QUANTITATIVE TREATMENT RESPONSE CHARACTERIZATION IN VIVO: USE CASES IN RENAL AND RECTAL CANCERS

by

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Use Cases in Renal and Rectal Cancers

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\(^1\)We certify that written approval has been obtained for any proprietary material contained therein.
"But as for you,
be strong and do not give up,
for your work will be rewarded."

– 2 CHRONICLES 15:7
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Abstract

Quantitative Treatment Response Characterization In Vivo: Use Cases in Renal and Rectal Cancers

Abstract

by

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Medical imaging has become widespread for evaluating treatment response in vivo by measuring changes in tumor size or contrast uptake. However, the imaging data alone is limited as it does not capture all information needed for a complete characterization of tumoral response to treatment. Recently, the use of image analysis has enabled strategies for more intelligently extracting information from imaging data. However, these strategies have been scarcely used for treatment response evaluation purposes. In this work, we investigate the development of two classes of image analysis called (1) radiomics for early treatment response characterization and (2) radiology-pathology fusion. Radiomics will enable the detection of more subtle changes in the tumor environment in vivo early into the treatment regime, and the fusion of radiology and pathology data will allow annotations of treatment effects seen on the pathology specimen to be spatially mapped onto the corresponding imaging.
1 Introduction

1.1 Problem Statement: Treatment Response Evaluation Using Imaging is Limited

As a multitude of new drugs and technologies for targeted therapy enter the cancer market each year, the need for measuring their effectiveness is also growing. While these advances in therapy regimes are being administered as new standards of care, the clinical protocol for evaluating treatment response has primarily remain unchanged. Treatment response assessment typically adheres to the following workflow: (1) a biopsy to measure levels of cancerous activity, (2) a scan using radiology to compute changes in tumor extent, and (3) examination of the pathological specimen after surgery.

Pathologic evaluation of excised specimens serves as the gold standard for treatment evaluation since all remaining cancer and treatment effects can be identified when examining the tissue ex vivo. Using histological staining, residual disease can be discriminated against benign confounding treatment effects, providing oncologists with the most informative information about tumoral response and patient prognosis. However, this pathology information is not available until after the patient has already
Introduction

undergone surgery to remove the tumor and surrounding tissue. Hence, noninvasive methods are needed to characterize treatment response pre-operatively.

The use of radiographic imaging technologies, including computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI), are becoming more widely used in assessing changes to the tumor \textit{in vivo}. For example, in solid tumors, treatment response with imaging is evaluated by measuring changes in the tumor size based on expert radiologist annotations of the tumor before and after treatment. While this enables a quantitative assessment of how much the tumor has changed volumetrically as a result of treatment, it is limited in providing any information about subtle changes in structure or function in tumor cells and lacks any criteria for discriminating between residual disease and benign treatment effects.

Tumor response characterization following treatment is vital for defining individualized treatment stratification and follow-up. If treatment response evaluation is incomplete, incorrect, or delayed until after surgical excision of the tumor, this may result in a substantial overtreatment or ineffective follow-up of many patients. Therefore, there is a need for more accurate and comprehensive methods to evaluate treatment response on \textit{in vivo} imaging.

1.2 Scope of this Work

Medical imaging is becoming more and more used to evaluate treatment response \textit{in vivo}, but has issues in capturing the entire spectrum of information needed for
completely characterizing treatment response. Image analysis strategies recently developed have demonstrated promise in extracting more information from the imaging data. However, the use of these strategies for treatment response evaluation is in need of further exploration. In this work, we investigate the development of (1) radiomics for capturing early treatment response in renal cell carcinoma and (2) radiology-pathology fusion for spatially mapping histopathologic treatment effects in rectal cancer onto the imaging.

1.3 Thesis Overview

This thesis presents methods for applying advanced image analysis methods for better characterizing treatment response on in vivo imaging in the context of renal cell carcinoma and rectal cancer. The methodologies and findings presented here are based on work published in Antunes et al (2016)\(^1\) and on work submitted for publication and currently under review. The rest of the thesis is organized as follows:

*Chapter 2: Targeted Therapy: Cytostatic Drugs* reviews the standard of care care in treating renal cell carcinoma, therapy motivating the need for more advanced imaging features for capturing treatment response.

*Chapter 3: Targeted Therapy: Chemoradiotherapy* reviews the standard of care care in treating rectal cancer, therapy motivating the need for spatially mapping pathology data onto radiology images.
Chapter 4: Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response presents an integrative framework between two modalities to identify imaging features which best capture early changes in the tumor environment due to treatment.

Chapter 5: Radiology-Pathology Fusion to Spatially Map Treatment Changes onto Imaging introduces a methodology for mapping annotations of treatment changes captured on pathology back onto the MRI for rectal cancer patients.

Chapter 6: Conclusions and Future Directions concludes the thesis by summarizing the most important discoveries in our work and proposing future directions for our work to promote better treatment response evaluation using in vivo imaging.
2 Targeted Therapy: Cytostatic Drugs

2.1 Current Trends in Renal Cell Carcinoma

Metastatic renal cell carcinoma (RCC) has a poor prognosis with a 5-year survival rate of 12.3%, and there will be an estimated 62,700 new cases of RCC in the U.S. in 2016\(^2\). Recently, tyrosine kinase inhibitors (TKIs) have been introduced as a cytostatic treatment for advanced RCC. For example, sunitinib is a TKI used as first-line therapy for patients with relapsed or medically unresectable RCC since it targets the vascular endothelial growth factor and its receptor\(^3,4\); inhibition of which has been shown to prolong progression-free survival in metastatic RCC\(^5,6\). Preclinical studies have shown that early treatment response as a result of sunitinib is a combination of both anti-angiogenesis as well as inhibition of tumor proliferation\(^7,8\).

2.2 Treatment Evaluation of Renal Cell Carcinoma

Fluorothymidine (FLT) has emerged as a promising positron emission tomography (PET) radiotracer for imaging cell proliferation\(^9\), and associated treatment effects. In Murakami et al (2013), FLT uptake levels in human RCC xenograft models decreased after only 7 days into cytostatic treatment\(^10\). Bao et al (2014) used a cytostatic
drug, sunitinib, in their application of treating human glioblastoma xenograft models and also saw decreased FLT uptake levels after only 7 days\textsuperscript{11}. Thus, in cytostatic treatments which attempt to inhibit tumor proliferation (contrary to conventionally used cytotoxic drugs whose effects can be measured via tumor volume reduction), FLT uptake may be a useful imaging biomarker for visualizing early treatment effects.

Magnetic resonance imaging (MRI) provides high-contrast structural and functional information for characterizing soft tissue, and has been examined extensively in both pre- and post-treatment settings. T2-weighted (T2w) MRI demonstrates pathological features arising from differences in water content within internal structures. Diffusion-weighted imaging (DWI) MRI captures changes in the cellular architecture of the tissue, based on differences in movement of water protons within different tissue regions. Diffusion can be quantified by generating an apparent diffusion coefficient (ADC)-map, which has previously demonstrated great promise as an imaging biomarker of treatment response\textsuperscript{12,13}. In therapeutic options that affect the vascularization of tumors (e.g. cytostatic treatment), and thus the anatomical structure of tissue, both T2w MRI and ADC maps may be expected to capture \textit{in vivo} changes due to treatment.

Furthermore, combining multiple imaging protocols (e.g. PET and MRI) holds great promise in clinical oncology applications, especially with the advent of newly developed hybrid scanners\textsuperscript{14}. MRI offers anatomical localization and an attenuation correction map for quantification of PET data due to concurrent acquisition within the same frame of reference, enabling an overlay of independent \textit{in vivo} structural, functional, and metabolic characterizations of tumor response.
Conventional assessment of \textit{in vivo} imaging is based on tumor morphology measurements, which have demonstrated a limited ability to identify treatment response. For example, the most widely used method in clinical standard of care to evaluate response to cytostatic drugs is the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1)\textsuperscript{15}. The RECIST v1.1 classification is solely based on unidimensional measurements on radiologic imaging to quantify changes in parameters such as the tumor diameter on MRI. However, morphological changes may only appear months into the treatment regimen, due to which RECIST v1.1 may not be able to identify any early treatment effects\textsuperscript{16}. Alternative characterization of tumor response using \textit{in vivo} imaging have involved measuring changes in contrast (for MRI), metabolic activity (for PET), or functional activity (for DWI). While there are guidelines for assessing treatment response on PET and MRI independently\textsuperscript{15,17,18}, to the best of our knowledge, there are no techniques for a joint combined assessment of PET/MRI to evaluate treatment response in oncological applications.

\section*{2.3 Renal Cell Carcinoma Management: Opportunities and Challenges}

In order to quantitatively characterize treatment-related changes in the tumor region on imaging modalities such as PET and MRI, we need to better capture these subtle changes in the tumor region before macroscopic changes in tumor morphology become visible. Further, by quantitatively combining parameters derived from PET, ADC, and T2w MRI, we expect an improved characterization of treatment response \textit{in vivo} compared to the individual imaging sequences.
In order to accurately quantify changes in the molecular and functional characterization of the tumor in the post-treatment setting, specific challenges must be overcome. First, the different imaging acquisitions (PET, T2w, ADC) must be transformed into voxel-wise correspondence in order to evaluate the spatial heterogeneity of associated parameters. By more rigorously evaluating treatment-related changes, we would expect to achieve greater sensitivity than region-based approaches (such as RECIST v1.1). Second, acquisition artifacts due to patient movement and image parameter drift\textsuperscript{19,20} must be corrected for, as these would significantly affect the ability of imaging parameters to capture subtle treatment-related changes in a voxel-wise approach. Finally, visual assessment of imaging parameters may not be able to identify subtle changes in the tumor region \textit{in vivo}; a problem further exacerbated when evaluating such changes early into treatment (a time-point at which these changes may be even less visually appreciable). This issue may be overcome by deriving computer-extracted features from radiographic images, termed radiomics\textsuperscript{21}. Radiomic features attempt to capture "image texture" through quantification of local changes in image intensity values in relation to their voxel-wise arrangement\textsuperscript{22–25}. This offers the ability to quantitatively capture sub-visual "textural" changes in the tumor region; changes that may characterize early treatment response but escape visual identification. Our radiomics framework and results are described in detail in \textit{Chapter 4: Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response}. 
3 Targeted Therapy: Chemoradiotherapy

3.1 Current Trends in Rectal Cancer

Rectal cancer has a relatively high local recurrence rate of 11% despite an aggressive standard-of-care treatment regime, and an estimated 49,190 people will die from the disease in 2016\textsuperscript{26}. Patients are first diagnosed using a biopsy which is analyzed for levels of a blood-based biomarker, carcinoembryonic antigen. Chemoradiotherapy is then administered to patients to reduce the spread of the cancer prior to surgery. Finally, a total mesorectal excision of the rectum and surrounding fatty tissue is conducted for all rectal cancer patients to ensure complete removal of any residual disease from the patient.

3.2 Treatment Evaluation of Rectal Cancer

Following neoadjuvant chemoradiation treatment, biopsies are acquired and analyzed to estimate tumor response to treatment. Pre-operative magnetic resonance imaging (MRI) is then used to visualize changes in tumor extent and restage the tumor for surgical planning. Following a total mesorectal excision, histological slides of the rectal
tissue are curated for a detailed assessment of residual disease and treatment effects. This enables the assignment of a tumor regression grade (TRG), which characterizes the amount of regressive changes after cytotoxic treatment, and is evaluated based on the extent of different therapy induced effects (i.e. fibrosis) in relation to the residual tumor\textsuperscript{27}. The prognostic value of TRG has been shown to exceed those of currently used staging systems\textsuperscript{28}, including tumor-node-metastasis (TNM) staging, which is often done on the pre-operative MRI. If TRG could be computed pre-operatively, this could assist in surgical planning, administering pre-operative adjuvant therapy, or post-chemoradiotherapy surveillance.

Using T2-weighted (T2w) MRI, radiologists are able to annotate the extent of tumor characterized by differences in high-contrast structural and functional information in soft tissue regions. This enables pre-operative localization of the tumor along the rectum as well as quantification of tumoral response based on shrinkage in diameter or volume using RECIST guidelines\textsuperscript{15}. However, the T2 signal decay produced by benign regions of confounding treatment effects often masks the T2 signal generated by residual cancer (Figure 3.1). Since TRG requires the localization of residual cancer and treatment effects, it is currently not possible to extract from \textit{in vivo} imaging and can only be computed on the pathological specimen. Thus, there is a need to discriminate different pathological classes of treatment response on the pre-operative MRI.
Figure 3.1. Radiology and pathology images of a rectal cancer patient who underwent chemoradiotherapy prior to surgical resection of the mesorectum. (a) 2D MR image of rectum acquired after chemoradiotherapy (b) Expert radiologist delineation of estimated residual rectal cancer based on changes in MRI signal pre and post treatment. (c) 2D pathology slide of same patient obtained after surgical removal of rectum and surrounding rectal tissue. (d) Expert pathologist delineation of different treatment effects seen on pathology image, which cannot be seen on radiology. Pathology report indicated that patient had complete tumoral response to radiation therapy (no residual rectal cancer).

### 3.3 Rectal Cancer Management: Opportunities and Challenges

Histological staining on the pathological specimen of rectal tissue enables differentiation of residual cancer and treatment effects. Pathologists are then able to make delineations of these different regions of response to chemoradiotherapy on the tissue, enabling a more comprehensive treatment response evaluation. By registering pathology images to their corresponding MR images, it is possible to spatially map the deep annotations from pathology to radiology, thereby enabling a noninvasive way to discriminate residual cancer and confounding treatment effects for a more complete and accurate treatment response characterization.

Previous studies have demonstrated using corresponding landmarks of the rectum between MR images and histology slides in order to correlate imaging features with regions of pathologic response\(^ {29,30} \). Co-registration of pathology and imaging data
for mapping spatial extent of disease onto pre-operative imaging has been previously performed by several other groups in the context of prostate cancer. However, these studies only spatially mapped delineations of cancer from the pathology to the radiology, enabling the distinction of residual disease from benign tissue, but limited in discriminating all regions of treatment effects on *in vivo* imaging.

In order accurately localize and quantify different regions of treatment effects within the tumor environment *in vivo*, specific challenges must be overcome. First, excised tissue specimens must be carefully analyzed for different regions of treatment effects. Next, 2D slides of pathological tissue and 2D radiology slices must be transformed into a pixel-wise correspondence, or co-registered. Then, annotations of different classes of pathology must be spatially mapped onto the radiologic images. Once annotations of different tissue regions are identified on radiology, image signals within these annotations can be compared across different regions and among different patients. Our solution to these challenges are described in *Chapter 5: Radiology-Pathology Fusion to Spatially Map Treatment Changes onto Imaging*.
Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response

4.1 Overview

The goal of this study was to demonstrate feasibility for performing radiomics analysis on integrated PET/MRI to characterize early treatment response in metastatic renal cell carcinoma (RCC) undergoing sunitinib therapy. Two patients with advanced RCC were imaged using an integrated PET/MRI scanner. [18F] fluorothymidine (FLT) was used as the PET radiotracer, which can measure the degree of cell proliferation. Image acquisitions included test/re-test scans prior to sunitinib treatment and one scan three weeks into treatment using [18F] FLT-PET, T2-weighted (T2w) and diffusion-weighted imaging (DWI) protocols, where DWI yielded an Apparent Diffusion Coefficient (ADC) map. Our framework to quantitatively characterize treatment-related changes involved the following analytic steps: (1) intra-acquisition and inter-acquisition registration of protocols to allow voxel-wise comparison of changes in radiomic features, (2) correction and pseudo-quantification of T2w images to remove acquisition artifacts and examine tissue-specific response, (3) characterization of information captured by T2w MRI, FLT-PET, and ADC via radiomics, and (4) combining multi-parametric information to create a map of integrated changes from PET/MRI.
radiomic features. Standardized Uptake Value (from FLT-PET) and ADC textures ranked highest for reproducibility in a test/re-test evaluation as well as for capturing treatment response, in comparison to high variability seen in T2w MRI. The highest ranked radiomic feature yielded a normalized percentage change of 63% within the RCC region and 17% in a spatially distinct normal region, relative to its pre-treatment value. By comparison, both the original and post-processed T2w signal intensity appeared to be markedly less sensitive and specific to changes within the tumor. Our preliminary results thus suggest that radiomics analysis could be a powerful tool for characterizing treatment response in integrated PET/MRI.

4.2 Materials and Methods

4.2.1 Data Description

Two patient studies were obtained from a prospective study evaluating the use of FLT-PET/MRI in monitoring anti-angiogenic therapy (PI: Dr. N. Avril, MD ClinicalTrials.gov Identifier: NCT02055586) at the Case Comprehensive Cancer Center Cleveland. Prior to imaging, patients had been diagnosed with advanced metastatic RCC and were scheduled to begin sunitinib therapy. Before starting treatment, patients were imaged on consecutive days (termed "test"/"re-test"). Patient 1 was additionally imaged three weeks into the treatment regimen (termed "mid"). Image acquisitions were acquired using the Ingenuity TF PET/MR (Philips Healthcare, Cleveland, OH). Imaging sequences considered in each acquisition set included a respiratory-triggered T2w turbo spin echo (RT T2w TSE) MRI, DWI spin echo (DWIse) and a whole body
Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response

(WB) PET protocol. DWI yielded an ADC-map (b-values = 0, 400, 800). [18F] labeled FLT was used as the PET radiotracer. Annotations of the tumor region (RCC) as well as a homogenous region of healthy tissue (termed "norm") were obtained on the T2w volumes via an expert radiologist.

![Figure 4.1. Overview of workflow and radiomics analysis methodology for capturing early treatment response in renal cell carcinoma.](image)

4.2.2 Intra-acquisition Spatial Alignment

T2w, PET, and ADC-map image volumes within each acquisition set were spatially aligned to one another using the T2w volume as a reference. This was done by
resampling and cropping PET and ADC-map volumes to the same field of view (as the T2w volume) in order to account for imaging differences in protocol-specific image dimensions and voxel sizes. This enabled a voxel-wise correspondence between the 3 PET/MRI protocols under consideration.

### 4.2.3 Bias Field Correction

Intensity inhomogeneity is an MR image acquisition artifact which refers to the gradual intensity variation within a tissue region over the image domain. This occurs due to imaging instrumentation radio-frequency non-uniformity or static field inhomogeneity, and is known to significantly affect image analysis methods and radiomic feature extraction. Intensity inhomogeneity is visualized as a significant variation in gray level intensities across the MR image, and was found to be present in the T2w images upon visual inspection. Thus, prior to registration, T2w volumes were bias field corrected for this image intensity non-uniformity by convolving the images with a Gaussian low pass filter, resulting in uniform intensities across the volume.

### 4.2.4 Intra-acquisition Co-registration

Acquisitions acquired on each of the 3 days were found to be misaligned due to organ and patient movement. 3-dimensional (3D) affine co-registration followed by a
3D deformable co-registration was applied between T2w volumes across the different acquisition sets in order to enable per-voxel correspondence and comparison between test/re-test and test/mid-treatment acquisitions. Image registration was driven using mutual information as the similarity measure, and was performed using the ITK framework in Elastix. The transformation matrices obtained from each registration T2w pairing were then applied to the corresponding PET and ADC-maps (i.e. the matrix to transform re-test T2w into alignment with test T2w was then applied to re-test PET and re-test ADC-map.) Thus, all 3 protocols across all 3 acquisition sets were in the same frame-of-reference with the ability to perform voxel-wise correspondences.

Separate annotation volumes had been created for each of test/re-test/mid-treatment T2w acquisitions. The transformation matrices from the co-registration step were applied to these annotations, following which a minimum overlapping volume (MOV) mask was made from the intersection of test, re-test, and mid-treatment acquisition annotations. This was done separately for annotations of healthy tissue and RCC regions, which were then used as regions of interest for the remainder of the analysis.

4.2.5 Intensity Standardization

T2w images visualize different in vivo tissue regions as having different levels of intensity contrast, within the same field of view. However, the image intensities in T2w MRI lack a fixed tissue-specific meaning, even when protocol, scanner, and patient parameters are held constant. This artifact is termed intensity non-standardness, which implies that differences in image intensities between corresponding voxels within the same tissue region, but from 2 different T2w acquisitions, cannot be assigned
Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response

Figure 4.2. Transformation of RCC volumes into a voxel-wise correspondence across acquisitions. (Top Row) Annotations of RCC region on original T2w volumes. Note slight differences in annotated region between each column. (Middle Row) Results of 3D rigid plus deformable co-registration shown as checkerboard images. Note contiguity of structures across checkerboard, indicating accuracy of registration. (Bottom Row) Creation of MOV mask from intersection of transformed annotations of co-registered RCC region across acquisitions to ensure voxel-wise comparison within tumor region. Note annotated region and structures are consistent across all 3 columns. Dice similarity coefficients between test/re-test and test/mid-treatment volumes were calculated as 0.90 and 0.83, respectively (note ideal Dice value between 2 volumes should be 1).

quantitative meaning. Thus, T2w volumes were standardized to align intensity distributions across acquisitions, using the method presented by Nyul et al (2000)\(^{41}\). Intensities of the homogenous healthy tissue regions from each T2w acquisition were
used to generate standardization maps which were then applied to the entire T2w volume. The output of standardization is a pseudo-quantitative T2w value at every voxel, which has tissue region-specific meaning. Intensity standardization was performed after registration as registration was found to exacerbate drift between intensity distributions (possibly due to implicit interpolation).

4.2.6 Radiomics Analysis

For each imaging protocol, a specific set of quantitative features (where \( f \) is the set of all features) was extracted within the annotated regions. Standard Uptake Value (SUV) was measured from FLT uptake in PET volumes using a decay-correction intensity computer algorithm\(^{42}\). ADC-map values were automatically generated from the magnitude of diffusion of water molecules in DWI volumes (b-values = 0, 400, 800).

Texture features were extracted from both the post-processed T2w and ADC volumes. A total of thirty 1\(^{st}\) and 2\(^{nd}\) order statistical features were used, including non-steerable gradient (gray level, Kirsch, Sobel)\(^{43}\) and Haralick\(^{44}\) operators. Radiomic analysis on PET data has demonstrated limited robustness and reproducibility due to the low spatial resolution of PET (in comparison to T2w, ADC)\(^{45,46}\). Based on findings in a recent validation study, we decided to only extract entropy and difference average texture features from FLT-PET\(^{47}\). In total, a set of 66 radiomic features were implemented (raw T2w signal, post-processed T2w, 30 post-processed T2w textures, raw ADC map, 30 ADC textures, SUV, and 2 PET textures).
4.2.7 Statistical Analysis

In order to rank features, the distribution of feature intensities within the RCC and healthy tissue regions across acquisition sets were compared. Differences in intensity distributions \(D\) were measured using the Bhattacharyya distance\(^{48}\). Bhattacharyya values \(B\) were normalized from \(0 \leq B \leq 1\), to represent perfectly aligned and perfectly misaligned intensity distributions, respectively.

A two-part scoring function was implemented

\[
S_1 = B(D^\text{test}_{\text{norm}}, D^\text{re-test}_{\text{norm}}) \quad (4.1)
\]
\[
S_2 = B(D^\text{test}_{\text{RCC}}, D^\text{mid}_{\text{RCC}}) \quad (4.2)
\]

where \(S_1\) quantifies the variability of each parameter between test/re-test acquisitions (i.e. a measure of specificity) and \(S_2\) quantifies the ability of a parameter to capture early response between test/mid-treatment acquisitions (a measure of sensitivity). Intensity distributions of a homogenous healthy region between test/re-test acquisitions are expected to be aligned (i.e. ideally, \(S_1 = 0\) implies low variability), whereas intensity distributions of the RCC region are expected to be markedly misaligned are a result of sunitinib treatment (i.e. ideally, \(S_2 = 1\) implies maximal response). Features were ranked based on a combination of the feature’s performance in each of the two parts of the scoring function based on a weighted ratio,

\[
S_{\text{overall}} = \frac{S_{w2}}{S_{w1}} \quad (4.3)
\]
The weighting factors were set as $w_1 = w_2 = 0.5$ in order to attribute equal importance to both terms of the scoring function $S_{overall}$ was computed for each feature in $f$.  

### 4.2.8 Multi-parametric Map

Percent difference (denoted $\% \Delta$) for each feature $f_i \in f$, $i = 1, ..., 66$ was measured on a per-voxel basis between test/mid-treatment acquisitions. A weighted difference image ($\bar{T}$) was then computed by combining $\% \Delta$ from a subset of features $\bar{f}_j$ using a weighted summation as follows,

$$\bar{T} = \sum_{j=1}^{n} a_{\bar{f}_j} \times \% \Delta_{\bar{f}_j}$$

where the weighted contribution factor $a_{\bar{f}_j}$ for each feature $\bar{f}_j$ is based on how well $S_{overall}$ for each feature $\bar{f}_j$ ranked relative to other features. The number of features used in generating $\bar{T}$ was set as $n = 3$, where SUV, the top-ranked ADC feature, and the top-ranked T2w feature were combined to yield a MP-PET/MRI difference image.

### 4.3 Experimental Results and Discussion

Table 4.2 summarizes the top 25 PET/MRI radiomic features for each patient, ranked in the descending order, based on the scoring function $S_1$ alone. It may be observed that SUV and ADC Haralick features are relatively highly ranked in both patients, suggesting low variability in a test/re-test evaluation. By comparison, ADC gradient features and the original T2w signal intensity ranked relatively high for Patient 2 only, and therefore may not be as reliable. Most T2w radiomic features, including
the post-processed T2w signal intensity, the raw ADC value, and both PET texture features were among the lowest ranked features, implying relatively high variability in a test/re-test evaluation. It has previously been shown that in a comparison of feature reliability, ADC texture features have ranked higher than T2w features in demonstrating more consistent changes in healthy tissue regions. In a previous case study that presented some of the data utilized in this work, FLT-PET was shown to have an overall high repeatability (i.e. low variability) between test/re-test studies. DWI-ADC was found to be less repeatable, likely due to respiration artifacts affecting image quality, while T2w MRI as well as radiomic features were not evaluated.

Figure 4.3 visualizes boxplots of %Δ between test/mid-treatment within healthy tissue and RCC regions for the three top-ranked radiomic feature based on the scoring function $S_{overall}$ (in order: SUV, ADC Energy, and T2w Difference average) for Patient 1 alone, as well as for the original signal intensities (mid-treatment acquisition for Patient 2 was unavailable). The three top-ranked features were combined to yield a MP-PET/MRI map, for which boxplots of %Δ are also shown. SUV and both T2w and ADC texture features appear to be able to capture treatment-related changes, whereas the original T2w signal intensity does not appear to capture any significant change other than differences due to acquisition variability. The MP-PET/MRI map may be considered most reflective of a marked difference in the tumor region, but may not necessarily best reflect change due to treatment alone (due to a greater change captured in the healthy tissue compared to SUV). This indicates the need for either a better weighting of the different modalities or a modified scoring function to optimize the integrated map.
Table 4.2. Top 25 ranked PET/MRI radiomic features for each patient based on scoring function $S_1$ (quantifying test/re-test variability).

<table>
<thead>
<tr>
<th>$S_1$ Rank</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T2w Gradient Y</td>
<td>ADC Gradient X</td>
</tr>
<tr>
<td>2</td>
<td>ADC Energy</td>
<td>ADC Sobel YX</td>
</tr>
<tr>
<td>3</td>
<td>T2w Sobel Y</td>
<td>ADC Entropy</td>
</tr>
<tr>
<td>4</td>
<td>ADC Difference Average</td>
<td>ADC Sobel X</td>
</tr>
<tr>
<td>5</td>
<td>SUV</td>
<td>SUV</td>
</tr>
<tr>
<td>6</td>
<td>ADC Gradient X</td>
<td>ADC Sum Entropy</td>
</tr>
<tr>
<td>7</td>
<td>ADC Inverse Difference Moment</td>
<td>ADC Inverse Difference Moment</td>
</tr>
<tr>
<td>8</td>
<td>T2w Correlation</td>
<td>ADC Energy</td>
</tr>
<tr>
<td>9</td>
<td>ADC Sobel YX</td>
<td>ADC Sobel XZ</td>
</tr>
<tr>
<td>10</td>
<td>T2w Sobel YX</td>
<td>ADC Range</td>
</tr>
<tr>
<td>11</td>
<td>ADC Difference Entropy</td>
<td>ADC Difference Average</td>
</tr>
<tr>
<td>12</td>
<td>ADC Difference Variance</td>
<td>T2w Correlation</td>
</tr>
<tr>
<td>13</td>
<td>ADC Gradient Y</td>
<td>ADC Gradient Y</td>
</tr>
<tr>
<td>14</td>
<td>ADC Entropy</td>
<td>ADC Gradient Magnitude</td>
</tr>
<tr>
<td>15</td>
<td>ADC Sobel X</td>
<td>ADC Difference Average</td>
</tr>
<tr>
<td>16</td>
<td>T2w Gradient X</td>
<td>PET Difference Average</td>
</tr>
<tr>
<td>17</td>
<td>T2w Sobel XY</td>
<td>Raw T2w</td>
</tr>
<tr>
<td>18</td>
<td>ADC Inertia</td>
<td>ADC Sobel YZ</td>
</tr>
<tr>
<td>19</td>
<td>ADC Sum Entropy</td>
<td>ADC Sobel ZY</td>
</tr>
<tr>
<td>20</td>
<td>ADC Gradient Y</td>
<td>PET Entropy</td>
</tr>
<tr>
<td>21</td>
<td>ADC Sobel XY</td>
<td>ADC Information Metric 1</td>
</tr>
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<td>22</td>
<td>ADC Information Metric 1</td>
<td>ADC Information Metric 2</td>
</tr>
<tr>
<td>23</td>
<td>ADC Information Metric 2</td>
<td>T2w Gradient Y</td>
</tr>
<tr>
<td>24</td>
<td>T2w Sobel X</td>
<td>ADC Gradient Z</td>
</tr>
<tr>
<td>25</td>
<td>ADC Correlation</td>
<td>ADC Sobel Z</td>
</tr>
</tbody>
</table>

Figure 4.4 visualizes the difference maps between test/mid-treatment acquisitions for Patient 1. Displayed are percent difference images for the three top-ranked radiomic features based on $S_{overall}$ and for the original signal intensities. Hotter colors represent greater percent changes as a result of sunitinib treatment. While differences in uptake appear across the entire functional tumor volume of FLT-PET, regions of change captured by SUV appear to be rather homogeneous (minimal differences within a small neighborhood of voxels), reflecting marked changes being captured
Radiomics Analysis on FLT-PET/MRI to Capture Early Treatment Response

by molecular imaging due to treatment, as well as the limited spatial resolution of PET. The regions of change seen on the difference maps of the original ADC and T2w images are visually much more heterogeneous (marked differences within a small neighborhood of voxels), indicative of these modalities capturing voxel-wise changes at the anatomic or structural level. Each of the different PET/MRI protocols appears to capture unique information regarding early treatment response, and the MP-PET/MRI map appears to combine these complementary sources of information to yield a comprehensive characterization of response due to TKI treatment.

The raw T2w value and post-processed T2w signal intensities were among the lowest ranking features based on $S_{overall}$, reflecting high test/re-test variability as well as not optimally capturing treatment-related changes. By comparison, T2w texture features appear to yield an improved performance in capturing treatment-related changes due to cytostatic treatment. Similarly, ADC texture features also appeared to capture additional information related to treatment change compared to the raw ADC value. In contrast, texture features from PET (not shown) appear to exhibit high test/re-test variability and unable to capture treatment related changes. This however is somewhat expected due to the limited spatial resolution of PET.

SUV was the highest ranking feature overall.

T2w radiomic features have previously been demonstrated to be able to differentiate tumor and healthy regions based on appearance of the prostate. Additionally, in cytotoxic applications, such as laser ablation, both T2w and ADC radiomic features were able to detect treatment-related changes in the ablation zone of the prostate. A subset of the radiomic features examined in these previous studies were utilized in this study to characterize treatment response in RCC based on changes in appearance
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before and during treatment. Since we are examining changes only three weeks into
treatment, qualitative changes on intensity images were hard to observe. However,
our initial results suggest that T2w and ADC radiomic features may be capturing
sub-visual changes in anatomic texture, thus quantifying changes in the microscopic
structures of the tumor as a result of sunitinib treatment.

While we have evaluated a large number of radiomic features to quantitatively
characterize treatment response on FLT-PET/MRI, the goal was to determine which
radiomic features best reflected treatment-related change while being resilient to
acquisition-related artifacts. The results of this study could therefore help guide
future studies for selecting the appropriate radiomics feature set to examine for treat-
ment evaluation in the context of different body regions and diseases.

![Figure 4.3. Boxplots showing %Δ between test/mid-treatment acquisi-
tions for Patient 1 in both the RCC and healthy tissue regions for top-rank-
 ranked PET/MRI radiomic features (based on scoring function $S_{overall}$)
as well as the original signal intensities.](image-url)
Figure 4.4. Percent difference maps based on treatment related changes between corresponding voxels in test/mid-treatment acquisitions for Patient 1 in the (a) healthy tissue and (b) RCC regions for PET/MRI radiomic features. (c) Difference maps of SUV, ADC energy, and T2w difference average are combined based on each feature’s weighted contribution factor to compute an integrated MP-PET/MRI map, reflecting a comprehensive characterization of early treatment related changes and response.

4.4 Concluding Remarks

In summary, we have presented a first attempt of using radiomics analysis for evaluating treatment-related changes in vivo after early sunitinib treatment for RCC via FLT-PET/MRI. Our experimental results suggested that PET/MRI radiomic features, namely SUV, ADC Energy, and T2w Difference Average, might be able to identify early structural and functional response to cytostatic treatment in metastatic...
RCC, but our conclusions are limited due to the fact that only two studies were considered in this work. A test/re-test evaluation indicated that SUV and ADC Haralick features appeared to display low variability across acquisitions for each patient. By comparison, the original T2w signal intensity and T2w radiomic features seemed inconsistent across acquisitions, even after intensity standardization. T2w and ADC texture features appeared to yield an improved performance in capturing treatment-related changes compared to the original T2w and ADC signal intensities, whereas PET texture features seemed to degrade in performance. While SUV was the highest ranking individual feature for overall sensitivity and specificity, the integration of top-performing radiomic features yielded an even higher sensitivity by combining complementary information from the different PET/MRI protocols. As this was only a proof-of-concept study, further investigation is needed to validate our findings on a larger cohort of data. Additional studies are also needed to correlate early treatment changes with progression free survival. Through the presented workflow, we also hope to perform radiomics analysis to identify imaging features predictive of treatment response in other cancers via PET/MRI.
5 Radiology-Pathology Fusion to Spatially Map Treatment Changes onto Imaging

5.1 Overview

The purpose of this experiment was to develop and quantitatively evaluate a radiology-pathology fusion method for spatially mapping post-chemoradiotherapy changes in rectal cancer onto the pre-operative MRI. The study included 6 subjects with rectal cancer treated with chemoradiotherapy who were pre-operatively imaged with a T2-weighted magnetic resonance imaging (T2W MRI) and later had the pathological tissue of the rectum curated. Delineations of residual disease, ulcers, fibrosis, muscularis, mucosa, fat, inflammation, and pools of mucin were made by an expert pathologist on 2-dimensional (2D) slides of the specimen. We fused the radiology and pathology information by using landmark-based registration to spatially map the deep annotations from 2D pathology slides onto corresponding 2D MRI slices. Qualitative assessment of registered pathology and annotations onto the MRI revealed good alignment of structures with those visible on the MRI. Overall deviation of selected anatomical landmarks was $1.50 \pm 0.63$ mm, less than 2% error relative to the average
annotation size of treatment effects (155.95 mm$^2$). Moreover, the T2w intensity distributions were markedly different when comparing fibrotic tissue to perirectal fat. Our fusion methodology enabled the successful and accurate localization of post-treatment effects onto in vivo MRI. This integration of radiology-pathology data enables the potential for a more comprehensive treatment response evaluation using pre-operative imaging.

5.2 Materials and Methods

5.2.1 Data Description

The institutional review board approved this retrospective study and waived the requirement of informed consent. In the period from September 01, 2009, to July 01, 2015, a total of 96 patients who were diagnosed with rectal cancer and treated with 2–12 months of neoadjuvant chemoradiotherapy underwent total mesorectal excision of the rectum and surrounding fatty tissue at University Hospitals Case Medical Center, 87 of whom were excluded because of equipment availability and time constraints, allowing us to digitize histological slides for only 9 of the 96 patients. Of these 9 patients, all 6 patients from 2011–2013 with non-fragmented rectal tissue specimens and who underwent MR imaging prior to surgery were included (all males, age range, 52–82 years, median, 57). Our cohort comprised of 1 patient with complete tumoral response, 4 patients with incomplete tumoral response, and 1 patient with poor tumoral response to chemoradiotherapy (response groupings based on pathological T stage with N0 and M0 controlled for$^{51}$). The time between MR imaging and total mesorectal excision ranged from 1.5–5 weeks (mean, 3 weeks).
5.2.2 Acquisition of in vivo Imaging

MR imaging was acquired following chemoradiotherapy and pre-operatively using a 3 Tesla T2w turbo spin echo MRI protocol (MAGNETOM Verio, Siemens Healthcare, Malvern, PA) with average repetition times/echo times of TR/TE = 1000/80 ms. The T2w volumes were reconstructed with in-plane resolution of 0.63–1.00 mm (256 x 256 in-plane pixels) and the distance between slices was 3mm.

5.2.3 Digitization of Surgical Specimens and Annotations of Treatment Effects

Following resection, tissue was marked using blue (parenchyma) and black ink (pleura), fixed in formaldehyde, and stained with Hematoxylin and Eosin. Pathology slides were scanned at 40x magnification (pixel size: 0.24 µm, 153470 x 105207 in-plane pixels). An expert pathologist assessed the tissue for the presence of residual cancer, ulcers, fibrosis, muscularis, mucosa, fat, inflammation, and pools of mucin, carefully delineating each region present for each patient (Aperio ImageScope, Leica Biosystems). Disease extent ranged from 1.26–170.04 mm$^2$ per patient and regions of treatment effects ranged from 1.40–2314.15 mm$^2$ per patient.

5.2.4 Co-registration of Radiology-Pathology to Spatially Map Deep Annotations onto MRI

Our fusion approach comprised pre-processing steps prior to co-registration, and the workflow is illustrated in Figure 5.1 The same workflow was replicated across all patients.
First, single T2w slice from the entire T2w volume which corresponded to the annotated pathology slide image was identified jointly by an expert radiologist and pathologist sitting together. All slices selected were in the plane axial to the tumor. Digitized pathology slides were then down-sampled to 0.5x magnification (pixel size: 18.68 µm, 1643 x 2397 in-plane pixels) and T2w slices were up-sampled by a factor of 6 (in-plane resolution of 0.11 – 0.17 mm, 1536 x 1536 in-plane pixels) using bilinear interpolation resampling in order to bring the rectum on the T2w slice into the same order of magnitude of size, in pixels, with the rectal tissue on the pathology slide. Pathology slides were flipped and rotated on a case-by-case basis in order to ensure gross alignment relative to the T2w slice.

After image resampling and additional operations, spatial misalignment still existed between the up-sampled T2w slice and down-sampled pathology slide due to differences in image orientations, image sizes and resolutions, and often incomplete cuts of histopathologic specimens (resulting in the rectal tissue folding). In order to correct these differences and bring these images into a pixel-wise correspondence, 2D co-registration was applied between these images, using radiology as the fixed image and pathology as the moving image. Thin-plate Spline method using an in-house registration platform, Prostalign, was implemented to drive registration by deforming the pathology slide around 5–7 fixed corresponding anatomical landmarks seen on both the pathology slide and T2w slice for each patient (landmark identification done jointly by expert radiologist and pathologist simultaneously).
Finally, the transformation matrices obtained from registration were then applied to the corresponding pathology annotations for that patient. Thus, original annotations of different treatment effects made on pathology data have now been mapped onto their corresponding spatial location on the up-sampled T2w slices.

Figure 5.1. Radiology-Pathology fusion methodology enables the mapping of chemoradiotherapy treatment effects onto MRI.
5.2.5 Evaluation Strategy

For each patient, image co-registration was first evaluated qualitatively by overlaying the registered pathology image and the registered pathology mask onto the up-sampled T2w image. Visual inspection was performed concurrently by an expert radiologist and pathologist to validate that corresponding regions of the rectum that can be seen on both imaging modalities were correctly localized (i.e. fat, residual cancer, lumen). T2w intensity distributions with the co-registered annotations of perirectal fat were compared between each patient to qualitatively assess overall registration across the cohort, as T2w MRI has a distinct signal characteristic in fatty tissue.

Additionally, we evaluated the accuracy of spatial-mappings by comparing the T2w intensity distributions between the mapped fibrotic and fatty tissues, with the hypothesis being that if the spatial mapping of these two regions had been performed accurately, the corresponding signal intensity distributions within these compartments should be different.

Quantitative evaluation was also performed for each patient using total registration error (TRE) by measuring the Euclidean distance between 5 corresponding anatomical landmarks between the pathology and radiology images (see Figure 5.2). All landmarks used to evaluate co-registration were independent of landmarks used to drive registration.
Figure 5.2. Co-registration evaluation strategy. Corresponding landmarks on (a) pathology slide and (b) T2w slice images selected to drive co-registration. Independent set of evaluation landmarks on (c) registered pathology and (d) T2w slice to measure TRE. Visual inspection of co-registration result using (e) registered pathology slide image onto T2w slice image, (f) spatial comparison of corresponding landmarks used for evaluation, and (g) overlay of registered pathology annotations onto T2w slice image. (h) Bar plots of registration error for each landmark used for evaluation, yielding a patient TRE of $1.30 \pm 0.46$ mm.

5.3 Results

Figure 5.3 shows the co-registration landmarks used and the co-registration results for 4 patients. Using only 5 landmarks to drive registration (except for one patient for whom the cut rectal tissue was extremely folded and required 2 additional landmarks), overlays of pathology onto radiology appear to accurately align the inside and outside borders of the rectal wall tissue for all patients. The pathology annotations of treatment effects spatially mapped onto the T2w images all correctly lined up inside the rectal wall tissue which is where we expect to capture different tissue responses.
from the targeted radiation therapy. The pathology annotation of perirectal fat is also correctly spatially mapped onto the fat surrounding the rectum on the T2w image for each patient. This is further validated by histogram distributions of the T2w fat intensities shown in Figure 5.4, which is reflecting a similar T2w intensity profile from within the spatially mapped perirectal fat regions for each patient. Patient 4 is the only patient with a shifted T2w fat intensity profile, which can be visually seen and understood from Figure 5.3h, where perirectal inflammation has leaked into the fatty tissue.

Figure 5.3. Results of remainder of co-registration experiments. (a, c, i, m) Original pathology slides and anatomical landmarks used for registration. (b, f, j, n) T2w slice and anatomical landmarks used for registration, corresponding with pathology slide in the column left of the T2w slice. (c, g, k, o) Registered pathological specimen overlaid onto T2w slice. (d, h, l, p) Spatially mapped histopathologic annotations (with region labels in legend) projected onto T2w slice.
Figure 5.4. Histogram distributions of T2w signal intensity from perirectal fat region across all patients. All but one patient (Patient 4) have a very similar fat intensity profile, indicative of successful spatial mapping of pathology fat annotation onto radiology.

The quantitative evaluation of the co-registration was performed on a total of 5 landmarks per patient, with all landmarks being independent from the set of landmarks to drive registration. The overall TRE was computing using Euclidean distance between the corresponding landmarks on the registered pathology and T2w image was calculated to be $1.50 \pm 0.63$ mm across the 6 patients. Given that the average annotation size of treatment effects was $155.95$ mm$^2$, this corresponds to less than 2% error in our mappings of spatial extent of pathological regions.
A comparison was made of the distribution of the intensity values within the spatially mapped regions of fibrosis and perirectal fat on the T2w image. Figure 5.5 shows violin plots of T2w intensity distributions within region of fibrotic tissue and perirectal fat, side-by-side on the same y-axis scale, for each patient. The result appears to indicate that fibrosis has a markedly different T2w imaging signature than the fat, supporting our previously stated hypothesis that the corresponding signal intensity distributions within these compartments should be different if the spatial mapping of these regions was performed correctly. Moreover, there appears to be a greater difference in fibrosis and fat T2w intensities for patients with good response to radiation therapy as opposed to patients with poor response to radiation therapy.

5.4 Discussion

The important prognostic value of analyzing treatment effects has contributed to the emerging need for localizing these regions on the pre-operative imaging. Analyzing these regions in vivo may enable better clinical decision-making for surgical planning or adjuvant treatment. In this study, we introduced a radiology-pathology fusion methodology that allowed us to spatially map annotated regions on the histopathologic specimen onto the pre-operative MRI in the context of rectal cancer. Challenges in discriminating residual disease and treatment effects on imaging were address by using co-registration methods and computerized tools to extract intensity distributions within the spatially mapped regions on the MRI.

This study presented a promising methodology for co-registering and registration evaluation of radiology-pathology data. Our registration error was on the order of
Figure 5.5. Side-by-side violin plots comparing T2w signal intensity distributions from the fibrosis region (red) to the perirectal fat region (blue) across all patients. The fibrosis region appears to have a markedly different signal intensity than the fat region after spatially mapping the pathology annotations onto the T2w images. That difference in signal intensities becomes harder to discriminate in cases of poorer response to treatment.

millimeters and was limited by in-plane resolution on the MRI, but was comparable to error in other radiology-pathology studies which implemented landmark-based registration\textsuperscript{31–33}. Only 5–7 anatomical landmarks were used to drive the registration, less than the average amount used in these other studies. Additionally, the T2w intensity differences between the regions of fibrosis and fat supports other studies which
have demonstrated the use of imaging features to discriminate imaging signatures of fibrosis and clusters of tumor cells\textsuperscript{53,54}. This indicates that our pathology annotation mapping may prove useful in identifying additional unique imaging signatures between regions of treatments effects in addition to residual disease.

Our study did however have its limitations. Of the original 96 patients who underwent a total mesorectal excision, only 6 had histological specimens of the rectal tissue curated and intact enough to be included in our work. Future studies applying this methodology should carefully curate complete histology cuts of the excised specimen in correspondence with the imaging slices. Additionally, the accuracy could be slightly improved by increasing the number of landmarks used to drive registration, at the cost of reducing the number of identifiable anatomical landmarks to evaluate the registration. Finally, in our comparison of T2w intensity distributions within the same region of fat across our patient cohort, differences in intensity values could be related to the fact that the T2w signal is unstandardized and often requires correction before analysis\textsuperscript{41}.

### 5.5 Concluding Remarks

In conclusion, this work presented a radiology-pathology fusion methodology which allowed different tissue responses to targeted radiation therapy to be spatially mapped from histopathologic specimens onto an MRI. Future directions of this work include using spatially mapped regions of treatment effect to identify radiomics features which differentiate residual disease from treatment effects on \textit{in vivo} imaging in rectal cancer applications. This might have implications for a more complete treatment response.
assessment prior to surgery, thus improving the standard-of-care for patient follow-up to chemoradiotherapy.
6 Conclusions and Future Directions

Technological advances in cancer management has led to more improved targeted therapies. Evaluation of the efficacy of these treatments is crucial to identify (1) patients who have complete tumoral response to treatment and do not require surgery or (2) patients who do not respond well to treatment and should be referred to a different therapy. Thus, there is a clear need for accurate and objective strategies for evaluating treatment response.

In this work, we presented two strategies for extracting more information from \textit{in vivo} imaging to enable a more complete treatment response characterization pre-operatively. First, we presented an integrated radiomics analysis for evaluating treatment-related changes \textit{in vivo} after early sunitinib treatment for RCC via FLT-PET/MRI. Our experimental results suggested that certain radiomic features might be able to identify early structural and functional response to cytostatic treatment in metastatic RCC whereas the radiologist is limited in capturing any differences using visual inspection of the T2w MRI signal alone. Second, we showcased a radiology-pathology fusion method which allowed different tissue responses to targeted radiation therapy to be spatially mapped from histology specimens onto an MRI. Our approach
demonstrated success in registering pathology and radiology images, enabling the radiologist to differentiate regions of residual design from benign treatment effects on the pre-operative T2w MR images.

Image analysis methods such as radiomics feature extraction and image co-registration enable a more robust, quantitative analysis of medical images than using qualitative visual inspection alone or using current standards of measuring tumor size. Such methods capture subtle, sub-visual changes in areas affected by treatment, and provide oncologists with more detailed information for adjuvant treatment or surgical planning purposes. Envisioned future directions and potential implications of this work are as follows:

- Radiomic features may be used in evaluating treatment response in the context of other cancers, enabling earlier assessment of how well treatment is working for each individual patient
- Spatially mapping of pathology annotations onto radiology images may be utilized to build feature sets which discriminate between different regions of treatment effects
References


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