tests; signed rank tests were used for paired data, and Wilcoxon rank sum tests for unpaired comparisons. Wilcoxon rank sum tests were also used to assess differences in values derived from separate Gleason scores. As these comparisons were within the same parameter, e.g., T2 in normal versus T2 in malignant tissue, the use of mean MRI values (to better reflect %areas) was not necessary. Thus, median MRI parameter values, more appropriate for non-parametric testing, were determined and used for tumour versus normal and Gleason score analyses. With the exception of the mixed-effect modeling, all statistical analyses were performed in Matlab 7.

5.4 Results

Linear mixed-effect modeling included values from 102 ROIs for ADC and T2 \( (n_{\text{PZ}} = 42, n_{\text{CG}} = 38, n_{\text{Tumour}} = 22) \), and 99 ROIs for \( K^{\text{trans}} \) and \( v_e \) \( (n_{\text{PZ}} = 42, n_{\text{CG}} = 36, n_{\text{Tumour}} = 21) \). Sample regression results are shown in Figures 5.3 and 5.4, and the slopes for all MRI parameter:component pairs tabulated in Table 5.1. The average inter-observer variation in component segmentation from all five cases was <3%, with a maximum discrepancy of 6.9% occurring in one case for %area\(_{\text{nuclei}}\). Sample histology and parameter maps are in Figure 5.5. ADC and
T2 were significantly negatively related to the $\%\text{area}_{\text{nuclei}}$ ($p \leq 0.001$) or $\%\text{area}_{\text{cytoplasm}}$ ($p \leq 0.01$) in prostate tissue, and had a significantly positive slope versus $\%\text{area}_{\text{lumen}}$ ($p \leq 0.001$). Neither parameter was significantly related to $\%\text{area}_{\text{stroma}}$ ($p = 0.32$ for ADC, $p = 0.92$ for T2). Conversely, $K_{\text{trans}}$ increased as the proportion of nuclei or cytoplasm increased ($p < 0.001$), decreased as $\%\text{area}_{\text{lumen}}$ increased ($p < 0.001$), and was also negatively related to $\%\text{area}_{\text{stroma}}$ ($p = 0.01$). Significant relationships were determined between $v_e$ and $\%\text{area}_{\text{nuclei}}$ (negative slope, $p = 0.05$) and $\%\text{area}_{\text{stroma}}$ (positive slope, $p = 0.008$).

Figure 5.3 – ADC versus $\%\text{area}$ for each tissue component, z-score normalized with regression result. Each data point represents corresponding mean ADC and $\%\text{area}$ values ((a) nuclei, (b) cytoplasm, (c) stroma, and (d) lumen), where $\times$ – peripheral zone, $+$ – central gland, $o$ – tumour. Main windows contain the z-scored normalized data used during modeling, as well as the regression result. For reference, the insets contain the original data prior to normalization. $p$-values are for the slope, compared to zero. No intercept values were significantly different from zero ($p > 0.7$).
Figure 5.4 – T2 relaxation versus %area for each tissue component, z-score normalized with regression result. Each data point represents corresponding mean T2 and %area values ((a) nuclei, (b) cytoplasm, (c) stroma, and (d) lumen), where x – peripheral zone, + - central gland, o – tumour. Main windows contain the z-scored normalized data used during modeling, as well as the regression result. For reference, the insets contain the original data prior to normalization. p-values are for the slope, compared to zero. No intercept values were significantly different from zero (p > 0.7).
Of the twenty-four patients in the study cohort, two were not included in tumour analysis; one patient’s cancer consisted of micro-foci only, and one patient’s tumour was confined to the central gland. The twenty-two peripheral zone tumours assessed consisted of fourteen Gleason score 6 and eight Gleason score 7 lesions. The median and range of values determined in tumour and normal peripheral zone ROIs are summarized in Table 5.2 for all MRI parameters and in Table 5.3 for cellular component values. Measurements of the apparent diffusion coefficient, ADC, and T2 relaxation were significantly lower in malignant peripheral zone (median values: $1.28 \times 10^{-3} \text{ mm}^2/\text{s}$ and 90 ms respectively) than in normal (1.56 $\times 10^{-3} \text{ mm}^2/\text{s}$ and 113 ms) in both paired ($p < 0.001$) and unpaired testing ($p < 0.005$). The dynamic contrast-enhanced MRI parameters, the volume transfer constant ($K_{\text{trans}}$) and the extravascular extracellular space ($v_e$), were significantly higher in tumours compared to normal peripheral zone (median values of 0.36 min$^{-1}$ and 0.32 respectively in tumour, 0.29 min$^{-1}$ and 0.31 in normal) when assessed with paired tests – i.e., comparing each tumour median against normal values from the same patient ($p \leq 0.05$), but not in unpaired testing ($K_{\text{trans}}$: $p = 0.34$; $v_e$: $p = 0.21$). The relative cross-sectional areas of nuclei and cytoplasm was higher in tumours compared to normal peripheral zone ($\%\text{area}_{\text{nuclei}}$: 24% versus 15% ($p < 0.001$), tumour versus normal; $\%\text{area}_{\text{cytoplasm}}$: 22% versus 17% ($p \leq 0.001$, tumour versus normal), and the proportion of luminal space was lower (13% in tumour, 30% in normal, $p < 0.001$). These results were true for both paired and unpaired comparisons. There were no significant differences in the relative cross-sectional areas of stromal tissue observed in tumour versus normal ($p = 0.49$ paired, $p = 0.63$ unpaired). Neither median MRI parameter values (ADC, T2, $K_{\text{trans}}$, or $v_e$) nor cellular composition ($\%\text{area}$ of: nuclei, cytoplasm, stroma, or luminal space) was significantly different between Gleason 6 and Gleason 7 tumours ($p > 0.36$).
Table 5.1 – Mean Slope ± Standard Error for MRI Parameter versus Proportion of Cellular Component

<table>
<thead>
<tr>
<th></th>
<th>nuclei</th>
<th>cytoplasm</th>
<th>stroma</th>
<th>luminal space</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC</td>
<td>-0.50 ± 0.11 (0.001)</td>
<td>-0.47 ± 0.13 (0.001)</td>
<td>0.13 ± 0.12 (0.32)</td>
<td>0.57 ± 0.09 (0.001)</td>
</tr>
<tr>
<td>T2</td>
<td>-0.42 ± 0.12 (0.001)</td>
<td>-0.39 ± 0.15 (0.01)</td>
<td>0.01 ± 0.14 (0.92)</td>
<td>0.52 ± 0.11 (0.001)</td>
</tr>
<tr>
<td>$K_{\text{trans}}$</td>
<td>0.34 ± 0.08 (&lt;0.001)</td>
<td>0.38 ± 0.10 (&lt;0.001)</td>
<td>-0.24 ± 0.09 (0.01)</td>
<td>-0.28 ± 0.07 (&lt;0.001)</td>
</tr>
<tr>
<td>$v_e$</td>
<td>-0.20 ± 0.10 (0.05)</td>
<td>0.13 ± 0.12 (0.26)</td>
<td>0.28 ± 0.10 (0.008)</td>
<td>-0.09 ± 0.09 (0.33)</td>
</tr>
</tbody>
</table>

p-value (brackets) for non-zero slope.
No units (all data z-score normalized prior to fitting with linear mixed-effect model).

Figure 5.5 – Representative histologic sections (a,b) with corresponding ADC (c) and $K_{\text{trans}}$ (d) maps. The tumour, located between the two “*” in (a), is seen on H&E as a region with increased density, and corresponds to an increase in cytoplasm and nuclei in (b) (orange), a decrease in ADC (c), and increase in $K_{\text{trans}}$ (d). (patient age, 59 y; prostate-specific antigen level, 2.93 ng/mL; pathologic stage, T2c; Gleason score, 7)
Table 5.2 – Summary MRI Parameter Values for Tumour and Normal Prostate in Peripheral Zone Tissue

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal median [range]</th>
<th>Tumour median [range]</th>
<th>p-values for tumour versus normal using a Wilcoxon rank sum and b paired signed rank tests, and p-value for *Wilcoxon rank sum test of Gleason 6 versus 7 tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC (x10^{-3} mm^2/s)</td>
<td>1.56 [1.05 – 2.09]</td>
<td>1.28 [0.85 – 1.69]^{a,b &lt; 0.001}</td>
<td>Gl.6 – 1.29 [1.04 – 1.46]^{0.47} Gl.7 – 1.17 [0.85 – 1.69]</td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>113 [72 – 227]</td>
<td>90 [68 – 139]^{a = 0.004, b &lt; 0.001}</td>
<td>Gl.6 – 89 [68 – 121]^{0.52} Gl.7 – 94 [73 – 139]</td>
</tr>
<tr>
<td>$K_{trans}$ (min^{-1})</td>
<td>0.29 [0.09 – 0.87]</td>
<td>0.36 [0.16 – 1.28]^{a = 0.34, b = 0.05}</td>
<td>Gl.6 – 0.36 [0.20 – 1.28]^{0.74} Gl.7 – 0.35 [0.16 – 0.69]</td>
</tr>
<tr>
<td>$v_e$ (no units)</td>
<td>0.31 [0.14 – 0.41]</td>
<td>0.32 [0.19 – 0.48]^{a = 0.21, b = 0.01}</td>
<td>Gl.6 – 0.32 [0.19 – 0.48]^{1.0} Gl.7 – 0.33 [0.20 – 0.48]</td>
</tr>
</tbody>
</table>

Table 5.3 – Summary Values for the Percentage Area of Cellular Components in Tumour and Normal Peripheral Zone Tissue

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal median [range]</th>
<th>Tumour median [range]</th>
<th>p-values for tumour versus normal using a Wilcoxon rank sum and b paired signed rank tests, and p-value for *Wilcoxon rank sum test of Gleason 6 versus 7 tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuclei</td>
<td>14.5 [7.9 – 25.0]</td>
<td>23.7 [13.8 – 45.7]^{a,b &lt; 0.001}</td>
<td>Gl.6 – 22.4 [13.8 – 35.3]^{0.36} Gl.7 – 25.4 [18.2 – 45.7]</td>
</tr>
<tr>
<td>cytoplasm</td>
<td>16.7 [8.6 – 33.1]</td>
<td>22.5 [11.4 – 37.2]^{a,b &lt; 0.001}</td>
<td>Gl.6 – 22.7 [11.4 – 37.2]^{0.76} Gl.7 – 21.9 [12.7 – 28.0]</td>
</tr>
<tr>
<td>stroma</td>
<td>38.2 [25.8 – 53.9]</td>
<td>38.6 [25.1 – 56.1]^{a = 0.63, b = 0.49}</td>
<td>Gl.6 – 37.3 [27.0 – 56.1]^{0.71} Gl.7 – 39.8 [25.1 – 43.1]</td>
</tr>
<tr>
<td>luminal space</td>
<td>29.6 [15.9 – 43.9]</td>
<td>13.5 [7.4 – 35.1]^{a,b &lt; 0.001}</td>
<td>Gl.6 – 13.5 [7.4 – 35.1]^{0.66} Gl.7 – 12.9 [9.8 – 18.0]</td>
</tr>
</tbody>
</table>

Percentage values (%) reported.

All p-values are significant at the 0.05 level.
5.5 Discussion

This chapter determines a number of significant relationships between MRI parameter values (i.e., the apparent diffusion coefficient (ADC), T2 relaxation, volume transfer constant ($K^{trans}$), and the extravascular extracellular space ($v_e$)) and the cellular composition of the corresponding tissue—regardless of malignant status. Additionally, the relative areas of a number of cellular components (% area of nuclei, cytoplasm, stroma, or luminal spaces) are significantly different in malignant versus benign peripheral zone tissue. Taken together, these two results suggest mechanisms that may influence the detection of prostate cancer with MRI. While the relationships themselves do not necessarily imply causation—both changes in relative area of a component and MR-parameter value may be due to underlying factors not identified in this study—this work presents an association between specific alteration in tissue composition and MRI.

Earlier works have also explored histopathologic correlation to tissue. Scoutt et al. determined that low T2-weighted signal in the uterus, imaged ex vivo, corresponded to the junctional zone, which was then determined to have a 3-fold increase in nuclear area compared to surrounding tissues (Scoutt et al. 1991); nuclear area measurements were obtained through image segmentation, similar to the methods employed in this chapter, however this was only performed on selected regions of histology, and only for nuclear area. Correlates to T2-weighted MRI have also been presented for prostate; Schiebler et al. reported the normalized T2-weighted signal intensity for various pathological tissue types (e.g., sclerotic benign prostatic hyperplasia, tumour, cystic benign prostatic hyperplasia, etc.), and then used point-counting methods in histology (examining high-power fields under a microscope using an eye-piece having multiple cross-hairs; the cell type (stromal, glandular, or open space) at each cross-hair was reported) to assess the composition of each pathologic tissue type (Schiebler et al. 1989). Although they drew some general comparisons between MRI and cellular composition, these relationships were never directly
measured. In addition to T2-weighted MRI, studies including contrast-enhanced and diffusion-weighted MRI have also been performed. Esserman et al. measured correlation between contrast enhancement features on DCE-MRI and pathologic characteristics for ductal carcinoma in situ (Esserman et al. 2006). Muraoka et al. investigated ADC in pancreatic cancer, and, in addition to assessing tissue composition in tumour versus noncancerous tissue, also found a significant negative correlation between ADC and the proportion of collagenous fibers (Muraoka et al. 2008). In the brain, Guo et al. compared both the diffusion coefficient and the cellularity (measured as the nuclear–cytoplasmic ratio) for lymphomas versus high-grade astrocytomas, with their results suggesting that higher cellularity contributed to lower ADC values (Guo et al. 2002). More recently, a significant negative correlation between ADC or T2 and cellular density (analogous to %area\_nuclei) has been reported in the prostate by a number of groups (Gibbs et al. 2009, Wang et al. 2009, Zelhof et al. 2009), as was an increase in cellular density and decrease in ADC in malignant versus normal peripheral zone tissue. The results of this chapter are consistent with the associations noted in the literature, and also extend these investigations in a number of ways: for the prostate, MRI parameters outside of ADC and T2 relaxation (i.e., DCE-MRI derived parameters $K_{\text{trans}}$ and $v_e$) were measured; image segmentation of histology included tissue components in addition to nuclear area such as %area of cytoplasm; finally, all analyses – both imaging and histology – were performed on the entire axial or whole-mount section, respectively, as opposed to small regions of interest that may be subject to bias or not be representative of the tissue of interest.

Tissue composition is heterogeneous and, as the MRI voxel size is considerably larger than the sub-cellular structures, a measured parameter value is an average that reflects the water diffusion in each of the tissue compartments. Each component contribution is weighted by its relative volume in the voxel, approximated by the %area determined in this study, and thus the measured value for a parameter should be sensitive to alterations in the proportions of the compartments. In the prostate, these compartments include: the intracellular
space from the epithelial lining of the glands, consisting of nuclei and cytoplasm; the interstitial extracellular space, consisting of the stroma; and the luminal spaces (Figure 5.2). Water diffusion is most-restricted in the intracellular space and least-restricted in the lumen, with diffusion in the interstitial extracellular space between these two extremes. T2 relaxation should exhibit similar sensitivities as ADC to changes in tissue composition, i.e., higher values for free water in luminal spaces and lower for the intracellular compartment, but may also have dependencies on macromolecular content unrelated to diffusion properties (Mitchell et al. 1987). The associations determined in this study – significant increases in both ADC and T2 with increases in the %area of luminal space, and decreases in the MRI parameters with %area of nuclei or cytoplasm – thus agree with the changes expected theoretically (Figures 5.3 and 5.4). However, the similar or greater sensitivity of ADC and T2 to the %area\textsubscript{nuclei}, compared to %area\textsubscript{cytoplasm}, is in disagreement with the work of Grant et al. who measured higher diffusion in the nucleus versus the cytoplasm, attributed to an increase in viscosity and tortuosity of the cytoplasm due to the structure of the cytoskeleton and presence of organelles (Grant et al. 2001). This discrepancy from the findings of Grant et al. is also present in the studies described above (Gibbs et al. 2009, Wang et al. 2009, Zelhof et al. 2009), and is likely an indication that it is not the water diffusion in the nucleus that is being directly measured, but rather the increase in cellular density, seen as an increase in the number of nuclei. A significant relationship was not observed between ADC or T2 and stroma, however this result may have been influenced by the inclusion of both smooth muscle and loose connective tissue in the segmentation of stromal components.

The interpretation of the relationships between the DCE-derived parameters and tissue composition is challenging, as the volume transfer constant, $K^{\text{trans}}$, should relate only to properties of the vasculature (vessel permeability and blood flow), and the extravascular extracellular space, $v_e$, to the interstitial leakage space. This latter relationship is reproduced in this study, with a weak but significant positive association determined between $v_e$ and %area\textsubscript{stroma}. However, the reported relationships for $K^{\text{trans}}$ are more likely due to
an indirect effect; specifically, changes in relative areas of cellular components may occur in pathologies associated with angiogenesis. Both prostate cancer and prostatic intraepithelial neoplasia are characterized histologically by enlarged nuclei, the presence of amphophilic cytoplasm, and increases in cellular density in the epithelial lining of the lumen (Humphrey 2003); they may also have greater microvascular density (Sinha et al. 2004), which has also been correlated in some studies to DCE-MRI (Schlemmer et al. 2004, Ren et al. 2008). Thus, pathologically there is some supporting evidence for the association of vasculature and changes in tissue composition.

The differences in the %area of nuclei, lumen, and cytoplasm for malignant relative to benign tissue were in agreement with the parameter:component relationships and changes in MRI values between tumour and normal, suggesting histologic factors influencing image contrast during prostate cancer imaging. However, in addition to cancer, nucleomegaly and increased cellular density can also be indicative of inflammation and prostatic intraepithelial neoplasia, and lumen size can be altered in cystically dilated glands, regions of atrophy, or fibrosis. These benign pathologies have been implicated as a source of false-positives in MRI (Quint et al. 1991, Jager et al. 1996, Shukla-Dave et al. 2004, Cheikh et al. 2009) or poor radiologic-pathologic volumetric correspondence (Sommer et al. 1993). This study quantitatively demonstrates the sensitivity of MRI to the underlying histologic changes, regardless of malignant status of the tissue. The slopes of the significant relationships were similar in absolute value to one another (Table 5.1); however, it is noted that, overall, ADC tended to be the most sensitive, with the steepest slope for all components except %area\textsubscript{stroma}. This sensitivity does not, however, infer greater specificity for detecting structural alterations occurring in prostate cancer, as these changes may also occur in non-malignant pathologies.

The study cohort was derived from patients for whom surgery was the recommended course of treatment; thus, the range of Gleason scores was limited. Additionally, the sample size was small, preventing full investigation of
differences in cellular components or MRI with Gleason score. Reports of correlation between MRI and Gleason score have been mixed, with some (Engelbrecht et al. 2003, Zakian et al. 2005, deSouza et al. 2008, Tamada et al. 2008), but not all (Padhani et al. 2000, Wang et al. 2009, Zelhof et al. 2009) studies demonstrating significance. As the proportion of each Gleason pattern within a single tumour can vary significantly, e.g., a Gleason score 7 tumour can be composed of anywhere from 5 – 95% Gleason pattern 4, establishing accurate relationships between imaging and Gleason score will be challenging without sub-lesion radiologic/pathologic registration and/or a large study cohort. However, early studies are demonstrating relationships between histologic features and Gleason score, with the nuclei in Gleason pattern 4 regions of Gleason score 7 tumours found to be larger when the primary pattern was 4, versus 3 (Venkataraman et al. 2009). Additionally, imaging characteristics derived from H&E-stained histopathology have also been identified as contributing significantly to a predictive model of recurrence (Donovan et al. 2008). Therefore, the findings presented here are encouraging that these distinctions in Gleason grades or other features predictive of long-term outcomes may eventually be appreciable on MRI, and contribute significantly to patient management either by reducing biopsy-sampling bias, or by direct estimation of prostate cancer aggressiveness.

The use of anatomically-derived ROIs facilitated objective registration between histology and MRI; however, these ROIs were necessarily large, and the averaging occurring in heterogeneous regions such as the central gland likely reduced the statistical significance of the measurements. Additionally, the image segmentation algorithm was unable to separate the effect of an increase in number from an increase in size of each component – e.g., changes in cellular density versus nucleomegaly. However, despite these limitations, it was possible to measure some of the underlying relationships between MRI and histology, including normal peripheral zone and central gland tissue as well as peripheral zone tumours in the analyses. Prospective studies investigating MRI and patient outcome require significant follow-up time; by relating clinical MRI to H&E-stained
pathology, it may be possible to more rapidly harness findings from larger-scale retrospective studies of historical biopsy data.

5.6 Summary

This chapter elucidates a number of significant relationships between MRI parameter (quantitative T2, ADC, $K^{\text{trans}}$ and $v_e$) and tissue composition (%area of nuclei, cytoplasm, stroma, and lumen) pairs. Additionally, there were significantly different MRI and component values between malignant versus normal, consistent with the overall measured trends. This represents a step towards improved characterization of both malignant and normal prostate tissue using MRI. In conclusion, the proportion of individual histological components significantly affects quantitative MRI. As these proportions are altered in malignant versus normal peripheral zone tissue, and potentially in benign pathologies, improved understanding of these fundamental dependencies may have significant clinical impact through improved characterization of prostate cancer with MRI to guide biopsy or therapy, or inform treatment decisions.
Chapter 6

Discussion
6.1 Summary and Implications

Prostate cancer has a direct impact on a significant portion of the male population. As life expectancy increases and methods of early detection improve, the number of men living with prostate cancer will continue to grow. These men face the difficult choice of opting for aggressive treatments such as radical prostatectomy and external beam radiation therapy, which have good long-term survival but high incidence of side-effects, or selecting active surveillance or focal therapy, options that have fewer associated morbidities but may be insufficient if the disease has not been accurately characterized. Improvement of patient selection for both aggressive and conservative approaches – i.e., identifying which patients are at risk of dying from their disease if radical treatment is not undertaken, versus patients for whom there is no immediate need to incur the risk of side-effects, provided their disease progression is monitored or the tumour is treated focally – could have a tremendous impact on the overall quality of life for a significant cohort of diagnosed patients.

Since the onset of this work, the need for and feasibility of a shift in the management of prostate cancer patients – from screening through to therapy – has been increasingly supported in the literature. Two recently published large-scale screening trials evaluated the impact of prostate-specific antigen (PSA) testing in prostate cancer patients and found little or no impact to death rates (Andriole et al. 2009, Schroder et al. 2009). Although the European trial did find a reduction in prostate cancer deaths with PSA-based screening, this benefit was associated with a high risk of overdiagnosis; i.e., as a result of screening, many men would be treated – incurring the risk of side-effects – for disease that would not have lead to the patient’s death. The ability to differentiate between lethal versus indolent cancers would thus have significant clinical benefit. Additionally, the results of the phase I trial presented by Lindner et al. demonstrates that focal therapy of localized prostate cancer with minimum adverse effects is possible (Lindner et al. 2009). One patient in the study subsequently required radical
prostatectomy for significant residual disease, which was performed without complication. This suggests that focal therapy could be used as an initial, less-morbid, treatment option; but, in the event of treatment failure, its use may not complicate further treatment with traditional therapies.

Moving forward, non-invasive characterization of localized prostate cancer through imaging could have a significant role in improving patient care. Image-guided biopsy could ensure that malignant tissue is sampled at the most-aggressive location, and accurate mapping of prostate cancer location and extent could be used in determining tumour progression or for treatment planning. Magnetic resonance imaging (MRI) currently provides the best visualization of prostate anatomy and good localization of prostate cancer; however, better performance is required to maximize impact on patient management. This thesis has described contributions made towards: 1) the development of multi-parametric MRI suitable for prostate cancer mapping; 2) understanding the characteristics of prostate cancers that may be inherently undetectable with some MRI measurements; and 3) determining the underlying relationships between tissue composition and MRI-derived parameter values, beyond malignant status of the tissue. Taken together, the work first presents a multi-parametric MRI method based on clinically relevant datasets to differentiate between normal and malignant tissue, then explores the strengths and weaknesses of the contributing parameters by investigating potential sources of false positive results and the correlation of signal with prostate histology.

The addition of MRI protocols such as diffusion weighted imaging (DWI) and dynamic contrast-enhanced (DCE) MRI to standard T2-weighted MRI is leading to improvements in prostate cancer localization. There has thus been interest in combining multiple techniques to further enhance disease characterization. Although multi-parametric MRI is showing potential, to date implementation has not been ideal. The qualitative methods proposed by Kozlowski et al., Tanimoto et al., and Chen et al., take advantage of radiologists’ expertise in interpreting each dataset, and avoid issues such as registration. But
the results are subjective, onerous for large-scale studies, and do not produce quantitative output. The broad application of qualitative methods to clinical use is thus limited. van Dorsten et al., Reinsberg et al., and Mazaheri et al. propose quantitative techniques that address some of these issues; parameter values are combined to yield a binary or continuous value, reflecting the malignant status of each voxel. However, these studies included magnetic resonance spectroscopic imaging (MRSI) data which, due to the large voxel sizes inherent to spectroscopy, limit the utility of maps generated from this data; in fact, tumour mapping was not attempted in these works. The multi-parametric model and techniques developed in Chapter 3 were designed from the onset to be appropriate for mapping the location and extent of prostate cancer in the peripheral zone, and feasible in a clinical setting. The suitability of the technique for generating maps of tumour probability was demonstrated in Figures 3.3 and 3.4, and, despite the fact that the results did not demonstrate a clear advantage of the multi-parametric model over diffusion weighted imaging, the work represents progress in multi-parametric MRI towards achieving the clinical goals.

In evaluating the performance of any imaging task, both false-positive and false-negative results are expected; for cancer detection, a false-positive refers to incorrectly identifying normal tissue as malignant, and false-negative refers to failing to identify the presence of tumour. For the task of characterizing the full extent of tumour, this definition may also extend to sources that may lead to poor volumetric correlation between imaging and pathologic findings. Chapter 4 investigated measurements of apparent diffusion coefficient (ADC) and T2 in tumours containing primarily malignant glands or dense stroma (‘dense’), tumours that were dominantly composed of normal peripheral zone tissue (‘sparse’), and normal peripheral zone tissue. The results indicated that ADC and T2 in dense tumours was significantly lower than that of normal peripheral zone tissue, but there was no significant difference between the ADC and T2 values of sparse tumours and normal. To minimize the accuracy required for radiologic-pathologic registration, tumours were first assessed regionally on pathology only, and given an overall category of ‘dense’ or ‘sparse’ based on
which tissue type dominated the cross-sectional area for that section. Fifty-one percent of tumours (20 of 39) had components of both tumour types, which is encouraging from the perspective of tumour detection or localization, but also suggests that accurate mapping of the full extent of prostate cancer with ADC or T2 may be limited. Although Quint et al., Jager et al., and Schiebler et al. have all commented on the effect that the presence of normal tissue interdigitated with malignant glands has on detectability of the full extent of tumour in the prostate, the study presented in Chapter 4 developed specific criteria on a larger cohort to demonstrate definitively that sparse tumours or regions within tumours may be inherently undetectable compared to surrounding normal tissue by some forms of MRI measurement. Thus, in addition to contributing to potential improvements in prostate cancer localization (Chapter 3), the thesis has also defined conditions under which tumour mapping with some forms of MRI measurement remains limited.

Although the ability to differentiate between normal and malignant peripheral zone regions was tested for sparsely malignant tissue in Chapter 4, the clinical impact of 'sparse' tumours is currently unknown. In the study cohort, all sparse tumours tended to have more favourable clinical characteristics, i.e., a Gleason score of 6 with no evidence of extracapsular extension or seminal vesicle invasion. It is possible that the presence of high proportions of normal peripheral zone tissue within a tumour is reflective of a less aggressive malignancy. This should be investigated in a larger cohort with a significant follow up time in order to ascertain any differences in mortality; if a clinical difference between sparse and dense tumours is eventually identified, the results of Chapter 4 suggest that MRI may provide a non-invasive means to differentiate between the two.

As the goal of MRI for tumour localization is primarily to differentiate between normal and malignant tissue, results (e.g., lower ADC or T2 values, or higher measurements of the volume transfer constant ($K_{\text{trans}}$) in tumour versus normal) are often attributed solely to malignant status. Although this
classification is useful for identifying regions of tumour, the signal or parameter values are in fact reflective of all properties of the tissue being imaged. Further elucidation into the underlying relationships between MRI parameters and tissue components could serve to improve image interpretation, building on the results presented in Chapter 4 to depend on measurements in histology versus more subjective estimates of proportions of each tissue type. Thus, the study presented in Chapter 5 was developed to investigate the overall relationships between features identified in histology and MRI-derived parameter values, including objectively-defined regions in normal peripheral zone, normal central gland tissue, and peripheral zone tumours. The study by Quint et al. demonstrates that the tissue optical density, which depends on the percentage area of luminal spaces, differs between prostate cancer and normal peripheral zone tissue; however, they did not extend their work beyond testing the significance of the difference between summary values – i.e., the overall relationship between optical density and MRI signal was not explored. The recent works by Wang et al., Zelhof et al., and Gibbs et al., all published in 2009, use image segmentation of limited fields in the histologic samples to derive estimations of the cellular density, and develop an overall relationship between ADC or T2 and cell density. There is a thus growing interest in improving the understanding of how MRI signal relates to histology. In Chapter 5, the entire whole-mount section was digitized at a resolution suitable for histologic review, which expands investigation beyond limited fields-of-view in histology which may not accurately reflect variation in tissue composition. Additionally, investigation of overall parameter:component relationships was extended to include previously unstudied tissue components such as the relative area of cytoplasm or stroma. Finally, the dataset analysed in this thesis includes maps of dynamic magnetic resonance (DCE) MRI parameters, which have, to date, not been studied. Although the results are potentially limited by issues such as registration and a small patient cohort, understanding the strengths, weaknesses, and sensitivities of each MRI parameter to the underlying histology could ideally be used to improve multi-parametric MRI in achieving specific tasks.
6.2 Limitations and Future Work

The goal of the three presented studies was to ultimately improve the characterization of the location and extent of prostate cancer. However, although the multi-parametric model, LR-3p, had higher area under the ROC curve ($A_z$) compared to each of the single parameters tested (apparent diffusion coefficient (ADC), T2 relaxation, volume transfer constant ($K^{\text{trans}}$), or extravascular extracellular space ($v_e$)), these results did not always achieve significance – such as in the case of LR-3p versus ADC. MR imaging methods were not developed as part of the thesis. Instead, research focused on combining measurements obtained from imaging protocols used in the clinic at the University Health Network, and comparing those to histology. Improvements to any of the MRI datasets, either at the time of acquisition or through more accurate generation of the parametric maps, would likely directly benefit all of the performed studies. In Chapter 3, measurements of apparent diffusion coefficient provided the best discrimination of any of the single parameters for differentiating between malignant and normal peripheral zone tissue. Additionally, in Chapter 5 it was seen that ADC was the most sensitive parameter to changes in tissue composition for all components except %area of stroma. Thus, improvements to the measurement of ADC, in particular improving specificity to malignant processes, may significantly improve the characterization of prostate cancer with MRI.

The range and number of b-values included during diffusion weighted imaging impacts the calculated ADC value, and the sensitivity of the measurement to different aspects of tissue physiology. In the prostate, Riches et al. demonstrated that the inclusion of multiple b-values (eleven values between 0 – 800 s/mm$^2$ were used) facilitates the separation of fast- and slow-ADC components, which may be sensitive to changes in perfusion and diffusion, respectively (Riches et al. 2009). Further work from the same group suggests that this separation may be useful in correlating ADC to prognosis, with both $\text{ADC}_{\text{slow}}$ and $\text{ADC}_{\text{fast}}$ significantly lower in value for high- versus low-risk groups,
whereas ADC\textsubscript{overall} (i.e., using a mono-exponential decay fit to all b-values) was not able to differentiate between risk groups (deSouza \textit{et al.} 2008). Riches \textit{et al.} also suggested that ADC\textsubscript{fast} may be highly variable compared to ADC\textsubscript{slow}, and that ADC\textsubscript{slow} can be well-characterized using a mono-exponential fit to two b-values, providing $b > 20$ s/mm\textsuperscript{2}. This suggests that a good compromise between acquisition time and clinical impact may be reached if diffusion weighted data are acquired using two b-values having minimum values of 20 s/mm\textsuperscript{2}, thus better isolating ADC\textsubscript{slow}.

Currently, conventional ADC measurement cannot differentiate between the various sources of diffusion restriction, e.g., increases in cellular density versus changes to nuclear size. These distinctions may be useful as correlates to disease aggressiveness or progress (Donovan \textit{et al.} 2008, Venkataraman \textit{et al.} 2009). Recently, Xu \textit{et al.} have numerically demonstrated the feasibility of using an oscillating gradient spin echo method to reduce diffusion time compared to conventional pulse gradient spin echo methods (Xu \textit{et al.} 2009). The short diffusion time causes the measurement of ADC to be more sensitive to intracellular changes, and may provide increased specificity to malignant pathologies.

In the patient cohort studied in this thesis, the pharmacokinetic parameters (volume transfer constant ($K^{\text{trans}}$) and the extravascular extracellular space ($v_e$)) were poorly correlated to ADC and T2 (Figure 3.4). Additionally, $K^{\text{trans}}$ and $v_e$ had significantly lower areas under the receiver operating characteristic curve, compared to ADC and T2. Yet, $K^{\text{trans}}$ contributed significantly to the multi-parametric model, and Figure 3.9 showed an example where the tumour was visible on the map of $K^{\text{trans}}$, but not on the maps of ADC or T2. The relatively low performance of DCE-MRI in this thesis leaves appreciable room for improvement, which may then have a dramatic impact on the performance of a multi-parametric model for characterizing prostate cancer.
Accurate determination of pharmacokinetic parameters with DCE-MRI is challenging, with many options presented in the literature for measurement techniques and model choices. To identify tumour in the prostate, the primary goal is to produce parameter maps that are capable of differentiating between malignant and normal tissues, and suitable for inter-subject studies (i.e., values are consistent between patients). Secondly, measurements that provide insight into the underlying physiology of the tissue will likely prove more useful in contributing to an overall characterization of the disease. The two-compartment model summarized by Tofts has been widely used in oncology, facilitating the determination of the volume transfer constant, \(K_{\text{trans}}\), and the volume fraction, \(v_e\). This model requires estimation of the contrast agent concentration flowing into the tissue of interest and, as determination of a patient-specific arterial input function (AIF) can be problematic, an assumed, population-derived AIF was employed. However, an assumed AIF cannot compensate for variations expected between patients, e.g. differences in cardiac output, or variability in contrast agent delivery. Inter-patient differences in parameter values caused by acquisition and modeling variations are not present during determination of ADC and T2, and thus the poor performance of \(K_{\text{trans}}\) and \(v_e\) reported in Chapter 3 may be partially explained by these factors. Reliable methods to improve the accuracy of the pharmacokinetic modeling or to normalize between patients are worth pursuing as part of ongoing research. However, it is important to note that any changes to data acquisition that affect parameter values or their distribution will require redevelopment of the model.

All methods for model development and subsequent evaluation in Chapter 3 were developed around using individual voxel values, ignoring any information regarding spatial correlation between voxels; i.e., each voxel was treated independently, despite the fact that a single voxel in a large area of suspected tumour is more likely to be malignant, compared to a suspicious voxel in isolation. This information is included during visual evaluation of any image or parametric map by an experienced observer, and thus the results of Chapter 3 may not reflect true clinical performance. Proper assessment should therefore
include observer studies. However, stronger evidence for improvement of a multi-parametric technique over single-parameter MRI is desirable before proceeding. Visual evaluation of prostate cancer also incorporates features related to shape. For example, a nodular lesion in the peripheral zone of the prostate is typically considered more suspicious of cancer compared to a wedge/crescent defect. Thus, the addition of texture analysis performed on any MRI datasets, or of an initial multi-parametric result, could be explored to replicate aspects of human interpretation, potentially improving tumour localization. This could be performed as a post-processing step, and therefore not require the acquisition of additional datasets.

By using voxel values for model training, noise characteristics and spatial coherence of parameter values were maintained. If each parameter is sensitive to a different physiologic property of a tumour, this strategy may improve the characterization of a lesion with multi-parametric MRI over a single parameter map, although this was not explicitly studied in this thesis. However, other strategies may also be useful, such as model development using summary measures derived from a percentage of the most-malignant values in regions of tumour. This may have potential to increase technique sensitivity for identifying aggressive regions within a tumour, and is an alternative approach that could be useful for applications such as guiding biopsy.

Much of the work in this thesis was focused on peripheral zone tumours and normal tissue. The majority (~70%) of tumours arise in peripheral zone tissue (McNeal et al. 1988, Chen, M. E. et al. 2000), and thus, although exclusion of central gland tumours is a limitation of the studies, the results are still relevant to a significant portion of patients. Central gland tumours were not studied because there was a limited number present in the patient cohort – five, versus thirty-eight peripheral zone tumours. Both the imaging and the histologic properties of the central gland differ from the peripheral zone, and so a suitable, and separate, cohort is required for investigation. Accurate detection and characterization of central gland prostate cancers thus remains a challenge for
the future. The benefit to patient care could be significant; due to the anterior location of the central gland (Figure 1.2), the false-negative rate of biopsy is higher in this zone compared to peripheral zone tumours (Wefer et al. 2000). Localization with MRI could therefore lead to improved detection, and guide biopsy in an area that is otherwise not always optimally sampled. Although assessment of central gland tumours was not performed in this thesis, Chapter 5 did include regions of interest in the central gland as part of development of the overall relationships between MRI parameters and tissue composition, and thus represents an initial attempt at characterizing central gland tissue.

In investigating the relationships between MRI parameters and tissue components in Chapter 5, the percentage area of each tissue component was extracted using colour-based image segmentation of digitized whole-mount hematoxylin and eosin (H&E) stained sections. Although the segmentation was not optimal, e.g. the algorithm cannot currently differentiate between smooth muscle and connective tissue, the ability to perform quantitative, objective measurements on large histologic samples should assist in further elucidation of relationships between imaging and histology. The majority of clinical pathology review for prostate cancer is performed on H&E-stained sections, as the nuclear morphology and glandular patterns can be well visualized for assessment of Gleason grade. As pathologic Gleason score is a strong predictor of patient outcome, differences in MRI parameters or tissue composition between Gleason 6 and 7 tumours were assessed for significance as part of Chapter 5. As noted in the discussion of that chapter, there is a large degree of heterogeneity in the composition of Gleason 7 tumours, which potentially confounds determination. Thus, a large cohort of patients, with a range of Gleason scores and sub-divided according to their Gleason grade composition, is necessary to accurately investigate relationships between Gleason score and imaging. The small size of the patient cohort investigated in the work presented in the thesis, coupled with the fact that all patients were derived from a surgical cohort, limited the study of any results related to Gleason score. However, using similar methodology, there is tremendous potential to continue exploration into any correspondence between
MRI and other histologic properties, including biomarkers for proliferation, inflammation, or oxidative stress.

Registration – either MRI-MRI or MRI-pathology – is important for all three of the studies presented in the thesis. For MRI datasets acquired for the same patient, during the same session, there are typically two sources of misregistration: patient motion during or between image acquisition; and distortion artifact. Motion was reduced by injecting hyoscine butylbromide to reduce peristalsis in patients with no contraindications for the drug, however bulk motion, i.e., motion of the patient, may still be present. Thus, Chapter 3 included a step to align all parameter maps for the slice location being assessed. The outer contour of the prostate was delineated for each parameter map, and the contours aligned manually. This corrects for in-plane, but not through-plane, shifts. But, as the slice thickness (3 mm) is much larger than the effective voxel size used in the study (0.78 mm per side), and motion is more likely in the left-right or anterior-posterior directions that define the plane, versus superior-inferior of the slice-direction, this alignment accounts for the majority of the expected motion. Despite facilitating development of the multi-parametric model for investigative purposes, this methodology restricts clinical implementation. One motivation for choosing a quantitative technique was to eliminate the need for individual review of all input datasets – a benefit that is negated if manual alignment is necessary.

To reduce distortion artifact due to susceptibility differences between tissue and the interior of the endorectal coil, air in the coil was replaced with a barium sulphate suspension in the latter portion of the patient cohort. This minimizes, but does not completely eliminate, distortion. The performance of multi-parametric MRI may be enhanced if the spatial coherence of voxels between parameter maps is improved. In the future, methods such as the characterization of the appropriate distortion maps (Jezzard et al. 1998, Shen et al. 2004) or post-processing techniques such as deformable registration (described below) may provide some benefit. However, their accuracy must be
extensively validated and their benefit to performance demonstrated prior to inclusion.

All three studies depended on the accurate translation of pathologist-defined regions of interest from whole-mount histology to imaging, performed by Dr. Haider, a radiologist with extensive experience interpreting prostate MRI. The translation could not be performed automatically, as considerable deformation of the histologic sample occurred during fixation and generation of the whole-mount section. Deformable registration methods have the potential to facilitate direct comparison between histology and imaging, which could improve this process as well as potentially facilitate sub-regional analyses (e.g., investigation of sparse regions within tumours, or regional distributions of Gleason grades). Thus, the dataset described in Chapter 2 was made available to collaborators specializing in deformable registration using finite element modeling (Dr.s Andrea L. McNiven and Kristy K. Brock). Preliminary results are showing promise, with sample data shown in Figure 6.1 and estimates of average registration-accuracy on the order of 1 mm in left-right or anterior-posterior directions (1.1 mm ± 0.6 and 1.2 mm ± 0.8, respectively), and 2.4 mm ± 1.2 in the superior-inferior direction. The feasibility of another method has also been recently demonstrated for the prostate; each row and column on the histologic section is resized to match MRI, improving the overlap between histology and imaging, and having an average accuracy of 2 – 3 mm (Noworolski et al. 2009). Although true deformable modeling will likely produce better registration, the latter technique is suitable for use on single-sections versus the entire volume, and may prove to be simpler to implement for applications where accuracy requirements are not as stringent.
6.3 Conclusion

This thesis details several important contributions to the imaging of prostate cancer with MRI. The multi-parametric model in Chapter 3 was derived using statistical methods to combine parameter values from maps of T2, apparent diffusion coefficient, and volume transfer constant to identify malignant voxels in peripheral zone prostate tissue. This was achieved using methods suitable for prospectively producing maps of suspected tumour location. Chapter 4 identifies a property, the presence of significant proportions of normal peripheral zone tissue within the tumour boundary, which limits the ability of MRI to detect prostate tumours, or regions of tumours. Finally, Chapter 5 presents the
individual sensitivities of each of the MRI parameters to the composition of the tissue being imaged. The results of all of the studies in the thesis represent advances in the in vivo characterization of prostate cancer, and provide a framework to impact patient care through the improvement in cancer localization, understanding the current limitations in MRI, and the foundation for novel investigation into tissue characterization.
References


correlation of imaging findings with whole-mount step section histopathology.” AJR Am J Roentgenol 188(1): 84-90.


