FULL ARTICLE

Investigating FTIR based histopathology for the diagnosis of prostate cancer

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Prostate cancer is the most common gender specific cancer. The current gold standard for diagnosis, histopathology, is subjective and limited by variation between different pathologists. The diagnostic problems associated with the correct grading and staging of prostate cancer (CaP) has led to an interest in the development of spectroscopic based diagnostic techniques. FTIR microspectroscopy used in combination with a Principal Component Discriminant Function Analysis (PC-DFA) was applied to investigate FTIR based histopathology for the diagnosis of CaP. In this paper we report the results of a large patient study in which FTIR has been proven to grade CaP tissue specimens to a high degree of sensitivity and specificity.

A representation of the use of infrared light to classify cancerous prostate tissue.

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1. Introduction

Prostate cancer (CaP) is the most common cancer and the second commonest cause of cancer related deaths of men in the UK, with 10 164 death in 2003 [1]. Despite it being a major public health problem, there are still many uncertainties relating to the in-

ability to discriminate accurately between clinically aggressive and non-aggressive cancer phenotypes and to the ability of the clinicians and pathologists to identify and treat aggressive disease at an early stage, when the condition is potentially curable.

The ability to detect clinically significant disease early has many obvious benefits. It facilitates early

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intervention with therapeutic strategies and with this comes the potential for significant reduction of mortality or morbidity. Added precision in diagnosis will also avoid unnecessary and inappropriate treatment of cancers with low biopotential and will potentially allow focussing of more aggressive therapies on disease sub-types with the highest likelihood of progression. The identification of new indicators of disease could also be used to monitor the response to therapy [2].

The current diagnostic methods involve clinical examination of the prostate by digital rectal examination (DRE) and the use of PSA as indicators for prostate biopsy. DRE is used for detecting palpable malignant characteristics whilst blood testing involves measurement of circulating prostate specific antigen (PSA) levels. Whilst this latter test has revolutionised the diagnosis and management of CaP by enabling early detection of disease it does not provide a definite diagnosis of CaP [3] and furthermore, it has led to the increased detection of cancers which may not necessarily have progressed to death in many men. The increased screening for “abnormal” PSA levels has caused a continuous rise in new cases of CaP [4] but it has yet to be shown categorically to decrease prostate cancer mortality [5].

A prostate biopsy is required for definitive diagnosis and the histopathological evaluation centres around two main observations, the change in architecture of the tissue, cell or internal constituents and the identification of immunohistochemical alterations in the tissue which relate to markers of particular cell types and/or changes in protein expression. The instrument for determination of biopotential of individual tumours is a tumour grading system known as Gleason grading [6]. This is the universally accepted method for the grading of prostate cancer [7] and consequently is accepted in the World Health Organisation (WHO) classification [8]. This method is based upon the complex continuum of glandular architectural changes that occur during CaP progression. However it has significant flaws, particularly intra-observer and inter-observer error. In a study of 390 CaP biopsies Lattouf *et al.* [9] found that different pathologists misgraded 70.8% of tumours and reproducibility increased to 49% when the same pathologist graded the tissue. Thus, the method is a very observer dependent test and this limits its usefulness in clinical decision-making. Several studies have assessed the observer dependence and reliability of the Gleason grading system [10–13]. This is a well documented problem into which a lot of directed research occurs but as yet no suitable replacement or addition to Gleason grading has been discovered.

This diagnostic problem is compounded by the variable natural history of CaP. There is a clear trend using Gleason grading wherein high grade disease is more aggressive and low grade disease is more indolent.

However, there is a great deal of crossover in the middle area (Gleason score 6 and 7), with many Gleason 7 cases failing to progress and conversely, many Gleason 6 cases progressing in an unpredictable way [14]. Thus current staging modalities are unable to predict the natural history of CaP sufficiently and accurately, resulting in inappropriate patient management with increased patient morbidity.

The limitations of these diagnostic modalities have led to an interest in the developments of spectroscopic analytical techniques for cancer diagnosis. IR spectroscopy is a quick, cost-effective, simple to operate, reagent free technique that requires simple sample preparation.

Infrared spectroscopy has been used and is still being investigated in its application to the diagnosis and staging of several cancerous and non-cancerous conditions [15–20]. In CaP it has been shown possible to discriminate benign tissue, prostate cancer and PIN (prostatic intraepithelial neoplasia) to a sensitivity of approximately 80%. This work proved that FTIR microspectroscopy coupled with PCA-fed-LDA is able to discriminate between benign and cancerous tissue [21]. Another study has shown the use of tissue microarray based FTIR imaging for a high throughput approach towards histological classification [22, 23]. However there is a need for a large patient study which examines CaP aggressiveness to build on this pilot study work focussed on cancerous versus non-cancerous tissue.

Our previous research has shown that FTIR spectroscopy coupled with multivariate analysis is a promising means of reinforcing the Gleason scoring system of grading prostate cancer [24–27]. FTIR spectra from CaP tissue biopsy samples were analysed using linear discriminant analysis (LDA) to construct a grading model. Results showed a low correlation of 20% between the biospectroscopic FTIR-LDA score and the histologic Gleason score. However, a much higher correlation was obtained according to the three-band Gleason score criteria: Gleason score less than 7 ($GS < 7$) equal to 7 ($GS = 7$) and greater than 7 ($GS > 7$). This is a more clinically oriented scale based upon the use of Gleason score 7 as an indication to the aggressiveness of the tumour, which reflects the reality of prostate cancer progression and the clinical triggers which initiate the decision to treat radically or to adopt a surveillance schedule.

This paper discusses the recent results of an important relationship in translational research between basic chemical science (Analytical Chemistry at the University of Manchester) and clinical translational research (the Genito-Urinary research group at The Christie NHS Foundation Trust and Paterson Institute for Cancer Research). The overall aim of this collaboration is to develop novel diagnoses for CaP that are highly sensitive and sufficiently specific

to enable CaP to be diagnosed and staged correctly and to identify clinically aggressive from non-aggressive disease. This will hopefully allow better direction of treatment in this distressing condition. Herein we describe the use of FTIR microspectroscopy combined with principal component – discriminant function analysis (PC-DFA) to evaluate a large patient group using analysis of formalin fixed archival prostate cancer tissue based on Gleason score and Tumour, Node, Metastases (TNM) staging system. The aim of this study is to determine a spectral signature that identifies specific sub-types of prostate cancer and to relate this to the observer dependent criterion of Gleason grading, in addition to examining spectral signatures based upon the observer independent TNM staging system. This has the potential to create a clinically important system of spectroscopic grading scale for CaP.

2. Materials and Methods

2.1. Primary Tissue Preparation

With full ethical committee approval, Trent MREC 01/4/061, tissue was collected from 39 consenting patients undergoing trans-urethral resection of the prostate (TURP) for bladder outflow obstruction. 40 prostate cancer (CaP) tissue biopsy specimens from 39 men were obtained as paraffin-embedded blocks (Genito-Urinary Cancer Research Group, Paterson Institute for Cancer Research) from patients with CaP. The tissue was fixed in 4% formalin for 24 hours then placed onto a Thermoshandon Excelsior processor which passes 20%, 90% and four 100% ethanol aliquots through the tissue for an hour each, followed by three changes of xylene for an hour at a temperature of 40 °C, followed by three changes of paraplast for an hour each at 62 °C. The tissue was embedded in a mould of molten wax and cooled on an ice plate for an hour before storage.

Serial sections were collected at 10 µm thickness from each specimen, one of which was mounted onto a BaF₂ plate (Linkham Scientific Ltd) with the adjacent section mounted onto a glass slide and stained with hematoxylin and eosin (H&E). An experienced histopathologist with a sub specialist interest in genitourinary pathology, including participation in the UK national prostate histopathology external quality (EQA) scheme assigned Gleason scores to areas of malignancy identified within the H&E sections. There is evidence to suggest that a single pathologist with an interest in urologic pathology is likely to give the most consistency of grading required for the evaluation of a diagnostic method [9, 28]. The complementary sections of the same can-

Figure 1 (online colour at: www.biophotonics-journal.org) Examples of Gleason score 7 biopsies with cancerous areas circled.

cer region were mounted on BaF₂ plates and washed on an orbital mixer with CitrocLEAR for 6 minutes to remove the paraffin and then acetone at 4 °C for a further 6 minutes before being air-dried for one hour under ambient conditions. The anatomical features identified from the H&E sections were used as landmarks to position the IR beam upon the malignant lesions of the unstained adjacent section. Figure 1 shows two examples of needle core biopsies of CaP tissue Gleason score 7 with cancerous areas marked.

2.2. FTIR-Microspectroscopy

FTIR spectra of Gleason graded primary prostate tissue were collected in transmission mode using a Nicolet Magna system 550 spectrometer, coupled to a NicPlan infrared microscope equipped with a liquid nitrogen cooled MCT/A detector. The microscope also incorporated a video camera in order to view optical images ($\times 150$ magnification) of the sampling area and a programmable computerised $x - y$ stage. The spectra were collected in transmission mode using an aperture size of $60 \times 60 \mu\text{m}$ to enable good quality spectra to be acquired whilst allowing the sample area to completely fill the analysis window. FTIR spectra represent an average of 512 scans in the mid-IR Wavenumber range $750 - 4000 \text{ cm}^{-1}$ with a spectral resolution of 4 cm^{-1} and took approximately 4 minutes each to collect. Background scans were obtained from a region outside

the sample field and ratioed against the sample spectrum. In total, 395 spectra were obtained from the 40 CaP tissue biopsies from 39 patients with at least 5 spectra recorded from each patient. The total number of spectra acquired from each patient was dependent on the size of the tumour lesion within the tissue section as well as positive identification of tumour glands in the unstained tissue section.

2.3. Data Processing

Data processing was carried out in MatlabTM using in-house written software. The spectral region used was 750–4000 cm⁻¹ with the region from 1847–2809 cm⁻¹ removed since it contains no diagnostically useful information. This resulted in 1188 spectral data points for analysis. The spectra were vector-normalised a standard pre-processing routine in which each variable, each input, is squared, the square root of the sum of these squares is calculated and each original input is divided by this square root to correct for baseline shifts and variations in sample thickness [29].

Principal Component – Discriminant Function Analysis (PC-DFA) was used to discriminate the spectra. PC-DFA used Principal Component Analysis (PCA) to reduce the dimensionality of the data prior to DFA. DFA then discriminates on the basis of the resulting principal components (PCs) and the *a priori* knowledge of the group memberships that are fed in the DFA algorithm. Maximising the inter-group variance and minimising the intra-group variance achieves this.

As PC-DFA is a supervised technique, a way of calculating the optimum number of principal components is needed. The only robust way of estimating the correct number of PCs is by carrying out some method of cross validation, in this case training set/test set validation [30]. Essentially a PC-DFA model is built for each PC up to a maximum number of m PCs. The optimum number of PCs is the one that provides maximal group separation and correct identification of classes. The problem occurs when deciding the maximum number of PCs. Dixon [31] and McFie *et al.* [32] state that DFA can only be performed if $(N_s - N_g - 1 > N_v)$ where N_s is the number of independent specimens, N_g is the number of groups and N_v the number of variables. Manipulating this equation and stipulating that each tissue biopsy equals an independent specimen, this allows us to use a maximum of 36 principal components.

PC-DFA is a supervised technique, the model is supplied with information about group membership, so any result produced by the model needs to be tested. The testing was carried out by retaining patient's spectra as an independent test set and then

supplying the spectra to the model as an independent test set and observing where the model places the spectra on a graphical output. Error ellipses with 95% confidence are added to the discriminant function plots. This was achieved using `error_ellipse.m` written by AJ Johnson and obtained from Matlab central file exchange. Covariance matrices were calculated from the discriminant function analysis scores matrix for each grouping, where the centroid was defined as the mean of the discriminant function analysis scores matrix for each grouping.

3. Results

3.1. FTIR Biospectroscopic Gleason Score

The FTIR PC-DFA diagnostic model was derived from 40 CaP tissue biopsies. The 40 biopsies correspond to 395 spectra (90 spectra from 10 biopsies for $GS < 7$, 118 spectra from 11 biopsies for $GS = 7$ and 187 spectra from 19 biopsies for $GS > 7$). Spectra were divided into a training set consisting of 233 spectra (39 spectra from 5 biopsies for $GS < 7$, 63 spectra from 6 biopsies for $GS = 7$ and 131 spectra from 12 biopsies for $GS > 7$) and a randomly chosen independent test set (51 spectra from 5 biopsies for $GS < 7$, 55 spectra from 5 biopsies for $GS = 7$, 56 spectra from 7 biopsies for $GS > 7$). One patient from each group was used to determine the optimum number of PCs and then incorporated into the independent test set. Importantly, in previous work the test set consists of spectra randomly selected from the entire data set of spectra, so spectra from one area of a biopsy may be in the training set and spectra from a different area of the same biopsy may be in the test set. In this present study spectra from one group of patients randomly selected make up the training set and the spectra from the remaining patients make up the independent test set. Thus a given patient cannot be represented in both training and test sets of spectra. This represents a much more stringent test model.

The Gleason score is the sum of the predominant and highest Gleason grades observed within biopsy. When the biopsy only has one Gleason grade this grade is doubled to produce the resulting Gleason score [3]. A 3-band Gleason score criteria was used, which divided specimens into groups corresponding to $GS < 7$, $GS = 7$ and $GS > 7$. This method is a more clinically oriented scale in which Gleason scores of < 7 are less aggressive, Gleason score $= 7$ are of intermediate aggressiveness and $GS > 7$ are the most likely to progress. This grouping has been shown to previously be characterised by specific FTIR spectral characteristics [25]. Table 1 shows sen-

Table 1 Sensitivities and specificities for the vector normalised Gleason Score model.

	GS < 7	GS = 7	GS > 7
Sensitivity (%)	70.6	83.6	69.6
Specificity (%)	88.2	86.0	71.7

sitivity and specificity observed for each of the bands in the criterion at 95% confidence limits. In the case presented here sensitivity refers to the proportion of biopsies with a particular GS, which have a positive test result for that score and specificity refers to the proportion of biopsies with a particular GS, which have a negative test result for that score. Figure 2 shows the discriminant function plot for this model based upon training set data (empty shapes) and blind set data (full shapes) with a 95% confidence limit where the green circle = GS < 7, red square = GS = 7 and cyan diamond = GS > 7.

The vector normalised Gleason score model achieves sensitivities as high as 83.6% and specificities as high as 86%. The axes of the discriminant function plots have positive and negative directions (Figure 2). These correspond to the positive and negative directions of the loadings plots. Discriminant function 1 is discriminating GS = 7 from GS > 7 and GS < 7. Discriminant function 2 is discriminating GS < 7 from GS = 7 and GS > 7, therefore it is being used to separate the less aggressive cancer from the more aggressive cancers. Figure 3 shows the loading plots for discriminant function 1 and discriminant function 2. The peaks in the positive direction of discriminant function 1 are those relating to GS = 7 and the peaks in the negative direction relate to GS > 7 and GS < 7. The peaks in positive direction of discriminant function 2 are those relating to GS < 7 and those in the negative direction relate to GS = 7 and GS > 7. Table 2(A) shows the major spectral peaks responsible for the Gleason score and proposed biomolecular assignments for discriminant function 1 and Table 2(B) for discriminant function 2.

Figure 2 (online colour at: www.biophotonics-journal.org) Discriminant function plot for the vector normalised Gleason score model based upon training set data (empty shapes) and blind set data (full shapes) with a 95% confidence limit where the green circle = GS < 7, red square = GS = 7 and cyan diamond = GS > 7.

3.2. FTIR Biospectroscopic Clinical Stage

The clinical stage is taken from the TNM (tumour/node/metastases) classification system [33]. T stages are classified as T1 and T2 tumours (confined to the prostate), T3 (breaching the prostatic capsule or invading the seminal vesicle) and T4 tumours (extending beyond the prostate and seminal vesicle to invade local pelvic structures). N stages are classified as N0, Nx (nodal status unknown) or N+ (nodal status positive) and M stage as M0 or M1.

Clinical staging information was available for 33 out of 39 patients. This corresponds to 347 spectra (191 spectra from 18 biopsies for T1 & T2 and 156 spectra from 15 biopsies for T3 & T4). Definitive lymph node staging was not available in T1 and T2 patients: these were designated N0. For the same reasons most low stage and grade tumours were designated Mx.

Figure 3 (online colour at: www.biophotonics-journal.org) Loading plots for (A) discriminant function 1 and (B) discriminant function 2 with major peaks labelled.

Table 2 Major spectral peaks responsible for the Gleason score discrimination and proposed biomolecular assignments for (A) discriminant function 1 and (B) for discriminant function 2. Spectral assignments taken from Refs. [36–41].

A		
Direction	Wavenumber (cm ⁻¹)	Proposed Biomolecular Assignment
+ve	1261	Amide III (NH bend (55%), CC stretch (19%), CN stretch (15%), CO bend (11%)).
+ve	1265	Amide III ((NH bend (55%), CC stretch (19%), CN stretch (15%), CO bend (11%))
+ve	1541	or PO ₂ ⁻ stretch, RNA, DNA
+ve	1541	Amide II of α -helical structures (NH bend (43%), CN stretch (29%), CO bend (15%), CC stretch (9%), NC stretch (8%))
+ve	2910	C–H stretch (asymmetric) of >CH ₂ in fatty acids, lipids, proteins
–ve	1460	CH ₃ antisymmetric bend
–ve	1685	Amide I of antiparallel β -sheets/aggregated strands protein structures (CO stretch (76%), CN stretch (14%), CCN deformation (10%))
–ve	2848	C–H symmetric stretch of >CH ₂ in fatty acids, lipids and proteins
–ve	2955	C–H asymmetric stretch of –CH ₃ in fatty acids, lipids and proteins

B		
Direction	Wavenumber (cm ⁻¹)	Proposed Biomolecular Assignment
+ve	1460	CH ₃ antisymmetric bend
+ve	1473	CH ₂ scissoring
+ve	1537	Amide II of β -sheets (NH bend (43%), CN stretch (29%), CO bend (15%), CC stretch (9%), NC stretch (8%))
+ve	2918	C–H stretch (asymmetric) of >CH ₂ in fatty acids, lipids, proteins
–ve	1350	Amide III (NH bend (55%), CC stretch (19%), CN stretch (15%), CO bend (11%)).
–ve	1541	Amide II of α -helical structures (NH bend (43%), CN stretch (29%), CO bend (15%), CC stretch (9%), NC stretch (8%))
–ve	1652	
–ve	1684	Amide I of antiparallel β -sheets/aggregated strands protein structures (CO stretch (76%), CN stretch (14%), CCN deformation (10%))
–ve	2845	C–H symmetric stretch of >CH ₂ in fatty acids, lipids and proteins
–ve	2912	C–H stretch (asymmetric) of >CH ₂ in fatty acids, lipids, proteins

In our study a 2 band criterion was used that divided specimens into either T1 & T2 (confined to the prostate) or T3 & T4 (extensions outside the prostate). The spectra were split into a training set consisting of 237 spectra (136 spectra from 12 biopsies for T1 & T2 and 101 spectra from 8 biopsies for T3 & T4) and a randomly chosen independent test set of 110 spectra (55 spectra from 6 biopsies for T1 & T2 and 55 spectra from 7 biopsies for T3 & T4). One patient from each group was used to determine the optimum number of PCs and then incorporated into the independent test set. Figure 4(A) shows the discriminant function plot for this model based upon training set data (empty shapes) and blind set data (full

shapes) where the red circle = T1 & T2 and green square = T3 & T4. As only one discriminant function is used, the number of discriminant functions is the number of groups minus one, no confidence ellipse is available. Figure 4(B) shows the loadings displaying the spectral differences for this vector-normalised model. The peaks in the positive direction of discriminant function 1 separate prostate confined cancer (T1 & T2) from extraprostatic cancer (T3 & T4) whereas the peaks in the negative direction separate invasive cancer from prostate confined cancer. Table 3 shows the major spectral peaks responsible for the Gleason score and proposed biomolecular assignments for discriminant function 1.

Table 3 Major spectral peaks responsible for the T stage discrimination and proposed biomolecular assignments for discriminant function 1. Spectral assignments taken from Refs. [36–41].

Direction	Wavenumber (cm ⁻¹)	Proposed Biomolecular Assignment
+ve	1350	Amide III (NH bend (55%), CC stretch (19%), CN stretch (15%), CO bend (11%))
+ve	1566	Amide II (NH bend (43%), CN stretch (29%), CO bend (15%), CC stretch (9%), NC stretch (8%))
+ve	1653	Amide I of α -helical or unordered structures (CO stretch (76%), CN stretch (14%), CCN deformation (10%))
+ve	2910	C-H stretch (asymmetric) of >CH ₂ in fatty acids, lipids, proteins
-ve	1460	CH ₃ antisymmetric bend
-ve	1473	CH ₂ scissoring
-ve	1558	Amide II (NH bend (43%), CN stretch (29%), CO bend (15%), CC stretch (9%), NC stretch (8%))
-ve	1656	Amide I of α -helical or unordered structures (CO stretch (76%), CN stretch (14%), CCN deformation (10%))
-ve	1686	Amide I of turns or antiparallel β -sheets structures (CO stretch (76%), CN stretch (14%), CCN deformation (10%))
-ve	2848	C-H symmetric stretch of -CH ₂ in fatty acids, lipids and proteins

4. Discussion

4.1. FTIR Biospectroscopic Gleason Score

Gleason grading of CaP biopsies is subject to both intra and inter observer variability, which reduces any prognostic value arising from the grading system [9–13]. In order to reduce the variability in Gleason grade between specimens we graded our specimens using a single histopathologist to assign Gleason scores to CaP tissue sections.

Whilst Gleason grading is a useful clinical method there are clear difficulties with inter-observer variation. Additional diagnostic modalities may provide an important adjunct to diagnosis and in particular, to the identification of aggressive cancers [34]. We have shown in this and previous studies [24–27] that chemometric analysis using vibrational spectroscopy is a viable tool for assessing paraffin embedded formalin fixed CaP biopsies on a large patient base.

One of the major limitations of this work is the basis upon the observer dependent Gleason grading system. This methodology is flawed, as it will tend to incorporate problems in the Gleason system into the new system. However, it was felt important to investigate this novel area in this manner to establish proof of principle. Now this goal has been achieved it is necessary to look forward to future work. A study utilising archival tissue from several different tissue banks, increasing the number of cases, would provide a greater test for the discrimination. Future work will now concentrate upon the development of a spectral grading scale in anticipation that this approach may yield alternative and potentially comple-

mentary methods for the assessment of prostate cancer.

The spectral differences between the Gleason scores (Table 2(A) and (B) and Figure 2) occur in the Amide, C-H, CH₂ and CH₃ regions. Previous work in the field has also found the spectral regions to be discriminatory [15–17, 26]. However the majority of work has focussed upon the ability to discriminate cancerous from non-cancerous tissue. In this study we have been able to grade individual cancers and the model has predicted a projection of previously unseen data from a completely blind data set. The prediction of aggressiveness is of particular importance in prostate cancer; where there is a pressing need to differentiate between the indolent and aggressive forms of the disease. The former represents the majority of cancers diagnosed through screening, whilst the latter is responsible for the large number of male cancer deaths [35, 42].

4.2. FTIR Biospectroscopic Clinical Stage

This study shows the successful first steps of tumour identification utilising an observer independent criterion. We have shown that it is possible to discriminate prostate confined cancer from extraprostatic cancer utilising FTIR spectral signatures and that the spectral peaks again reside in the amide and lipid regions of the spectrum (Figure 4(B) and Table 3). This is a potentially important finding, which may be of particular utility in treatment planning, where pathological upstaging of disease previously thought to be of a lower stage is a common prob-

Figure 4 (online colour at: www.biophotonics-journal.org) **(A)** discriminant function plot for this model based upon training set data (empty shapes) and blind set data (full shapes) where the red circle = T1 & T2 and green square = T3 & T4 and **(B)** loadings plot with major peaks labelled.

lem [35] and the ability to show that an individual patient's disease is of a higher clinical stage than is currently possible using existing criteria is of special importance. Such information, if it were available reliably would potentially alter the type of treatment offered to individual patients dramatically. This study has shown there are valid biochemical differences between these two states of prostate cancer, opening the way for further studies to validate the utility of this approach in prostate cancer staging.

In conclusion this study utilises a well-planned and constructed discriminatory test with patients being used as an independent test set. The utilisation of whole patients as an independent test set means the model is projecting and diagnosing previously unseen data. We report the use of FTIR combined with PC-DFA to discriminate CaP tissue based upon a 3-band Gleason criterion to sensitivities and specificities as high as 83.6% and 86.0% respectively. This study also reports the first steps towards independent spectral testing by utilising an observer independent criterion of locally confined compared to locally invasive prostate cancer. These findings have significant potential importance for the development of better techniques for diagnosis, prognostication and treatment planning in this disease.

5. Future Prospects

There are numerous small scale studies that have shown that infrared spectroscopy can be used to discriminate areas of cancerous from non cancerous tissue, and discriminate other histological types of tissue [15, 16, 18–23]. In this study we have demonstrated the more challenging task of grading cancer tissue and with some success have shown that IR data can give an indication of the stage of the

disease. Importantly both models have been blind tested using patients that were not included in the training set. Future work in this field involves carrying out larger scale trials possibly multi-centre studies with a much larger number of samples. Furthermore, due to developments in infrared technology, rather than using a microscope with a single point detector, as used here, future work will almost certainly be carried out using infrared microscopes fitted with linear array (typically 16 detector elements) or focal plane array (typically 64×64) for tissue imaging. Using a pixel resolution of $25 \times 25 \mu\text{m}^2$, it has recently been shown that large areas of tissue (few mm^2) can be imaged rapidly in a few hours [20]. This is important since it means that the whole biopsy can be sampled in an acceptable time frame. New rapid scanning 128×128 focal plane array detector systems promise to reduce these times further and also decrease the spatial (pixel) resolution to approximately $5 \times 5 \mu\text{m}^2$. These advances open up the possibility of routinely analysing large numbers of samples that will be required for this technology to reach the clinical environment. Similarly, with this in mind, imaging tissue micro arrays coupled with fast computational methods will help in the automation of the diagnostic process, again leading to a more rapid analysis of samples [22, 23, 43]. The combination of this new technology with the analysis methods presented in this paper will hopefully allow FTIR based histopathology to achieve its full potential in the clinical environment in the future.

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Peter Gardner obtained a Ph.D. in surface vibrational spectroscopy in 1988 from the University of East Anglia. He then spent four years at the Fritz Haber Institute (MPG) in Berlin using vibrational spectroscopy to monitor oscillatory

reaction phenomenon on surfaces. In 1992 he joined the Chemistry Department at the University of Cambridge studying catalytic systems and then in 1994 moved to the Chemistry Department at UMIST. In 2004, with the formation of the University of Manchester he joined the School of Chemical Engineering and Analytical Science but his research is carried out at the newly opened Manchester Interdisciplinary Biocentre. Since 2000 he has built a successful biomedical spectroscopy group and in collaboration with Noel Clarke has focussed on using vibrational spectroscopy in the diagnosis of prostate cancer. His group also have a keen interest in understanding the fundamental caused of spectroscopic discrimination and separating biochemical and physical spectral influences.

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