Functional Imaging and Texture Analysis
in Radiotherapy Planning

(FiNiTe RT)

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Submitted for the Degree of
Doctor of Philosophy from the
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October 2016

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I would like to dedicate this thesis to the dearest to my heart, my loving parents . . .
Acknowledgements

And I would like to thank the following...

My PhD supervisors, Phil Evans, Andy Nisbet, Chris South and Sarah McQuaid for sharing their knowledge and expertise, providing guidance, support and encouragement throughout the four years of my studies.

James Scuffham, for useful advice and help in retrieving data.

Veni Ezhil, for valuable clinical discussions and her support of this project and for providing patient data.

Vineet Prakash, for sharing his knowledge, contouring tumours and his enthusiasm about this research project.

Philip Webster and the Alliance Medical Group, for logistic support and providing access to imaging equipment.

Iain Philips, for useful clinical discussions and contouring bronchial tree.

Donna Rickard and Mo Hussein, for helping with treatment planning.

Michelle James, for kindly booking the room for my monthly meetings.

CVSSP administrative team, Liz, Anna and Nan for all their help and support.

Past and present members of the CVSSP, most notably the medical imaging group, for all their support, encouragement and help.

I would like to particularly thank my parents, Ahmad and Fatma for their unconditional love and support over the years of pursuing my education away from them, my sisters and my brother, for their non-stop encouragement, and my nieces and nephews for always putting a smile on my face and distracting me from the pressures of the PhD.

Finally, I would like to thank the Kuwaiti Government for sponsoring this PhD.
Abstract

Texture analysis has been proposed recently as an imaging biomarker in the field of oncology. The previously published work has shown promising results in relating tumour heterogeneity to patient survival. Yet, a standardised texture analysis method has not been established, and the physiological implications of the extracted textural features are not well understood. An understanding of tumour image texture is crucial before such a method could be utilised in clinical settings.

In this thesis, the author presents methodology developed to generate optimised three-dimensional voxel-based CT texture maps (3D-VTM) to examine regional heterogeneity information within tumours and their relation to tumour metabolism measured as 18F-fluoro-deoxy glucose (18F-FDG) Positron Emission Tomography (PET) distributions. Ten patients diagnosed with advanced non-small cell lung cancer (NSCLC) were investigated. For optimal texture information decoding, an optimised quantisation method is presented. The texture feature that reflects heterogeneity and which showed correlation with patients survival was chosen for this thesis. To account for respiratory motion effects, an in-house designed phantom was used to characterise the effects of motion on texture analysis and consequently adapt our method in that regard.

To quantify the relationship between CT tumour image heterogeneity and the 18F-FDG uptake distribution, the overlap fraction (OF) and Dice similarity coefficient (DSC) were calculated. The 3D-VTMs produced from CT tumour images using our proposed image information optimisation method have shown a clear textural pattern within tumours that correlates with the 18F-FDG distribution. These produced 3D-VTMs showed that regions with low CT heterogeneity within the tumour overlap with regions of high 18F-FDG uptake (>50% SUVmax). The OF between >50% SUVmax volume and low entropy region is as high as 84% with a mean of 65%±12 and the DSC is 75% with a mean of 60%±11.

The potential clinical application of our proposed method in radiotherapy dose painting is demonstrated. Three dose painting plans based on i) CT tumour image heterogeneity, ii) tumour 18F-FDG uptake distribution and ii) the combination of both were created. The dose painting plans were compared to the current standard of uniform dose plan and against each other. The results demonstrated the feasibility of dose escalation in advanced NSCLC conforming to normal tissue clinical constraints where the difference in dose received by the organs at risk (OARs) in the created plans were not
statistically significant. Moreover, the results showed the possibility of covering the 95% of the high 18F-FDG uptake regions within the tumour with 95% of the prescribed dose when planning based on intratumoural heterogeneity measured from CT images.

Keywords: Texture Analysis, Radiotherapy, Dose Painting.

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## Table of contents

List of figures · xiii  
List of tables · xxi  

### Chapter 1 - Introduction and Background · 1

#### 1.1 Introduction · 1  
#### 1.2 External Beam Radiotherapy · 3

- 1.2.1 Radiotherapy Treatment for Lung Cancer · 3  
#### 1.3 The Main Steps of Radiotherapy Treatment · 6

- 1.3.1 Imaging and Volume Definition · 6  
- 1.3.2 Treatment Planning · 6  
- 1.3.3 Treatment Delivery · 10  
#### 1.4 Imaging for Radiotherapy · 14

- 1.4.1 Anatomical Imaging · 14  
- 1.4.2 Functional Imaging · 19  
#### 1.5 Dose Painting · 22  
#### 1.6 Texture Analysis · 26

- 1.6.1 Texture Analysis in CT · 30  
- 1.6.2 Texture Analysis in PET · 33  
- 1.6.3 Texture Analysis in MRI · 35  
- 1.6.4 Challenges in Texture Based Radiotherapy Planning · 35
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7</td>
<td>Radiomics</td>
<td>38</td>
</tr>
<tr>
<td>1.8</td>
<td>Conclusions</td>
<td>38</td>
</tr>
<tr>
<td>1.9</td>
<td>Thesis Overview</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>First-order Statistics Texture Analysis for Survival Prediction</td>
<td>43</td>
</tr>
<tr>
<td>2.1</td>
<td>Introduction</td>
<td>43</td>
</tr>
<tr>
<td>2.2</td>
<td>Feasibility Study</td>
<td>44</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Methods</td>
<td>44</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Results</td>
<td>50</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Discussion and Conclusions</td>
<td>59</td>
</tr>
<tr>
<td>2.3</td>
<td>Validation study</td>
<td>60</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Methods</td>
<td>60</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Results</td>
<td>61</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Discussion and Conclusions</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Effects of Respiratory Motion on Textural Features: A Phantom Study</td>
<td>67</td>
</tr>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>67</td>
</tr>
<tr>
<td>3.2</td>
<td>Methods</td>
<td>68</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Phantom Design</td>
<td>68</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Experimental Design</td>
<td>70</td>
</tr>
<tr>
<td>3.2.3</td>
<td>Data Acquisition</td>
<td>70</td>
</tr>
<tr>
<td>3.2.4</td>
<td>Data Analysis</td>
<td>70</td>
</tr>
<tr>
<td>3.3</td>
<td>Results</td>
<td>81</td>
</tr>
<tr>
<td>3.3.1</td>
<td>The Modulation Transfer Function of The CT Scanner</td>
<td>81</td>
</tr>
<tr>
<td>3.3.2</td>
<td>The Modulation Transfer Function of The In-house Implemented LoG Filters</td>
<td>84</td>
</tr>
<tr>
<td>3.4</td>
<td>Discussion and Conclusions</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Methodology For Optimised Volumetric Voxel Based Texture Mapping</td>
<td>93</td>
</tr>
</tbody>
</table>
Table of contents

4.1 Introduction ......................................................... 93
  4.1.1 Second Order Statistics Texture Analysis ......................... 93
4.2 Volumetric Voxel-based Texture Mapping ............................. 97
  4.2.1 Voxel Based GLCM .............................................. 97
  4.2.2 Feature Extraction .............................................. 98
  4.2.3 Quantisation of Tumour Image Information ....................... 98
4.3 Code Development .................................................. 102
4.4 Discussion ....................................................... 102
4.5 Conclusions .................................................... 103

5 CT Volumetric Voxel Based Texture Mapping and 18F-FDG Uptake Distributions 107

5.1 Introduction ....................................................... 107
5.2 Methods .......................................................... 108
  5.2.1 Patients Cohort ................................................ 108
  5.2.2 Image Acquisition ............................................. 109
  5.2.3 Region of Interest Segmentation ................................ 110
  5.2.4 Comparison with 18F-FDG Uptake Distributions .................. 113
5.3 Results ............................................................ 114
  5.3.1 Image Registration ............................................. 114
  5.3.2 Tumour Image Quantisation .................................... 116
  5.3.3 3D Voxel Based Texture Maps .................................. 119
  5.3.4 Comparison with 18F-FDG Distribution ........................ 124
  5.3.5 Registration and 3D-VTMs ..................................... 130
5.4 Discussion ....................................................... 131
5.5 Conclusion .................................................... 132

6 Radiotherapy Planning Based on Functional Imaging and Texture Analysis 133

6.1 Introduction ....................................................... 133
# Table of contents

6.2 Methods ................................................................. 135  
6.2.1 Patients Data ....................................................... 135  
6.2.2 Treatment Planning Study ........................................... 136  
6.2.3 Analysis of The Plans ............................................... 144  
6.3 Results ................................................................. 145  
6.3.1 Target Volumes ...................................................... 145  
6.3.2 Treatment Planning Study .......................................... 148  
6.4 Discussion ............................................................. 165  
6.5 Conclusions ............................................................ 167  

7 Discussion, Conclusions and Future Work 169  
7.1 Overview ............................................................... 169  
7.2 Textural Features as Biomarkers for Patient Survival ................. 169  
7.3 Spatial Measurement on Intratumoural Heterogeneity ................. 170  
7.3.1 Texture Filter Design ................................................ 170  
7.3.2 Image Quantisation .................................................. 171  
7.3.3 Choice of Extracted Feature ....................................... 173  
7.4 Intratumoural Heterogeneity and Tumour Function ..................... 175  
7.5 Clinical Applications .................................................. 175  
7.6 Conclusions ............................................................ 177  
7.7 Future work ............................................................ 178  

References 181  

Appendix A FiNiTe Project Protocol 195  

Appendix B B-spline Deformable Registration 207  

Appendix C List of Publications 213
List of figures

1.1 An example of a modern linear accelerator showing the main parts of the linac [146]. 4

1.2 A schematic illustration of the branching of the bronchus which is known as the bronchial tree [26]. 5

1.3 A schematic illustration of the target volumes in radiotherapy. 7

1.4 A radiotherapy plan showing the isodose lines superimposed on the CT images in three views. The green region is the PTV. The orange isodose line represent the volume that is covered by the 95% of the prescribed dose. The top view is the axial image, the bottom right in the sagittal image and the bottom left is the coronal image. 8

1.5 The survival fraction curve for early responding tissue (tumour tissue) and late responding tissue (normal tissue) [105]. 12

1.6 The phases of cell cycle [110]. 13

1.7 The Fourier Slice Theorem. The Fourier Slice Theorem described the relationship between an image and its views in the frequency domain. In the spatial domain, each view is found by integrating the image along rays at a particular angle. In the frequency domain, the spectrum of each view is a one-dimensional "slice" of the two-dimensional image spectrum [123]. 16

1.8 The geometry of Radon transform [1]. The angle between the projection line and the x-axis is $\theta$. 17

1.9 The main steps in filtered back projection [1]. 19

1.10 Imaging principle of PET: (a) The annihilation of a positron and an electron; (b) The line of response (LOR) [44]. 20
2.1 The in-house implemented LoG filters (a) LoG2 which is \( 4 \times 4 \text{mm}^2 \) with \( \sigma = 1.0 \), (b) is LoG3 which is \( 6 \times 6 \text{mm}^2 \) with \( \sigma = 1.5 \), (c) is LoG4 which is \( 8 \times 8 \text{mm}^2 \) with \( \sigma = 1.8 \), (d) is LoG5 which is \( 10 \times 10 \text{mm}^2 \) with \( \sigma = 2.0 \), (e) is LoG6 which is \( 12 \times 12 \text{mm}^2 \) with \( \sigma = 2.5 \). 51

2.2 An example of the TexRAD output for one of the investigated patient where (A) shows the delineated tumour. (B) Is the tumour filtered image using filter ssf2 (fine scale). (C) Is the tumour filtered image using filter ssf4 (medium scale). (D) Is the tumour filtered image using filter ssf6 (coarse scale). 52

2.3 An example of the output for the in-house implemented LoG filters for one of the investigated patient where (A) shows the segmented tumour. (B) Is the tumour filtered image using filter LoG2 (fine scale). (C) Is the tumour filtered image using filter LoG4 (medium scale). (D) Is the tumour filtered image using filter LoG6 (coarse scale). 53

2.4 Survival as a function of entropy for TexRAD filters and corresponding filters developed in-house. (a) Fine scale filter (\( 4 \times 4 \text{mm}^2 \)) results. (b) Medium scale filter (\( 6 \times 6 \text{mm}^2 \)) results. (c) Coarse scale filter (\( 12 \times 12 \text{mm}^2 \)) results. 54

2.5 An example of the calculated ratio between entropy values measured from TexRAD filters (\( \text{Entropy}_{\text{TexRAD}} \)) to entropy values measured from the corresponding LoG filters (\( \text{Entropy}_{\text{LoG}} \)) for the medium size filter (ssf3). 55

2.6 Bland Altman (difference plot) for TexRAD filters and corresponded filters generated in-house. (a) Fine scale filter (\( 4 \times 4 \text{mm}^2 \)). (b) Medium scale filter (\( 6 \times 6 \text{mm}^2 \)). (c) Coarse scale filter (\( 12 \times 12 \text{mm}^2 \)). 57

2.7 Kaplan-Meier curves generated for normalised entropy values measured after applying TexRAD filters where group 1 refers to low normalised entropy (\( < \text{threshold of 1.233} \)) and group 2 refers to high normalised entropy (\( > \text{threshold of 1.233} \)) (a) results for using filters developed in-house (b) results from using TexRAD software. 58

2.8 Kaplan-Meier curves generated for normalised entropy values, group 1 refers to low normalised entropy (\( < 1.233 \)) and group 2 refers to high normalised entropy (\( > 1.233 \)). (a) Using the median entropy value as a threshold. (b) Using the previously published \([153]\) threshold of 1.233. 62

2.9 (A) Tumour CT image with low normalised entropy and associated with poor survival group while (B) is a tumour CT image with high normalised entropy and associated with good survival group. (1) Is the filtered tumour image using small-sized filter. (2) Is filtered tumour image using medium-sized filter. (3) Is filtered tumour image using larger-sized filter. 63
3.1 The MUSS phantom design. The phantom has four regions that can be filled with contrast agent to exhibit a desired pattern of high and low contrast regions.

3.2 The MUSS phantom solution filling arrangement showing in red. The filling arrangement was designed to produce a bar pattern to conduct the MTF measurements.

3.3 (a) The Experimental Set-up. (b) A Close-up of the MUSS phantom during the experiment.

3.4 A schematic illustration of MTF calculation using the edge method.

3.5 An illustration of the ROI regions and Bg region used to calculate the $MTF_{LoG+scanner}$ for the in-house implemented LoG filters.

3.6 The re-sampled CT image of the MUSS phantom for the static acquisition.

3.7 The re-sampled CT image of the MUSS phantom for the motion acquisition.

3.8 The ERF of the static and the moving acquisition of the MUSS phantom.

3.9 The LSF of the static and the moving acquisition of the MUSS phantom. PDF is the probability density function.

3.10 $LSF_{Fitted\_static}, LSF_{Fitted\_motion}$ and $LSF_{Predict\_motion}$. PDF is the probability density function.

3.11 The MTF measured from the static and the moving acquisition of the MUSS phantom.

3.12 The static phantom CT images filtered by the LoG filters. (a) Image filtered with LoG2. (b) Image filtered with LoG3. (c) Image filtered with LoG4. (d) Image filtered with LoG5. (e) Image filtered with LoG6. Refer to table (3.1) for filter sizes.

3.13 The moving phantom CT images filtered by the LoG filters where the direction of motion is top to bottom. (a) Image filtered with LoG2. (b) Image filtered with LoG3. (c) Image filtered with LoG4. (d) Image filtered with LoG5. (e) Image filtered with LoG6. Refer to table (3.1) for filter sizes.

3.14 MTF for the LoG filters in static using the FT and SD methods. (a) MTF of LoG2, (b) MTF of LoG3, (c) MTF of LoG4, (d) MTF of LoG5, (e) MTF of LoG6.

3.15 MTF for the scanner and the LoG filters in static and in presence of respiratory motion. (a) MTF of LoG2, (b) MTF of LoG3, (c) MTF of LoG4, (d) MTF of LoG5, (e) MTF of LoG6.

3.16 The limiting resolution quoted in terms of MTF50 for the scanner and the LoG filters during static and the moving acquisition.
3.17 The limiting resolution quoted in terms of MTF10 for the scanner and the LoG filters
during static and the moving acquisition. ................................. 91

4.1 Schematic diagram of coordinates planes and 2D displacement vectors. ............... 94
4.2 Schematic diagram showing all 13 possible direction of connecting voxels. .......... 95
4.3 Schematic diagram showing angles between spherical polar coordinates. .......... 95
4.4 A flow chart of our proposed methodology. ................................ 105

5.1 (a) Fused 18F-FDG PET/CT image showing a hypoxic/necrotic region within the
tumour. (b) The corresponding CT image. (c) The corresponding 18F-FDG PET image. 110
5.2 The work flow and results of the deformable registration steps between PET/CT and
CTPlan for a patient from our cohort (Patient 6). .......................... 112
5.3 Difference image between the static and the registered image. ....................... 115
5.4 An example of tumour image normalised histograms at different quantisation levels N.
(a) Is the normalised histogram using the original intensity range within the tumour
without quantisation. (b) Is the normalised histogram using 8 quantisation levels.
(c) Is the normalised histogram using 16 quantisation levels. (d) Is the normalised
histogram using 32 quantisation levels. (e) Is the normalised histogram using 64
quantisation levels. (f) Is the normalised histogram using 128 quantisation levels. (g)
Is the normalised histogram using 256 quantisation levels. ........................ 117
5.5 An example of tumour image normalised histograms at different quantisation levels N.
(a) Is the normalised histogram using the original intensity range within the tumour
without quantisation. (b) Is the normalised histogram using 8 quantisation levels.
(c) Is the normalised histogram using 16 quantisation levels. (d) Is the normalised
histogram using 32 quantisation levels. (e) Is the normalised histogram using 64
quantisation levels. (f) Is the normalised histogram using 128 quantisation levels. (g)
Is the normalised histogram using 256 quantisation levels. ........................ 118
5.6 An example of a 2D slice from the produced 3D-VTM using the UQ method for
Patient 10 with different quantisation levels N. ............................ 120
5.7 An example of a 2D slice from the produced 3D-VTM using the LMQ method for
Patient 10 with different quantisation levels N. ............................ 121
5.8 An example of a 2D slice from the produced 3D-VTM using the LMQ method for
Patient 10 at different filter sizes. ............................................. 122
5.9 18F-FDG PET/CT images for three patients are shown in (a), (b) the produced texture map using the uniform quantisation, (c) the produced texture map using the proposed LMQ method, (d) The 18F-FDG uptake distribution within the GTV. Images displayed using computational environment for radiotherapy research (CERR) and itk-snap software [43][163]. .......................................................... 123

5.10 (a) The Overlap Fraction between regions of zero entropy within the GTV and SUV for different SUV% thresholds. b) The DSC between regions of zero entropy within the GTV and SUV for different SUV% thresholds. Solid lines are patients with homogeneous 18F-FDG distribution within tumours while dashed lines are patients with heterogeneous necrotic tumours. .......................................................... 125

5.11 Two examples of 3D-VTM performance from Patient 10 PET/CT scan (A) Demonstrates the performance of the 3D-VTM in the case of necrotic tumour tissue. (B) Illustrates the implication of including normal homogeneous structures within the GTV. (1) Is the CT<sub>PET</sub> image, (2) is the 18F-FDG uptake distribution, and (3) is the produced texture map using the proposed LMQ method. .......................................................... 127

5.12 The 3D representation of the GTV, zero entropy volume and SUV≥50% volume demonstrating the overlap between them for patients characterized with a homogeneous 18F-FDG uptake. .......................................................... 128

5.13 The 3D representation of the GTV, zero entropy volume and SUV≥50% volume demonstrating the overlap between them for patients characterized with a heterogeneous 18F-FDG uptake. .......................................................... 129

6.1 The Bronchial tree as delineated on the planning CT. The axial view is shown on the left, the sagittal view is shown on the top right and the coronal view is shown on the bottom right. .......................................................... 140

6.2 An example of the GTV<sub>primary</sub>, GTV<sub>entropy</sub> and GTV<sub>FDG</sub> for a patient from our investigated cohort (Patient 3). .......................................................... 145

6.3 An example of a dose distribution from the four created radiotherapy plans for Patient 7 from the investigated cohort. (a) Is the uniform dose plan. (b) Is the Boost<sub>entropy</sub> Plan. (c) Is Boost<sub>FDG</sub> Plan. (d) Is the Boost<sub>entropy+FDG</sub> Plan. The axial view is shown on the left, the sagittal view is shown on the top right and the coronal view is shown on the bottom right. .......................................................... 148

6.4 An example of the OARs DVH from the four treatment plans for Patient 7 from our investigated cohort. .......................................................... 149
6.5 The volume of the normal lung that is receiving 5Gy (V5) in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume. 150

6.6 The volume of the normal lung that is receiving 10Gy in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume. 151

6.7 The volume of the normal lung that is receiving 20Gy in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume. 152

6.8 The volume of the heart that is receiving 60Gy (V60) in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume. 153

6.9 The maximum dose to 0.5 cm$^3$ of the oesophagus in the four treatment plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance dose. 154

6.10 An example of a DVH from the four created radiotherapy plans for Patient 5 from the investigated cohort. 155

6.11 The dose received by 95% of the PTV$_{primary\_Uncrop}$ and PTV$_{primary}$ volume in the uniform dose plan. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose. 156

6.12 The dose received by 95% of the PTV$_{entropy}$ volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose. 158

6.13 The dose received by 95% of the PTV$_{FDG}$ volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose. 159
6.14 The dose received by 95% of the PTV_{entropy+FDG} volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b)Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose. .................................................. 160

6.15 The dose received by 95% of the GTV_{FDG} volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b)Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose. .................................................. 161

6.16 The maximum dose to 0.5cm³ of the bronchial tree in the four treatment plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b)Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance dose. .................................................. 163

7.1 (a) A follow-up CT image of the thorax three years post treatment. (b) The corresponding 3D-VTM for the whole lung. (c) The corresponding 3D-VTM with incorporating the HU information showing the tumour recurrence as a high intensity region (white). 179
List of tables

1.1 First-Order Texture Features: Based on average pixel value $i$ where $N$ is the number of grey levels in the histogram. Intensity histogram analysis. ........................................ 27
1.2 Second-Order Texture Features: Based on grey level co-occurrence matrices. .... 28
1.3 High-Order Texture Features: Based on neighbourhood grey tone difference matrices. 29

2.1 Size of the TexRAD Laplacian of Gaussian Filters. .............................................. 45
2.2 Summary of regression analysis and correlation significance between entropy values and survival for all TexRAD filters. ................................................................. 56
2.3 Receiver operating characteristics (ROC) analysis results with p-value for survival analysis using Kaplan-Meier curves for TexRAD filters notated as ssf and in-house implemented filters notated LoG. The median entropy value was used as the threshold value for the KM analysis. (AUC = area under the curve) ............................................. 59
2.4 p-value results from Log-rank test on KM curves using two different threshold .... 61

3.1 Size of the in-house implemented LoG filters where $\sigma$ is a scaling factor related to the smoothing effect of the filter. ................................................................. 77
3.2 A list definitions of ERF, LSF and MTF abbreviations used in this study. .......... 80

4.1 2D displacement angles in GLCM. ................................................................. 94
4.2 Volumetric angle displacement in 13 possible directions in GLCM calculation. .... 96

5.1 Patients characteristics of the group of ten patients investigated in this study. The patients are divided into two sub-groups; those diagnosed with non-necrotic tumours (1-5) and those whose tumours were necrotic (6-10), each sub-group was ordered by tumour size. (SCC=squamous cell carcinoma, Adeno=adenocarcinoma) .......................... 109
5.2 Dice Similarity Coefficient (DSC) for Patient 8. ........................................ 130
5.3 Overlap Fraction (OF) for Patient 8. ......................................................... 130

6.1 Patients characteristics of the group of ten patients investigated in this study. The patients are divided into two sub-groups; those diagnosed with non-necrotic tumours (1-5) and those whose tumours were necrotic (6-10), each sub-group was ordered by tumour size. (SCC=squamous cell carcinoma, Adeno=adenocarcinoma) ......... 135
6.2 The dose constraints to organs at risk used in this study. .......................... 137
6.3 List of definitions of GTV, PTV and treatment plans abbreviations used in this study. ................................................................. 143
6.4 The GTV size for the ten investigated patients. For definitions of the listed GTV structures, please refer to table (6.3). The results for the mean±SD are quoted in significant digits. ......................................................... 146
6.5 The PTV size for the ten investigated patients. For definitions of the listed PTV structures, please refer to table (6.3). The results for the mean±SD are quoted in significant digits. ......................................................... 147
6.6 Sizes of the PTV_{entropy} in cm³ and the size after cropping away from the BT. PTV_{entropy} C_{BT} %Vol = the percentage cropped volume from PTV_{entropy}. The results for the mean±SD are quoted in significant digits. ......................................................... 164
6.7 Sizes of the PTV_{FDG} in cm³ and the size after cropping away from the BT. PTV_{FDG} C_{BT} %Vol = the percentage cropped volume from PTV_{FDG}. The results for the mean±SD are quoted in significant digits. ......................................................... 164
6.8 Sizes of the PTV_{entropy+FDG} in cm³ and the size after cropping away from the BT. PTV_{entropy+FDG} C_{BT} %Vol = the percentage cropped volume from PTV_{entropy+FDG}. The results for the mean±SD are quoted in significant digits. ......................................................... 165
Chapter 1

Introduction and Background

1.1 Introduction

Radiotherapy has seen rapid development in the last few decades with delineation of target volumes becoming more precise, due to improvements in medical imaging modalities [117]. In the field of radiation oncology, medical images are used to diagnose, stage, plan and assess the response of tumours to treatment [117]. Today, using computed tomography (CT) to guide radiotherapy planning is considered to be the gold standard. In addition to its high image resolution (≈1mm), radiotherapy treatment planning systems (TPS) rely on Hounsfield units (HU) obtained from CT for dose calculations. Yet, CT based radiotherapy planning relies on qualitative information from CT images where quantitative measurements are usually confined to determining tumour size in three dimensions. Even though tumours have been shown to be biologically heterogeneous, radiotherapy planning is still often based on TNM staging. The TNM system is used to stage cancer: a letter and a number are assigned to each letter in the TNM system, where T stands for the tumour, referring to its size; N stands for node and represents the status of lymph node involvement; and M is for metastasis, providing information on whether the cancer has spread to other parts of the body [26]. Given the increased evidence of intra-tumoural heterogeneity and the observed diversity of patients’ response to treatment, the field of oncology has been striving for personalized treatment, based on prognostic information of a tumour’s response to treatment.
In recent years, nuclear medicine imaging, especially positron emission tomography (PET) has been increasingly utilised in the field of oncology as a source of quantitative measurements regarding tumour biology. Fluoro-deoxy-glucose labeled with flourine-18 (18F-FDG) PET imaging has probably made a greater contribution to radiotherapy planning in regards to assessing response to treatment than other functional imaging modalities including functional magnetic resonance imaging (fMRI). The wide availability, high sensitivity and the relatively well established protocols and quantification methods of 18F-FDG PET have led to its subsequent popularity in the field of oncology [112]. Even though several studies have reported 18F-FDG PET to be a predictor for a tumour’s response to treatment [23][64][158][19][109][65][81][144][111][63], the standardised uptake value (SUV) remains semi-quantitative, challenged by changes in tumour volume, uptake time and kinetics of the uptake and subsequently the distribution of the tracer [63][78].

Limitations in existing imaging modalities and the concept that radiological images hold more information than is being utilised has led to increased interest in the field of radiomics. Radiomics refers to quantitative feature extraction from radiological images and their use to generate meaningful data [85]. In cancer therapy, assessing tumour heterogeneity in relation to survival by extracting textural features has emerged in the past few years as a potentially useful tool. Algorithms have been proposed in the literature to increase the predictive power of existing imaging modalities by extracting tumour textural features based on intensity values. The findings showed that tumour textural heterogeneity correlates with treatment outcome and overall patient survival [10]. Yet, the field suffers from a lack of a standardised approach to perform texture analysis leading to confounding results. Image quantisation is one of the crucial factors in extracting textural information and a validated standardised method has not been established in the published literature. Moreover, texture analysis is challenged by the limited clinical validation and the unclear physiological mechanism underpinning such findings [10]. Even though the proposed methodologies are still in their early stages of development, suffering from expected shortcomings and faced with various challenges, the presented results are promising. The benefits of employing a quantitative post-processing algorithm on routinely acquired patients images to predict response to treatment or to provide meaningful information about tumour function, if established to be valid, would be numerous.

In this chapter, I present an overview of the main concepts in the field of radiotherapy including the clinical process of radiotherapy treatment and treatment planning techniques. An overview of CT
1.2 External Beam Radiotherapy

Radiotherapy is one of the main treatment modalities in the field of oncology. In radiotherapy, ionising radiation is used to kill malignant cells. Cell killing is caused by DNA damage to the cancer cells. The aim of radiotherapy is to deliver a radiation dose as high as possible to the tumour while minimising the radiation dose to the surrounding healthy tissue to minimise tissue damage [128]. In external beam radiotherapy (EXBRT) or teletherapy, a linear accelerator (LINAC) is used to generate a beam of ionising radiation (photon or electron) of energy range of typically 4-20 Megavoltage (MeV) [132]. The radiation output from the LINAC is delivered in terms of monitor units (MU), 1 MU from the LINAC is measured as 1cGy (Gy is the unit for radiation dose as absorbed in unit mass) using a monitor chamber in a 10x10 cm\(^2\) field size at a depth point in a water phantom [105].

The therapeutic use of X-rays started with the use of the conventional X-ray machines with kilovoltage energies treating only superficial lesions. With the advances in technology, the modern linear accelerators are able to deliver higher energies of typically 4-20 MeV and treat deeply located tumours. Furthermore, most modern LINACS are equipped with an image guiding system based on cone beam CT (CBCT) to obtain an image of the patient before delivering the treatment allowing precise treatment delivery. An example of a modern linear accelerator is presented in figure (1.1).

1.2.1 Radiotherapy Treatment for Lung Cancer

Lung cancer is the second most common cancer in men and women and is the leading cause of cancer mortality in the UK [26]. Based on the pathology, lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). In the UK, SCLC comprises 12% of lung cancer diagnosis while NSCLC comprises 87%. For inoperable non-small cell lung cancer (NSCLC), the treatment of choice is a combination of chemotherapy and radiotherapy. For early stage inoperable peripheral NSCLC, the treatment of choice is stereotactic ablative radiotherapy (SABR). SABR is an external beam radiotherapy technique where accelerated schedules (known as hypofractionation)
Fig. 1.1 An example of a modern linear accelerator showing the main parts of the linac [146].
1.2 External Beam Radiotherapy

Fig. 1.2 A schematic illustration of the branching of the bronchus which is known as the bronchial tree [26].

for delivering the dose is employed: 3 fractions of 18Gy, 5 fractions of 12Gy or 8 fractions of 7.5Gy [74]. This technique requires high precision in tumour localisation in order to reduce the uncertainty and allow a delivery of a very high radiation dose to the tumour. Tumour images are acquired before each session in SABR to ensure an accurate delivery of the dose. The local control for patients treated with SABR has been reported to be as high as 90% at 3 years [28]. However, SABR treatment is only the standard treatment for peripheral tumours not central lung tumours. Central lung tumours are defined as tumours within 2cm of the region surrounding the branching of the bronchus, known as the bronchial tree as shown in figure (1.2). A high radiation dose to the bronchial tree has been reported to cause high grade toxicity in patients treated with SABR for central tumours [28] [89].

Thus, for inoperable central NSCLC, the treatment of choice is the conventional chemoradiotherapy treatment with a dose of 64Gy delivered in 32 fractions. The prognosis of advanced inoperable NSCLC treated with conventional EXBRT is as low as 10%. 
1.3 The Main Steps of Radiotherapy Treatment

The delivery of radiotherapy treatment involves three main steps; imaging and volume definition, treatment planning and treatment delivery. These steps will be discussed in the following sections.

1.3.1 Imaging and Volume Definition

Imaging is the first step in radiotherapy and it is fundamental in ensuring accurate treatment planning and delivery. In imaging, the patient position is decided taking into account the treatment area. For example, lung patients are usually positioned supine (lying facing upwards) with their arms up away from the treatment area. Reference marks or tattoos are used to ensure position reproducibility through the treatment course. During this step, patient tumour images are acquired to be used for treatment planning. Currently, the treatment planning and dose calculations are based on CT images, thus a planning CT scan is acquired for the patient in the treatment position [79].

For volume definition, the radiation oncologist contours the tumour and the critical structures known as organs at risk (OAR) on the obtained CT images. The tumour is delineated as a part of three volumes; the gross tumour volume (GTV), the clinical target volume (CTV) and the planning target volume (PTV). GTV is the radiologically visible tumour while the CTV includes the visible tumour and the sub-clinical microscopic extension of the malignant disease. Although a margin of $\approx 5\text{mm}$ around the GTV is often used to countour the CTV, the CTV can be adjusted based on clinical judgment. To account for set-up uncertainties, internal organ motion and patient movement, a margin of typically $1-2\text{cm}$ is added to the CTV to create the planning target volume (PTV) [2][76]. Alternatively, an internal margin (IM) can be added to the CTV to compensate for physiological variations due to internal organ movement; this volume is known as the internal target volume (ITV). The PTV is then grown from the ITV with a margin designed to account for set up uncertainties and patient movement only. A schematic illustration of the target volumes is presented in figure (1.3).

1.3.2 Treatment Planning

In this step, the planning CT scan and the contoured structures are transferred into the treatment planning system (TPS). In the computerised TPS, radiation fields are arranged in a way that delivers
the prescribed dose to the PTV while keeping the normal tissue dose within the acceptable limit. In 3D conformal radiotherapy, the TPS sets the radiation fields based on the 3D projection of the PTV in the direction of the radiation beam. These fields are physically achieved by the programmable multi-leaf collimator (MLC) component of the linac. The MLC is a device that is attached to the linac’s gantry head and it is made of "leaf" pairs. The leaves are made of a high atomic number material mostly tungsten. Modern linacs could have 80-160 leaves with a typical leaf width of 5\text{mm}. The leaves are programmed to produce the radiation field. The beams angles are determined in a way that the overlap between the PTV and the surrounding tissue is minimal [79].

The dose deposition in tissue is calculated based on the electron density inferred from the CT numbers. The delivered dose is visualised by isodose line; line connecting points receiving the same dose; those lines are superimposed on the CT images. To measure the dose received by each structure volume, dose volume histograms (DVH) are generated. Figure (1.4) demonstrates an example of a typical 3D plan for lung cancer showing the target volume and the superimposed isodose lines [79].
Fig. 1.4 A radiotherapy plan showing the isodose lines superimposed on the CT images in three views. The green region is the PTV. The orange isodose line represent the volume that is covered by the 95% of the prescribed dose. The top view is the axial image, the bottom right in the sagittal image and the bottom left is the coronal image.
1.3.2.1 Intensity Modulated Radiotherapy

3D conformal radiotherapy has the ability to shape the dose distribution yet it is limited by the high dose regions caused by the overlapping of the conformal beams. To overcome this issue, in the late 1990s intensity modulated radiotherapy (IMRT) was introduced. IMRT allows the dose to vary within the treatment field to better shape the dose distribution preventing high overlap regions. In IMRT, the radiation fluence is modulated across the beam in such a way it conforms to the 3D shape of the target while keeping the dose to normal tissue to a minimum. This is achieved by using multiple non-uniform beams to deliver a uniform dose to the target by either using multiple static fields with different shapes and angles (step-and-shoot technique) or by dynamically changing the MLC leaves while irradiating (sliding window technique) [105].

In IMRT planning, the directions, shapes and weights of the beams are optimised in such a way that the combination of the non-uniform beams will add up to a uniform dose distribution within the target volume. Due to the complexity of the task at hand, this optimisation is achieved using inverse planning. In inverse planning, firstly the planning objectives of dose constraints for normal tissues and target volume are set. An iterative optimisation algorithm is implemented to adjust the beams parameters to generate a dose distribution that conforms to the desired objectives. The agreement between the set objectives and the generated dose distribution is assessed by an objective function. The desired solution to this optimisation problem is a dose distribution that minimises the objective function [79][105].

IMRT has wide applications in most aspects of radiotherapy because of its ability to create multiple targets and multiple avoidance structures allowing the treatment of different targets simultaneously to different doses. By delivering radiation with greater precision, IMRT has been shown to minimise acute treatment-related morbidity, making dose escalation feasible which may ultimately improve local tumour control [105].

1.3.2.2 Volumetric Modulated Arc Therapy

Even though IMRT has a huge impact on the precision of treatment planning and improvement of local control, it suffers from challenging limitations. IMRT treatment planning and quality assurance is more complex and requires more time. The longer treatment sessions of IMRT compromises
patients comfort and the stability of their position. Furthermore, IMRT requires more monitor units to be delivered from the linac which raises concerns about exposure to low dose radiation with risks of radiation-induced malignancy. Volumetric Modulated Arc Therapy (VMAT) is a form of an IMRT treatment that has been introduced recently to overcome some of these limitations. In VMAT, the intensity modulated radiotherapy is delivered while the gantry is dynamically rotating around the patient. VMAT benefit over IMRT is the reduction of treatment time, increasing treatment efficiency and the reduction of monitor units [129]. The treatment planning process used in VMAT is similar to that of IMRT.

For advanced lung tumours, the reduction in treatment time offered by VMAT could be beneficial in regards to minimising the intrafraction respiratory motion effects on treatment delivery [129]. Moreover, for advanced lung cancer treatment, a study by Jiang et al. 2011 [75] reported that using partial arcs in VMAT showed more sparing (lower dose) to normal lung tissue than using a single arc or IMRT.

1.3.3 Treatment Delivery

The course of radiotherapy treatment is delivered over a number of sessions known as fractions. Fractionation is a significant principle in radiotherapy and it is based on the five R’s of radiobiology.

1.3.3.1 The Five R’s of Radiobiology

Fractionation is mostly governed by five principles known as the five R’s of radiobiology or radiotherapy; repair, reoxygenation, repopulation, redistribution and radiosensitivity.

**Repair** refers to post irradiation cell damage repair, and it is the basis of fractionation in radiotherapy. In the linear quadratic (LQ) theory, the cell will be inactivated due to DNA damage caused by ionising radiation. The cell kill is caused by two components: a component where the kill is proportional to the total dose $D$ (linear) and a component that is proportional to the square of the dose $D^2$ (quadratic). The linear component is represented by $\alpha D$ and the quadratic components is represented by $\beta D^2$. In these terms, $\alpha$ and $\beta$ are constants where $\alpha$ accounts for the irreparable cell damage and $\beta$ for repairable damage. The probability of cell surviving ($S$) an irradiation event can be expressed by Poisson statistics as shown in equation (1.1).
1.3 The Main Steps of Radiotherapy Treatment

\[ S = \exp(-\alpha D - \beta D^2) \]  

(1.1)

The ratio \( \alpha/\beta \) gives the dose when the linear and quadraic components are equal. By taking the log of \( S \), \( \alpha D = \beta D^2 \) and consequently \( D = \alpha/\beta \). Having a high \( \alpha/\beta \) (5-10Gy) indicates cells with low capability of repairing sublethal damage which is a characteristic of tumour cells. While a low \( \alpha/\beta \) (1-4Gy) indicates cells with the ability to repair sublethal damage which is a characteristic of normal tissue. This difference between tumour cells and normal tissues is the rationale for fractionation. The survival curves in figure (1.5) shows the crossover between the two types of tissue is around 5Gy, meaning, a delivery of a dose higher than that will cause damage to the normal tissue. Yet, a higher dose is needed to kill tumour cells thus, a higher radiation dose is delivered in low dose fractions. This leaves enough time for normal tissue to repair the sublethal damage while the sublethal damage of tumour cells will accumulate over fractions. Based on clinical data, 3Gy is an accepted value for \( \alpha/\beta \) for many normal tissue and 10Gy \( \alpha/\beta \) for typical tumours, yet these values vary between tissue and tumour types. The LQ model is the most used clinically since it is shown to fit experimental data and works well for low doses (<10Gy per fraction). Yet, the accuracy of the LQ model at high doses is debatable [79].

**Reoxygenation**, oxygen is a known radiosensitiser thus cells that are oxygen deprived (hypoxic) are more resistant to radiation. It has been reported in the literature that a large proportion of tumours are hypoxic, this leads to the oxygenated tumour cells being destroyed first hence increasing the surviving fraction of hypoxic tumour cells. In-vivo experiments showed that allowing time for tumour cells to reoxygenate decreased the numbers of the hypoxic cells making the tumour more sensitive to radiation [79].
Fig. 1.5 The survival fraction curve for early responding tissue (tumour tissue) and late responding tissue (normal tissue) [105].
1.3 The Main Steps of Radiotherapy Treatment

Repopulation, tumour cells repopulate much faster than normal tissue, thus the treatment fractionation should be scheduled in a way that will allow the normal tissue to repair while minimising the tumour repopulation effect [79].

Redistribution, cell division happens in a cycle that has four phases as explained in figure (1.6). Studies have shown cells to be more sensitive to radiation in the G2 and mitotic phase while being most resistant at the synthesis phase. Redistribution benefits can be achieved if the radiation dose was delivered when the tumour cells are in the mitotic phase when they are most sensitive. Yet, this is usually not accounted for when designing fractionation since it is not practically obtainable [79]. However, treating at several different time-points increases the probability of catching each cell at it most sensitive at some point during the treatment course.

Radiosensitivity refers to the intrinsic radiosensitivity of cells meaning the ability of a cell to sustain radiation damage. Tissues vary in their ability to reproduce; cells with high division and metabolic rate that are non-specialised, such as lymphoid, bone marrow and reproductive (ovaries and
testes) organs are most intrinsically radiosensitive, while highly specialised tissues such as muscle and mature bones are least radiosensitive.

### 1.3.3.2 Image Guided Radiotherapy

In radiotherapy treatment delivery, image guided radiotherapy (IGRT) is usually employed. In IGRT, the patient is imaged before and/or during treatment allowing a precise verification of treatment position and treatment delivery. Most modern linacs are equipped with an image guiding system based on cone beam CT (CBCT). The main difference between CBCT and the conventional diagnostic systems is that CBCT uses a cone radiation beam. The conventional CT scanners uses a fan beam to acquire multiple slices of the patient, while the CBCT images a whole volume in one rotation [127]. Furthermore, CBCT provides the option of using a low energy beam (range of 40-125kVp) compared to the higher energy range used in the conventional CT scanners [8].

Although IGRT ensures the geometric accuracy of treatment delivery and offers the possibility of re-optimising the treatment plans, the increased time on the treatment couch and increased radiation exposure to the patients are the main disadvantages.

### 1.4 Imaging for Radiotherapy

#### 1.4.1 Anatomical Imaging

Following the clinical introduction of CT in the 1970s, radiotherapy treatment has become mainly based on CT images for diagnosis/staging, planning and follow up [40]. Nonetheless, CT images have sub-adequate soft tissue contrast, are negatively affected by the presence of metal implants and expose the patient to ionising radiation. Hence, the use of Magnetic Resonance Imaging (MRI) became a more popular modality in the field of radiotherapy. MRI uses electromagnetic radiofrequency (RF) to image tissues. The proton in the hydrogen nucleus is positively charged and has an intrinsic spin. When the hydrogen atom is placed within a strong magnetic field, the hydrogen proton spin will align with the magnetic field. The MR machine delivers a RF pulse that perturbs the alignment of the hydrogen nuclei. When the RF pulse is suspended, the hydrogen atoms in the human body will start to re-align to the external field (recovery). Hydrogen atoms in different tissues have a different recovery...
1.4 Imaging for Radiotherapy

time and that provides the MRI contrast. MRI has the advantage of using non-ionising radiation and having an excellent soft tissue contrast. However, MRI is costly, not as available as CT and doesn’t provide electron density information needed for radiotherapy planning. Yet, in recent years there has been great interest in integrating MRI into the radiotherapy workflow with the emergence of MRI based planning systems [137]. Currently, CT imaging stays the gold standard for radiotherapy treatment planning.

CT scanners provide anatomical 3D images displayed using Hounsfield Units. Hounsfield Units are obtained by linearly transforming the X-ray attenuation coefficients (\( \mu \)) of tissue based on the \( \mu \) of water as shown in equation (1.2).

\[
HU = 1000 \times \frac{\mu_{\text{tissue}} - \mu_{\text{water}}}{\mu_{\text{water}}}
\]  

(1.2)

The current CT systems are volumetric systems with an X-ray source and multiple detector rows allowing the scanning of an entire volume in seconds. Modern CT scanners are spiral CTs; the X-ray projection takes a spiral path while the table moves axially which allows volumetric fast scanning. The CT system collects the X-ray transmission profiles through the patient from multiple views. The numbers in the CT image matrix are related to the X-ray attenuation of the tissue. Using a filtered back projection (FPB) algorithm, cross-sectional CT images are reconstructed based on the acquired X-ray transmission profiles through the patient. In FPB, the scanned data are converted from the object space into the Radon space in what is known as a sinogram. In the sinogram, each profile projection angle from the spatial domain is represented by one line in the frequency domain known as the Fourier slice theorem [40]. An illustration of the Fourier slice theorem is shown in figure (1.7).

For an image \( f(x, y) \), Radon transform (R) could be simply explained as a series of offset lines integral for each angle in the image as shown in figure (1.8).
Fig. 1.7 The Fourier Slice Theorem. The Fourier Slice Theorem described the relationship between an image and its views in the frequency domain. In the spatial domain, each view is found by integrating the image along rays at a particular angle. In the frequency domain, the spectrum of each view is a one-dimensional "slice" of the two-dimensional image spectrum [123].
Fig. 1.8 The geometry of Radon transform [1]. The angle between the projection line and the x-axis is $\theta$. 
Mathematically, the Radon transform could be expressed as in equation (1.3), where the slope of the line projection is \( p \) and the intercept is \( \tau \).

\[
R(p, \tau) = \int_{-\infty}^{\infty} f(x, px + \tau) dx
\]  (1.3)

The more applicable form of Radon transform using the delta function \( \delta \) is presented in equation (1.4). In equation (1.4), the angle and the offset of the projection line are \( \theta \) and \( r \) respectively.

\[
R(r, \theta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) \delta(x \cos \theta + y \sin \theta - r) dx dy
\]  (1.4)

The sinogram (the output of the Radon transform) is then filtered with a high pass filter (such as ramp filter) to reduce blurring before it is back projected to reconstruct the image. Back projection is done using the inverse Radon transform presented in equation (1.5), where \( f' \) is the filtered data. Figure (1.9) shows the main steps in FBP.

\[
f(x, y) = \int_0^{\infty} f'(x \cos \theta + y \sin \theta) d\theta
\]  (1.5)
1.4 Imaging for Radiotherapy

1.4.2 Functional Imaging

CT imaging provides anatomical and morphological information but given that tumour’s sensitivity to radiation is affected by a number of biological factors (oxygenation level, clonogenic density and rate of proliferation) [150], medical imaging techniques that allow visualisation and mapping of a certain biological or functional process has great potential in the field of radiation oncology. Although some CT imaging technique such as CT perfusion and Blood-oxygen-level dependent contrast magnetic resonance imaging (BOLD-MRI) provides functional information, Positron Emission Tomography (PET) has made a greater contribution to radiotherapy treatment than the other functional imaging modalities.

PET involves injecting the patient with a positron-emitting radiopharmaceutical and detecting the resultant emissions to image a biochemical process. The decaying radiopharmaceutical causes electron- positron annihilations where each annihilation produces 2 gamma rays (each 511 keV and at an angle of 180° from each other). The resultant pair of gamma (γ) rays is detected by two opposing detectors in the PET scanner; this is known as true coincidence. The line along which the event must have occurred is termed line of response (LOR). By combining the parallel LOR’s, a projection of the radioactivity distribution can be acquired and stored in a sinogram. The final sinogram is a collection of counts detected at each angle at a specific radial distance in the field of view (FOV). Figure (1.10) shows an illustration of the imaging principles in PET.
To obtain the final image and quantify the radioactivity concentration from the measured projections, a reconstruction algorithm is applied. The most common reconstruction algorithm for PET images is Order Subsets Expectation Maximization (OSEM). OSEM is an iterative reconstruction method that is an accelerated version of the Maximum Likelihood-Expectation Maximisation (ML-EM) algorithm. The EM is used to find the estimate that will maximise the likelihood that leads to the observed emissions. The ML-EM estimates the annihilation events distribution rather than directly reconstructing the image. The ML-EM starts with initial sets of model parameters regarding the emissions, usually a uniform distribution. The estimated projections are then compared to the measured projections. Based on the ratio between the two, a correction term is calculated and used for the new estimate parameters. This process is repeated until a solution is converged leading to a very slow process. In OSEM, the acquired projections are grouped into subsets based on the projection angles, where the image is updated with each iteration until a solution is converged. The image is updated as many times as the number of the subsets. OSEM has the advantage of faster process time compared to ML-EM algorithm [71].

The acquired PET images are usually displayed superimposed with CT images to complement the functional information with anatomical structure. PET/CT scanners are equipped with a CT component. The PET and CT images are acquired sequentially at the same session and at the same
patient position. More importantly, the acquired CT scan is used for attenuation correction for the PET image. The attenuation correction is needed due to the loss of detection of the annihilated electron-positron $\gamma$-rays true coincidence which can be caused by the absorption of the $\gamma$-rays in the body or their scattering outside the field of view of the detector. This leads to decreased image counts and consequently increased noise, distortion and artefacts in the image. Since attenuation is more likely in the areas deeper in the body, those areas will have decreased activity while regions of low attenuation like the surface of the body will have a higher activity count. This is corrected for by obtaining an attenuation correction map from the CT component of the PET scan by calculating the attenuation coefficients from the CT transmission profiles at the energy of the radioactive source emissions. Consequently, the attenuation correction map is an emission attenuation map calculated from the CT image. The attenuation correction map is saved into a sinogram and incorporated into the PET image reconstruction process [13].

In the field of radiation oncology, most commonly, the radioisotope fluorine-18 is used to label fluorodeoxyglucose, resulting in the radiotracer $^{18}$F-FDG which reflects glycolysis and metabolism. $^{18}$F-FDG PET imaging is currently accepted within the field of oncology for staging tumours and evaluating response to treatment given tumours are highly metabolic and will have a high uptake. The voxel intensity of a PET image is a measure of the radioactivity concentration in the imaged region. To quantify the radioactivity concentration, the Standardised Uptake Value (SUV) is calculated as shown in equation (1.6). This corrects for the variations in the injected activity and patients’ weight.

$$\text{SUV} = \frac{\text{Radioactivity concentration in tissue (MBq/Kg)}}{\text{Activity injected (MBq)/body weight (Kg)}}$$  \hspace{1cm} (1.6)

The SUV value within a region is often reported as either the maximum value (SUV$_{\text{max}}$) or the mean value (SUV$_{\text{mean}}$) within the region of interest. SUV$_{\text{max}}$ is less affected by intra-interobserver variability than SUV$_{\text{mean}}$ yet it is more affected by noise [151]. Generally, an SUV $\geq 2.5$ is considered
malignant tissue [67]. However, given that 18F-FDG reflects glycolysis, high metabolic tissues within the body will have a high uptake such as the brain and the myocardium. Moreover, inflammatory or infectious tissue will also have a high uptake which might lead to false positives. Another consideration is the partial volume effect; it is observed when the size of the lesion is less than two to three times the resolution of the scanner, this usually causes the lesion to appear less active. Considering the patient history and the corresponding CT image when interpreting PET images is fundamental [67].

Despite the non-specificity of 18F-FDG, it is the most common radiotracer used in cancer imaging due to its high sensitivity and wide availability [112]. 18F-FDG PET imaging has been validated extensively in the literature and has shown a correlation to treatment outcome, hence it has been suggested to be the imaging modality to inform dose painting in radiotherapy [23][64][158][19][109][65][81][144][111][63][6].

1.5 Dose Painting

Traditionally, delivery of a homogenous dose distribution to the tumour was desirable. However, tumours have been shown to be biologically heterogeneous in regards to their cellular density, proliferation and oxygenation [148][91]. To account for the tumours’ biological heterogeneity in radiotherapy planning, Ling et al. [91] proposed the concept of biological dose painting in 2000. The authors hypothesized delivering a heterogeneous dose distribution to the tumour volume based on the spatial variation of the biological characteristics of the tumour (cellular density, proliferation and oxygenation). In biological dose painting, the dose distribution will be sculptured to escalate dose to sub-volumes of increased tumour burden and increased resistance to radiation within the tumour. The authors referred to these sub-volumes as biological tumour volumes (BTV). To identify BTV, the authors suggested the utilisation of upcoming molecular and functional imaging modalities such as PET and magnetic resonance imaging and spectroscopy (MRI/MRS). The authors argue that dose escalation to the BTV while conforming to normal tissue constraints is achievable by the use of the IMRT planning technique. IMRT is an inverse planning technique that has a superior physical conformity to conventional techniques in delivering higher dose to the target volume while minimising dose to normal tissue. The physical conformity of IMRT allows the delivery of a non-uniform dose distribution which conventional techniques cannot achieve [91]. Overall, the proposed concept by
Ling et al. [91] did not address the applicability and technical feasibility of biological dose painting. Rather, it was a theoretical concept in need of extensive investigations and clinical validations.

Yet, since the introduction of biological dose painting, the field of radiotherapy has been striving for personalized radiotherapy treatment planning based on patient specific radiobiological parameters. In 2002, an inverse planning approach incorporating functional imaging data into the dose optimisation algorithm was proposed by Xing et al. [155]. The authors suggested dose escalation to abnormally metabolic regions within the tumour. To achieve an inhomogeneous dose distribution, dose is assigned to voxels based on their functional importance (FI) level [155]. Studies by Chao et al. [29] and Alber et al. [9] investigated the possibility of prescribing a heterogeneous dose distribution based on signal intensity obtained from PET studies using hypoxia tracers copper-diacetyl-bismethylthiosemicarbazone (Cu-ATSM) and 18F-fluoromisonidazole (FMISO) respectively. While Chao et al. [29] suggested to selectively boost the dose to the hypoxic sub-volume, Alber et al. [9] proposed an optimisation algorithm where dose is distributed based on dose efficiency distribution obtained from tumours’ biological images where each voxel is assigned a number in the target volume based on expected effect of radiation on tumour tissue at that point. The presented algorithm will shift the dose towards a low dose efficiency point [9].

The above studies [155][29][9] suggested a linear dose escalation to sub-volumes based on functional image signal intensity while conforming to normal tissue constraints. This approach is based on the argument that increasing dose to the tumour will increase cell killing and consequently improve tumour control probability (TCP). While the argument is commonly accepted, the level of dose escalation required to achieve a significant increase in TCP is yet to be determined.

The first theoretical framework integrating spatial variation of radiobiological parameters known to influence radiotherapy treatment (cell density, proliferation, and oxygenation) into IMRT planning was presented in 2005 by Yang and Xing [159]. The authors suggested a continuous dose distribution to maximise cell killing, where the overall TCP is a product of the TCP of each individual voxel in the tumour. This framework was adopted by South et al. in 2008 [125]. The authors [125] proposed an analytical approach to derive patient-specific radiobiological parameters (cell density, proliferation and oxygenation) so the approximation of the radiobiological parameters by Yang and Xing is eliminated [159][125]. The effect of varying biological parameters values on dose per voxel was investigated by [125]. As reported by the authors, oxygenation has the most significant effect on dose per voxel.
Introduction and Background

when compared to proliferation rate and clonogen density. The level of dose necessary to overcome hypoxia resistance was estimated by quantifying oxygen distribution per voxel from the uptake of hypoxia specific PET tracer FMISO. The oxygenation level was correlated to FMISO uptake and dose level using animal data and simulation studies [113][131]. The authors showed that TCP can be improved by re-distributing the dose to areas exhibiting high level of hypoxia. The maximum TCP was shown to be achieved using 2-5 dose levels. Yet, in some cases of high level of hypoxia in head and neck tumours, maximum TCP could not be achieved due to normal tissue constraints [125][126][124]. Even though the study presents a very promising optimisation technique based on improving TCP per voxel, where a relationship between hypoxia and dose level is established, it presented a relatively high level of uncertainty given the extracted mathematical relationship between FMISO uptake, oxygenation and dose is based on animal and simulation studies. Furthermore, the reported results are based on a small cohort (3 patients) and lacks clinical validation.

Given that tumour hypoxia has been shown to be mostly related to loco-regional failure and resistance to radiation [108][149], several studies [33][62][161] have been focusing on dose painting based on boosting hypoxic sub-volumes. In addition, the considerable availability and validation of hypoxia radiotracers such as 18F-fluoromisonidazole (FMISO), 18F-fluoroazomycin (FAZA), and Cu-ATSM enhanced their application in biological dose painting studies [130][166].

Previously discussed studies [155][29][9][159][125][33][62][161] suffer from similar common pitfalls of biological dose painting. Biological dose painting based on specific radiobiological parameters is hindered by the lack of available clinical data to establish a mathematical relationship between tracer uptake, radiobiological parameter and the required radiation dose. Furthermore, reoxygenation and repopulation processes are usually neglected. In addition, limitations of the available tracers in regards to their sensitivity and specificity would influence the resulted distribution and should be accounted for.

Faced with challenges in using a specific biological radiotracer, multiple studies have been investigating the concept of biological dose painting based on 18F-FDG. Some researchers have attempted to associate 18F-FDG uptake to a specific biological phenomenon. A correlation of 18F-FDG uptake and hypoxia was investigated by [115][31][88]. The results showed no significant association. Another attempt was conducted by Sauter et al. [118] to correlate 18F-FDG uptake to blood volume and blood flow measurements from CT perfusion scans for patients diagnosed with
1.5 Dose Painting

NSCLC. The authors reported no relation between perfusion and metabolism of glucose in large tumours yet an inverse correlation with mediastinal lymph nodes was found. The authors concluded that 18F-FDG uptake in tumours’ cells is a complex process between hypoxia, perfusion and glucose metabolism and cannot be attributed to a specific biological phenomenon [118].

Although a quantitative relationship between 18F-FDG uptake and the biology of the tumour could not be established, Aerts et al. [6] suggested it to be a suitable tracer for dose painting. The authors base the claim on the wide availability of 18F-FDG, well established standardised protocols of 18F-FDG PET imaging, and the stability exhibited by tracer uptake during radiotherapy treatment [111][6]. The authors argue that given the high post treatment overlap rate (> 70% in NSCLC) exhibited by high 18F-FDG uptake regions identified prior to radiotherapy, targeting such regions with higher dose could be beneficial [5]. Furthermore, studies on mice showed an improved TCP when escalating dose to areas with high 18F-FDG uptake [120]. Researchers [41][145][93][111] explored the possibility of linear dose escalation to sub-volumes within tumours based on 18F-FDG uptake. Yet, the benefit of such escalation is not yet clear. Currently, a clinical trial on adaptive dose painting investigating boosting sub-volumes with high 18F-FDG uptake in non-metastatic head and neck cancer is being conducted by De Neve [17]. Moreover, a phase II randomised trial by De Ruysscher and Belderbos on boosting active 18F-FDG areas in NSCLC up to at least 72 Gy compared to escalating dose to the whole tumour volume is currently ongoing [143].

Due to the low resolution of PET (5-7mm) and the necessity to register the images with CT for anatomical definition, some researchers suggested the use of MRI for dose painting in radiotherapy planning [20][142][69]. MRI has the advantage of superior soft tissue contrast when compared to CT images [142]. Diffusion weighted MRI provides information regarding tissue cellular density while dynamic contrast enhanced MRI (DCE-MRI) gives information regarding blood flow and microvasculature permeability. Both parameters affect radiosensitivity of the tumour and could be used to guide dose painting [142].

Despite the attractiveness of MRI guided dose painting, there are many challenging aspects to overcome. Target delineation based on functional MRI is still not fully explored where published research studies investigating quantification of MRI functional parameters and target delineation are relatively scarce when compared to PET imaging [69]. In addition, the standardisation of functional MRI quantification is still not well established. Furthermore, accurate and reproducible positioning is
considered more challenging in MRI based radiotherapy planning. Coils used in MRI machines would compromise the position of the patients especially in the cases of head and neck cancer. Moreover, MRI images are subject to distortion and lack information on electron density and still need to be co-registered with CT images [69][80].

The applicability of MRI guided dose painting was explored by Bates et al. [14] who conducted a simulation study to investigate whether incorporating ventilation and perfusion data from Hyper-polarized helium-3 MRI (3He-MRI) has any benefit to NSCLC patients (stage IIIA-B) receiving IMRT treatment. The simulated clinical scenarios included patients with functional lung deficits. The authors reported a median reduction of 27% in dose-volume parameters for functional lung when accounting for functional data in IMRT planning [14]. Currently, a randomised phase III trial is being conducted by Haustermans where multiple techniques of functional MRI (T1 and T2 diffusion weighted and DCE MRI) are used to boost focal lesions within the prostate [92].

Based on reviewed literature, PET is most likely to be the leading imaging modality in dose painting thus it is the imaging modality considered in this project.

1.6 Texture Analysis

The texture of an image can be generally defined as the spatial variation in pixel intensity levels. Texture can be assessed using statistical methods (histogram and grey level dependence matrices), model based methods such as fractal models or transform based methods such as the Fourier and the wavelet transform [97]. In medical imaging, statistical based texture analysis has made the most significant contribution.

Intensity histograms are a representation of the distribution of pixel intensities $i$ in an image and they are considered first order statistical methods where global features such as mean and standard deviation can be extracted. The features that can be extracted from intensity histograms are presented in table (1.1) [97].

Second order statistics textural features are extracted by the analysis of grey level co-occurrence (dependence) matrices (GLCM) [97][32]. GLCM represents the joint probability density function $P(i, j; \theta, d)$ of the number of times an intensity level $i$ and an intensity level $j$ occur in a certain
Table 1.1 First-Order Texture Features: Based on average pixel value $i$ where $N$ is the number of grey levels in the histogram. Intensity histogram analysis.

<table>
<thead>
<tr>
<th>Global Texture Features</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean $\mu$: average intensity values in an image.</td>
<td>$= \sum^N ip(i)$</td>
</tr>
<tr>
<td>Variance $\sigma^2$: the spread or variation around the mean.</td>
<td>$= \sum^N (i - \mu)^2 p(i)$</td>
</tr>
<tr>
<td>Skewness: symmetry of intensity values in an image. Skewness is zero if the histogram is symmetrical</td>
<td>$= \sigma^{-3} \sum^N (i - \mu)^3 p(i)$</td>
</tr>
<tr>
<td>Kurtosis: indication of histogram flatness.</td>
<td>$= \sigma^{-4} \sum^N (i - \mu)^4 p(i)$</td>
</tr>
<tr>
<td>Energy: measures uniformity of intensity values.</td>
<td>$= \sum^N [p(i)]^2$</td>
</tr>
<tr>
<td>Entropy: represents irregularity of intensity values distribution.</td>
<td>$= -\sum^N p(i) \log(p(i))$</td>
</tr>
</tbody>
</table>

direction with $\theta = 0^\circ, 45^\circ, 90^\circ$, or $135^\circ$ at specified distance $d$ [61]. The features that can be extracted from GLCM are presented in table (1.2) [61], where $p_x$ is the probability matrix obtained from summing the rows in $p(i, j)$ and $p_y$ is the probability matrix from summing the columns in $p(i, j)$. The means and standard deviation of $p_x$ and $p_y$ are represented by $\mu_x$, $\mu_y$, $\sigma_x$ and $\sigma_y$ respectively. The number of grey levels in an image is $N$.

Textural parameters extracted from neighbourhood grey-tone (intensity) difference matrices (NGTDM) are considered higher order parameters which are based on the relation between a pixel and neighbouring pixels. Run length matrices (RLNM) are considered higher order statistical methods where RLNM consists of the number of consecutive pixels that have the same intensity level $i$ and which occur in a specified direction [50]. The features that can be extracted from RLNM are presented in table (1.3) [50].
Introduction and Background

Table 1.2 Second-Order Texture Features: Based on grey level co-occurrence matrices.

<table>
<thead>
<tr>
<th>Local Texture Features</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angular Second Moment (energy or uniformity): measures homogeneity of intensity value distribution in an image.</td>
<td>$= \sum_{i,j}^N</td>
</tr>
<tr>
<td>Contrast: measures amount of local variation in intensity values.</td>
<td>$= \sum_{i,j}^N (i-j)^2 p(i,j)$</td>
</tr>
<tr>
<td>Homogeneity (inverse difference moment): measures the homogeneity of the intensity values of the pixel pair.</td>
<td>$= \sum_{i,j}^N \frac{p(i,j)}{1-(i,j)^2}$</td>
</tr>
<tr>
<td>Correlation: measures the linear dependencies of intensity values in an image.</td>
<td>$= \sum_{i,j}^N \frac{(i,j) p(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y}$</td>
</tr>
<tr>
<td>Entropy: measure of randomness of intensity values in an image.</td>
<td>$= -\sum_{i,j}^N p(i,j) \log(p(i,j))$</td>
</tr>
<tr>
<td>Sum of Squares (Variance)</td>
<td>$= \sum_{i,j}^N (i-\mu)^2 p(i,j)$</td>
</tr>
<tr>
<td>Sum Average</td>
<td>$= \sum_{i=2}^{2N} ip_{x+y}(i)$</td>
</tr>
<tr>
<td>Sum Entropy</td>
<td>$= -\sum_{i=2}^{2N} p_{x+y}(i) \log(p_{x+y}(i))$</td>
</tr>
<tr>
<td>Sum Variance</td>
<td>$= -\sum_{i=2}^{2N} (i - \text{sum entropy})^2 p_{x+y}(i)$</td>
</tr>
<tr>
<td>Difference Variance</td>
<td>$= \text{Variance of } p_{x+y}$</td>
</tr>
<tr>
<td>Difference Entropy</td>
<td>$= -\sum_{i=0}^{N-1} p_{x-y}(i) \log(p_{x-y}(i))$</td>
</tr>
<tr>
<td>Information Measures of Correlation</td>
<td>$= \frac{HXY - HXY1}{\max {HX, HY}} (1 - \exp[ -2.0 (HXY2 - HXY) ]^2$ where $HXY = -\sum_{i,j=1}^N p(i,j) \log(p(i,j))$ $HX \text{ and } HY \text{ are entropies of } p_x \text{ and } p_y$ $HXY1 = -\sum_{i,j=1}^N p(i,j) \log(p_x(i) p_y(j))$ $HXY2 = -\sum_{i,j=1}^N p_x(i) p_y(j) \log(p_x(i) p_y(j))$</td>
</tr>
<tr>
<td>Maximal Correlation Coefficient</td>
<td>$(\text{Second Largest Eigenvalues of } Q)^{1/2}$ where $Q(i,j) = \sum_k \frac{p(i,k)p(j,k)}{p_x(i) p_y(k)}$</td>
</tr>
</tbody>
</table>
### Table 1.3 High-Order Texture Features: Based on neighbourhood grey tone difference matrices.

<table>
<thead>
<tr>
<th>Local Texture Features</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complexity: measures amount of information (primitives) in texture.</td>
<td>$= \sum_{i,j} G_{h} \left[ \frac{(</td>
</tr>
<tr>
<td>Busyness: measure the rate of change in intensity values.</td>
<td>$= \left. \frac{\sum_{i,j} G_{h} p(i) s(i)}{\sum_{i,j}</td>
</tr>
<tr>
<td>Contrast: measures the variation of intensity values in an image.</td>
<td>$= \left[ \frac{1}{N(N-1)} \sum_{i,j} G_{h} p(i) \cdot p(j) \left( i-j \right)^2 \right] \times \left[ \frac{1}{n^{2}} \sum_{i} G_{h} s(i) \right]$</td>
</tr>
<tr>
<td>Coarseness: measures the density of edges in an image.</td>
<td>$= \left[ \varepsilon + \sum_{i} G_{h} s(i) \right]^{-1}$</td>
</tr>
<tr>
<td>Texture strength: measures how definable (distinguishable) texture primitive is.</td>
<td>$= \left. \frac{\sum_{i,j} G_{h} \left( p(i)+p(j) \right) \left( i-j \right)^2}{\varepsilon + \sum_{i,j} G_{h} \frac{p(i)+p(j)}{n^{2}}} \right.$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regional Texture Features</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-Runs Emphasis: measures the run length distribution emphasizing short runs by dividing by the square of run length value.</td>
<td>$= \frac{\sum_{i,j} p(i,j)}{\sum_{i,j} p(i,j)^2}$</td>
</tr>
<tr>
<td>Long-Runs Emphasis: measures the run length distribution emphasizing long runs by multiplying by the square of run length value.</td>
<td>$= \frac{\sum_{i,j} i^2 p(i,j)}{\sum_{i,j} p(i,j)}$</td>
</tr>
<tr>
<td>Grey level non-uniformity: represents the similarity of intensity values in an image.</td>
<td>$= \frac{\sum_{i,j} \left[ \sum_{i,j} p(i,j) \right]^2}{\sum_{i,j} \sum_{i,j} p(i,j)^2}$</td>
</tr>
<tr>
<td>Run Length non-uniformity: measure the run lengths similarity.</td>
<td>$= \frac{\sum_{i,j} \left[ \sum_{i,j} p(i,j) \right]^2}{\sum_{i,j} \sum_{i,j} p(i,j)^2}$</td>
</tr>
<tr>
<td>Run Percentage: ratio of total numbers of runs to the total number of possible runs measuring the homogeneity of runs. For images with most linear structure, the value of run percentage is lowest.</td>
<td>$= \sum_{i,j} p(i,j)$</td>
</tr>
</tbody>
</table>
Tables (1.1, 1.2 and 1.3) present a full set of the textural features that could be measured from first order, second order and higher order statistics. In texture analysis, the order of the extracted feature refers to the relationship between the pixels of the extracted features. First order statistics are extracted from the average of pixels intensity and second order statistical features are measured based on the relationship between two pixels where higher order statistical features are based on the relationship between more than two pixels. Therefore, first order statistical methods do not convey spatial information, whereas second and higher order statistical methods maintain spatial information.

Textural features such as mean, variance, entropy, energy, contrast, coarseness are well understood in regards of the information they provide of an image’s textural properties and have been heavily used to quantify image texture in the literature. Yet, the information extracted about image texture properties from other features such as sum entropy, sum variance, and information measures of correlation are not clear in regards to the clinical aspect. However, these features are presented as they could prove to be clinically important in the future and to provide the complete set of statistical texture measures.

Traditionally, textural analysis in the medical field has been used for tissue classification such as lung pathologies, breast lesions, head and neck tumours, and gliomeural tumours. Furthermore, NGTDM based textural analysis was suggested by Huan et al. for automatic delineation of target volumes in head and neck tumours for radiotherapy planning. In recent years, statistical based texture analysis has been suggested to have prognostic value in radiotherapy. Several textural features could be extracted from grey level dependence matrices and neighbourhood grey tone difference matrices, yet based on reviewed literature, features that describe heterogeneity such as contrast, energy, entropy and homogeneity have shown the most significant results in relation to patients’ survival.

1.6.1 Texture Analysis in CT

The initial implementation of texture analysis to predict patient survival was suggested by Ganeshan et al. in 2007. The authors suggested band-pass filtering of CT images using the Laplacian of Gaussian (LoG) filter to detect features at different anatomical scales. The LoG filter is a two-dimensional (2D) spatial filter. It is a combination of a smoothing filter (Gaussian) to reduce noise
and an edge detector filter (Laplacian). The Laplacian detects areas in the image where a prominent change in the intensity occurs, hence it is mostly used as an edge detection method. When the filter is convolved with the image, the region where the response of the filter passes through zero (zero crossing) is the region where an intensity change is detected.

Extracting texture parameters from CT image histograms post the application of the LoG filter was recommended by the authors given their un-complex and rotationally invariant nature despite their spatial insensitivity. The authors performed texture analysis on CT images using in-house software which is commercially available by the name of TexRAD (TexRAD, UK). A number of studies using this software have been published [51][52][102][53][42][55][54][56][162]. The group suggested the use of five LoG filters ranging from fine to coarse textures (filter widths fine = 4 image pixels and coarse = 12 image pixels) and quantifying tumours heterogeneity by measuring the mean, standard deviation, skewness, mean of positive pixels value (MPP), uniformity and entropy from the CT slice with the largest cross section of the tumour. The group reported uniformity and entropy, to show the most significant results in predicting patient survival.

A publication using TexRAD investigated whether first order textural features extracted from staging PET/CT images, for 66 NSCLC patients were related to patient survival [153]. By quantifying the entropy of the image which represents the pixel intensity distribution within the image, tumour heterogeneity was assessed. To correlate the heterogeneity of the tumours to patient survival, Kaplan-Meier (KM) curves were generated. The KM analysis is based on cumulative conditional probability to estimate surviving a duration of time. The overall survival time is split into small time intervals based on the observed survival time of the investigated group. For each time interval, the survival probability $P(t(q))$ is measured as the number of surviving subjects at that specific time interval $t(q)$ divided by the sample size as shown in equation (1.7) [11].

$$P(t(q)) = \frac{\text{Number of survivals past } t(q)}{\text{Sample size}}$$ (1.7)
To generate KM curves, the subjects were divided into two groups based on the value of tumour image entropy. In KM analysis a cut-off value (threshold value) is used to dichotomise the subjects into the two groups. The Log-Rank test was used to test the significance of the investigated feature on survival. In Win et al. 2013 [153], patients were assigned to groups of low and high entropy based on the optimum threshold value obtained from a feasibility study including 54 NSCLC patients. The entropy value that was shown to be optimum in predicting survival was a normalised value taken from medium-filter/coarse-filter entropy values. The authors reported tumour normalised entropy to be the only parameter significantly related (p = 0.027) to survival for patients in the curative-intent group while tumours entropy, stage and permeability showed significant association with survival for the palliative patients group with p = 0.042, p = 0.020 and p = 0.003 respectively.

Although the published studies using the TexRAD software [51][52][102][53][42][55][54][56][162][153] have shown some correlation between survival and global textural parameters, they have not explained the significance of choosing the optimum filter width and how that relates to anatomical feature extraction. Moreover, TexRAD based studies have not validated the uniformity and entropy threshold values used for generating KM curves. In addition, the proposed methodology by the TexRAD group is based on using a single slice CT image with the largest cross section of the tumour, and not considering volumetric measurements, which leads to loss of spatial information and consequently may significantly affect texture quantification.

Correlation of patients’ survival to second order textural parameters extracted from CT data was investigated by Vaidya et al. in 2012 [139]. The study included 27 patients diagnosed with NSCLC confined to the thorax, where baseline 18F-FDG PET and correspondent CT were analysed. The endpoints were set to be local recurrence with at least 6 months follow up. To account for patient breathing artefacts, an algorithm based on the inverse filtering process was applied to acquired images. Textural features extracted from PET/CT data included energy, contrast, entropy and local homogeneity from co-occurrence matrices calculated for the 3D volume of the tumour. Textural features extracted from CT images showed some correlation with loco-regional failure, yet none of the features reached significance. The de-blurring method was applied to PET images not CT images, so whether motion artefacts affected the power of extracted features was not determined. The authors reported extracted textural features from PET images to have the lowest correlation with loco-regional failure when compared to intensity volume histogram (IVH) and gross tumour volume (GTV) and
SUV measurements. The de-blurring of PET images showed little improvement on the correlation coefficient $r$ (from 0.114 to 0.18 and p-value from 0.28 to 0.17) for local homogeneity. Textural features showed a stronger correlation with local control rather than loco-regional failure whereas IVH showed the opposite trend as reported by the authors [139].

Textural features were reported by Mattonen et al. [98][99] to predict recurrence for early stage NSCLC patients who underwent stereotactic ablative radiotherapy (SABR). The authors reported standard deviation, (reported as the variation of HU within the ground-glass opacity (GGO) regions which are regions where the normal lung parenchyma density is increased with visible vessels), are able to significantly discriminate ($p = 0.0078$) between patients with radiation induced injury and patients with recurrence in a follow up CT scan acquired at 9 months with an error of 26% [98]. The authors explored the ability of second-order textural features to predict recurrence by generating GLCM from GGO for follow up CT scans taken at 6 months for a group of 22 patients. The texture features calculated from the 2D averaged GLCM were energy, entropy, correlation, inverse difference moment, inertia (contrast), cluster shade, and cluster prominence. Cluster prominence and cluster shade are measures of pixel pair grouping quantifying the symmetry (skewness in GLCM) of grey levels in an image. The authors reported energy, entropy, and inertia to be significantly different between groups with radiation induced injury and recurrence, with $p = 0.036$, $p = 0.034$ and $p = 0.036$ respectively. Respiration, co-morbidities, and any scanning factors that would affect CT density were not considered [99]. Given that injury to lung tissue such as radiation fibrosis and pneumonitis, post radiotherapy, resembles tumour recurrence on CT images a more analytical quantitative approach to predict recurrence is needed.

### 1.6.2 Texture Analysis in PET

GLCM based texture analysis of 18F-FDG PET data has been reported to provide significant and superior results to SUV measurements in predicting complete response (CR), partial response (PR) and non-response (NR) to therapy, for patients diagnosed with oesophageal carcinoma as reported by Tixier et al. [134] in 2011. The study included 41 patients where SUV measurements were compared to global, regional and local PET textural features. The authors reported both global features and SUV measurements to be sufficient predictors of CR but could not distinguish NR from PR. Regional entropy was reported to be the most significant predictor in identifying and distinguishing NR, CR,
Introduction and Background

and PR. The images of the tumour were quantised by resampling the intensity values to 16, 32, 64 or 128 set of discrete values to reduce noise and to normalise the intensities across the image. The authors assessed the variation in quantising GLCM to discrete number of values and found no significant difference (p>0.05) [134].

The reproducibility of PET textural analysis was evaluated by Tixier and colleagues [134][135] comparing two baseline 18F-FDG PET scans taken 2-7 days apart for 16 patients diagnosed with oesophageal cancer. Results showed the mean percentage difference (%DIFF) between the two studies to be 4.7% ± 19.5% and 5.5% ± 21.2% for SUV mean and SUV maximum respectively, confirming previous studies. Local heterogeneity parameters showed a better reproducibility with mean %DIFF of -2% ±5.4% for entropy and 1.8% ±11.5% for homogeneity, where other textural parameters showed lower reproducibility ranging from 40.9% for lower limit and 62.7% for upper limit [134][135].

Extraction of higher order textural features from NGTDM was investigated by Cook et al. [36] using 48 baseline 18F-FDG PET scans for NSCLC patients treated with definitive chemoradiotherapy. The response was assessed 12 weeks after treatment on CT using Response Evaluation Criteria in Solid Tumours (RECIST). Coarseness, contrast, busyness and complexity were extracted from NGTDM and compared with SUV measurements (mean, maximum, and peak), metabolic tumour volume (MTV) and total lesional glycolysis (TLG) as predictors of response. The authors reported none of the SUV parameters nor TLG and MTV to be a significant predictor of responders and non-responders. Coarseness, contrast and busyness measurements were significantly different between responders and non-responders with area under the curve (AUC) of 0.8, 0.82, and 0.72 respectively indicating a fair distinction ability. Nevertheless, the authors did not report whether any of the texture parameters could distinguish or predict partial response and complete response [36].

Even though recent publications have shown a correlation between heterogeneity measurements and tissue response, the accuracy and precision of PET textural analysis is yet to be explored. Limitations due to spatial resolution, noise, motion artefacts, image acquisition parameters and reconstruction methods are expected to lead to a degradation of the extracted textural features and should be addressed. A review on texture analysis in PET by Cook et al. [37] focusing on the technical factors and clinical application supports this conclusion.
1.6.3 Texture Analysis in MRI

Assessing tumour heterogeneity by utilising dynamic contrast enhanced MRI (DCE-MRI) in defining tumours functional risk volume (FRV) to predict treatment outcome in cervical cancer was investigated by Mayr et al. [100] in 2012. FRV is defined as a region of low contrast uptake where the signal intensity is < 2.1 when compared to the image before contrast injection. The authors presented a heterogeneity characterisation approach that consisted of generating relative signal intensity (RSI) distributions based on the perfusion levels of individual voxels which were then tabulated in an RSI histogram. The study included 102 patients diagnosed with cervical cancer stage IB2-IVA based on the International Federation of Gynaecology and Obstetrics (FIGO) staging system of cervical cancer. The total FRV was derived from perfusion heterogeneity assessment prior to chemo-radiotherapy and during treatment in weeks 2-2.5 and 4-5. The FRV2 was measured at 2-2.5 weeks during the chemo-radiotherapy treatment course while the FRV3 was measured at 4-5 weeks during the chemo-radiotherapy treatment course. FRV’s were associated with disease specific survival endpoints and primary tumour control. Patients’ median follow up was reported to be 6.8 years. The results reported by the authors suggest FRV to be a significant early predictor of outcome in the long term. Pre-treatment FRV was least predictive where the power of prediction increased from 24.3% to 42.5% for FRV2 and 45.2% for FRV3 in discriminating 6-year actuarial tumour control rate and recurrences of primary tumour [100][101]. GLCM based texture analysis in DCE- MRI [7] was reported to be successful in discriminating responders and non-responders for 89 patients diagnosed with locally advanced breast cancer and receiving neoadjuvant chemotherapy, its predictive value for patients undergoing radiotherapy is yet to be determined. Nevertheless, from 14 extracted features only contrast, difference variance and difference entropy reached significance [7].

1.6.4 Challenges in Texture Based Radiotherapy Planning

Whilst a customised radiotherapy plan based on prediction of tumour response to treatment is an attractive idea, texture analysis is hindered by multiple challenges. Uncertainties in patient positioning, organ motion, inter-observer variability, image acquisition parameters, reconstruction algorithms, and texture extraction methods affect the extracted textural features and all must be investigated and addressed. Yet, the major question is the biological origin of image texture.
To date, the biological basis of textural analysis remains not fully understood. In vivo studies relating the apparent heterogeneity of tumours extracted from medical images to biological phenomena have not been attempted yet. Ganeshan et al. [56] attempted to investigate an association of CT textural analysis with angiogenesis and hypoxia using pimonidazole staining and hypoxia marker GLUT-1 on surgically removed lesions. The analysis showed a correlation between the standard deviation and the mean value of positive pixels (MPP) in the region of interest and pimonidazole staining. Uniformity of the distribution of positive grey-level pixel values showed an inverse association with GLUT-1 while MPP showed an inverse association with CD34 expression (which represents angiogenesis) in both contrast and non-contrast CT [56]. The study was based on measurements taken outside the human body on surgically removed lesions (ex-vivo) and comprised of a small cohort (14 patients); hence a strong relationship could not be inferred.

Another challenging aspect in texture analysis is the confounding results presented in the literature. A study by Chalkidou et al [27] investigated the probability of type-I error (false positive) for studies published between years 2000-2013 correlating textural features extracted from CT or PET images to prognosis. Fifteen studies were corrected for adjusting the significance level and optimising the cut-off value for survival analysis by using the Benjamini-Hochberg method. When applying the correction factors, the results showed a 76% (range 34-99%) probability of selection bias and false positive. Also, the majority of the published studies did not reach statistical significance after correcting for bias.

Other factors that challenges the accuracy and reliability of texture analysis are respiratory motion and tumour delineation.

1.6.4.1 Respiratory Motion

Although a study by Vaidya et al. [139] reported no significant difference in p-values in associating textural features extracted from motion corrected PET and non-corrected PET images to tumour local failure, respiratory motion leads to image blurring, inaccurate representation of tumour volume and potentially mislocalization of lesions especially for tumours located in the thorax. A publication by Aristophanous et al. [12] investigated target delineation in lung tumours on 4D and 3D PET images. The authors reported the mean difference between tumour volume in 3D PET (VOL3D) and tumour
volume in 4D PET (VOL4D) for lesions located in the lower lobe and lower mediastinum to be 50% while the upper lobe difference was 10%. In addition, the lesions exhibiting motion > 3mm showed a larger difference (approximately 54%) than lesions moving < 3mm (%DIFF 14%). The authors concluded that the benefit of 4D PET volume definition is dependent on tumour location and the range of motion [12]. While recently available PET/CT scanners have respiratory gating, there is not a currently established method for 4D MRI in radiotherapy. Many methods have been proposed in the literature for investigating the feasibility of 4D-MRI [25][138][136][18] yet none has been clinically implemented. Overall, studies focusing on tumour motion are challenged by the lack of ground truth where target delineation is subject to inter-observer variability. Further, changes in patients’ breathing patterns and irregular breathing are complicating factors as well as patients’ movement between consecutive scans. The magnitude of the effect of tumour motion on textural analysis is yet to be investigated since change in the apparent tumour volume would lead to a change in measured texture especially when the extent of motion is similar in scale to studied textural features.

1.6.4.2 Tumour Delineation

Tumour delineation critically influences treatment outcome, but whether it affects the power of extracted textural features is still under debate. According to Kazarski et al. [107] a large cross sectional area of the tumour is sufficiently representative and provides comparable results to whole tumour analysis when applying histogram-filtration methods to predict survival. Yet, other studies using texture analysis in tissue classification have reported volumetric texture analysis to be superior to 2D analysis due to loss of spatial information [94][156][30]. Whether the precision of spatial information is needed in predicting survival and response is still to be explored further since most of the studies in the literature have adopted one methodology without comparison of other methods. On the other hand, it has been reported by Mattonen et al. [99] that delineation of GGO in follow up CT scans using expansion and contraction of 1-2 mm of the GGO had minimal effect on extracted textural features, leading to the assumption of lower impact of delineation variability in predicting recurrence [99].

Nevertheless, inter-observer variability in target delineation should be minimised, and semi-automated and fully automated segmentation approaches have been suggested in the literature. Many of the research studies have been published investigating multiple PET and MRI segmentation methods
such as clustering, active contours and thresholding [83]. On the other hand, multimodality image segmentation methods were suggested by El Naqa et al. [106] and Chowdhury et al. [34]. The authors presented frameworks to link structures of interest across different imaging modalities to aid in target delineation for radiotherapy planning. Based on the published literature, the proposed methods suffer from shortcomings such as the lack of ground truth and their heavy dependency on image quality, thus a recommendation cannot be easily reached. Yet, image segmentation is crucial in radiotherapy planning and should be implemented while considering the achievable accuracy level and the technical feasibility. Manual delineation of tumours is still considered the gold standard in radiotherapy planning.

1.7 Radiomics

Even though a biological or a physiological basis of tumour image texture has not been established nor a standarised methodology for performing texture analysis, in the past two years, there has been a great interest in the field of radiomics. In radiomics, all possible quantitative image features (400+) including shape, intensity and including but not exclusively textural features are extracted. Studies investigating radiomics based features are not discussed given the focus of this thesis is statistical texture analysis of tumour images. Multiple studies have been published in regards to building predictive or prognostic models based on radiomics features extracted from CT and or PET images yet the published studies are still suffering from the same challenges as texture analysis [37][140][38][154][121][119][72][39].

1.8 Conclusions

Based on the reviewed literature, it is postulated that CT will be the leading imaging modality in texture analysis given its superior resolution and its good integration into the field of radiotherapy compared to PET. Moreover, PET’s poor resolution, high noise and tracer uptake and quantification challenges under-powers the extracted features and could lead to pseudo-texture. MRI based texture analysis seem an attractive idea due to its superior soft tissue contrast, yet there are many challenging aspects to overcome. Target delineation based on functional MRI is still not fully explored where published
research studies investigating quantification of MRI functional parameters and target delineation are relatively scarce compared to CT imaging. In addition, the standardisation of functional MRI quantification is still not well established. Functional MRI is still not widely available and not as fully integrated into radiotherapy planning as CT and PET.

In order to integrate texture analysis into radiotherapy planning, a standardised method of conducting texture analysis must be established and a validation of the reported findings in correlating image texture to patient survival is fundamental. Moreover, an understanding of the physiological implications of tumour image texture is crucial for clinical applications. Extracting quantitative image features that are indicative of tumour biology or function could be highly beneficial in the field of radiotherapy especially in guiding dose painting.
1.9 Thesis Overview

The main aim of the work presented in this thesis was to investigate whether i) tumour CT images have genuine texture defined as intratumoural heterogeneity that is characteristic of the underlying tissue ii) whether intratumoural heterogeneity provides useful or meaningful information in regards to tumour function and iii) whether this information is clinically relevant and could be used to guide dose painting.

In this thesis, I present an optimised method to visualise volumetric voxel based maps of intratumoural texture variations. I then show the relationship between the spatial textural information obtained from the generated maps and tumour function. This method is optimised based on a texture feature that was shown to correlate with patient survival. Furthermore, I investigate a characterisation of the effects of motion on texture analysis. Finally, I present the application of this proposed methodology in guiding radiotherapy dose painting. The patient cohort consist of patients diagnosed with advanced NSCLC. Due to the poor prognosis (5 years survival <10%) of this group of patients, investigating new methods to aid in dose painting could be beneficial.

The work in this thesis has been carried out in collaboration with Royal Surrey County Hospital (RSCH), Guildford Diagnostic Imaging Centre (GDI) and Alliance Medical Limited. This study obtained local ethics approval from the Royal Surrey County Hospital NHS Foundation Trust, for the submitted project proposal please refer to Appendix (A).

I started the work in this thesis by surveying the first commercially available software dedicated to correlating tumour texture to patient survival; TexRAD (TexRAD, UK). In chapter 2, I present our in-house implementation of the TexRAD algorithm and a comparison between results generated using the two systems. I also present a validation study of the published texture analysis parameters that were shown to be predictive of patient survival in NSCLC.

In chapter 3, a phantom study to characterise the effects of respiratory motion on extracting textural features from CT images is presented. In the phantom study, the modulation transfer function (MTF) was calculated for the CT scanner and for different size texture filters in static and moving acquisitions. The results from the MTF analysis provides information about the optimum filter size that allows maximum information to be extracted from images that are affected by respiratory motion.
Based on the results from chapter 2 and chapter 3, I conceptualised a method that allows for voxel based texture mapping of tumour image. In chapter 4, I present our novel methodology of designing a voxel based texture mapping filter including; choice of texture filter, our proposed novel method for image quantisation and choice of filter size.

The application of the volumetric voxel based texture mapping methodology on CT images of NSCLC is presented in chapter 5 along with an investigation of the relationship between the produced volumetric voxel based texture maps and tumour metabolism obtained from 18F-FDG PET images for a set of ten patients diagnosed with advanced NSCLC is presented and analysed.

In chapter 6, the findings of the relationship between tumour CT image heterogeneity and 18F-FDG uptake distribution presented in chapter 5 are used to guide dose painting for the investigated patients. The methods and results of a comparative dose painting planning study are presented in chapter 6. The planning study includes creating four radiotherapy treatments plans for each patient based on: the current treatment standard of uniform dose to the tumour, a dose painting plan based on tumour CT image heterogeneity, a dose painting plan based on tumour 18F-FDG uptake distribution and a dose painting plan based on the combination of tumour CT image heterogeneity and FDG uptake distribution.

In the last chapter, chapter 7, the implications of the work in this thesis are discussed in addition to a discussion of the main conclusions and future work.
Chapter 2

First-order Statistics Texture Analysis for Survival Prediction

2.1 Introduction

One of the main objectives in this thesis is to investigate the application of texture analysis in the field of radiation oncology hence, a survey of the existing texture analysis methods in the literature was undertaken. In this chapter, an evaluation and a validation of the first and currently, the only available commercial software, TexRAD (TexRAD, UK), dedicated to correlation of patient survival with tumour’s CT first-order textural features is presented [51][52][102][53][42][55][54][56]. TexRAD software uses five Laplacian of a Gaussian (LoG) filters of different sizes to measure the entropy of tumour images and correlates this with patients survival as has been discussed in (1.6.1). This chapter presents two new studies: a feasibility study and a validation study. In the feasibility study, I implemented in-house Laplacian of a Gaussian (LoG) filters that corresponds to the filters suggested by the TexRAD group. The in-house implemented filters performance is validated against TexRAD software filters by analysing a dataset using the two systems. In the validation study, the texture analysis methodology and parameters using the LoG filters, that has been shown to correlate to patients survival in non small cell lung cancer (NSCLC) and published by Win et al. [153], are investigated. The survival analysis results from the validation study in regards to correlating first-order textural
features extracted from tumour images after the application of the LoG filters are compared to what is published by Win et al. 2013 [153].

2.2 Feasibility Study

2.2.1 Methods

2.2.1.1 Dataset

18F-FDG PET/CT images of 20 patients diagnosed with NSCLC were collected retrospectively in addition to other patients information (disease stage, histopathology, survival). The patients group had a mixed histology of eight squamous cell carcinoma, five adenocarcinoma and seven NSCLC of unknown histology. The staging for the investigated patients was locally advanced where the TNM staging ranged between T2N0 to T4N2. The non-contrast PET/CT images were acquired using a GE Discovery LS scanner (GE Healthcare, UK); images were reconstructed using filtered back projection (FBP) for the CT images. The acquisition parameters for CT images were 150kVp and 100mAs. The reconstructed transaxial image size was 512x512 with 0.98x0.98x5.00 mm³ voxel size. This study obtained local ethics approval from the Royal Surrey County Hospital NHS Foundation Trust.

2.2.1.2 TexRAD Software

CT images for the investigated dataset were anonymised and imported into TexRAD software. The region of interest (ROI) for analysis was defined as the primary tumour at the largest cross-section within the CT scan as suggested by the published literature from the TexRAD group [54]. A trained observer (the author) delineated the ROI under the supervision of a consultant radiologist (VP). Using TexRAD software, the five available Laplacian of a Gaussian (LoG) filters were applied to the CT tumour image. The applied LoG sizes and \( \sigma \) are presented in table (2.1). First-order textural features of mean, standard deviation, entropy, kurtosis, and skewness were measured from the filtered tumour images. The produced texture parameters were exported from TexRAD software for further analysis.
2.2 Feasibility Study

Table 2.1 Size of the TexRAD Laplacian of Gaussian Filters.

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Filter size (mm$^2$)</th>
<th>$\sigma$ size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssf2</td>
<td>4x4</td>
<td>1.0</td>
</tr>
<tr>
<td>ssf3</td>
<td>6x6</td>
<td>1.5</td>
</tr>
<tr>
<td>ssf4</td>
<td>8x8</td>
<td>1.8</td>
</tr>
<tr>
<td>ssf5</td>
<td>10x10</td>
<td>2.0</td>
</tr>
<tr>
<td>ssf6</td>
<td>12x12</td>
<td>2.5</td>
</tr>
</tbody>
</table>

2.2.1.3 In-house Implementation of TexRAD Methodology

LoG filters that corresponds to the TexRAD filters were implemented in-house by the author based on the information released by TexRAD group in addition to private discussion with the company’s science director. This information only included filter size $h$ and $\sigma$ for each of the LoG filters. The author developed the corresponding filters in Matlab software (The MathWorks, Inc, Natick, MA). The filters were developed by first creating a 2D symmetrical matrix $(x, y)$ using equation (2.1) where $h$ is the filter size. The produced matrix $(x, y)$ was used to create the five LoG filters with $\sigma = 1.0, 1.5, 1.8, 2.0, 2.5$ using equation (2.2). The value of $\sigma$ is a scaling factor related to the smoothing effect of the filter, a larger $\sigma$ will lead to a larger smoothing effect (2.2). The in-house implemented filters are referred to as LoG2, LoG3, LoG4, LoG5, LoG6 which corresponds to ssf2, ssf3, ssf4, ssf5 and ssf6 respectively (refer to table (2.1)). The LoG filters were then summed to zero to null the constant component of the image as presented in equation (2.3).

\[
(x, y) = \left( \frac{-(h - 1)}{2} : \frac{h - 1}{2}, \frac{-(h - 1)}{2} : \frac{h - 1}{2} \right)
\]  

(2.1)
\[ \text{LoG}(x, y) = -\frac{1}{\pi\sigma^4}[1 - \frac{x^2 + y^2}{2\sigma^2}] \exp \frac{-x^2 + y^2}{2\sigma^2} \] (2.2)

\[ \text{LoG} = \text{LoG} - \sum_{(x,y)/h^2} \] (2.3)

The same dataset of patients images analysed in the TexRAD software was analysed using the in-house implemented LoG filters. The CT scans for the investigated patients were anonymised and imported into itk-snap software [163]. The ROIs were delineated by the same trained observer (the author) by replicating what was delineated in TexRAD software. The ROIs were copied and imported into Matlab software (The MathWorks, Inc, Natick, MA) to apply the in-house implemented LoG filters and to measure texture features. LoG filters are spatial filters; the filtered image is a result of convolving the tumour CT image with the LoG filter.

2.2.1.4 Statistical and Survival Analysis

Studies published using TexRAD by groups investigating texture analysis in NSCLC [56][153], showed global entropy (E_{global}) measured from tumour CT image post the application of LoG filters correlates with patients survival. Hence, tumour E_{global} as shown in equation (2.4) was measured from filtered CT tumour images. In equation (2.4), \( p \) is the probability of a voxel with intensity value \( i \) occurring in the image and \( N \) is the number of grey levels.
2.2 Feasibility Study

\[ E_{global} = -\sum_{i}^{N} p(i) \log(p(i)) \]  \hspace{1cm} (2.4)

Pearson’s correlation (R) was used to investigate the relationship between tumour entropy (calculated post the application of both TeXRAD filters and in-house implemented LoG filters) and patients survival. To test the null hypothesis that there is no correlation between entropy and survival, regression analysis was conducted using the F-test. To calculate the 95% confidence interval (CI) for R, R was transformed to \( z \) which has a normal distribution using equation (2.5). The 95% CI is then calculated as shown in equation (2.6) where \( n \) is the sample size. To test the significance of the observed correlation, tables that show critical values for R where p-value would be <0.05 were used [11].

\[ z = \frac{1}{2} \log \left( \frac{1 + R}{1 - R} \right) \]  \hspace{1cm} (2.5)

\[ 95\% \ CI \ (R) = z \pm 1.96 \frac{1.96}{\sqrt{n - 3}} \]  \hspace{1cm} (2.6)
In regression analysis, the slope \( b \) indicates the strength of the relationship, if any, between variables. To calculate the 95% CI for the slope \( b \), equation (2.7) was used. \( t_{0.975} \) is the value of \( t \) distribution at \( n - 2 \) degrees of freedom and \( Se(b) \) is the standard error in the slope \( b \) [11]. The standard error in the slope \( Se(b) \) is related to the standard deviation of the residual \( (S_{res}) \) between observed and fitted values, it is calculated using equation (2.8) [11].

\[
95\% \text{ CI (b)} = b \pm t_{0.975}Se(b) \tag{2.7}
\]

\[
Se(b) = \frac{\text{residual standard deviation}(S_{res})}{\sqrt{\text{sum of squares for entropy}}} \tag{2.8}
\]

Receiver operator curves (ROC) were generated to examine the ability of entropy values to predict patients’ survival for more than the median survival time of 30 months. The relationship between entropy and patients survival was assessed using Kaplan-Meier (KM) analysis in IBM SPSS for Windows version 22 (SPSS Inc, Chicago US). The median entropy value was used to dichotomise subjects into groups of low and high entropy. Due to the differences between centres in regards to CT scanners, reconstruction algorithms and scanning protocols, an absolute threshold entropy value is difficult to establish. Win et al. 2013 [153] suggested a normalised entropy value by dividing the entropy value measured from LoG medium filter of \( \sigma=1.5 \) and coarse filter of \( \sigma=2.5 \). In this study, this normalised entropy method suggested by [153] was also tested for significance using the TexRAD
filters and in-house implemented LoG. The subjects that have not experienced a terminal event were
censored in the KM analysis, meaning the total survival time cannot be accurately estimated for that
patient hence their survival time will not be considered when dividing the survival time into intervals.
Log-Rank test was performed on the KM curves to assess the significance in survival time [11],
p-value < 0.05 is considered significant. The Log-rank test calculates the distribution $X^2$ which is the
sum of the squared difference between the observed events $O$ (terminal event) and expected events $E$
(survived subjects) divided by $E$ as shown in equation (2.9). For a sample size of $n$, the p-value is
obtained for the $X^2$ distribution with $n - 1$ degrees of freedom.

$$X^2 = \text{sum}(O - E)^2 / E \quad (2.9)$$

Given TexRAD is commercial software, detailed information about the algorithms implemented
within it are not available, an exact agreement between the absolute values of measured entropy using
TexRAD filters and in-house implemented LoG filters is challenging. To assess the agreement and
consistency between entropy measurements generated by TexRAD software and in-house implemented
filters, Bland-Altman plots were generated. The difference $d$ in the entropy value measured from
the TexRAD filter (Entropy$_{\text{TexRAD}}$) and from the corresponding in-house implemented LoG filter
(Entropy$_{\text{LoG}}$) is plotted against the mean of the two values (Entropy$_{\text{TexRAD}}$+Entropy$_{\text{TexRAD}}$/2). The
plot will show the systematic difference between the two measurements. The difference should ideally
be zero, yet systematic difference between the two measurement within the 95% confidence limit is
considered acceptable. The 95% confidence limits are calculated as $d \pm 2s$ where $s$ is the standard
deviation between Entropy$_{\text{TexRAD}}$ and Entropy$_{\text{TexRAD}}$. Moreover, the ratio between Entropy$_{\text{TexRAD}}$
and Entropy$_{\text{LoG}}$ was calculated.
2.2.2 Results

2.2.2.1 TexRAD Results in Comparison to In-house Implemented Filters

Figure (2.1) shows the five in-house implemented LoG filters that corresponds to the standard TexRAD filters of sizes ranging from $4 \times 4 \text{mm}^2$ to $12 \times 12 \text{mm}^2$. Examples of a filtered CT image from TexRAD software and from Matlab using the in-house implemented LoG filters are presented in figure (2.2) and figure (2.3) respectively. Figure (2.2) and figure (2.3) show the response of the filters when there is an intensity change within the image, a bright area suggests a strong change in intensity. The histogram of the filtered image is then used to calculate the textural features of the image, hence the filtered image is not an image of texture.
Fig. 2.1 The in-house implemented LoG filters (a) LoG2 which is 4x4 mm$^2$ with $\sigma=1.0$, (b) is LoG3 which is 6x6 mm$^2$ with $\sigma=1.5$, (c) is LoG4 which is 8x8 mm$^2$ with $\sigma=1.8$, (d) is LoG5 which is 10x10 mm$^2$ with $\sigma=2.0$, (e) is LoG6 which is 12x12 mm$^2$ with $\sigma=2.5$. 

2.2 Feasibility Study
Fig. 2.2 An example of the TexRAD output for one of the investigated patient where (A) shows the delineated tumour. (B) Is the tumour filtered image using filter ssf2 (fine scale). (C) Is the tumour filtered image using filter ssf4 (medium scale). (D) Is the tumour filtered image using filter ssf6 (coarse scale).

The results of $E_{\text{global}}$ measured from the filtered CT image with the largest cross sectional area of the tumour are presented in figure (2.4). Figure (2.4) shows the results from TexRAD fine, medium and coarse filters (ssf2, ssf3, and ssf6) and the corresponding in-house implemented LoG filters (LoG2, LoG3 and LoG6). For coarse filter (ssf6), entropy measurements could not be generated for 4 patients as the filter could not detect changes in intensity within the ROI.
2.2 Feasibility Study

Fig. 2.3 An example of the output for the in-house implemented LoG filters for one of the investigated patient where (A) shows the segmented tumour. (B) Is the tumour filtered image using filter LoG2 (fine scale). (C) Is the tumour filtered image using filter LoG4 (medium scale). (D) Is the tumour filtered image using filter LoG6 (coarse scale).
Fig. 2.4 Survival as a function of entropy for TexRAD filters and corresponding filters developed in-house. (a) Fine scale filter (4x4 mm$^2$) results. (b) Medium scale filter (6x6 mm$^2$) results. (c) Coarse scale filter (12x12 mm$^2$) results.
2.2 Feasibility Study

Fig. 2.5 An example of the calculated ratio between entropy values measured from TexRAD filters (Entropy$_{TexRAD}$) to entropy values measured from the corresponding LoG filters (Entropy$_{LoG}$) for the medium size filter (ssf3).

The ratio between entropy values measured from using TexRAD filters (ssf2, ssf3, ssf4, ssf5, ssf6) to the entropy values measured from the corresponding LoG filters (LoG2, LoG3, LoG4, LoG5, LoG6) were 0.65±0.04, 0.63±0.03, 0.60±0.04, 0.57±0.07 and 0.56±0.07 respectively. The mean of the ratios means was 0.60±0.03. An example of the calculated ratio between Entropy$_{TexRAD}$ to Entropy$_{LoG}$ for the medium size filter (ssf3/LoG3) is presented in figure (2.5).

Regression analysis results conducted using the F-test are presented in table (2.2). A description of the straight line fit between entropy values and survival including regression coefficient R, and the 95% confidence levels (CI) on R and slope are provided in table (2.2). The results in table (2.2) shows no strong correlation between entropy values extracted from tumours using TexRAD filters and patients survival. The observed values of R doesn’t exceed the critical values of R that leads to $p<0.05$. For example, for ssf2 R=0.03, for this value to be significant, R needs to be $>0.45$ which is not the case. Moreover, the 95% CI for slope $b$ goes through zero indicating no strong correlation between the variables.

Despite the discrepancy in the absolute entropy values measured from the application of TexRAD filters and in-house generated filters as shown in figure (2.4), the results from Bland Altman plots presented in figure (2.6) demonstrates the two methods agree within the 95% CI.
Table 2.2 Summary of regression analysis and correlation significance between entropy values and survival for all TexRAD filters.

<table>
<thead>
<tr>
<th>Filter</th>
<th>Slope (b)</th>
<th>Intercept</th>
<th>R</th>
<th>95% CI (R)</th>
<th>95% CI (slope)</th>
<th>R values for p&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssf2 (fine)</td>
<td>0.002</td>
<td>4.39</td>
<td>0.03</td>
<td>-0.44 to 0.45</td>
<td>-0.29 to 0.29</td>
<td>0.455</td>
</tr>
<tr>
<td>ssf3</td>
<td>-0.01</td>
<td>4.47</td>
<td>0.39</td>
<td>-0.42 to 0.49</td>
<td>-0.07 to 0.05</td>
<td>0.468</td>
</tr>
<tr>
<td>ssf4</td>
<td>-0.01</td>
<td>4.32</td>
<td>0.38</td>
<td>-0.42 to 0.47</td>
<td>-0.13 to 0.11</td>
<td>0.468</td>
</tr>
<tr>
<td>ssf5</td>
<td>-0.004</td>
<td>3.84</td>
<td>0.07</td>
<td>-0.46 to 0.47</td>
<td>-0.39 to 0.38</td>
<td>0.482</td>
</tr>
<tr>
<td>ssf6 (coarse)</td>
<td>-0.01</td>
<td>3.78</td>
<td>0.17</td>
<td>-0.48 to 0.51</td>
<td>-0.65 to 0.62</td>
<td>0.497</td>
</tr>
</tbody>
</table>

The KM results using the median entropy value as the cut-off value obtained from each investigated filter is presented in table (2.3). The p-value calculated using the Log-rank test reached significance (P <0.05) for medium texture filters (ssf4) and in-house implemented filter (LoG3 and LoG4). KM results for the medium size filters show the low entropy group to have better survival than the high entropy group. The results from the ROC curves indicate that entropy is not a significant prognostic parameter given that the area under the curve (AUC) is <0.6 as shown in table (2.3). AUC values presented in this thesis are comparable to what is reported by [55].

The p-value for the TexRAD filters and the in-house implemented filters were 0.019 and 0.04 respectively when using the normalised cut-off value of 1.233 from Win et al. [153]. The KM results for normalised entropy values for both generated from TexRAD and in-house implemented filters are presented in figure (2.7). For normalised entropy, the group with low normalised entropy (high overall entropy) has a poor prognosis while the group with normalised entropy higher (low overall entropy) than the threshold value has better survival. The censored cases in the KM plots refer to subjects that have not experienced a terminal event.
2.2 Feasibility Study

Fig. 2.6 Bland Altman (difference plot) for TexRAD filters and corresponded filters generated in-house. (a) Fine scale filter (4x4 mm$^2$). (b) Medium scale filter (6x6 mm$^2$). (c) Coarse scale filter (12x12 mm$^2$).
Fig. 2.7 Kaplan-Meier curves generated for normalised entropy values measured after applying TexRAD filters where group 1 refers to low normalised entropy (<threshold of 1.233) and group 2 refers to high normalised entropy (>threshold of 1.233) (a) results for using filters developed in-house (b) results from using TexRAD software.
Table 2.3 Receiver operating characteristics (ROC) analysis results with p-value for survival analysis using Kaplan-Meier curves for TexRAD filters notated as ssf and in-house implemented filters notated LoG. The median entropy value was used as the threshold value for the KM analysis. (AUC = area under the curve)

<table>
<thead>
<tr>
<th>Filter</th>
<th>AUC</th>
<th>P-value</th>
<th>Log Rank</th>
<th>KM Threshold Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssf2 (fine)</td>
<td>0.52</td>
<td>0.53</td>
<td></td>
<td>4.4</td>
</tr>
<tr>
<td>LoG2 (fine)</td>
<td>0.60</td>
<td>0.15</td>
<td></td>
<td>6.9</td>
</tr>
<tr>
<td>ssf3</td>
<td>0.50</td>
<td>0.06</td>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td>LoG3</td>
<td>0.41</td>
<td>0.02</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>ssf4</td>
<td>0.50</td>
<td>0.04</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>LoG4</td>
<td>0.50</td>
<td>0.02</td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>ssf5</td>
<td>0.53</td>
<td>0.12</td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>LoG5</td>
<td>0.40</td>
<td>0.06</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>ssf6 (coarse)</td>
<td>0.53</td>
<td>0.16</td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td>LoG6 (coarse)</td>
<td>0.50</td>
<td>0.60</td>
<td></td>
<td>6.6</td>
</tr>
</tbody>
</table>

2.2.3 Discussion and Conclusions

The initial results obtained from TexRAD software suggest no strong prognostic correlation between tumour entropy and patients survival. Yet, p-values obtained from KM curves reached significance indicating a relationship between entropy and survival. The group of patients with low entropy (high normalised entropy) is characterised by better survival than the group of patients having high entropy (low normalised entropy). While entropy values measured from the application of in-house implemented LoG filters are different to the TexRAD values, results from Bland Altman plots and KM curves illustrated that the in-house implemented LoG filters provide comparable results. Furthermore, it is expected for the results to vary due to the lack of information regarding any image processing that is performed within the TexRAD software. Given the technical differences in acquiring the CT data between centres such as scanner manufacturers, reconstruction algorithms and scanning protocols [10], it is presumed that a normalised entropy value is needed to allow for comparison and validation between centres. The presented results showed the possibility of normalised entropy to have a prognostic role in NSCLC. Yet, due to the small number of patients, additional data are needed to validate the presented results and draw final conclusions. In the next section, a larger dataset is used to validate the presented normalised entropy results.
2.3 Validation study

2.3.1 Methods

2.3.1.1 Dataset

For this study, diagnostic 18F-FDG PET/CT scans for 49 patients diagnosed with NSCLC were collected retrospectively. The patient group has a heterogeneous histology and TNM staging. The group consists of 28 squamous cell, 12 adenocarcinoma and 9 of unknown NSCLC histology. The TNM staging range was between T2N0 to T4N3. The CT scans were acquired with a peak tube voltage of 140kVp and a current of 100mAs, and were reconstructed onto an image matrix in which each slice contained 512x512 voxels with a voxel size of 0.98x0.98mm². The slice thickness was 5mm. This study obtained local ethics approval from the Royal Surrey County Hospital NHS Foundation Trust.

2.3.1.2 Texture Analysis

The collected scans were anonymised and imported into the TexRAD software. The ROI was delineated at the largest cross-sectional area of the primary tumour by a trained observer (the author) under the supervision of a consultant radiologist (VP). The LoG filters available in TexRAD (table (2.1)) were then applied to the delineated ROI. E\textsubscript{global} was then calculated from the histogram of the filtered tumour image. The entropy values were exported into Matlab software (The MathWorks, Inc, Natick, MA) for analysis. The normalised entropy (medium-filter value/coarse-filter value) as suggested by Win et al. 2013 [153] was calculated.

2.3.1.3 Survival Analysis

To dichotomise the patients into groups of good and poor survival, KM survival analysis was conducted using IBM SPSS version 22.0. The threshold value for normalised entropy was set to two levels; the median of the dataset entropy values and to the value previously published by [153] of 1.233. The statistical significance of the differences between the KM survival curves for each group was then
2.3 Validation study

<table>
<thead>
<tr>
<th>Threshold Type</th>
<th>Median</th>
<th>Win et al [153]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold Value</td>
<td>1.167</td>
<td>1.233</td>
</tr>
<tr>
<td>P-value</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

assessed using the log-rank test, with the null hypothesis of no difference between the two groups. p-values < 0.05 is considered significant.

2.3.2 Results

2.3.2.1 Survival Analysis

In the dataset of patients, at the time of analysis, the mean survival time was 21.5 months (range 3.0 to 52.0) with 34% of patients alive. Figure (2.8) shows the KM curves for the two investigated threshold values. Table (2.4) shows the threshold values and their corresponding log-rank p-values. Both threshold, the median value from this study dataset and the previously published threshold value from [153] reached significance of 0.02 and 0.03 respectively. The KM curves in figure (2.8) show patients with tumours of high normalised entropy >threshold of 1.233 (low overall entropy denoted as group 2 in figure (2.8)) to have better survival. While patients with tumours of low normalised entropy <threshold of 1.233 (high overall entropy denoted as group 1 in figure (2.8)) were categorised to have poor survival. Figure (2.9) is an example of filtered CT images from two patients. The patient in figure (2.9A) is diagnosed with squamous cell NSCLC stage T3N2 with a known survival of 3.05 months where the patient B in figure (2.9B) was also diagnosed with squamous cell NSCLC stage T4N2 with a survival time of 35.4 months. The filtered tumour CT images shown in figure (2.9) highlight the different textures associated with the tumours. The patient B whose tumour had high normalised entropy (low overall entropy) was categorised into the group with good prognosis using both investigated threshold levels. Conversely, patient A with low normalised entropy (high overall entropy) value was categorised into the group with poor prognosis using the investigated threshold levels.
Fig. 2.8 Kaplan-Meier curves generated for normalised entropy values, group 1 refers to low normalised entropy (<1.233) and group 2 refers to high normalised entropy (>1.233). (a) Using the median entropy value as a threshold. (b) Using the previously published [153] threshold of 1.233.
Fig. 2.9 (A) Tumour CT image with low normalised entropy and associated with poor survival group while (B) is a tumour CT image with high normalised entropy and associated with good survival group. (1) Is the filtered tumour image using small-sized filter. (2) Is filtered tumour image using medium-sized filter. (3) Is filtered tumour image using larger-sized filter.

### 2.3.3 Discussion and Conclusions

In this chapter, an analysis of entropy as a measure of texture from CT tumour images as a potential prognostic tool in NSCLC was presented. A validation of the in-house generated LoG filters against the first commercial software (TexRAD) to measure tumour texture from CT images was carried out. In the feasibility study, I showed the performance of our in-house implemented LoG filters is comparable to that of TexRAD.

Previously published studies using the TexRAD software used optimised parameters to dichotomise patients into good and poor survival groups. The application of these threshold values outside the investigated dataset was not assessed due to the variation of the obtained entropy values given the technical differences (scanner manufacturers, reconstruction algorithms, scanning protocol) between centres. In this chapter, I investigated the application of a derived normalised entropy value from the study published by Win et al. [153] on a different cohort from a different centre. To test this hypothesis, the methodology used by Win et al. [153] was applied to a new group of patient data and the same value of normalised entropy was used as a threshold. In addition, the median value of normalised entropy as a threshold value was also tested for significance to allow for a fair dichotomisation of the data. The study by Win et al. [153] had a patients cohort of 66 diagnosed with
NSCLC (16 squamous cell, 32 adenocarcinoma, 18 unspecified NSCLC) with mixed staging starting at stage 1A to stage 4 where our patient cohort didn’t include stage 1 NSCLC. Although the PET/CT scanner manufacturer is similar between the two studies (GE Healthcare), the scanner model and scanning acquisition parameters are different. The Win et al.[153] study acquired the images using a VCT-XT Discovery GE scanner with 100-150mAs, 80kVp and 3.27\,mm slice thickness. Images for our study were acquired on a GE Discovery LS scanner with 100mAs, 150kVp and 5.00\,mm slice thickness. Yet, the survival analysis using the optimally derived normalised entropy value from Win et al. [153] was shown to yield significance at p<0.05 level, indicating that survival was significantly longer in the patients whose normalised entropy was greater than 1.233. This shows that the Win et al. [153] parameters would have yielded predictive power with this dataset. This is an important finding, since it implies that parameters obtained from one study could potentially be used generically to predict survival for other patients, despite the differences in patients cohort and in imaging equipment, etc. Furthermore, using the median value of the normalised entropy as a threshold yielded significant results as well. The preliminary results from this chapter raises a question about the physiological underlining of tumour CT texture and whether the measured entropy value correlates with tumour function.

To try to better understand tumour CT image texture, I propose investigating tumour image texture on a voxel based level. Given that the texture analysis method presented in this chapter using the LoG filters is a global method that is applied to a single CT slice and does not analyse spatial variation in texture, I propose the use of the second-order statistical texture analysis method of grey level co-occurrence matrices (GLCM). I propose the use of GLCM to generate a volumetric map of tumour texture to allow the assessment of spatial variation in tumour texture in a CT image. GLCM has been shown in the literature to discriminate between normal and abnormal (disease and cancerous) tissue [147][15]. Furthermore, entropy measured from GLCM was reported to be significantly different for patients who recurred after receiving stereotactic ablative radiotherapy (SABR) for NSCLC by Mattonen et al [98] [99]. To investigate the relationship between CT tumour texture and tumour function, I propose the comparison between the tumour’s 3D texture map and functional images of 18F-FDG PET. To do so, a novel method of generating volumetric voxel based tumour texture maps was developed. Given that respiratory motion will degrade image quality and consequently affects the results of texture analysis, a phantom study was conducted to determine the effect of respiratory motion on the performance of the the different size filters. The phantom study is presented in chapter...
(3). The results from chapter (3) strongly influenced the development of our voxel-based GLCM filter that will be presented in chapter (4). The comparison between the generated volumetric voxel based texture maps using our developed methodology and functional data is presented in chapter (5).
Chapter 3

Effects of Respiratory Motion on Textural Features: A Phantom Study

3.1 Introduction

In recent years, the use of texture analysis in predicting tumour response to treatment has been suggested \[10\]. Yet, texture analysis in CT is dependent on image spatial resolution which is deteriorated by respiratory motion. The aim of this work was to characterise the effects of respiratory motion on the performance of the in-house implemented Laplacian of Gaussian (LoG) filters in extracting textural features. Features extracted from such filters have been shown in the literature \[53\] \[56\] and in chapter (2) to correlate with patient survival in non-small cell lung cancer (NSCLC). To do so, we designed a phantom experiment to characterise the performance of the CT scanner and the LoG filters at different spatial frequencies in the presence of respiratory motion. The performance of the CT scanner and the effects on the LoG filters were characterised by measuring the Modulation Transfer Function (MTF). The MTF provides a description of the sharpness of the imaging system. Also, the LoG filters response at different spatial frequencies allows a better understanding of the structure size of the extracted textural features. Moreover, characterising the response of the LoG filters of different sizes at different spatial frequencies in the presence of respiratory motion allows us to choose the optimum filter size to design our proposed texture mapping filter.
In this chapter, we present our multi-spatial scale (MUSS) in-house developed phantom designed specifically to measure the effects of spatial heterogeneity. CT images of the MUSS phantom were acquired while static and in-motion and the MTF of the scanner system was measured using these images. The performance of the LoG filters was also measured for the static and in-motion acquisition.

3.2 Methods

3.2.1 Phantom Design

The multi-spatial scale (MUSS) phantom was made of Polymethyl methacrylate (PMMA). PMMA is a water equivalent material extensively used in constructing radiation dosimetry phantoms [66]. The MUSS phantom designed for this work has a size of $131\times121\times30\text{mm}^3$. The phantom has four sections, each section contains a square lattice of a different size made to be able to resemble a checker-board pattern. The square lattice sections included hollow cubes where the cubes can be filled with a solution to allow arbitrary density variations to be imaged. The sizes of the cubes in the four sections are $4\times4\text{mm}^2$, $6\times6\text{mm}^2$, $8\times8\text{mm}^2$, $10\times10\text{mm}^2$ where $4\times4\text{mm}^2$ cube is the smallest feasible size to manufacture and fill. The MUSS phantom design is shown in figure (3.1). The phantom has a PMMA top that attaches to prevent the solution that fills the lattice from spilling. A plastic extension was designed to attach the MUSS phantom to a system that simulates respiratory motion.
Fig. 3.1 The MUSS phantom design. The phantom has four regions that can be filled with contrast agent to exhibit a desired pattern of high and low contrast regions.
3.2.2 Experimental Design

To measure the MTF of the system, for each of the four sections within the phantom, every other cube in the square lattice was filled to produce a pattern with alternate cubes left empty as seen in figure (3.2). This allowed measurement of the MTF as discussed in section (3.2.4). The cubes were filled with a solution of sucrose and high purity water mixture to simulate tissue. The solution had 8% sucrose concentration, the percentage concentration of the solution was calculated as the fraction by weight of the sucrose.

To conduct the experiment, the phantom was attached to the motor of a dynamic thorax phantom (CIRS Company, Virginia, USA). The dynamic thorax phantom platform was controlled via a computer to simulate respiratory motion as shown in figure (3.3). The programmed respiratory motion followed a sine wave with a period of 4 seconds and an amplitude of $1.00 \text{cm}$ with a total displacement of $2\text{cm}$ in the superior-inferior direction. During the experiment, the phantom was placed on solid water slabs to raise it to the height of the motor attachment as shown in figure (3.3).

3.2.3 Data Acquisition

The MUSS phantom was scanned using a GE discovery CT scanner (GE healthcare, Ohio, USA) with 120kVp and 78mAs acquisition parameters. The reconstructed transaxial image size was 512x512 with $0.98 \times 0.98 \times 1.25 \text{mm}$ voxel size. The reconstruction algorithm was Filtered Back Projection (FBP). The phantom was first aligned using the scanner’s alignment lasers and then scanned while static, and then in-motion using the same scanning parameters.

3.2.4 Data Analysis

The data were re-sampled in the direction of motion (coronal view) and the centre slice was chosen for the analysis. The phantom was moving in the superior-inferior direction while being scanned, hence the data needed to be re-sampled in this direction to analyse the effects of motion on the data. To characterise the effects of motion on the scanner spatial resolution, the MTF of the static and the moving data were calculated using the edge method to provide a numerical description of the imaging system response at different spatial frequencies [77]. To evaluate the performance of the LoG filters
Fig. 3.2 The MUSS phantom solution filling arrangement showing in red. The filling arrangement was designed to produce a bar pattern to conduct the MTF measurements.
Fig. 3.3 (a) The Experimental Set-up. (b) A Close-up of the MUSS phantom during the experiment.
at different spatial frequencies in the presence of motion, the MTF of the LoG filters was calculated using the Fourier Transform (FT). The performance of the LoG filters in combination with the scanner resolution was assessed by measuring the MTF of the phantom filtered images using the Standard Deviation method [46].

### 3.2.4.1 The Modulation Transfer Function of The CT Scanner

The MTF is the Fourier space amplitude of the response to a delta function input whereas the line spread function (LSF) is the equivalent response in the spatial domain. MTF is the modulus (magnitude) of the normalised frequency response of the imaging system, also known as the optical transfer function (OTF) as shown in equation (3.1) where $u$ and $v$ are the spatial frequency variables.

\[
MTF(u,v) = |OTF(u,v)| \quad (3.1)
\]

The OTF describes the ability of an imaging system to pass information and it consists of MTF (the magnitude) and the imaginary part known as the phase transfer function (PTF) as shown in equation (3.2).

\[
OTF(u,v) = MTF(u,v)e^{iPTF(u,v)} \quad (3.2)
\]
Effects of Respiratory Motion on Textural Features: A Phantom Study

The MTF can be measured by taking the FT of the line spread function (LSF). Traditionally, the LSF is calculated by differentiating the edge response function (ERF). The ERF is obtained by taking a line profile through an imaged edge. In this experiment, the imaged edge was the transition region between the sucrose-water mixture and air (filled and unfilled cube). Measuring the transition between the high to low density region (bright to dark area on CT image) can provide an estimate of the spatial impulse response of an imaging system. Calculating the FT of this impulse response will provide the frequency response of the system as the MTF. A schematic illustration of MTF calculation using the edge method is presented in figure (3.4).

To calculate the MTF of the scanner, the ERF was obtained by taking a line profile through an edge between filled and unfilled cubes within the phantom. The ERFs were measured for the static and in-motion data experimentally by plotting the raw data points obtained from the phantom CT images. The scanner ERF for the static acquisition is labelled as $\text{ERF}_{\text{Exp,static}}$ and the scanner ERF for the moving acquisition is labelled as $\text{ERF}_{\text{Exp,motion}}$. Due to the noise in the acquired data, fitted ERFs were obtained by iteratively fitting a sigmoid function to the raw data points of the $\text{ERF}_{\text{Exp,static}}$ and $\text{ERF}_{\text{Exp,motion}}$. The sigmoid function is shown in equation (3.3), where $B_{\text{max}}$ is the maximum value for the curve, $t$ is the slope of the curve, $w_{50}$ is the midpoint of the curve and $w$ is the point on the sigmoid curve.
3.2 Methods

\[ ERF_{\text{fitted}} = \frac{B_{\text{max}}}{1 + e^{-t(w_{50}-w)}} \]  

(3.3)

The goodness of fit was assessed by calculating the root mean squared error (RMSE) as presented in equation (3.4). In equation (3.4), the measured value is \( y_i \), the fitted value is \( y'_i \) and \( N \) is the sample size [11].

\[ RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - y'_i)^2} \]  

(3.4)

The modelled ERFs are labelled ERF\text{Fitted}_{\text{static}} and ERF\text{Fitted}_{\text{motion}} respectively. The ERF\text{Exp}_{\text{static}} and the ERF\text{Exp}_{\text{motion}} were differentiated to obtain the LSF\text{Exp}_{\text{static}} and LSF\text{Exp}_{\text{motion}} respectively; the ERF\text{Fitted}_{\text{static}} and ERF\text{Fitted}_{\text{motion}} were differentiated to obtain the LSF\text{Fitted}_{\text{static}} and LSF\text{Fitted}_{\text{motion}}.

To validate the calculated LSF\text{Fitted}_{\text{motion}}, a predicted LSF in the presence of motion (labelled LSF\text{Predict}_{\text{motion}}) was calculated by convolving the LSF\text{Fitted}_{\text{static}} with the probability density function (PDF) of the programmed sine wave (equation(3.5)). The amplitude of the sine wave is termed \( R \) which in this experiment is equal to 1 cm while \( x \) is a sampling data point.
Effects of Respiratory Motion on Textural Features: A Phantom Study

\[ PDF_{sine} = \frac{1}{\pi \sqrt{R^2 - x^2}} \]  

(3.5)

The agreement between the LSF\textsubscript{Fitted\_motion} and LSF\textsubscript{Predict\_motion} was evaluated in the terms of the full width half maximum (FWHM) measured from the two distributions. The FWHM is measured as the width of a distribution at half of its maximum value. The FWHM of LSF is a measure of an imaging system’s resolution. The scanner MTFs during the static and the in-motion acquisition were calculated by taking the FT of the LSF\textsubscript{Fitted\_static}, LSF\textsubscript{Fitted\_motion} and LSF\textsubscript{Predict\_motion}. The calculated MTFs were labelled MTF\textsubscript{Fitted\_static}, MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} respectively. The agreement between the MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} was measured by conducting a two sample t-test and calculating the p-value for significance. The calculated MTFs were normalised by dividing the MTF curve by its maximum value. The MTF measurement was analysed in terms of MTF50 and MTF10. MTF50 is the spatial frequency where the MTF is reduced to 50% and MTF10 is spatial frequency where the MTF is reduced to 10%. Traditionally, MTF50 and MTF10 are quoted given they have been found to be good indicators of the imaging system resolution [133].

3.2.4.2 The Modulation Transfer Function of The In-house Implemented LoG Filters

The MTF of the LoG filters was calculated by taking the FT of the filters to evaluate their performance. The LoG filters are 2D isotropic filters; the diagonal of the LoG filter was used to calculate the MTF of the LoG filters. As presented in chapter (2), the widths of the investigated LoG filters are presented in table (3.1). The MTF curves of the LoG filters for the static acquisition are labelled MTF\textsubscript{LoG\_static}. The MTFs of LoG filters in the presence of motion (MTF\textsubscript{LoG\_motion}) were calculated by multiplying the MTF\textsubscript{LoG\_static} with the scanner’s MTF\textsubscript{Fitted\_motion} as shown in equation (3.6).
Table 3.1 Size of the in-house implemented LoG filters where $\sigma$ is a scaling factor related to the smoothing effect of the filter.

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Filter size ($mm^2$)</th>
<th>$\sigma$ size</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoG2</td>
<td>4x4</td>
<td>1.0</td>
</tr>
<tr>
<td>LoG3</td>
<td>6x6</td>
<td>1.5</td>
</tr>
<tr>
<td>LoG4</td>
<td>8x8</td>
<td>1.8</td>
</tr>
<tr>
<td>LoG5</td>
<td>10x10</td>
<td>2.0</td>
</tr>
<tr>
<td>LoG6</td>
<td>12x12</td>
<td>2.5</td>
</tr>
</tbody>
</table>

$$MTF_{\text{LoG motion}} = MTF_{\text{LoG static}} * MTF_{\text{Fitted motion}}$$ (3.6)

To validate the measured $MTF_{\text{LoG static}}$, the $MTF_{\text{LoG+scanner}}$ were measured for the LoG filters using the standard deviation (SD) method by Droge and Morin 1982 [46]. The $MTF_{\text{LoG+scanner}}$ is measured from the filtered images of the phantom (the image is convolved with the LoG filter); hence, it is a measure of the LoG filters performance in combination with the scanner response. In the SD method, the $MTF_{\text{LoG+scanner}}$ measurement depends on calculating the modulation $M(f)$ as the SD of the voxels’ Hounsfield unit (HU) values within a region (ROI) of a cyclic bar pattern. It has been shown by Coltman [35] that MTF can be measured by measuring the output amplitude $A(f)$ from the images of a cyclic bar pattern. The output of imaging a cyclic bar pattern is a sinusoidal wave with amplitude $A_0$. As reported by Coltman, the MTF (labelled as $MTF_{\text{LoG+scanner}}$) is expressed as in equation (3.7). In equation (3.7), the square wave is considered as a sum of frequencies $f, 3f, 5f, \ldots, fc$.
from the sinusoidal component of the square wave where $f_c$ is the frequency where the MTF is zero [46].

$$MTF_{\text{LoG+scanner}} = \frac{\pi A(f)}{4A_0}, \quad f \geq f_c/3 \quad (3.7)$$

Given that the square wave variance $M_0^2 = A^2$, and the sinusoidal signal variance $M(f)^2 = \frac{1}{2}A^2$ [46], equation (3.7) simplifies to

$$MTF_{\text{LoG+scanner}} = \frac{\pi \sqrt{2}}{4} \frac{M(f)}{M_0} \quad (3.8)$$

The $M(f)$ is corrected for noise by substracting the SD of a uniform region (Bg) in the phantom. The input modulation $M_0$ is measured as the difference between the mean of the CT numbers of the highest response and the lowest response regions in the image [46].

To calculate $MTF_{\text{LoG+scanner}}$ for the LoG filters, each filter was convolved with the phantom image and then the filtered images were used to carry out the calculations. $MTF_{\text{LoG+scanner}}$ was calculated for each of the four sections in the MUSS phantom representing four spatial frequencies of 0.08, 0.1, 0.12, and 0.166 mm$^{-1}$. An illustration of the background region (Bg) and the ROIs are presented in figure (3.5). The $MTF_{\text{LoG+scanner}}$ was calculated for each section of the square lattice in four different regions within the lattice and the average value was used. The error was calculated as $\pm 1$ standard
3.2 Methods

Fig. 3.5 An illustration of the ROI regions and Bg region used to calculate the MTF_{LoG+scanner} for the in-house implemented LoG filters.

deviation of the four measurements. The calculated MTFs and MTF_{LoG+scanner} were normalised by dividing the MTF curve by the highest MTF value. The MTF measurements were quoted in terms of MTF50 and MTF10. A list of the ERF, LSF and MTF terms used in this study are summarised in table (3.2).
Table 3.2 A list definitions of ERF, LSF and MTF abbreviations used in this study.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERF</strong></td>
<td></td>
</tr>
<tr>
<td>$\text{ERF}_{\text{Exp, static}}$</td>
<td>Obtained by taking a line profile through an edge in the static phantom image</td>
</tr>
<tr>
<td>$\text{ERF}_{\text{Exp, motion}}$</td>
<td>Obtained by taking a line profile through an edge in the moving phantom image</td>
</tr>
<tr>
<td>$\text{ERF}_{\text{Fitted, static}}$</td>
<td>Obtained by fitting a sigmoid function to the raw data points of the ERF_{Exp, static}</td>
</tr>
<tr>
<td>$\text{ERF}_{\text{Fitted, motion}}$</td>
<td>Obtained by fitting a sigmoid function to the raw data points of the ERF_{Exp, motion}</td>
</tr>
<tr>
<td><strong>LSF</strong></td>
<td></td>
</tr>
<tr>
<td>$\text{LSF}_{\text{Exp, static}}$</td>
<td>Obtained by differentiating the ERF_{Exp, static}</td>
</tr>
<tr>
<td>$\text{LSF}_{\text{Exp, motion}}$</td>
<td>Obtained by differentiating the ERF_{Exp, motion}</td>
</tr>
<tr>
<td>$\text{LSF}_{\text{Fitted, static}}$</td>
<td>Obtained by differentiating the ERF_{Fitted, static}</td>
</tr>
<tr>
<td>$\text{LSF}_{\text{Fitted, motion}}$</td>
<td>Obtained by differentiating the ERF_{Fitted, motion}</td>
</tr>
<tr>
<td>$\text{LSF}_{\text{Predict, motion}}$</td>
<td>Obtained by convolving LSF_{Fitted, static} and the PDF_{sine}</td>
</tr>
<tr>
<td><strong>MTF</strong></td>
<td></td>
</tr>
<tr>
<td>$\text{MTF}_{\text{Fitted, static}}$</td>
<td>Obtained by taking the FT of the LSF_{Fitted, static}</td>
</tr>
<tr>
<td>$\text{MTF}_{\text{Fitted, motion}}$</td>
<td>Obtained by taking the FT of the LSF_{Fitted, motion}</td>
</tr>
<tr>
<td>$\text{MTF}_{\text{Predict, motion}}$</td>
<td>Obtained by taking the FT of the LSF_{Predict, motion}</td>
</tr>
<tr>
<td>$\text{MTF}_{\text{LoG, static}}$</td>
<td>Obtained by taking the FT of the LoG filter</td>
</tr>
<tr>
<td>$\text{MTF}_{\text{LoG, motion}}$</td>
<td>Obtained by multiplying MTF_{LoG, static} by MTF_{Fitted, motion}</td>
</tr>
<tr>
<td>$\text{MTF}_{\text{LoG+scanner}}$</td>
<td>Obtained by calculating the MTF using the SD method from the filtered phantom image</td>
</tr>
</tbody>
</table>
3.3 Results

3.3.1 The Modulation Transfer Function of The CT Scanner

The MUSS phantom re-sampled (coronal view) CT images for the static and the moving acquisitions are presented in figure (3.6) and figure (3.7) respectively. Figure (3.8a) shows $\text{ERF}_{\text{Exp}\_\text{static}}$ and $\text{ERF}_{\text{Fitted}\_\text{static}}$ for the static acquisition. $\text{ERF}_{\text{Exp}\_\text{motion}}$ and $\text{ERF}_{\text{Fitted}\_\text{motion}}$ from the moving phantom CT images are presented in figure (3.8b).

Fig. 3.6 The re-sampled CT image of the MUSS phantom for the static acquisition.

Fig. 3.7 The re-sampled CT image of the MUSS phantom for the motion acquisition.
The experimental and modelled LSFs for the static and in-motion data are presented in figure (3.9). The FWHM measured from $\text{LSF}_{\text{Fitted static}}$ and $\text{LSF}_{\text{Fitted motion}}$ were 1.3mm and 2.7mm respectively. $\text{LSF}_{\text{Fitted static}}$, $\text{LSF}_{\text{Fitted motion}}$ and $\text{LSF}_{\text{Predict motion}}$ are presented in figure (3.10). As hypothesized, the presence of respiratory motion degrades the resolution of the CT scanner as shown in figure (3.10). The FWHM of the $\text{LSF}_{\text{Predict motion}}$ (which is obtained by convolving the PDF of the programmed sine wave with the $\text{LSF}_{\text{Fitted static}}$) was 2.7mm which in agreement with the FWHM of the $\text{LSF}_{\text{Fitted motion}}$. Although there is some discrepancy between the shape of the $\text{LSF}_{\text{Fitted motion}}$ and $\text{LSF}_{\text{Predict motion}}$, the FWHM of these two LSFs agree where both were measured as 2.7mm.

![Fig. 3.8 The ERF of the static and the moving acquisition of the MUSS phantom.](image1)

![Fig. 3.9 The LSF of the static and the moving acquisition of the MUSS phantom. PDF is the probability density function.](image2)
3.3 Results

Fig. 3.10 LSF\textsubscript{Fitted\_static}, LSF\textsubscript{Fitted\_motion} and LSF\textsubscript{Predict\_motion}. PDF is the probability density function.

MTF\textsubscript{Fitted\_static}, MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} are presented in figure (3.11). The MTF curves were calculated by taking the FT of LSF\textsubscript{Fitted\_static}, LSF\textsubscript{Fitted\_motion} and LSF\textsubscript{Predict\_motion} respectively. The MTFs presented in figure (3.11) behave as predicted where the response of the system is superior when the phantom is static and it degrades with motion due to image blurring as expected. The limiting resolution of the scanner measured in spatial frequency in terms of MTF\textsubscript{50} is 0.4\textit{mm}\textsuperscript{−1}, 0.2\textit{mm}\textsuperscript{−1} and 0.15\textit{mm}\textsuperscript{−1} for MTF\textsubscript{Fitted\_static}, MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} respectively. The MTF\textsubscript{10} was measured as 0.6\textit{mm}\textsuperscript{−1}, 0.3\textit{mm}\textsuperscript{−1} and 0.4\textit{mm}\textsuperscript{−1} for MTF\textsubscript{Fitted\_static}, MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} respectively. Given MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} appears slightly different, there is a drop in the MTF\textsubscript{Fitted\_motion} due to the value going to negative before taking the modulus of the calculated FT. To assess the statistical significance of the difference, a two sample t-test was used. The p-value was calculated between MTF\textsubscript{Fitted\_motion} and MTF\textsubscript{Predict\_motion} and was found to be 0.6 which is statistically insignificant.
Effects of Respiratory Motion on Textural Features: A Phantom Study

3.3.2 The Modulation Transfer Function of The In-house Implemented LoG Filters

Figure (3.12) shows results of the filtered CT images of the static phantom (images convolved with the LoG filters). The results of convolving the moving phantom CT images with the LoG filters are presented in figure (3.13). As shown in figure (3.12), the LoG filters performance in detecting regions of intensity changes is highest when the filter width matches the size of the structure detected. For example, in figure (3.12b) the image was convolved with filter LoG3 which is $6\times6\text{mm}^2$ and the response of the filter was highest for the region with $6\times6\text{mm}^2$ square lattice (bottom right region). However, structures smaller than the filter size can still have a high response if their size is close to that of the filter. The response of the filter is considered highest when the entire structure is detected (bright region) by the filter not just the edges of a structure. Figure (3.13) demonstrates how the filter performance is affected by motion; the blurring of structures limits the filter’s detection ability. The smearing of the structures due to motion hinders the performance of the filters which will affect the extraction of textural features.
Fig. 3.12 The static phantom CT images filtered by the LoG filters. (a) Image filtered with LoG2. (b) Image filtered with LoG3. (c) Image filtered with LoG4. (d) Image filtered with LoG5. (e) Image filtered with LoG6. Refer to table (3.1) for filter sizes.
Fig. 3.13 The moving phantom CT images filtered by the LoG filters where the direction of motion is top to bottom. (a) Image filtered with LoG2. (b) Image filtered with LoG3. (c) Image filtered with LoG4. (d) Image filtered with LoG5. (e) Image filtered with LoG6. Refer to table (3.1) for filter sizes.
3.3 Results

The MTF_{LoG, static} measured using the FT method and the MTF_{LoG+scanner} measured using the SD method are presented in figure (3.14). The error bars for the MTF_{LoG+scanner} represents ±1 standard deviation of the mean values of the MTF_{LoG+scanner} measurements. The results in figure (3.14a) for MTF_{LoG, static} (blue dashed line) indicates that small-size LoG filter covers a wider frequency range than larger filters. Yet, the small LoG filter performance is limited by the scanner’s MTF given that the scanner’s MTF covers a narrower frequency range than the small LoG filter as seen in figure (3.14a). The medium-size LoG filter appears to cover the frequency range allowed by the scanner’s MTF as seen in figure (3.14b). The results of the MTF_{LoG+scanner} (black points) shows that the scanner’s resolution largest effect on the LoG performance was prominent in the high spatial frequency (0.166 mm\(^{-1}\)) for the larger filters as seen in figure (3.14d,e). The medium-size filters MTFs in figure (3.14b,c) show the best agreement between the MTF measured using the FT and the SD method indicating a minimal effect of scanner’s resolution on the performance of the LoG filters.

The MTF_{LoG, motion} results for the LoG filters measured by multiplying the MTF_{LoG, static} and the scanner MTF_{Fitted, motion} are presented in figure (3.15). The results in figure (3.15) show that the performance of the LoG filters is limited by the scanner resolution in the presence of motion. Calculating the MTF_{LoG, motion} using the SD method was challenging due to difficulties in ROI selection given the motion effects hence it was not included in the analysis.
Fig. 3.14 MTF for the LoG filters in static using the FT and SD methods. (a) MTF of LoG2, (b) MTF of LoG3, (c) MTF of LoG4, (d) MTF of LoG5, (e) MTF of LoG6.
3.3 Results

Fig. 3.15 MTF for the scanner and the LoG filters in static and in presence of respiratory motion. (a) MTF of LoG2, (b) MTF of LoG3, (c) MTF of LoG4, (d) MTF of LoG5, (e) MTF of LoG6.
The MTF50 and MTF10 results are quoted in the spatial domain (\textit{mm}) to contextualise the spatial scale of structures that can be detected within an image. The limiting resolution of the scanner, measured as MTF50 and MTF10, was 3.3\textit{mm} and 1.6\textit{mm} for the static acquisition and 6.6\textit{mm} and 3.3\textit{mm} for the moving acquisition respectively. The MTF50 and MTF10 for the LoG filters static and in the presence of motion are presented in figure (3.16) and figure (3.17) respectively. The results demonstrated a loss of information when using small-size filters due to the limiting resolution of the scanner. As seen in figure (3.16) and figure (3.17), the static MTF50 and MTF10 (blue points) for the 4x4\textit{mm}$^2$ LoG filter (MTF50=2.8\textit{mm}, MTF10=1.3\textit{mm}) were lower than the scanner’s MTFs (MTF50=3.3\textit{mm}, MTF10=1.6\textit{mm}) indicating it can detect structures smaller than the scanner resolution. In the presence of motion the small filters MTFs (MTF50=7.1\textit{mm}, MTF10=3.8\textit{mm}) were higher than the scanner’s MTF (MTF50=6.6\textit{mm}, MTF10=3.3\textit{mm}). These results show that the smaller filter is most affected by motion in comparison to the medium-size filters (6x6\textit{mm}$^2$ and 8x8\textit{mm}$^2$). The medium-size filters 6x6\textit{mm}$^2$ and 8x8\textit{mm}$^2$ static MTF50 were 4.0\textit{mm} and 5.0\textit{mm} respectively and the in-motion MTF50 were 6.6\textit{mm} and 6.8\textit{mm} respectively. The medium-size filters MTFs are the closest to the scanner’s MTF in the presence of motion. The larger-size filters are less affected by motion due to their narrower bandwidth as shown in figure (3.16) and figure (3.17) where the 12x12\textit{mm}$^2$ filter’s MTF50 and MTF10 are the same in static and in presence of motion.
3.4 Discussion and Conclusions

The aim of the experiment was to characterise the effects of respiratory motion on the resolution of CT images and the consequent performance of the LoG filters. The characterisation of respiratory motion effects on different size of LoG filters allowed the conceptualization of the optimal filter size to design a voxel based texture mapping filter to continue this project. To do so, the MTF was measured for the CT scanner and the LoG filters from data acquired during the static and the moving acquisition of an in-house designed phantom (MUSS phantom). The MUSS phantom has a multi-spatial scale square lattice to reflect a desired spatial heterogeneity.

The results from the experiment presented in this chapter supports the hypothesis of a degraded imaging system resolution when respiratory motion is present. The MTF of the in-house implemented LoG filters were measured using the FT and the SD method. The MTF\textsubscript{LoG+scanner} results showed that the scanner’s resolution had affected the performance of the larger-size filters particularly in the high frequency range. Also, MTF\textsubscript{LoG+scanner} calculations could have been affected by the region selection process: to measure the MTF\textsubscript{LoG+scanner}, the ROI was selected manually which could have affected the results. Moreover, the MTF\textsubscript{LoG+scanner} measurement could have been affected by image noise.
Although the $M(f)$ was corrected for noise by subtracting the standard deviation of the Bg region which is the uniform PMMA phantom, the PMMA material is not completely homogeneous.

The MTF results for the LoG filters showed that the small-size LoG filters will cover a wider spatial frequency range when compared to the larger filters. However, the frequency range covered by small-size LoG filters is not fully achieved due to the limiting resolution of the scanner as shown in figure (3.14a). When motion is present, the larger filters’ frequency range will get narrower as shown in figure (3.15e). The medium-size filters appear to behave better and cover the frequency range allowed by the MTF of the scanner when motion is introduced.

The LoG filter is a combination of a smoothing Gaussian filter for noise reduction and a Laplacian for intensity changes detection, the filters with larger widths (larger sigma) will have a greater smoothing effect which is in combination with the blurring due to motion will further blur the image. The small-size filters’ performance was shown to be mostly affected by motion in comparison as shown in figure (3.16) and figure (3.17). The medium-size filters appeared to cover the frequency range allowed by the combined MTF of the scanner and respiratory motion. Based on the results presented in this chapter, a medium-size filter will be mainly investigated in mapping tumour texture as it will be discussed and presented in chapter (4) and chapter (5).
Chapter 4

Methodology For Optimised Volumetric Voxel Based Texture Mapping

4.1 Introduction

The purpose of this chapter is to outline and explain the proposed methodology for producing volumetric voxel based texture maps (3D-VTM). Details of the texture filter developed by the author for optimally generating a tumour texture map to visually assess tumour heterogeneity is presented. An investigation of the optimum method to quantise tumour images prior to texture analysis allowing optimum texture information extraction is introduced.

4.1.1 Second Order Statistics Texture Analysis

Grey Level Co-occurrence Matrices

Second order textural features extracted from images account for the effects of the spatial location of a pixel intensity relative to another. These features can be calculated using the grey level co-occurrence matrix (GLCM) [61]. The GLCM provides spatial information and has been reported in the literature to discriminate between normal and abnormal tissue [147][15]. Furthermore, Mattonen et al. [98][99] reported textural features measured from GLCM to be significantly different for patients
who experienced disease recurrence after receiving stereotactic ablative radiotherapy (SABR) for NSCLC. I propose the use of GLCM to assess tumour heterogeneity on a voxel by voxel basis by producing volumetric voxel based maps of the tumour image.

The GLCM $P_{\theta d}^\theta(i, j)$ is a two dimensional (2D) histogram of grey values $i$ and $j$. $P_{\theta d}^\theta(i, j)$ specifies the probability that a pixel with a grey level $j$ is located at a distance $d$ and direction $\theta$ from a pixel with grey level $i$ at a total of $N$ grey levels. For analysis in 2D images, $\theta = 0^\circ, 45^\circ, 90^\circ, 135^\circ$ as seen in figure (4.1) and table (4.1) [61]. The diagonal of the GLCM represents the probabilities that 2 neighbour pixels have the same grey value. A total of 14 textural features can be extracted from the co-occurrence matrix as reported by [61] and listed in table (1.2) in chapter (1).
In this thesis, the traditional 2D GLCM was extended to three dimensional (3D) space to accommodate the volumetric imaging data. To do so, the GLCM was calculated from the 3D volume in 3 directions \((x, y, z)\) as seen in figure (4.2). The displacement between voxels position will be denoted as \(d(x, y, z)\). Given that a voxel is surrounded by 26 neighbouring voxels in the 3D space, the displacement between voxels can be calculated in 13 directions. Using the Euclidean distance at \(d=1\), the displacements can be characterized using 2 angles; \(\theta\) is the angle between the \(x\) and \(y\) plane and \(\Phi\) is the angle between the voxels of interest and the \(z\) plan as shown in figure (4.3). \(\Phi\) is calculated using equation (4.1). The displacement vectors for all 13 possible directions are summarized in table (4.2).

\[
\cos \Phi = \frac{x_1 x_2 + y_1 y_2 + z_1 z_2}{\sqrt{x_1^2 + y_1^2 + z_1^2} \sqrt{x_2^2 + y_2^2 + z_2^2}} 
\]  

(4.1)
Table 4.2 Volumetric angle displacement in 13 possible directions in GLCM calculation.

<table>
<thead>
<tr>
<th>Direction $(\theta, \Phi)$</th>
<th>Displacement</th>
<th>Euclidean Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(0^\circ, 90^\circ)$</td>
<td>$\pm(d,0,0)$</td>
<td>$d$</td>
</tr>
<tr>
<td>$(90^\circ, 90^\circ)$</td>
<td>$\pm(0,d,0)$</td>
<td>$d$</td>
</tr>
<tr>
<td>$(-, 90^\circ)$</td>
<td>$\pm(0,0,d)$</td>
<td>$d$</td>
</tr>
<tr>
<td>$(45^\circ, 90^\circ)$</td>
<td>$\pm(d,d,0)$</td>
<td>$\sqrt{2}d$</td>
</tr>
<tr>
<td>$(135^\circ, 90^\circ)$</td>
<td>$\pm(-d,d,0)$</td>
<td>$\sqrt{2}d$</td>
</tr>
<tr>
<td>$(90^\circ, 45^\circ)$</td>
<td>$\pm(0,d,d)$</td>
<td>$\sqrt{2}d$</td>
</tr>
<tr>
<td>$(90^\circ, 135^\circ)$</td>
<td>$\pm(0,d,-d)$</td>
<td>$\sqrt{2}d$</td>
</tr>
<tr>
<td>$(0^\circ, 45^\circ)$</td>
<td>$\pm(d,0,d)$</td>
<td>$\sqrt{3}d$</td>
</tr>
<tr>
<td>$(0^\circ, 135^\circ)$</td>
<td>$\pm(d,0,-d)$</td>
<td>$\sqrt{2}d$</td>
</tr>
<tr>
<td>$(45^\circ, 54.6^\circ)$</td>
<td>$\pm(d,d,d)$</td>
<td>$\sqrt{3}d$</td>
</tr>
<tr>
<td>$(135^\circ, 54.6^\circ)$</td>
<td>$\pm(-d,d,d)$</td>
<td>$\sqrt{3}d$</td>
</tr>
<tr>
<td>$(45^\circ, 125.3^\circ)$</td>
<td>$\pm(d,d,-d)$</td>
<td>$\sqrt{3}d$</td>
</tr>
<tr>
<td>$(135^\circ, 125.3^\circ)$</td>
<td>$\pm(-d,d,-d)$</td>
<td>$\sqrt{3}d$</td>
</tr>
</tbody>
</table>
4.2 Volumetric Voxel-based Texture Mapping

4.2.1 Voxel Based GLCM

Traditionally, GLCM is used as a single-value method where one GLCM is calculated for an image and a single textural feature value is extracted from it. In this thesis, the GLCM method was extended to 3D \((x, y, z)\) and implemented as a voxel based method. The GLCM was calculated in 13 different directions for each voxel in the tumour to generate a 3D voxel based map (3D-VTM). The textural features were extracted from each voxel’s GLCMs and are then averaged and assigned to the voxel to generate tumour texture maps. Three sizes of the GLCM neighbourhood region (filter size) in \((x, y, z)\) are proposed: 3x3x3 voxels (small), 5x5x3 voxels (medium) and 7x7x3 voxels (medium). Given that the CT data are acquired where the slice thickness is a few \(mm\) thick, the smallest \(z\) which was 3 voxels is chosen for all filter sizes. Using a small neighbourhood region to calculate GLCM statistics is arguably preferable to extract most textural information in the image. Yet, based on the results from chapter (3), the small-size filter performance is hindered by the effects of respiratory motion. Thus, a medium-size GLCM neighbourhood regions of (5x5x3 voxels) and (7x7x3 voxels) are suggested to overcome noise and blurring due to respiratory motion. The GLCM neighbourhood region will not filter the image, it will calculate the occurrences of intensity value pair within that region to create the GLCM and then the feature can be calculated from it. For simplicity, the GLCM neighbourhood region size is referred to as GLCM filter size in this thesis. This method was implemented in-house using Matlab software (The MathWorks, Inc, Natick, MA).

Given that volumetric voxel-based texture mapping calculates textural information for each voxel relative to its neighbours, edge voxels will not have enough neighbouring voxels for the analysis. For this reason, the region of interest, was dilated in the 3D space by a dilation factor by voxels \((dfv)\) as presented in equation (4.2) where \(h\) is the filter size. This added margin was removed after the texture map was produced.

\[
dfv = \frac{h - 1}{2} \tag{4.2}
\]
4.2.2 Feature Extraction

Several textural features could be extracted from GLCM, yet based on the reviewed literature [98][99][10] and the results from the study presented in chapter (2), entropy has shown the most significant results in relation to patients’ outcome and overall survival. Textural features extracted from images are a measure of the spatial variation in intensity which is a reflection of the information content in the image. Measuring entropy (equation 4.3) is a robust method to characterise image information [73]. In this study, entropy is proposed as a surrogate for texture to produce texture maps representing image structure information. Entropy is a measure of disorder and it is suitable to assess intratumoural heterogeneity.

\[
Entropy = - \sum_{i=1}^{N} \sum_{j=1}^{N} P(i,j) \log P(i,j)
\]  

(4.3)

4.2.3 Quantisation of Tumour Image Information

To reduce noise and computation power when using the GLCM method, the image region of interest was quantised to N number of grey levels. This process involved 2 steps; the choice of the N levels and the choice of quantisation method. These will be discussed in the following sections.

Quantisation Level N

Traditionally, the quantisation level N is commonly a power of two (8, 16, 32, 64, 128, 256) when using the GLCM method. In previously published studies in texture analysis in relating textural features to treatment outcome, the choice of the quantisation level was either set to the highest possible number hypothesizing it will better encode the image information [48] or multiple N levels were investigated to yield the best results [70]. In machine learning studies, a training data set is used to
choose the quantisation level $N$ that provides the best result for the required output [70]. In this thesis, a novel method of choosing an optimum quantisation level is presented. In image information theory, the image data are compressed into bits to reduce redundancy and represent the image without loss of information. The information content in an image is given by equation (4.4) where $p$ is the probability of an event and in this case a grey level occurring in an image. Thus, to calculate the optimum number of effective quantisation level $N$, I propose the use of $\log_2$ of the Region of Interest Intensity Range $(\text{max}_i - \text{min}_i)$ as shown in equation (4.5). The maximum intensity value within the region of interest is $\text{max}_i$ while the minimum intensity value is $\text{min}_i$. Although one might argue using the whole intensity range to quantise the ROI in GLCM, the ROI contains statistical noise and blurring thus fluctuation in voxels intensity values for the same tissue is expected; hence, quantisation is required.

\[ I(p) = \log_2(p) \]  

(4.4)

\[ N_{\text{effective}} = \lfloor \log_2(\text{max}_i - \text{min}_i) \rfloor \]  

(4.5)

Quantisation Methods

In previously published studies [10], uniform quantisation (UQ) was implemented to quantise the image prior to texture analysis. Uniform quantisation scales the image $IMG(x)$ based on the minimum ($\text{min}_i$), maximum ($\text{max}_i$) and the range of the intensity values $i (\text{max}_i - \text{min}_i)$ within the tumour.
image as presented in equation (4.6) [73]. If $IMG(x) = \text{min}_i$, $V(x)$ is set to 1.

$$V(x) = \left\lfloor N \times \frac{IMG(x) - \text{min}_i}{\text{max}_i - \text{min}_i} \right\rfloor$$

(4.6)

I propose the use of optimum Lloyd Max quantisation (LMQ) method to quantise the ROI before GLCM calculations. The LMQ is an iterative non uniform quantiser where multiple combinations of transition levels $t_k$ and reconstruction levels $r_k$ are investigated to determine the optimal quantisation level $t_L$. The quantiser maps the continuous variable $x$ into a discrete variable $x'$ where the boundaries of the bins are determined by the transition levels $t_k$ and the value for the voxels assigned to the bins are determined by the reconstruction levels $r_k$. The decision of these levels are based on minimising voxel to voxel mean square error $\varepsilon$ as presented in equation (4.7), where $t_L$ is the investigated quantisation levels and $p_x(x)$ is a continuous probability density function [73].

$$\varepsilon = \int_{t_1}^{t_{L+1}} (x - x')^2 p_x(x) dx$$

(4.7)

Equation (4.7) is re-written as equation (4.8) where $L$ is the investigated quantisation level.

$$\varepsilon = \sum_{i=1}^{L} \int_{t_i}^{t_{i+1}} (x - r_i)^2 p_x(x) dx$$

(4.8)
to minimise $\varepsilon$, it is differentiated in regards to $t_k$ and $r_k$ and the partial derivatives is set to zero.

$$\frac{\partial \varepsilon}{\partial t_k} = (t_k - r_{k-1})^2 p_x(t_k) - (t_k - r_k)^2 p_x(t_k) = 0 \quad (4.9)$$

$$\frac{\partial \varepsilon}{\partial t_k} = 2 \int_{t_k}^{r_{k+1}} (x - r_k) p_x(x) dx = 0, \cdots \cdots 1 \leq k \leq L \quad (4.10)$$

Given $t_{k-1} \leq t_k$ it leads to

$$t_k = \frac{(r_k + r_{k-1})}{2} \quad (4.11)$$

$$r_k = \frac{\int_{t_k}^{r_{k+1}} x p_x(x) dx}{\int_{t_k}^{r_{k+1}} p_x(x) dx} \quad (4.12)$$

Equations (4.11) and (4.12) will be solved simultaneously to find the optimum transition levels $t_k$ and reconstruction levels $r_k$ that minimize the $\varepsilon$ between the original distribution $x$ and the quantised distribution $x'$. Equations (4.11) and (4.12) demonstrate that the optimum $t_k$ will be centrally located between the optimum $r_k$. This optimisation process was stopped when the mean squared error (MSE) reached $< 10^{-7}$ between iterations.


4.3 Code Development

The volumetric voxel based texture mapping methodology proposed by the author in this chapter was implemented in Matlab software (The MathWorks, Inc, Natick, MA) version R2012a by the author. The code starts with segmenting the region of interest in 3D from the image. The same region of interest is then segmented with an added margin by dilating the original region of interest using the dilation factor by voxels. The $N_{\text{effective}}$ is then calculated from the original region of interest without the added margin. The LMQ is then applied with a $N_{\text{effective}}$ number of bins, this will iteratively search for multiple combinations of transition levels $t_k$ and reconstruction levels $r_k$ to minimise the MSE between the original and the quantised image. The LMQ is stopped when the MSE reaches $< 10^{-7}$ between iterations. Once the optimised combinations of transition levels $t_k$ and reconstruction levels $r_k$ are found, they are used to quantise the region of interest plus the added margin. The volumetric voxel based texture mapping is then conducted on the quantised region of interest plus the margin using the in-house written software by the author by iteratively taking each voxel in the region of interest and calculating the GLCM between that voxel and the voxels in the 3D neighbourhood region using a for loop. A total of 13 GLCMs are calculated for each voxels where the code written by the author rotates the neighbourhood region in 13 different possible ways in the 3D space. From each GLCM, entropy is calculated and then the value of the average entropy measured from the 13 GLCMs are assigned to the voxel of interest. Finally, the added margin is removed and the volumetric voxel based map is saved in a mat. file. The code written by the author requires an average of 0.02 seconds per voxel for GLCM calculations on an Intel(R) Core(TM) i5-2500 CPU@3.30GHz.

4.4 Discussion

An optimised method and a framework to produce volumetric voxel based texture maps was presented as shown in a flow diagram in figure (4.4). This 3D-VTM method allows the visualisation and investigation of textural spatial variation within tumours. The proposed methodology suggests the application of a spatial textural filter GLCM that has been proven in the literature to be able to distinguish between different tissue types [98][99][147][15]. The size of the GLCM filter proposed is based on the results from chapter (3) which shows that filter performance will be limited by the
scanner resolution and the presence of motion, hence, a filter size that is larger than the scanner resolution in the presence of motion is required.

An important step in conducting texture analysis is image quantisation. Tumour image quantisation encodes the information content within the image which may significantly affects the texture analysis results. The image quantisation consists of two steps, the quantisation method and choice of the optimum quantisation levels. The texture analysis work published in medical imaging used a uniform quantisation method where the image is quantised based on the minimum and maximum intensity value within the image not considering where most of the intensity values reside within the image histogram. I proposed the use of the optimum LMQ method and given the non-linearity of the LMQ this will allow a better sampling of where most of the image information occurs. Previously published work [48] investigated multiple quantisation levels of power two to report results without offering an un-biased method to choose the optimum number of quantisation levels. In machine learning approaches, all possible combinations of textural features and quantisation levels are investigated to reach the desired results. Yet, to independently examine tumour texture, an optimised standardised method is needed to avoid bias and that is what we presented in this chapter.

Furthermore, measuring tumour texture at the edge of the tumour image where there is a lack of neighbouring voxels was not addressed in the literature. In this thesis, we suggest an added margin equal to $h - 1/2$ that we termed a dilation factor by voxels (dfv). Even though we choose to assess tumour heterogeneity on a voxel basis by measuring tumour image entropy for reasons discussed in section (4.2.2), our proposed methodology can be used to extract any other textural features and the framework we presented can be adapted to use other textural filters.

## 4.5 Conclusions

The emerging proposition of texture analysis of tumour images as a biomarker for survival and local control in the field of radiotherapy calls for a thorough investigation of tumour image texture. This investigation requires the establishment of an un-biased independent standardised method to conduct texture analysis on tumour images. In this chapter, we presented our proposed methodology to generate volumetric voxel based texture maps of tumour images to visualise and assess the spatial variation of heterogeneity within tumour images. The implementation of our method on patients
tumour images and the relation of the produced 3D-VTMs to tumour function will be presented in the
next chapter. In chapter (5), we will also evaluate the benefits of our proposed methodology over the
standard methods.
4.5 Conclusions

Fig. 4.4 A flow chart of our proposed methodology.
Chapter 5

CT Volumetric Voxel Based Texture Mapping and 18F-FDG Uptake Distributions

5.1 Introduction

Work published in the literature in recent years has shown evidence that tumour image texture correlates with patients survival [10]. Furthermore, entropy measured from tumour CT images has been shown to correlate with patients survival [51][52][102][53][42][55][54][56], and see also chapter (2). A deeper understanding of tumour CT image entropy and its relationship to the physiology of the tumour is much needed. In the previous chapter (chapter 4) I presented our novel optimised method to measure and visualise tumour image texture on a voxel by voxel basis which provides information about the textural spatial variations within the tumour image. This chapter presents the implementation of the proposed volumetric voxel based texture maps (3D-VTM) methodology.

3D voxel based heterogeneity maps were generated and analysed for ten patients diagnosed with non-small cell lung carcinoma (NSCLC) and presented in this chapter. We also evaluate the value of our proposed optimised quantisation algorithm over the standard approach. We then present a quantitative comparison of 3D-VTMs with tumour $^{18}$F-fluoro-deoxy glucose (18F-FDG)
uptake distributions from PET scans to assess the relationship between CT intratumoural textural heterogeneity and tumour function measured as glucose metabolism. In this chapter, we present the first evidence that CT intratumoural heterogeneity measured from 3D-VTM provides potentially useful information on biological variations.

5.2 Methods

5.2.1 Patients Cohort

Data for a group of ten patients diagnosed with advanced NSCLC between years 2009-2012 were collected retrospectively. This group of patients had been treated with radical radiotherapy for primary tumours ranging in size from 34 to 372 cm$^3$, with a mean of 135.4 cm$^3$. Patient characteristics are summarised in table (5.1). This study obtained local ethics approval from the Royal Surrey County Hospital NHS Foundation Trust.

The patients investigated in this study can be divided into two sub-groups; those diagnosed with necrotic tumours and those whose tumours were not necrotic. Fast growing solid tumours cannot fulfil the metabolic needs of the growing tumour, thus part of the tumour will lack blood supply which induces hypoxia (lack of oxygenation). Hypoxia has been associated with tumour resistance to treatment and poor prognosis [108]. This state of hypoxia will eventually lead to tumour necrosis; tumour tissue that lacks functionality [103][114][104]. The relationship between hypoxia and 18F-FDG uptake is complex and contradicting results have been published in the literature on whether 18F-FDG uptake correlates to tumour hypoxia. It has been reported that hypoxic tumour requires more glucose leading to a higher 18F-FDG uptake within the tumour. However, conflicting results have been reported on whether there is an overlap between hypoxic regions and high 18F-FDG uptake regions [90]. Given that a prolonged state of hypoxia will eventually lead to necrosis, tumours can progress from having a high-density, high-metabolism, high 18F-FDG core (brightest at the centre on PET), to having a low 18F-FDG necrotic core with surrounding shell of 18F-FDG avid cells (a bright ring surrounding dark centre on PET). A non 18F-FDG avid region within the tumour could indicate lack of disease, hypoxic region or could indicate that disease has progressed to the point of necrosis. Necrotic areas may include or be adjacent to areas containing chronically hypoxic but
Table 5.1 Patients characteristics of the group of ten patients investigated in this study. The patients are divided into two sub-groups; those diagnosed with non-necrotic tumours (1-5) and those whose tumours were necrotic (6-10), each sub-group was ordered by tumour size. (SCC=squamous cell carcinoma, Adeno=adenocarcinoma)

<table>
<thead>
<tr>
<th>Pt.no</th>
<th>Age</th>
<th>Gender</th>
<th>TNM Stage</th>
<th>Tumour Size cm³</th>
<th>Histology</th>
<th>18F-FDG Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>Male</td>
<td>T2N0M0</td>
<td>34.7</td>
<td>SCC</td>
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<tr>
<td>2</td>
<td>74</td>
<td>Male</td>
<td>T2N1M0</td>
<td>37.4</td>
<td>SCC</td>
<td>Intense Homogenous</td>
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<tr>
<td>3</td>
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<td>Male</td>
<td>T2N1M0</td>
<td>52.7</td>
<td>Adeno</td>
<td>Homogenous</td>
</tr>
<tr>
<td>4</td>
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<td>Male</td>
<td>T2N2M0</td>
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</tr>
<tr>
<td>5</td>
<td>59</td>
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<td>T2N2M0</td>
<td>152.5</td>
<td>SCC</td>
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</tr>
<tr>
<td>6</td>
<td>76</td>
<td>Male</td>
<td>T3N0M0</td>
<td>71.5</td>
<td>SCC</td>
<td>Heterogeneous necrosis/hypoxic</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Male</td>
<td>T2aN2M0</td>
<td>113.5</td>
<td>SCC</td>
<td>Cavitated + necrosis/hypoxic</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>Female</td>
<td>T3N2M0</td>
<td>123.6</td>
<td>SCC</td>
<td>Patchy Heterogeneous/hypoxic</td>
</tr>
<tr>
<td>9</td>
<td>69</td>
<td>Male</td>
<td>T4N2M0</td>
<td>339.9</td>
<td>SCC</td>
<td>Heterogeneous necrosis/hypoxic</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>Male</td>
<td>T3N2M0</td>
<td>372.8</td>
<td>SCC</td>
<td>Heterogeneous with necrosis/hypoxic</td>
</tr>
</tbody>
</table>

still viable tumour cells, and it is impossible to clearly demarcate the transition [59]. Hypoxic and necrotic tissues are identified by the lack of 18F-FDG uptake within the solid tumour and a decrease in CT Hounsfield units (HU) in comparison to the other regions of the tumour. An example of a hypoxic/necrotic region from one of our cohort’s PET/CT images is shown in figure (5.1).

### 5.2.2 Image Acquisition

All ten patients underwent a radiotherapy planning CT scan in addition to a non-contrast CT scan on a 18F-FDG PET/CT scanner; these data were collected retrospectively for analysis. I denote the planning CT scan by $CT_{plan}$, and the CT component of the PET/CT scan as $CT_{PET}$. For the $CT_{plan}$, a GE LS RT16 scanner (GE Healthcare, UK) was used with 120kVp and 331mAs acquisition parameters. The reconstructed transaxial image size was 512x512 with 0.98x0.98x2.5mm³ voxel size. The reconstruction algorithm was Filtered Back Projection (FBP). The patients underwent the radiotherapy planning CT 55 days (mean) after the PET/CT scan. The PET/CT images were acquired using a GE Discovery LS scanner (GE Healthcare, UK); images were reconstructed using an Ordered Subset Expectation Maximisation (OSEM) algorithm for PET and FBP for $CT_{PET}$. The
acquisition parameters for CT\textsubscript{PET} were 150kVp and 100mAs. The reconstructed transaxial image size was 512x512 with 0.98x0.98x5.00 mm\textsuperscript{3} voxel size for the CT\textsubscript{PET} images and 128x128 image size with 3.91x3.91x5.00 mm\textsuperscript{3} voxel size for the PET images. The injected activity for 18F-FDG PET imaging was 400±40 MBq. The 18F-FDG PET images were converted to SUV maps by multiplying the uptake in each voxel (Bq ml\textsuperscript{-1}) by the patient’s weight divided by the decay-corrected injected activity [164].

5.2.3 Region of Interest Segmentation

The primary tumour was segmented from the CT\textsubscript{PET} and 18F-FDG PET images to generate the 3D-VTMs as the CT\textsubscript{PET} and 18F-FDG PET images have been acquired at the same time point; this will enable a meaningful comparison between 3D-VTMs and 18F-FDG uptake distributions. To segment the primary tumour, which is represented by gross tumour volume (GTV), the GTV contour [2] delineated by a consultant radiation oncologist was transferred from CT\textsubscript{plan} to the PET/CT scan. This was carried out by registering the CT\textsubscript{plan} and CT\textsubscript{PET} scans using a B-spline deformable registration method [122].
5.2 Methods

5.2.3.1 Image Registration

To segment the primary tumour represented by the GTV from the CT\textsubscript{PET} and the 18F-FDG PET images, the CT\textsubscript{PET} was registered to the CT\textsubscript{plan}. The obtained transformation matrix was then applied to the 18F-FDG PET images where the GTV was segmented from the PET images to segment the GTV from the 18F-FDG PET images and consequently allow the comparison between the produced 3D-VTMs and 18F-FDG uptake distribution. CT\textsubscript{plan} and the PET/CT scan were both obtained with patients arms up, yet the CT\textsubscript{plan} was conducted on a flat table while the CT\textsubscript{PET} table was curved. The registration process included two steps; an initial rigid registration step and then a deformable registration step. Rigid registration was initially performed to align the images centers and linearly interpolate the image size as described in [45]. Deformable registration was then carried out using B-spline method [116][122]. A detailed description of B-spline deformable registration is presented in appendix (B). The Plastimatch C++ library was used to conduct the registration process [122].

Validation of Image Registration

The image registration results were validated by obtaining a difference image between the CT\textsubscript{plan} and the registered CT\textsubscript{PET}. Given the CT\textsubscript{PET} and the 18F-FDG PET images are obtained at the same patient position at the same time points, the two scans are already registered. A qualitative assessment of the registered scans and the difference images was carried out by an experienced consultant radiologist (VP) and the author. The lack of structure in the difference image indicates a good registration. Very small structure in the difference image away from where the tumour occurred was considered acceptable since it is away from the region of interest. The work flow of the deformable registration process is presented in figure (5.2) using images from our patients cohort.
Fig. 5.2 The work flow and results of the deformable registration steps between PET/CT and CT_{Plan} for a patient from our cohort (Patient 6).
5.2 Methods

5.2.3.2 Generating Volumetric Voxel Based Texture Maps

Volumetric Voxel Based Texture Maps (3D-VTMs) were generated for the tumours segmented from the CT\textsubscript{PET} using our proposed methodology presented in chapter (4). Prior to tumour segmentation, the primary tumour volume (GTV) was dilated by the dilation factor dfv (refer to equation (4.2)). The tumour volume was quantised using the standard uniform quantisation (UQ) method used in the published literature and our proposed optimum LMQ quantiser. Different quantisation levels $N$ were investigated; using our proposed method of $log_2$ of Tumour Intensity Range and using the traditional power of 2 quantisation levels (8, 16, 32, 64, 128, 256) for both of the quantisation methods. The three proposed GLCM filter sizes (3x3x3 voxels, 5x5x3 voxels and 7x7x3 voxels) were implemented and used to generate the 3D-VTMs. As discussed before in (4.2.2), to assess intratumoural heterogeneity, local entropy (entropy per voxel) was calculated from each GLCM per voxel and assigned to that voxel to generate the 3D-VTMs.

5.2.4 Comparison with 18F-FDG Uptake Distributions

The SUV maps generated from the tumour 18F-FDG distribution were thresholded to 34%, 40%, 50%, 60%, 70% and 80% of SUV\textsubscript{max} (these volumes were labelled SUV\textsubscript{34}, SUV\textsubscript{40}, SUV\textsubscript{50}, SUV\textsubscript{60}, SUV\textsubscript{70}, and SUV\textsubscript{80}). The thresholded SUV maps were overlaid on the produced texture maps to investigate the relationship between 18F-FDG uptake and CT intratumoural heterogeneity. The 34% threshold of SUV\textsubscript{max} has been used as the minimum threshold as previous studies have validated this threshold for tumour delineation in PET imaging for lung tumours [141][5]. The Overlap Fraction (OF) and Dice Similarity Coefficient (DSC) were calculated to quantify the overlap and the similarity of the 18F-FDG distribution and CT intratumoural spatial heterogeneity. OF is calculated as the overlap volume divided by the SUV threshold volume as shown in equation (5.1). DSC is twice the overlap volume divided by the sum of the two volumes of interest as shown in equation (5.2) [4][73].

To test the validity of the findings, the 3D-VTMs images were rotated by 45° and the DSC was re-calculated. In this case the coefficient should decrease substantially. Furthermore, the DSC between the primary tumour GTV contour and SUV threshold values were calculated to test the sensitivity of our proposed method. To assure the registration did not affect the analysis, a 3D-VTM was produced.
for a patient from our cohort from the un-registered CT\textsubscript{PET}, the OF and DSC were calculated between the produced 3D-VTM and the 18F-FDG uptake from the original PET scan.

\begin{equation}
OF = \frac{Vol_1 \cap Vol_2}{Vol_{SUV}}
\end{equation}

\begin{equation}
DSC = \frac{2 \times (Vol_1 \cap Vol_2)}{Vol_1 + Vol_2}
\end{equation}

### 5.3 Results

#### 5.3.1 Image Registration

A difference image between the CT\textsubscript{plan} and the registered CT\textsubscript{PET} for each of the ten patients is presented in figure (5.3). As seen in figure (5.3), there is lack of structure (image mostly uniform) in the difference image which indicates a good registration. There is a little structure (bright area) where the scanner couch is and that is due to the fact that the PET/CT was conducted on a curved couch where as, the CT\textsubscript{plan} was conducted on a flat couch. Yet, in the region where the tumour is located, the registration algorithms performance is considered acceptable.
5.3 Results

Fig. 5.3 Difference image between the static and the registered image.
5.3.2 Tumour Image Quantisation

The range of the intensity values within the segmented tumours was 970-1788 Hounsfield units (HU) with a mean of 1357 HU. Using $\log_2$ Tumour Intensity Range, the optimum quantisation level of power two is 8 levels. The histograms calculated from using the UQ and LMQ methods to quantise the tumour image for two patients from the investigated cohort are presented in figure (5.4) and figure (5.5). Figure (5.4 a) and figure (5.5 a) are the normalised histograms using the whole intensity range within the tumour. The LMQ method optimises the transition $t_k$ and reconstruction levels $r_k$ to minimise the MSE between the original and the quantised histogram. Whilst the UQ method will uniformly assign the intensity values between the maximum and minimum of the image intensity range. The histograms calculated by using the UQ and LMQ methods to quantise the tumour image using the optimised quantisation level 8 are presented in figure (5.4 b) and figure (5.5 b). When the tumour image was quantised to 8 levels, the UQ method led to most of the information in the image to be deposited in one bin whilst the LMQ method allowed a better representation of image information as shown in figure (5.4 b) and figure (5.5 b). As the quantisation level increases to 16, the two histograms generated from the UQ and LMQ methods started to be more similar. The similarity between the two methods histograms increased with increasing the quantisation level until they looked almost identical at quantisation level 256 as seen in figure (5.4 c-d) and figure (5.5 c-d).
5.3 Results

Fig. 5.4 An example of tumour image normalised histograms at different quantisation levels N. (a) Is the normalised histogram using the original intensity range within the tumour without quantisation. (b) Is the normalised histogram using 8 quantisation levels. (c) Is the normalised histogram using 16 quantisation levels. (d) Is the normalised histogram using 32 quantisation levels. (e) Is the normalised histogram using 64 quantisation levels. (f) Is the normalised histogram using 128 quantisation levels. (g) Is the normalised histogram using 256 quantisation levels.
Fig. 5.5 An example of tumour image normalised histograms at different quantisation levels N. (a) Is the normalised histogram using the original intensity range within the tumour without quantisation. (b) Is the normalised histogram using 8 quantisation levels. (c) Is the normalised histogram using 16 quantisation levels. (d) Is the normalised histogram using 32 quantisation levels. (e) Is the normalised histogram using 64 quantisation levels. (f) Is the normalised histogram using 128 quantisation levels. (g) Is the normalised histogram using 256 quantisation levels.
5.3 Results

5.3.3 3D Voxel Based Texture Maps

Figure (5.6) shows the produced 3D-VTMs for the segmented tumour using the standard uniform quantisation method for a patient from the investigated cohort using different quantisation levels. Figure (5.7) shows the produced 3D-VTMs for the segmented tumour using our proposed LMQ method for the same patient using different quantisation levels. As shown in figure (5.7), using the optimised quantisation level (N=8) in combination with the optimum LMQ method led to a clear textural pattern within the 3D-VTM while increasing the quantisation levels did not. Furthermore, as shown in figure (5.6), using the standard uniform quantisation method did not yield any noticeable textural pattern at any of the investigated quantisation levels.

The produced 3D-VTMs using our proposed optimised quantisation method at the three investigated filter sizes are presented in figure (5.8) for one of the patients from this study cohort. As hypothesized, the small-filter size (3x3x3 voxels) will lead to a noisier texture map as shown in (5.8 a) while the larger-size (7x7x3 voxels) filter will have more smoothing effect as shown in figure (5.8 c). The 3D-VTMs produced from the medium-size filter (5x5x3 voxels) were used to continue the analysis of the maps. Given the slice thickness of the CT image is 2.5\text{mm}, the medium filter size in \text{mm} will be 5x5x7.5\text{mm}^3.

Figure (5.9), presents examples of the generated 3D-VTMs for three of the ten patients with different imaging characteristics. Patient 4 had a small tumour with homogeneous 18F-FDG uptake, Patient 7 had a medium-sized tumour with necrotic centre, and Patient 10 had a large tumour exhibiting heterogeneous 18F-FDG uptake with necrotic regions. Figure (5.9 a) shows the 18F-FDG PET/CT images with the delineated GTV contour used to segment the primary tumour region. When generating the 3D-VTMs using the uniform quantisation method, most of the CT tumour information was scaled into a single bin which leads to a limited amount of information as shown in Figure (5.9 b). Figure (5.9 c) is the 3D-VTM generated using the optimum LMQ method allowing a better sampling of the intensity range within the tumour which led to a clear textural pattern. Areas of low entropy value (dark) represent homogeneous regions where brighter areas of high entropy values represent heterogeneous regions. The 18F-FDG uptake distribution is shown in Figure (5.9 d). From visual inspection of Figure (5.9 c,d), there is a clear visual overlap between low entropy (zero entropy) and high 18F-FDG uptake.
Fig. 5.6 An example of a 2D slice from the produced 3D-VTM using the UQ method for Patient 10 with different quantisation levels N.
5.3 Results

(a) Delineated Tumour

(b) N=8

(c) N=16

(d) N=32

(e) N=64

(f) N=128

(g) N=256

Fig. 5.7 An example of a 2D slice from the produced 3D-VTM using the LMQ method for Patient 10 with different quantisation levels $N$. 
Fig. 5.8 An example of a 2D slice from the produced 3D-VTM using the LMQ method for Patient 10 at different filter sizes.
Fig. 5.9 18F-FDG PET/CT images for three patients are shown in (a), (b) the produced texture map using the uniform quantisation, (c) the produced texture map using the proposed LMQ method, (d) The 18F-FDG uptake distribution within the GTV. Images displayed using computational environment for radiotherapy research (CERR) and itk-snap software [43][163].
5.3.4 Comparison with 18F-FDG Distribution

The results for assessing the degree of correlation between regions of zero entropy and 18F-FDG distribution by calculating OF and DSC are presented in figure (5.10). The OF and DSC results show that regions with high 18F-FDG uptake ($\geq 50\%$ SUVmax) correlate with zero entropy regions. The correlation between $\geq 50\%$ SUVmax volume and zero entropy region is as high as 84% for OF and 75% for DSC. The results presented in figure (5.10 a) shows a clear trend of an OF increase as the SUV threshold increases in 9 of the 10 patients (Patient 10 is an outlier). The mean OF between zero entropy regions and SUV34 for the 9 patients is 53±15%. The mean OF gradually increases to reach 65±12% at SUV50 and 86±20% for SUV80, indicating the high SUV regions ($\geq 50\%$ of SUVmax) are encompassed within the zero entropy regions while the SUV34 region is extending outside the zero entropy region.

The DSC between zero entropy regions and 18F-FDG distributions in figure (5.10 b) shows an interesting trend where tumours having a homogeneous 18F-FDG uptake (solid lines in figure (5.10 b) as stated in table (5.1) had a DSC peak around SUV50 with a mean of 60±11% and then decreased to reach a mean of 25±25% for SUV80, whilst tumour volumes expressing a heterogeneous 18F-FDG uptake with necrotic regions (dashed line in figure (5.10)) had the highest DSC for SUV34 with a mean of 53±10% and continued to decrease as the SUV threshold volume increased.
5.3 Results

Fig. 5.10 (a) The Overlap Fraction between regions of zero entropy within the GTV and SUV for different SUV% thresholds. b) The DSC between regions of zero entropy within the GTV and SUV for different SUV% thresholds. Solid lines are patients with homogeneous 18F-FDG distribution within tumours while dashed lines are patients with heterogeneous necrotic tumours.
For Patient 10, the OF between zero entropy region and high 18F-FDG region fell to 30% even though the high SUV regions occur in the vicinity of zero entropy region as was shown in figure (5.9). The discrepancy between zero entropy region and 18F-FDG uptake for Patient 10 occurs mostly in the inferior part of the tumour. Figure (5.11) illustrates 2 slices as examples from the inferior part of the tumour for patient 10 where the discrepancy between the 3D-VTM and 18F-FDG uptake occurred. This discrepancy is due to the necrotic region in the tumour where there is a minimal 18F-FDG uptake yet the 3D-VTM recognises the necrotic region as part of the tumour as seen in figure (5.11 a2) and (5.11 a3). Based on discussions with the consultant nuclear medicine radiologist (VP), the large mass on the CT image shown in figure (5.11 a1) which has low 18F-FDG uptake (figure (5.11a2)) could be attributed to either collapsed lung or necrotic tissue. In this case, the location of the non 18F-FDG avid mass on CT and the density changes measured in HU across this mass suggests this part of the tumour is hypoxic/necrotic. This explains the observed trend in DSC in figure (5.10 b) between zero entropy region and 18F-FDG distribution for necrotic tumours with heterogeneous 18F-FDG uptake. The 3D-VTM recognises the necrotic/hypoxic regions in the solid mass as part of the tumour while the 18F-FDG uptake is minimal as demonstrated in Figure (5.11 a).

Furthermore, for Patient 10, the delineation of the GTV included a part of the liver to accommodate the extent of the tumour as explained by the consultant radiation oncologist (VE). This is shown in figure (5.11 b1); this region appears homogeneous with zero entropy in the 3D-VTM which introduces a bias in the OF and DSC results.

The validity of the findings was tested by rotating the 3D-VTMs 45° and re-calculating the DSC between zero entropy regions and differently thresholded regions of SUVmax. The DSC values decreased greatly to below $10^{-3}$ indicating the strength of the correlations observed. Moreover, the DSC between the primary tumour contour and the SUVmax thresholded regions was highest for SUV34 with a mean of $53\pm10\%$ and continued to drop without the peak previously observed for the zero entropy region for all the patients. The mean DSC between the primary tumour contour GTV and SUV50 was $36\pm10\%$.

The 3D representation of the overlap between GTV, zero entropy region and $\geq50\%$ SUVmax region for the ten patients investigated in this study is presented in figure (5.12) and figure (5.13).
Fig. 5.11 Two examples of 3D-VTM performance from Patient 10 PET/CT scan (A) Demonstrates the performance of the 3D-VTM in the case of necrotic tumour tissue. (B) Illustrates the implication of including normal homogeneous structures within the GTV. (1) Is the CT\textsubscript{PET} image, (2) is the 18F-FDG uptake distribution, and (3) is the produced texture map using the proposed LMQ method.
Fig. 5.12 The 3D representation of the GTV, zero entropy volume and SUV $\geq 50\%$ volume demonstrating the overlap between them for patients characterized with a homogeneous 18F-FDG uptake.
5.3 Results

Fig. 5.13 The 3D representation of the GTV, zero entropy volume and SUV ≥ 50% volume demonstrating the overlap between them for patients characterized with a heterogeneous 18F-FDG uptake.
5.3.5 Registration and 3D-VTMs

To ensure the registration did not have a large effect on our results, a 3D-VTM was generated from the unregistered 18F-FDG PET/CT scan for one patient from our cohort. The GTV was delineated on the CT\textsubscript{PET} by a trained observer (the author) using the contour from the CT\textsubscript{plan} as a guide. The results for the DSC analysis for the registered and unregistered 3D-VTM are presented in table (5.2). The results in table (5.2) indicates that the registration didn’t affect the similarity between zero entropy region and SUV\textsubscript{max} thresholded regions, the mean difference was $0.7 \pm 0.6$. The OF analysis between the unregistered 3D-VTM and 18F-FDG uptake are presented in table (5.3). The mean difference between the OF for the registered and un-registered 3D-VTM and the different thresholded SUV regions was $2.6 \pm 1\%$. The results in table (5.2 and 5.3) show the registration had a minimal effect on the DSC and OF.

Table 5.2 Dice Similarity Coefficient (DSC) for Patient 8.

<table>
<thead>
<tr>
<th>SUV Threshold Percentage</th>
<th>Registered 3D-VTM</th>
<th>Unregistered 3D-VTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>34%</td>
<td>57.0%</td>
<td>57.0%</td>
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<td>40%</td>
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<td>54.0%</td>
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</tr>
<tr>
<td>80%</td>
<td>6.1%</td>
<td>5.7%</td>
</tr>
</tbody>
</table>

Table 5.3 Overlap Fraction (OF) for Patient 8.

<table>
<thead>
<tr>
<th>SUV Threshold Percentage</th>
<th>Registered 3D-VTM</th>
<th>Unregistered 3D-VTM</th>
</tr>
</thead>
<tbody>
<tr>
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<td>40%</td>
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</tr>
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<td>80%</td>
<td>76 %</td>
</tr>
<tr>
<td>80%</td>
<td>80%</td>
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</tr>
</tbody>
</table>
5.4 Discussion

The growing interest in utilising texture analysis to predict tumour response to treatment is likely to benefit from a deeper understanding of tumour texture. To date, the published studies in quantifying tumour texture in relation to treatment response and patient survival are based on single-value texture analysis methods. These methods are based on extracting a single value for textural features from the tumour volume and testing possible correlations. Moreover, an optimum method of quantising tumour images prior to texture analysis which is crucial in measuring texture is not presented in previously published studies.

In this study, I presented a novel method of producing a visual 3D-VTM from CT tumour images using a designed optimised quantisation method where the benefit of this quantisation method is clearly presented in addition to the significance of the produced texture maps. The non-linearity of the proposed LMQ method allows a better sampling of where most of the intensity values (HU) exist whilst the standard uniform quantisation method does not. Our results showed a clear CT textural pattern within the solid tumour using our algorithm. The results highlight the significance of optimised quantisation of tumour volume in texture analysis which has not been addressed in previous studies in this field. Furthermore, the presented method in this study does not require a machine learning approach to optimise parameters and achieve the presented results.

Our results show regions with zero entropy value within the primary tumour correlate with high 18F-FDG uptake regions where as the primary tumour contour did not. Factors such as mis-registration and respiratory motion could cause some discrepancies between the 3D-VTM and the 18F-FDG distribution. Yet, the presented OF and DSC results shows a good agreement between the high SUV and zero entropy regions where the high SUV regions are mostly encompassed by the zero entropy regions. In addition, our OF and DSC results based on unregistered CT scans and the correlation between tumour local entropy and 18F-FDG distribution demonstrates how the registration effect was minimal.

Furthermore, as shown in figure (5.11 a) our proposed 3D-VTM method has the promising potential to detect necrotic tissue within the tumour where there is a lack of 18F-FDG uptake. This potential of our method to detect parts of the tumour that are non 18F-FDG PET avid has significant implications in the field of radiation oncology. Since the distinction between hypoxic and necrotic
tissues in 18F-FDG PET/CT is not possible, there could be a possible benefit of dose escalation to those hypoxic/necrotic regions given the possibility of viable tumour cells within hypoxic/necrotic regions. Hence, to extract the maximum information from tumour images, the combination of the 3D-VTM and PET and CT images may be needed. Furthermore, CT images are routinely acquired for cancer patients in diagnosis, staging, radiotherapy planning and follow up; establishing a standardised CT based texture analysis method has the potential to be highly beneficial.

In the next chapter, I present the application of my method in dose painting in radiotherapy. In chapter (6), I present the results of a dose escalation radiotherapy planning study to regions with zero entropy within the tumour image in comparison to dose escalation based on regions of high SUV (≥50%SUV) and the combination of both volumes. I also compare the dose escalation planning studies to the current standard of uniform dose planning.

5.5 Conclusion

Our study presents a method to generate a visual 3D map of variations in CT image texture within a tumour using an optimised quantisation method to visualise the textural contents within tumours without the use of machine learning techniques. The preliminary results of implementing our proposed 3D-VTM method show a clear textural pattern within CT tumour images correlating to tumour glucose metabolism and hypoxic/necrotic region detection suggesting valuable information from tumour CT texture analysis, particularly for hypoxic/necrotic tumours. Using our presented method to visualise tumour texture could have multiple applications in radiotherapy especially in dose boosting which could provide information that complements the conventional CT images and functional images.
Chapter 6

Radiotherapy Planning Based on Functional Imaging and Texture Analysis

6.1 Introduction

Patients diagnosed with advanced non-small cell lung carcinoma (NSCLC) have poor prognosis, with five years survival at <10% despite the application of new radiotherapy techniques [26]. In recent years, targeting regions within the tumour with a higher radiation dose based on the biological properties of the tumour has been suggested to improve local control in aggressive cancer types [16][6]. This technique is called dose painting. Given that 18F-FDG PET is widely used in cancer imaging and studies have shown a correlation between high 18F-FDG uptake regions and treatment resistance, it has been suggested to escalate the radiation dose to high 18F-FDG uptake regions within the tumour [6][5]. The results we presented in chapter (5) show a good agreement between areas of low CT heterogeneity (zero entropy) and high 18F-FDG uptake in tumour. Consequently, CT texture mapping could be used to define regions for dose escalation in NSCLC. Furthermore, the presented results showed that the 3D-VTM may detect necrotic/hypoxic regions within the tumour that are non 18F-FDG avid. Thus, dose escalation to these necrotic/hypoxic regions could be beneficial due to the possibility of viable tumour cells within these regions.
In this chapter, I present the application of the 3D-VTMs method in guiding dose painting by contours (simultaneous integrated boost) in NSCLC. I conducted a study comparing simultaneous integrated boost (SIB) radiotherapy plans to uniform dose plans for patients diagnosed with advanced NSCLC. The boost volumes were identified based on: i) CT intratumoural spatial heterogeneity obtained from the 3D-VTMs, ii) 18F-FDG uptake distribution within the tumour, and iii) the combination of both. The SIB plans were compared against each other and against a uniform dose plan in regards to clinical feasibility and conforming to normal tissue dose constraints. The main aims of this study were as follows:

- To evaluate the feasibility of dose escalation in advanced NSCLC while keeping normal tissue dose within the acceptable limits.

- The possibility of delivering an adequate dose to the high 18F-FDG uptake regions by planning based on the 3D-VTMs.

- To investigate the feasibility of dose boosting based on the combined information from the 3D-VTMs and the tumour’s 18F-FDG uptake.

This was achieved by the following steps:

- Creating a dose optimisation template; includes upper and lower radiation dose limits to target volumes and normal tissue. This template was used to create the uniform dose plans and the SIB plans.

- Using the optimised template, a uniform dose plan of 64Gy in 32 fractions (the standard current practice) for ten patients diagnosed with advanced NSCLC was created.

- Delineation of three boost volumes based on tumour CT image entropy, 18F-FDG uptake distribution and the combination of both.

- Using the optimised template, for each of the three boost volumes, a SIB plan was created to deliver 84Gy to the boost volumes and 64Gy to the remaining planning target volume (PTV) in 32 fractions for the same ten patients.

- An analysis of the four treatment plans created was conducted.
Table 6.1 Patients characteristics of the group of ten patients investigated in this study. The patients are divided into two sub-groups; those diagnosed with non-necrotic tumours (1-5) and those whose tumours were necrotic (6-10), each sub-group was ordered by tumour size. (SCC=squamous cell carcinoma, Adeno=adenocarcinoma)

<table>
<thead>
<tr>
<th>Pt.no</th>
<th>Age</th>
<th>Gender</th>
<th>TNM Stage</th>
<th>Tumour Size cm$^3$</th>
<th>Histology</th>
<th>18F-FDG Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>Male</td>
<td>T2N0M0</td>
<td>34.7</td>
<td>SCC</td>
<td>Homogenous</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>Male</td>
<td>T2N1M0</td>
<td>37.4</td>
<td>SCC</td>
<td>Intense Homogenous</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>Male</td>
<td>T2N1M0</td>
<td>52.7</td>
<td>Adeno</td>
<td>Homogenous</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>Male</td>
<td>T2N2M0</td>
<td>55.4</td>
<td>Adeno</td>
<td>Homogenous</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>Male</td>
<td>T2N2M0</td>
<td>152.5</td>
<td>SCC</td>
<td>Homogenous</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>Male</td>
<td>T3N0M0</td>
<td>71.5</td>
<td>SCC</td>
<td>Heterogeneous necrosis/hypoxic</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Male</td>
<td>T2aN2M0</td>
<td>113.5</td>
<td>SCC</td>
<td>Cavitated + necrosis/hypoxic</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>Female</td>
<td>T3N2M0</td>
<td>123.6</td>
<td>SCC</td>
<td>Patchy Heterogeneous/hypoxic</td>
</tr>
<tr>
<td>9</td>
<td>69</td>
<td>Male</td>
<td>T4N2M0</td>
<td>339.9</td>
<td>SCC</td>
<td>Heterogeneous necrosis/hypoxic</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>Male</td>
<td>T3N2M0</td>
<td>372.8</td>
<td>SCC</td>
<td>Heterogeneous with necrosis/hypoxic</td>
</tr>
</tbody>
</table>

6.2 Methods

6.2.1 Patients Data

The data from the patient cohort investigated in chapter (5) were used in this study. The patient cohort included ten patients diagnosed with inoperable advanced NSCLC (T2-T4, stage II-III). As previously presented in chapter (5), the investigated patient characteristics are summarised in table (6.1). This group of patients had received chemo-radiotherapy in the Royal Surrey County Hospital between year 2009-2012. The data from the radiotherapy planning CT scan, and the PET/CT scan for the ten patients were collected retrospectively. The planning CT scan is labelled (CT$_{plan}$) and the CT part of the PET/CT scan is labelled CT$_{PET}$. For details of image acquisition parameters for the planning CT scan and the PET/CT scan please refer to (5.2.2). The generated 3D-VTM$s$ in chapter (5) for this group of patients were used in this study. This study obtained local ethics approval from the Royal Surrey County Hospital NHS Foundation Trust (RSCH).
6.2.2 Treatment Planning Study

The radiotherapy contours [2] as delineated by a consultant radiation oncologist (VE) were transferred from CT\textsubscript{plan} to the PET/CT scan by registering the CT\textsubscript{plan} and CT\textsubscript{PET} scans using a B-spline deformable registration method [122]. For a detailed description of the registration process, please refer to (5.2.3.1).

To create the treatment plans, firstly, uniform dose plans of 64Gy in 32 fractions were created for the ten investigated patients. The PTV for the uniform dose plan was already delineated by the radiation oncologist. For the boost plans, the boost volumes were first identified and then a margin was added to create the boost planning target volumes. The prescribed dose in the boost plans was 84Gy to the boost volume and 64 Gy to the remaining PTV in 32 fractions. The organs at risk (OAR) dose constraints for the uniform dose plans and the boost plans were decided based on the local clinical protocol of the RSCH which is based on published literature and guidelines [86][49][82][152][95][96] as will be discussed in the following sections. These organs at risks are spinal cord, oesophagus, normal lung (both lungs-CTV), and heart. Although the bronchial tree is not an established OAR for the 64Gy in 32 fractions radiotherapy course or for boosting in lung cancer, I investigated the tolerance dose to the bronchial tree given the high dose escalation in the boost plans. A detailed description of the treatment planning methodology is presented in the following sections.

6.2.2.1 Uniform Dose Plans

The CT\textsubscript{plan} scans for the ten investigated patients combined with the radiotherapy contours were imported into the treatment planning system (TPS) Eclipse version 13.6 (Varian Medical Systems, Palo Alto, USA) at the RSCH. For the uniform dose plan, the dose was prescribed to the original planning target volume (PTV\textsubscript{primary_UnCrop}) as delineated by the consultant oncologist (VE). The PTV\textsubscript{primary_UnCrop} includes the radiologically visible primary tumour known as the gross tumour volume (GTV) plus a 0.5cm margin to account for microscopic disease extension (clinical target volume (CTV)) and a 1cm margin to account for setup uncertainties. These margins are based on the RSCH local protocol; the CTV margin is not necessarily isotropic and it can be adjusted based on clinical judgement. Given that the investigated patient cohort had central tumours, the
PTV_{primary UnCrop} was cropped 10\text{mm} away from the spinal cord (SC) and 3\text{mm} away from the oesophagus to avoid high dose to these structures. The new target volume is labelled as PTV_{primary}.

The uniform dose plans were created by the author under the supervision of the head of radiotherapy dosimetry in the RSCH (CS). Using volumetric modulated arc therapy (VMAT) planning technique, two half arcs were used to create the VMAT plans with a prescribed dose of 64Gy in 32 fraction (the current standard practice) to the PTV_{primary} for the ten investigated patients. An optimisation planning template was created by setting the tolerance dose to the organs at risk (OARs) and the prescribed dose to the PTV_{primary}. The dose constraints to the OARs were set according to the local protocol of the RSCH. The protocol is derived from published guidelines and literature [86][49][82][152][95][96]. Table (6.2) presents the dose constraints used in this study.

The optimisation planning template was iteratively adjusted on using two of the ten investigated patients and once an acceptable plan was created using the template, the optimised template was used to create the plans for the rest of the patient cohort. The algorithm used for dose calculation was Analytical Anisotropic Algorithm (AAA) version 11.0 and the optimisation algorithm was Photon optimiser version 13.6. These algorithms are implemented in the TPS and a detailed description is not publicly available by the software manufacturer.

Table 6.2 The dose constraints to organs at risk used in this study.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Volume</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal Cord</td>
<td>0%</td>
<td>Dose &lt;45Gy</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>0.5cm$^3$</td>
<td>Dose &lt;60Gy</td>
</tr>
<tr>
<td>Normal Lung</td>
<td>45%</td>
<td>Dose &lt;10Gy</td>
</tr>
<tr>
<td></td>
<td>35%</td>
<td>Dose &lt;20Gy</td>
</tr>
<tr>
<td>Heart</td>
<td>33%</td>
<td>Dose &lt;60Gy</td>
</tr>
</tbody>
</table>
6.2.2.2 Simultaneous Integrated Boost Plans

The simultaneous integrated boost (SIB) is a treatment technique where different doses per fraction are delivered to different target volumes simultaneously. In this study, there were two target volumes; PTV_{primary} and the boost volume.

For the SIB plans, three boost volumes were delineated within the gross tumour volume (GTV_{primary}). The boost volume based on the 3D-VTMs was delineated as the region of zero entropy within the 3D-VTMs and was labelled GTV_{entropy}. GTV_{entropy} was delineated (using automatic thresholding) on the 3D-VTM of the tumour CT image and then the contour was transferred to the CT plan. The boost volume based on the 18F-FDG uptake was delineated as the region of the tumour’s PET image having \( \geq 50\% \) of SUVmax and was labelled GTV_{FDG}. GTV_{FDG} was delineated (using automatic thresholding) on the 18F-FDG PET tumour image and then the contour was transferred to the CT plan. The two boost volumes; GTV_{entropy} and GTV_{FDG} were combined into the third boost volume labelled GTV_{entropy+FDG}. The boost volumes delineation was achieved using an in-house written Matlab software (The MathWorks, Inc, Natick, MA) in combination with the computational environment for radiotherapy research (CERR) software [43]. The boost volumes structure set were imported into the TPS.

The boost volumes; GTV_{entropy}, GTV_{FDG} and GTV_{entropy+FDG} were smoothed by filling any cavities \( 0.5 < cm^3 \). A margin of 3\,mm was added to the GTV_{entropy}, GTV_{FDG} and GTV_{entropy+FDG} to get the boost PTVs to account for physical uncertainties. The boost volumes plus the added margin were labelled; PTV_{entropy-UnCrop}, PTV_{FDG-UnCrop} and PTV_{entropy+FDG-UnCrop} respectively. Given the tumours are mostly central tumours (close to the mediastinum), the boost PTVs were cropped 10\,mm away from the spinal cord (SC) and 3\,mm away from the oesophagus allowing dose escalation while conforming to the OARs’s constraints. The modified PTVs were labelled PTV_{entropy}, PTV_{FDG} and PTV_{entropy+FDG}.

For each patient, three SIB plans were created based on the three boost volumes; PTV_{entropy}, PTV_{FDG} and PTV_{entropy+FDG} and were labelled Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG} respectively. The SIB plans were created using the VMAT technique with two half arcs and a prescribed dose of 84Gy in 32 fractions to the boost volume and 64Gy in 32 fractions to the PTV_{primary}. The prescribed dose 84Gy was advised by the collaborator consultant radiation oncologist (VE) as it
is the highest safe escalation dose in NSCLC reported in a phase II trial [21]. The dose planning optimisation template for the OARs from the uniform dose plan was used to create the SIB plans. A dose objective for the boost target volumes was added to the existing template. To ensure the part of the PTV<sub>primary</sub> not included in the boost volume received an adequate dose (95% of 64Gy), a new structure was created by subtracting the boost volume from the PTV<sub>primary</sub> and was labelled PTV<sub>primary-Boost</sub>. The PTV<sub>primary-Boost</sub> was set to receive 64Gy. A ring region was created 2 cm around the PTV<sub>primary</sub> with objective dose of ≤95% of 64Gy allowing dose escalation to the boost volumes while keeping the dose to the surrounding area to a minimum. The uniform dose plans and the SIB plans were discussed, assessed and approved by the collaborator consultant radiation oncologist (VE).

### 6.2.2.3 Dose to The Bronchial Tree

The Bronchial tree (BT) is the branching of the main airway to the lungs known as the bronchus; an example of a BT delineation on a CT scan is presented in figure (6.1). High radiation dose to the BT has been shown to cause high grade toxicity for patients treated for central tumours when treated with image guided stereotactic ablative radiation therapy (SABR). SABR is the delivery of a very high radiation dose to a small image-defined lesion in a small number of fractions; maximum of 60Gy in 8 fractions for NSCLC.

In NSCLC, SABR is the standard practice to treat small peripheral tumours and not central tumours given that the phase II trial showed an 83% freedom from high toxicity at two years for peripheral tumours compared to 54% for central tumours. The side effects included bronchial stenosis (narrowing of the bronchus) and thus a dose avoidance region (no fly zone) of 2 cm around the BT is needed for treating NSCLC with SABR. Since central tumours are in a very close proximity to the BT, SABR treatments are difficult to deliver and the standard treatment is still the conventional 2-3Gy per fraction [28][89].

A dose constraint or limit for the BT in the conventional 64Gy in 32 fractions or for dose escalation studies in central lung tumours has not been published in the literature. Yet, given the high dose in the boost plans (84Gy in 32 fractions), I investigated the dose tolerance for the BT as an additional consideration in this study. An extrapolated dose limit was calculated from the SABR guidelines to the conventional fractionation to evaluate the dose received by the BT. To do so, the biologically
effective dose was calculated based on the linear quadratic (LQ) model of the probability of cell survival (S) post irradiation. The LQ equation for cell survival (S) is presented in equation (6.1) where \( n \) is the number of fractions and \( d \) is the dose per fraction [79].

\[
S = \exp(-\alpha nd - \beta nd^2)
\]  

(6.1)

The biological effect of the irradiation \( E \) is represented by the term \( (\alpha nd + \beta nd^2) \) where \( \alpha \) and \( \beta \) are parameters representing irreparable and repairable cell damage respectively (refer to 1.3.3.1). To get the biologically effective dose (BED), \( E \) is divided by \( \alpha \). This simplifies to:

\[
\text{BED} = \frac{E}{\alpha} = \frac{\exp(-\alpha nd - \beta nd^2)}{\alpha}
\]
From equation (6.2), the total equivalent dose $nd$ can be calculated using equation (6.3).

$$\frac{BED}{1 + \frac{d}{\alpha/\beta}} = nd$$  \hspace{1cm} (6.3)

To calculate the total equivalent dose ($EQD$) in a different fractionation scheme, equation (6.4) is used.

$$EQD = nd = \frac{BED}{1 + \frac{d}{\alpha/\beta}}$$  \hspace{1cm} (6.4)
The dose limit in the SABR guidelines states that 0.5\(cm^3\) of the BT should receive <44Gy in 8 fractions [89][28]. To calculate the dose limit for the BT in 84Gy in 32 fractions, equation (6.2) was used as seen in equation (6.5).

\[
n_1d_1 \left[1 + \frac{d_1}{\alpha/\beta}\right] = n_2d_2 \left[1 + \frac{d_2}{\alpha/\beta}\right]
\]  

(6.5)

For equation (6.5), an \(\alpha/\beta\) of 3Gy was used, \(d_1 = 44\text{Gy}/8\), \(n_1 = 8\) and \(n_2 = 32\) and solved for \(d_2\). The calculated dose limit for the BT for 84Gy in 32 fractions is 71.5Gy to 0.5\(cm^3\) of the BT.

Given the tumours in our patient cohort are central tumours and very close to the BT, it is hypothesized that the BT may receive a dose higher than 71.5Gy for the SIB plans but not for the uniform dose plan where the prescribed dose is 64Gy. New boost volumes were created by cropping the PTV\(_{\text{entropy}}\), PTV\(_{\text{FDG}}\) and PTV\(_{\text{entropy+FDG}}\) 3\(mm\) away from the BT to investigate the possibility of delivering an escalated dose while limiting the dose to the BT. The new boost volumes were labelled PTV\(_{\text{entropy-BT}}\), PTV\(_{\text{FDG-BT}}\) and PTV\(_{\text{entropy+FDG-BT}}\). This extrapolated dose limit was not considered in the dose optimisation template for creating the four treatment plans rather it was an exploratory analysis.

The definitions of the different GTV, PTV and plan abbreviations used in this study are summarised in table (6.3).
Table 6.3 List of definitions of GTV, PTV and treatment plans abbreviations used in this study.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTV_{primary}</td>
<td>The gross tumour volume which represents the visible primary tumour.</td>
</tr>
<tr>
<td>GTV_{entropy}</td>
<td>The zero entropy sub-volume within the GTV_{primary}.</td>
</tr>
<tr>
<td>GTV_{FDG}</td>
<td>The $\geq 50%$SUV_{max} sub-volume within the GTV_{primary}.</td>
</tr>
<tr>
<td>GTV_{entropy+FDG}</td>
<td>The combination of the GTV_{entropy} and GTV_{FDG}.</td>
</tr>
<tr>
<td>GTV_{Overlap}</td>
<td>The overlap between the GTV_{entropy} and GTV_{FDG}.</td>
</tr>
<tr>
<td>PTV_{primary,UnCrop}</td>
<td>The planning target volume of the primary tumour.</td>
</tr>
<tr>
<td>PTV_{primary}</td>
<td>PTV_{primary,UnCrop} cropped 10mm away from SC and 3mm away from the oesophagus.</td>
</tr>
<tr>
<td>PTV_{entropy,UnCrop}</td>
<td>The planning target volume of the zero entropy sub-volume within the GTV_{primary}.</td>
</tr>
<tr>
<td>PTV_{entropy}</td>
<td>PTV_{entropy,UnCrop} cropped 10mm away from SC and 3mm away from the oesophagus.</td>
</tr>
<tr>
<td>PTV_{FDG,UnCrop}</td>
<td>The planning target volume of the $\geq 50%$SUV_{max} sub-volume within the GTV_{primary}.</td>
</tr>
<tr>
<td>PTV_{FDG}</td>
<td>PTV_{FDG} cropped 10mm away from SC and 3mm away from the oesophagus.</td>
</tr>
<tr>
<td>PTV_{entropy+FDG,UnCrop}</td>
<td>The combination of PTV_{entropy} and PTV_{FDG}.</td>
</tr>
<tr>
<td>PTV_{entropy+FDG}</td>
<td>PTV_{entropy+FDG,UnCrop} cropped 10mm away from SC and 3mm away from the oesophagus.</td>
</tr>
<tr>
<td>PTV_{Overlap}</td>
<td>The overlap between the PTV_{entropy} and PTV_{FDG}.</td>
</tr>
<tr>
<td>PTV_{entropy-BT}</td>
<td>PTV_{entropy} cropped 3mm away from the BT.</td>
</tr>
<tr>
<td>PTV_{FDG-BT}</td>
<td>PTV_{FDG} cropped 3mm away from the BT.</td>
</tr>
<tr>
<td>PTV_{entropy+FDG-BT}</td>
<td>PTV_{entropy+FDG} cropped 3mm away from the BT.</td>
</tr>
<tr>
<td>Uniform Dose Plan</td>
<td>Treatment plan where a uniform dose of 64Gy is prescribed to the PTV_{primary}, considered the current standard treatment for NSCLC.</td>
</tr>
<tr>
<td>Boost_{entropy} Plan</td>
<td>SIB plan with an escalated dose of 84Gy to the PTV_{entropy}.</td>
</tr>
<tr>
<td>Boost_{FDG} Plan</td>
<td>SIB plan with an escalated dose of 84Gy to the PTV_{FDG}.</td>
</tr>
<tr>
<td>Boost_{entropy+FDG} Plan</td>
<td>SIB plan with an escalated dose of 84Gy to the PTV_{entropy+FDG}.</td>
</tr>
</tbody>
</table>
6.2.3 Analysis of The Plans

The size of the generated boost volumes and the overlap volume between PTV_{entropy} and PTV_{FDG} were measured. The dose distribution was calculated for the four created treatment plans. A dose volume histogram (DVH), which provides information about the dose received by a volume of an organ, was generated for each of the OARs and the target volumes from each of the four plans [79]. The dose to the OARs and their compliance with the dose constraints presented in table (6.2) were calculated from each plan. The statistical significance between dose received by the OARs in the four treatments plans was measured using Wilcoxon signed rank test. The p-value was adjusted using Bonferroni correction for multiple comparisons (0.05/3=0.017), the difference is considered statistically significant if p<0.017.

To quote and compare the dose received by the target volumes, the dose received by 95% (D95) of the PTV_{primary}, PTV_{entropy}, PTV_{FDG} and PTV_{entropy+FDG} volumes were calculated. Although a 100% coverage of the target volume with the prescribed dose is desired [76] [2], in IMRT planning, it has been reported that allowing a small part of the target volume to receive slightly less dose leads to a better dose distribution that conforms to the shape of the critical organs adjacent to the target volume [22][160][58]. A “near-minimum” dose is considered a more robust and clinically meaningful measure of target coverage than a point minimum [68]: in this study, I calculated the dose received by 99%, 98% and 95% of the target volumes. Given that the calculated dose at the target boundaries between tissues of different densities is strongly affected by uncertainties in dose calculations, the D95 may be the most robust of these [68][60]. The D99, D98 and D95 all showed similar trends between plans; I therefore present here the D95 as an indicator of target coverage for each plan.
6.3 Results

6.3.1 Target Volumes

The sizes of the GTVs and PTVs for each of the ten investigated patients are presented in table (6.4) and table (6.5) respectively. The mean size of GTV\textsubscript{primary} for the ten investigated patients was 140±120 cm\(^3\), the mean size of GTV\textsubscript{entropy} was 30±20 cm\(^3\), the mean size of GTV\textsubscript{FDG} was 20±20 cm\(^3\) and the mean size of the GTV\textsubscript{entropy+FDG} was 40±30 cm\(^3\). The mean size of the overlap volume between GTV\textsubscript{entropy} and GTV\textsubscript{FDG} (GTV\textsubscript{Overlap}) was 10±10 cm\(^3\). As discussed in (5.3.4), the mean overlap between GTV\textsubscript{entropy} and GTV\textsubscript{FDG} was 65±12\% for patients 1-9. Patient 10 was considered an outlier due to the reasons discussed in (5.3.4). An example of the delineated GTV\textsubscript{primary}, GTV\textsubscript{entropy} and GTV\textsubscript{FDG} for one of the patients from our cohort is presented in figure (6.2).

![Example of delineated GTVs](image_url)

Fig. 6.2 An example of the GTV\textsubscript{primary}, GTV\textsubscript{entropy} and GTV\textsubscript{FDG} for a patient from our investigated cohort (Patient 3).
Table 6.4 The GTV size for the ten investigated patients. For definitions of the listed GTV structures, please refer to table (6.3). The results for the mean±SD are quoted in significant digits.

<table>
<thead>
<tr>
<th>Pt.no</th>
<th>$GTV_{primary}$</th>
<th>$GTV_{entropy}$</th>
<th>$GTV_{FDG}$</th>
<th>$GTV_{entropy+FDG}$</th>
<th>$GTV_{Overlap}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.7</td>
<td>4.4</td>
<td>3.8</td>
<td>6.3</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>37.4</td>
<td>11.1</td>
<td>7.9</td>
<td>12.0</td>
<td>7.1</td>
</tr>
<tr>
<td>3</td>
<td>52.7</td>
<td>10.5</td>
<td>9.1</td>
<td>13.4</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>55.4</td>
<td>16.0</td>
<td>10.0</td>
<td>17.6</td>
<td>8.7</td>
</tr>
<tr>
<td>5</td>
<td>152.5</td>
<td>50.0</td>
<td>29.6</td>
<td>57.0</td>
<td>23.5</td>
</tr>
<tr>
<td>6</td>
<td>71.5</td>
<td>26.8</td>
<td>21.6</td>
<td>35.1</td>
<td>13.7</td>
</tr>
<tr>
<td>7</td>
<td>113.5</td>
<td>20.9</td>
<td>19.1</td>
<td>31.4</td>
<td>9.6</td>
</tr>
<tr>
<td>8</td>
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<td>56.4</td>
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<td>15.7</td>
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Mean±SD | 140±120 | 30±20 | 20±20 | 40±30 | 10±10 |

Median | 92.5 | 23.8 | 17.6 | 32.2 | 10.9 |
For the PTV_{primary,UnCrop}, the mean size for the ten investigated patients was 387.7 cm$^3$ with a range of 147.4-819.3 cm$^3$. Cropping the PTV_{primary,UnCrop} 10 mm from the spinal cord and 3 mm away from the oesophagus compromised the PTV_{primary,UnCrop} for eight of the ten patients (for Patient 1 and Patient 6 the PTV_{primary,UnCrop} was not compromised). The mean size of the PTV_{primary} for the eight patients was 380.5 cm$^3$ with a range of 147.4-802.1 cm$^3$. The mean % volume cropped from the PTV_{primary,UnCrop} was 1.2% with a range of 0.02-4.2%. For the three boost volumes, cropping away from the spinal cord and oesophagus only affected Patient 5 (cropped volume was 2.7%) for PTV_{entropy}, Patient 10 for PTV_{FDG} (cropped volume of 0.2%) and consequently Patient 5 and Patient 10 for the PTV_{entropy+FDG} (cropped volume of 0.3% each).

For the planning target volumes, the mean size of the PTV_{primary} was 380±210 cm$^3$, the mean size of the PTV_{entropy} was 60±50 cm$^3$, the mean size of the PTV_{FDG} was 50±30 cm$^3$ and the mean size of the PTV_{entropy+FDG} was 80±60 cm$^3$. The mean size of the overlap volume between the PTV_{entropy} and PTV_{FDG} (PTV_{Overlap}) was 30±20 cm$^3$.

Table 6.5 The PTV size for the ten investigated patients. For definitions of the listed PTV structures, please refer to table (6.3). The results for the mean±SD are quoted in significant digits.

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<th>Pt.no</th>
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6.3.2 Treatment Planning Study

The four plans (uniform dose plan, Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG}) created for each patient using the optimised template were shown to be clinically feasible. The dose to OARs were within the tolerance limits and the target volume received an adequate dose as will be discussed in the following sections. An example of the calculated dose distributions for one of the patients from our cohort is presented in figure (6.3).

Fig. 6.3 An example of a dose distribution from the four created radiotherapy plans for Patient 7 from the investigated cohort. (a) Is the uniform dose plan. (b) Is the Boost_{entropy} Plan. (c) Is Boost_{FDG} Plan. (d) Is the Boost_{entropy+FDG} Plan. The axial view is shown on the left, the sagittal view is shown on the top right and the coronal view is shown on the bottom right.
6.3 Results

6.3.2.1 Dose to Organs at Risk

The dose volume histogram (DVH) was generated for the OARs by calculating the dose received by the organ to ensure compliance with the set dose restrictions. An example of a DVH of the OARs from the four treatment plans for a patient from our cohort is presented in figure (6.4).

Fig. 6.4 An example of the OARs DVH from the four treatment plans for Patient 7 from our investigated cohort.
The dose to the spinal cord was <45Gy for all the patients in all the four plans conforming to the tolerance dose. The mean sizes of the volume receiving 5Gy (V5) for the normal lung were 46.8±12.7%, 47.0±12.6%, 45.7±12.3% and 46.8±12.7% for the uniform dose plan, Boost_{entropy} plan, Boost_{FDG} plan and Boost_{entropy+FDG} plan respectively. The V5 for the normal lung for each patient in the four treatment plans is presented in figure (6.5).

**V5 of The Lungs**

(a) Non Necrotic/Hypoxic Tumours

(b) Necrotic/Hypoxic Tumours

Fig. 6.5 The volume of the normal lung that is receiving 5Gy (V5) in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume.
6.3 Results

The mean size of the volume receiving 10Gy (V10) for the normal lung was 28.9±8.1%, 29.5±8.4%, 29.0±7.8% and 28.8±8.1% for the uniform dose plan, Boost\textsubscript{entropy} plan, Boost\textsubscript{FDG} plan and Boost\textsubscript{entropy+FDG} plan respectively. The V10 for the normal lung for each patient in the four treatment plans is presented in figure (6.6).

![V10 of The Lungs](image)

Fig. 6.6 The volume of the normal lung that is receiving 10Gy in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume.
The mean size of the volume receiving 20Gy (V20) for the normal lung was 21.1±5.8%, 21.0±5.8%, 21.0±6.0% and 20.9±5.9% for the uniform dose plan, Boost_{entropy} plan, Boost_{FDG} plan and Boost_{entropy+FDG} plan respectively. The V20 for the normal lung for each patient in the four treatment plans is presented in figure (6.7).

Fig. 6.7 The volume of the normal lung that is receiving 20Gy in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume.
The mean size of the V60 (volume receiving 60Gy) of the heart was 1.3±2.0%, 1.4±2.2%, 1.3±2.0% and 1.4±2.3% for the uniform dose plan, Boost\textsubscript{entropy} plan, Boost\textsubscript{FDG} plan and Boost\textsubscript{entropy+FDG} plan respectively. The V60 for the heart for each patient in the four treatment plans is presented in figure as shown in figure (6.8). The dose received by 0.5cm$^3$ of the oesophagus for all the patients in the four treatment plans is presented in figure (6.9). Figure (6.9) shows that the dose to the 0.5cm$^3$ of the oesophagus is within the tolerance limit (<60Gy) for all the patients in the four treatment plans.

**V60 of The Heart**

Fig. 6.8 The volume of the heart that is receiving 60Gy (V60) in the four treatment plans. (a) Is the volume calculated from the non-necrotic/hypoxic tumours plans. (b) Is the volume calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance volume.
Fig. 6.9 The maximum dose to 0.5 cm$^3$ of the oesophagus in the four treatments plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance dose.

The dose received by the spinal cord, normal lungs, heart and oesophagus are all within the set tolerance dose for all the patients in the four treatments plans. For Patient 10, the lung V5 was slightly higher (mean 73.7%±1.7) than the tolerance volume of 65%. This is due to the large size of the tumour (largest tumour in our cohort) yet, it was still considered clinically acceptable. The Wilcoxon rank test showed that there was no significant statistical difference between the dose received by the OARs in the SIB plans compared to the uniform dose plan, all p-values were >0.017. The results show that the dose to the OARs in the four treatment plans are all within the tolerance limits. Furthermore, the dose escalation in the boost plans was achieved without a significant increase in the dose to the OARs in comparison to the uniform dose plan.
6.3 Results

6.3.2.2 Dose to The Planning Target Volumes

The DVH for each of the planning target volumes in each of the four treatment plans was calculated. An example of the generated DVH for PTV\textsubscript{primary}, PTV\textsubscript{entropy}, PTV\textsubscript{FDG} and PTV\textsubscript{entropy+FDG} in the uniform dose plan, Boost\textsubscript{entropy} plan, Boost\textsubscript{FDG} plan and Boost\textsubscript{entropy+FDG} plan are presented in figure 6.10.

Fig. 6.10 An example of a DVH from the four created radiotherapy plans for Patient 5 from the investigated cohort.
The D95 of the PTV\textsubscript{primary} was $\geq 60.8\text{Gy}$ (95\% of 64Gy) in the uniform dose plan for all the patients with a mean of 61.8$\pm$0.7Gy. The mean D95 of the PTV\textsubscript{primary UnCrop} was 61.5$\pm$0.8Gy. The D95 of the PTV\textsubscript{primary UnCrop} was $\geq 60.8\text{Gy}$ (95\% of 64Gy) for nine of the ten patients. For Patient 5, the PTV\textsubscript{primary UnCrop} was compromised by 4\% of it’s volume, consequently, the D95 for the PTV\textsubscript{primary UnCrop} for this patients was 93\% of the prescribed dose (59.9Gy). Yet, for the other nine patients, the D95 of the PTV\textsubscript{primary UnCrop} and PTV\textsubscript{primary} received 95\% of the prescribed dose as shown in figure (6.11). The D95 of the PTV\textsubscript{primary UnCrop Boost} and PTV\textsubscript{primary Boost} in the SIB plans were $\geq 60.8\text{Gy}$ (95\% of 64Gy) for all the patients.

![Graph](image-url)
For the SIB plans, 95% of the PTV_{entropy}, PTV_{FDG} and PTV_{entropy+FDG} received $\geq 79.8\text{Gy}$ (95% of 84Gy) for all the ten patients in their corresponding plans; Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG} with a mean of 82.3±0.2Gy, 82.35±0.37Gy and 82.14±0.3Gy respectively. The results in figure (6.12-6.14) shows the D95 of the boost PTVs; PTV_{entropy}, PTV_{FDG} and PTV_{entropy+FDG} in each of the three SIB plans (Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG}). The mean D95 of the PTV_{entropy} was 82.1±0.8Gy, 68.9±5.5Gy, 82.5±0.2Gy in the Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG} plans respectively. For the PTV_{FDG}, the mean D95 was 75.2±4.4Gy, 82.3±0.4Gy, 82.4±0.4Gy in the Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG} plans respectively and D95 for the PTV_{entropy+FDG} was 76.5±4.1Gy, 69.6±5.9Gy, 82.1±0.3Gy in the Boost_{entropy}, Boost_{FDG} and Boost_{entropy+FDG} plans respectively. Given the boost volumes are within the PTV_{primary}, they received the same dose as the PTV_{primary} in the uniform dose plan as discussed previously.

Figure (6.12) shows that when boosting based on PTV_{FDG} (red squares), the D95 of the PTV_{entropy} for only one patient received $\geq 79.8\text{Gy}$ (95% of 84Gy) which is expected given the boost volumes based on entropy were larger than the boost volumes based of 18F-FDG uptake for all the investigated patients (refer to table 6.4 and 6.5). When boosting based on the combination of entropy and 18F-FDG uptake boost volumes (PTV_{entropy+FDG}, the green triangles), both PTV_{entropy} and PTV_{FDG} D95 was $\geq 79.8\text{Gy}$ (95% of 84Gy) as presented in figure (6.12 and 6.13). Given that PTV_{entropy+FDG} was larger than PTV_{entropy} and PTV_{FDG}, when boosting based on the PTV_{entropy} (blue circles), the D95 of the PTV_{entropy+FDG} was $\geq 79.8\text{Gy}$ for only four patients and for only one patient when boosting based on PTV_{FDG} (red squares) as shown in figure(6.14). As seen in figure (6.13), when boosting based on the PTV_{entropy} (blue circles), the PTV_{FDG} D95 received a dose of $\geq 79.8\text{Gy}$ for three patients while the other seven patients received 80-92% of the prescribed dose. Since one of the aims of this study was to assess whether the high 18F-FDG regions receives an adequate dose when boosting based on the tumour’s entropy, figure (6.15) shows that when boosting based on the PTV_{entropy}, the GTV_{FDG} D95 was covered with $\geq 79.8\text{Gy}$ for nine of the ten patients. Patient 10 is considered an outlier due to the reasons discussed in (5.3.4).
Fig. 6.12 The dose received by 95% of the PTV\textsubscript{entropy} volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose.
6.3 Results

Fig. 6.13 The dose received by 95% of the PTV$_{FDG}$ volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose.
Fig. 6.14 The dose received by 95% of the PTV\textsubscript{entropy+FDG} volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose.
6.3 Results

D95 of The GTV_{FDG}

(a) Non Necrotic/Hypoxic Tumours

(b) Necrotic/Hypoxic Tumours

Fig. 6.15 The dose received by 95% of the GTV_{FDG} volume in the three created SIB plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the 95% of the prescribed dose.
6.3.2.3 Dose to The Bronchial Tree

The dose received by 0.5 cm$^3$ of the bronchial tree (BT) for each patient in the four treatment plans is presented in figure (6.16). For the uniform dose plan, the dose received by the BT was within the tolerance dose. For the SIB plans, the BT dose was within the calculated limit for Patient 2 and Patient 6 only while it exceeded the extrapolated dose limit for the rest of the patients. As per the discussion with the collaborator radiation oncologist (VE), a higher dose to the BT could be clinically acceptable to achieve a full coverage of the boost volumes which are considered to be the more aggressive part of the tumour. Moreover, since a tolerance dose to the BT or toxicity from BT irradiation in the conventional 64Gy in 32 fractionation or dose escalation studies in lung cancer were not reported or investigated in the literature, modification of the created treatment plans was not considered clinically necessary. However, as an exploratory assessment, I investigated the effects of modifying the boost volumes to avoid a high dose to the BT.

When modifying the boost PTVs (cropped 3mm away from the BT), the mean size of the cropped volume for $\text{PTV}_{\text{entropy}}$ ($\text{PTV}_{\text{entropy}} \times_{\text{BT}} \% \text{Vol}$) and $\text{PTV}_{\text{entropy+FDG}}$ ($\text{PTV}_{\text{entropy+FDG}} \times_{\text{BT}} \% \text{Vol}$) for the eight patients was 3.2% with a range of 0.6-7.5% and 3.8% with a range of 1.0-7.5% respectively. In the case of $\text{PTV}_{\text{FDG}}$, only six volumes needed to be modified, the mean size of the cropped volume for $\text{PTV}_{\text{FDG}}$ ($\text{PTV}_{\text{FDG}} \times_{\text{BT}} \% \text{Vol}$) for the six patients were 4.4% with a range of 1.1-9.2%. The results for the boost volumes sizes after cropping away from the BT are presented in table (6.6, 6.7 and 6.8).

The plan was re-optimised for one of the patients to test the feasibility of achieving a high boost dose with restricting the dose to the BT. A total dose of 84Gy in 32 fractions was prescribed to the $\text{PTV}_{\text{entropy-BT}}$. The dose to the BT was reduced to 70.7Gy to 0.5cm$^3$ of the BT by under-dosing 4.5% of the $\text{PTV}_{\text{entropy}}$. The results from this patient shows that it is feasible to deliver an escalated dose to central tumours with restricting dose to the BT but with the expense of compromising the target volume. However, even the compromised area of the boost target still receives a dose higher than that in the standard treatment.
6.3 Results

Dose to The Bronchial Tree

Fig. 6.16 The maximum dose to 0.5cm$^3$ of the bronchial tree in the four treatment plans. (a) Is the dose calculated from the non-necrotic/hypoxic tumours plans. (b) Is the dose calculated from the necrotic/hypoxic tumours plans. The dashed line is the tolerance dose.
Table 6.6 Sizes of the PTV_{entropy} in $cm^3$ and the size after cropping away from the BT. PTV_{entropy} C_{BT} %Vol = the percentage cropped volume from PTV_{entropy}. The results for the mean±SD are quoted in significant digits.

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Table 6.7 Sizes of the PTV_{FDG} in $cm^3$ and the size after cropping away from the BT. PTV_{FDG} C_{BT} %Vol = the percentage cropped volume from PTV_{FDG}. The results for the mean±SD are quoted in significant digits.

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6.4 Discussion

Table 6.8 Sizes of the $\text{PTV}_{\text{entropy+FDG}}$ in $cm^3$ and the size after cropping away from the BT. $\text{PTV}_{\text{entropy+FDG}} C_{\text{BT}} \%\text{Vol} =$ the percentage cropped volume from $\text{PTV}_{\text{entropy+FDG}}$. The results for the mean±SD are quoted in significant digits.

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</table>

6.4 Discussion

In the previous chapter, I showed the relationship between the volumetric voxel based heterogeneity (local entropy) measured from CT images of NSCLC tumours and tumour’s 18F-FDG uptake measured from PET images. Tumour’s 18F-FDG distribution have been suggested to guide dose painting in NSCLC given that 18F-FDG uptake have been shown in the literature to relate to tumour’s recurrence. In this chapter, I investigated the possibility of using the generated 3D-VTMs from NSCLC CT images in dose painting as a surrogate for tumour metabolism. To do so, I created three simultaneous boost plans (SIB) based on tumour metabolism (18F-FDG uptake), tumour CT image entropy (3D-VTMs) and the combination of both. A dosimetric analysis and a comparison between the plans was conducted. Moreover, I investigated the clinical feasibility of dose boosting in NSCLC by comparing the boost plans to a uniform dose plan.

The SIB plans were compared against a uniform dose plan of 64Gy in regards to dose to organs at risk (OARs). The results illustrated the possibility of dose escalation in NSCLC without exceeding the clinical dose limit to the OARs. In this study, I also considered the dose to the BT. Although the BT doesn’t have an established dose restriction in the published literature for 64Gy in 32 fractions or dose escalation studies in central lung tumours, high radiation dose to the BT has shown to cause high grade toxicity in SABR trials. A dose limit for the BT was extrapolated from SABR guidelines. The
results showed that in the SIB plans, the dose to the BT exceeded the extrapolated limit. Modifying the boost PTVs decreased the dose to the BT to the acceptable level yet it led to a compromised coverage of the boost PTVs. Based on the discussion with the consultant radiation oncologist (VE), given this group of patients have a chance of preserving lung function, a total high dose to the BT could be accepted in order to adequately cover the boost volumes. Overall, the three SIB plans have been shown to be clinically feasible without a significant increase in the dose to OARs compared to the uniform dose plan.

A dosimetric comparison was conducted between the three SIB plans (Boost\text{entropy}, Boost\text{FDG} and Boost\text{entropy+FDG}) to assess the dose received by the high 18F-FDG regions when boosting based on the tumour CT image local entropy. The boost volume PTV\text{FDG} were found to be smaller than the boost volume PTV\text{entropy} for all the ten investigated patients hence, 95\% of PTV\text{entropy} was not covered by 95\% of the prescribed dose when boosting based on PTV\text{FDG} except in one case. When boosting based on PTV\text{entropy}, the D95 of the PTV\text{FDG} was 95\% of the prescribed dose for three patients while the others received between 80-92\% of the prescribed dose. Yet, the D95\% of the GTV\text{FDG} was covered by 95\% of the prescribed dose for all the investigated patients excluding Patient 10 due to reasons discussed in section (5.3.4). The results indicates that when boosting based on tumour CT image local entropy, the high 18F-FDG regions will receive an adequate dose. Since CT images are the basis for radiotherapy planning and dose calculation, these results are quite crucial in showing the potential of our proposed method to be used for dose painting in NCSLC.

Moreover, dose escalation in Boost\text{entropy+FDG} plans for all the investigated patients have been shown to be clinically feasible and conform to OARs dose constraints despite the larger boost volume. Due to uncertainties in 18F-FDG PET imaging such as partial volume effect and registration errors and the challenges in volumetric texture mapping (discussed in (5.3.4)), boosting the combination of the boost volume identified by the two methods with an adequate margin might be desired to cover what could be the most aggressive part of the tumour. In chapter (5), I showed a preliminary evidence of the possibility of the 3D-VTM to detect the necrotic/hypoxic regions within the tumour hence, boosting based on PTV\text{entropy} or PTV\text{entropy+FDG} could be beneficial since it covers the high 18F-FDG regions and the necrotic/hypoxic part of the tumour that might still have viable cells within it.
6.5 Conclusions

In conclusion, dose escalation to boost volumes based on tumour CT image local entropy, tumour 18F-FDG uptake and the combination of both are shown to be clinically feasible without significantly increasing the dose to OARs. The results presented in this chapter demonstrated one application of our proposed volumetric texture mapping method. I demonstrated that high 18F-FDG regions within the tumour can be covered with at least 95% of the prescribed dose when boosting based on tumour CT image local entropy, thus showing the potential of our method in the area of dose painting. Yet, boosting based on the combination of tumour CT entropy and 18F-FDG uptake could be more desirable to cover both metabolically active and necrotic/hypoxic region within the tumour.
Chapter 7

Discussion, Conclusions and Future Work

7.1 Overview

In this final chapter, the key results and the implications of the presented work are discussed. In addition, the main conclusions and proposed future work are presented.

7.2 Textural Features as Biomarkers for Patient Survival

The emerging proposition in the field of oncology of using tumour image features as biomarkers for patient survival, calls for thorough investigation and validation. Initial results relating tumour image texture to treatment outcome and patient survival are promising. Predicting tumour response prior to radiotherapy could potentially allow stratification of patients to high and low risk groups where high risk groups may be targeted with more aggressive treatment courses. In addition, predicting recurrence earlier from follow-up scans would be valuable for planning medical intervention as early as possible. However, the field of texture analysis lacks standardisation of methods leading to contradictions in the reported findings and difficulties in comparing results. Furthermore, the number of textural features that can be extracted from radiological images are numerous, yet only some showed an association, or correlation, with patient survival or response to treatment. The significance of these
textural features should be investigated and the causal relationship between these features and outcome prediction/survival should be understood before moving forward. Whilst the aim of this work was not to correlate textural features to patient survival, a validation of the optimised, normalised threshold value for entropy (a texture feature that showed correlation to patients survival in NSCLC in the published literature) was carried out. The aim of this was to assess and evaluate the robustness of the optimised normalised entropy threshold value as a prognostic tool for NSCLC patient survival. Our results reached statistical significance when using the threshold value from the published literature on our patients cohort. This is an important finding implying that parameters obtained from one study could potentially be used generically to predict survival for other patients, despite the differences in patients cohort and in imaging equipment. In addition, these results validate entropy as a potential biomarker for patient survival.

7.3 Spatial Measurement on Intratumoural Heterogeneity

The studies presented in the literature correlate a single-value texture feature to treatment outcome, or patient survival, without taking into account the spatial information within the tumour image. To investigate whether the tumour CT image has textural variations within it, I developed a methodology to map tumour image texture volumetrically on a voxel by voxel basis. In the process, key challenges were addressed as will be discussed in the following sections.

7.3.1 Texture Filter Design

Based on the reviewed literature, GLCM has been shown to be able to distinguish between cancerous and normal tissue [147][15]. Moreover, features extracted from GLCM were reported to be significantly different for lung cancers that recurred compared to those that did not [98][99]. GLCM provides spatial information and can be implemented as a volumetric voxel based method which was the goal of this work.

To choose the GLCM neighbourhood region size (filter size), many aspects needed to be acknowledged. The filter needed to be an odd number of voxels so each voxel can have a uniform number of neighbouring voxels. This leads to the smallest possible 3D filter size of 3x3x3 voxels. However, the
size of the extracted texture features is limited by the scanner resolution. Also, image blurring due to respiratory motion will degrade the image quality. To tackle this, an in-house designed phantom was constructed to reflect different levels of spatial scale. The phantom was used in an experiment to characterise and quantify the resolution of the CT scanner and the effects of respiratory motion on extracting texture features. The results from the experiment were used to make a decision about the optimum filter size. Hypothetically, one would argue that the use of the smallest filter size is desirable yet due to technical limitations of the scanner resolution and the effects of respiratory motion, small sized filters are not necessarily favourable. Thus, medium sized filters offer a trade-off between extracting the maximum textural information on the smallest spatial scale possible and corresponding to the scanner resolution.

Measuring tumour image texture at the edge of the tumour, where there is a lack of neighbouring voxels, is another challenging issue. In this thesis, I suggested an added margin equal to the size of the filter subtracted by one and divided by two. This margin is to be removed after the 3D-VTM is generated. However, edge voxels may have an enhanced response (higher entropy value) especially in the case of lung tumours due to the different tissue properties between normal lung and tumours. Another approach was to generate the 3D-VTMs without adding a margin and then removing the edge voxels from the generated map however, this might lead to loss of information.

### 7.3.2 Image Quantisation

An important step in conducting texture analysis is image quantisation. Image quantisation encodes the information content within the image which may significantly affect the texture analysis results. Most of the published studies have used a uniform quantisation method which scales the tumour image into an equal number of bins between the minimum and maximum intensity. The investigated number of bins reported in the literature was a power of two (8, 16, 32, 64, 128, 256). These quantisation levels were explored and the one that yielded significant results in regards to patient survival and outcome prediction was reported. A standard method of choosing the quantisation level is not established.

A study by Leijenaar et al. [87] attempted to address this issue by theoretically suggesting a method for quantising 18F-FDG PET tumour image specifically (and not CT). In [87], two methods of quantising the SUV values prior to feature extraction were investigated. The two investigated methods
were using a fixed bin size (B) or using a fixed number of bins (D). The discretisation values used for the bin width B were: 0.05, 0.1, 0.2, 0.5 and 1.0 SUV and the discretisation values for bin number D were: 8, 16, 32, 64 and 128. Forty-four textural features from grey-level matrices were extracted from pre-treatment 18F-FDG PET tumour images of 35 patients diagnosed with NSCLC. To assess the consistency of the extracted textural features values, the pairwise intra-class correlation coefficient (ICC) was calculated between the feature values extracted using the two investigated quantisation methods. To evaluate the interpretation of the texture feature, patients were ranked (high to low texture feature value) based on the texture value extracted from pre-treatment images and ranked again based on the texture feature changes between pre-treatment and during treatment images (during the second week on radiotherapy treatment). The difference in patients ranking was quantified by calculating pairwise correlation. Spearman’s rank correlation coefficient (ρ) was calculated between pairwise correlation of patients rankings with ρ > 0.9 considered an acceptable agreement. Leijenaar et al. [87] reported that ρ for the inter-class correlation was <0.85, thus all textural features depend on SUV discretisation. Furthermore, they reported only 20 features to be unaffected by the discretisation method in regards to patient ranking. The authors did not justify the suggested quantisation levels D or bin width B rather it was stated as an arbitrary selection. Furthermore, consistency of the extracted feature value does not necessarily mean accuracy as it could be a reflection of the properties of the feature calculation itself rather than the quantisation technique. The authors proposed the fixed bin width method to maintain a constant intensity resolution within the quantised image, yet this is only advised for SUV quantisation and can not be generalised to other modalities. The authors justification of this approach is that the 18F-FDG PET image does not have a set range of SUV values rather it is patient dependent, whilst CT values are normalised based on the attenuation coefficients of water and air, where the same range of values exist for all scanned patients [87]. Moreover, the authors did not provide any evidence that their proposed quantisation technique leads to meaningful or significant results while our proposed quantisation methodology justifies the use of the quantisation level and shows the benefits in producing meaningful texture maps.

Another study by Vallieres et al. 2015 [140] used the Lloyd-Max quantiser to quantise fused PET MR images and extract single texture feature values from soft tissue sarcoma images. The textural features were also extracted from non fused 18F-FDG PET images and T1 and T2 MR images. The authors investigated different quantisation levels (8, 16, 32 and 64) and reported 64 levels to yield the highest Spearman’s rank correlation in predicting lung metastasis for the MR images and the fused
PET MR images. For the 18F-FDG PET images, the use of 16 quantisation levels yielded the highest Spearman’s rank correlation in predicting lung metastasis. Moreover, Vallieres et al. 2015 [140] reported that using the Lloyd-Max quantiser for the 18F-FDG PET images, T1 and the fused PET MR images yielded the highest Spearman’s rank correlation with the p-value reaching significance. However, for the T2 MR images, the highest Spearman’s rank correlation was observed when using the uniform quantiser yet the p-value did not reach significance. The authors did not provide an explanation of why the reported quantisation levels and quantisation methods yielded significant results for some type of images while it did not for others. It is important to acknowledge that the texture features were extracted from images that have been pre-processed using wavelet band-pass filtering, isotropic resampling and were also normalised before the use of the quantiser [140]. These heavy pre-processing steps makes assessing the benefits of the implemented quantisation methodology and the produced results difficult.

On the other hand, our proposed quantisation method addresses the two aspects of quantisation i) choice of quantisation level and ii) quantisation method where justifications and evidences of the benefits of our proposed methodology were provided.

### 7.3.3 Choice of Extracted Feature

The number of textural features that can be extracted from an image is numerous, some studies have reported up to forty features. However, most of the extracted textural features are not well understood in regards to the information they provide. Some studies investigated all possible texture features, some investigated selected features and the reported prognostic power of the investigated features varied in the literature. Regardless of the lack of standardisation in performing texture analysis and a validation or an explanation of the reported findings, a move towards the field of prognostic radiomics has taken place in the last two years. Radiomics encompasses all quantitative features that can be extracted from an image including, but not exclusively texture features. Other features include shape and intensity features. Up to 440 features have been reported in the literature.

A study by Aerts et al. [3] assessed the prognostic value of 440 radiomic features extracted from CT images of lung, and head and neck tumours obtained from 878 patients of mixed TNM staging. In [3], based on the feature characteristics, the radiomics features were split into four groups: tumour
image intensity, shape, texture, and multiscale wavelet. The investigated patients dataset was split into one training dataset and three validation datasets. The training set was labelled Lung1 and had 422 NSCLC patients. The validation datasets were labelled as follows: Lung2 had 225 NSCLC patients, H&N1 had 136 patients diagnosed with head and neck small cell carcinoma and H&N2 had 95 patients diagnosed with head and neck small cell carcinoma obtained from a different centre [3]. The CT images were quantised by setting an equal bin width of 25 HU. The median value of the extracted feature was used as a threshold value for Kaplan-Meier analysis on the training dataset Lung1. The same median value was used to validate the prognostic power of the extracted features on the validation datasets. The authors [3] reported only sixty-six (15%) features from the 440 investigated features to yield significant survival results in the three validation sets (Lung2, H&N1, H&N2). The authors also reported that features describing tumour heterogeneity were correlated with worse survival outcome in all the investigated patients. To build a prognostic model and remove feature redundancy, the authors choose the best performing feature from the four groups of the radiomic features. These features were: energy (describes tumour density) from the image intensity group, shape compactness (how compact the tumour shape is) from the shape group, grey level non-uniformity (describes tumour heterogeneity) from the texture group and grey level non-uniformity (also describes tumour heterogeneity) from the multi-scale wavelet group. The generalisation of the area under the curve from ROC known as the concordance index (CI) was used to validate the four features on the validation datasets. The results reported a CI of 0.65, 0.69 and 0.69 for Lung2, H&N1 and H&N2 respectively. The CI for tumour volume was 0.63, 0.68 and 0.65 for Lung2, H&N1 and H&N2 respectively for the prognostic radiomics model. The CI for the TNM staging was 0.60, 0.69 and 0.66 Lung2, H&N1 and H&N2 respectively. Combining the radiomics features with the volume or TNM staging did not increase the reported CI except for H&N1 when radiomics was combined with TNM (CI was 0.70). The reported results did not correct for tumour size or volume which may affect the quantification of the extracted features. Moreover, an explanation of the observed correlation between the extracted features and prognosis was not provided by the authors.

In this thesis, the decision was to investigate entropy which is a well understood and a robust feature that describes the information content in an image. Moreover, entropy is a measure of heterogeneity and it is a suitable feature to show intratumoural spatial heterogeneity. Also, entropy measured from tumour CT images has been shown to correlate with patient survival in this thesis and in multiple studies for different tumour sites.
7.4 Intratumoural Heterogeneity and Tumour Function

The physiological origin that could be the cause of the reported correlation between tumour image texture and survival is yet to be understood. Some authors justified the observed correlation as a reflection of the tumour biological heterogeneity [42]. As texture can be roughly defined as the spatial variation within an entity, tumour texture can be argued to be representative of tumour heterogeneity. Tumour heterogeneity can be recognised on a cellular level and on a structural level. CT imaging has a resolution of ≈1mm, thus cellular heterogeneity can not be observed from CT images. Given that tumours invade nearby tissue, this could lead to tumour structural heterogeneity which may be reflected on a CT image. The results from the implementation of our proposed methodology shows that radiologically uniform tumours have regional textural variations. I hypothesized that this observed intratumoural heterogeneity is structural tumour heterogeneity. This is supported by the comparison between the 3D-VTMs and 18F-FDG PET uptake images. As the high 18F-FDG uptake regions within the tumours overlapped with the homogeneous regions of the 3D-VTMs, this may indicate that the more aggressive parts of the tumour are structurally different than the less metabolically active parts on CT images.

7.5 Clinical Applications

The non-invasive nature of medical imaging in informing about tissue characteristics provides great potential and applications in the clinical settings. CT imaging is a well-established modality in the oncological diagnosis/staging, treatment and follow up work-flow. However, the assessment of CT images have been mostly qualitative and subjective. The introduction of fast computers and advanced technology made rapid archiving, processing and quantitative analysis of medical images an attainable reality. Since then, numerous studies investigated the use and integration of quantitative tumour image analysis into clinical practice. The presented results are encouraging, yet on the other hand, an understanding and a thorough validation of the reported findings is needed. In this thesis, I proposed a methodology of visualising 3D voxel based texture maps of tumours with a novel application in guiding dose painting. The presented results demonstrated high FDG regions within the tumour volume are covered with 95% of the prescribed dose when boosting based on the image of intratumoural heterogeneity from the CT scan.
Extracting quantitative information that is indicative of tumour function from CT images is highly beneficial given CT imaging is routinely acquired for cancer patients and widely available and integrated into the clinical practice. Yet, a validation on a larger dataset is needed before such an approach is realised. Also, validating the biological origin of the observed tumour image texture is challenging, relating tumour image texture to tumour pathology by collecting tissue samples may make this possible.
7.6 Conclusions

- Tumour heterogeneity measured as entropy from a CT image showed a correlation with overall patient survival for those diagnosed with NSCLC. An established threshold value in the literature from a different study was utilised in this work. This suggests the possibility of using parameters from a different study to predict patient survival despite differences in patient cohort and imaging parameters.

- Intratumoural spatial heterogeneity measured from CT images of NSCLC showed a clear textural pattern relating to tumour glucose metabolism, therefore, suggesting valuable information may be obtained from this approach.

- Image quantisation has been shown to significantly affect texture analysis. The proposed optimised image quantisation method in this thesis allowed the visualisation of meaningful textural content within tumour images without the use of machine learning or classification techniques.

- The scale of the extracted textural features has been demonstrated to be limited by the imaging system resolution. Moreover, image blurring due to motion has been demonstrated to measurably affect the performance of the texture analysis. Respiratory gating or motion correction methods are needed to minimise the loss of information resulting from this.

- The clinical application of our proposed methodology in radiotherapy dose painting demonstrated that high 18F-FDG regions within the tumour can be covered with an adequate dose when boosting based on CT image intratumoural heterogeneity. Furthermore, dose escalation in NSCLC was shown to be clinically feasible while keeping the dose to the OARs within accepted limits.
7.7 Future work

- Investigating other textural features using the volumetric voxel based texture mapping method and their relationship to tumour function.

- Investigating the relationship between intramural heterogeneity measured from the 3D-VTMs and hypoxia by comparing the 3D-VTMs to hypoxia tracer uptake distributions imaged with PET.

- Investigating and validating the proposed methodology on a larger dataset and other tumour sites such as head and neck.

- The use of motion corrected data to avoid the motion effects for both generating the 3D-VTMs and for target volume delineation.

- Investigating the relationship between the zero entropy region within the 3D-VTMs and tumour recurrence and treatment outcome.

- Incorporating machine learning techniques into volumetric voxel based texture mapping especially for feature selection.

- The application of 3D-VTMs in longitudinal treatment follow-up data to discriminate between scar tissue (fibrosis) post irradiation and tumour recurrence. This is currently a part of an ongoing MD project of Iain Phillips in the Royal Surrey County hospital using the methodology developed in this thesis. This new project aims to investigate whether the 3D-VTMs can distinguish between fibrosis and tumour recurrence for patients who have received SABR treatment for early stage lung cancer. The 3D-VTMs are generated for the whole lung volume due to difficulties in delineating region of interest in post-irradiation lung. To quantise the whole lung, the quantisation reconstruction levels and partitions are based on the quantisation of the original tumour volume prior to treatment. Moreover, the HU information is combined with the 3D-VTMs to provide additional information about tissue properties. Example preliminary results are presented in figure (7.1).
Fig. 7.1 (a) A follow-up CT image of the thorax three years post treatment. (b) The corresponding 3D-VTM for the whole lung. (c) The corresponding 3D-VTM with incorporating the HU information showing the tumour recurrence as a high intensity region (white).
References


References


Appendix A

FiNiTe Project Protocol

FiNiTe RT

Functional Imaging and Texture Analysis in Radiotherapy Dose Painting

Chief Investigator: Dr Veni Ezhil MRCP FRCR

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Contents:
1 Background ...................................................................................................................................................
2 Study design ...................................................................................................................................................
3 Objectives .....................................................................................................................................................
  3.1 Primary objectives ....................................................................................................................................
  3.2 Secondary objectives ................................................................................................................................
4 Statistical consideration ..............................................................................................................................
  4.1 Endpoints ..................................................................................................................................................
  4.2 Analysis methods ......................................................................................................................................
  4.3 Sample size ..............................................................................................................................................
5 Study Organisation ........................................................................................................................................
6 References ......................................................................................................................................................
Objectives: To determine whether functional imaging (FI) and texture analysis (TA) may be used in planning radiotherapy dose painting for patients diagnosed with non-small cell lung cancer (NSCLC) who are treated with external beam radiotherapy.

Hypothesis: Dose painting based on the combination of functional imaging (FI) and texture analysis (TA) will produce a non-uniform dose distribution that is measurably and significantly different to dose painting based on FI alone and to dose distribution produced by the current standard of prescribing a uniform dose distribution.

Study Population Summary: The proposed service evaluation study population is 10 patients who have been treated for non-small cell lung cancer (NSCLC) with external beam radiotherapy in the past two years.

Endpoints:
Primary Agreement of dose painting dose distribution based on functional imaging combined with texture analysis to dose painting based on FI alone and the current standard of a uniform dose distribution. The level of agreement will be quantified in terms of difference in target volumes and mean dose to the target volume and normal tissues.
Secondary To model the change in tumour control probability (TCP) and normal tissue complication (NTCP) as a result of FI with TA based dose painting.
To test whether FDG PET and texture analysis provide equivalent information or the combination of both produce significantly different results.
To investigate the level of confidence for observed difference in tumour’s texture between complete responders and partial responders six months post treatment.
1 Background A retrospective study using staging PET scan, planning CT scan, and follow up CT scan of patients treated for non-small lung cancer (NSCLC) with external beam radiotherapy. The study will evaluate the use of FDG functional imaging (FI) and texture analysis (TI) in radiotherapy dose painting against the current planning techniques. This is a computer modelling and image analysis study that uses scan data of a set of patients.

1.1 Dose Painting in Radiotherapy Traditionally, a uniform dose distribution to the tumour has been prescribed in radiotherapy treatment. However, it has been shown that tumours are biologically heterogeneous in regards to their cellular density, proliferation and oxygenation [1-2]. To account for the tumour’s biological heterogeneity, the delivery of a non-uniform dose distribution by applying a biological dose painting method has been suggested in the literature. The escalation of dose to sub-volumes of increased tumour burden and heterogeneity is hypothesized to increase tumour control probability (TCP). Molecular and functional imaging, such as positron emission tomography (PET) and magnetic resonance imaging (MRI), are strong candidates to guide the dose painting and enable dose escalation. The recent advancement in inverse treatment planning for radiotherapy seen in intensity modulated radiotherapy (IMRT) has made the delivery of a non-uniform dose distribution with a superior physical conformity achievable [2]. Dose painting can be achieved by two main approaches, dose painting by contours (DPBC) and dose painting by numbers (DPBN). In DPBC, one or more boost volumes are delineated which would represent biologically active sub-regions within the tumour. Boost volumes will receive a higher dose than the rest of the tumour volume either by redistributing the dose or by prescribing additional dose [3]. DPBN is achieved by prescribing the dose on pixel by pixel basis, assuming a linear relationship between pixel intensity and dose [4]. Meijer et al. [5] reported the maximum dose achieved with DPBC and DPBN was comparable in a retrospective study for NSCLC patients. Although DPBN has the advantage of delivering high peak doses at certain pixels, South et al. [6] has shown that by redistributing the dose in head and neck cancer patients using two to five compartments, the maximum achievable TCP is reached.

Dose painting based on Fluorodeoxyglucose (18F-FDG) has been suggested to be most suitable due to the moderately well-established and extensively validated protocols for the use of 18F-FDG PET in oncology compared to other PET tracers and functional MRI. Although 18F-FDG is a non-specific radiotracer that cannot be attributed to a specific biological phenomenon, it reflects glycolysis and tumour metabolism with a relatively high sensitivity. Moreover, it is widely available and the most commonly used radiotracer in cancer imaging compared to other radiotracers. The use of alternative
PET tracers suffer from limitations due to the lack of available clinical data and limitations in regards to their sensitivity and specificity [7], plus their limited availability and high cost.

1.2 Texture Analysis Textural analysis is an emerging method and it is used to assess tumour heterogeneity by extracting textural features (e.g., contrast, skewness, entropy, homogeneity, uniformity) from intensity histograms and co-occurrence matrices of CT, PET and MRI images. The Intensity histogram is a representation of the distribution of pixel intensities in an image from where global features of the image can be extracted such as the mean and standard deviation. On the other hand, second order statistics texture analysis is achieved by co-occurrence matrices. Co-occurrence matrix consist of the number of times an intensity level x and an intensity level y occur in a certain direction [8]. Another method for extracting higher order textural features is run length matrices. Run length matrices consist of the number of consecutive pixels that have the same intensity level and which occur in a specified direction [9]. Co-occurrence matrix based textural analysis has been used in the field of medical imaging for tissue classification [10]. Recently, it has been proposed that texture analysis has prognostic or predictive roles in cancer therapy (chemotherapy, radiotherapy or both) [11]. Tumours heterogeneity features have been reported to have a superior predictive power than PET or MRI measurements alone [11-13]. Yet, whether textural features can be used to identify areas of increased tumour burden or resistance to radiation is still to be determined. Moreover, the applicability of textural analysis in radiotherapy planning and dose painting is yet to be investigated. And even though recent studies have shown a correlation between heterogeneity measurements and tissue response, the accuracy and precision of textural analysis based on PET data is yet to be explored.

1.3 Current Standard and Proposed Method Patients diagnosed with NSCLC are treated with external beam radiotherapy where a uniform dose distribution is prescribed to the target volume. A planning CT scan is used to guide the radiotherapy treatment planning. An FDG PET scan is often only used for staging at time of diagnosis. The proposed service evaluation study will determine whether the combination of functional information obtained from PET scans and texture measurements extracted from CT and PET scans may be used to guide radiotherapy planning. Prescribing a non-uniform dose distribution where the regions of increased heterogeneity or tumour burden, will receive a higher dose relative to other regions in the tumour volume will be modelled. The local failure for late stage NSCLC patients who received radiotherapy treatment as per current standard is reported to be as high as 70% [14]. Dose painting by contours in radiotherapy is a technique that has the potential to allow
targeted dose escalation in NSCLC radiotherapy treatment where TCP is not presently good with the expectation of improved outcome. Furthermore, DPBC can be planned using commercially available treatment planning system. The goal of this work is to produce a radiotherapy treatment planning method for NSCLC that has the potential to be used to treat RSCH patients, using the imaging and treatment technology available at this centre.

2 Study Design: A retrospective study evaluating the use of functional imaging (FI) and texture analysis (TA) for dose painting in non-small-cell lung cancer patients (NSCLC) who are treated with external beam radiotherapy against the current standard.

2.1 Study Procedures As a retrospective service evaluation study, no procedures will be carried out on patients involved. The study will involve computer modelling and image analysis of patients’ PET and CT scans. The service evaluation study will involve obtaining existing staging FDG PET images; radiotherapy planning CT scans, and follow up CT scans for 10 patients who have been treated for NSCLC with external beam radiotherapy. These scans are obtained as part of standard patient management.

After retrospectively seeking consent for predetermined candidates, we will allocate a code to all images prior to inclusion to ensure anonymity. The staging FDG PET scan will be extracted and encoded and a quantitative analysis will be carried out to determine the tracer uptake variation. A dose distribution based on the measured uptake variations will be prescribed. The PET scan and the planning CT will be analysed to extract texture information regarding tumour heterogeneity. Intensity histograms from two dimensional (2D) images will be used to extract first order statistics texture measurements while co-occurrence matrices and run length matrices will be used as measurements of higher order statistics. The project will involve the extension of the traditional 2D texture measurements to three dimensional (3D) texture analyses to be used in identifying sub-volumes of increased heterogeneity and tumour burden referred to as boost volume. Using the combination of uptake distribution with texture features a boost volume will be delineated and a dose distribution will be prescribed (for planning study, not to be used clinically). Another dose distribution will be prescribed where the boost volume delineation will be based on functional information alone. The dose will be prescribed so that the boost volume will receive an escalated dose to a level where the normal tissues dose limits are not exceeded. A treatment plan (not to be used clinically) will be generated for the different dose prescriptions discussed above. The dose distribution for each plan will be evaluated
based on the mean dose delivered to target volume and boost volume, the volume of the tumour target and the boost volume, tumour control probability (TCP), normal tissue complication probability (NTCP) models and dose volume histograms (DVH). The results will test the hypothesized benefit of dose painting. The patients’ coded data will be transferred into a password secured server at the University of Surrey’s Centre for Vision, Speech, and Signal Processing (CVSSP) where access will only be granted to members of the study group.

3 Objectives: To determine whether functional imaging (FI) and texture analysis (TA) may be used in radiotherapy dose painting for patients diagnosed with non-small lung cancer (NSCLC) and who are treated with external beam radiotherapy.

Primary

- To determine whether the planned distribution for dose painting based on FI with TA is significantly different to the distribution for dose painting based on FI alone and the current standard of uniform dose distribution.

Secondary

- To model the change in tumour control probability (TCP) and normal tissue complication (NTCP) as a result of FI and TA based dose painting.
- To test whether FDG PET and texture analysis provide equivalent information or both are needed for optimal planning of dose painting.
- To investigate the level of confidence for observed difference in tumour’s texture between complete responders and partial responders.

4 Statistical Consideration:

4.1 Endpoints

Primary

- Level of agreement of dose painting dose distribution based on FI with TA to dose painting based on FI alone and the current standard of uniform dose distribution. The planned dose distributions will be compared with respect to the boost volume delineated and the maximum achievable dose to that volume.
Secondary

• Change in tumour control probability (TCP) and normal tissue complication (NTCP) as a result of FI and TA based dose painting.

• Determine whether FDG PET and texture analysis provide equivalent information or both are needed.

• Investigate the statistical level where a significant change is seen in tumour’s texture between complete responders and partial responders.

4.2 Analysis Method

Primary endpoint: the boost volume (regions to receive an escalated dose) delineated based on FI alone, and FI with TA, as well as the maximum delivered dose to those regions will be reported as significantly different if the variation falls outside the 95% confidence limits (P less than 0.05).

Secondary endpoint: relationship between areas of increased heterogeneity in pre-radiotherapy scans and areas of partial-respondance in follow-up scans using functional information alone, and texture analysis with functional information using similarity coefficient.

The change in calculated TCP using linear quadratic model and NTCP for normal lung and other mediastinal organs (oesophagus, heart, … etc) for dose painting plans based on FI, FI with TA, and the standard treatment.

4.3 Sample Size Based on the previous work of Bradley et al. [15] the mean percentage difference between the delineated tumour volume for NSCLC when using CT alone and using CT/PET was reported to be 57%. Hence, a percentage difference equal to or above 50% with power = 80% can be observed with sample size N = 10 patients [16].

The study will compare radiotherapy plans based on FI alone and FI with TA against the standard planning technique. The difference between the two dose painting plans will be reported significant if the minimum difference falls outside two standard deviations (k = 2) of the mean value at the 95% confidence level.
5 Study Organisation:

**Chief Investigator:** Dr Veni Ezhil MRCP FRCR

**Co-Investigators:**
- Prof Philip Evans
- Ms Sheaka Alobaidli
- Dr Chris South
- Dr Sarah McQuaid
- Prof Andrew Nisbet
- Dr Vineet Prakash

Dr Chris South and Dr Sarah McQuaid will provide access to CT and PET data and will allocate a study number to each patient to anonymize patient images. Miss Alobaidli will be responsible for image analysis and generating dose distributions and treatment plans (not to be used clinically) while assisting with data collection and anonymization.
References


Appendix B

B-spline Deformable Registration

The B-spline deformable registration algorithm maps the voxels in the moving image $M$ (CT\textsubscript{PET}) to the static image $S$ (CT\textsubscript{Plan}) generating a displacement field $g$ using cubic B-spline curves in such

$$M(x,y,z) = g(S(x,y,z))$$

B-spline registration has two steps, 1) B-spline interpolation 2) cost-function gradient computation. In B-spline interpolation the image volume is partitioned into equal sized regions by aligning a control grid over the voxel grid. The algorithm calculates control points to describe the voxel’s movement between the moving and the static image. These control points are uniformly spaced and aligned with the voxel grid. Interpolation using piecewise continuous B-spline basis functions between control points was used to generate displacement vectors. The algorithm minimises the mean squared cost function $C$ shown in equation (B.1). The cost function $C$ calculates the square of the intensity difference between static image $S$ and moving image $M$ via numeric optimisation [116][122].
\[ C = \frac{1}{N} \sum_{x} \sum_{y} \sum_{z} (S(x, y, z) - M(x + v_{x}, y + v_{y}, z + v_{z}))^{2} \]  \hspace{1cm} (B.1)

To minimise \( C \), the algorithm calculates the spline coefficients \( T \). The spline coefficient \( T_{x,i,m,n} \) in equation (B.2) defines the x component of the displacement vector for one of the control points that influence the voxel movements. Equation (B.2) calculates the x component of the displacement vector for a voxel located at \((x, y, z)\)

\[ v_{x}(x, y, z) = \sum_{i=0}^{3} \sum_{m=0}^{3} \sum_{n=0}^{3} \beta_{i}(u)\beta_{m}(v)\beta_{n}(w)T_{x,i,m,n} \]  \hspace{1cm} (B.2)
\( \beta \) is the uniform cubic B-spline basis function shown in equation (B.3).

\[
\beta_l(u) = \begin{cases} 
\frac{(1-u)^3}{6} : l = 0 \\
\frac{3u^3 - 6u^2 + 4}{6} : l = 1 \\
\frac{-3u^3 + 3u^2 - 3u + 1}{6} : l = 2 \\
\frac{u^3}{6} : l = 3,
\end{cases}
\]

In equation (B.2), \( \beta_l(u) \) is the basis function along the x-direction and it is calculated using equation (B.3). Where \( u \) is equal to

\[
u = \frac{x}{N_x} - \left\lfloor \frac{x}{N_x} \right\rfloor
\]
The dimensions of the gridded region that is segmented within the volume by B-spline are $N_x, N_y, N_z$ and the indices of the voxel of interest that is located at $(x, y, z)$ are $x_t, y_t, z_t$. Similarly, to calculate $\beta_m(v)$ and $\beta_n(w)$, $v$ and $w$ are calculated as

$$v = \frac{y}{N_y} - \left\lfloor \frac{y}{N_y} \right\rfloor$$

$$w = \frac{z}{N_z} - \left\lfloor \frac{z}{N_z} \right\rfloor$$

The displacement vector field is termed $\vec{v}$. A vector field can be defined using coefficients $T$ of finite number of control points. Registration is an optimisation problem where a cost function can be defined to quantify the similarity between the images of interest. B-spline registration is performed where $T$ coefficients are iteratively defined at the control points then B-spline interpolation is conducted and the cost function $C$ is evaluated. To generate the next set of coefficients $T$, the change in cost function $C$ termed cost function gradient $\frac{\partial C}{\partial T}$ is calculated for each control point. The cost function gradient $\frac{\partial C}{\partial T}$ equation is presented in (B.4-B.7). The gradient is calculated for control point at this grid coordinates $(k, \lambda, \mu)$. The voxel coordinates within the region grid are termed $(a, b, c)$. 


\[
\frac{\partial C}{\partial T_{k,\lambda,\mu}} = \frac{1}{64} \sum_{(x,y,z)} \frac{\partial C}{\partial \vec{v}(x,y,z)} \frac{\partial \vec{v}(x,y,z)}{\partial T} \tag{B.4}
\]

\[
\frac{\partial \vec{v}(x,y,z)}{\partial T} = \sum_{l=0}^{3} \sum_{m=0}^{3} \sum_{n=0}^{3} \beta_l(u) \beta_m(v) \beta_n(w) \tag{B.5}
\]

\[
\frac{\partial C}{\partial \vec{v}(x,y,z)} = 2 \times \left[ S(x,y,z) - M(x + v_x, y + v_y, z + v_z) \right] \nabla M(x,y,z) \tag{B.6}
\]

\[
\frac{\partial C}{\partial T_{k,\lambda,\mu}} = \frac{1}{N} \sum_a^{N_x} \sum_b^{N_y} \sum_c^{N_z} \frac{\partial C}{\partial \vec{v}(x,y,z)} \sum_{l=0}^{3} \sum_{m=0}^{3} \sum_{n=0}^{3} \beta_l \left( \frac{a}{N_x} \right) \beta_m \left( \frac{b}{N_y} \right) \beta_n \left( \frac{c}{N_z} \right) \tag{B.7}
\]

To find the \( T \) coefficient values that minimises the cost function \( C \), the limited memory Broyden-Fletcher-Goldfarb-Shanno (L-BFGS), quasi-Newton optimiser is used [165]. Detailed description of the L-BFGS method is presented in [24] and [165]. The coefficients are updated for each iterations and the cost and gradients are computed until the cost function \( C \) has converged.
Appendix C

List of Publications

• Alobaidli S, Mcquaid S, South C, Prakash V, Evans P, Nisbet A.
The role of texture analysis in imaging as an outcome predictor and potential tool in radiotherapy treatment planning.

• Alobaidli S, McQuaid S, Scuffham J, South C, Nisbet A, Evans P.
Effect of respiratory motion on extracted textural features in tumour CT images. Radiotherapy and Oncology.
Radiother Oncol 2016;119, Supplement 1:S877-S878.

• Alobaidli S, South C, McQuaid S, Scuffham J, Phillips I, Prakash V, Ezhil V, Nisbet A, Evans P.
Dose Painting Study Based on CT Intratumoural Heterogeneity vs. FDG PET Uptake in NSCLC.
Under Review.

• McQuaid S, Scuffham J, Alobaidli S, Prakash V, Ezhil V, Nisbet A, South C, Evans P.
Factors influencing the robustness of P-value measurements in CT texture prognosis studies.
Under Review.
Awards:
Winner of the "Three Minute Research Competition" in the Faculty of engineering and Physical Science Festival of Research 2016.