The Role of Vibrational Spectroscopy in the Diagnosis of Brain Tumours

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Abstract

With the incidence of many cancers, including brain tumours, increasing worldwide, the diagnostic pathway and new innovative treatments have often failed to keep pace. The main stay of cancer diagnosis remains at the Histopathologists' microscope, with little change in light microscopy within recent times. Research promises many new diagnostic tools, aiming to improve turn around times and provide instant accurate answers. One such tool is vibrational spectroscopy. This thesis aims to use spectroscopy as a proof of concept within brain tumour diagnostics to demonstrate its abilities within the cancer diagnostic pathway.

Proof of concept studies aimed at targeting both biofluid and tissue diagnosis of primary and metastatic brain tumours has been performed, along with involvement of Patients' and Clinicians' to aid development of diagnostic tools. Spectrochemical methods including Raman and attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR) have shown an ability to diagnose primary and metastatic tumours; with fresh frozen tissue ATR-FTIR proved superior with a classification accuracy of non-tumour brain *versus* primary brain tumours of 97.2%, though this decreased when comparing tumour types (79% accuracy); when differentiating metastatic brain tumours from formalin fixed tissue accuracy was similar for both spectroscopic techniques at 60% for colorectal adenocarcinomas, 68% for lung adenocarcinomas and 70% for melanoma; finally, with biofluids, using ATR-FTIR to determine a primary *versus* metastatic tumour and the type of each tumour, accuracy was low at non tumour 85%, high grade glioma 92%, low grade glioma 61%, meningioma 43%, melanoma metastasis 21%, colorectal adenocarcinoma metastasis 50% and lung adenocarcinoma metastasis 24%. The final, novel study, trialled a handheld Raman spectrometer within the histopathology department at Royal Preston Hospital, Lancashire Teaching Hospitals NHS Trust, to determine if the benefits of spectroscopy lay within the intraoperative diagnosis. The final results demonstrate accuracies from 64-94% depending on tumour type, demonstrating that with further training, Raman spectroscopy may provide a clinically useful diagnostic tool within the operating theatre, to replace the need for intraoperative smear preparations and diagnosis by a Neuropathologist.

Overall, this thesis highlights the need to involve Patients' and Clinicians' within research to ensure uptake and accurately targeted diagnostic tools. It also demonstrates the potential of spectroscopy, when well targeted within the diagnostic pathway. Moving forward, further work to move Raman spectroscopy into the operating theatre, is likely to prove beneficial to patients.

Contents

Acknowledgements

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To those above and many others, thank you, I appreciate everything you have done to help and support me and I hope, in part, this thesis demonstrates that.

List of Figures

List of Tables

List of Abbreviations

Alk	Anaplastic lymphoma kinase
ANOVA	Analysis of variance
Anti-EpCam/BerEP4	Anti-epithelial cell adhesion molecule
ATR-FTIR	Attenuated total reflectance-Fourier-transform infrared spectroscopy
BCC	Basal cell carcinoma
BMS	Biomedical Scientist
Ca125	Cancer antigen 125
CDX-2	Homeobox protein CDX-2
CEA	Carcinoembyronic antigen
CIN	Cervical intraepithelial neoplasia
CK7	Cytokeratin 7
CK20	Cytokeratin 20
CNS	Central Nervous System
CRUK	Cancer Research UK
CT	Computerised tomography
CTC	Computerised tomography colonography
DVD	Digital video disc

EGFR	Epidermal growth factor receptor
FAP	Familial adenomatous polyposis
FFPE	Formalin fixed paraffin embedded
FNA	Fine needle aspiration
FOBT	Faecal occult blood test
FTIR	Fourier-transform infrared spectroscopy
GI	Gastrointestinal
GP	General Practitioner
H&E	Haematoxylin and Eosin
HPV	Human papilloma virus
IDH1	Isocitrate dehydrogenase 1
IHC	Immunohistochemistry
IR	Infrared
KRAS	Kirsten rat sarcoma virus
Melan A	Melanoma antigen recognised by T cells 1
MGG	May-Grünwald Geimsa
NICE	National Institute for Health and Care Excellence
NGS	Next Generation Sequencing
NHS	National Health Service
NRES	National Research Ethics Service

PAP	Papanicolaou
PCA-LDA	Principal component analysis- linear discriminant analysis
PDL1	Programmed death-ligand 1
PSA	Prostate specific antigen
P53	Tumour protein 53
PoC	Point of Care
SERS	Surface enhanced Raman Spectroscopy
SOP	Standard operating proceedure
SSMM	Superficial Spreading Malignant Melanoma
STP	Sustainability and transformation partnerships
TTF1	Thyroid transcription factor 1
WHO	World Health Organisation

Chapter 1

1. Introduction

The diagnosis and treatment of cancer has taken great steps forward in recent years, with earlier diagnosis and improved treatment, though little has changed around the pathological diagnostic process. Techniques invented hundreds of years ago are still in use today and are held up as the gold standard. Many new diagnostic tools are now in production aiming to improve accuracy, reproducibility and speed of diagnosis. The aim of this thesis is to use one such tool as a proof of concept within brain tumour diagnostics to demonstrate that whilst new developments may be useful as an adjunct within the diagnostic pathway, the gold standard of histopathology remains as important as ever.

1.1 Pathology

Pathology is a medical speciality whose name refers to the study of disease. It is derived from three Greek words meaning tissue, suffering and study of (IvyRose Holistic, accessed 23/1/18). It encompasses nineteen disciplines, including; histopathology, virology, microbiology, biochemistry and immunology. Histopathology as a sub-speciality has been around for many years and is practised by medical doctors with specialist training to become a Histopathologist. It encompassed the microscopic examination of tissue, often used to diagnose disease. Tissue is examined, following fixation with formalin, and then processed and embedded within wax. Following this, sections several microns thick are cut and stained with Haemotoxylin and Eosin (H&E). The sections are then examined using conventional light microscopy, a technique that has changed little since the time of Virchow, widely known as the 'Father of modern pathology' in the mid 1800's (Schultz, 2008). Histopathology usually provides the patient with a diagnosis and is a crucial stage within most patient pathways, with 70% of all diagnoses within the NHS attributable to pathology (RCPath, 2017). Figure 1.1 shows the flow of specimens through a pathology laboratory prior to examination.

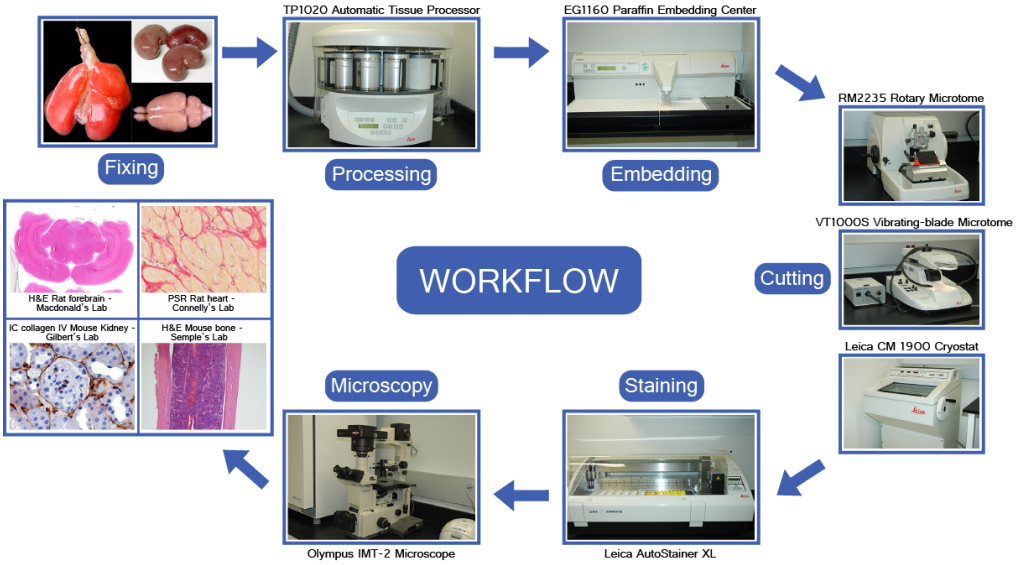

Figure 1.1: A flow diagram demonstrating the workflow through a pathology department. Starting with formalin fixation, which takes 24-48 hours. This is followed by processing and embedding which for large specimens takes another 24 hours. This is then cut and stained, ready for microscopy. (St Michaels Hospital, accessed 12/12/17)

This process has been in place for many years and results in a tissue sample being out of the patient for over 48 hours before it can be adequately examined by a pathologist and a report issued. In contrast to this frozen sections were brought in to circumvent this process for an urgent specimen with diagnosis provided during an operation. This is used primarily as an intraoperative aid, often to determine margin clearance or if the area resected contains a malignant tumour. This cannot provide as detailed an answer as full fixation and examination, but it does, in most cases, answer the question of benign versus malignant in under half an hour of the tissue arriving into the pathology laboratory. This is achieved via snap freezing the tissue and cutting it in a cryostat which allows the tissue to be cut frozen without defrosting. This is then stained in the same manner as a fixed tissue section with H&E.

Formal histological examination often provides a definitive diagnosis and the paraffin tissue block created can then be used for molecular testing if needed. During the diagnostic process, special techniques such as immunohistochemistry (IHC) are often used in order to support the morphological examination. IHC uses antibodies directed at specific antigens in order to detect their presence within a specific tissue. When used to determine the origin of a tumour a panel is used to encompass those expected to be positive and negative to ensure accuracy. A reagent (such as DAB, 3.3'-diaminobenzidine) indicates a positive presence with a brown stain, within either the cell

cytoplasm or nucleus (see Table 1.1, page 15 and table 1.2, page 16). The combination of positive and negative stains, chosen depending on the tissue type, helps to determine the tissue of origin when combined with morphological appearances.

One of the main pitfalls within histopathology is the widely reported inter-observer error. As so much of pathology is performed independently it can be subject to human error. In complex cases or difficult areas, such as dysplasia in the upper GI tract, cases are shown to colleagues, which can lead to disagreement. These cases are then sent to specialists, who tend to look solely at this area of pathology in order to reach a consensus. In some cases, this may still not be possible and clinical correlation is crucial to provide the best management for the patient. Given these complexities, it is not surprising there are intra and interobserver differences. These can range from a change in dysplasia grade to difficulty in identifying early invasive lesions (Coco *et. al.,* 2011). This can be demonstrated within the thyroid with discrepancy between invasive and non-invasive lesion within 57% of minimally invasive thyroid follicular carcinomas (Franc *et. al.,* 2003).

In order to meet Government targets, for example for cancer care, histopathology output is measured in turn around times. This is the time taken from the tissue being removed from the patient to the time for an authorised report within the hospitals system. The Royal College of Pathologists make suggestions as to how long this should take based upon the sample and the urgency determined by the requesting Clinician. This can range from 5-10 working days depending on if this is a biopsy (*e.g.* a small piece of tissue, usually less than 5mm) to a resection specimen (*e.g.* a segment of bowel) and if this is deemed clinically urgent by the requesting Clinician.

This contrasts with cytology, which is the study of cells. Cytology specimens can be taken from anywhere in the body, they usually are from abnormal fluid collections, such as pleural effusions or from fine needle aspiration (FNA) of a lump, lymph node or organ *e.g.* thyroid. The cells are either smeared directly onto a slide and fixed with an alcohol spray prior to staining or they are suspended in an alcohol based fixative, such as cytorich red, and then spun using a centrifuge onto a slide. Special inserts are used within the centrifuge to ensure the cells are focused onto one spot. This technique is known as a cytospin preparation (see Figure 1.2). This is then traditionally stained with a Papanicolaou (PAP) stain. Direct smear preparations are stained with either May-Grünwald Geimsa (MGG) or PAP stain. This is done to best visualise the nuclei of the cells and the cytoplasm. These specimens have no architecture to assess and the diagnosis is made purely on the appearance of the cells and nuclei. This can be prepared

in 24 hours, improving turn around times. It has its own limitations due to lack of architecture and the need for further work in order to be able to perform immunohistochemistry. Though as a sub specialist area the development of cell blocks (a technique that produces a formalin fixed paraffin embedded block for IHC, similar to that used for histology and processed in the same manner) has brought cytological diagnosis forward and its role as a diagnostic tool is increasing.

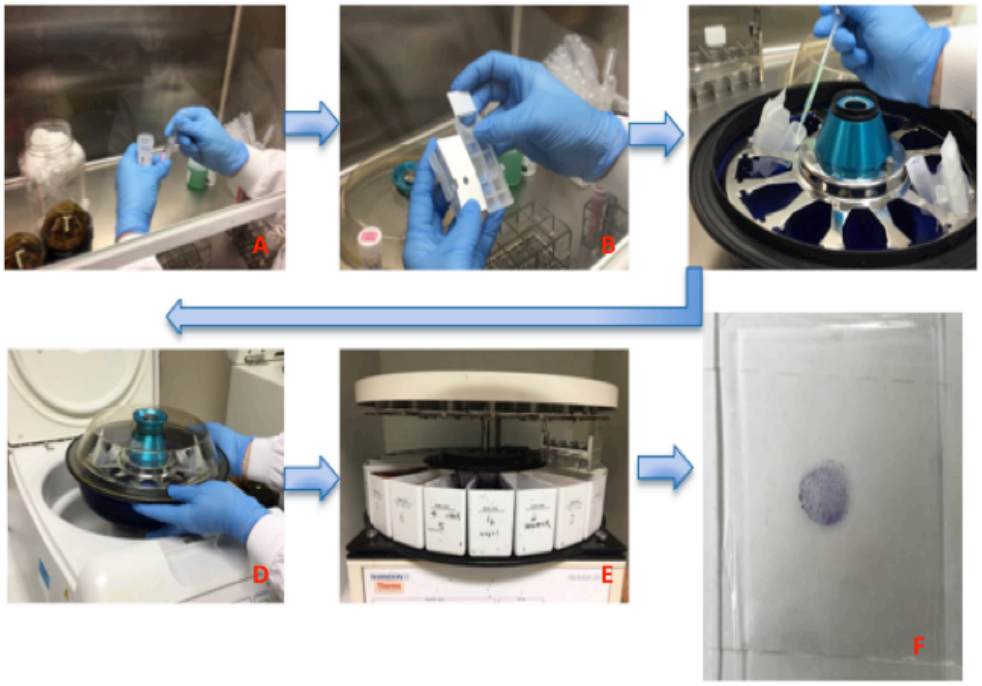

Figure 1.2: Pictures demonstrating the preparation of a cytology slide. The cells are suspended in an alcohol based fixative fluid before being spun onto a circular area of the slide using a centrifuge and a stencil. This is then stained using a Papanicolaou (PAP) stain before microscopy. (A) the specimen is transferred into a spinning tube and spun down, (B) the glass slide is placed into the centrifuge container to focus the cells onto a spot, (C) the sample that has been spun down into a pellet is now placed into the centrifuge container, (D) the centrifuge is closed, (E) after the centrifuge the slide is placed into the auto-stainer, (E) a PAP stained slide.

1.1.1 Pathology Services in the UK

The last Government led review of pathology in the UK took place in 2006. This was undertaken as a seminal paper to highlight the need to improve UK pathology services. It recognised UK pathology deals with over 17 million slides covering both histology and cytology a year (Carter, 2006). The majority of NHS based hospitals have a pathology

department, though over recent years pathology as a speciality is being forced to streamline departments and merge to form large specialised centres, where possible, to reduce the cost of the service (Carter, 2006). This has followed the subspecialisation of surgery, which has resulted in a hospital specialising in, for example, cardiothoracic surgery. This is highlighted by Blackpool Victoria Hospital, which covers all lung resection specimens for Lancashire and South Cumbria (shown in Figure 1.3), comprising over 1.7 million patients (Healthier Lancashire and South Cumbria, 2016). This results in a department dealing with specimens for patients over a large demographic area of the UK and the need for crosslinking computer systems to allow important clinical information not to be missed is crucial.

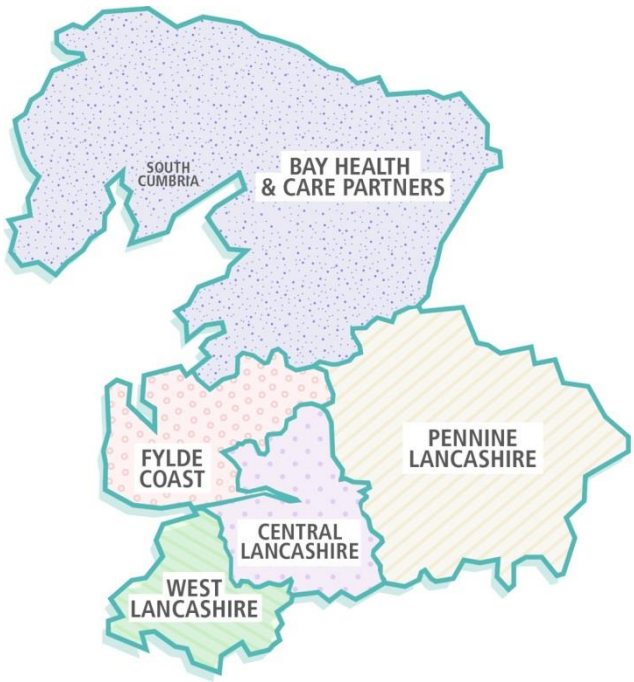

Figure 1.3: The Lancashire and South Cumbria geographical area as dictated by the regional sustainability and transformation partnership (STP) (Healthier Lancashire and South Cumbria, 2016)

Pathology is also facing a recruitment crisis. There are more Consultants retiring than trainees both within the training programme and completing training. This has led to some departments relying on out-sourcing cases for reporting to private firms. These firms provide a diagnostic service on a pay scale depending on the complexity of the case. Within the NHS itself, a routine biopsy from the GI tract costs around £60 (Shepherd *et. al.,* 2014). Other options being trialled to improve staffing levels, involves the training of biomedical scientists (BMS) to perform some roles normally done by a pathologist. This often includes cut up (the selection of tissue of from areas of interest

from a large specimen in order to process into tissue blocks for examination) and in some cases reporting basic specimens with strict guidelines (Lishman and Sturdgess, 2017). This has been met with some resistance, as the BMS is not a medically trained professional and opened a large debate into who should be allowed to undertake various roles within the laboratory. In the United States of America, their histopathology departments are run in a similar fashion with hospital based pathologists paid for the work performed via insurance companies as opposed to the NHS.

1.1.2 Pathology Worldwide

Within the wider developing world, histopathology services are rudimentary at best. There is a marked shortage in staff. Within North America and Europe there are from 14-40 pathologists per million populations, whereas in the developing world this ranges from 0-low single digits. For example Tanzania has 15 pathologists for 38 million people (Benedikttson *et. al.*, 2007). Services such as the American Forces Institute have been cut, again reducing availability of the developing world to histopathology services (Humphreys *et. al.*, 2010). Training in these regions is also variable, along with many hospitals relying on machines donated from the Western world, which may not be fit for purpose in developing countries with marked climate differences (Benedikttson *et. al.,* 2007). To compound this, within these parts of the world, cancer diagnostics often occurs at a late stage and treatment is unlikely to be readily available. Therefore offering an easy to use technology is likely to aid diagnostics greatly, especially if the technology is low cost to run (Benedikttson *et. al.,* 2007). Additions to diagnostics within this forum could revolutionise diagnosis, however, without the treatment to follow on, its use is again limited.

1.1.3 Pathology Moving Forward

Within the developed world, in an effort to ever specialise departments and to meet the growing demand for 'personalised medicine', pathologists are tending to work in a niche market of a single organ system to accommodate the need for in-depth specialist knowledge. Compounding this there is a new Government aim of a cancer diagnosis within 4 weeks of visiting your GP by 2020 (Karakusevic *et. al.*, 2016). This again

highlights the need for fast, accurate diagnostics. There are two complicating factors to this; one is the increasing demand and pressure on overstretched and under resourced pathology departments, and secondly the impending recruitment crisis within pathology as fewer trainees apply and the aging consultant body retires (RCPath, 2015). To help combat this, new diagnostic tools are required to aid the pathologist in ensuring accurate diagnostics whilst improving and decreasing turn around times. This aids the pathology department by improving workflow and allows other areas of clinical work to meet the new Government targets of 4 weeks to diagnosis by providing the diagnosis is a timely manner (Karakusevic *et. al.*, 2016).

A tool to allow the clinician to make an accurate diagnosis in clinic without the need for a biopsy, leaving the pathologist to focus on the resection and staging may be very beneficial. However, given tumours can change their appearances following chemo-radiotherapy, a biopsy prior to treatment, if this is the patient's first port of call, may still be required. Being able to re-examine a biopsy in the context of examining the resection specimen or subsequent biopsies can be very helpful in challenging cases.

1.2 Cancer

Over 350,000 people are diagnosed with cancer each year; of these over 50% comprise breast, prostate, lung or bowel cancer (CRUK A, accessed 1/12/16). The vast majority of these patients will require samples to pass through a histology department for diagnosis. New statistics show that 1 in 2 people born after 1960 will be diagnosed with some form of cancer in their lifetime, demonstrating the importance of innovative new technology to assist already busy pathology departments (CRUK B, accessed 3/11/17).

1.2.1 Primary brain tumours

Primary central nervous system (CNS) tumours comprise approximately 3% of all cancers, of which less than 5% are hereditary. Almost half of these tumours are astrocytic in origin, with another 20% meningothelial in origin. Of the astrocytic tumours, glioblastomas are more common in men, whereas mengingiomas are more common in women. For the latter this is thought to be due to the oestrogen driver of

some of the meningiomas (CRUK C, accessed 3/11/17, CRUK D, accessed 3/11/17, Ellison *et. al.,* 2013).

Around half of all patients are alive one year after diagnosis, showing the relatively poor outcome of the majority of primary CNS tumours. These tumours present with a range of features, including; epilepsy, focal neurological defects and non-specific features such as mood change.

Diagnosis is often made initially intraoperatively using a smear preparation by a Neuropathologist (see Figure 1.4). This affords the surgeon more information on the tumour such as its lineage and can help plan the extent of the resection. Following on from this the tissue is then formalin fixed to provide a definitive specimen on which to perform immunohistochemistry and to grade the tumour according to the World Health Organisation (WHO) grading system for CNS tumours (Ellison *et. al.,* 2013).

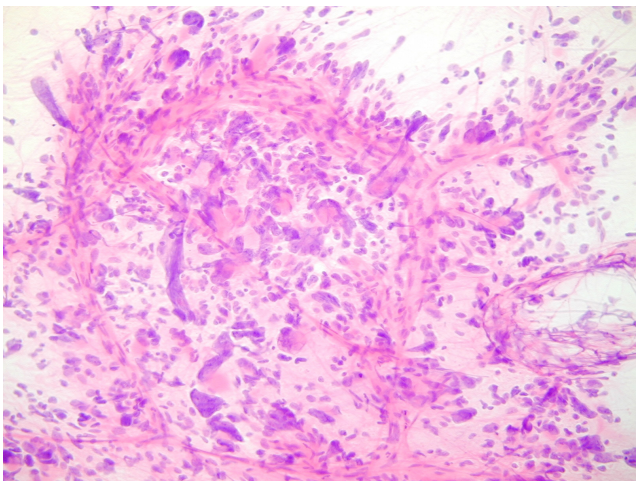

Figure 1.4.: Photomicrograph of a H&E stained brain smear, demonstrating the lack of architecture seen in a smear preparation. It highlights the cells and nuclei but no tissue structure is seen.

1.2.1.1 Astrocytomas

Astrocytomas are glial in origin, and usually occur in the 3rd-4th decades of life. They arise within either; the neural stem cells, progenitor cells or differentiated glial cells. They are graded based on WHO recommendations from 1 to 4, with 1 and 2 comprising low-grade tumours and 3 and 4 high grade (Louis *et. al.*, 2016). Grade 4 corresponds to a glioblastoma. Grades 2 – 4 are most common in adults within the cerebrum. Numerous genetic mutations occur with early mutations within isocitrate dehydrogenase 1 (IDH1)

and tumour protein 53 (p53), and allelic loss in chromosome 10 common in glioblastomas. Up to 50% of all gliomas are glioblastomas at diagnosis. Of the lower grade tumours 50-70% progress to glioblastomas, usually over 3-5 years. This transformation is associated with increasing genetic abnormalities, examples of which can be seen in the pathway below (Figure 1.5) (Ellision *et. al.,* 2013).

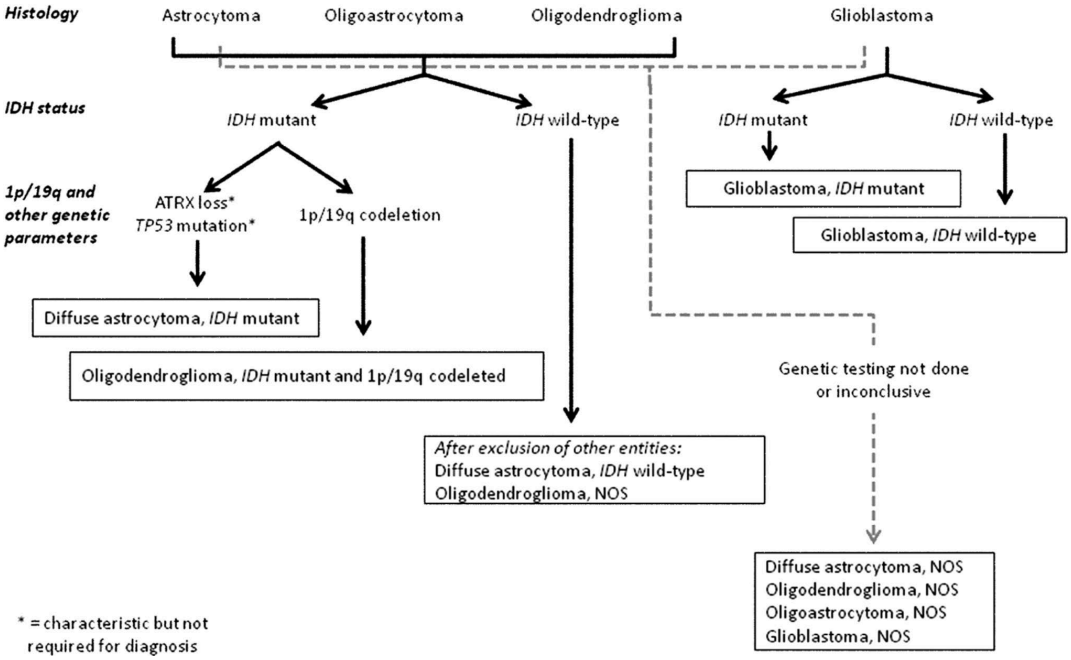

Figure 1.5: A pathway demonstrating the different mutations seen in primary and secondary glioblastomas based on the WHO Classification system (Louis *et. al.*, 2016).

Radiologically, astrocytomas of differing grades show different appearances. These are again different to those seen within metastasis. This rule, can however, be broken with metastasis mimicking primary tumours and vice versa. Radiologically, primary brain tumours are usually seen as peripherally enhancing lesions (see Figure 1.6).

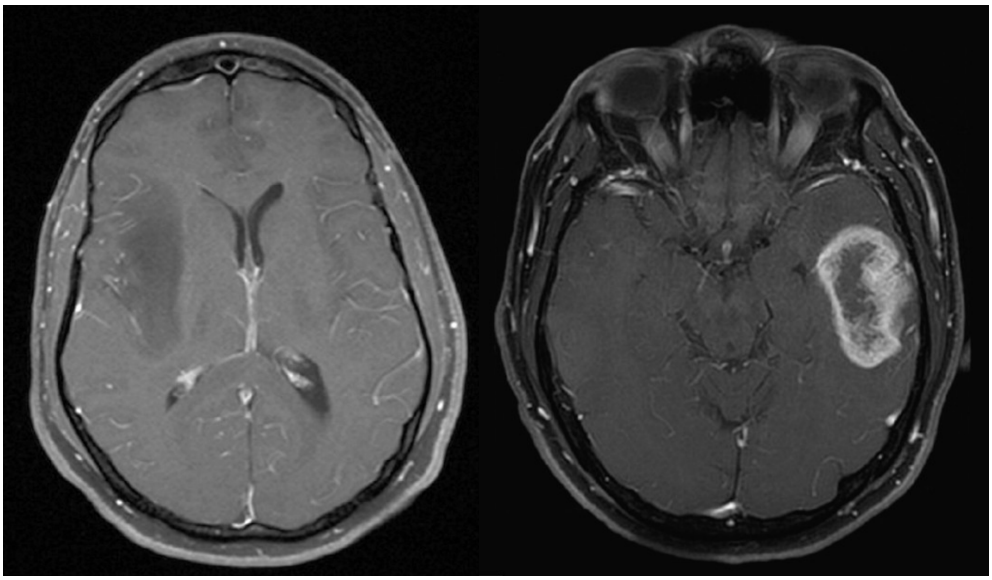

Figure 1.6: Radiological images of a low grade astrocytic tumour (A) demonstrating a non enhancing, ill defined lesion and a high grade astrocytic tumour (glioblastoma) (B) showing a peripherally enhancing lesion (Ellision *et. al.*, 2013).

Pathologically as the grade of the tumour increases, so does the cellularity, pleomorphism of the cells, mitoses, necrosis and vascular proliferation (Figure 1.7). This enables the pathologist to grade the tumour, providing important information to the Clinician to guide treatment and prognosis.

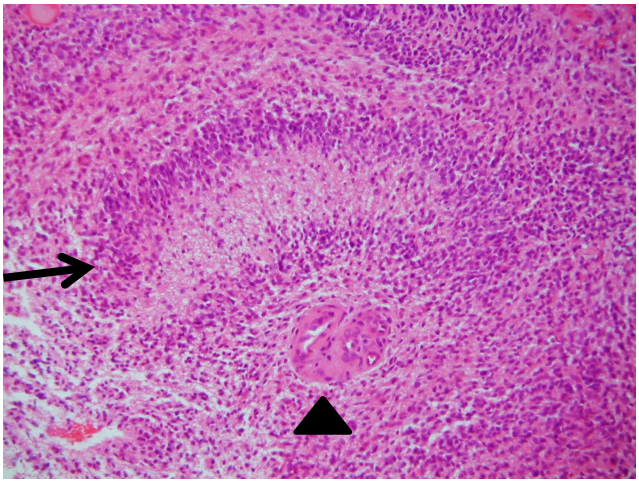

Figure 1.7: Photomicrograph of a glioblastoma demonstrating palisading tumour necrosis (black arrow) and vascular proliferation (arrow head).

1.2.1.2 Meningiomas

Meningiomas arise from the mengingothelial cells within the leptomeningies and can occur anywhere in the CNS. The site will often determine the ease and completeness of resection. Up to 15% of those thought to be completely resected will recur. The incidence of these tumours increases with age with a female:male ratio of 3:2. They are associated with oestrogen dependant carcinomas of the breast and endometrium. A wide range of histological patterns are seen as cells can have either epithelial or mesenchymal differentiation, with the WHO grading these from 1 -3 see Figure 1.8 below) (Ellision *et. al.,* 2013).

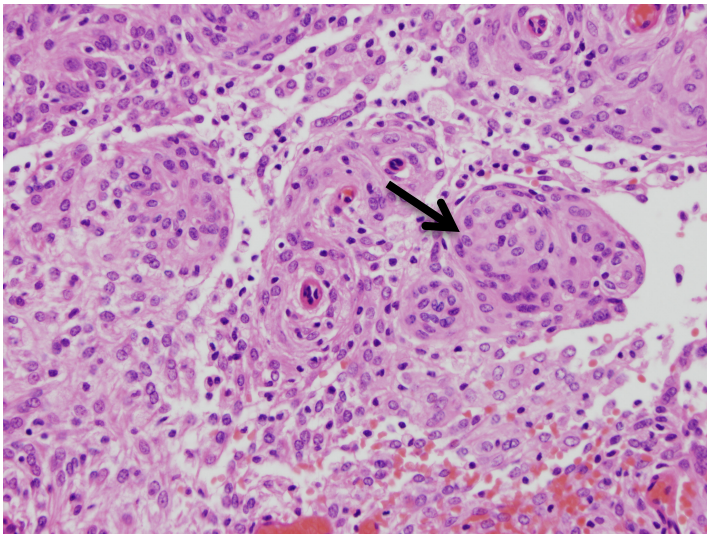

Figure 1.8: Photomicrograph of a meningioma demonstrating cleared nuclei and whorls, indicative of meningothelial cells. The arrow indicates a whorl, within which the nuclei show clearing.

1.2.1.3 Metastatic Brain tumours

Metastatic brain tumours are usually the end point in a persons' journey through cancer. The number of metastatic brain tumours identified each year is difficult to quantify, however they out number primary tumours by roughly 3:1, with the majority of metastases from primary lung tumours (Davis *et. al.*, 2012, Huang *et. al.*, 2013, Renfrow *et. al.,* 2013). Interestingly, less than 9% of colorectal tumours metastasize to brain, in contrast to melanoma, which shows a predilection for the CNS (Sanghvi *et. al.,* 2017). Whilst 80% of patients have a known primary, for some patients, the identification of

metastasis may be the initial presentation of the primary tumour (Bekaert *et. al.,* 2017). It is thought that the number of brain metastases is higher than reported as some may go undiagnosed. Most metastasis are found at the grey white matter junction (these are areas with a dual blood supply). They are usually discrete masses though occasionally show a more diffuse, infiltrative growth pattern mimicking primary CNS tumours. For those who undergo metastectomy for diagnosis or symptom relief, the tissue, once removed is sent for histopathological analysis to determine the origin of the primary tumour.

In order to reach a diagnosis this often comprises a mix of morphological appearances and IHC tests. The combination of IHC tests enables the pathologist to give either a single or group of organs from which the primary tumour likely arises. Morphologically these tumours can look remarkably similar (see Figure 1.9). For example, when differentiating adenocarcinomas from melanomas a combination of epithelial and specific protein markers are used (please see also Table 1.1). These include; anti-epithelial cell adhesion molecule (Anti-EpCam/BerEP4), a marker of epithelial cells and therefore expressed in the majority. Cytokeratin 7 (CK7) and cytokeratin 20 (CK20), these are cytokeratin's expressed in different patterns throughout glandular and transitional epithelial tissues within the body. The pattern of CK7 and CK20 helps to localise the primary tissue site, for example in lung tumours CK7 is positive and CK20 negative. This is a similar pattern to that seen in the upper GI tract. However in the lower GI tract CK7 is negative and CK20 positive. This is then followed with more specific markers, such as homeobox protein CDX-2 (CDX-2), which indicates origin in the GI tract, or thyroid transcription factor 1 (TTF1), which would suggest a primary lung tumour. There is some overlap with staining hence some lung adenocarcinomas will express CDX-2, when they demonstrate a more intestinal phenotype. The combination of staining patterns allows the user to determine primary site, in combination with clinical and radiological information. For melanoma, these stains will be negative and a more limited range of stains is positive, these include S100 protein and melanoma antigen recognized by T cells 1 (Melan A). The table below highlights the positive and negative staining patterns of lung and colorectal adenocarcinomas and melanoma when tested for a panel of immunohistochemical stains.

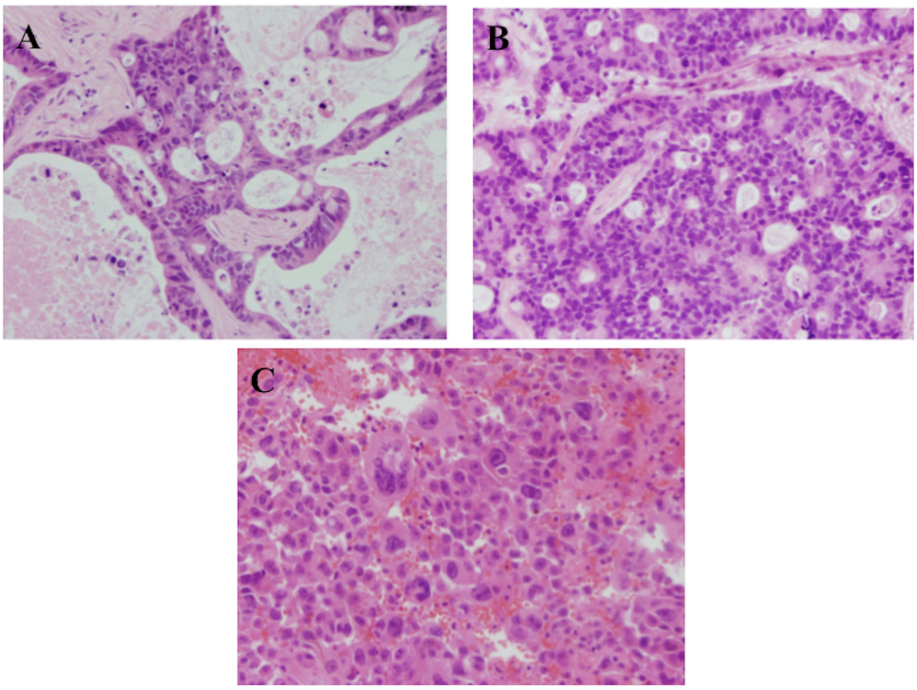

Figure 1.9: Photomicrographs of brain metastasis from colorectal and lung adenocarcinomas and a melanoma metastasis. (A) is a metastasis from a colorectal adenocarcinoma (H&E ×200 objective); (B) is a metastasis from a lung adenocarcinoma (H&E ×200 objective); and, (C) is a metastasis from a malignant melanoma (H&E ×200 objective).

Table 1.1: A representative immunohistochemical panel for the differentiation of lung and colorectal adenocarcinomas from melanoma. (Key: +=positive, -=negative, +/-=variable)

Immunohistochemical Stain	Lung Adenocarcinoma	Colorectal Adenocarcinoma	Melanoma
BerEP4	+	+	-
CK7	+	-	-
CK20	-/+	+	-
TTF1	+	-	-
CDX-2	-/+	+	-
S100	-	-	+
Melan A	-	-	+

1.2.2 Lung Carcinoma

In 2014 there were 46,403 people were diagnosed with lung cancer; of these 5-8% where still alive after 5 years (CRUK E, accessed 3/11/7, CRUK F, accessed 1/5/17). The development of lung cancer is multifactorial. Whilst the contribution from tobacco and smoking is no longer disputed, there are a growing proportion of tumours, up to 10-15%, developing in women who have never smoked. These tumours are increasingly adenocarcinomas, harbouring specific genetic mutations, with over half of all lung cancers in women being adenocarcinomas. However, the majority of cases (over 90%) are in adults over 40 years, and most common in men (CRUK E, accessed 3/11/17).

Histologically, lung cancer is divided primarily into small cell and non-small cell carcinoma. This is done on the basis of morphology and immunohistochemical testing. The distinction is important for two main reasons; firstly the chemotherapy regimes differ greatly and secondly with the advent of molecular pathology, the genetic mutations are tested for on the basis of the histological diagnosis as a means to stratify those most likely to harbour treatment targetable mutations.

Non-small cell carcinoma is further subdivided into either squamous cell carcinoma, or adenocarcinoma. This is often done with the help of IHC as squamous cell carcinoma and adenocarcinoma demonstrate different staining patterns due to their different cells of origin (see Table 1.2). Approximately three quarters of all lung adenocarcinomas express TTF1, making this IHC stain very valuable. Half of all lung tumours in women are adenocarcinomas.

Table 1.2: A representative immunohistochemical panel for the differentiation of non small cell tumours of the lung into lung adenocarcinomas and squamous cell carcinomas (Key: +=positive, -=negative, +/-=variable)

Tumour	CK7	TTF1	CK5/6	p40/p63
Adenocarcinoma	+	+	-	-
Squamous cell carcinoma	-/+	-	+	+

1.2.3 Colorectal Adenocarcinoma

Colorectal cancer is the most common malignancy in northwest Europe and North America. It affects 1:14 men and 1:19 women during their lifetime, with most over the age of 75 (CRUK G, accessed 3/11/17). Survival has more than doubled in the last 40 years with over 57% alive at 10 years (CRUK H, accessed 3/11/7). There are many contributing factors to the development of colorectal cancer. These can be split into environmental; including diet, as diets high in animal fats and red meat have been shown to increase risk though this relationship is complex, and genetic; for example familial adenomatous polyposis (FAP) and Lynch syndrome.

The presenting features often include; change in bowel habits, rectal bleeding, weight loss or urgent hospital admission with bowel obstruction. Widely used as part of the diagnostic work up, is carcinoembryonic antigen (CEA) level. This marker is known to be increased in patients with colorectal cancer, and levels return to normal following surgery. They are then shown to increase on recurrence. However, this has its limitations. It is not appreciably increased in the development stages of colorectal cancer nor is it specific. CEA has found to be increased in a range of cancers, including; stomach, lung

and pancreatic cancer. Colorectal cancer most often metastases to the liver and lymph nodes with brain metastases being relatively rare.

As the development of colorectal cancer has become more understood with the adenoma carcinoma pathway (please see Figure 1.10), the development of a screening test has been possible. Faecal occult blood testing is now commonplace in the over 60's in England and is increasing the number of cancer detected earlier, enabling improved outcomes (Public Health England, 2015).

As for lung cancer, colorectal cancer has its own targetable mutations. Testing for mismatch repair genes and Kirsten rat sarcoma virus (KRAS) testing is now commonplace for colorectal cancer cases in order to guide treatment.

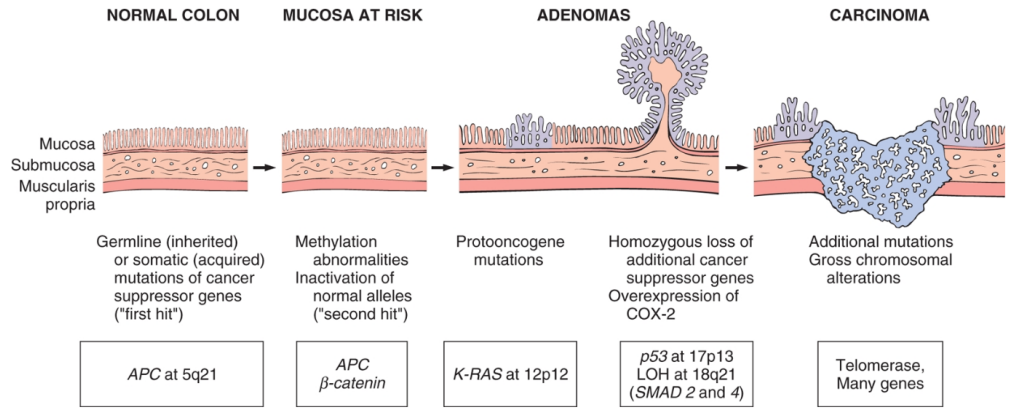

Figure 1.10: The adenoma/carcinoma sequence in the development of colorectal carcinoma. (Kumar *et. al.*, 2010).

1.2.4 Melanoma

A melanoma is a malignant tumour of the melanocytes, most commonly occurring in the skin. Melanocytes are found in the basal layer of the epidermis, this is the innermost layer of the epidermis closest to the dermis. There are many types of malignant melanoma but the four major types are: lentigo maligna melanoma which is found on chronically sun-damaged skin and is most common in older generations that have had much more sun exposure, they are commonly elevated from the skin once they invade and have a brown colour; acral lentiginous melanomas occur on the soles of the feet,

palms and under the nails and are the most common form of melanoma in Asian and Afro-Caribbean population; superficial spreading malignant melanoma (SSMM), which can occur in any age group, are most common in the middle-aged population, they appear flat to the skin or slightly raised with an irregular border and can be a variety of colours including brown, white and blue; finally, nodular melanomas can occur on any body surface and invade early, they can occur in any age range but are most common in middle age, are raised from the skin and most commonly black in colour.

Melanoma is a cancer on the increase worldwide. It almost exclusively affects Caucasians with approximately 9,000 new cases of melanoma diagnosed in the UK each year. Although skin cancer is more common in the over 50s, melanoma is the most common cancer in the 15-34 age group. The highest rates of melanoma in the world are found in Australia and New Zealand (CRUK I, accessed 3/11/17, CRUK J, accessed 3/11/17).

1.3 Personalised medicine/Molecular pathology

The advent of molecular pathology has allowed various tumour types to be sub-classified further and for the production of genetically targeted therapy. One of the first tumours to benefit was breast cancer with the discovery of Herceptin (NHS choices, accessed 23/1/17). This led to changes in breast cancer reporting and the requirement for all cases to be sent for hormone status analysis, including Her-2 status, the gene targeted by Herceptin. Breast cancer is now moving forward again with at risk patients, defined based upon the stage of their disease, being tested using a tool called Oncotype Dx. This is a genetic test, performed in the USA, examining activity of 21 pre-defined genes, which gives a risk stratification as to the risk of recurrence or new primary and the benefit to chemotherapy (Genomic Health Inc., accessed 12/12/17). This is now in widespread use within the NHS. Other examples include the ground breaking changes in lung cancer using anaplastic lymphoma kinase (Alk) and epidermal growth factor receptor (EGFR) mutation status analysis and therefore allowing targeted treatments, which in some cases have been shown to extend life by 9-12 months respectively (Lindeman *et. al.*, 2013, Vincent *et. al.,* 2012). For lung cancer, this is a marked improvement, given 5 year survival has only increased from 5% to 8% in the last 40 years (CRUK F, accessed 1/5/17). This has driven the drug companies to find new targets for potential therapies. One such target is programmed death-ligand 1 (PDL1);

this has multiple immunohistochemistry tests depending on which drug company is funding the test, though concordance between them is not known (Cree *et. al.*, 2016). This requires uniformity, especially if it is to be in general use in departments around the country. The main challenge can be for the NHS to keep up with the pace of development and for NICE to approve these new drugs. They are often very expensive, given the research and development required, which can lead to a rejection on the grounds NICE do not feel it meets their cost effectiveness levels. This has recently led to a spate of crowd funding for treatment and more people looking abroad for new ground-breaking therapies. Therefore in order to support the development of targeted medicine, not only do new mutations need to be identified, but easy, quick, reliable and importantly cheap methods for their identification are required.

1.4 Patient centred care

Healthcare has come a long way from the old model of the doctor and patient interaction where the doctor takes on a paternal role and dictates the patients' treatment plan. The patients of the 21st century are taking a much more active role in their diagnosis, treatment and management. A study by the King's fund demonstrated that as medical professionals we are not reaching far enough to help patients understand their disease and hence treatment options available to them (Mulley *et. al.,* 2012). A recent study has also shown that even with a 1% risk of a cancer diagnosis, patients would want a referral for further investigations (Banks *et. al.,* 2014). Giving someone a cancer diagnosis is a major life changing moment for them and their families. The importance of the setting and delivery of the information cannot be underestimated. In a description of patient preferences it was shown that the most important factors to the patient were time spent talking and ability to ask questions, be treated as an individual and to feel the doctor was supporting them (Thorne *et. al.,* 2010). All of these factors together help form the basis of the doctor-patient relationship moving forward and the memories the patient takes away of their diagnosis. It is crucially important that this is done well to help the patient moving forward and to develop the level of trust needed to help treat the person successful. A recent report from The Brain Tumour Charity highlighted the importance of recognition of symptoms with 62% of people being diagnosed as an emergency following repeated visits to GPs and doctors and wide ranging referral patterns. The majority of diagnoses were done by neurologists, with only 60% rated as having very good or excellent communication skills (MHP Health, 2013). Correcting these statistics

would be a crucial step towards early diagnosis and building trust with patients through communication.

For many years we have understood the importance of informed consent, however, how well informed are patients? How much do they understand of the treatment options given and how are their decisions being made and to that end how much do medical professionals guide the decisions. Decisions regarding treatment both given and received are made on an individual basis with the best information available at the time. The importance of the way risks are presented either as figures or pictorial representations are hugely important as patient understanding is the basis upon which an informed choice can be made. An understanding of their disease and how it progresses is also crucial to enable a truly informed choice (Elmore *et. al.,* 2010). Patients are likely to search for information surrounding their diagnosis and often bring this to their physician to discuss treatment options (Lewis N. *et. al.,* 2009). Patients also benefit from interactions with others with similar cancers and can find experiential information and support from these sources invaluable (Hartzler *et. al.,* 2011). However, alongside this there must be a human element, experiences both of the doctors and patients will also play a role no matter how small. Gattellari *et. al.* (2001) showed how by giving patients increased roles in the decision-making process anxiety levels were decreased and satisfaction levels increased (Gattellari *et. al.,* 2001). Alongside this, a study by Wessels *et. al.* (2010) demonstrated good concordance between doctors and patients surrounding treatment preferences. Though patients' valued expertise of the health professional higher than doctors thought they would (Wessels *et. al.,* 2010). In an interesting study from the doctors' perspective, Shepherd *et. al.* (2011) interviewed doctors treating a variety of cancers to discuss their views on patient involvement in treatment decisions. It highlighted that the doctors recognised the need for patient involvement in areas where there was a choice to be made, such as the treatment of low grade lymphomas where many treatment options are available and in cases of disfiguring surgery, for example a mastectomy. Yet they note how if the situation is clear cut, i.e. emergency surgery, there is much less patient involvement. Interestingly they found that the type of cancer also influenced patient based decision making, as those with breast cancer were far more likely to want to be involved in treatment decisions due to the high profile of their cancer, the nature of the surgery and the push for women to be more informed (Shepherd *et. al.,* 2011). Along-side this Keating *et. al.* (2010) found patient influence on a treatment decision varied depending upon the evidence behind a decision. In situations where there was good evidence for or against and when there was equivocal evidence, patients played a larger part in decision making. However, when there was no evidence

or in the treatment of metastatic disease physicians took the decision making control (Keating *et. al.,* 2010). Involved in all these studies there are many confounding factors. Underpinning it all in a number of cases is the fact that the patient does not wish to be involved in the decision and trusts the doctor to manage their disease appropriately. The flip side of this is the growing number of patients who wish to understand their disease, make informed choices and position themselves at the cutting edge of treatment to reach for a cure.

Also important to consider is the impact carers have upon patient decisions. Zhang *et. al.* (2010) have conducted several studies looking at differing choices made by the patient and their care-giver. They report how carers are more likely to push for continued treatment until side effects become too great, and how they are more willing to discuss palliative situations. Whereas the patient is more likely to agree with the doctor on stopping treatment which is no longer working, and are more reluctant to discuss palliative care (Zhang *et. al.,* 2010). At the opposite end of the spectrum to this are the elderly people living alone with terminal cancer. There are often feelings of 'being a burden' and not wanting to impose upon medical professionals' time. They may feel it is difficult to access care without someone to help them to get to appointments or their GP (Hanratty *et. al.,* 2013). Finally, Dow *et. al.* (2010) showed Lamont and Siegler's original paradox (patients are more willing to discuss end of life care with a physician they have never met) still holds true, they also found that over half of patients would actually prefer to discuss this with their oncologist if they must have such a conversation (Dow *et. al.,* 2010). This must be brought into consideration when planning future cancer diagnostic and treatment aids.

Two interesting reviews encompassing studies between 1966-2009 left more questions than answers by demonstrating how patients often wished to be more involved in decision making, their perception of involvement varied and they also wished for different levels of involvement depending on where they were in their cancer journey. This also varied by cancer with colorectal cancer reporting the lowest active involvement (6%) and prostate the highest (as high as 84%) (Hubbard *et. al.*, 2008, Tariman *et. al.,* 2010). This shows how difficult it is for doctors to gauge level of involvement in decision making, or indeed what treatment the patient would want when deciding for them in certain situations. Alongside this, after treatment consultations with patients regarding whom they would like to manage their follow up care resulted in over half (52%) indicating they would rather remain seeing a cancer specialist than their primary care physician, as they felt they are better placed to understand their cancer, their care and any future management required (Hudson *et. al.,* 2012). It has also been

demonstrated that a patients' need for information remains at a high level during treatment at follow up appointments, particularly when patients have a low literacy level (Douma *et. al.,* 2012). Finally, a study by Atherton *et. al.* (2013) demonstrated that patients one year on from a cancer diagnosis reported lower quality of life scores in both physical and emotional domains when they perceived they had less input in their treatment decision than they wanted (Atherton *et. al.,* 2013).

This places a greater emphasis on the need for doctors to involve patients in decisions regarding their treatment as perhaps it improves mood and possibly outcome. When combined this makes the area a minefield for doctors and researchers alike. However, what is clear is there is a growing number of patients' for whom their diagnosis and their subsequent treatment is an area over which they like to have an impact upon any decisions made. For this reason involving patients in the first steps of research is becoming an essential part of investigating cancer and developing diagnostic and clinically useful tools to aid future diagnostics and management.

1.4.1 Patients Perception of Diagnosis and Screening

Screening for cancer has made large steps towards reducing the number of late stage cancers found and aims to reduce the associated mortality. Patients' perception of screening and their experiences play a role in their attendance and adherence to a screening programme. It is also possible that their emotional state prior to the initiation of the screening programme can dictate their adherence (Hinojosa-Lindsey *et. al.,* 2013). Of great importance is the need to listen to patients. Providing information, awaiting their decision and then dismissing this in favour of a personal opinion does not adhere to the belief of an informed choice (Lin *et. al.,* 2012).

Colorectal cancer has involved patients in several of its diagnostic steps. First in the uptake of screening, Miller *et. al.* (2011) tested the use of a web based multimedia programme at increasing the uptake of colorectal cancer screening and found an increases in expressing a screening preference (84% *vs.* 55% $p<0.0001$), and increase in readiness to receive screening (52% *vs.* 20% $p=0.0001$) and an increase in ordering and completing the screening tests (Miller *et. al.,* 2011). This group also performed a follow up study analysing a different population against a non-interactive format and again found that there was an increase in uptake up to 24weeks post exposure to the web media (Ruffin *et. al.,* 2007). However, Smith *et. al.* (2010) used a leaflet and DVD to inform

patients about colorectal cancer screening and found that whilst this increased patient knowledge (6.50 *vs.* 4.10 out of 12) and informed decision making, it led to a reduction in the uptake of screening (51% *vs.* 65%) (Smith *et. al.,* 2010). Schroy *et. al.* (2012) were also able to demonstrate a modest (8%) increase in colorectal cancer screening rates when using a decision aid (Schroy *et. al.,* 2012). Jibara *et. al.* (2011) looked specifically at the Hispanic population in the USA and found that colorectal cancer screening rates were lower amongst people who had lived in the USA for a longer time period and were more fearful of colonoscopy (Jibara *et. al.,* 2011). This emphasises the point that careful explanation of the procedure is required to increase the uptake of screening, though it may also lead to some people failing to take on the screening test. Following on from this many studies have been conducted around the screening tests used, most importantly the use of colonoscopy versus the use of computed tomography colonography (CTC). CTC involves the patient undergoing a CT scan whilst the bowel is insufflated with gas. The main focus of this research is the public perception and acceptability of traditional colonoscopy to see if CTC is preferable and would therefore increase uptake. There are several studies highlighting the preference of the general population towards CTC, with Pooler *et. al.* (2012) questioning those undergoing CTC. The majority of which found it acceptable and 30% stated they would not have attended screening had only traditional colonoscopy been the chosen method (Pooler *et. al.,* 2012). Ghanouni *et. al.* (2012) used focus groups to gauge public perception; pre discussion preference was for CTC (75%), however following an explanation of both procedures, the risks, benefits and sensitivity the final outcome showed an almost identical split with 46% preferring colonoscopy and 42% CTC (Ghanouni *et. al.,* 2012). This highlights the importance of clear explanation and spending time ensuring basic understanding (all terms used such as 'bowel prep' were discussed prior to the focus groups and procedural explanations) to allow for an informed discussion and final decision. Howard *et. al.* (2011) demonstrated patients preferred a colonoscopy over CTC in a population who had experienced both using a discrete choice preference. They showed an increase in number of patients preferring traditional colonoscopy based upon the risk of missing a polyp in CTC and the need for a second investigation if CTC found a lesion. The main limiting factor was bowel preparation, with a large increase in patients favouring CTC if minimal bowel preparation was possible (Howard *et. al.,* 2011). Similar results were also found by Imaeda *et. al.* (2010) with 62% choosing colonoscopy and only 10% CTC (Imaeda *et. al.,* 2010).

These studies highlight the need for patient information and discussion. They demonstrate, taking colorectal cancer as an example, that whilst CTC is less invasive and

more acceptable to patients on the surface, once the procedure is explained alongside traditional colonoscopy the latter increases in popularity. This is mainly due to the need for one investigation instead of two as colonoscopy can be used for diagnosis and treatment, reducing the need for repeat visits to hospital which has obvious benefits. The other factor to come out of these studies is the increase in uptake of screening when patients have greater medical input into their decision and perceive benefits from the screening programme (Hawley *et. al.,* 2012).

Studies comparing faecal occult blood tests to either flexible sigmoidoscopy or total colonoscopy have also demonstrated patients are more willing to undergo the latter based upon risk data when this is clearly explained (Hol *et. al.*, 2010, Wong *et. al.,* 2010). Overall, the most important factor within this is by increasing patient knowledge surrounding screening tests, for example in colorectal cancer, you enable them to make an informed choice (Jerant *et. al.,* 2013). This provides an interesting insight into patient decision-making, which can be of use to the researcher. Whilst it may appear that non-invasive tests are of great benefit, patients place the need for a quick, accurate diagnosis and balance of risk over that of an invasive test.

Decision aids aimed solely at the elderly population are rare. Two studies, one by Lewis *et. al.* (2010) and the other Mathieu *et. al.* (2007) have both developed tools in colorectal and breast cancer to demonstrate the pros and cons for screening. Lewis *et. al.* (2010) showed that whilst knowledge increased from 4% to 41% ($p<0.01$), only 7 people changed their minds (5 against and 2 for) ($p=0.76$) (Lewis *et. al.*, 2010). Mathieu *et. al.* (2007) also showed an increase in patients making an informed decision (48.8% up to 73.5% $p<0.01$), however there was no change in the participation in screening (Mathieu *et. al.,* 2007). I think this demonstrates that whilst knowledge is increased, which is certainly important, it does not impact upon screening choices in the elderly population. There are other factors at play that these studies have not identified that drive peoples decision with regards screening. Lewis *et. al.* (2013) followed up their study by demonstrating that physicians look at the health of their elderly patients prior to recommending colorectal cancer screening (Lewis *et. al.*, 2013). This demonstrates one hurdle to the screening policy for elderly patients and would represent a barrier to the uptake of diagnostic innovations if used in a screening capacity.

1.4.2 Treatment Options

The King's report highlighted the importance of obtaining a patients perspective on their treatment and what treatment they would like to receive (Mulley, 2012). Treatment for many cancers involves surgery. This can range from excision of skin cancers to major internal surgery involving removal of one or more organs or large portions of the gastrointestinal tract. This surgery is not without risk nor does it leave the patient without noticeable differences in their lives. Surgery for gastro-oesophageal cancers involves removal of part of the oesophagus and/or the stomach depending on its location. This then affects the way patients eat and digest their food. It is major surgery involving opening of both the chest and abdomen and has a lengthy recovery time along with risk of numerous complications. It is therefore important patients are well counselled before surgery. A study of post-operative oesophagectomy patients by found that they valued quality of life and cure rates over the hospital in which they had surgery and the surgeons' reputation. They found it more important they knew and trusted their surgeon. The doctors surveyed also gave similar results (Thrumurthy *et. al.,* 2011). This study serves to demonstrate the factors patients find important when being counselled for such life changing operations and gives an insight into the factors they find important when making life-changing decisions. Although, on the flip side a study of men who underwent radical prostatectomy for prostate cancer stated that the consequences, including urinary incontinence and sexual dysfunction, were acceptable in view of curing the cancer (Eliat-Tsanani *et. al.,* 2013).

When it comes to the use of pre-operative chemo and radiotherapy it has been shown that patients value outcome in terms of function. Kennedy *et. al.* (2011) conducted interviews to determine if people would accept pre-operative therapy based upon the attached side effects, including sexual dysfunction. They demonstrated that 54% of patients would only accept pre surgical therapy if the risk of local recurrence was <5%, interestingly 8% said they would not accept pre surgical therapy even if the risk of recurrence was 0% (Kennedy *et. al.,* 2011). A study by Lee *et. al.* (2012) in breast cancer patients showed 18% did not receive the treatment they preferred when asked (mastectomy versus breast conserving surgery). The study also highlighted the information gap with patients not understanding the important differences in the two treatment modalities such as the difference in margin positivity in breast conserving surgery versus a mastectomy (Lee *et. al.,* 2012). Whilst this study was conducted post-surgical intervention and it is to be expected patients will forget some of the information provided; only 26-45% of patients could recall the difference in margin positivity which is an important factor in the final

determination of treatment (Lee *et. al.,* 2012). It is important that patients receive full and clear information provided in both oral and written forms in order for them to be able to retain this information and make a balanced decision. It is also disappointing that so many people stated they did not receive the treatment they wanted as it is important that the patients' beliefs and choices are strongly represented when planning cancer treatment.

Chemotherapy and radiotherapy form the basis for treatment for many cancers, with or without surgery. They both have side effects and may affect patient quality of life. Some chemotherapy drugs are more toxic than others and therefore cause more side effects. Studies surrounding patients' perception of toxicity versus survival have shown patients are willing to accept the toxic side effects of chemotherapy to attempt curative treatment or to lengthen life, even if this is for a short time only (Duric and Stockler, 2001, Duric *et. al.,* 2008). Brotherston *et. al.* (2013) reported that 69% of patients chose chemo-radiotherapy over radiotherapy alone to prevent a difference in survival rate of <5%. Even though 80% of patients said based upon their experiences they would wish to avoid chemotherapy (Brotherston *et. al.,* 2013). In a study looking at older breast cancer patients they showed where the patient would accept chemotherapy for life extension <12months they were 3.9 times more likely to receive chemotherapy than those who would only accept treatment for a benefit of >12months. Good communication also demonstrated a higher chemotherapy rate, as did attending the appointment with a family member (Mandelblatt *et. al.*, 2010, 2012). Gerber *et. al.* (2012) looked at lung cancer patients to examine their comprehension and attitudes towards maintenance chemotherapy versus a treatment break. They found through focus groups that the patients were able to weigh up the pros and cons and understand the concept of maintenance chemotherapy and discuss their thoughts surrounding it and the reasons behind this option for treatment (Gerber *et. al.,* 2012). I think this demonstrates how patients are interested in their treatment options and when it is carefully explained they are able to weigh up the risks and benefits and come to a conclusion that fits best with them. Be this a treatment break allowing them time to feel better without chemotherapy side effects or continuing with maintenance therapy and feel they are possibly able to extend their lives. In addition to this a study by Zafar *et. al.* (2013) of colorectal cancer patients with metastatic disease found that 82% of patients that consulted an oncologist received chemotherapy. Interestingly a group of patients felt that chemotherapy was unlikely to extend their life or improve their symptoms yet they still received treatment (Zafar *et. al.,* 2013). This raises interesting questions surrounding the basis of the patients' decision; did they defer to the oncologist whom recommended treatment or

whilst they believed the views they expressed did they hope they may confer some benefit from the treatment? The importance of hope is also expressed in a study by Tomlinson *et. al.* (2011) which looked at parents of children with palliative cancer and physicians. They found that parents were much more likely to opt for chemotherapy in a palliative situation than a physician ($p<0.0001$) and that this held true even if the chemotherapy would reduce survival time and quality of life. Parent ranked 'hope' and 'quality of life' as the most important factors in making this decision (Tomlinson *et. al.,* 2011). Interestingly the views held by the parents are very similar to adults with palliative cancer and their carers. This is something very important to be considered when discussing treatment with families and ensuring their thoughts and wishes are translated when planning treatment.

Carey *et. al.* (2012) conducted a study focused on haematological patients as they believed concordance with treatment decisions may be different in this patient population as compared to the solid cancers. They found 46% of patients preferred a passive role in treatment planning and that 56% reported they had an exact match between their preferred and perceived involvement in treatment planning (Carey *et. al.,* 2012).

In patients with low risk cancers such as basal cell carcinomas (BCC), the treatment takes two common themes, surgery or imiquimod cream. A study by Tinelli *et. al.* (2012) used a discrete choice experiment to determine which patients preferred. They showed all patients, with and without previous experience of BCC treatment preferred imiquimod as it would not cause a scar (Tinelli *et. al.,* 2012).

This demonstrates that patients overall are often happy to accept high risks and side effects in order to improve outcome. What is concerning and difficult is to ensure understanding and how many patients say they did not receive the treatment they wanted. It would be interesting to understand how they had come to this conclusion. As perhaps pre-treatment counselling may help them come to terms with the risks they are agreeing to and accepting as often people as a whole hope they will be the lucky one.

1.4.3 Clinical Trials

Patients enter clinical trials for many different reasons: the hope for a cure, helping others or simply prolonging life. Jenkins and Fallowfield (2000) conducted a study using a questionnaire to assess cancer patients' perception of clinical trials. Overall, 72%

accepted entry to a trial, with the two main reasons given being 'helping others (23.1%) and 'trust in the doctor' (21.1%) (Jenkins and Fallowfield, 2000). This raises an interesting dilemma surrounding clinical trials as it demonstrates how powerful the doctors' suggestion of a trial can be. Brown *et. al.* (2011) developed a tool to aid patients being recruited to a clinical trial in order to help them ask questions and gain sufficient information to make a decision (Brown *et. al.,* 2011). The National Cancer Research Institute figures from 2010 show that 1 in 6 adults diagnosed with cancer enter a clinical trial (NCR Institute, 2010). Dear *et. al.* (2012) also report anecdotal evidence that a website giving information regarding clinical trials helped to increase rates of discussions about trials, though they did not find this statistically significant, nor did they find an increased rate of uptake of clinical trials after visiting the site (Dear *et. al.,* 2012). Important in the development of these aids such as online websites is the need to consult the end user. Patients are best placed to comment on the ease of use and accessibility of information and can provide valuable insights to the developer hopefully to improve use over time (Atkinson *et. al.,* 2011).

There is also a move towards keeping patients informed of the outcomes of clinical trials. In a study by Mancini *et. al.* (2010) an internet website method of giving out results was trialled. They found that there was a significantly greater understanding of the results in the Internet group (18.8% *vs.* 5.6%, p=0.039), however the preferred method of obtaining trial results remained in a consultation with an oncologist. They also found that patients felt more comfortable discussing positive trial results with family, but were more reluctant to discuss negative results, and were more likely to discuss these to obtain reassurance (Mancini *et. al.,* 2010). When people enter trials it is important they are informed how and when the results will be available. Whilst a proportion will choose not to access them it remains an important source of information for patients regarding any treatment or intervention they may have received.

1.4.4 Outside of Cancer

Cancer treatment is not the only area of medicine involving patients in research. An interesting study carried out by Jayasooriya *et. al.* (2011) demonstrated that patients when provided with an information booklet detailing the management options for asymptomatic carotid stenosis (a leading cause of ischaemic strokes) with the risks discussed, a split in treatment decisions almost identical to that seen in a New England Journal of Medicine online poll completed by Clinicians was seen. From the patient

survey 22% chose carotid artery stenting compared to 19.5% of Clinicians, 48 % patients chose best medical therapy compared to 48.5% of Clinicians and 30% of patients chose carotid endarterectomy compared to 32% of Clinicians (Jayasooriya *et. al.,* 2011). The patient groups may have different reasons for their choices such as 'not wanting a scar' (Jayasooriya *et. al.,* 2011) as compared to the Clinicians, however involving patients in decisions regarding their care can only help improve outcomes as they will be making the choice they are more likely to be able to manage after weighing up the risks to their health.

1.4.5 Moving forward, involving patients earlier

These studies in a variety of settings using combinations of focus groups, web based multimedia and questionnaires have all demonstrated how increasing patient knowledge leads to an increase in uptake of testing, with more invasive tests such as colonoscopy, increasing in preference as the risks and benefits as compared to other tests are explained. The use of visual aids, including decision boards, helps to impart knowledge to patients and aid understanding. This can allow for more effective discussion with clinicians as increasing the number of appointments to allow for greater discussion is not always possible (Politi *et. al.,* 2012). Allowing patients to dictate the future direction of medical research is a beneficial process allowing researchers to circumvent the need to determine if their new technology will be acceptable to patients and clinicians. It is also a crucial step in allowing patients to understand disease, the importance of screening tests and demonstrating whilst a test may be invasive it may be the best way to reach a diagnosis reducing time to diagnosis and multiple investigations. This understanding for researchers also enables them to target tools for use both during screening, diagnosis and intra operatively by taking on board the needs of the Clinician as well as patient preference. This helps bridge the gap between the laboratory and the hospital as well as allowing each group a greater understanding of each other's role along with potential benefits and pitfalls of any new diagnostic tool.

1.5 Cancer diagnosis

1.5.1 Patient Pathway

Patients attend their GP for any number of reasons; ranging from a well person check to sinister symptoms suggestive of an underlying malignancy. There is a clearly defined patient care pathway for suspected cancer, which is site specific and aims to pre select high risk patients and move them to treatment within 62 days (NICE, 2005). This has been in place for over 20 years, yet no currently proposed plans improve it, nor is the target consistently met (NHS Interim Management and support, accessed 12/1/18). Looking at the current patient referral pathway (please see Figure 1.11), it is possible to see areas that could be improved with new technology and streamline the process further, such as reduced turnaround times within pathology departments. However, integrating a new test to aid or perform diagnostics can pose significant challenges to scientists and developers alike (Srivastava and Gopal-Srivastava, 2002). For example, the path to introducing a new biomarker, as suggested by Cancer Research UK (CRUK) contains many hurdles over which a new marker is required to jump in order to demonstrate its superiority in the field of clinical medicine (see Figure 1.12, CRUK K, accessed 12/1/18). This pathway is challenging as most biomarkers used in current clinical practise, for example Ca125 may aid diagnosis but are not used as a stand alone test. Correlation with pathology and radiology is always required. That said one of the most important questions it asks is 'does the use of the biomarker reduce cancer mortality' (CRUK K, accessed 12/1/18). It is well known that early cancer diagnosis saves lives, importantly for the current state of the NHS, earlier cancer diagnosis can, in the long term, also save money. Therefore the aim of any new biomarker or diagnostic tool must be to improve mortality. The question then becomes at which point in the diagnostic pathway can a new tool such as spectroscopy be targeted. Many studies have been performed using spectroscopy in a variety of formats to diagnose many forms of cancer.

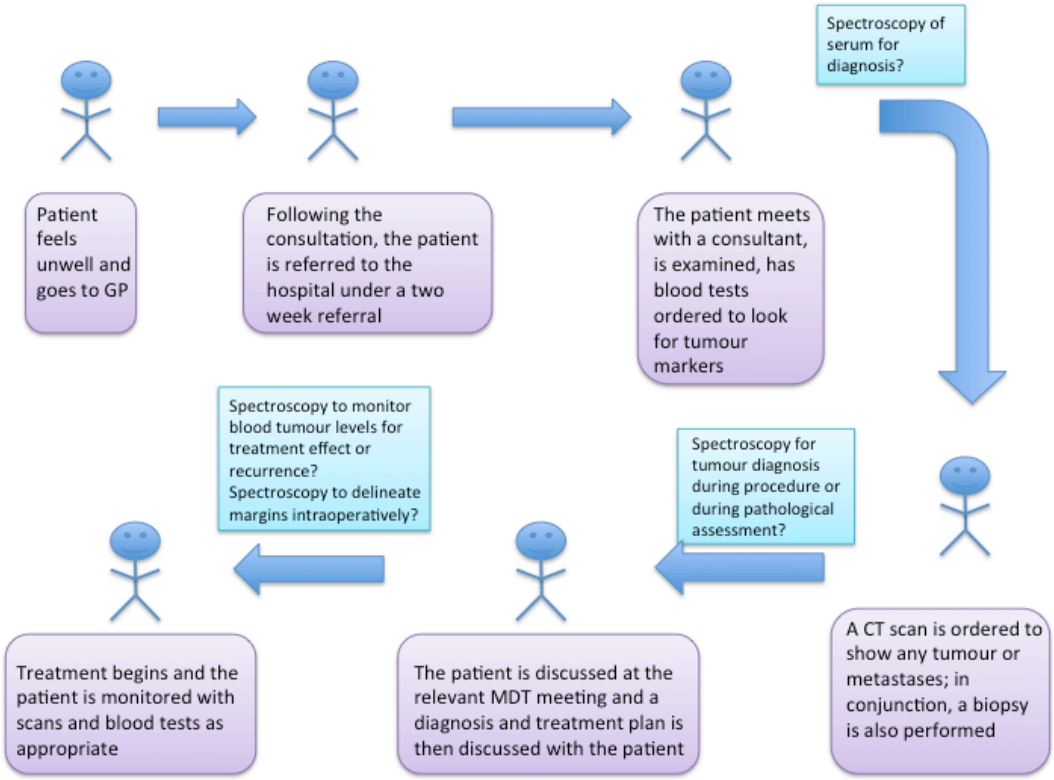

Figure 1.11: The current patient pathway with areas new technology could target to improve the time taken within the pathway (Bury *et. al.*, 2018)

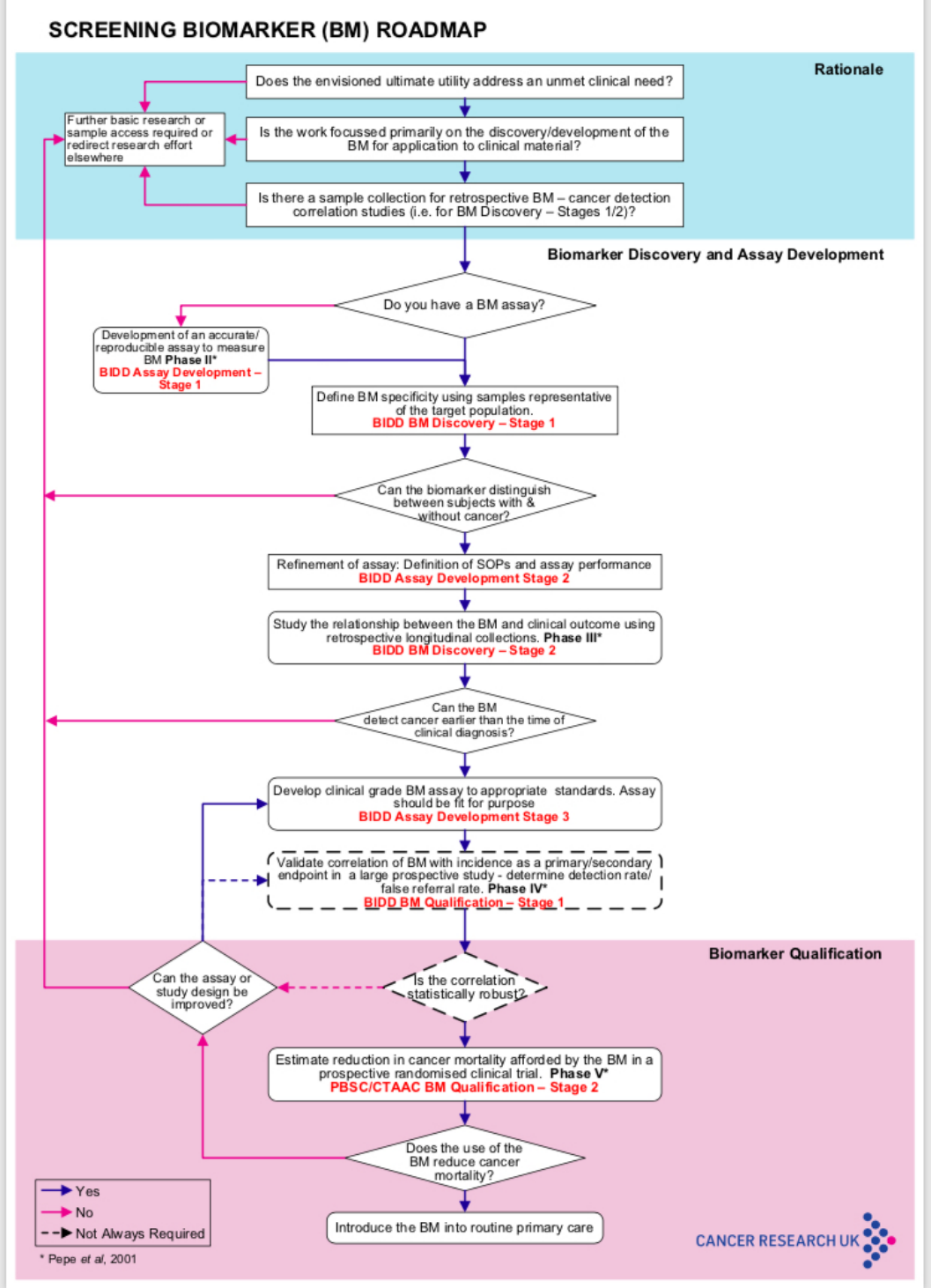

Figure 1.12: The CRUK new biomarker roadmap to aid scientists in introducing new tests (CRUK K, accessed 12/1/18).

1.5.2 New Diagnostic Tools

Patients who attend their GP practise with symptoms such as abdominal pain are expecting some form of examination and investigation. This predominantly occurs within secondary care, with pathways developed to allow timely diagnosis whilst providing patients time to come to terms with their diagnosis. Many people find blogging a useful way of explaining to others about their form of cancer and use it as a coping mechanism. It becomes evident when reading them that there is a point at which a person becomes concerned, they are attending their doctor as they feel unwell and are worried (Pancreatic Cancer UK, 1/5/17). The diagnosis still comes as a shock and surprise, but then reading their experiences of the current diagnostic pathway and involvement with the cancer specialist nurses it is possible to see everybody's role working as it has been designed and guiding a patient through their cancer diagnosis and treatment. To take a diagnosis back to an unsuspecting person attending for a well person check may be a step to far. In order for any tool to become common use it would clearly need to have high sensitivity and specificity. It would in essence become a screening test and the need to then understand if the test was detecting 'cancer' as a whole or a specific form becomes crucial.

Interestingly researchers in Swansea have developed a technique that detects changes in the proteins on red blood cells which it says can be used to detect oesophageal cancer (Thompson, accessed 1/10/16). This has not yet been published, however it offers an exciting insight into the possibility of the holy grail of biomarkers; one that can detect small quantities of mutated proteins and link this to a cancer diagnosis. This does however bring back the question of what are these tests being devised in laboratories all around the world capable of? This example has focused on oesophageal cancer and time will tell if this is possible to detect, and if so, if this is specific to this cancer type or has a more general ability to detect the presence or absence of 'cancer'. The potential of the so-called "liquid biopsy" (a blood test) has been under investigation for many years and includes a company in the USA called 'Grail', with the board of directors listing an ex Google team member (Grail, accessed 1/10/17). There is also a team in Cambridge working on a fluorescence endoscopic tool to detect oesophageal malignancy (Fitzgerald, accessed 1/10/17). I think this is where the potential of new technology lies. The patient has already attended the hospital and is expecting an investigation as opposed to a diagnosis provided to an unsuspecting patient who went to their GP for another reason. The potential of the Swansea research could again be placed into secondary care as an aid to diagnostics or a monitoring tool to perhaps replace the need for repeat CT scans.

The importance lies in what the tool is expected to detect, cancer as a whole, or a specific form of cancer? If the aim is the later, will this be all types of cancer or confined to the top ten/twenty types of cancer? The writer believes the later is impossible and impractical. To be able to train a diagnostic tool to detect all forms of cancer is unlikely to be possible. Some are incredibly rare, for example, thymic cancer. In 2014, breast, prostate, lung and bowel cancer accounted for over half of all new cancer diagnoses (CRUK A, accessed 1/12/16). Therefore, perhaps a tool to detect these may be useful. However, others may be too few in number to offer a reasonable sensitivity and specificity.

1.5.3 Developing a new diagnostic tool – for screening and beyond

The WHO identifies the need for a screening test to be sensitive and specific. For cancer diagnostics, it must focus on those cancers, which are most prevalent in the general population (Andermann *et. al.,* 2008). Therefore any test developed using spectroscopy is most useful within high-risk cancer groups, for example breast and lung cancer, due to the high number of new diagnoses and the known benefit to early surgery. Those cancers such as brain tumours with a much lower prevalence rate are more likely to benefit from intraoperative aids then diagnostics given the lack of a defined pathway for patients to follow for screening. This is to enable any system to be adequately trained and develop a robust algorithm to provide high sensitivity and specificity. It is worth remembering the introduction of faecal occult blood testing (FOBT) has identified many patients at risk of colorectal cancer and directed them for further, more invasive, diagnostic testing. The FOBT is non-specific. However, it has been used as a method to find those at increased risk of cancer and direct them to a more invasive procedure (usually colonoscopy). The uptake for bowel cancer screening initially was just over half of those invited (Logan *et. al.,* 2012). This has not prevented its use, and in fact, in studies its use has been shown to reduce mortality by 16% in those offered screening and 25% in those accepting screening, and on-going analysis of the bowel cancer screening programme has continued to support its use (Logan *et. al.,* 2012). From this we can see a new point of care test does not require the perfect sensitivity and specificity wanted in an ideal world. If the test is meant as an indicator to lead onto further, more definitive diagnostic testing, *i.e.* a colonoscopy as follows the FOBT, lower accuracy can be accepted. The importance is the test is acceptable to patients and they therefore enrol in screening. If you compare the cervical cancer screening programme, they use risk versus benefit to prioritise

detecting cervical intraepithelial neoplasia (CIN) and cervical cancer. CIN is divided into grades 1 to 3 depending on the level of dysplasia in the epithelium, which is spilt into thirds; bottom third grade 1, middle grade 2 and full thickness grade 3. They aim to detect CIN 2 and above with a higher sensitivity and maintain a high specificity rather than focus on human papilloma virus (HPV) changes and CIN1 (Smith and Patnick, 2013). This is also seen in breast cancer screening with 2-3 women avoiding death from breast cancer from every 1000 women that are screening for 20 years (Loberg *et. al.,* 2012).

Hence spectroscopy may be able to play a role as an indicator, alerting the clinician to the possible presence of a cancer. Though, if this is the route sought, an investigation plan must be produced. A whole body computed tomography (CT) exposes a patient to a large volume of radiation, equating to roughly 6 years of background radiation (Radiation info, accessed 1/12/17). Therefore the test would need to provide an indication of the site of the tumour in order to allow focused diagnostic investigation without exposing the general population, who may in fact be well, to a large dose of unnecessary radiation. Whole body CTs are also not without risk and the possibility of picking up multiple incidental findings that themselves induce worry and require follow up without ever resulting in actual illness is of the order of 30% (Lumbreras *et. al.,* 2010). Aside from CT scanning, invasive tests, such as colonoscopy are themselves not without risk, though this risk is often considered minimal, with one non screening population study finding a 0.5% risk of serious event (Lumbreras *et. al.,* 2010). Therefore careful consideration is required to determine what spectroscopy is able to detect and how accurate this is. Can it be definitive and therefore the next treatment steps are planned, histology confirmation is not required. Or is it remaining at the suggestive phase, such as prostate specific antigen (PSA) and Ca125, indicating a possibility and giving a hints as to the primary location to allow directed investigation. This decision requires a dialogue between the scientist and the clinician. The clinician must be clear on what is required and at what threshold it would be accepted. Just as the scientist must explain the techniques capabilities and limitations, what can be offered and in a reasonable time frame? Given the prevalence of some of these tumours, it perhaps makes sense to focus these new diagnostics tests, at least initially, on the majority of cancers being diagnosed, *e.g.* breast and colon cancer.

1.5.4 Point of care testing

With the point of care (PoC) industry projected to be worth over 36 billion US dollars by 2021, the development of new diagnostic tests is moving at pace (Market and Market, accessed 1/12/16). Cancer research UK has spent 15 million pounds in the last financial year (15/16) in order to develop 3 hubs within which research into early diagnostics will be focussed (CRUK L, 2016). Spectroscopy as a technique has been in use for many years, predominantly within a research setting in the context of the medical world. Many studies have been performed using various cancer types with varying success. Medpally *et. al.* (2017) looked a serum of prostate cancer patients using Raman to try to improve throughput and remove the use of expensive substrates whilst still improving on the sensitivity and specificity of the current PSA test (Medipally *et. al.,* 2017). However, for their normal controls they used a mix of men and women over a different (lower) age range to that of their cancer patients. How useful this 'normal'data is could be open to debate, firstly as woman cannot get prostate cancer and secondly, given the younger age range of the control population, it may be that other contributing factors that develop as people age providing the difference between the two groups and not the presence of a cancer. Other studies, such as that by Owens *et. al.* (2014) have shown a 93.3% accuracy to use infrared (IR) and 74% accuracy with Raman spectroscopy when classifying patients with ovarian cancer based on whole blood testing (Owens *et. al.,* 2014). This requires minimal sample preparation as well as a small amount of blood to test. Given this is a pilot study, much larger studies would be required to validate this technique. However, these figures do sit amongst others produced by different research groups, such as that demonstrated by Hands *et. al.* (2014) They obtained sensitivities and specificities up to 87.5% and 100% respectively when using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) on serum from normal and patients with glioblastomas. This was used in conjunction with Bioplex assays (Hands *et. al.*, 2014). It provided a diagnosis within 5 hours, which is much quicker than current techniques. These studies are both detecting changes in different proteins, some of which encode oncogenes (Owens *et. al.,* 2014). However given the small scale of the study it is clear much larger, controlled studies would be required to move this technique forward. Indeed it may be difficult given the low prevalence of brain tumours to introduce this as a screening test.

1.6 Diagnostics

1.6.1 Clinical Use

Within clinical use are various tools to support and guide cancer diagnosis. Starting with clinical examination, which enables a clinician to carefully examine a patient and determine if any masses are palpable. Moving on from this are blood tests to look for specific tumour markers within the blood such as PSA and CA125. These tools are neither specific nor sensitive but can add support to the clinical examination and raise suspicion levels. Next is radiological imaging, in the form of plain xray, ultrasound scan or CT scan. This can enable visualisation of any tumours and metastasis. To complete this there is the gold standard of a biopsy. This is often required prior to surgery to enable the patient to make an informed decision and in the case of metastatic disease allows the oncologist to target chemotherapy.

1.6.2 Pathological Diagnosis

As discussed above, to support the diagnostic process a pathologist has many tools to hand. Starting firstly in the cut up room with the ability to examine and dissect the specimen. This then allows the production of H&E stained slides. In complex cases, immunohistochemistry or molecular techniques may be required to provide a definitive diagnosis. However in a small number of cases this may still not yield an answer. This is especially true in challenging small biopsies, hampered by the limited tissue available. Also prevalent within histopathology is inter-observer error. Given the subjectivity of some areas of pathology, such as dysplasia in Barrett's oesophagus, which at the low-grade end of the spectrum can often cause discussion amongst expert pathologists. For example, Coco *et. al.* (2011) found a kappa score of 0.44 for 6 experts looking at dysplasia in Barrett's oesophagus (Coco *et. al.,* 2011). Kappa scores are used to assess agreement between observers, taking into account agreement occurring by chance. Scores usually range from 0 to 1, with 0 indicating a chance results and 1 perfect agreement. Scores below 0 do not often occur in clinical fields (Sim *et. al.,* 2005). Therefore, this highlights relatively poor agreement between the pathologists. In order to well train any new diagnostic tool concordance from histopathology experts will be crucial.

1.6.3 New methods

Multiple techniques are currently in development, with varying degrees of success. This ranges from smart phone apps to whole genome testing (Balch, Genomics England, accessed 1/5/17). In order for these to be introduced into clinical practise they must be cost efficient and aid the clinical diagnostic process. One such technique that has been in the spotlight recently is vibrational spectroscopy. This encompasses many different types of spectroscopy, developed to better aid diagnostics. It is expensive to set up, but once running can offer cheap, reagent free results, in a short time span, most importantly faster than immunohistochemistry and a specialist opinion. If confirmed, faster than a H&E and tissue processing, possibly even taking it one step further back into the clinical domain allowing diagnosis whilst the patient waits in a clinic room.

In recent years the use of next generation sequencing (NGS) has been increasing with the costs associated starting to fall. The main challenges now are the interpretation of the information obtained; how should this be used and how the variety of genetic differences and missense mutations interact in order to produce the clinically evident disease (Landsverk and Wong, 2013). This next step is now required in order to determine the utility of sequencing beyond single gene testing, as has previously been done, in order to determine specific changes (Organisation for Economic Co-operation and Development, 2011).

Also increasing is the number of biomarkers detected by "omics" research. Again, many challenges are being encountered; none more crucial than funding. Which biomarkers should be studied and who is footing the bill are crucial to progression (Armstrong *et. al.,* 2014). It is difficult to engage clinicians and sell the benefits of a new biomarker without determining and proving its utility. Evidence based medicine is at the heart of medical training and without evidence of benefit to the patient it can be difficult to secure funding and garner enthusiasm (Armstrong *et. al.*, 2014, Byrne *et. al.,* 2015). These new biomarkers however, do herald the new era of personalised medicine and with them new treatments can be developed that target specific markers found in specific patients (Armstrong *et. al.,* 2014). As time progresses and these areas of research expand it is likely NGS and novel biomarkers will form the new basis for cancer treatment.

1.7 Vibrational spectroscopy

Vibrational spectroscopy comprises two main complimentary techniques; Raman spectroscopy and infrared spectroscopy. These provide information regarding molecular structure and produce a 'fingerprint' of the tissue or fluid analysed. Comparison of these fingerprints allows differentiation of, for example, tumour or tissue types. Infrared spectroscopy uses polychromatic light to detect the point at which bond vibration occurs within a sample, whereas Raman uses monochromatic light to detect inelastically scattered photons. Each component of the specimen, for example proteins, vibrate at a different wavelength allowing the production of the distinctive fingerprint (Butler *et. al.,* 2016). An example of a Raman and infrared spectra of polystyrene is shown in figure 1.13 below.

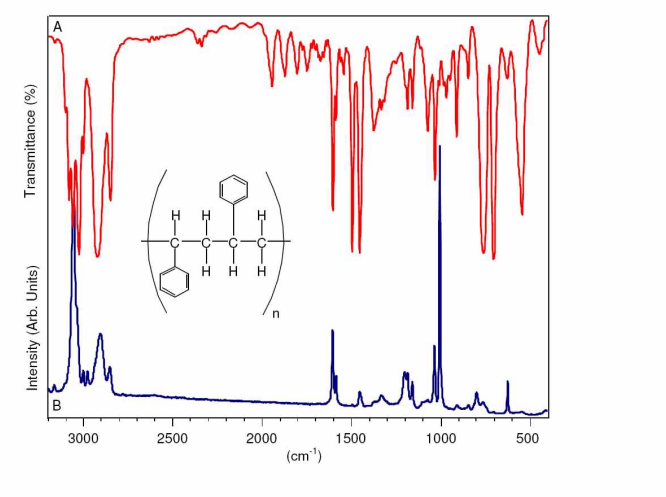

Figure 1.13: Example of Raman and infrared spectra of polystyrene showing the different appearances of both techniques on the same material. (Raman = blue, Infrared = red) (School of Chemistry, accessed 1/5/17).

One success of spectroscopy its low running costs. This includes the use of aluminium foil wrapped glass slides. These have been found to be as effective as calcium fluoride slides and cost markedly less, increasing the price of a simple glass slide very little (Cui *et. al.,* 2016). The same team also produced a tool kit called iRootlab in order to enable easy analysis of spectra within a MATLAB environment. This provides benefits given technique and analysis can be optimised and reproduced in order to unify analysis (Trevisan *et. al.,* 2013). It does however require agreement throughout the field on the best method for analysis, which while attempted by Butler *et. al.*, has yet to be accepted across the board (Butler *et. al.,* 2016).

Spectroscopy, in a medical context, can be used to analyse either tissue or biofluids. There is a large body of research showing use of both mediums with improving results. Body sites examined have included skin, oesophagus, ovary and cervix with varying degrees of success (Barr *et. al.*, 2011, Kendall, *et. al.*, 2010, Lyng *et. al.*, 2007, Gajjar *et. al.,* 2012). The differentiation of normal tissue/fluid *vs.* cancer has been done with high degrees of accuracy, for example; Gajjar *et. al.* (2013) separated serum from ovarian and endometrial from normal with accuracies of 96 and 81% respectively (Gajjar *et. al.*, 2013). Whilst some studies have started to compare differing tumour types, such as primary and metastatic brain tumours, the analysis has focused on direct questions, *i.e.* normal versus primary tumour, normal versus metastasis (Hands *et. al.*, 2016).

Also in development are spectroscopic driven tools to aid the surgeon. Liu *et. al.* (2012) trialled a wearable device fitted with near infrared fluorescence for surgeons operating on a mouse model to determine if this could be used to delineate tumour from non tumour more accurately than the human eye. It was found to be useful as a 2D model, but was unable to fully assess topography in a timely manner, therefore whilst it added some information, crucial parts were missed out. It was also a bulky system that based on pictures would take some training for a surgeon to operate with one eye covered by the device (Lui *et. al.,* 2012). There are many other tools also in development using nanoparticles loaded into "Spectropens" (a combined near-infrared laser and detector) to allow surgeons to visualise tumour cells more readily. These have shown promise in the laboratory and only time will tell if these techniques can be brought effectively into the clinical arena (Patlak, 2011).

Spectroscopy has been used to demonstrable effect within the laboratory, however translational studies moving into the clinical forum have been few and far between. In order for this technology to move forward, engagement with the medical world is required to move this technology into an area from which patient and clinicians can benefit.

1.7.1 Nanoparticles

Nanoparticles, specifically metallic nanoparticles, can also be used in conjunction with vibrational spectroscopic techniques. The nanoparticles, made of gold or silver, are placed on top of the tissue or fluid being analysed and have been shown to enhance the Raman signal. This is known as surface enhanced Raman spectroscopy (SERS). Within

biological samples this enhancement is seen at the level of one or two orders of magnitude, which whilst not as great as that seen on non-biological samples still offers an improvement in signal (Fogarty *et. al.,* 2014). This can therefore possibly aid with distinguishing the small differences seen within samples.

1.7.2 Towards a molecular future

One of the roles for spectroscopy, is in either frozen section work or treatment follow up. Targeting the tissue that would have previously been sent for frozen section and instead using spectroscopy to analyse it in the theatre would save at least half an hour. It would also allow multiple points to be examined and hopefully give answers as accurately as a pathologist. It may not be able to determine tumour type, as often occurs with a lung mass, but would be very useful to delineate tumour margins from inflammation. Alternatively as a tool to monitor treatment follow up, as biomarkers such as CA125 are inherently subjective, Moss *et. al.* (2005) found 80% of women in their study had raised CA125 due to reasons other than ovarian malignancy (Moss *et. al.,* 2005). There are also many cancers for which there is no marker, and therefore a tool that allows scan free follow up would be greatly beneficial. It would free up radiology scan spaces for new diagnostic work and reduce the exposure of patients to radiation.

Thus far spectroscopy has been used in a controlled manner, studies have been performed on blood and tissue of known cancer types and control populations, with varying success (Mitchell *et. al.,* 2014). Suggestions have been raised about its use in cancer of unknown primary diagnostics, however, no studies have yet been performed on multiple cancer types and controls in order to determine if spectroscopy can detect the different cancer types and determine a primary location (Hughes and Baker, 2016). It would be interesting to see the results of a study combining multiple types of cancer versus a control population. Following on, it would also be interesting to determine if spectroscopy could be able to define the primary tumour site from a number of adenocarcinomas or squamous cell carcinomas of various origin sites as current morphological and immunohistochemical methods struggle, particularly with the latter. Krafft *et. al.* (2006) demonstrated they could differentiate between brain tumour metastasis from a variety of locations, they also found large overlap between the lung and colorectal cancer metastasis. They did find greater differences with the renal cell carcinoma metastasis as compared to the lung and colorectal cancer (Krafft *et. al.,* 2006). This is not surprising as these tumours have markedly different morphological

appearances histologically, different immunohistochemical profiles and are from a different cell lineage. Gajjar *et. al.* (2012) has shown that it is possible to differentiate between brain tumours of different lineage. They examined normal brain, meningiomas, gliomas and metastasis and using ATR-FTIR were able to separate these tumour types into groups (Gajjar *et. al.,* 2012).

Therefore, defining the remit for spectroscopy and setting expectations is crucial and will benefit both the clinical and scientific worlds.

1.8 Conclusion

Overall, it is clear there is room for improvement in the cancer diagnostic pathway. However, there is still time required for patients to come to terms with their diagnosis and to understand and consent to treatment moving forward. Given the pressure on the NHS and the volume of patients any tool that can assist and perhaps remove the need for a step, such as a follow up scan after treatment, are greatly beneficial. Therefore careful planning of where a new technique will best fit within the diagnostic pathway is almost as crucial to its survival and uptake as the sensitivities and specificities it offers. On-going discussions between the scientific and clinical communities are the foundations required to develop tests and techniques that can benefit patients and provide clinicians with the tools they need to improve services. Techniques that imitate those available, but provide less data may add to the pathway as opposed to reducing and improving it and therefore may add a cost too great for the NHS and NICE to justify. Whereas carefully focused developments to aid tools already in use may unlock crucial information to help improve cancer treatment and improve mortality.

Therefore, it was felt important to consider if new PoC testing was wanted and where it may fit into the patient pathway. To start the patient pathway was considered (see Figure 1.11) and developed into a correspondence (See appendix 9.1). This then led to the development of several studies, incorporating varying elements of the current diagnostic pathway, to determine how viable the use of new technology, in this case vibrational spectroscopy, was as compared to current techniques (see Figure 1.14).

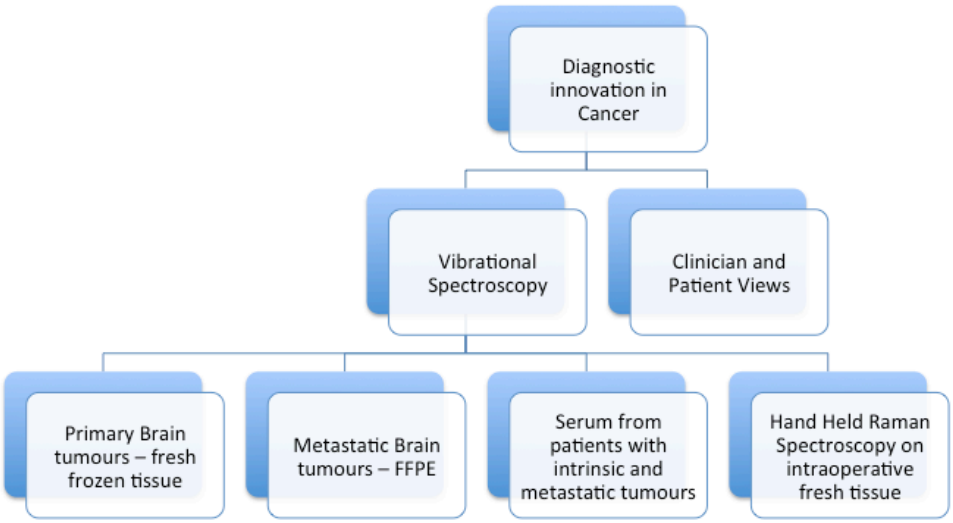

Figure 1.14: The overview of the PhD project, demonstrating how the components fit together to investigate the use of vibrational spectroscopy within the clinical field.

1.9 Aims and objectives

Aim: The aim of this thesis was to address both the use of vibrational spectroscopy in the diagnosis of cancer and how to develop this technology for clinical use.

Objectives of the Research:

Theme 1:

- Understand what makes a test acceptable to a patient and useful to a Clinician
- Establish patient and Clinician preferences surrounding cancer diagnosis
- Examine where new technology would fit into the clinical environment

Theme 2:

- Develop proof of concept for diagnosis of primary and metastatic brain tumours using a combination of
  - Formalin fixed paraffin embedded tissue
  - Fresh frozen tissue
  - Plasma and serum

Theme 3:

- Taking theme 1 output in conjunction with theme 2 to develop a working prototype for spectroscopic diagnosis of brain tumours on fresh tissue.

Chapter

2

2. Patient and Clinician Involvement Study

Declaration of Work

To Whom it May Concern,

Dr Danielle Bury designed the study, questionnaire and paperwork with in put from Dr Michelle McManus and Dr Matthew Baker. Dr Bury completed the application for the National Research Ethics via the integrated research application form and answered all questions following submission.

Dr Bury arranged and undertook all focus groups, recording and transcribing each anonymously. She also publicised the questionnaire, gaining assistance from local cancer specialist nurses for input into their support groups and canvasing local doctors. She then performed analysis of both questionnaire and focus groups, putting together the closing report form for the South West Wales Ethics committee and a paper for publication of the results with the support of Prof F Martin.

Signed

……. ……..

Prof F L Martin Dr D Bury

Introduction:

Healthcare has come a long way from the old model of the doctor and patient interaction where the doctor takes on a parental role and dictates the patients' treatment plan. The patients of the 21st century are taking a much more active role in their diagnosis, treatment and management. This study was designed to involve both Patients and Clinicians within the early steps of research project development. A combination of questionnaire and focus groups were used to determine thoughts surrounding time taken to cancer diagnosis and cancer diagnosis, as well as more general thoughts on screening and were they felt new diagnostic tools would be helpful. A copy of the questionnaires used is available within appendix 9.5 This qualitative approach was used to build an understanding of both Patients and Clinicians thoughts and ideas as to areas that could benefit from additional input from new diagnostic tools such as spectroscopy. A wide range of cancer support groups and clinicians, not just brain tumours were targeted to allow the study to explore limitations of cancer diagnosis within a variety of cancers and identify if there were any common themes through which the use of vibrational spectroscopy could be targeted.

Method:

In order to assess both patient and clinician views this study was conducted using both a questionnaire and focus group approach. This was to allow short answer questions in the form of the questionnaire and to allow expanded discussion *via* the focus groups. Ethical approval from the South-West Wales research ethics committee, reference 13/WA/0411 was granted for the study on 20/1/14 (available within appendix 9.4).

The survey was designed to use a combination of attitude scale questions using a 7 point-Likert scale, with additional questions allowing further exploration of key issues and grouping variables, such as demographic information. Open questions at the end of the survey were used to allow participants to give any additional free responses. The questionnaire was released on "Smart Survey" and distributed to medical professionals via medical trainee representatives and consultants. This allowed the survey to reach as wide a range of medical professionals at varying stages of their careers as possible. For patients, it was distributed *via* cancer specialist nurses from Lancashire Teaching Hospitals NHS Trust who work with support groups or advertised posters within the Rosemere Cancer Centre. The opening page displayed an explanation of the study, with consent for inclusion in the study being confirmed by completion of the questionnaire.

No names or identifiable details were obtained.

Focus groups were held at Lancashire Teaching Hospitals NHS Trust for consultants and at the University of Central Lancashire for patients. Patients were offered £10 as a gratuity for attending. Prior to the focus groups information was provided and any questions answered. All participants were consented with a pre-approved consent form prior to starting and focus groups were recorded and then transcribed anonymously to remove identifiers. As the idea was to allow free discussion around the topic of new technology in healthcare, questions were left open and kept to a minimum and used only to guide discussion.

Analysis was performed on the questionnaire answers to identify the most commonly selected responses and the free text 'other' boxes were ordered to look for common themes. Following transcription of the focus groups they were read and themes identified. These were then viewed in context with the questionnaire responses to allow conclusions to be drawn.

Results:

Overall 72 doctors completed the questionnaire and 6 attended during one of 2 focus groups. The patient questionnaire was completed by 93 people but the focus group was only attended by 1 person. Several patients were invited but cancelled due to ill health or lack of transport.

Clinicians:

Medical professionals ranged from foundation trainees to senior and retired consultants, from 26-66 years old, with responders equally split between male and female (48.6% and 51.4%). The majority identified themselves as British (66%) with 93% working full-time. The respondents predominantly work as Consultants with 61% dealing with cancer every day and 29% in regular contact with cancer patients. Approximately half are involved with cancer care. Of the total, 94% worked within a hospital environment. This may have led to bias within the results, given the high number of hospital based physicians. However, as the majority of patients are diagnosed and treated by hospital based physicians they are likely to be able to discuss limitations surrounding cancer diagnosis more accurately. General Practitioners were invited to complete the survey, however uptake was low.

Up to 54% of clinicians have previously attended screening with those who have not stating they are not yet old enough to be called for screening. They felt a hospital doctor should give a cancer diagnosis (94%), with up to half recognising a GP could give a cancer diagnosis. However, they felt overall a hospital consultant, who had organised the investigations and would be involved with further management and hence able to discuss treatment options and answer questions would be the most appropriate person. A GP would be helpful in providing assistance should time be an issue. They thought on the whole (69%) that patients would want their cancer diagnosis from a hospital doctor.

It was thought the areas requiring improvements focussed around the time taken to give the diagnosis (48%) and explanation of diagnosis and treatment plan (48%). Only 25% of respondents felt there was need for improvement in the method of diagnosis.

When the prospect of a new screening tool was raised, 68% would recommend it, providing it fulfilled Wilson's Criteria for screening and not just be a means to detect cancer without any survival benefits (*i.e.* a disease must have a early phase that can be detected allowing for effective treatment to prolong life) (CRUK N, accessed 11/12/17).

Clinicians felt the investigations suggested for cancer diagnosis, ranging from a clinical examination to a biopsy under general anaesthetic would have different acceptability's to a patient, from 82% down to 15%, as the investigations became more invasive (Figure 2.1a). This was interesting, when compared to the patients' results as both found different investigations acceptable (Figure 2.1b). Patients also found investigations more acceptable than clinicians thought they would be (Figure 2.1a and 2.1b).

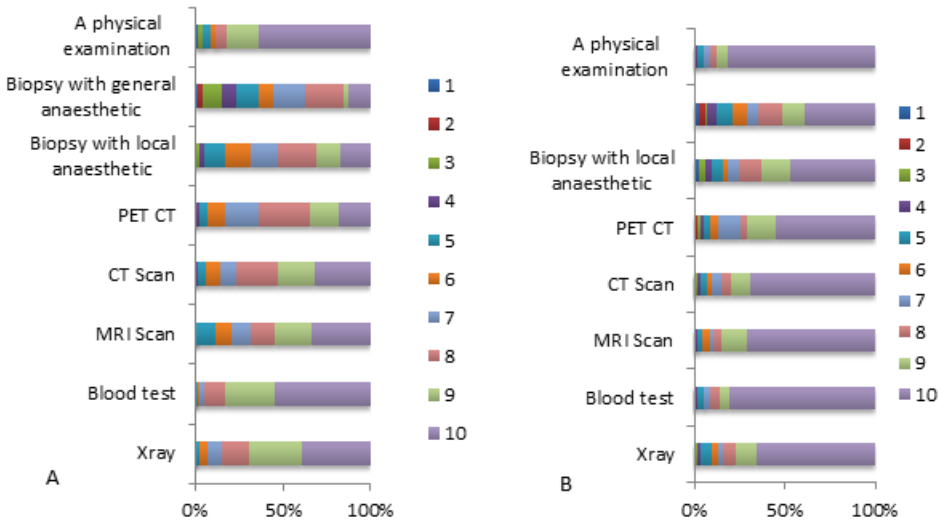

Figure 2.1: Which investigations are found acceptable and which are not? (by acceptable we mean you would be willing to accept the investigation and do not feel it is unreasonable when looking for cancer). (A) The clinicians' responses to which investigations they felt patients found acceptable. (B) The patient responses to which investigations they found acceptable. The scale ranges from unacceptable – 1 – to acceptable with no concerns – 10.

Clinicians felt that a cancer diagnosis should be expected within a week (43%) or a month (48%), however 52.8% would like a diagnosis before feeling unwell. They thought overall patients would be best-informed regarding risks and complications of treatment, see Figure 2.2.

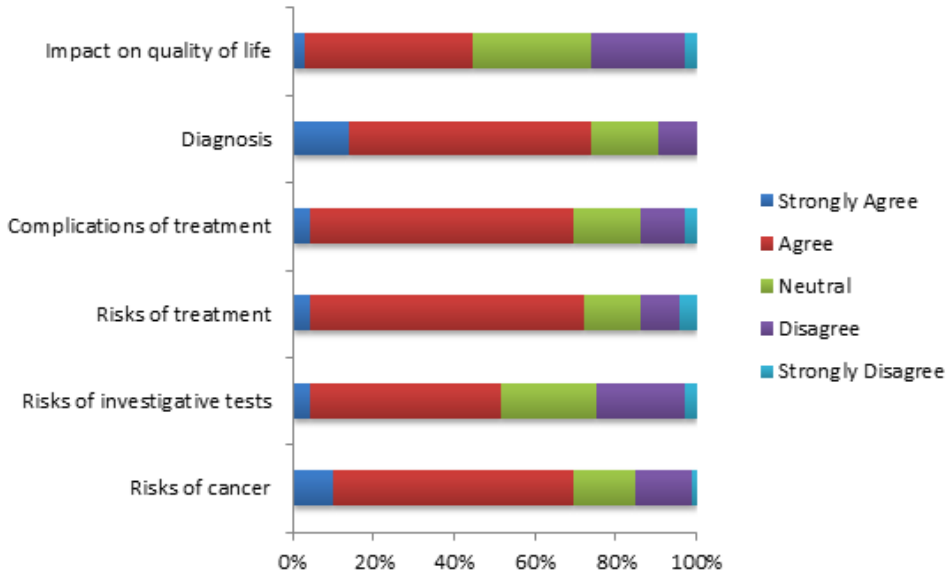

Figure 2.2: The clinicians' responses to the level at which they felt a patient is fully informed regarding their diagnosis and management.

Moving forward, clinicians felt early diagnosis along with improved molecular testing for new genetic alterations would best aid patients. Similar topics were also brought up within the focus groups. They felt the best use of a tool such as spectroscopy would be as an adjunct or replacement of frozen sections. As the turnaround, whilst often around 30 minutes, can leave surgeons waiting whilst the patient is on the operating table and if further sampling is required this can greatly increase the length of the operation and the patients' general anaesthetic.

One of the main concerns raised was the cost of bringing the equipment into the mainstream and the need for National Institute of Clinical Excellence (NICE) approval. They also felt that allowing home testing for cancer was a step too far. They felt that some patients may take any suggestion of a cancer diagnosis and not realise the sensitivities and specificities attached and end up committing suicide based on the result. This they thought would be unacceptable and that cancer diagnostics should remain with a health care professional.

Patients:

In comparison, the patient questionnaire which was completed by almost equal numbers of men (43%) and women (56%), ranging in age from 18-81 years, of which people worked in a variety of jobs demonstrated similar findings, but a greater willingness to accept investigations than the doctors thought (see Figure 2.1). Their main concern was the need for an early and accurate diagnosis and if an invasive investigation was required, then it was needed. Of the people completing the survey, 43% were employed full time. Employment ranged from unemployed to company directors with a ranging pay scale. Over half had experience of cancer, with one third personal and 50% close family. Screening had been attended previously by 43%, with reasons for non-attendance ranging from "not yet eligible", to "don't see the benefit" and those unaware screening programmes exist. The GP was still the most visited healthcare professional at 60%, with most people visiting a healthcare professional every 3 months (Figure 2.3). This may have been impacted by follow up times often provided within clinics, which are often set at 3 months.

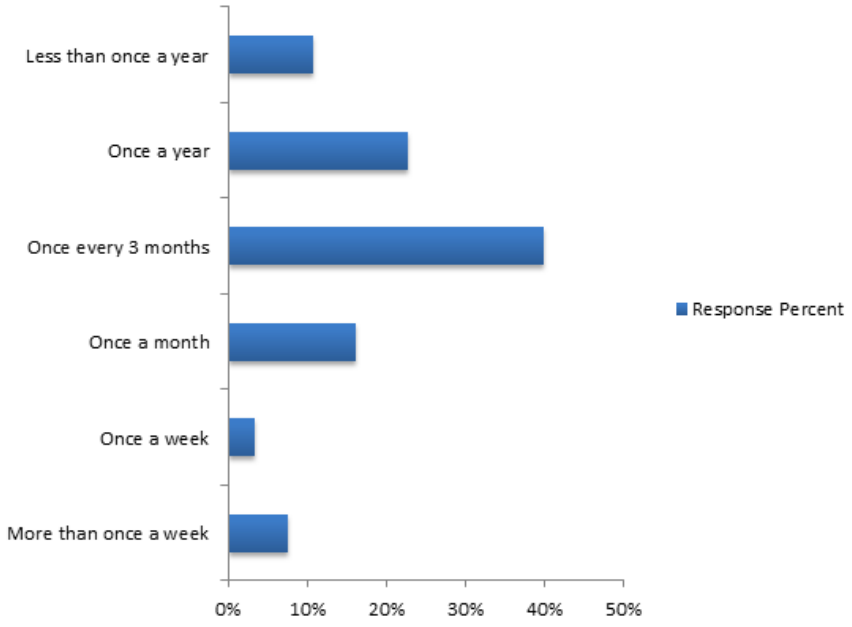

Figure 2.3: Patient responses to how often they saw a healthcare professional. This encompassed nurses through to doctors.

When asked where they would like to receive a cancer diagnosis, 53% said in hospital and only 14% their GP. This was based on hospitals being able to provide more

information and likely to have done the investigations. When asked who they would like to give the diagnosis, multiple answers were allowed, 72% said a hospital-based consultant and 43% their GP (Figure 2.4). Interestingly, the cancer specialist nurses, who are seen as having more time to sit and discuss things, where chosen more than a patients' GP (46% to 43% respectively). It was felt the GP would not have sufficient knowledge to be able to discuss the diagnosis and treatment plan moving forward. However, people did say they wanted an answer quickly after investigations and therefore if time constraints were applied their GP would be an acceptable option.

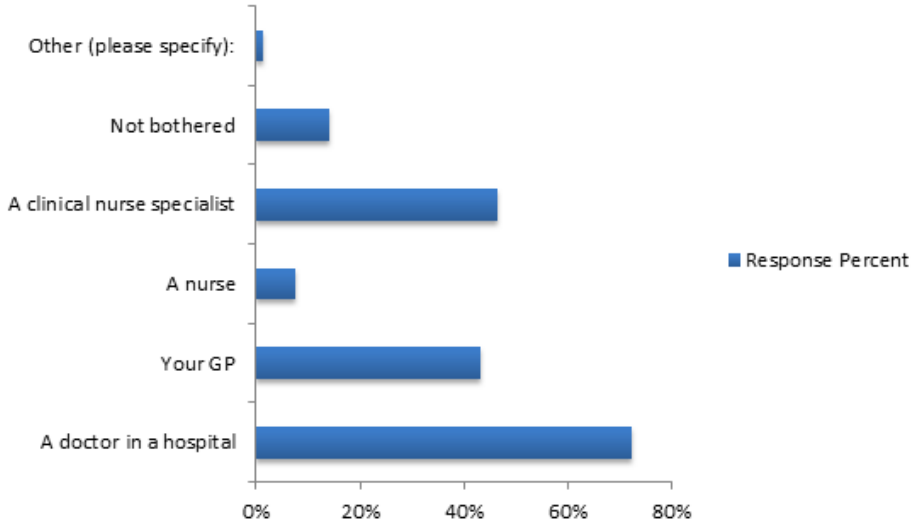

Figure 2.4: Patients responses to whom they feel should give a cancer diagnosis. Respondents were allowed to select as many answers as they felt appropriate.

Patients felt cancer diagnosis could be improved by increasing the time taken and ensuring the explanation is given in an empathetic manner, with clear guidance on next steps and treatment.

When screening was suggested 77% said they would be interested in attending as most would want to know if they had cancer. The majority of patients (82%, see Figures 2.1b and 2.5) where happy to accept all levels of intervention required to reach a cancer diagnosis. They felt this was necessary to achieve the end goal of an accurate diagnosis. Even though as one person described, 'I had all of the above tests and found the four endoscopies the worst but would endure them again.' and several said 'I would have any test' qualified with 'if there was any chance of catching cancer early' and 'either of the

invasive tests would put me off but investigation of a suspected cancer is worth the inconvenience'.

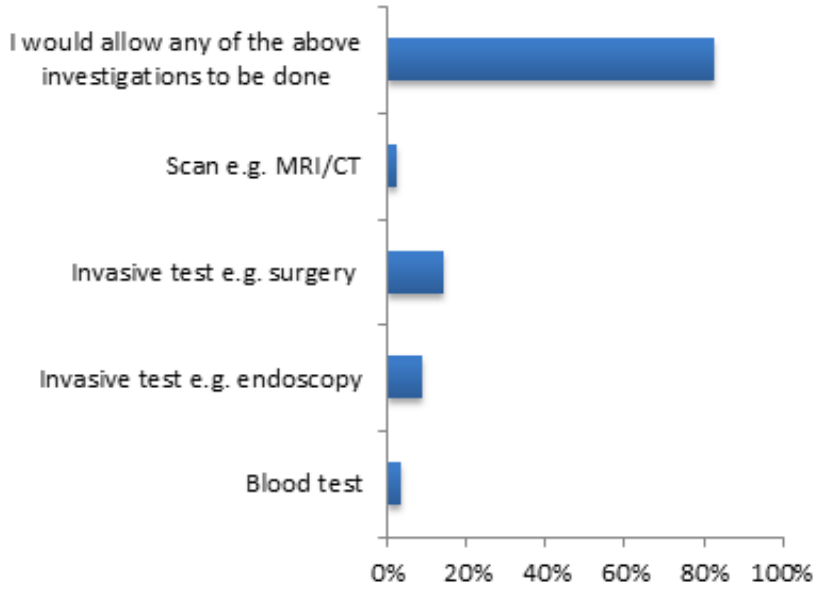

Figure 2.5: Patient responses to investigations they would not want during a diagnostic pathway.

Just under half of the group (46%) felt a diagnosis should be reached within a week of seeing a specialist (see figure 2.6), and half believed they could be fully informed and understand all aspects of diagnosis and treatment provided (see figure 2.7). Therefore this is an area that targeted vibrational spectroscopy may be able to develop, enabling the patient to have an earlier diagnosis.

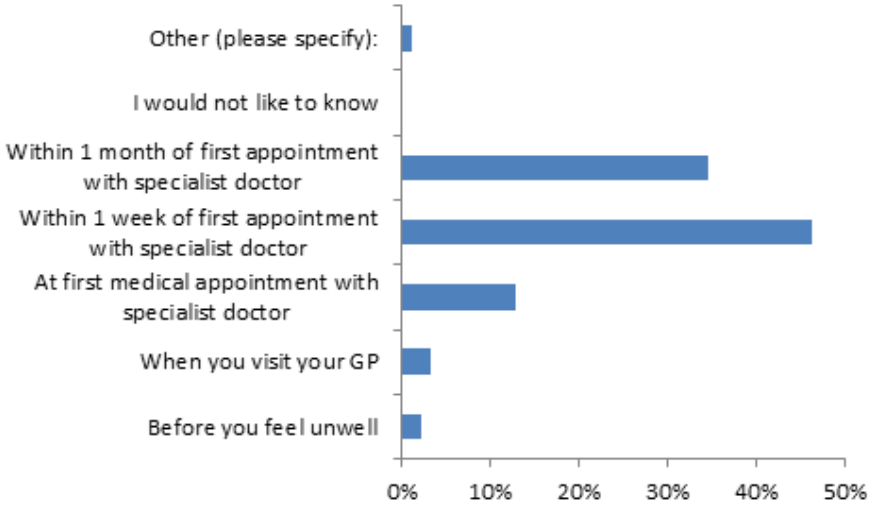

Figure 2.6: Patients were asked 'At what point would you EXPECT diagnosis of cancer to occur? Please select ONE response'. The majority expected a diagnosis within a week of seeing a specialist doctor.

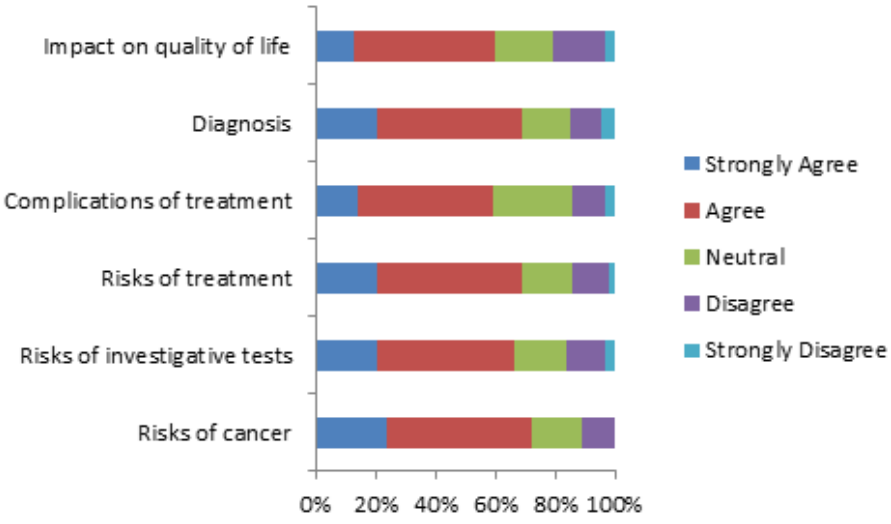

Figure 2.7: Patients were asked ' Do you think you are fully informed and/or can access information about' a range of topics, including impact of quality of life, diagnosis and complications. Over half felt they were well informed of all risks and complications.

The focus group consisted of only one patient who was very open about their cancer experience. The lack of other participants does make the results hard to draw conclusions from. However the main points to come through were again similar to those within the

questionnaire focussing on the need for clear, open and honest explanation and ensuring of understanding and the need for empathy. They felt the oncologist was one of the best people they met whom had been the most open with them. They too would want a diagnosis from a specialist consultant not their GP as they did not know their GP well and felt the consultant knew more about their cancer. The suggestion of a home test for cancer was met with reluctance, with the suggestion that its use and outcome would be very person dependant and that perhaps cancer diagnosis was best remaining in the hospital forum.

Discussion:

The questionnaire and focus group responses have all shown the crucial need for time and clarity when giving patients a cancer diagnosis. Whilst this has been an area where medical training has been focused over many years it still clearly needs work and remains at the heart of a persons' cancer journey. The most interesting point was the acceptance of investigations by patients. The range of results given shows how even some relatively simple investigations are considered less acceptable. Whilst the medical profession is trying to move ever closer to patient centred care and allowing the patient to make a decision, a large proportion of people are willing to accept whatever investigation is felt necessary by the medical team. As one respondent said 'if the specialist believed it to be the most effective way of diagnosing cancer early, I would accept it' along with others who stated 'whatever it takes'. The doctor is still in the most powerful position, yet I am not sure they all realise how willing patients are to undergo these investigations if required. Especially given the results above where they felt some investigations may not be as accepted by patients. There was quite a disparity between what the doctor believed was acceptable and what the patient felt was. It would be interesting to know if the patients have experienced these investigations, the responses clearly show some have, as perhaps the doctor may be more aware of the investigation and what is required. It would be interesting to repeat the work and identify what a patient understood by the investigation and how many had experienced it and understand the impact that had upon acceptability. It does however demonstrate to the medical profession the importance of any comment made and how despite the drive for patient involvement in care the emphasis is still placed on the medical recommendation.

Of interest, most patients would still rather receive a cancer diagnosis in secondary care, even with the time constraints placed upon it and the likelihood of having not met the doctor previously. Within primary care they have often used a GP much more often and may have a rapport with them, yet they recognise the treatment plan and prognosis is

more likely to be developed in secondary care. The use of clinical nurse specialists cannot be understated. They were chosen more often than a GP to give a cancer diagnosis. They are often seen as having more time to sit and talk to patients and explain a diagnosis. As their role develops it is crucial this time is kept, as they are often a great source of support for patients.

The main limitations of study surrounded number of both Patients and Clinicians involved, particularly within the focus groups. The Patient focus group was attended by only 1 person, therefore the data obtained is limited. Recruitment for this part of the study was challenging, if it were repeated providing transport for Patients may aid the number of people attending. This was also an issue with the questionnaire. It was available in paper format, but this was not requested at all. Some questionnaires were started and not completed, which may be due to the length and number of questions. These were not included in to the analysis. Questionnaires do not allow for lengthy discussions around thoughts and feeling and provide only a limit snap shot based on either a selection or short response question. Perhaps if the study were to be repeated it would be useful to perform the questionnaire first, then invite open discussion within focus groups based on the ideas arising out of the questionnaire answers (Beiske, 2002).

The results also highlight both slides are aligned with the need for screening and early cancer detection. Whilst it is concerning some small patient areas are not aware of screening pathways, overall the government drives surrounding cervical and colorectal cancer, for example, and the need for screening have increased uptake (CRUK N, accessed 11/12/17). The two groups were also both reluctant for a home-based test. Both describe the risks associated and the need for careful evaluation and avoiding unnecessary worry and potential harm. Again this is a very interesting point. The patient group recognised similar concerns to medics surrounding this and the potential risk to life if a patient became very upset based upon the results without a clear understanding of the accuracy of an investigation. It again highlights the need to involve patients early in research as whilst an at-home diagnostic tool may sound like a positive development in the laboratory, it may not actually be a positive idea in reality. Nor may patients be willing to use it.

Conclusion:

Overall this study has highlighted disparities between patient and clinician thoughts surrounding cancer diagnosis and treatment. It has also highlighted areas where both are aligned in thoughts on new diagnostics, showing that whilst a test may seem unacceptable, most would be willing to undertake it. It has demonstrated that any new

development within cancer diagnostics is likely to be most beneficial within secondary care as this is where patients felt they were most likely to receive information of benefit and understand their treatment plans moving forward. Demonstrating whilst a GP is crucial in supporting patients following diagnosis, most would still rather receive a diagnosis in a secondary care setting.

Chapter

3

3. Fresh Frozen Primary Brain Tumours

Declaration of Work

To Whom it May Concern,

Dr Danielle Bury designed the study in conjunction with Dr Matthew Baker. Dr Bury completed the application form for use of tissues from the Brain Tumour North West tissue bank. She arranged with Mrs K Ashton for samples. Collected these and performed all spectral acquisition. Dr Bury also reviewed and marked all matched H&E slides.

Following discussion with Prof F Martin, Mr C L M Morais assisted with results analysis. Dr Bury then produced a paper for publication of the results.

Signed

……. ……..

Prof F L Martin Dr D Bury

Introduction

Brain tumours account for 3% of all tumours diagnosed annually, with incidence rates increasing approximately 15% over the last decade (CRUK D, accessed 3/11/17). Whilst they comprise a small proportion of all cancer diagnoses per year, the difficulty of complete removal of the tumour is inherent. High-grade tumours can be infiltrative and when operating within the brain, the risk of removing crucial structures in a bid to free the patient of the tumour yet risk leaving them with significant neural deficit is ever present. Up to 75% of tumour resections are thought to leave behind viable tumour (Hollon *et. al.,* 2016). This study was designed to look at the potential of vibrational spectroscopy to be able to provide an intraoperative method of diagnosing tumours that would allow the surgeon to determine if tissue was in fact tumour and improve the ability to resect malignancy. Current techniques include the use of 5-aminolevulinic acid (5-ALA). This uses a fluorescent compound to fluoresce tumour cells to enable the surgeon to visualise them more easily. This allows real-time feedback and does not rely on repeat imaging on the operating table (Hadjipanavis *et. al.,* 2015.). This has a high success rate but can prove difficult at the edge of tumours where normal brain tissue can appear a similar lighter colour to that of neoplastic tissue (Hadjipanavis *et. al.,* 2015, Galli *et. al.,* 2017).

Both Raman and ATR-FTIR spectroscopic methods were used to detect non-tumour brain tissue from a variety of primary brain tumours using brain tissue that had been frozen after receipt from the surgical theatre. This was placed upon calcium fluoride slides and defrosted prior to use. Fresh frozen tissue was used to mimic as closely as possible fresh brain tissue and reduce artefact from paraffin embedded tissues.

Methods

Ninety-six cases of fresh frozen brain tissue comprising primary brain tumours both gliomas of varying grades and meningiomas, along with normal brain were selected from the Brain Tumour North West tissue bank, with ethical approval (NRES14/EE/1270). This tissue has been retrieved from the patient and then snap frozen on arrival within the

histopathology department. Sections are cut within a cryostat machine to ensure tissue remains frozen, sections are allowed to defrost prior to spectral acquisition. This tissue was chosen for analysis as it has not previously been formalin fixed and therefore is closest to fresh tissue allowable given the number of cases tested. The cases used in the study are shown in table 3.1 below, categorised by tumour type.

Table 3.1: Tumour samples selected for analysis, broken down by tumour type and WHO grade.

	N	WHO Grade 1	WHO Grade 2	WHO Grade 3	WHO Grade 4	No Grade
All Cases	96	25	11	14	33	5
Normal brain	8	N/A	N/A	N/A	N/A	N/A
Gliomas	54	1	6	11	33	3
Meningiomas	34	24	5	3	N/A	2

Ten-µm-thick frozen sections were cut and placed onto 25 × 25 × 1 mm Raman-grade calcium fluoride-coated slides (Cyrstan Ltd). A matched 4-µm-thick section stained with haematoxylin and eosin (H&E) was then cut to allow viable tumour areas to be marked and confirmed. This allowed points within the tumour tissue to be tested using spectroscopy, to prevent any contaminating spectra from background brain tissue or necrotic areas. Following this, spectrochemical measurements were performed using both Raman and ATR-FTIR spectroscopy, focussed on the viable tumour areas.

Raman spectroscopy

Spectra were taken from 20-25 random points within the tumour tissue area using a Horiba Jobin-Yvon LabRAM HR800 spectrometer. An air-cooled CLDS point mode diode 785 nm laser with a single edge filter (cut off to 100 cm^{-1}) and an output power of 300 mW. This was done with a confocal hole of 100 µm at a grating of 300 gr/mm and a ×50 objective. For each spectrum, 2 accumulations each over 30 seconds were acquired.

ATR-FTIR Spectroscopy

The ATR-FTIR spectroscopy measurements were performed on an Agilent Cary-600 Series FTIR spectrometer. Measurements were taken in transmission mode with 32 co-added scans over a range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1}. A background scan was taken prior to each sample with the same settings. Twenty random points were selected within each viable tumour area.

Computational analysis

Data collection and manipulation, was performed within a MATLAB R2014b environment (MathWorks Inc., USA) using PLS Toolbox 7.9.3 (Eigenvector Research Inc., USA) with specimens first assigned to training, validation and test groups using the Kennard-Stone algorithm, a method of dividing data into training, validation and test groups (see Table 3.2). Of the samples, 70% were placed into training and 15% each into validation and test groups. This method was chosen as it has been previously shown to be effective in spectral analysis (Lima *et. al.,* 2015). Youden's index was also calculated to determine the significance of the results, with 1 meaning no false positives or negatives and 0; occurring by chance.

Table 3.2 Number of samples within the training, validation and test groups based on the application of the Kennard-Stone algorithm.

Class	Training	Validation	Test
Normal	111	24	24
Meningioma	466	100	100
Glioma	739	158	159

Pre-processing using Savitzky-Golay smoothing followed by multiplicative scatter correction (MSC), baseline correction, and vector normalization were performed. The spectra were cut from 1800-500 cm^{-1} [see Supplementary Information (SI) Figures S1 and S5)]. Following on from this, principal component analysis with linear discriminant analysis (PCA-LDA) or quadratic discriminant analysis (PCA-QDA), and genetic algorithm with LDA (GA-LDA) or quadratic discriminant analysis (GA-QDA) were performed in order to determine the best analytical method (Lima *et. al.,* 2015). The training samples were used for model construction and the test set for the final classification evaluation. The optimum number of variables for GA-LDA/QDA was performed based on the average risk G of misclassification, which is calculated in the validation set as:

$$G = \frac{1}{N_v}\sum_{n=1}^{N_v} g_n \quad (1)$$

where N_v is the number of validation samples and g_n is defined as

$$g_n = \frac{r^2(x_n, m_{I(n)})}{\min_{I(m)\neq I(n)} r^2(x_n, m_{I(m)})} \quad (2)$$

where $I(n)$ is the index of the true class for the *n*th validation object x_n; $r^2\left(x_n, m_{I(n)}\right)$ is the squared Mahalanobis distance between object x_n (of class index $I(n)$) and the sample mean $m_{I(n)}$ of its true class; and $r^2\left(x_n, m_{I(m)}\right)$ is the squared Mahalanobis distance between object x_n and the sample mean $m_{I(m)}$ of its wrong class (28). The GA routine was carried out during 40 generations with 80 chromosomes each. Crossover and mutation probabilities were set to 60% and 10%, respectively. Moreover, the algorithm was repeated three times, starting from different random initial populations. The best solution (in terms of the fitness value) was employed. LDA and QDA were employed to the PCA scores and GA selected variables as follows (Siqueira *et. al.,* 2017):

$$L_{ik} = (\mathbf{x}_\mathrm{i} - \bar{\mathbf{x}}_k)^\mathrm{T} \boldsymbol{\Sigma}_{pooled}^{-1} (\mathbf{x}_i - \bar{\mathbf{x}}_k) - 2\log_e \pi_k \quad (3)$$

$$Q_{ik} = (\mathbf{x}_\mathrm{i} - \bar{\mathbf{x}}_k)^T \boldsymbol{\Sigma}_k^{-1} (\mathbf{x}_\mathrm{i} - \bar{\mathbf{x}}_k) + \log_e |\boldsymbol{\Sigma}_k| - 2\log_e \pi_k \quad (4)$$

where L_{ik} and Q_{ik} are the LDA and QDA classification scores, respectively; $\mathbf{x}_\mathrm{i}$ is the measurement vector containing the input variables for sample *i*; $\bar{\mathbf{x}}_k$ is the mean measurement vector for class *k*; $\boldsymbol{\Sigma}_{pooled}$ is the pooled covariance matrix between the classes; $\boldsymbol{\Sigma}_k$ is the variance-covariance matrix of class *k*; and π_k is a prior probability term, defined as the ratio between the number of samples in class *k* and the total number of samples in the training set.

Results

Raman Spectroscopy

From the 96 cases, 1911 spectra were collected. During pre-processing 30 spectra were removed due to poor quality, observed by a Hotelling T^2 *versus* Q residuals test. As in Table 2, tumours were classified by type rather than grade. Following pre-processing (figure 3.1) there were 159 spectra in the training class, 666 in the validation class and 1056 in test class. Firstly, comparison was done between normal and tumour tissue, grouping both meningiomas and gliomas together (Figure 3.2, Table 3.3). This demonstrates that 94% of the cases were correctly classified as either tumour or non-tumour brain tissue, with a sensitivity (*i.e.* the number of actual positives identified as positive) of 98.8% and specificity (*i.e.* the number of actual negatives identified as negative) of 41.7%.

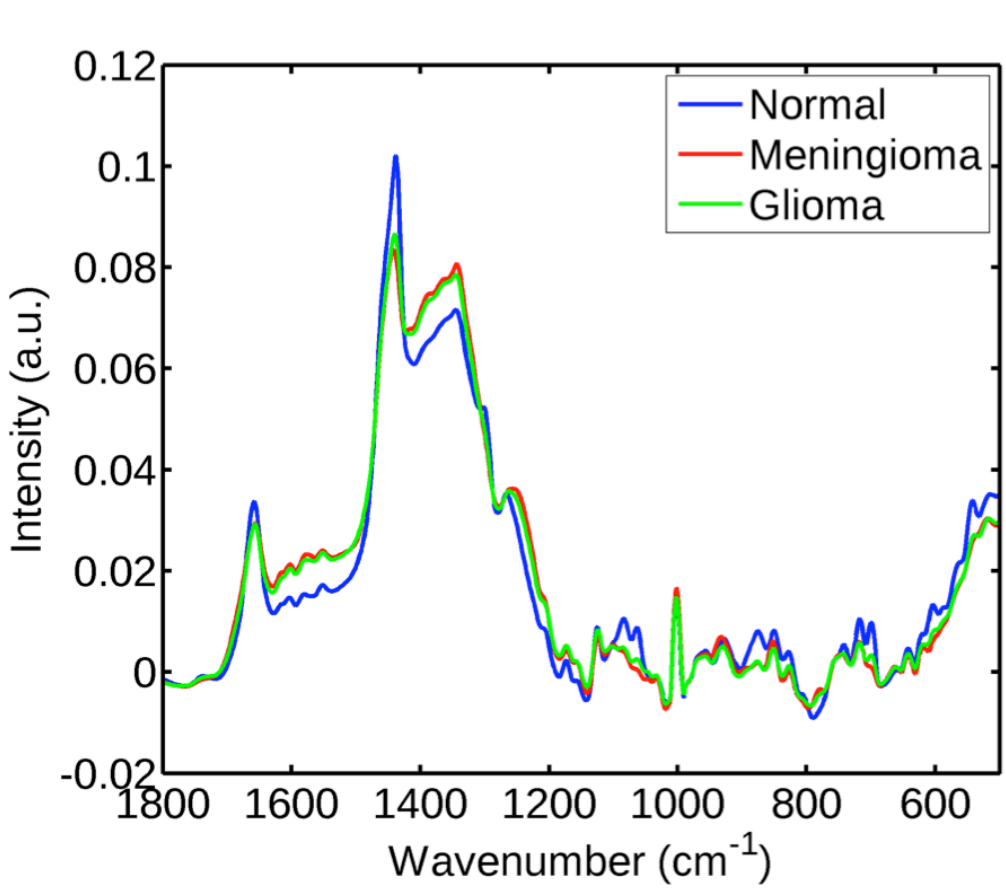

Figure 3.1: Pre-processed mean Raman spectra, averaged spectra for each tumour type.

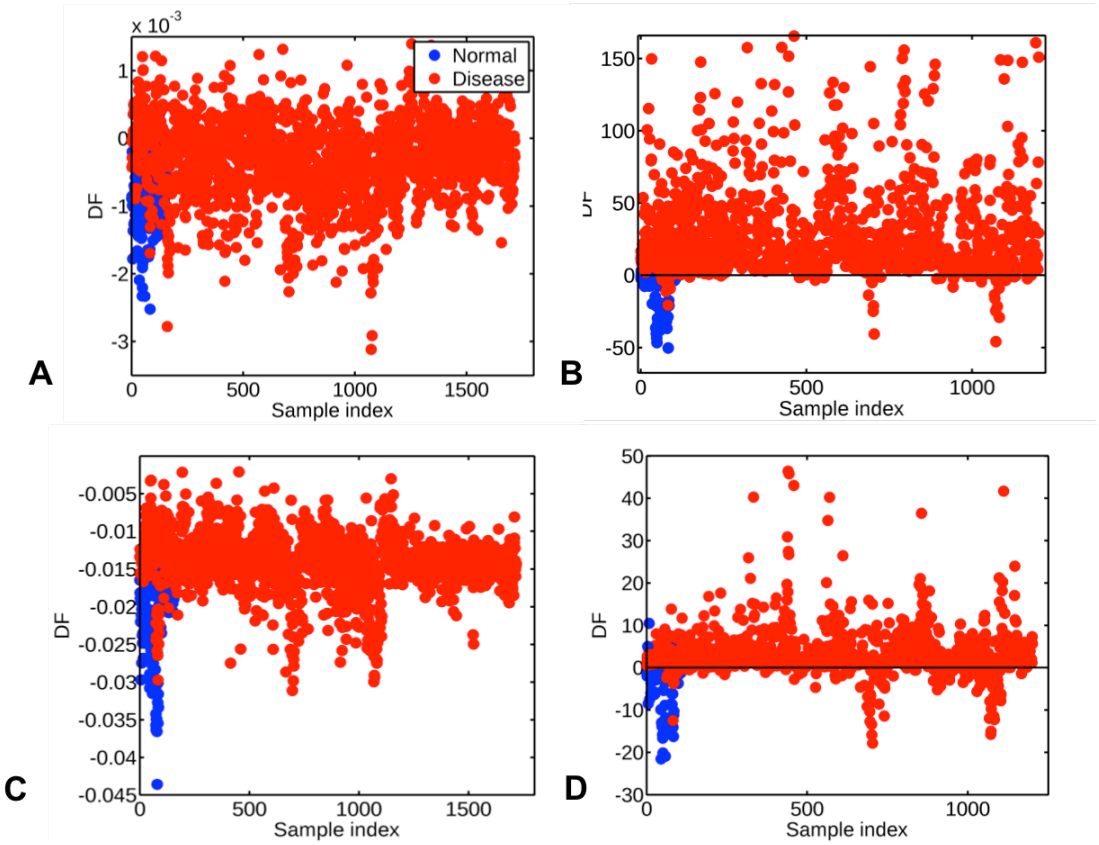

Figure 3.2 Discriminant function plots to demonstrate the separation of normal *versus* tumour (Meningioma and glioma) using Raman spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.3 Results for classification models for normal *versus* tumour (meningioma and glioma) using Raman spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	93.3	94.0	91.5	91.8
Sensitivity (%)	98.4	98.8	97.3	97.7
Specificity (%)	37.5	41.7	29.2	29.2
PPV (%)	94.4	94.8	93.7	93.7
NPV (%)	69.2	76.9	50.0	53.8
Youden's Index	0.36	0.41	0.27	0.27

Correct Classification (%)	Training	Validation	Test
PCA-LDA	85.5	91.4	93.3
PCA-QDA	93.4	94.3	94.0
GA-LDA	84.3	90.7	91.5
GA-QDA	86.6	91.8	91.8

Following on from this the model was tested to determine if it could identify; meningioma from glioma (figure 3.3, table 3.4), normal from meningioma (figure 3.4, table 3.5), normal from glioma (figure 3.5, table 3.6) and normal from meningioma from glioma (figure 3.6, table 3.7). When asked to determine tumour by type the overall classification accuracy fell to 63.1%. Normal brain tissue was still detected with an accuracy of over 90%.

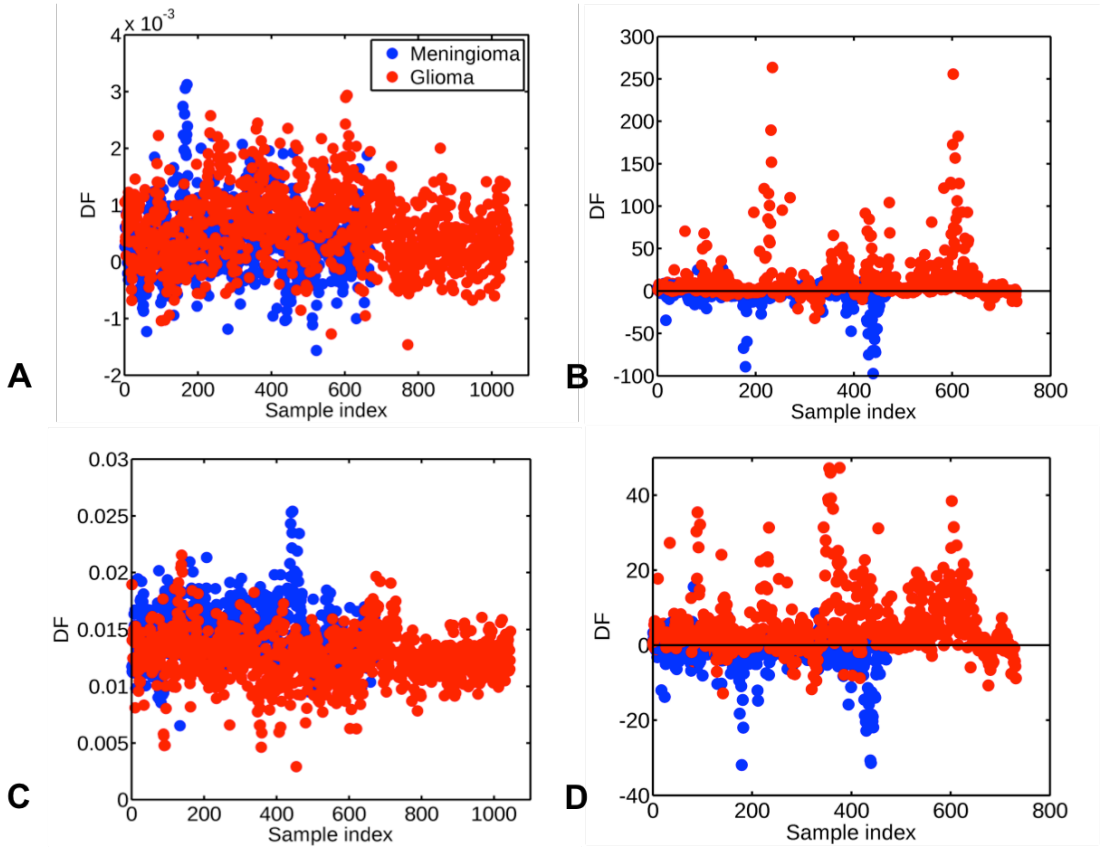

Figure 3.3: Discriminant function plots to demonstrate separation of meningioma versus glioma using Raman spectroscopy. ((A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.4: Results for classification models for meningioma versus glioma using Raman spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	67.1	66.7	67.4	66.7
Sensitivity (%)	83.4	94.3	88.5	89.8
Specificity (%)	41.6	23.8	34.7	30.7
PPV (%)	68.9	65.8	67.8	66.8
NPV (%)	61.8	72.7	66.0	66.0
Youden's Index	0.25	0.18	0.23	0.21

Correct Classification (%)	Training	Validation	Test
PCA-LDA	67.5	68.9	67.1
PCA-QDA	70.5	67.3	66.7
GA-LDA	73.4	73.1	67.4
GA-QDA	75.8	72.0	66.7

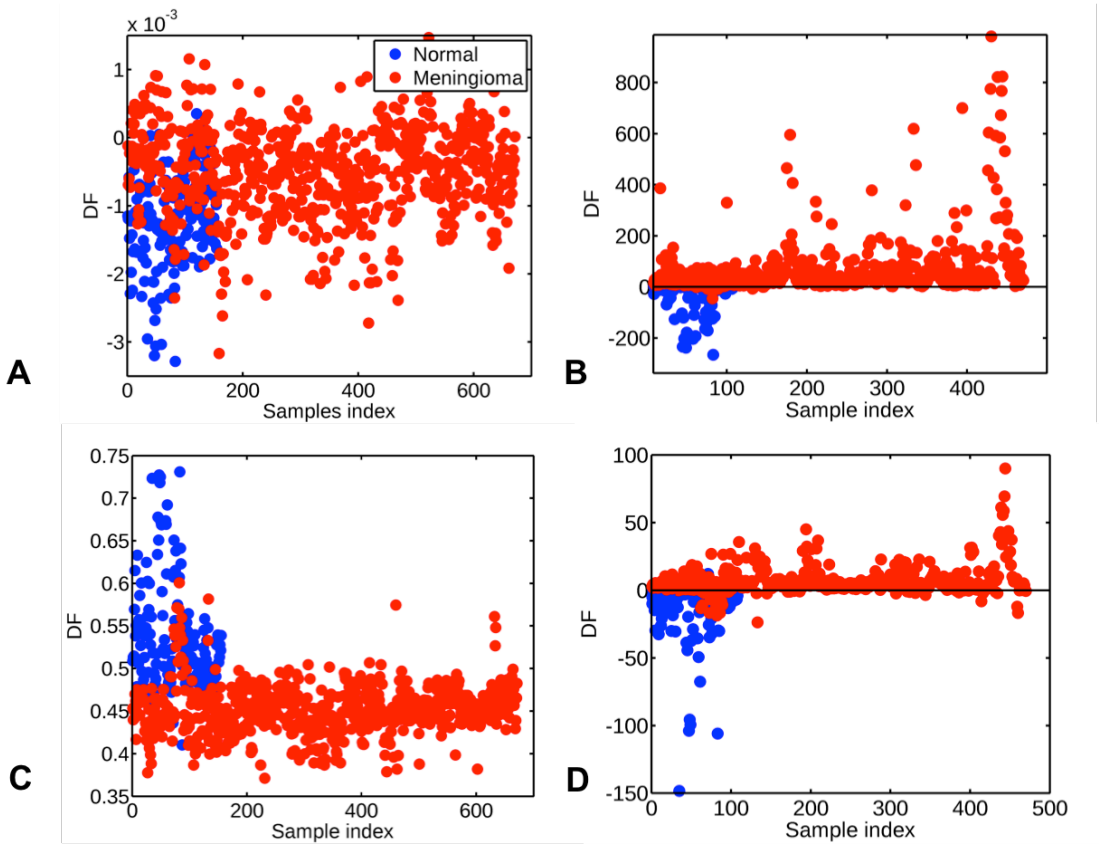

Figure 3.4: Discriminant function plots to demonstrate the separation of normal versus meningioma using Raman spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.5: Results for classification models for normal versus meningioma using Raman spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	89.6	90.4	88.8	95.2
Sensitivity (%)	98.0	99.0	97.0	95.0
Specificity (%)	54.2	54.2	54.2	95.8
PPV (%)	90.0	90.1	89.9	99.0
NPV (%)	86.7	92.9	81.2	82.1
Youden's Index	0.51	0.53	0.51	0.91

Correct Classification (%)	Training	Validation	Test
PCA-LDA	92.0	89.4	89.6
PCA-QDA	94.5	88.6	90.4
GA-LDA	92.0	90.2	88.8
GA-QDA	91.3	98.4	95.2

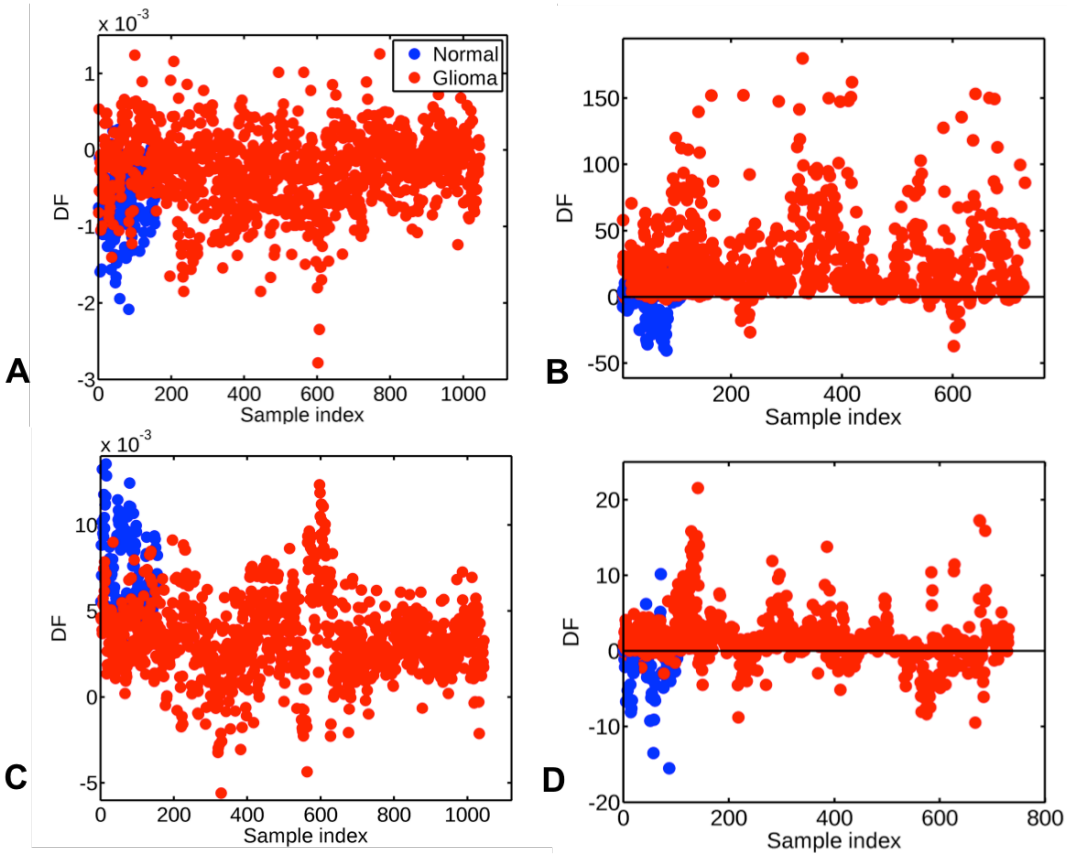

Figure 3.5: Discriminant function plots to highlight the separation of normal versus glioma using Raman spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.6: Results for classification models for normal versus glioma using Raman spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	90.1	91.7	90.6	88.4
Sensitivity (%)	99.4	100	98.1	96.2
Specificity (%)	29.2	37.5	41.7	37.5
PPV (%)	90.2	91.3	91.7	91.0
NPV (%)	87.5	100	76.9	60.0
Youden's Index	0.29	0.38	0.40	0.34

Correct Classification (%)	Training	Validation	Test
PCA-LDA	79.7	87.2	90.1
PCA-QDA	90.2	91.7	91.7
GA-LDA	82.7	90.0	90.6
GA-QDA	76.8	88.9	88.4

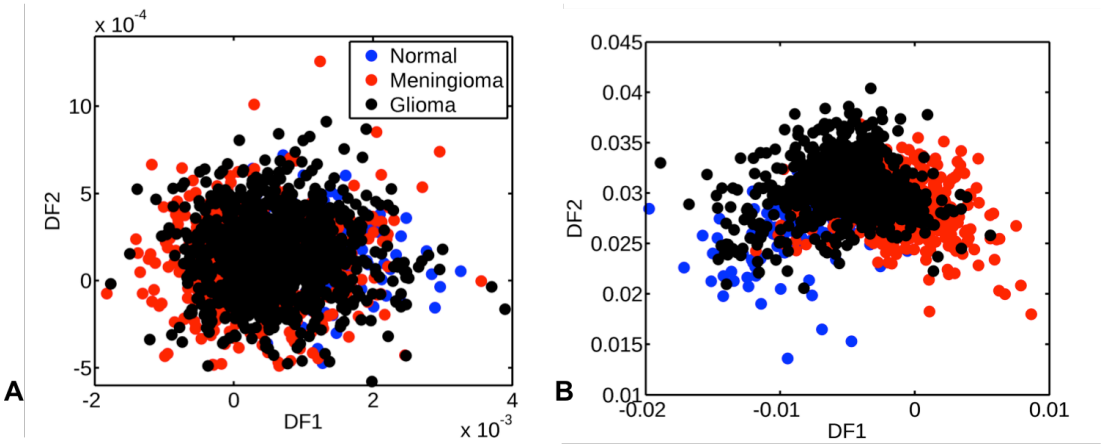

Figure 3.6 Discriminant function plots to demonstrate the separation of normal *versus* meningioma *versus* glioma using Raman spectroscopy. (A) Principal component analysis – linear discriminant analysis, (B) Genetic Alogorithmn-Linear discriminant analysis.

Table 3.7 Results for classification models of normal *versus* meningioma *versus* glioma using Raman spectroscopy.

	Normal		Meningioma		Glioma	
	PCA-LDA	GA-LDA	PCA-LDA	GA-LDA	PCA-LDA	GA-LDA
Accuracy (%)	92.9	92.6	69.5	68.4	63.6	62.4
Sensitivity (%)	33.3	29.2	33.7	36.6	86.6	82.8
Specificity (%)	98.4	98.4	89.5	86.2	35.2	36.8
PPV (%)	66.7	63.6	64.2	59.7	62.7	62.6
NPV (%)	94.1	93.7	70.7	70.9	67.7	63.0
Youden's Index	0.32	0.28	0.23	0.23	0.22	0.20

Correct Classification (%)	Training	Validation	Test
PCA-LDA	59.0	62.5	63.1
GA-LDA	66.1	68.9	61.7

ATR-FTIR Spectroscopy

The process was then repeated for ATR-FTIR spectroscopy. From the 96 cases, 1919 spectra were collected; again during pre-processing 38 spectra were removed due to poor quality, observed by a Hotelling T^2 *versus* Q residuals test. Spectra were divided as above. Following pre-processing (figure 3.7) there were 159 spectra in the training class, 666 in the validation class and 1056 in the test class. As for the Raman spectra, firstly, normal was compared to tumour (meningioma and glioma) with GA-QDA providing the best results, with a classification accuracy of 97.2% (Figure 3.8, Table 3.8). The sensitivity was 100% and specificity 66.7%. Following on from this the model was tested to determine if it could identify; meningioma from glioma (figure 3.9, table 3.9), normal from meningioma (figure 3.10, table 3.10), normal from glioma (figure 3.11, table 3.11) and normal from meningioma from glioma (figure 3.12, table 3.12).

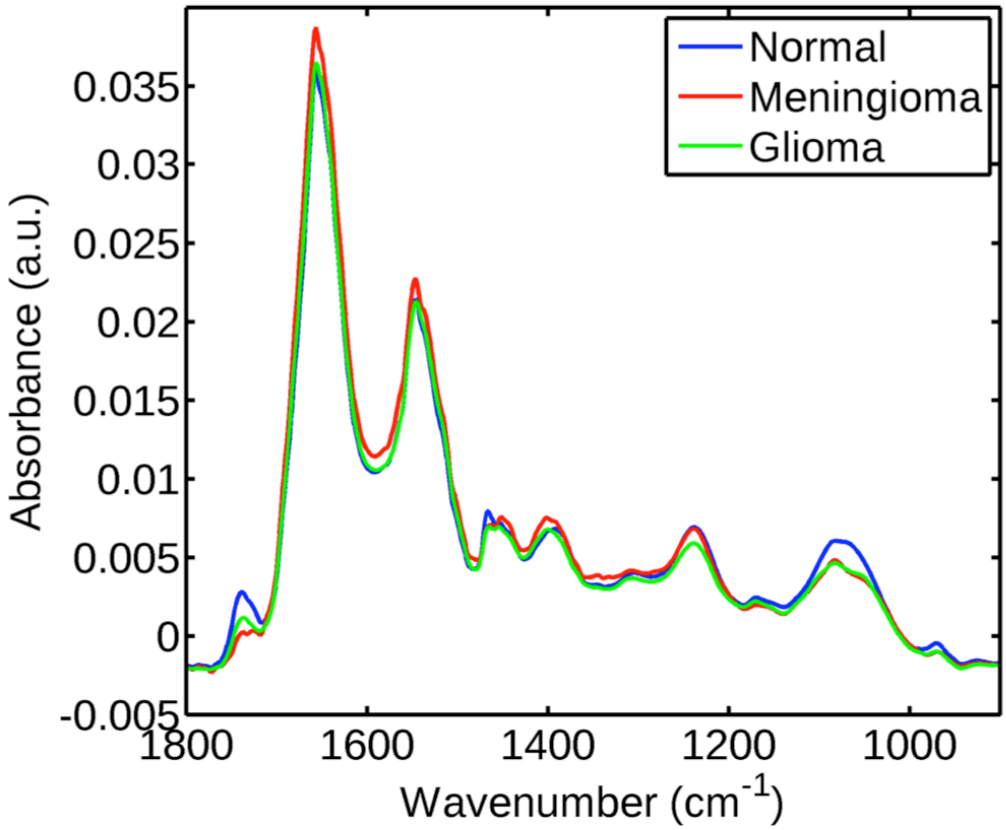

Figure 3.7: Mean pre-processed IR spectra

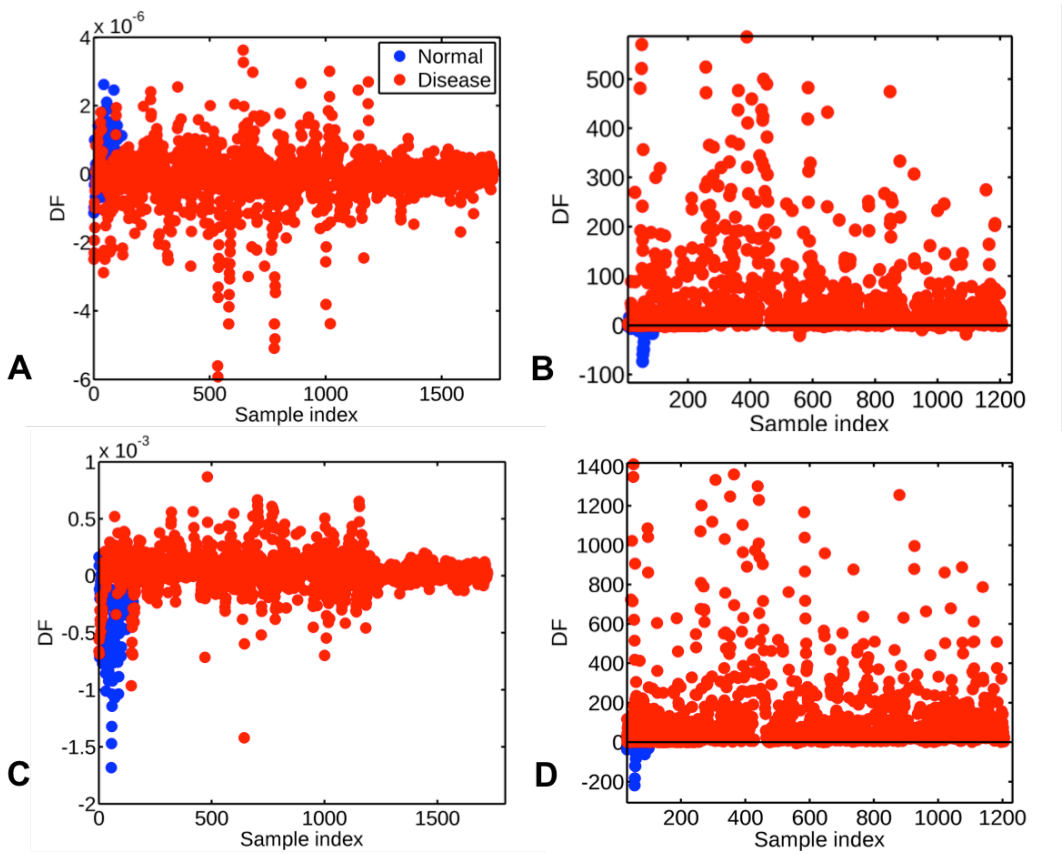

Figure 3.8 Discriminant function analysis to demonstrate the separation of normal *versus* tumour (meningioma and glioma) using IR spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis..

Table 3.8 Results of classification models for normal *versus* tumour (meningioma and glioma) using IR spectroscopy, with the best classification model highlighted in red.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	92.1	87.1	94.1	97.7
Sensitivity (%)	98.1	87.1	100	100
Specificity (%)	12.5	87.5	16.7	66.7
PPV (%)	93.7	98.9	94.1	97.5
NPV (%)	33.3	33.9	100	100
Youden's Index	0.11	0.75	0.17	0.67

Correct Classification (%)	Training	Validation	Test
PCA-LDA	81.1	92.9	90.5
PCA-QDA	93.3	86.2	84.5
GA-LDA	91.5	95.4	92.9
GA-QDA	96.7	97.9	97.2

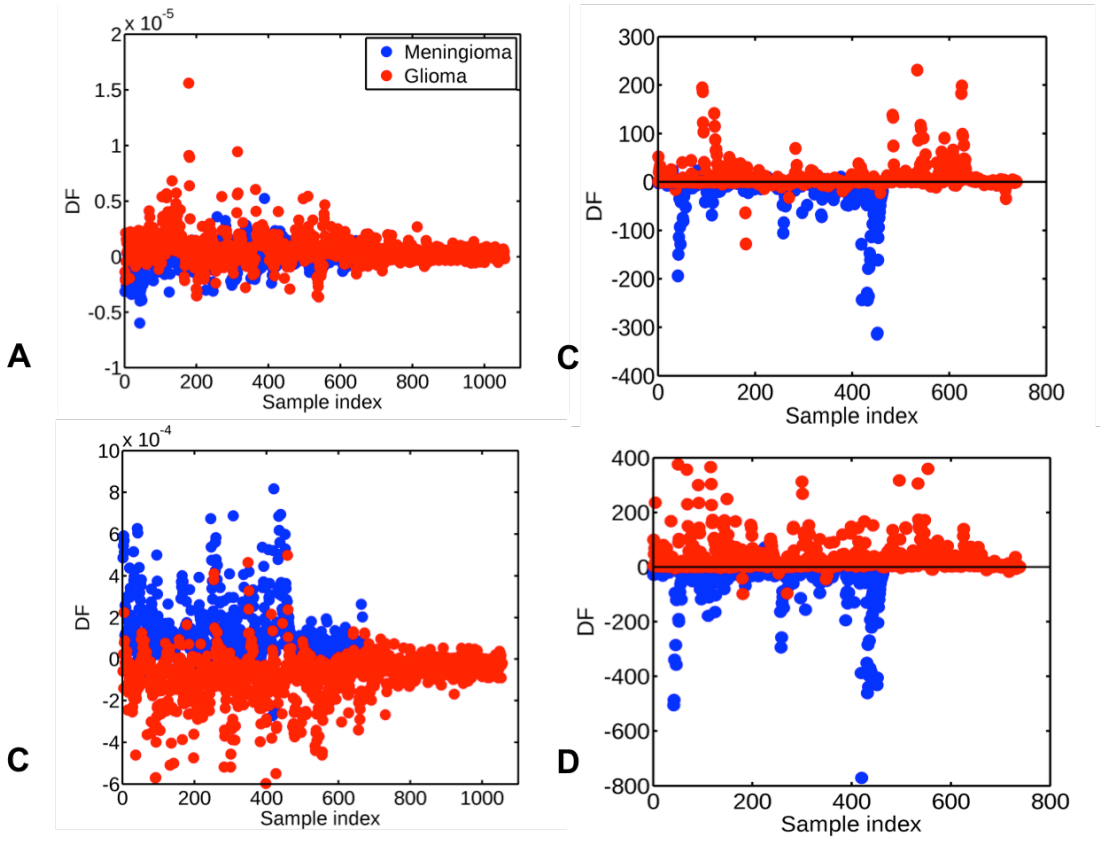

Figure 3.9: Discriminant function analysis to demonstrate separation of Meningioma versus Glioma using IR spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.9: Results for classification models for meningioma versus glioma using IR spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	74.5	74.1	81.5	82.2
Sensitivity (%)	97.5	88.1	94.3	92.5
Specificity (%)	38.0	52.0	61.0	66.0
PPV (%)	71.4	74.5	79.4	81.2
NPV (%)	90.5	73.2	87.1	84.6
Youden's Index	0.36	0.40	0.55	0.59

Correct Classification (%)	Training	Validation	Test
PCA-LDA	75.4	69.4	74.5
PCA-QDA	74.7	72.1	74.1
GA-LDA	88.0	84.9	81.5
GA-QDA	89.2	88.0	82.2

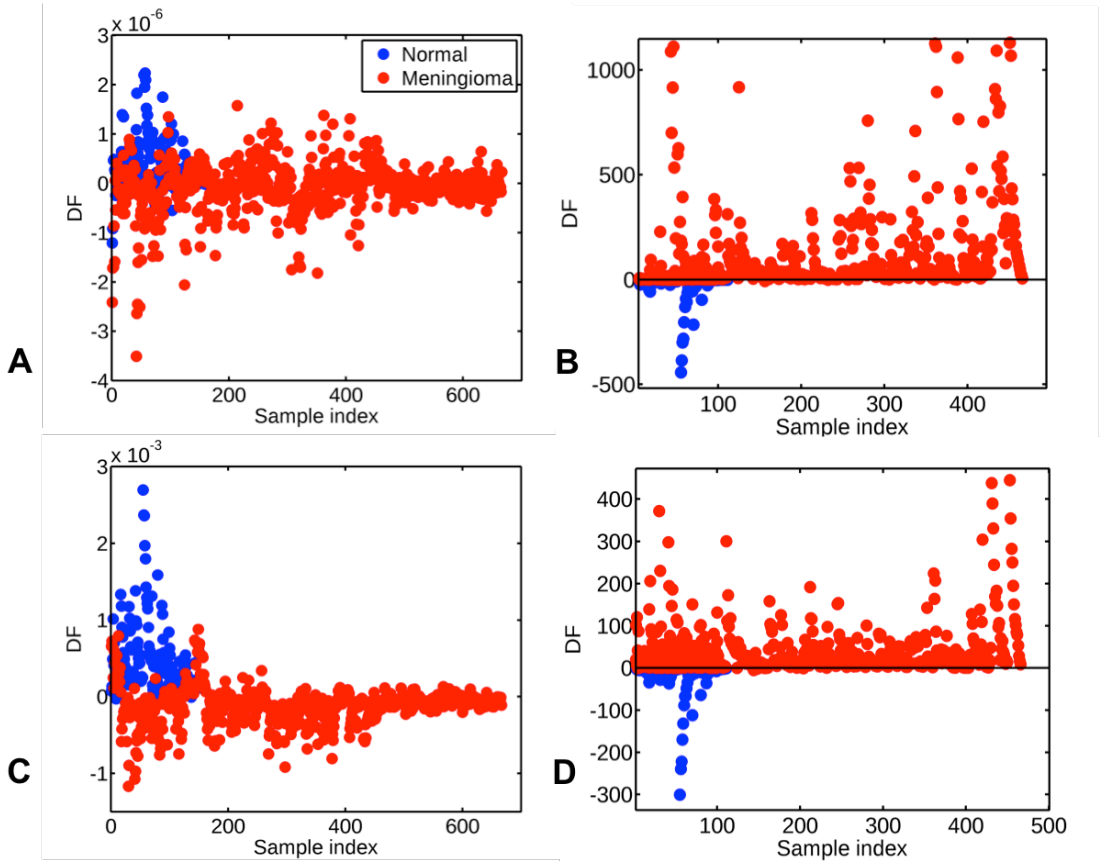

Figure 3.10: Discriminant function analysis to demonstrate separation of Normal versus meningioma using IR spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn- Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.10: Results for classification models for normal versus meningioma using IR spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	86.3	95.2	83.9	96.8
Sensitivity (%)	100	95.0	100	99.0
Specificity (%)	29.2	95.8	16.7	87.5
PPV (%)	85.5	99.0	83.3	97.1
NPV (%)	100	82.1	100	95.5
Youden's Index	0.29	0.91	0.17	0.87

Correct Classification (%)	Training	Validation	Test
PCA-LDA	89.3	91.1	86.3
PCA-QDA	95.8	91.9	95.2
GA-LDA	89.5	91.9	83.9
GA-QDA	96.5	98.4	96.8

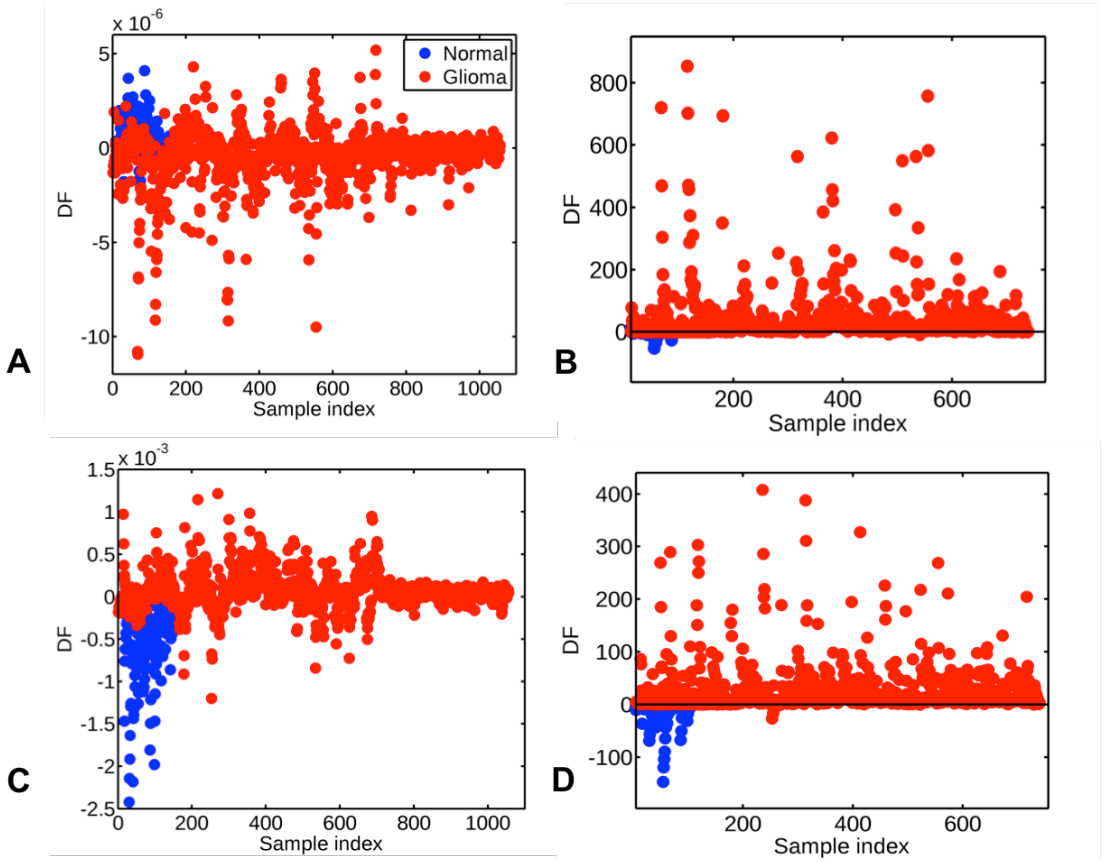

Figure 3.11: Discriminant function analysis to demonstrate separation of normal vs glioma using IR spectroscopy. (A) Principal Component Analysis-Linear Disciminant Analysis, (B) Principal Component Analysis-Quadratic Discriminant Aanalysis, (C) Genetic Algorithmn-Linear Disciminant Analysis, (D) Genetic Algorithmn- Quadratic Discriminant Aanalysis.

Table 3.11: Results for classification models for normal versus glioma using IR spectroscopy. Highlighted in red is the best classification model.

	PCA-LDA	PCA-QDA	GA-LDA	GA-QDA
Accuracy (%)	80.6	73.4	88.7	94.4
Sensitivity (%)	95.0	74.0	100	96.0
Specificity (%)	20.8	70.8	41.7	87.5
PPV (%)	83.3	91.4	87.7	97.0
NPV (%)	50.0	39.5	100	84.0
Youden's Index	0.16	0.45	0.42	0.84

Correct Classification (%)	Training	Validation	Test
PCA-LDA	76.9	90.7	86.9
PCA-QDA	92.1	80.2	82.0
GA-LDA	92.1	94.5	92.3
GA-QDA	96.2	95.6	96.2

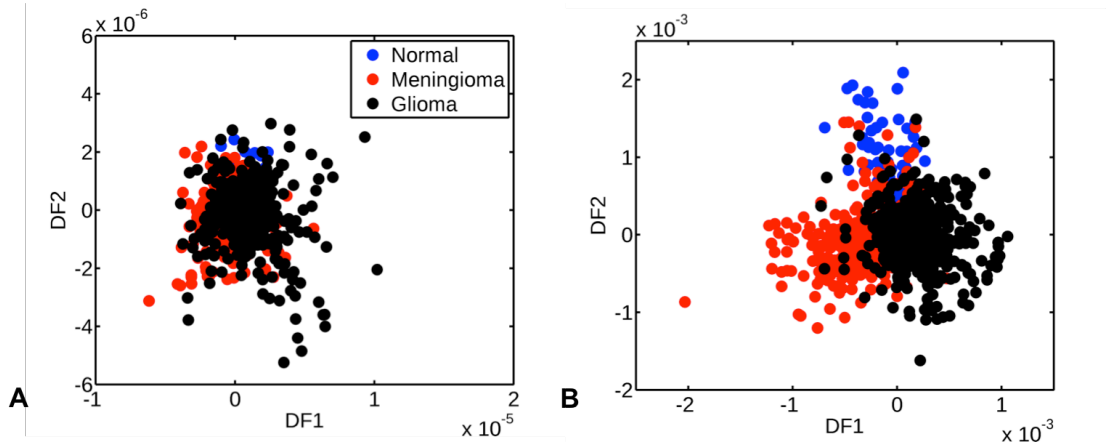

Figure 3.12 Discriminant function analysis to demonstrate separation of normal *versus* meningioma *versus* glioma using IR spectroscopy. (A) principal component analysis – linear discriminant analysis, (B) Genetic Alogrithmn-linear discriminant analysis.

Table 3.12 Results of the classifcation models for normal *versus* meningioma *versus* glioma using IR spectroscopy.

	Normal		Meningioma		Glioma	
	PCA-LDA	GA-LDA	PCA-LDA	GA-LDA	PCA-LDA	GA-LDA
Accuracy (%)	90.8	95.8	73.1	83.4	64.0	79.2
Sensitivity (%)	8.3	50.0	26.0	56.0	96.2	98.1
Specificity (%)	98.5	100	98.9	98.4	22.6	54.8
PPV (%)	33.3	100	92.9	94.9	61.4	73.6
NPV (%)	92.1	95.6	71.0	80.4	82.4	95.8
Youden's Index	0.07	0.50	0.25	0.54	0.19	0.53

Correct Classification (%)	Training	Validation	Test
PCA-LDA	62.8	61.7	64.0
GA-LDA	83.1	84.0	79.2

As the classification model becomes more complex, the accuracy drops; for example, when comparing if the classification model could correctly identify normal versus meningioma versus glioma the accuracy fell to 79.2%, however this was still above that achieved with the Raman spectroscopy (63.1%). FTIR also gave higher accuracy results when comparing tumour to no tumour, 97.7% compared to 94%. This may be due to the water content within the frozen samples interfering with the Raman spectra.

Discussion

The ability of vibrational spectroscopic techniques to detect brain tumours with both blood components (Owens *et. al.,* 2014, Gajjar *et. al.,* 2013, Hands *et. al.,* 2014, Hands *et. al.,* 2016) and formalin-fixed tissue (*Gajjar et. al.,* 2012) has been previously demonstrated with high accuracy levels. Studies using fresh frozen brain tissue are few and far between, with one study within the paediatric field showing an ability to detect different tumour types and a second trialling a hand held Raman machine intraoperatively slowly moving forward (Auner *et. al.,* 2013, Desroches *et. al.,* 2018, Desroches *et. al.,* 2015). This study aimed to compare both Raman and ATR-FTIR spectroscopy using fresh tissue, which had previously only been frozen, in order to determine which provided the most accurate classification results as a precursor to developing a tool for intraoperative detection of primary brain tumours. We have shown that as compared to normal brain tissue, ATR-FTIR and Raman spectroscopy can both detect normal from tumour tissue with a high degree of accuracy (97.7% and 94%, respectively). However, when asked to determine tumour type, the accuracy of both techniques drops (79.2 and 63.1%, respectively). FTIR spectroscopy was however, considerably higher than Raman, perhaps demonstrating it is better placed to differentiate between the tumour types. The accuracy does though remain greatly below that offered by a conventional intraoperative smear diagnosis and thus would require improvement in

order to be a useful, clinically diagnostic tool. Importantly, the sensitivity when comparing normal to tumour is high (87.1-100%), meaning we are not over diagnosing tumours. The specificities are lower, though in this situation where a surgeon is aware of the presence of a tumour, high sensitivity remains the priority. One limitation of the study is the low number of 'normal' *i.e*. non tumour cases tested (*n=8*) as the majority of patients undergoing neurosurgery have a tumour. This is due to the low number of normal fresh frozen cases available within the brain bank. Therefore, if used clinically, the ability to test more background non tumour brain is likely to improve the classification accuracy and specificity. One other consideration is if the use of spectroscopy to detect meningiomas is useful. Given the distinctive radiology and macroscopic appearances, it may be that surgical tumour detection is limited between normal and glial tumours. If mengiomas were to be excluded, this would increase the detection accuracy between normal brain and gliomas to over 90% (see table 3.11). Finally, glial tumours were detected as a group and not based on their WHO classification. This was done due to the relatively small numbers of low grade tumours (n=7/54, WHO Grades 1&2). Surgically, this is a useful distinction to make, therefore taking this study forward it would be necessary to differentiate the glial tumours into low and high grade based upon WHO grades.

Overall, we have shown spectroscopy may have potential in the diagnosis of intraoperative brain tumours; however, further work to improve classification would be required prior to clinical implementation. Further work to allow for comparison of primary to metastatic tumours would also prove useful in providing clinical useful information in real time.

Chapter

4

4. Metastatic Brain Tumours

Declaration of Work

To Whom it May Concern,

Dr Danielle Bury designed the study in conjunction with Prof F Martin. Dr Bury arranged with Mrs K Ashton for samples and examined all histological slides. She then collected these and performed all spectral acquisition and analysis of results.

Dr Bury then produced an abstract for the American Association of Clinical Oncologists summer meeting (see appendix 9.9) and a paper for publication of the results. This has been accepted within Analytical Methods, and available within appendix 9.2.

Signed

……. ……..

Prof F L Martin Dr D Bury

Introduction

Metastatic brain tumours are usually the end point in a persons' cancer journey, yet for some may represent the initial diagnosis. The background prevalence of metastatic brain tumours is difficult to quantify; however, those clinically detectable outnumber intrinsic tumours by roughly 3 to 1, with the majority of metastases arising from primary lung tumours (Davis *et. al.,* 2012, Huang and Ouyang, 2013, Renfrow and Lesser, 2013). In contrast, colorectal tumours comprise 4-8% of metastasis, yet less than 9% of all cases metastasise to the brain (Sanghvi *et. al.,* 2017). For those who undergo metastectomy for diagnosis or symptom relief, the tissue, once removed is sent for histopathological analysis to determine the location of the primary tumour. This study was designed to examine the capabilities of spectroscopy, both Raman and ATR-FTIR, in determining the primary location of a metastatic tumour. Tumours were chosen, firstly those that commonly metastasise to the brain, *i.e.* lung adenocarcinoma and malignant melanoma. Colorectal adenocarcinoma was then used as a tumour with morphologically similar appearances to lung adenocarcinoma but differing immunohistochemical profile. This was chosen in preference to breast carcinoma, as whilst this morphologically often appears different to lung adenocarcinoma, the immunohistochemical profiles of both tumours overlap significantly, often providing challenges to the Histopathologist.

The initial hypothesis was that the two adenocarcinoma groups would show similar spectral patterns and therefore would be difficult to differentiate as compared to the metastatic melanoma group, which would demonstrate a marked difference.

Methods

Formalin-fixed paraffin embedded tissue from twenty-one brain metastasis comprising colorectal adenocarcinoma metastasis (n=7), lung adenocarcinomas metastasis (n=7) and metastatic melanomas (n=7) were obtained from the Brain Tumour North West (BTNW) research tissue bank (RTB – ethics NRES14/EE/1270). Sections (10-µm-thick) were placed onto glass slides covered with aluminium foil. Foil covered slides have been previously demonstrated to be as effective as the more expensive CaF_2 slides significantly reducing the costs of this process (Cui *et. al.,* 2016). These were de-waxed prior to spectral acquisition by leaving overnight in fresh xylene. They were then washed in fresh xylene for 5 min. Following this, they were immersed in fresh ethanol at 100% twice and then 70% ethanol once, for 5 min each, and then allowed to air dry prior to

spectral acquisition. H&E-stained slides were viewed to delineate the tumour to be examined, to reduce contamination of spectra from background brain tissue.

Raman spectroscopy

A Renishaw InVia Raman spectrometer was used to collect 25 spectra per section using a 785 nm laser at 1200 g mm^{-1} grating with an acquisition time of 30 seconds for each sample. This was over a spectral range of 400-1600 cm^{-1}. A 50× objective with numerical aperture of 0.85 was used to focus the laser beam. The spectral sites were selected at random moving over the tissue.

Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectra were collected using a Bruker TENSOR 27 FTIR spectrometer with Helios ATR attachment containing a diamond crystal internal reflective element and a 45° incidence angle of IR beam. A new background spectrum was collected prior to each new sample, following cleaning of the crystal with distilled water. For each case 32 scans with 8 cm^{-1} spectral resolution were taken at 10 randomly selected points. The sampling aperture was 250 μm × 250 μm and the mirror velocity was 2.2 Hz.

Computational analyses

Computational analyses, including principal component analysis (PCA) with linear discriminant analysis (LDA) and linear discriminant classifier (LDC) was then performed within a MATLAB environment, using the IRootlab toolkit as a user interface (Trevisan *et. al.,* 2013). For classification spectra were pre-processed by cutting to the region of interest (Raman = 500-1800 cm^{-1}; IR = 900-1800 cm^{-1}), followed by polynomial baseline correction and vector normalisation. Spectra were then interrogated *via* PCA-LDA to generate scores plots and cluster vectors to determine points of variation between the spectra; PCA-LDC was then applied to calculate the classification accuracy as compared to the histopathological result. The top 6 spectral differences between the adenocarcinoma and melanoma groups were also determined.

Results

Analysis of the spectra has shown similar results for both Raman and IR spectroscopy. They demonstrate similar spectral appearances for both adenocarcinoma groups, with significant differences seen to the spectra of the melanoma (figure 4.1).

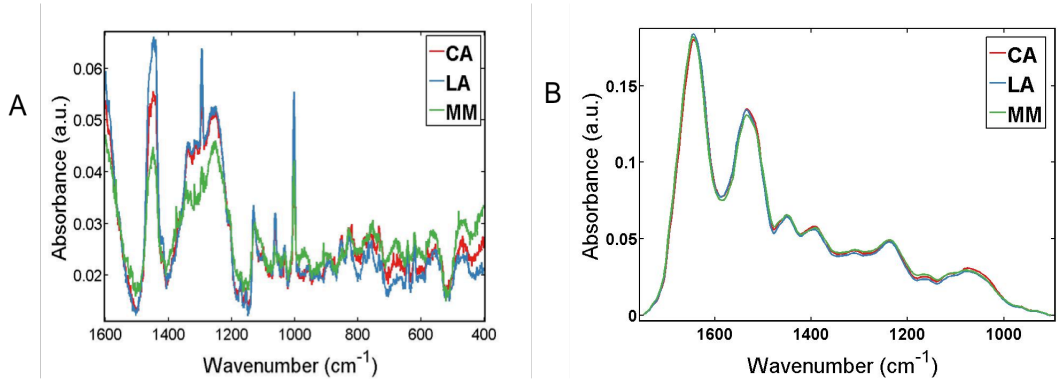

Figure 4.1 A graph demonstrating the mean pre-processed spectra from each tumour group using: (A) Raman spectroscopy (cut to the region of interest, polynomial baseline correction and vector normalisation); and, (B) IR spectroscopy (cut to the region of interest, rubberband baseline correction and vector normalisation). (KEY: CA=COLORECTAL ADENOCARCINOMA, LA=LUNG ADENOCARCINOMA, MM=MELANOMA).

PCA-LDA was carried out to determine the principal components and thus the factors that account for most variance between the three groups in order to classify them. It was demonstrated that the groups show a degree of overlap (figure 4.2), which is greatest between the two adenocarcinoma groups. The difference to the melanoma group is again highlighted. From this, cluster vectors were used to visualise the differences between the three groups. It can be seen (Figure 4.3) that the two adenocarcinoma groups are similar with small areas of variance. However, the melanoma groups show a marked difference. This is particularly demonstrated within panel (D) where melanoma is taken as the baseline. This shows how similar adenocarcinomas are despite their different primary locations.

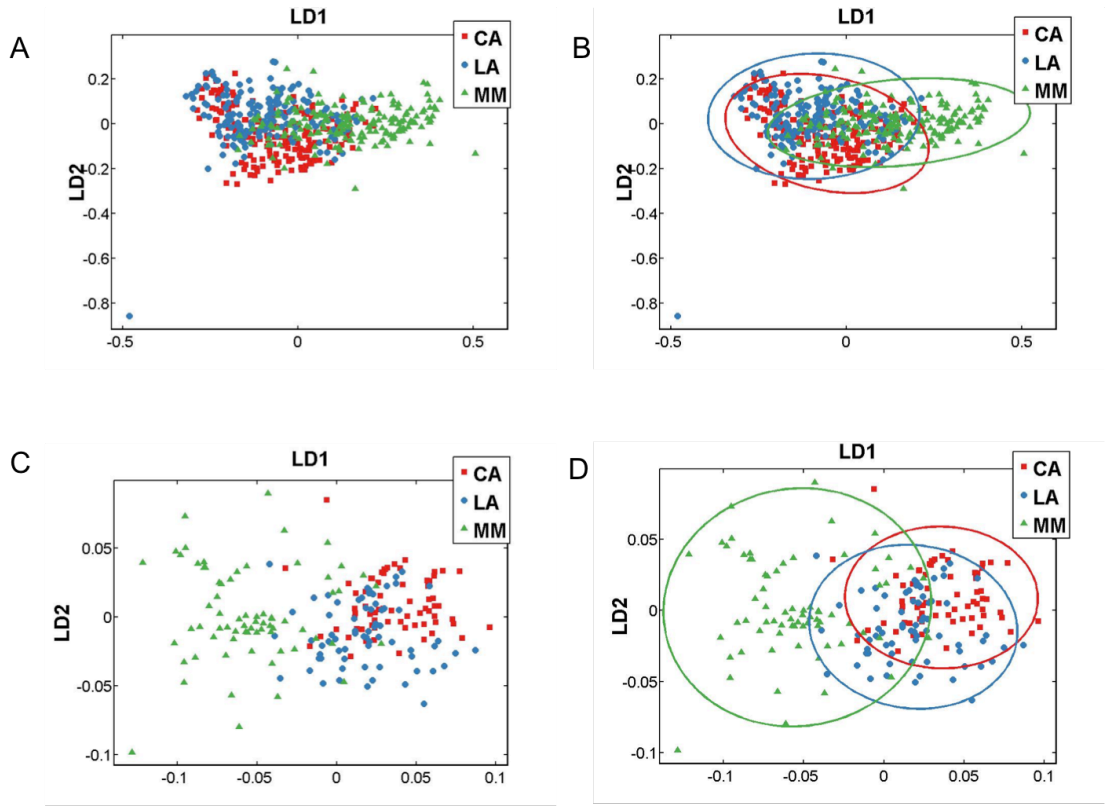

Figure 4.2 A graph demonstrating the PCA-LDA results for Raman and IR. The left side demonstrates the Raman results firstly without (A) and secondly with (B) 95% confidence intervals. This is then mirrored for IR, without (C) and with (D) 95% confidence intervals. (KEY: CA – COLORECTAL ADENOCARCINOMA, LA – LUNG ADENOCARCINOMA, MM – MALIGNANT MELANOMA)

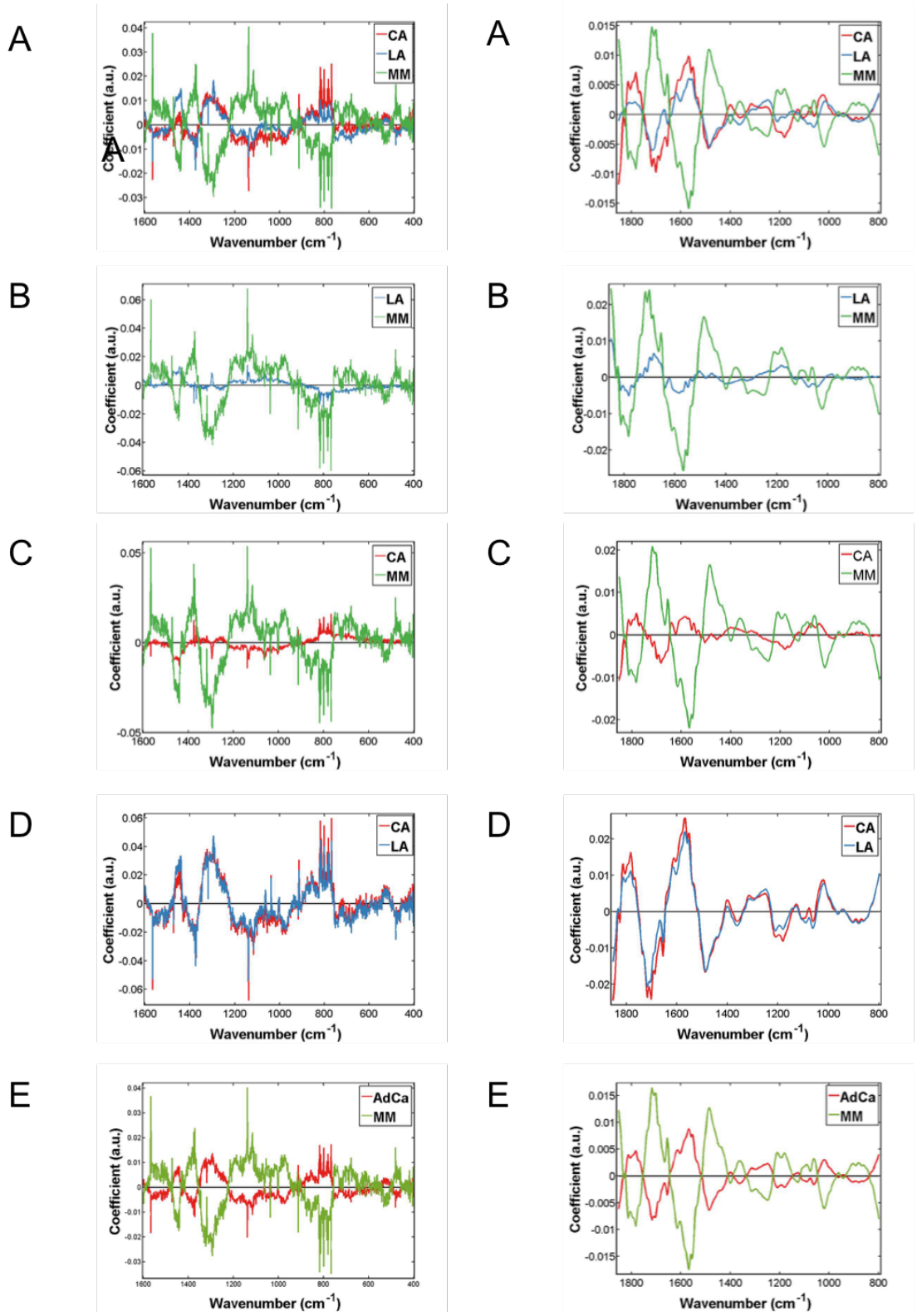

Figure 4.3 These graphs show the cluster vectors for Raman and IR. The left side displays the Raman results, starting with (A) all the groups, (B) CA is taken as the baseline, (C) LA taken as the baseline, (D) MM taken as baseline and (E) compares adenocarcinoma vs. MM. This is mirrored on the right for IR. (KEY: CA –

COLORECTAL ADENOCARCINOMA, LA – LUNG ADENOCARCINOMA, MM – MALIGNANT MELANOMA, ADCA – ADENOCARCINOMA).

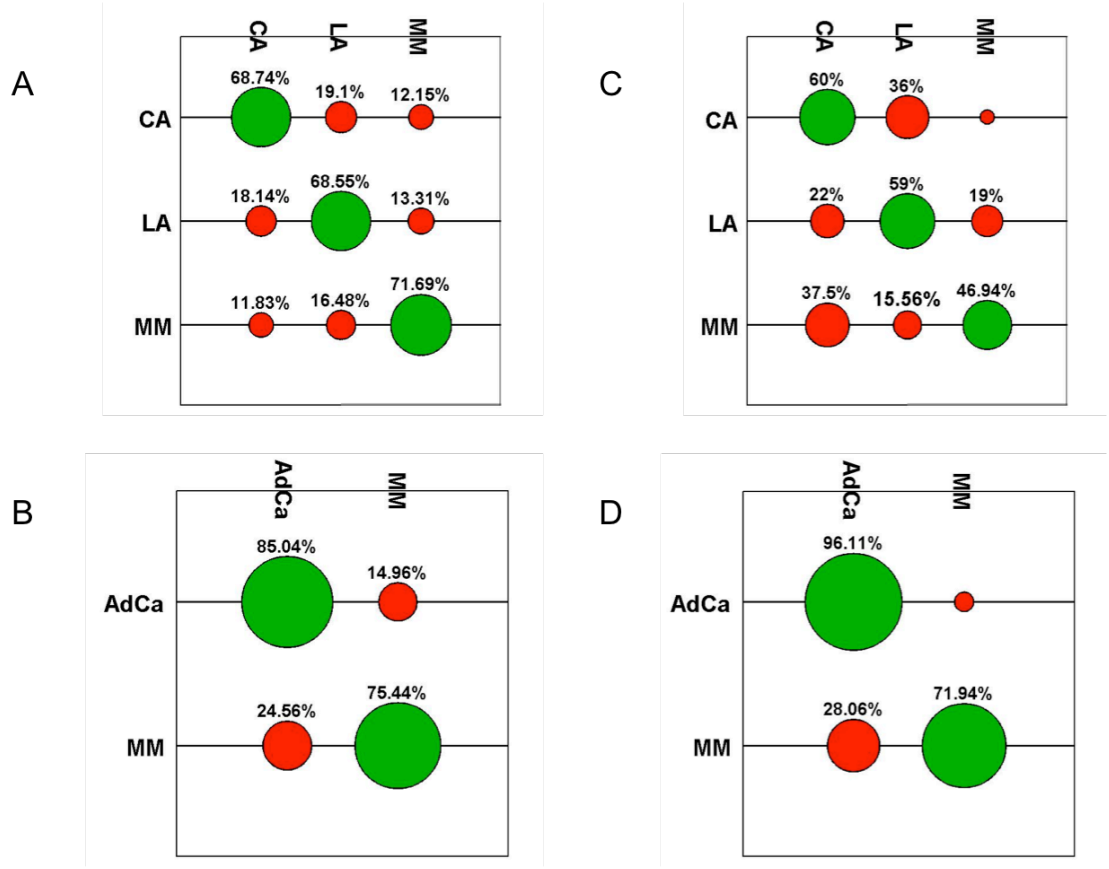

Figure 4.4 The confusion matrices display the percentage of the results assigned to the correct group (green) or another group (red). The Raman results are shown on the left with (A) displaying each of the three cancer groups separately, and (B) compares adenocarcinoma to MM. On the left are the IR results; (C) displays each of the three cancer groups separately and (D) again compares adenocarcinoma to MM. (KEY: CA – COLORECTAL ADENOCARCINOMA, LA – LUNG ADENOCARCINOMA, MM – MALIGNANT MELANOMA, ADCA – ADENOCARCINOMA).

A PCA-LDC, giving the classification accuracy for each group as compared to the final histological diagnosis, was then performed (Figure 4.4). This was run for three separate groups and then two (combining the two adenocarcinoma groups) groups to show the difficulty in separating the adenocarcinomas. When using three groups for Raman, the classification accuracy is 69% for colorectal adenocarcinoma, 69% for lung adenocarcinoma and 72% for melanoma. Using IR this is 60% for colorectal adenocarcinoma, 59% for lung adenocarcinoma and 47% for melanoma. If the two

adenocarcinoma groups are combined, classification accuracy markedly increases. With Raman this improves to 85% for adenocarcinoma and 75.4% for melanoma, and with IR 96% for adenocarcinoma and 72% for melanoma. This is, however, still below that found with traditional histopathology.

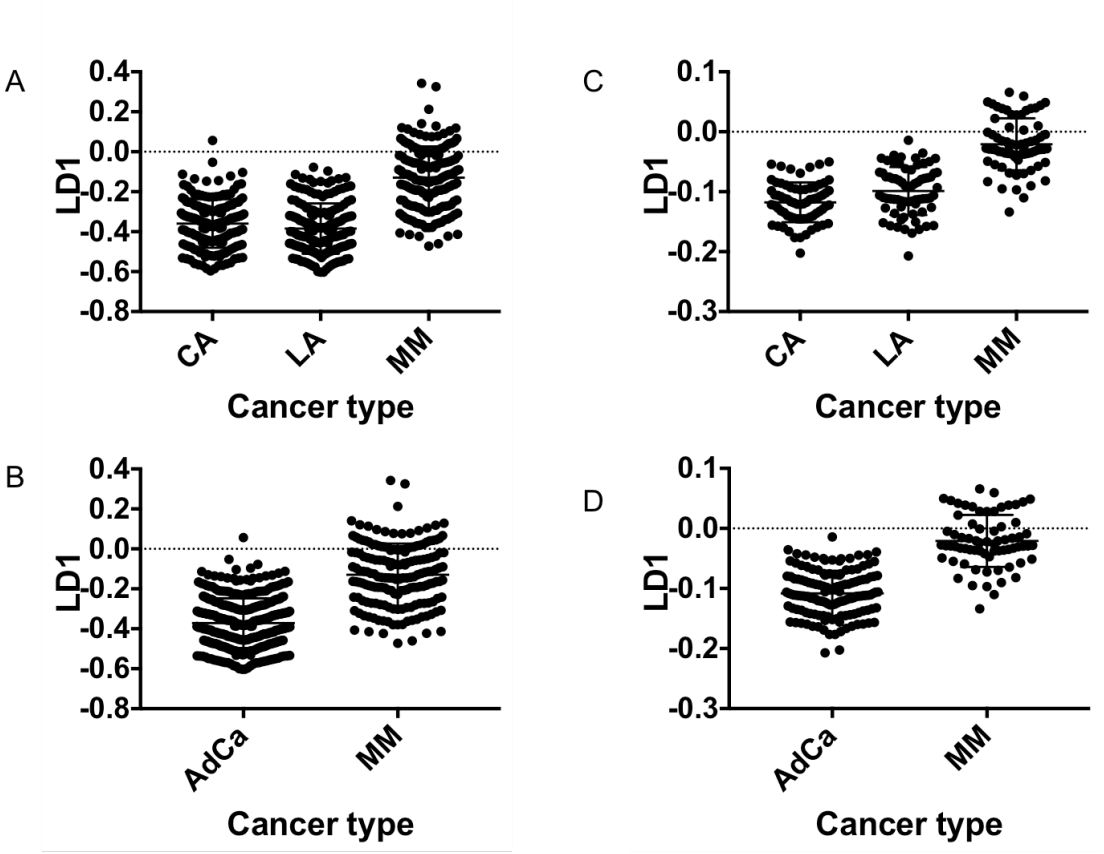

Figure 4.5 These graphs represent the results of both a one-way ANOVA and student's *t*-test scores plot for Raman and PCA-LDA. (A) shows the one way ANOVA for Raman with all three tumour groups, (B) the student's *t*-test for Raman with adenocarcinoma and MM. This is mirrored for IR with (C) showing the one-way ANOVA for IR with all three tumour groups and (D) the student's *t*-test for IR with adenocarcinoma and MM. (KEY: CA – COLORECTAL ADENOCARCINOMA, LA – LUNG ADENOCARCINOMA, MM – MALIGNANT MELANOMA).

Following this, a one-way ANOVA was performed for the three groups to assess if the differences seen between the spectra were significant. A student's *t*-test was performed on the merged 2 groups to assess significance due to the small numbers involved (Figure 4.5). This was performed on the PCA-LDA results using all spectra for each case. For the three Raman groups this was P=0.0016 at 95% confidence interval and for IR this was

not significant (*P*=0.08) (table 4.1). For two groups, this was again significant at <0.0001 for Raman and IR, with a 95% confidence interval (table 4.2).

Table 4.1 Results of one-way ANOVA for both Raman and IR to determine statistical difference between all three groups. (KEY: CA – COLORECTAL ADENOCARCINOMA, LA – LUNG ADENOCARCINOMA, MM – MALIGNANT MELANOMA)

Method	Group	Mean difference	95% CI	*P*-value
Raman	CA *vs.* LA	0.03	-0.01 - 0.06	0.1898
	CA *vs.* MM	-0.23	-0.26 - -0.20	**<0.0001**
	LA *vs.* MM	-0.25	-0.29 - -0.22	**<0.0001**
IR	CA *vs.* LA	-0.02	-0.03 - -0.00	0.0122
	CA *vs.* MM	-0.10	-0.11 - -0.08	**<0.0001**
	LA *vs.* MM	-0.08	-0.09 - -0.06	**<0.0001**

Table 4.2 :Results of a student's *t*-test for Raman and IR to determine statistical difference between adenocarcinoma and MM. (KEY: MM = METASTATIC MELANOMA, AD = ADENOCARCINOMA)

Method	Group	Difference between means	95% CI	*P*-value
Raman	MM *vs.* AD	0.24+/- 0.01	0.22 – 0.27	**<0.0001**
IR	MM *vs.* AD	0.09 +/- 0.01	0.08 – 0.10	**<0.0001**

The statistical significance between each group was also calculated using a one-way ANOVA (Table 4.1). This highlights the statistically significant differences found between adenocarcinoma and melanoma. There is no statistical difference between the two adenocarcinoma groups on either Raman or IR spectroscopy.

To conclude, the significant differences were calculated (figure 4.6) and tentative distinguishing wavenumbers assigned to those differences (Table 4.3). This was done to examine the points at which the tumours vary and to see which areas accounted for the variation.

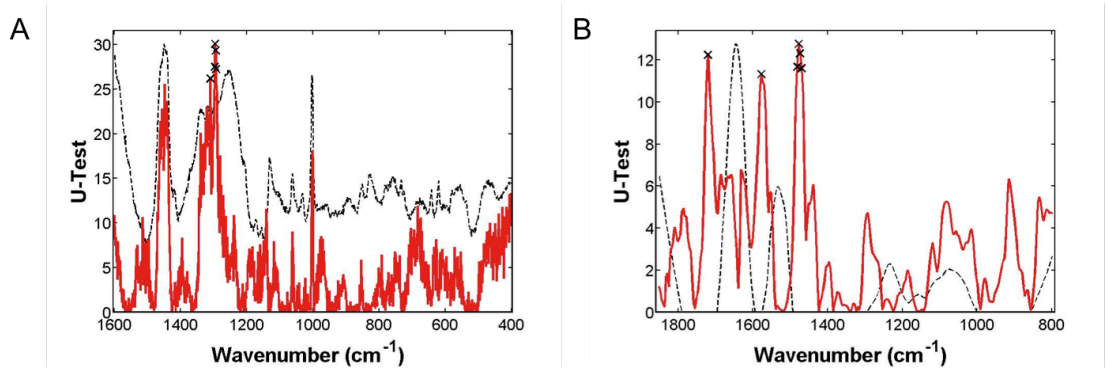

Figure 4.6 The significant wavenumber differences between the adenocarcinoma groups and melanoma. The red line indicates the U wave number curve and the black doted line the significance threshold. The X highlights the 6 most significant differences. A: Raman, B: ATR-FTIR

Table 4.3 The tentative assignments of significant points of difference for Raman and IR, using adenocarcinoma *vs.* melanoma (Movasaghi *et. al.*, 2007, Movasaghi *et. al.*, 2008).

Method	Wavenumber (cm^{-1})	Tentative assignment
Raman	1310	CH_3/CH_2 twisting or bending mode of lipid/collagen CH_3/CH_2 twisting, wagging &/or bending mode of collagens & lipids
	1297	CH_2 deformation/Palmitic acid, acyl chains, fatty acids
	1296	CH_2 deformation
	1295	Methylene twisting /CH_2 deformation
	1294	Methylene twisting
	1293	Cytosine/ Methylene twisting
IR	1720	C=O
	1578	Ring C-C stretch of phenyl
	1481	Amide II
	1477	CH_2 bending of methylene chains in lipids /Polyethylene methylene of deformation modes
	1474	CH_2 bending of methylene chains in lipids /Polyethylene methylene of deformation modes
	1470	CH_2 bending of methylene chains in lipids

Discussion

Both spectroscopic methods have been shown to be able to classify the different tumours by type (*i.e.*, adenocarcinoma *vs.* melanoma), providing similar results. However, accuracy is greatly diminished if it is used to classify the primary origin of the tumour type, specifically determining if the adenocarcinoma arose within the lung or colon. Minor differences are seen between the spectra of these two tumours (Figure 4.1); however, these differences are not statistically significant. This would, therefore, limit any clinical use, as it would not be able to provide as much information as traditional histopathology with H&E and IHC. These tumour types were chosen specifically to provide two similar tumours (*i.e.* the two adenocarcinomas, that require immunohistochemistry to differentiate) alongside one different tumour, both morphologically and immunohistochemically (*i.e.* melanoma). Both lung adenocarcinomas and melanomas frequently metastasis to the brain. It may be that such new tools may aid the clinician in determining tumour type intra-operatively, *i.e.* that the tumour is a metastasis and not a primary brain tumour, but formal histopathology with IHC would still be required for primary tissue origin identification. This, however, is also of interest given the marked spectral similarities between adenocarcinomas of different primary origins (Figure 4.2). This is useful for Clinicians, as it may help with cancer of unknown primaries (*i.e.* the primary site is unknown and traditional methods such as radiology and pathology are failing to determine the primary site) or in aiding the surgeon to distinguish a primary from metastatic tumour.

Within this study, confounding factors, such as the number or location of the brain metastasis, nor patient factors have been used to contribute to the accuracy of the results. As this was a comparison to conventional histopathology, these factors would not impact upon microscopy or immunohistochemistry, therefore it was felt not appropriate to be added into the diagnostic algorithm.

When evaluating the potential value of spectroscopy as a possible intraoperative tool its ability to determine cancer versus no cancer and suggest a tumour type would be required. To provide further information to that provided by intraoperative neuropathology, spectroscopy would need to differentiate the primary tumour origin for a metastasis. However, as can be seen, both Raman and IR spectroscopy are able to detect differences between the two tumour types, but not specify the primary tissue origin

accurately enough for treatment decisions. As the technique develops, it may replace frozen section, often performed intraoperatively to determine if a tumour is primary, *i.e.*, has arisen within the brain, or is a metastasis to guide the surgeon in relation to the extent of the resection he may perform, as has been suggested previously (Ji *et. al.,* 2013, Ji *et. al.,* 2015, Hollon *et. al.,* 2016). At which point, acknowledgement of a metastasis (from a primary tumour) would be the level required with histopathology completing the primary tumour origin determination as currently occurs. This would provide a potentially useful area for the technology to exploit as frozen section work can be challenging and potentially an area for error to be removed by use of spectroscopy. However, comparative work to normal brain tissue and primary tumours would be required to ensure the technique is able to differentiate all potential results. This study has highlighted spectroscopy would not be able to determine primary tissue origin of a metastasis in its current form.

As the technique develops, it may eventually be able to provide additional information to support the initial histopathological diagnosis, which may in the future provide treatment related or prognostic information once the spectra are fully understood in the years to come.

Chapter

5

5. *Detection of Primary and Metastatic brain tumours from biofluids*

Declaration of Work

To Whom it May Concern,

Dr Danielle Bury designed the study in conjunction with Prof F Martin. Dr Bury arranged with Mrs K Ashton for samples, collected these and performed all spectral acquisition. She then performed the analysis. Dr Bury also reviewed all histological slides.

Dr Bury then produced a paper for publication of the results, which has been published within Spectrachemica Acta Part a, please see appendix 9.3.

Signed

……. ……..

Prof F L Martin Dr D Bury

Introduction

Blood testing for cancer diagnostics is a popular ideal. It uses an acceptable patient test, *i.e.*, a blood test, which is non-invasive and machine analysed, can be run on a mass scale, and can target specific markers circulating in the blood. An example is prostate specific antigen (PSA) to allow detection of a suspected prostate cancer. For PSA this gives a quantitative figure that must be addressed in context with patient age to stratify risk. It has its flaws as a significant percentage of prostate cancers are non-secretors, meaning that there is no appreciable rise in PSA identifiable to indicate the underlying disease process. Conversely, benign conditions such as prostatitis can raise the PSA. However, tests such as PSA, or for women, CA125, are commonly used in clinical medicine, with beneficial outcomes, providing they are taken in context with the clinical and radiological picture. However, their sensitivity and specificity drops when used as a screening detection method, hence no mass-screening programme has yet been introduced using PSA or CA125. Many tumour types do not have specific blood markers. Therefore this study was designed to examine the potential of ATR-FTIR in analysing plasma samples from patients with a variety of primary and metastatic brain tumours. The metastatic tumours chosen were the same as those within the previous study for comparison. This approach aims to investigate whether ATR-FTIR spectroscopy is a useful tool to detect tumours within blood plasma when asked to differentiate on a wider-scale more akin to a typical clinical setting. If accuracy falls in low-grade and metastatic lesions against a backdrop of high-grade lesions and controls, this would limit clinical use.

Methods

Plasma from 50 patients comprising normal, *i.e.*, no known brain tumour (n=10), glioma high-grade (n=5) or low-grade (n=5), meningioma (n=10) and brain metastasis patients, a mix of lung adenocarcinoma (n=7), colorectal adenocarcinoma (n=7) and malignant melanoma (n=6) patients were obtained from the Brain Tumour North West tissue bank (BTNW). This was under ethical approval number (RTB - ethics NRES14/EE/1270). These were stored at -80°C and defrosted prior to use. From the samples, 50 µl of plasma was pipetted onto a glass slide wrapped in aluminium foil. This has previously been shown to be as effective as slides such as calcium fluoride-coated windows (Cui *et. al.,* 2016).

The slides were left to dry overnight prior to spectral acquisition. ATR-FTIR spectra were collected using a Bruker TENSOR 27 FTIR spectrometer with Helios ATR attachment containing a diamond crystal internal reflective element and a 45^{o} incidence angle of IR beam. For each case 32 scans with 8 cm^{-1} spectral resolution were taken at 10 randomly selected points. A new background spectrum was collected prior to each new sample, followed by cleaning of the crystal with distilled water. The sampling aperture was 250 μm × 250 μm and the mirror velocity was 2.2 Hz.

Computational analysis was then performed within a Matlab environment using IRootlab toolkit as a user interface (Trevisan *et. al.,* 2013). Spectra were then pre-processed by cutting to the region of interest (1850-800 cm^{-1}), followed by polynomial baseline correction and vector normalisation. Following this principal component analysis-linear discriminant analysis (PCA-LDA) to determine differences between the groups was performed, along with PCA-linear discriminant classifier (PCA-LDC) to calculate the classification accuracy of each group. Statistical significance was then determined using a one-way ANOVA within PRISM statistical analysis software.

Results

From the 50 cases 500 spectra (*i.e.*, 10 spectra per sample) were obtained. Following pre-processing a PCA-LDA was performed to identify if the groups (or categories) are significantly different based upon their spectra, along with PCA-LDC to generate confusion matrices to look at the accuracy of the spectra in detecting each tumour type. This was performed initially looking at normal *vs.* each tumour group and then combining all groups together to determine if they could be differentiated accurately from each other, as would occur in a typical clinical setting.

Figure 5.1 shows normal compared to low-grade and high-grade gliomas. It shows how well the spectra are separated based upon them being classed as normal (89%) or high-grade (98%) with some overlap between low-grade and normal dropping the low-grade classification accuracy to 84%. The misclassified high grade spectra were below the level of resolution for the analytical method.

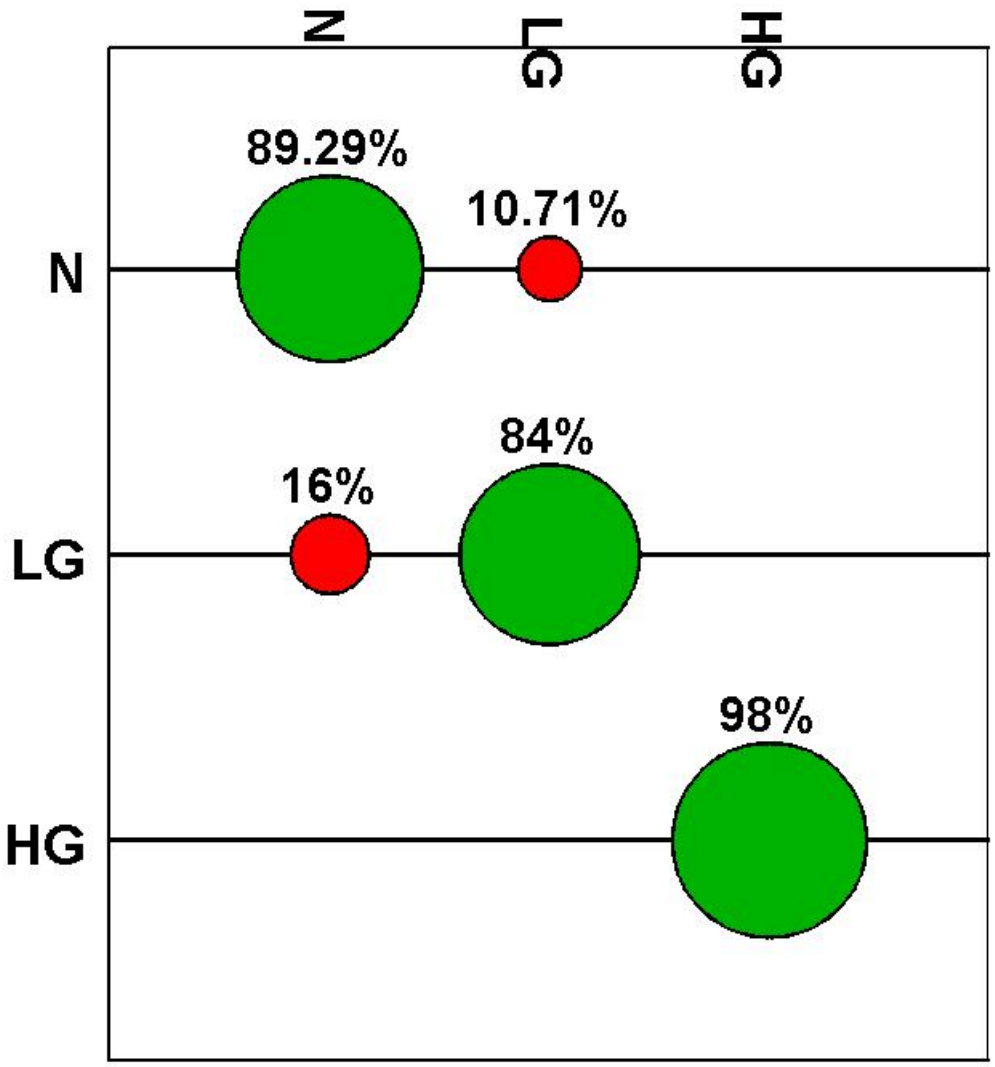

Figure 5.1 Confusion matrix comparing normal (N) to low-grade (LG) and high-grade (HG) gliomas. It shows the accuracy of classification for the three categories, with minimal overlap between normal and low-grade tumours. Green-filled circles represent accurate classification whereas red-filled circles represent inaccurate classification.

This differentiation between the three groups is statistically significant using a one-way ANOVA, as shown in Table 5.1. Similar results are seen for normal *vs.* meningioma categories (88% and 85%, respectively) and normal *vs.* metastasis categories (96% and 92%, respectively), though results were mixed for the metastasis group depending on the type of primary tumour, with lung adenocarcinoma giving the best accurate classification at 63% (Figure 5.2).

Table 5.1 A one-way ANOVA showing the differences between each of the normal, high-grade (HG) and low-grade (LG) glioma groups.

Tukey's multiple comparisons test	Mean Difference	95% CI of difference	Adjusted *P*-value
Normal *vs.* HG	0.05	0.05 to 0.06	<0.0001
Normal *vs.* LG	0.01	0.01 to 0.02	<0.0001
HG *vs.* LG	-0.04	-0.04 to -0.03	<0.0001

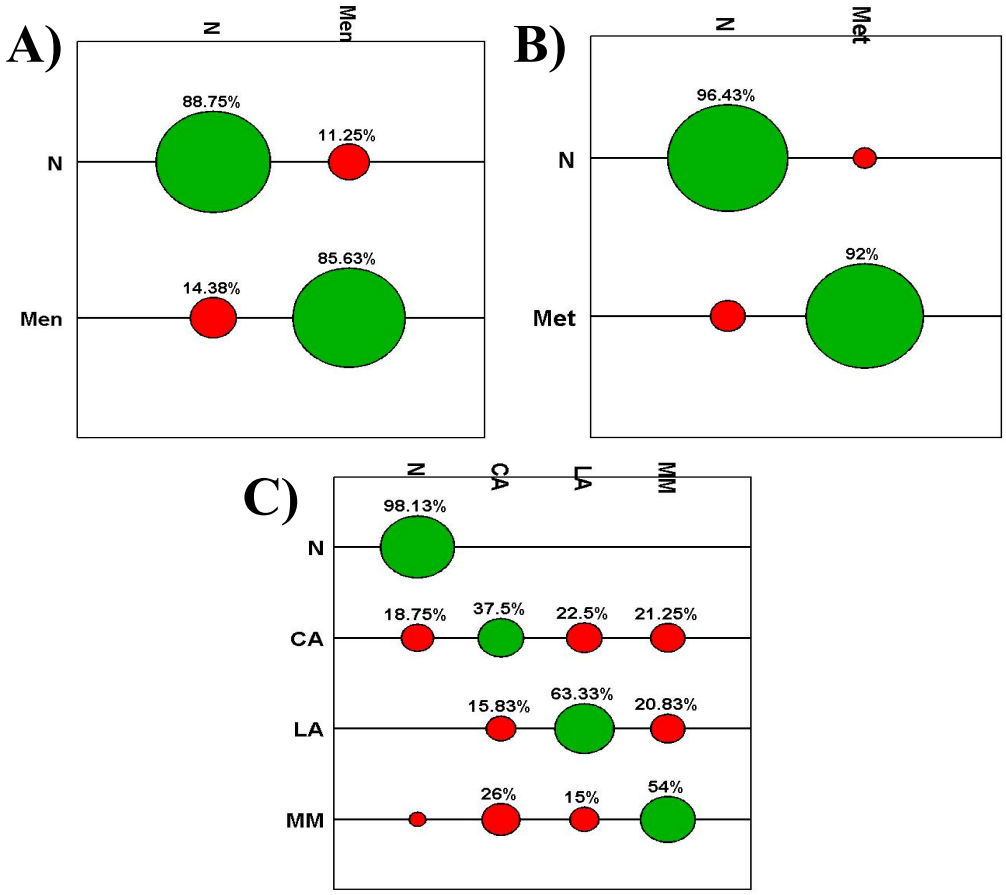

Figure 5.2 Confusion matrices for (A) control (N) *vs.* meningioma (Men); (B) control (N) *vs.* metastasis; and, (C) control (N) *vs.* the different metastatic groups, colorectal adenocarcinoma (CA), lung adenocarcinoma (LA) and melanoma (MM). Green-filled circles represent accurate classification whereas red-filled circles represent inaccurate classification.

For this spectrochemical approach to be a valid clinical test it is critically important for it to be able to differentiate all types of tumour from an initial plasma assessment. Therefore, following PCA-LDA for all 5 categories confusion matrices were generated. It shows that the accuracy of assigning categories drops significantly for low-grade glial tumours, meningiomas and metastasis (Figure 5.3).

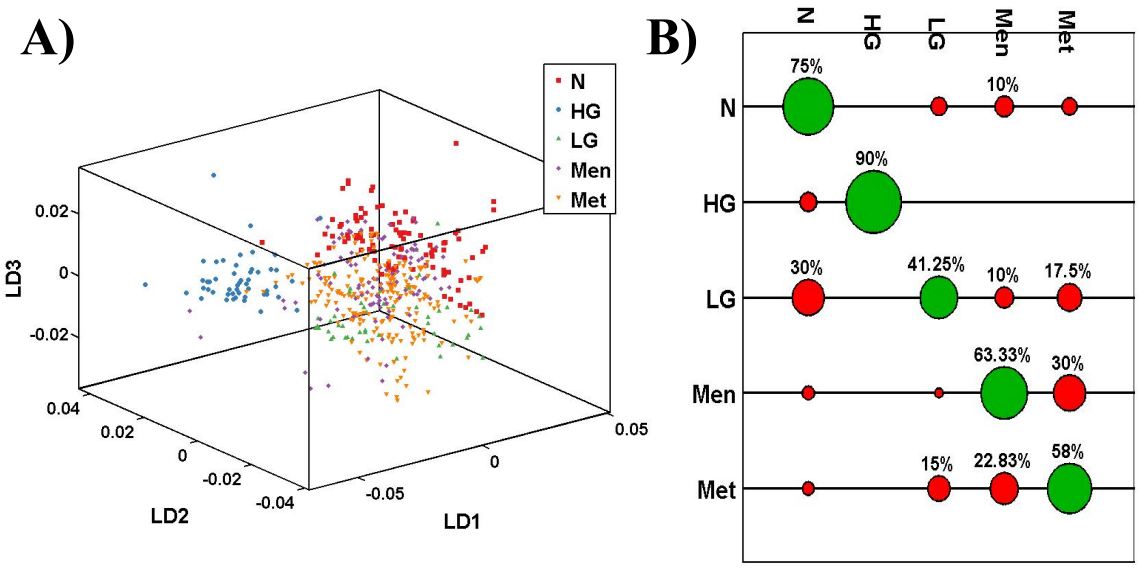

Figure 5.3 Three-D scores plot (A) and confusion matrix (B) for all separate categories, demonstrating overlap with low-grade gliomas, meningiomas and metastasis. Green-filled circles represent accurate classification whereas red-filled circles represent inaccurate classification. Key: N, control; HG, high-grade glioma; LG, low-grade glioma; Men, meningioma; and, Met, metastasis

Following this, a one-way ANOVA was performed to demonstrate the differences between the five categories (Figure 5.4, Table 5.2), which demonstrates statistical significance, p <0.001 at the 95% confidence interval.

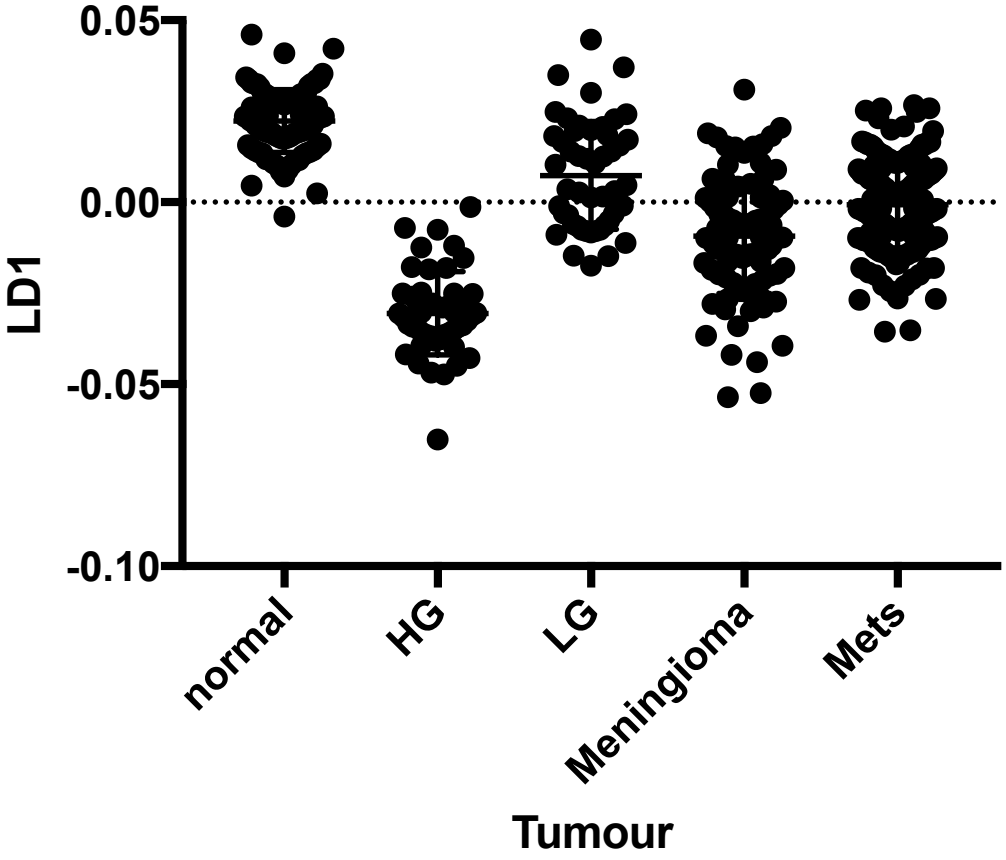

Figure 5.4 A one-way ANOVA was performed looking at the differences between the five categories. This demonstrates the first linear discriminant of each spectra (LD1). Key: N, control; HG, high-grade glioma; LG, low-grade glioma; Men, meningioma; Met, metastasis

Table 5.2 The results of the one-way ANOVA showing statistically significant comparisons between each group. LG, low-grade glioma; HG, high-grade glioma; Mets, metastasis to brain

Tukey's multiple comparisons test	Mean Difference	95% CI of difference	Adjusted *P*-value
Normal *vs.* HG	0.05	0.05 to 0.06	<0.0001
Normal *vs.* LG	0.01	0.01 to 0.02	<0.0001
Normal *vs.* Meningioma	0.03	0.03 to 0.04	<0.0001
Normal *vs.* Mets	0.02	0.02 to 0.03	<0.0001
HG *vs.* LG	-0.04	-0.04 to -0.03	<0.0001
HG *vs.* Meningioma	-0.02	-0.03 to -0.02	<0.0001
HG *vs.* Mets	-0.03	-0.04 to -0.02	<0.0001
LG *vs.* Meningioma	0.02	0.01 to 0.023	<0.0001
LG *vs.* Mets	0.01	0.00 to 0.01	0.0005
Meningioma *vs.* Mets	-0.01	-0.01 to -0.00	<0.0001

However, the classification accuracy drops further if the metastasis category is split by primary tumour location, with only the detection of high-grade glioma maintaining >90% (figure 5.5). Whilst statistical significance is maintained from normal to tumour category, it is lost between certain tumour categories such as the two adenocarcinoma groups. It fails also, however, to reach significance for melanoma *vs.* lung adenocarcinoma, which is surprising (Table 5.3).

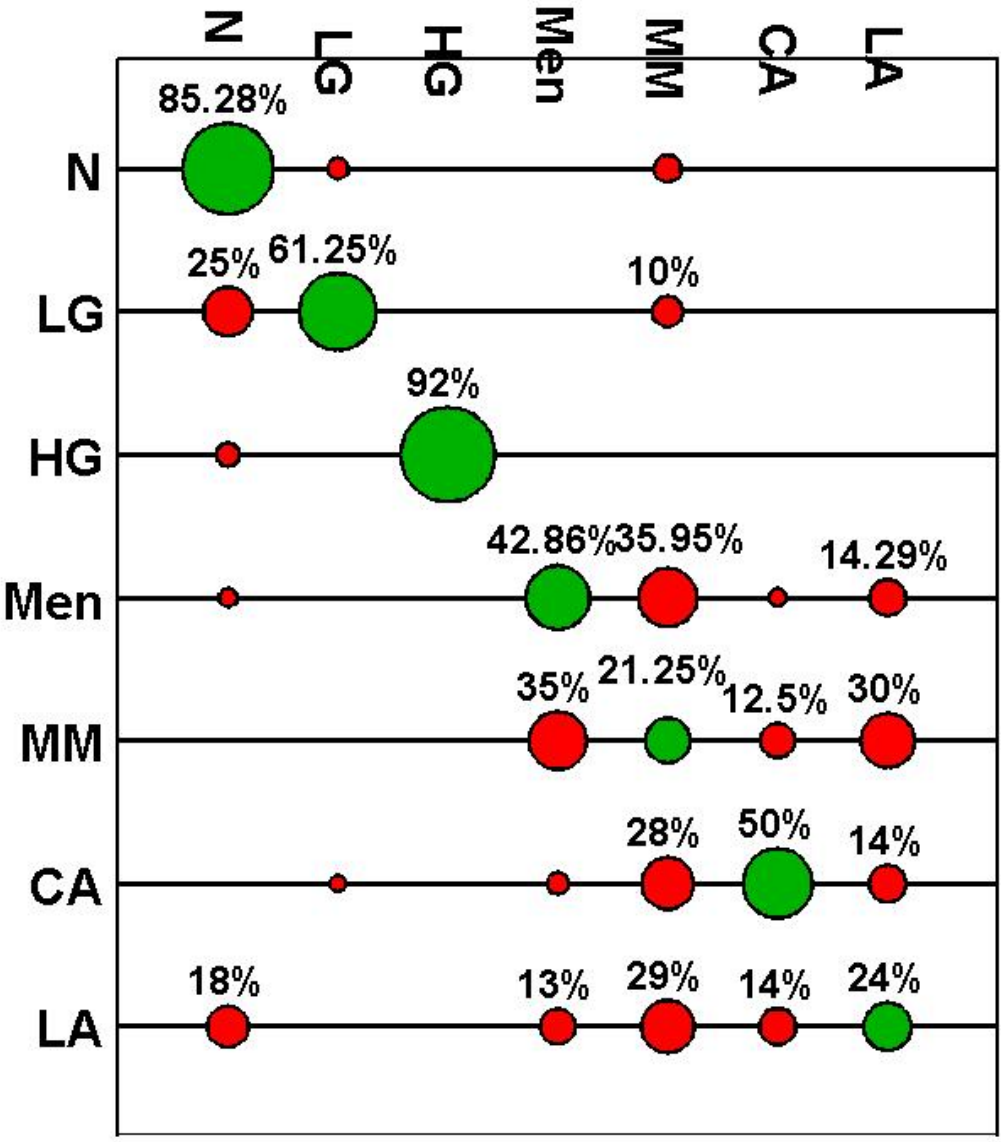

Figure 5.5 A confusion matrix showing the detection rates of all tumours compared to control cases with metastasis split into primary tumour site. Green-filled circles represent accurate classification whereas red-filled circles represent inaccurate classification. Key: N, normal; LG, low-grade; HG, high-grade; Men, meningioma; MM, melanoma metastasis; CA, colorectal adenocarcinoma metastasis; LA, lung adenocarcinoma metastasis

Table 5.3 Results of a one-way ANOVA comparing the tumours to look for statistically significant differences. Highlighted in red are those categories failing to reach statistical significance. Key: N, control; LG, low-grade; HG, high-grade; Men, meningioma; MM, melanoma metastasis; CA, colorectal adenocarcinoma metastasis; LA, lung adenocarcinoma metastasis

Tukey's multiple comparisons test	Mean Difference	95% CI of difference	Adjusted *P*-value
Normal *vs.* LG	0.02	0.01 to 0.02	<0.0001
Normal *vs.* HG	0.05	0.04 to 0.06	<0.0001
Normal *vs.* Men	0.03	0.03 to 0.04	<0.0001
Normal *vs.* MM	0.03	0.02 to 0.04	<0.0001
Normal *vs.* CA	0.02	0.02 to 0.03	<0.0001
Normal *vs.* LA	0.02	0.02 to 0.03	<0.0001
LG *vs.* HG	0.03	0.03 to 0.04	<0.0001
LG *vs.* Men	0.02	0.01 to 0.02	<0.0001
LG *vs.* MM	0.01	0.01 to 0.02	<0.0001
LG *vs.* CA	0.01	-0.00 to 0.01	0.2092
LG *vs.* LA	0.01	0.00 to 0.01	0.0190
HG *vs.* Men	-0.02	-0.02 to -0.01	<0.0001
HG *vs.* MM	-0.02	-0.03 to -0.01	<0.0001
HG *vs.* CA	-0.03	-0.04 to -0.02	<0.0001
HG *vs.* LA	-0.03	-0.03 to -0.02	<0.0001
Men *vs.* MM	-0.00	-0.01 to 0.00	0.4924
Men *vs.* CA	-0.01	-0.02 to -0.01	<0.0001
Men *vs.* LA	-0.01	-0.02 to -0.01	<0.0001

MM *vs.* CA	-0.01	-0.02 to -0.00	0.0002
MM *vs.* LA	-0.01	-0.01 to -0.00	0.0077
CA *vs.* LA	0.00	-0.00 to 0.01	0.9569

Discussion

In a typical clinical setting, patients present with a multitude of morbidities, real or perceived. The clinician's challenge is to diagnose especially life-threatening conditions such as cancer as soon as possible. Given the complexity of the clinical picture, this can be time-consuming, inaccurate and expensive. Consequently, there has been great effort invested in attempts to develop biomarker-led blood tests for disease. In the last number of years, there have been a lot of pilot studies using spectrochemical techniques such as ATR-FTIR or Raman spectroscopy to distinguish control and cancer-sourced samples. Whilst the results of these studies are promising, many have not been designed with the complexity of a typical clinical setting in mind.

Our results demonstrate that ATR-FTIR spectroscopy is able to detect patients with intrinsic or metastatic brain tumours using plasma samples from peripheral blood. However, this is most effective when used to detect high-grade glial tumours, or when asking a specific question, *i.e.*, high-grade *vs.* low-grade glial lesions or control *vs.* meningioma. Once more tumour types are introduced into a classification algorithm, accuracy drops along with the statistical significance of differences between the groups. Taking this back into the clinical setting suggested above, with the incidence of brain tumours within the general population being 10/100,000 population/year, therefore for every positive result there will be many negatives in any given clinic (MacDonald *et. al.,* 2000). Strokes and epilepsy along with headache disorders make up the majority of clinic attendees. In order for this test to be validated it would be crucial to test a wide range of disorders to ensure this would not affect the results. It would also not account for patients with other underlying cancer(s) and how this could be differentiated.

One possible confounding factor that may need to be considered in future work is that it is not known if there is tumour involvement of the dura, the exact location of the tumours or number of metastasis in the metastatic group. It is also not known how widespread metastases are within the body. Therefore the lack of such information may influence classification accuracy.

This study has shown that ATR-FTIR spectroscopy could play a role in plasma testing for both intrinsic and extrinsic brain tumours; however, this role is of limited value. Patients with high-grade intrinsic tumours and metastatic lesions are more likely to be detected *via* the conventional route of GP referral to secondary care or emergency admission at which point tumours would be identified. However, one use may be in accident and emergency when patients present with concerning symptoms; here, it might be used as a screening test for a high-grade glial lesion., as two thirds of patients with a high grade glioma attend A&E. The use of ATR-FTIR spectroscopy is unlikely to speed up the diagnostic process nor eliminate any of the current steps within the patient pathway; therefore, its usefulness as a biofluid screening tool may remain limited. Given its apparent weakness for low-grade lesions, for which it would be most beneficial, its clinical impact for pre-surgical diagnostics is limited.

This study demonstrates that ATR-FTIR spectroscopy is able to differentiate brain tumour types from blood plasma with a high degree of accuracy. However, this is most effective when a direct clinical question is asked. When confounded by increasing differential diagnoses, the classification accuracy of the system falls markedly for low-grade lesions and metastasis. Therefore, this likely makes the use of ATR-FTIR spectroscopy within a clinical setting of limited value. Further work is required to determine if there is a more appropriate point to harness the use of ATR-FTIR spectroscopy within the clincal pathway.

Chapter

6

6. *Fresh brain tumours tested using a hand held Raman probe, can it differentiate primary from metastatic tumours?*

Declaration of Work

To Whom it May Concern,

Dr Danielle Bury designed the study in conjunction with Prof F Martin. Dr Bury produced a standard operating procedure (SOP) document and risk assessment in conjunction with Mrs K Ashton. Dr Bury then taught Mrs Ashton how to use the Raman probe. Dr Bury analysed results from the probe with assistance from Mr C L M Morais.

Dr Bury then produced a paper for publication of the results.

Signed

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Prof F L Martin Dr D Bury

Introduction

Brain tumours account for 3% of all tumours diagnosed annually (CRUK D, accessed 3/11/17). Whilst they comprise a small proportion, the difficulty of complete removal of the tumour is inherent. High-grade tumours can be infiltrative and when operating within the brain the risk of removing crucial structures in a bid to free the patient of the tumour, yet risk leaving them with significant neural deficit is ever present. Up to 75% of tumour resections are thought to leave behind viable tumour, though there is a survival benefit to improved/complete resection (Hollon *et. al.,* 2016, Broadbent *et. al.,* 2016). Therefore, any new technique available to highlight residual tumour, thus improving outcome and resection, yet reducing the non-tumour tissue removed would be beneficial. Currently, the use of 5-aminolevulinic acid (5-ALA) does allow for fluorescence of tumour cells in order to aid resection; however, this is imperfect. It can be difficult to tell apart tumour from background fluorescence (Galli *et. al.,* 2017).

Therefore this novel study was designed to determine the potential of Raman spectroscopy using fresh brain tissue taken at the time of surgery and sent for an intraoperative smear diagnosis to determine the primary tumour type. The results were compared to both the intraoperative smear diagnosis and final fixed paraffin result.

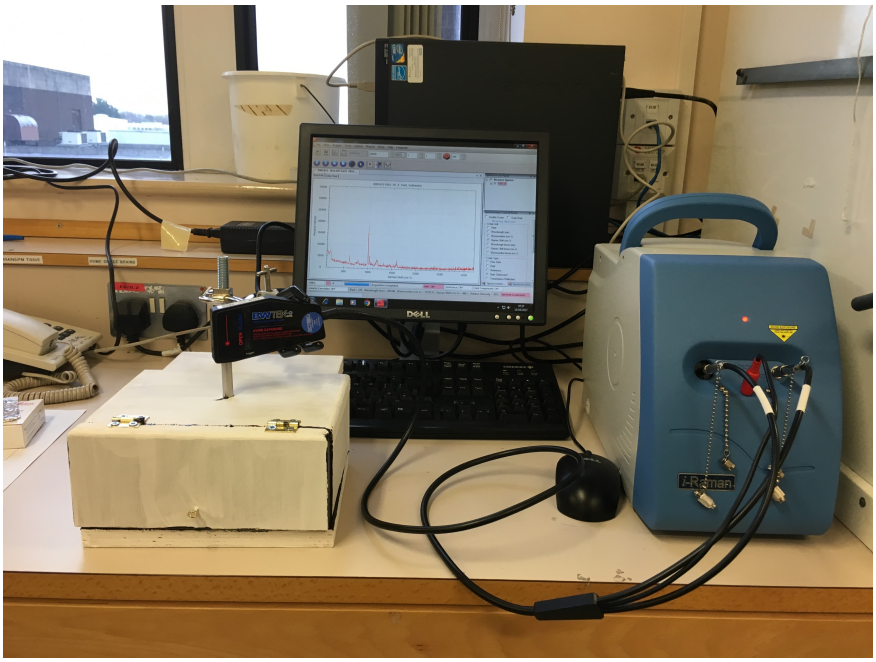
This was done, as the need to test fresh tissue is crucial, to overcome any spectral changes seen due to formalin fixation or freezing artefact (O'Faolain *et. al.,* 2005, Huang *et. al.,* 2003). The use of gold nanoparticles in conjunction with Raman spectroscopy has previously been shown to improve the Raman signal received, reducing signal to noise ratio and thus enhance the spectral quality (Bulter *et. al.,* 2015). Therefore due to the small sample size, the use of nanoparticles was performed to maximise spectra.

Methods

Prior to using the handheld Raman machine, a custom-built box was required to ensure darkness when analysing the tissues. As this was being placed into a working laboratory, it would not be possible to work in darkness and it would also need to fit into a category 2 fume hood for work with fresh tissue. With this is mind, a box was custom engineered using plywood. A stage was built within this box to allow the slide to be moved in the x and y planes with a custom cut out area for the slide to be held securely. This was to allow the tissue to be accurately positioned under the probe. A clamp was then secured to

the box to allow the probe to be moved in the z plane to allow it to be positioned at the correct height above the tissue. Thus allowing movement similar to a conventional light microscope. The box was painted with black paint on the inside to minimise reflection of any light entering it. It also enabled it to be wiped clean if required (Figure 6.1 and appendix 9.11.

A



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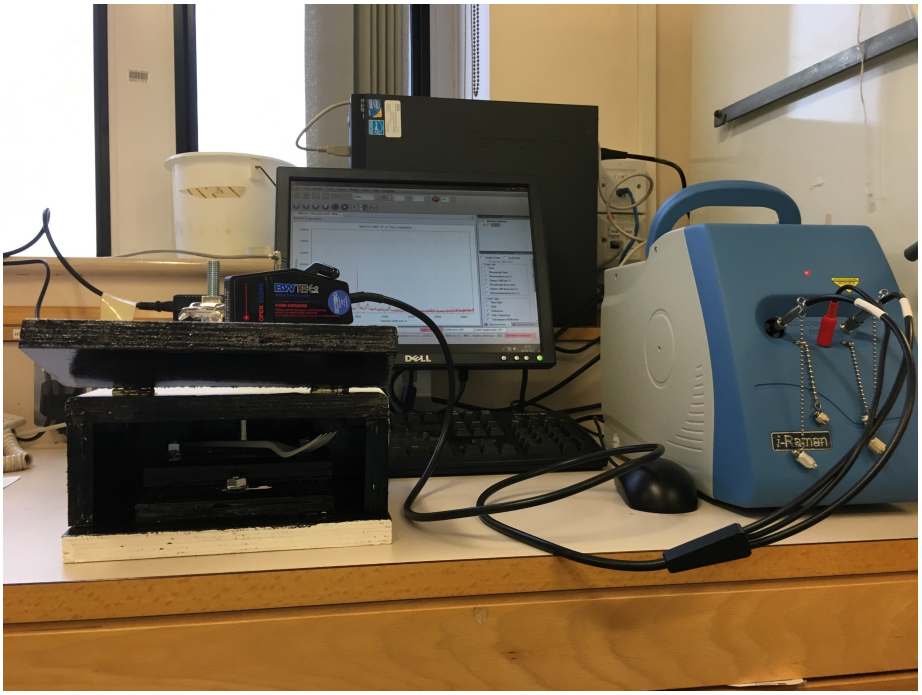


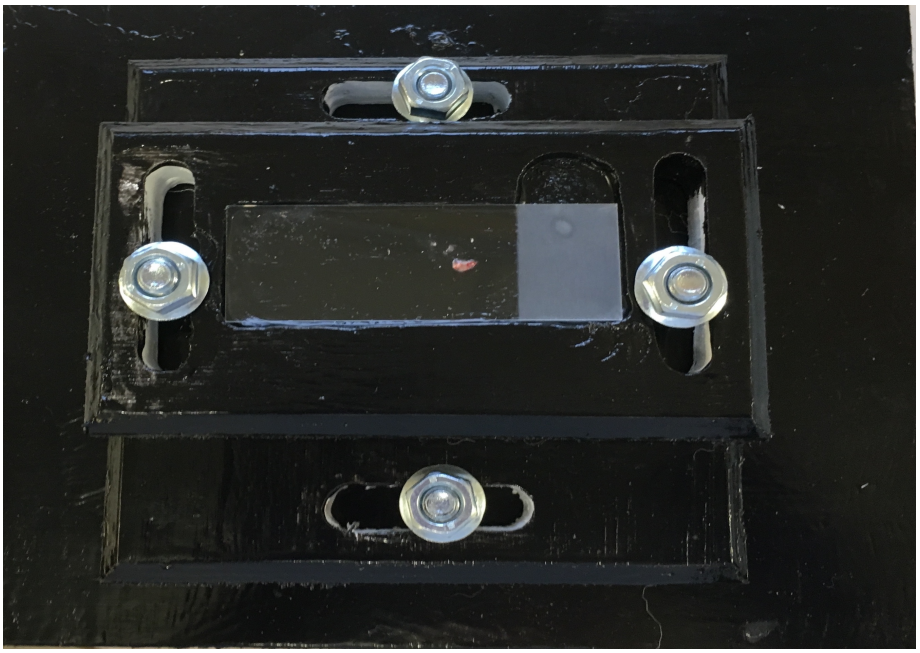
C 

Figure 6.1: The hand held Raman probe with purpose built box in situ in the Neuropathology department at Royal Preston Hospital. (A) and (B) show the full set up, with (C) highlighting the set up inside the box with example slide.

Fresh brain tissue samples sent to the laboratory for intraoperative smear preparations were tested over a 6-month period (table 6.1). Ethics approval was obtained from the BTNW brain bank (NRES14/EE/1270). We obtained *n*=29 samples, which were analysed using an i-Raman portable Raman system with BAC100/BAC102 lab-grade Raman probe from B&W Tek from Pacer International, with software version 4.1.

Table 6.1 Results of both intraoperative smear preparations and final formalin-fixed paraffin-embedded tissue for each case tested.

Case Number	Smear Result	Paraffin Result
1	Low-grade glioma	Glioblastoma
2	Meningioma	Meningioma
3	Metastasis	Ovarian serous carcinoma
4	High-grade glioma	Glioblastoma
5	High-grade glioma	Glioblastoma
6	Meningioma	Meningioma
7	Metastasis	Adenocarcinoma
8	High-grade glioma	Glioblastoma
9	High-grade glioma	Glioblastoma
10	Metastasis	Renal cell carcinoma
11	Metastasis	Lung adenocarcinoma
12	?no tumour	Glioblastoma
13	Low-grade glioma	Astrocytoma Grade 2
14	Inflammation	Astrocytoma Grade 2
15	Inflammation	Astrocytoma Grade 2
16	Metastasis	Ovarian serous carcinoma
17	High-grade glioma	Glioblastoma
18	High-grade glioma	Glioblastoma
19	High-grade glioma	Glioblastoma
20	High-grade glioma	Glioblastoma
21	High-grade glioma	Glioblastoma
22	?reactive ?Low-grade glioma	Low grade glioma
23	Intermediate-grade glioma	Glioblastoma
24	Low-grade glioma	Astrocytoma Grade 3
25	Lymphoma	High grade B cell lymphoma
26	Glioma	Astrocytoma Grade 2
27	No definite tumour	Astrocytoma Grade 2
28	Low- to intermediate-grade glioma	Astrocytoma Grade 2
29	High-grade glioma	Glioblastoma

Prior to sample analysis, a small amount of tissue (similar in size to that used for a smear preparation) (Ellison *et. al.,* 2013) was placed onto a glass slide covered with aluminium foil (Cui *et. al.,* 2016) and 100 µL of 5 µg/mL BioPure^{TM} 20 nm gold nanoparticles diluted in PBS was placed onto the sample and left for a few minutes to absorb prior to collecting 10 spectra per sample. Gold nanoparticles were used to enhance spectral quality. Each spectra had an acquisition time of 30 seconds at a laser power of 75% (see appendix 9.11 for set up analysis).

Data analysis was then conducted using MATLAB R2014b software (MathWorks Inc., USA) with an IRootlab toolkit (Trevisan *et. al.,* 2013). IRootlab was chosen as it provides an interface with MATLAB to ensure consistent analysis. The raw spectral data was initially pre-processed by cutting the region of interest, 1800-400 cm^{-1}, followed by polynomial baseline correction and vector normalisation. Thereafter, 10-fold cross-validated principal component analysis-linear discriminant classifier (PCA-LDC) was applied for classification of the datasets. PCA-LDC uses principal component analysis (PCA) as feature extraction method, where the original data is decomposed into a few number of principal components (PCs) representing the majority of the information in the original dataset. The scores on each PC are then used as input variables for linear discriminant analysis (LDA). LDA works by maximizing the between-class variance over the within-class variance in order to create a linear decision boundary between the classes that provides the optimum class segregation (Santos *et. al.,* 2017). Patient factors, including the location of the tumour and biopsy were not considered within this study as these factors would be unlikely to directly impact the histopathological analysis and thus may unnecessarily complicate the results algorithm.

Results

Over the 29 samples, 290 spectra were collected and analysed. From this, PCA-LDC was employed and receiver operating characteristic (ROC) curves generated. This was done to determine the classification accuracies of the Raman spectra as compared to both the intraoperative smear result and final FFPE histological diagnosis, followed by ROC curves to determine the accuracy of the classification model as well as its sensitivity and specificity were generated. Low-grade gliomas were considered WHO grades 1 and 2, and high-grade gliomas WHO grades 3 and 4. Meningiomas were classed as WHO grade 1. Metastatic tumours were grouped due to the range of different primary site s within the tumours tested, and as intraoperatively ‘metastasis’ is sufficient for intraoperative surgical planning. Example spectra are seen in figure 6.2.

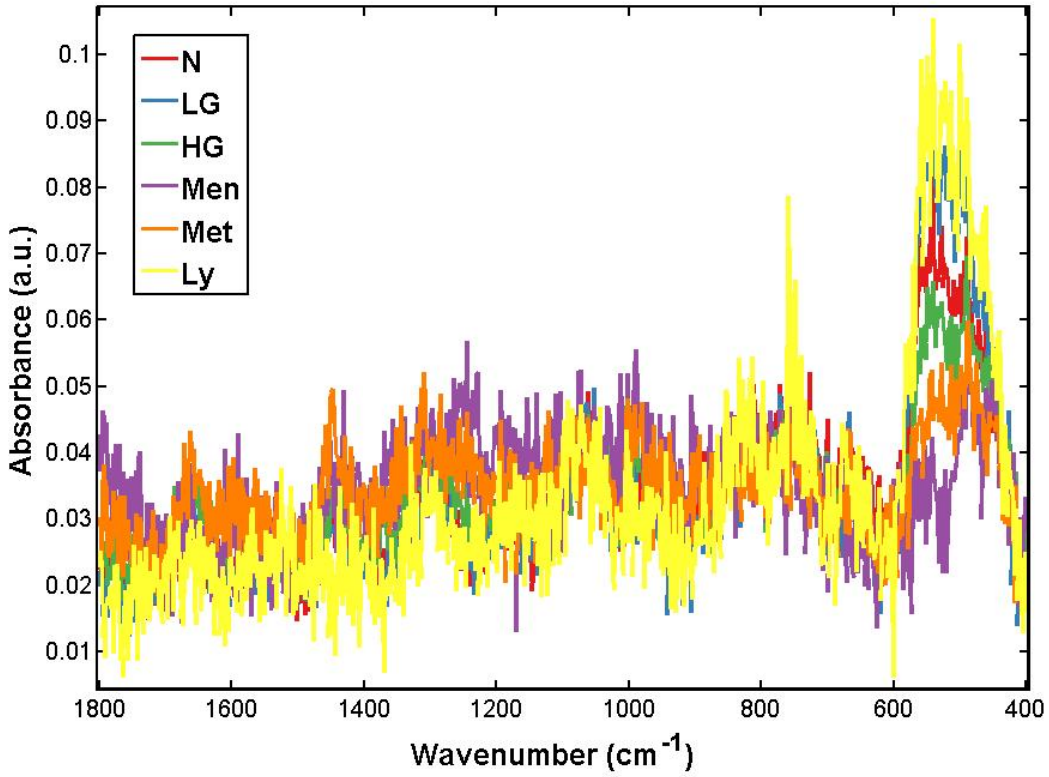


Figure 6.2: Example Raman Spectra, as compared to the smear results. Key: N; Normal brain tissue, LG; Low-grade Glioma, HG; High-grade Glioma, Men; Meningioma, Met: Metastasis, Ly; Lymphoma.

Raman results compared to intraoperative smear preparation

From Figure 6.3 it can be seen that the accuracy for detection of primary brain tumours was between 64% and 92%. The algorithm provided the lowest accuracy for meningioma (64%) with differentiation of glial tumours proving more robust (92.2 and 89.7%). The ROC parameters and curves (Figure 6.4, Table 6.2) demonstrate the sensitivities and specificities range from 64%-94% and 91%-100%, respectively, again with meningioma falling behind the other tumours for sensitivity. As the area under the curve is >0.8 for all tumour classifications it confirms the high accuracy of the classification model and presence of statistical significance (P <0.001). This is an important result if this model is to provide clinically useful information. With the exception of meningioma the positive and negative predictive values are consistently high (Table 6.3), with all negative predictive values over 95%.

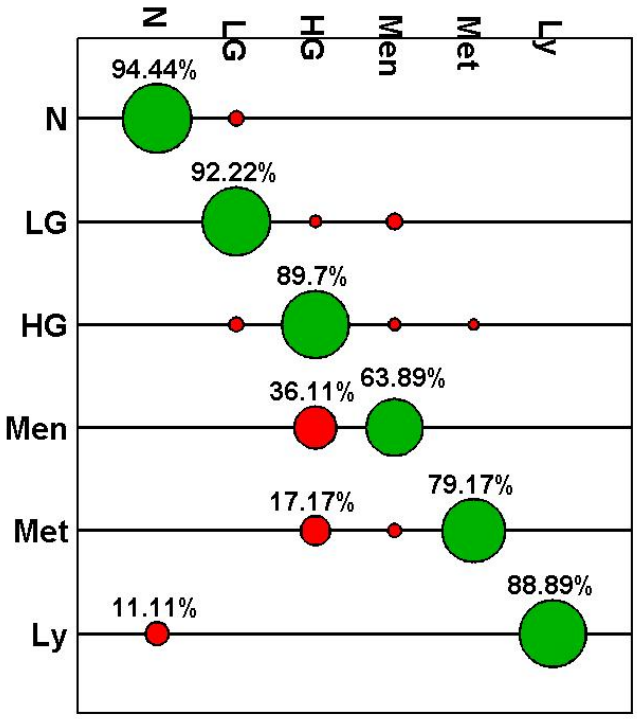


Figure 6.3. Graphical confusion matrix for PCA-LDC model using smear-based results. Key: N; Normal brain tissue, LG; Low-grade Glioma, HG; High-grade Glioma, Men; Meningioma, Met: Metastasis, Ly; Lymphoma.

Table 6.2. Figures of merit for PCA-LDC model using smear-based samples. Key: N; Normal brain tissue, LG; Low-grade Glioma, HG; High-grade Glioma, Men; Meningioma, Met: Metastasis, Ly; Lymphoma, PPV; positive predictive value, NPV; negative predictive value.

Class	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
N	98.6	94.4	99.5	97.7	98.8
LG	96.1	92.2	97.0	88.7	98.0
HG	90.3	89.7	90.6	83.5	94.4
Men	94.8	63.9	97.1	62.1	97.3
Met	95.4	79.2	98.8	93.3	95.8
Lv	99.6	88.9	100	100	99.6

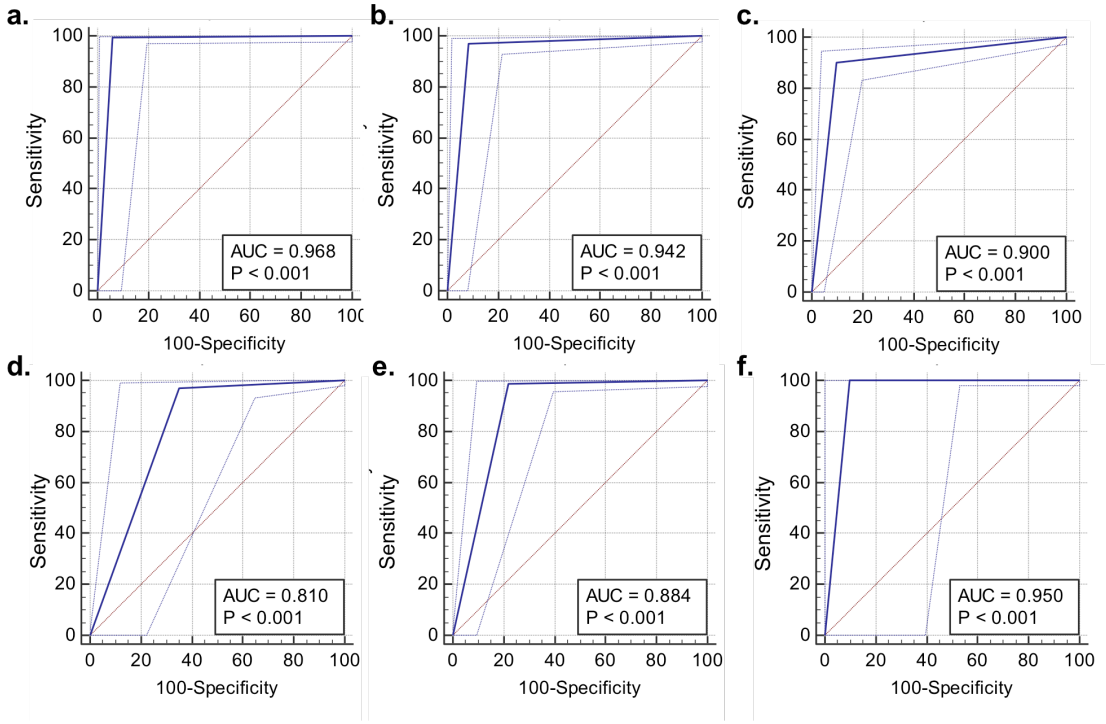


Figure 6.4; Receiver operating characteristic curves for smear-based samples: (a) Normal brain tissue; (b) Low Grade Glioma; (c) High Grade Glioma; (d) Meningioma; (e) Metastasis; and, (f) Lymphoma. (AUC: area under the curve).

Raman results compared to FFPE tissue results

When comparing the Raman results to the final FFPE diagnosis, the classification model also works with a high degree of accuracy. With the exception of metastatic tumours, the accuracy dips slightly for all cases as compared to the smear results (Figure 6.5, Table 6.3). This may be due to a variety of reasons, including normal brain tissue within the biopsy material or areas of necrosis. Given this is not possible to determine macroscopically by eye, this remains a limitation of the study. The reduction in classification accuracy is to be expected as the neuropathologist has many diagnostic tools to aid the final FFPE diagnosis such as tumour morphology, architecture and immunohistochemical testing. The ROC graphs though do continue to show the reliability and statistical significance of the classification model (Figure 6.6), highlighting the ability of Raman spectroscopy to differentiate the tumour types within this study.

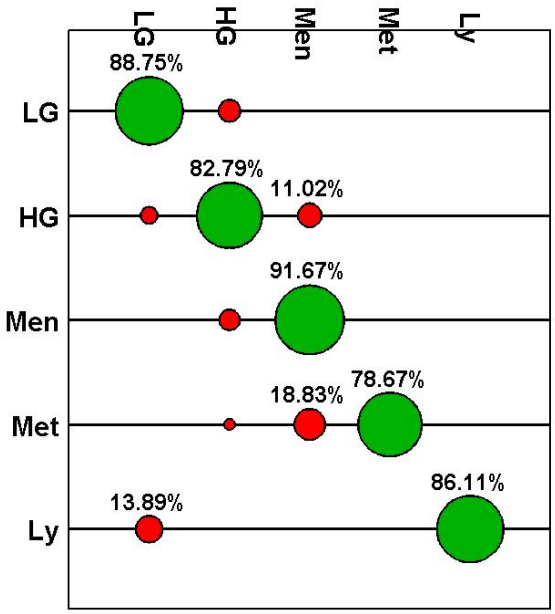


Figure 6.5. Graphical confusion matrix for PCA-LDC model using formalin fixed paraffin-embedded tissue results. Key: LG; Low-grade Glioma, HG; High-grade Glioma, Men; Meningioma, Met: Metastasis, Ly; Lymphoma.

Table 6.3. Figures of merit for PCA-LDC model using paraffin-embedded tissue results. Key: LG; Low-grade Glioma, HG; High-grade Glioma, Men; Meningioma, Met: Metastasis, Ly; Lymphoma, PPV; positive predictive value, NPV; negative predictive value.

Class	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LG	93.8	88.7	95.4	85.8	96.4
HG	88.0	82.8	92.8	91.6	85.1
Men	90.8	91.7	90.8	42.4	99.3
Met	96.3	78.7	100	100	95.7
Lv	99.5	86.1	100	100	99.5

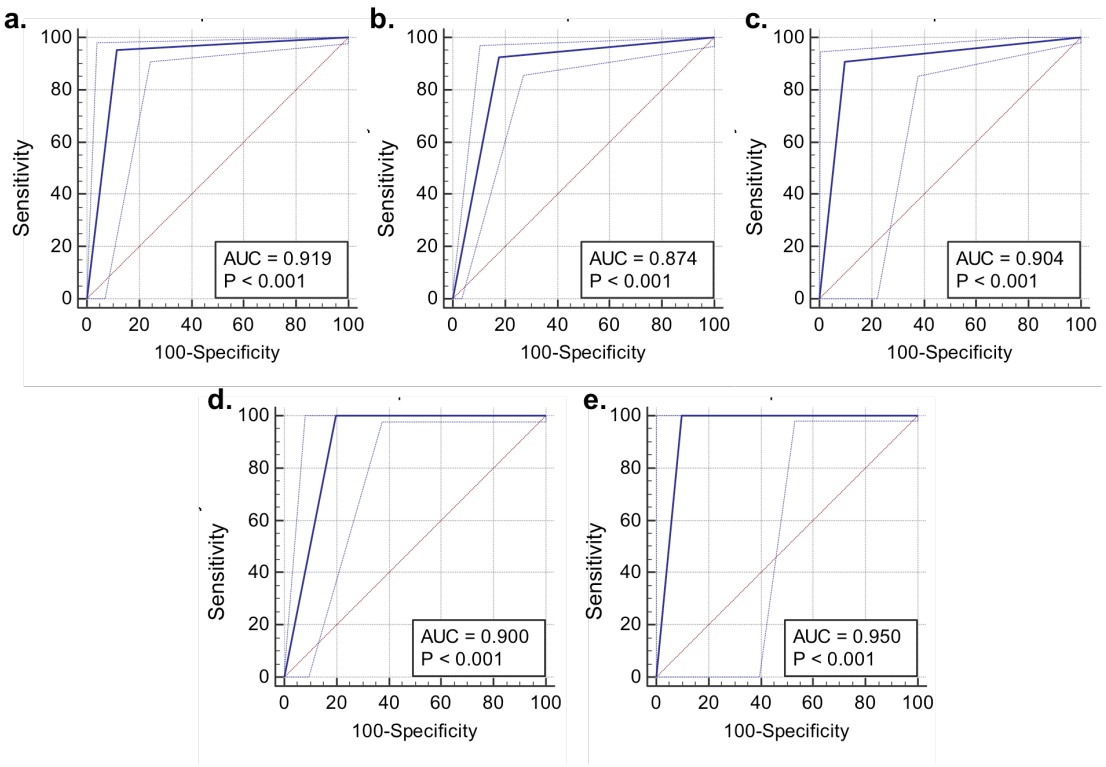


Figure 6.6. Receiver operating characteristic curves for formalin-fixed paraffin-embedded tissue results: (a) Low-grade Glioma; (b) High-grade Glioma; (c) Meningioma; (d) Metastasis; (e) Lymphoma. (AUC: area under the curve).

Discussion

Many Raman spectroscopic studies have been performed in recent years with the aim of introducing a clinically useful diagnostic tool that is easy to use and reagent-free. Much work has been performed towards standardisation of methodology and analysis, as this has previously led to criticism as many different techniques have been used (Butler *et. al.,* 2016). Previous work within the field has shown good discrimination between normal and cancerous tissue. For example, within brain tumours, prostate and ovarian cancer we have previously found potential using Raman spectroscopy to differentiate normal from tumour within both tissue and biofluids (Owens *et. al.,* 2014, Gajjar *et. al.,* 2012, Patel *et. al.,* 2011). The aim of this study was to determine if a handheld Raman probe could provide comparable results to both an intraoperative smear preparation and the final FFPE histological diagnosis. Comparable results would allow for further exploration of a Raman based probe for intraoperative use, particularly within the field of

neuro-oncology. The use of fresh tissue, within a neuropathology laboratory, testing samples sent for smear preparations demonstrates a novel approach within this field, moving spectroscopic assessment closer to the patient.

These results demonstrate the ability of a handheld Raman device, when combined with gold nanoparticles, to differentiate tumour types from fresh brain tissue. The results are comparable to both the intraoperative smear preparations and final FFPE diagnosis, with accuracy at detecting a variety of primary brain tumours and metastases ranging from 63.9-94.4% as compared to the intraoperative smear preparation, and 78.7-91.7% when compared to the FFPE diagnosis. With the exception of meningioma the sensitivities and specificities are above 75% throughout, with the majority over 90%. The PPV and NPV results are also consistently high. These results are also comparable to a recent study demonstrating the possible use of Raman to detect tumours prior to biopsy (Desroches *et. al.,* 2018). For a test to be clinically useful, especially intraoperatively, a high accuracy, PPV and NPV is needed. These results compare well to a study performed on intraoperative smears and the final results compared to the FFPE diagnosis, which yielded an accuracy of 95.25% with PPV of 95.3% and NPV of 95.1% (Sanjeev *et. al.,* 2016). This is an important step as it allows the results to be comparative to current techniques, possibly demonstrating an improvement. By adequately training the Raman probe these results demonstrate a possible improvement on the current method of intraoperative smear diagnosis, reducing the human element involved and decreasing time to reach a diagnosis. As the accuracy of the Raman probe is slightly reduced when results are compared to the FFPE diagnosis for the majority of tumours (see figures 6.2 and 6.4), the role for conventional neuropathology remains, with this tool focussed towards intraoperative diagnosis.

These positive findings indicate the possible benefits to having a handheld Raman device present within the neurosurgical theatre. As all tissue was preserved following spectral acquisition and fixed to aid final diagnosis, we have also shown that spectral acquisition and addition of nanoparticles have not harmed the tissue, nor prevented final histological diagnosis. This is an important step when bringing this technology into the clinical field. Patient factors were not considered within this study as they were felt unlikely to directly influence the histopathological assessment. As the technique is developed, it may prove useful to add patient characteristics into an algorithm to improve accuracy, particularly within the paediatric field as some tumours are inherent to certain age groups.

The strength of these results would suggest a handheld device within theatres, once properly trained, would be able to assist surgeons in removing tumour tissue without the need for an intraoperative smear preparation. This could reduce surgical time as no result is awaited and allow for improved surgical resection as small foci of tumour could be identified. Further work would be required to determine the minimum tumour volume needed for a positive result. This was not attempted within this study and would be an important step moving forward. Mapped margin biopsies would be required with histopathological analysis. As the classification model is able to determine tumour type this also would allow for further management steps to be completed, such as the addition of Gliadel wafers in the case of high-grade gliomas. The use of intracranial chemotherapy, such as Gliadel, is recommended by the National Institute for Clincial Excellence (NICE) under certain conditions, one of which is the diagnosis intraoperatively of a high-grade glioma by a neuropathologist (National Institute for Clinical Excellence, 2007). Raman spectroscopy could therefore be used to circumvent the need to involve the neuropathologist, streamlining processes within theatre. The identification of a metastatic tumour is also important, we have not used the results to determine primary tumour origin for metastatic tumours, as this has previously been shown to be challenging, particularly for cases such as adenocarcinomas from different primary sites (Krafft *et. al.,* 2006). Intraoperatively, the determination of a metastasis *versus* a primary brain tumour is the level required and offered from an intraoperative smear preparation. Therefore allowing conventional histopathology and immunohistochemistry to determine the primary site of origin is the most logical step.

Determination of surgical margins within breast cancer has been demonstrating using Raman spectroscopy (Haka *et. al.,* 2006). If developed, our classification model may also allow for other surgical sites to determine presence of absence of tumour intraoperatively, again removing the need for intraoperative frozen sections to be performed and improve resection clearance.

Overall, this study presents a novel approach to intraoperative brain tumour diagnosis and is one of the first studies to report results on intraoperative fresh brain tumour samples. The next step is to move this technology into theatre and continue to develop the classification model to allow for real time feedback to the surgeon and allow Raman technology to reach its full potential.

Chapter

7

7. Discussion

Brain tumours may account for a relatively small proportion of new cancer diagnoses per year (3%); however, their effect can be more devastating than most (CRUK M, accessed 18/2/18). The presenting symptoms may be vague, leading to multiple visits to a doctor prior to diagnosis (MHP Health, 2013) and the risks of surgery are great. The need to resect the tumour *versus* protecting functioning brain tissue is great. Therefore, any new medical tool that could potentially aid either diagnostics or intraoperative assessment would be clinically useful.

Through interaction with patients and clinicians the need for new diagnostic tools that diagnose cancer faster and more accurately is highlighted. Yet patients wish for their diagnosis to remain in secondary care, a crucial factor when considering any new diagnostic tool and where it can be targeted (see Figure 1.11). They also demonstrate a willingness to accept more invasive investigations than thought by the clinicians; an interesting point to consider when developing a diagnostic tool. From the work done with patients and clinicians, a series of proof of concept studies were developed to test a new innovative diagnostic method, in this case vibrational spectroscopy, to see if it could aid and improve the current NICE cancer care pathway (NICE, 2005).

As the aim of the project was to address the use of vibrational spectroscopy in the diagnosis of cancer and how to develop it for clinical use the studies were designed to target various points in the current patient pathway using spectroscopy and determine its viability as well as comparing the results to the gold standard of histopathology. Brain tumours can be either primary, *i.e.*, arise within the brain, or metastatic, *i.e.*, have spread to the brain from a primary point elsewhere in the body, *e.g.*, the lung. It was therefore important that the studies incorporated a combination of primary and metastatic tumours, as these would both be encountered within clinical practise, as metastases outnumber primary brain tumours 3:1 (Davis *et. al.,* 2012). A combination of FTIR and Raman spectroscopy was used for the studies either alone, or in combination to compare the diagnostic accuracy. For each study the accuracy was determined *via* comparison to the final histopathological diagnosis. This was for two main reasons, firstly, it provided a constant end point with the possibility to review histopathology slides and understand the distribution of the tumour and secondly it provided a constant known result. Other factors such as tumour size, distribution and location as well as location of biopsy sites were unknown, with review of the radiology beyond the scope of this project, as the project was focussed on diagnostics and the ability of spectroscopy to mimic

histopathology. Common primary brain tumours were also chosen as they provided sufficient tissue and make up the bulk of primary brain tumours.

The first two studies focussed on tissue based diagnosis towards an aim of either intraoperative diagnosis or aiding the pathologist during diagnosis as an alternative to immunohistochemistry. The first study was designed to target primary tumours, using a combination of both Raman and ATR-FTIR spectroscopy to see which is best able to classify common brain tumours using fresh frozen tissue. This would be able to provide insight into the ability of spectroscopy to diagnose brain tumours and help set up the later study using fresh tissue. It found both forms of spectroscopy were able to differentiate non-tumour brain tissue from gliomas and meningiomas. For normal *versus* tumour, Raman spectroscopy was able to correctly classify 94% of the cases, with a sensitivity of 98.8% and specificity of 41.7%, compared to FTIR spectroscopy which classified with an accuracy of 97.2% with sensitivity and specificity of 100% and 66.7% respectively. When asked to determine tumour by type (*i.e.*, glioma or meningioma) for Raman the overall classification accuracy fell to 63.1% and FTIR spectroscopy accuracy fell to 79.2%. The results demonstrated that both forms of spectroscopy were able to tell tumour from non-tumour tissue but within this study, struggled to differentiate tumour types, with accuracies lower than would be required clinically. It highlights that whilst spectroscopy has potential, further work surrounding classification of tumour types would be needed.

Following on, metastatic tumours were investigated again using a combination of Raman and ATR-FTIR spectroscopy, but this time on FFPE tissue. Metastases from common tumours were selected to mimic clinical medicine. Lung adenocarcinomas are one of the most common tumours to metastasise to the brain, (Huang *et. al.,* 2013), these were compared with colorectal adenocarcinomas in order to provide two metastatic tumours with similar phenotype, with the acceptance that colorectal adenocarcinomas are less likely to metastasise to the brain (approximately 9%) (Davis *et. al.,* 2012, Huang *et. al.,*2013, Renfrow and Lesser, 2013*,* Sanghvi *et. al.,* 2017). Melanoma metastases were then used to provide a markedly different metastatic tumour, both in tumour lineage, morphological appearance and immunohistochemical profile. This was done in order to determine if spectroscopy was able to determine the primary origin of a metastatic tumour, not just its presence. The two similar tumours were used as it was felt spectroscopy would struggle with this, as conventional histopathology requires immunohistochemistry in order to differentiate the primary origin. If successful this study would have shown the benefit of spectroscopy when compared to conventional histopathology and provided additional support to diagnosis. However, whilst both

Raman and ATR-FTIR spectroscopy were able to determine the presence of tumour tissue, the differences seen from the adenocarcinomas to the melanoma was much greater than the differences between the two adenocarcinoma groups. Given the similarities between the adenocarcinomas this was to be expected, however the accuracy fell from 85% and 96% for Raman and ATR-FTIR spectroscopy respectively when identifying an adenocarcinoma to 68.7% and 60% for colorectal adenocarcinomas, and 68.6% and 59% for lung adenocarcinomas. The identification melanoma was constant around 70% for both techniques. Both methods gave results much lower than that offered by conventional histopathology with immunohistochemistry and therefore were unlikely to be clinically useful or remove the need for histopathological diagnosis.

The third study looked at moving diagnosis back to the initial phases of patient work-up or screening potential, using biofluids, namely plasma as a contrast to the tissue based work. There have been suggestions of vibrational spectroscopy based screening test to detect cancer (Hughes *et. al.,* 2016) with promising results shown by various studies, predominantly using serum. Both Gajjar *et. al.* (2013) and Owens *et. al.* (2014) demonstrated the ability to detect endometrial and ovarian carcinoma with high success 81.67% and 71.47% respectively (Gajjar *et. al.,* 2013, Owens *et. al.,* 2014*)*. Hands *et. al.* (2014, 2016) also demonstrated good results with brain tumours, primarily primary tumours (Hands *et. al.*, 2014, 2016*)*. This study compared normal (*i.e.*, no known tumour) with primary brain tumours (high- and low-grade gliomas and meningiomas) and metastatic tumours (lung and colorectal adenocarcinomas and melanomas) using ATR-FTIR spectroscopy. This study demonstrated that when asked a specific question ATR-FTIR spectroscopy had a high degree of accuracy; for example normal *vs.* high-grade glioma *vs.* low-grade glioma; 89.3% to 84% to 98% respectively. However, when the question was expanded, as would be seen in a clinical setting for screening or initial diagnostics, to incorporate a more broad question, such as 'does this patient have a tumour, what is it and where is it from?' the accuracy of detection of the type of tumour, primary, secondary (with and without primary location), fell markedly in most areas (normal; 85.28%, low-grade glioma; 61.25%, high-grade glioma; 92%, meningioma; 42.86%, melanoma metastasis; 21.25%, colorectal adenocarcinoma metastasis; 50% and lung adenocarcinoma metastasis; 24%. This raises questions about the use of such a tool as a screening method, or within a clinic as it would need to be asked a more direct question. The location of the tumours within the brain is not known, nor for the metastatic cases is the number or location of metastasis (except for the location of one within the brain). This could be seen as a weakness to the study, as perhaps the tumours that are being detected are those that involve the dura or are in a particular location

within the brain. Moving forward with future work it would be interesting to develop a method whereby the radiological results could be added to spectroscopic output to determine if this provided a more accurate result. However, if taken back to the studies initial purpose; to determine clinical usefulness either in clinic or as a screening tool, the location of a tumour would not be known and clinically spectroscopy would need to detect any tumour, not just those in favourable locations. Conventional radiology would still be required to plan surgery and hence spectroscopic detection would not circumvent this. In cases were the radiology is known with a suggestion as to the tumour type based on radiological appearances, perhaps spectroscopy may play a role as a more direct question could be asked. However, this would need to form part of a larger study in order to combine radiology, spectroscopy and the final histology.

The final study was designed to compare a hand held Raman spectrometer to histopathological intraoperative smear diagnosis, being the first known study comparing the two results using fresh tissue. If successful, this study could demonstrate the ability of spectroscopy as a possible intraoperative aid for the surgeon. Recent studies have shown it can be used with high accuracy for targeting brain biopsy locations from tumour, though these results were developing using spectroscopy for tissue with high tumour burden (Desroches *et. al.,* 2018). This study did not put any restrictions on the tissue, it was simply that sent for a smear preparation as would be analysed by the neuropathologist. Elsewhere there are very few spectrometers within the clinical world with one being trialled in London (Optics.org, accessed 22/2/18). Some studies have been performed close to the operative theatre; therefore this was an exciting and novel experiment to perform (Horsnell *et.al.*, 2010, 2012 Haka *et. al.*, 2009, 2006). A handheld Raman spectrometer was therefore placed within the Neuropathology department at Royal Preston Hospital. There were several reasons for basing this within the pathology laboratory; firstly, it allowed the fresh tissue being sent for intraoperative smear preparations to be tested. This meant working on tissue freshly removed from the body with no preservatives, nor having been previously frozen or fixed in any way. Secondly, as Raman has to be performed with minimal light disruption, it allowed darkness to be achieved using a purpose built box thereby not impacting in any way upon surgery (see Figure 6.1).

As the spectroscopy was performed alongside the smear preparation there was also no delay in the intraoperative diagnosis thereby extending surgical time. By keeping the test within pathology, it allowed the Neuropathologists to see no damage was being done to the valuable tissue samples prior to fixation and enabled the development of a standard operating procedure (SOP) and safety protocol see Appendix 9.10) for spectroscopy

within the lab as well as a bank of spectra from cases consented for research. Running the study over several months allowed for the collection of spectra from both primary and metastatic tumours. The use of nanoparticles was in order to amplify the Raman signal given the use of a small probe and small amount of fresh tissue. The results were impressive. The ability to detect the different tumour types when compared to both the smear and paraffin results with accuracies ranging from 63-94% and sensitivities and specificities from 63.9-100%. It showed good resolution for low- and high-grade glial lesions and metastasis. It appeared to struggle most with the meningiomas, though accuracy greatly improved as compared to the paraffin report (63 to 91%). Though this could also be relatively to the small number of cases ($n=2$) or presence of necrosis within one sample. With the exception of meningiomas the accuracy based upon the smear result (Figure 6.3) would allow the use of a hand held intraoperative device in order to determine if tissue was neoplastic or not intraoperatively, its cell lineage and high grade nature. It may also be able to guide the surgeon as to the type of tumour present based upon these results, allowing for further management steps to be taken as would follow an intraoperative smear result. As the metastatic tumours came from a variety of origins they have not been subdivided for the analysis. However, intraoperatively, there is unlikely to be a pressing need to differentiate the primary tumour origin on the table, this would be able to wait for a final histological diagnosis. Given the previous results and the difficulty to separate adenocarcinomas of different primary origins it would probably be an unreasonable expectation to include this within an intraoperative tool and best left within the realms of the neuropathologist. Confirming a tumour as glial, meningothelial or metastatic is a more viable option. With the ability to differentiate high- and low-grade glial tumours in order to aid further management (such as Gliadel, or future therapy) is however a step forward (NICE, 2007). It would reduce the surgical waiting time for an intraoperative smear result and decrease pressure on ever-busier laboratories and pathologists time by removing the need. It would also enable a surgeon to have a result at any time of the day/night or weekend not only when the laboratory is open. When tested, it may also allow for detection of intraoperative surgical resection margins allowing for improved resection rates, particularly within challenging areas of the brain, thus aiming to improve long-term survival.

7.1 Moving Forward

These studies have highlighted both the strengths and weakness of vibrational spectroscopy as a clinically useful and viable tool. They have explored the patient and clinician views surrounding cancer and cancer diagnosis with interesting results that have enabled further thoughts surrounding what vibrational spectroscopy as a tool may be capable of providing to clinical medicine.

Firstly as a point of care testing device, its limitations are greatly felt to out-way any potential benefits. The need for specific clinical queries builds in a significant challenge that does not circumvent the need for any of the current steps of the clinical pathway therefore providing little benefit for the outlay. Given this would be trained based upon final pathological diagnosis, it also is unlikely to improve the current issues surrounding interobserver error. Challenging cases are always brought back to the histopathology with a clinical discussion surrounding treatment. Whilst spectroscopy may add to this, that is likely to be a long term goal not a short impact upon clinical management.

Ongoing clinical observation of patients with known tumours at risk of recurrence may benefit from spectroscopic monitoring. However, this would require much work with patients post operatively with recurrent testing and scanning to the point of recurrence to see if spectroscopy is able to detect those at risk of recurrent disease prior to it being visible on a conventional scan, *e.g.*, MRI. However, if this is unlikely to lead to earlier surgical or treatment input, its use is again limited. Within the biofluid study the health of patients outside of their cancer diagnosis was not considered. This was not an age-matched study. Therefore, it is possible other factors such as hypertension or medications patients may be taking that also have an impact upon the difference in the spectral results seen. This would all need to be considered prior to starting a larger clinical trial using spectroscopy.

Finally, the hand held intraoperative study gave the most promising results. This is an exciting step towards clinical inclusion of spectroscopy and highlighted it may be able to aid the surgeon and ease workload on pathology laboratories. It is not known where in the tumour these biopsies are taken from, nor the location of the tumour within the brain, therefore it is again not possible to correlate with radiological findings. However, the tissue sent to the pathologist for an intraoperative diagnosis is what has been tested therefore similar results to their diagnosis is exciting. It demonstrates the possibility of training a similar system for use intraoperatively to aid the surgeon to enable improved resection rates and to target neoplastic tissue more easily and with more confidence. This

in turn would hopefully improve survival rates, or at least disease free intervals (*i.e.*, the time taken from treatment to recurrence of disease). Therefore moving forward, this study should be used to form the basis of a classification model to enable this technology to be trialled within the neurosurgical operating theatre. Where possible, if the neuropathologists are agreeable, areas tested by the surgeon and deemed cancer or not could be placed into separate histology pots to enable matching with the final histological diagnosis. The non-neoplastic samples would correspond to areas the surgeon was removing based on clinical suspicion, even with the spectral result. This would allow improved training of the system and a learning period for the surgical team. If successful, the possibilities for this technology include expansion within the field of frozen section work. It could enable its use in a multitude of theatre settings, replacing frozen sections and providing almost instant feedback and results.

Overall, this thesis has shown vibrational spectroscopy could have a role to play in the field of clinical medicine in the future. The aims and objectives of this PhD have been met. The use of a handheld Raman spectroscopic device within the neuropathology department at Royal Preston Hospital has had some success. It has shown the ability to differentiate some tumour types and match the results given from the intraoperative smear provided to the surgeon. It has also developed a platform from which the use of spectroscopy within the operating field can be developed and strengthened. It is crucial that moving forward clinicians communicate and work closely with those in scientific research. Without the ability to develop new diagnostic tools and test them within more real world settings, it is unlikely that the technology itself will surpass the human input, in conjunction with long standing trusted diagnostic methods.

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9. Appendix

9.1 Lancet Correspondence, (Bury et. al., 2018).

WHO leadership is essential for the elimination of NTDs

The second director of the Department of Control of Neglected Tropical Diseases (NTDs) of WHO retired at the end of September, 2017. He was appointed in 2014 to ensure administrative stability after 9 years of innovative growth of this WHO department, which was established in 2005 after the retirement of the first director.[1] Sustaining the momentum for elimination of NTDs requires a timely appointment of a new director to lead an effective Department of NTDs in WHO.

The innovative vision of NTDs was developed by WHO between 2003 and 2005.[1] Young talents who are managing programmes related to NTDs in endemic areas are working for elimination of NTDs, and universities are researching and teaching about NTDs. New agreements between the pharmaceutical industry and WHO, global partners who are are working together against NTDs, and aid agencies of countries like the USA, the UK, Japan, and, more recently, China, have all committed resources to assure access to medicines to treat NTDs.[2] More than 1 billion doses of safe, quality-assured, single-dose treatments reach at-risk people from the poorest urban and rural communities of endemic countries every year.[3]

In April, 2017, 10 years after the first WHO partners meeting, health ministers, donors, philanthropists, and industry representatives met in Geneva, Switzerland to confirm their support to eliminate NTDs.[4] Although this meeting was an opportunity to review progress, it was also perceived as a moment of excessive self-gratification. Major challenges are still ahead including the eradication of guinea worm, supplying the capacity and resources to expand the delivery of preventive chemotherapy, and controlling the emergence and re-emergence of some NTDs, such as hookworm in the south of the USA[5] or urogenital schistosomiasis in Corsica.[6]

The success of the NTDs programme will lead to complacency if the immensity of the task ahead and the need of WHO leadership are not stressed. A WHO department with an energetic leader is necessary to gather evidence and the scientific community behind the control of NTDs, to issue new guidance, to highlight the crucial role of a central figure to identify populations in need, and to logistically coordinate resources to deliver treatments.

The Department of NTDs needs to regain the leadership of a complex open partnership and to rebuild momentum for delivery of the largest ever donations of essential medicines, as a component of universal health coverage. It is not well known that WHO is the only platform through which people affected by neglected conditions have access to free-of-charge, quality-assured treatment that would not otherwise be available (or even manufactured).

The appointment of a new leader to direct a specific department for NTDs under the current Director General, Dr Tedros Adhanom Ghebreyesus, who comes from a country that is committed to fight NTDs, will be the best guarantee to regain momentum to eliminate NTDs by 2030, in line with the targets set up by the UN Sustainable Development Goals.

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Are new technologies translatable to point-of-care testing?

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The point-of-care testing (PoCT) market is rapidly expanding and its predicted worth by 2021 is US$36·96 billion.[1] This market has many facets, one of which is tumour and cancer markers. To develop a new test for clinical use, a biomarker needs to be identified and a quick and simple detection method developed. This biomarker then goes through many steps before clinical use including the all-important step—can it detect cancer earlier than existing methods?

Variants of emerging technologies, such as vibrational spectroscopy or nuclear magnetic resonance spectroscopy, show promise for their use in the clinical forum. However, the point at which these interventions might fit into the diagnostic pathway remains unclear (appendix). For example, many proof-of-concept studies have investigated various uses of vibrational spectroscopy, including biofluids.[2] The uptake of this technology has been slow in the clinical environment[3] and it has

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not yet improved on existing clinical methods, with cases misclassified and malignancy missed.[4]

No clear use has been found that is superior to the existing clinical practice of intraoperative frozen sections and formal histopathological examination. Scientists developing these technologies clearly need direction. With the government's push to reduce the time to diagnosis of cancer patients, will PoCT be a useful adjunct or are the sensitivities and specificities suboptimal? The clinical pathway allows for a specialist-led, personalised plan for patients (appendix) that focuses on the individual—PoCT puts diagnosis back in the general practitioner surgery and places a lot of pressure on the physician to deal with hopes and expectations handled by a practised secondary care team. Not only will the physicians' information be limited to a simple indicator of PoCT, radiology and an appropriate oncology clinician giving treatment information will not be available.

Therefore, it is difficult to see how technology designed to circumvent the diagnostic process and provide instant answers fits into the clinical pathway. Although point-of-care testing is crucial in some areas of cancer diagnostics, careful thought is required to ensure that valuable research funding is correctly distributed for the development of clinically useful tools in the areas that need and require them. Appropriate allocation will only be possible with open communication between scientists and clinicians; neither professional can make new technology work alone.

We declare no competing interests.

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Changes to NHS charges: what does this mean for our most vulnerable patients?

August, 2017, saw the introduction of new regulations on health-care charges to migrants and overseas visitors in England.[1] Patients who are unable to prove entitlement to free care will receive an estimated treatment bill, which must be fully paid before receipt of care, and might increase exponentially. Urgent treatment, as defined by the treating clinician, should be provided and billed for afterwards. These regulations are the outcome of only 418 responses obtained by the Department of Health from their consultation exploring the extension of charging overseas visitors and migrants who use the National Health Service (NHS).[2]

These measures will increase barriers to accessing health care, which leaves groups such as refugees, asylum seekers, and homeless people at risk of not getting the health care they need. Evidence shows that this increasingly hostile environment is preventing such patients from accessing care, the majority of whom are entitled to it,[3] and that restricting access to health care on the basis of immigration status could further compromise the health of vulnerable individuals.[4]

This new system to check patient eligibility could have unintended consequences, with identity checks potentially giving rise to racial profiling, and it must be implemented with a comprehensive assessment to ensure that denial of health care at one point does not result in worse future health outcomes.

Recovery of costs is the main justification for these new regulations. However, there is little evidence regarding the anticipated financial saving; it is estimated to be just 0·00016% of the NHS's annual budget.[4] The government's cost recovery assessment fails to value the time necessary for staff to review identity documents and assess eligibility to free care. Confusion about eligibility for free care could delay diagnosis and treatment, which could incur substantial long-term costs to the NHS.[5]

It's unclear who will carry out identity checks, and how clinicians' roles will be compromised by acting as border guards, potentially denying care. Doctors of the World have launched a campaign for health-care professionals to push back against these reforms. The new rules for upfront immigration checks and charges will make the current climate of fear among our patients even worse. Hospitals should provide a safe environment for vulnerable women and children, not subject them to further intimidation.

The NHS was founded to provide comprehensive care to all, regardless of their ability to pay. We are seeing this sentiment gradually diminish as new legislative caveats are introduced, risking profit, rather than patients, being at the heart of the NHS.

We declare no competing interests.

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Phenotyping Metastatic Brain Tumors Applying Spectrochemical Analyses: Segregation of Different Cancer Types

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ABSTRACT

Metastatic brain tumors represent a significant proportion of tumors identified intraoperatively. A rapid diagnostic method, circumventing the need for histopathology studies, could prove clinically useful. As many spectroscopic studies have shown ability to differentitate between different tumor types, this technique was evaluated for use within metastatic brain tumors. Spectrochemical approaches [Raman and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) spectroscopy] were applied to determine how readily they may identify the primary site for the metastatic tumor. Metastases were from primary adenocarcinomas of lung ($n = 7$) and colorectum ($n = 7$), and for comparison, metastatic melanoma ($n = 7$). The objective was to determine if Raman or ATR-FTIR spectroscopy could delineate the origin of the primary tumor. The results demonstrate that there are marked similarities between the two adenocarcinoma groups and whilst Raman and ATR-FTIR can distinguish the three groups with limited success, classification accuracy is greatly improved when combining the adenocarcinoma groups. The use of such techniques in the clinical setting is more likely to be found intraoperatively, determining the presence of a tumor and suggesting the tumor class; however, traditional histopathology would still be needed to identify the primary origin of the tumor.

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Introduction

Metastatic brain tumors are usually the end-point in a persons' battle with cancer, yet for some may represent the initial diagnosis. The background prevalence of metastatic brain tumors is difficult to quantify; however, those clinically detectable outnumber intrinsic tumors by roughly 3 to 1, with the majority of metastases arising from primary lung tumors (Davis et al. 2012, Huang and Ouyang 2013, Renfrow and Lesser 2013).

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Spectral classification for diagnosis involving numerous pathologies in a complex clinical setting: A neuro-oncology example

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ABSTRACT

Much effort is currently being placed into developing new blood tests for cancer diagnosis in the hope of moving cancer diagnosis earlier and by less invasive means than current techniques, *e.g.*, biopsy. Current methods are expected to diagnose and begin treatment of cancer within 62 days of patient presentation, though due to high volume and pressures within the NHS in the UK any technique that can reduce time to diagnosis would allow reduction in the time to treat for patients. The use of vibrational spectroscopy, notably infrared (IR) spectroscopy, has been under investigation for many years with varying success. This technique holds promise as is would combine a generally well accepted test (a blood test) with analysis that is reagent free and cheap to run. It has been demonstrated that, when asked simple clinical questions (*i.e.*, cancer *vs.* no cancer), results from spectroscopic studies are promising. However, in order to become a clinically useful tool, it is important that the test differentiates a variety of cancer types from healthy patients. This study has analysed plasma samples with attenuated total reflection Fourier-transform IR spectroscopy (ATR-FTIR), to establish if the technique is able to distinguish normal from primary or metastatic brain tumours. We have shown that when asked specific questions, *i.e.*, high-grade glioma *vs.* low-grade glioma, the results show a significantly high accuracy (100%). Crucially, when combined with meningiomas and metastatic lesions, the accuracy remains high (88–100%) with only minimal overlap between the two metastatic adenocarcinoma groups. Therefore in a clinical setting, this novel technique demonstrates potential benefit when used in conjuction with existing diagnostic methods.

1. Introduction

Blood testing for cancer diagnostics is a popular ideal. It uses an acceptable patient test, *i.e.*, a blood test, which is minimally-invasive and machine analysed, can be run on a mass scale, and can target specific markers circulating in the blood. Whilst some cancers can be identified by the use of biomarkers, there are currently no such markers for primary or metastatic brain tumours, nor are any biomarkers yet involved in a mass-screening programme [1]. Brain tumours, both primary and metastatic, often present with a range of non-specific symptoms. The diagnostic process involves a combination of history taking, examination and radiology to determine the presence of a tumour and its possible origin [2]. There are specific radiological appearances that can help differentiate between primary and metastatic brain tumours; however, these rules do not always hold true [3]. A brain tumour may also be the first presentation of a metastatic cancer from elsewhere within the body; this accounts for up to a quarter of brain tumours [4]. Currently, a combination of radiological imaging and histology is used to detect the primary origin of a brain tumour. When metastatic, pathologists can apply immunohistochemical stains to formalin-fixed paraffin-embedded (FFPE) tissue, within which a combination of positive and negative stains can help determine a primary site of origin.

Over recent years the potential of vibrational spectroscopy has been touted as an 'inexpensive, high throughput and reagent-free' cancer diagnostic tool. *In vivo* studies have shown great promise using both tissue and blood component analysis with detection of cancer *vs.* non-cancer in many pilot studies showing promising results [5,6]. When considering biofluids, predominantly serum has been analysed for brain cancer, using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, with varying sample methods used [7]. This is due to the erythrocyte component in whole blood providing a strong interfering spectroscopic signal, likely masking the underlying changes seen in cancer *vs.* non-cancer patients [8]. The main limitations of these studies focus around different methods of sample preparation and analysis. No universal method of spectral analysis has yet been agreed. Butler

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9.4 *Cover letter of approval from the Research Ethics Service*

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Yn rhan o seilwaith ymchwil Cymru a ariannir gan y Sefydliad Cenedlaethol ar gyfer Ymchwil Gofal Cymdeithasol ac Iechyd, Llywodraeth Cymru

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NISCHR
Gwasanaeth Moeseg Ymchwil | RES | Research Ethics Service

South West Wales Research Ethics Committee
Floor 8
36 Orchard Street
Swansea
SA1 5AQ

Telephone : 01792 607416
Fax : 01792 607533
E-mail : penny.beresford@wales.nhs.uk
Website : www.nres.nhs.uk

20 January 2014

Dr Danielle Bury
ST2 Histopathology
Acute Pennine Deanery
Royal Blackburn Hospital
Haslingdon Road
Blackburn
BB2 3HH

Dear Dr Bury

Study title:	**Delivering Innovative Technologies into the Diagnostic Process for Patient Benefit**
REC reference:	**13/WA/0411**
Protocol number:	**N/A**
IRAS project ID:	**141389**

Thank you for your correspondence of 16/01/2014, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Ms Penny Beresford, penny.beresford@wales.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

9.5 Clinician and Patient study approved documents.

CONSENT FORM – CLINICIAN FOCUS GROUP

Title of Project: Diagnostic Innovation in Cancer

Name of Researcher:
Please initial box

1. I confirm that I have read and understand the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

3. I understand that discussions held during the focus group will be recorded to be transcribed but this will be done anonymously. I give permission for this to be done. ☐

4. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file.

Diagnostic Innovation in Cancer

Clinician Information Leaflet – Focus Group

We are conducting research into Clinician preferences surrounding cancer diagnosis and we would like to invite you to participate, however you are under no obligation to become involved. Below is some information relating to the study that will help you decide whether or not you would like to be involved. A member of the research team can be contacted to answer any of your questions (see below).

What is the study about?

At the University of Central Lancashire we are performing research into cancer and how to diagnose it and we would like to discuss the current and new diagnostic methods with you to better understand a Clinician's opinion. This is to help us direct our future studies.

What is the aim of the study?

The aim of the study is to look at patients and doctors opinions of the current cancer diagnosis pathway and possible new diagnosis methods.

What will happen if I agree to take part in this research?

You will attend a focus group led by 2 members of the research team with up to 10 participants to discuss your opinions surrounding cancer diagnosis, and for example where it should take place and who should be delivering the news. We will start by asking you to sign a consent form agreeing to participate. This is to allow us to transcribe the focus group anonymously. If you have any questions about taking part we will be able to answer these either prior to the focus group or on the day.

The focus group will start with a short presentation about cancer diagnosis and then as a group this will be discussed. We will record the conversation but this will be written up anonymously and then the tapes will be deleted to protect privacy.

The focus group will last for up to 2 hours, refreshments including lunch will be provided. There will be no further time requirements beyond this.

What happens if I change my mind?

If you decide not to attend the focus group you do not have to provide a reason and we will not contact you to attend a different day unless you ask to do so. On the day if you want to leave during the focus group you are free to do so without explanation. If you no longer wish for us to use your comments we will not. Once the focus group results have been written up as they are anonymous we will no longer be able to remove your comments as we will not know which they are.

Where will the study take place?

The focus groups will be held at Royal Preston Hospital. The aim is to run the focus groups at a convenient time so as not to impact on clinical commitments.

What are the risks associated with taking part in the research?

There are no risks associated with taking part in this study.

What are the benefits of taking part in the research?

There are no direct benefits to yourself from taking part in this study. We are asking you to help us in order for us to use your thoughts to help shape our future research.

How will we ensure any personal information used during the research is kept confidential?

If you come to a focus group we will ask you to sign a consent form to allow us to keep a record of the comments. We will however not identify you personally within these notes. Your consent forms will be kept securely at the University of Central Lancashire. No other personally identifiable information will be collected. Your taking part in this research will be kept confidential.

Are there any reasons why I might not be eligible to take part in the research?

For the focus groups we require Clinicians involved in any part of the cancer diagnostics pathway.

Complaints

We hope you take part and find our study interesting. However we realise problems may arise. If you have any concerns, please contact the researchers listed below. We will do our best to answer any problems. If you are not happy with the outcome please contact Prof R Lea whose details are available from Dr M Baker.

The Rosemere Cancer Foundation, a local cancer charity for Lancashire and South Cumbria, has funded this study.

This study will form part of Dr Danielle Bury's PhD project.

As with all studies in the NHS it has been looked at by an independent group of people who form the Research Ethics Committee. This is done to protect your interests. The South West Wales Research Ethics Committee has reviewed this study

You may decide to withdraw from the study at any time without need for an explanation.

Thank you for taking the time to read this leaflet.

For further information or to confirm your attendance please contact either Dr Danielle Bury on debury@uclan.ac.uk or Dr Matthew Baker on mjbaker@ulcan.ac.uk or 01772 893209.

Many Thanks,

Dr Danielle Bury and Dr Matthew Baker

University of Central Lancashire

CONSENT FORM – PATIENT FOCUS GROUP

Title of Project: Diagnostic Innovation in Cancer

Name of Researcher:
Please initial box

1. I confirm that I have read and understand the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that discussions held during the focus group will be recorded to be transcribed but this will be done anonymously. I give permission for this to be done. ☐

4. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file.

Diagnostic Innovation in Cancer

Participant Information Leaflet – Focus Group

We are conducting research into patient preferences surrounding cancer diagnosis and we would like to invite you to participate, however you are under no obligation to become involved. Below is some information relating to the study that will help you decide whether or not you would like to be involved. A member of the research team can be contacted to answer any of your questions or explain any details further (see below).

What is the study about?

At the University of Central Lancashire we are performing research into cancer and how to diagnose it and we would like to discuss current and new ways of diagnosing cancer with you to better understand a patients' opinion. This is to help us direct our future studies.

What is the aim of the study?

The aim of the study is to look at patients and doctors opinions of the current cancer diagnosis pathway and possible new diagnosis methods.

What will happen if I agree to take part in this research?

You will attend a focus group led by 2 members of the research team with up to 10 participants, all with personal experience of cancer, to discuss your opinions of cancer diagnosis. Examples of the questions are; where diagnosis should take place and who should be delivering the news, etc. We will start by asking you to sign a consent form agreeing to participate. This is to allow us to collect the results of the focus group anonymously. If you have any questions about taking part we will be able to answer these either prior to the focus group or on the day. We will not tell any of the medical team treating you or your GP that you have taken part.

The focus group will start with a short presentation about cancer diagnosis and then as a group this will be discussed. We will record the conversation, but this will be written up anonymously and then the tapes will be deleted to protect privacy.

The focus group will last for up to 2 hours, refreshments will be provided. There will be no further time requirements beyond this. All travel expenses will be refunded and participants will receive £10 for the time and inconvenience experienced.

What happens if I change my mind?

If you decide not to attend the focus group you do not have to tell us why and we will not contact you to come a different day unless you ask to do so. On the day if you want to leave during the focus group you are free to do so without explanation. If you no longer wish for us to use your comments we will not. Once the focus group results have been written up as they are anonymous we will no longer be able to remove your comments as we will not know which they are.

Where will the study take place?

The focus groups will be held at the University of Central Lancashire. Parking will be available for those who would like it. Three focus groups will be run at different times of the day so people at work will be able to attend a session run later in the day.

What are the risks associated with taking part in the research?

There are no risks associated with taking part in this study.

What are the benefits of taking part in the research?

There are no direct benefits to yourself from taking part in this study. We are asking you to help us in order for us to use your thoughts to help shape our future research.

How will we ensure any personal information used during the research is kept confidential?

If you come to a focus group we will ask you to sign a consent form to allow us to keep a record of the comments. We will not identify you personally within these notes. Your consent forms will be kept securely at the University of Central Lancashire. No other personally identifiable information will be collected. Your part in this research will be kept confidential.

Are there any reasons why I might not be eligible to take part in the research?

For the focus groups we need patients over the age of 18 years who are able to consent for themselves and that have had experience of cancer or the cancer diagnostic pathway.

Complaints

We hope you take part and find our study interesting. However we realise problems may arise. If you have any concerns, please contact the researchers listed below. We will do our best to answer any problems. If you are not happy with the outcome please contact Prof R Lea whose details are available from Dr M Baker.

The Rosemere Cancer Foundation, a local cancer charity for Lancashire and South Cumbria, has funded this study.

This study will form part of Dr Danielle Bury's PhD project.

As with all studies in the NHS it has been looked at by an independent group of people who form the Research Ethics Committee. This is done to protect your interests. The South West Wales Research Ethics Committee has reviewed this study.

If you decide you do not wish to take part in the research or you decide to withdraw from the study

Thank you for taking the time to read this leaflet.

For further information or to attend a focus group please contact either Dr Danielle Bury on debury@uclan.ac.uk or Dr Matthew Baker on mjbaker@uclan.ac.uk or 01772 893209.

Many Thanks,

Dr Danielle Bury and Dr Matthew Baker

University of Central Lancashire

Diagnostic Innovation in Cancer

Participant Information Leaflet - Questionnaire

We are conducting research into patient preferences surrounding cancer diagnosis. You have been identified as a potential participant in this research; however you are under no obligation to become involved. Below is some information relating to the research that will help you decide whether or not you would like to be involved. A member of the research team can be contacted to answer any of your questions (see below).

What is the study about?

At the University of Central Lancashire we are performing research into cancer and how to diagnose it and we would like to discuss the current and new wasys of diagnosing cancer with you to better understand a patients' opinion. This is to help us direct our future studies.

What is the aim of the study?

The aim of the study is to look at patients and doctors opinions of the current cancer diagnosis pathway and possible new diagnosis methods.

What will happen if I agree to take part in this research?

Below is the web address to access an anonymous questionnaire online. We would like you to complete this questionnaire, it should take approximately 30 minutes. If you would like to but are unable to do it online or need it in a different language please contact us below and we will send out a paper copy with a stamped addressed

envelope for you to return it. On finishing the questionnaire you will be given details to access the results of this study when it is finished.

By filling in the questionnaire after reading this leaflet you are agreeing that you understand the information provided and agree to us analysing the answers you give. **We ask if you do not agree to this then please do not submit your questionnaire answers.**

If you have any further questions please do not hesitate to contact us. As the questionnaire is completed anonymously once submitted we will not be able to retrieve and delete your answers as we will not know which they are.

What are the risks associated with taking part in the research?

There are no risks associated with taking part in this study.

What are the benefits of taking part in the research?

There are no direct benefits to yourself from taking part in this study. We are asking you to help us in order for us to use your thoughts to help shape our shape our future research.

How will we ensure any personal information used during the research is kept confidential?

No personally identifiable information will be needed to complete the questionnaire. You will be asked for your age and employment to allow us to fully analyse the data.

If you request a paper copy your details will not be held on any record and will be destroyed once the questionnaire has been sent out.

Are there any reasons why I might not be eligible to take part in the research?

We require patients over the age of 18 years.

Complaints

We hope you take part and find our study interesting. However we realise problems may arise. If you have any concerns, please contact the researchers listed below. We will do our best to answer any problems. If you are not happy with the outcome please contact Prof R Lea whose details are available from Dr M Baker.

The Rosemere Cancer Foundation, a local cancer charity for Lancashire and South Cumbria, has funded this study.

This study will form part of Dr Danielle Bury's PhD project.

As with all studies in the NHS it has been looked at by an independent group of people who form the Research Ethics Committee. This is done to protect your interests. The South West Wales Research Ethics Committee has reviewed this study.

If you decide you do not wish to take part in the research at any time your medical care will not be affected.

Thank you for taking the time to read this leaflet.

To visit and complete the questionnaire please go to XXXXXXX. It will be open from XX to XX.

For further information or to request the questionnaire in a different format please contact either Dr Danielle Bury on debury@uclan.ac.uk or Dr Matthew Baker on mjbaker@uclan.ac.uk or 01772 893209.

Many Thanks,

Dr Danielle Bury and Dr Matthew Baker

University of Central Lancashire

Lancashire Teaching Hospitals NHS Trust

rosemere CANCER FOUNDATION
Reg. Charity No: 1131583

uclan
University of Central Lancashire

NHS

Would you be interested in taking part in cutting edge research?

- We are looking for people to complete an online questionnaire about your preferences around cancer diagnostic testing in order to help us direct our research into the development of a new diagnostic testing device to help patients of the future.
- You must be over 18 years old to complete the questionnaire.
- The questionnaire will take approximately 30minutes to complete and is completely anonymous.
- **Completion of the questionnaire is not compulsory and neither your doctor nor GP will be informed.**
- By completing the questionnaire you agree to our research team analysing your results. As this is all done anonymously and once completed your answers cannot be removed.
- More information can be found at the start of the questionnaire.
- For further questions, or if you would like to access the questionnaire in a different format, for example a paper copy, please contact either Dr Danielle Bury on debury@uclan.ac.uk or Dr Matthew Baker on mjbaker@uclan.ac.uk or call 01772 893209.
- Otherwise please visit XXXXXX to complete the questionnaire! Thank you for your input into our research.

This study has been reviewed by the South West Wales Research Ethics Committee.

Clinician Questionnaire

Q1: Gender – Male/Female

Q2: Age (in years)

Q3: Ethnicity - choices given

Q4: Employed

Full time

Part time

Retired

Student

Not employed

Q5: Job title

Q6: Household income – bracket figures given

Q7: Have you ever attended a cancer screening programme? – Yes or No

If No – is there a reason why you have not attended a cancer screening programme?

Q8: Who do you think should give a cancer diagnosis? Please select as many as are applicable.

A doctor in a hospital

Your GP

A nurse

A clinical nurse specialist

Not bothered

Other (specify)

Who would prefer to give a cancer diagnosis? Select one from above.

Q9: From your experience of cancer diagnosis and treatment, do you feel there is particular area that requires improvement? Please select as many as appropriate.

Method of diagnosis

Time taken for diagnosis

Ensuring complete removal of cancer at surgery

Explanation of diagnosis and treatment plan

None

N/A

Other (specify)

Please explain your response

Q10: How often do you come into contact with cancer patients?

Every day

Once a week

Once every 2 weeks

Once a month

Less often than once a month

Q11: How often do you diagnose cancer?

Every day

Once a week

Once every 2 weeks

Once a month

Less often than once a month

Q12: Are you involved with cancer treatment?

Yes or No

Q13: Where do you think patients should be diagnosed with cancer?

At home

GP surgery

Hospital

Other – Please specify

Q14: Where do you think patients would like to be diagnosed with cancer?

At home

GP surgery

Hospital

Other – Please specify

Q15: If we were to offer you a screening test that may diagnose a cancer for which a patient had no symptoms and did not know they had, would you recommend this to patients? Please explain your response.

Q16: Which investigations do you think patients find acceptable and which are not? (By acceptable we mean you would be willing to accept the test and do not feel it is an unreasonable test when looking for cancer). On the scale provided please indicate your level of acceptability for each of the tests. 1 indicates 'Not acceptable, to 10 indicating 'acceptable with no concerns'.

Xray

Blood test

MRI scan

CT scan

PET CT – this is a type of CT scan that involves injecting a dye to highlight any abnormalities – not all cancers react to this dye

Biopsy with local anaesthetic (local anaesthetic is the use of a drug which is injected into the biopsy site to make it numb so no pain is felt during the biopsy, you are awake during this)

Biopsy with general anaesthetic (this is a biopsy where you are put to sleep)

A physical examination by a doctor eg listening to your chest

Q17: At what point would you EXPECT diagnosis of cancer to occur? Please select one response - time scales given, please explain your response

Q18: At what point would you LIKE (in a ideal world) diagnosis of cancer to occur? Please select one response. - time scales given, please explain your response

Q19: Do you believe you are fully informed and/or can access information about: Strongly agree to strongly disagree.

Risks of cancer

Risks of investigative tests

Risks of treatment

Complications of treatment

Diagnosis

Impact of quality of life

Q20: Based on your role within the cancer care pathway, where do you feel research can most benefit patients? For example, blood test diagnosis, detecting microscopic cells at surgery to ensure complete clearance? Etc.

Diagnostic Innovation in Cancer – Patient Questionnaire

Q1: What is your gender?

 Male

 Female

Q2: What is your age (in years)?

…….

Q3: What is your ethnicity?

White

☐ British

☐ Irish

☐ Other

Asian or Asian British

☐ Indian

☐ Pakistani

☐ Bangladeshi

☐ Any other Asian background

Mixed

☐ White and Black Caribbean

☐ White and black African

☐ White and Asian

☐ Any other mixed background

Black or Black British

☐ Caribbean

☐ African

☐ Any other black background

Other Ethnic Group

☐ Chinese

☐ Any other Ethnic Group

☐ I do not wish to disclose my ethnic origin

Q4: What is your employment status?

☐ Full time

☐ Part time

☐ Retired

☐ Student

☐ Not employed

Q5: What is your job title?

…….

Q6: What is your household income?

☐ Less than £10,0000

☐ £10,000 to £19,999

☐ £20,000 to £29,999

☐ £30,000 to £39,999

☐ £40,000 to £49,999

☐ £50,000 to £59,999

☐ £60,000 to £69,999

☐ £70,000 to £79,999

☐£80,000 to £89,999

☐ £90,000 to £99,999

☐ £100,000 to £149,999

☐ £150,000 or more

Q7: Do you have experience of cancer? Please select as many as are applicable

☐ Personal

☐ Close family (eg parents, children, siblings)

☐ Distant family (eg aunts/uncles, grandparents)

☐ Friend

☐ None of the above

Q8: Do you have a family history of cancer? Please select as many as are applicable

☐ 1st degree relative – this may be your mother, father, brother or sister

☐ 2nd degree relative – this may be your grandparents, aunts/uncles, nephews/nieces

☐ Known genetic abnormality – have you ever had a test from a doctor that has shown a change in one of your genes that gives you a higher change of getting cancer?

☐ Not applicable

☐Other (Please specify)

……..

Q9: Have you ever attended a cancer screening programme?

☐Yes

☐ No

If No – is there a reason why you have not attended a cancer screening programme?

……

Q10: If you needed to attend an appointment testing for cancer, what is the travel time to your nearest hospital that offers cancer tests?

☐ Less than 30 minutes

☐ Between 30 mins to an hour

☐ More than 1 hour

☐ Don't know

Q11: On average, how often do you come into contact with healthcare professionals? – by this we mean a GP, a doctor in a hospital, or a nurse (including cancer specialist nurse). Please select one.

☐ More than once a week

☐ Once a week

☐ Once a month

☐ Once every 3 months

☐ Once a year

☐ Less than once a year

Q12: Who is the healthcare professional you come into contact with most frequently?

……

Q13: Where would you prefer to be informed of your diagnosis? Please choose one

☐ At home

☐ GP Surgery

☐ Consultant Clinic

☐ At Optician/dentist/pharmacy

☐ Not bothered

☐ Other (please specify)

……

Please explain your response:

……

……

……

Q14: Who do you think should give a cancer diagnosis? Please select as many as applicable

☐ A doctor in a hospital

☐ Your GP

☐ Nurse

☐ Clinical Nurse Specialist (a nurse who deals solely with patients that have one type of cancer or disease)

☐ Not bothered

☐ Other (specify)

……..

Who would you prefer to give a cancer diagnosis? Select one of the above and explain.

……..

……

……

Q15: From your experience of cancer diagnosis and treatment, do you feel there is a particular area that requires improvement? Please select as many as are applicable.

☐ Method of diagnosis

☐ Time taken for diagnosis

☐ Ensuring complete removal of cancer at surgery

☐ None

☐ Not applicable

☐ Other (please specify)

…….

Please explain your answer:

……..

……

……

Q16: If we were to offer you a screening test that may diagnose a cancer for which you have had no symptoms and did not know you had, would you take up the offer?

☐ Yes

☐ No

☐ Don’t know

Please explain your response.

……..

……

……

Q17: Which investigations do you find acceptable and which are not? (By acceptable we mean you would be willing to accept the test and do not feel it is an unreasonable test when looking for cancer). Please indicate your level of acceptability for each of the tests. 1 indicates 'Not acceptable, to 10 indicating 'acceptable with no concerns'.

☐ Xray

☐ Blood test

☐ MRI scan

☐ CT scan

☐ PET CT – this is a type of CT scan that involves injecting a dye to highlight any abnormalities – not all cancers react to this dye

☐ Biopsy with local anaesthetic (local anaesthetic is the use of a drug which is injected into the biopsy site to make it numb so no pain is felt during the biopsy, you are awake during this)

☐ Biopsy with general anaesthetic (this is a biopsy where you are put to sleep)

☐ A physical examination by a doctor eg listening to your chest

Q18: Which of the investigations below would you NOT want to have if you were being investigated for cancer? Please select as many as are applicable.

☐ Blood test

☐ Invasive test eg endoscopy – this involves a camera being inserted for example into your bowel to look at the bowel and take a biopsy

☐ Invasive test eg surgery – this would involve putting you to sleep to look closely at a suspected cancer

☐ Scan eg MRI/CT

☐ I would allow any of the above investigations to be done

Please explain your response

……..

……

……

Q19: At what point would you EXPECT diagnosis of cancer to occur? Please select one response

☐ Before you feel unwell

☐ When you visit your GP

☐ At first medical appointment with a specialist doctor

☐ Within 1 week of first appointment with specialist doctor

☐ Within 1 month of first appointment with specialist doctor

☐ I would not like to know

Please explain your response

……..

……

Q20: At what point would you LIKE (in a ideal world) diagnosis of cancer to occur? Please select one response.

☐ Before you feel unwell

☐ When you visit your GP

☐ At first medical appointment with a specialist doctor

☐ Within 1 week of first appointment with specialist doctor

☐ Within 1 month of first appointment with specialist doctor

☐ I would not like to know

Please explain your response

……..

……

……

Q21: In your experience (personal or from someone you know), what was the time frame from first visit to GP to diagnosis of cancer?

☐ Less than 2 weeks

☐ 2-4 weeks

☐ 4-8 weeks

☐ Not applicable/not known

☐ More than 2 months – please specify

……

Q22: Do you believe you are fully informed and/or can access information about: Strongly agree to strongly disagree.

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Risks of cancer					
Risks of investigative tests					
Risks of treatment					
Complications of treatment					
Diagnosis					
Impact of quality of life					

Thank you for taking the time to answer this survey!

If you have any concerns or questions about this survey, please contact the researchers listed below. We will do our best to answer any problems. If you are not happy with the outcome please contact Prof R Lea whose details are available from Dr D Bury.

The Rosemere Cancer Foundation, a local cancer charity for Lancashire and South Cumbria, has funded this study. As with all studies in the NHS it has been looked at by an independent group of people who form the Research Ethics Committee. This is done to protect your interests. The South West Wales Research Ethics Committee has reviewed this study. If you decide you do not wish to take part in the research at any time your medical care will not be affected.

For further information or to request the questionnaire in a different format please contact Dr Danielle Bury on debury@uclan.ac.uk or Dr Michelle McManus on mamcmanus@uclan.ac.uk or 01772 894154. The School of Forensic and Investigative Sciences (FIS) office can be contacted on 01772 895687

9.6 *Brain Tumour North West Ethical Approval*

BRAIN TUMOUR NORTH WEST

uclan · University of Wolverhampton · Lancashire Teaching Hospitals NHS · The Walton Centre NHS Foundation Trust · Clatterbridge Centre for Oncology NHS Foundation Trust

Application for clinical samples/data from the Brain Tumour North West and the Walton Research Tissue Banks

Applicant: D. Bury Duration of Project: 6 years

Name DANIELLE BURY

Address DEPT. OF NEUROPATHOLOGY
ROYAL PRESTON HOSPITAL

Tel: 01772 52 2140
Email: dieb11@doctors.org.uk

Principal Investigator / Student *(delete as appropriate)*

Project Title: DIAGNOSTIC INNOVATION IN CANCER

Is the application for samples to support the project being made to:
~~**Brain Tumour North West (BTNW) Tissue Bank / Walton Research Tissue Bank (WRTB) /**~~
Joint application to both banks *(delete as required)*

Funding:
Is the funding for the project
a) external (eg funded through a grant application)
b) internal (eg funded from a researchers laboratory or institutional budget)
c) other *(please give details)*

If the project has been submitted as a grant application for external funding?
Was the application successful? yes / no
Was the project externally peer-reviewed? yes / no

External Funding body: ROSEMERE CANCER FOUNDATION

Details of Funding: Total £ 55,385 (staff .....and consumables .....)

Research Sponsor: R+D DEPARTMENT LTHTR.

Approvals:
Ethics Approval
Does the project have ethics approval? yes / no
If yes, please supply reference number .....and date of approval .....

Both Tissue Banks have generic ethical approval to supply tissue/data for projects conducted by internal applicants, without the need for further ethics approval. External researchers wishing to use tissue or data supplied by the bank need to apply to REC for individual ethical approval.

Research Governance
a) Are the applicants employed by NHS establishment(s)? yes / no
b) Does the project involve research activity using the anonymised samples/data in an NHS establishment? yes / no

If yes to a or b, the project may require research governance approval and applicants should consult the Research Governance Manager at their hospital

Co-applicants:	**Name**	**Affiliation**
	PROF T DAWSON	LTHTR
	DR M BAKER	UCLAN

Outline of Project

Please give a brief outline of the project under the headings below (not more than 1-3 A4 pages) or attach the external funding application

Scientific Background

Hypothesis and Study Aim

Plan of Project

(number of cases including samples from other banks)

Experimental Methodology,

Data Analysis and Statistics

PLEASE SEE EXTERNAL FUNDING ~~APPLICATION~~ APPLICATION - THEME 2.

Tissue Required – *Please indicate whether you require paraffin embedded tissue, fresh frozen tissue, cellular component of blood, plasma or serum and how many samples you require.*

Please indicate if all samples are required at the start of the project or if further applications for samples will be made in the light of initial findings.

WE PLAN TO RUN A PILOT STUDY USING UP TO 150 FRESH FROZEN SAMPLES (2 SLIDES OF EACH, 1 H&E & 1x10 µm SECTION) COMPRISING MENINGIOMAS, GLIOMAS AND NORMAL TISSUE. BASED ON THE RESULTS THE REMAINDER OF THE WORK WILL BE CARRIED OUT ON FRESH TISSUE. THIS PART OF THE STUDY WILL BE ON-GOING ~~FOR~~ FROM THE CONCLUSION OF THE PILOT STUDY UNTIL THE END OF THE PHD (2018) AND WILL INCLUDE AS MANY FRESH SAMPLES AS POSSIBLE RUNNING PROSPECTIVELY.

Please also provide a brief lay summary (maximum 200 words)

THIS RELATES TO THEME 2 ~~WITH~~ WITHIN THE PROJECT ~~PROPOSAL~~ OUTLINE AND M7 ON THE GANTT CHART.

For further information please contact:
BTNW Tissue Bank: Prof T Dawson email: Timothy.Dawson@lthtr.nhs.uk
Walton Research Tissue Bank: Dr C Walker email: carol.walker@thewaltoncentre.nhs.uk

Please email completed applications to:
BTNW Tissue Bank: Prof T Dawson email: Timothy.Dawson@lthtr.nhs.uk
Walton Research Tissue Bank: Dr C Walker email: carol.walker@thewaltoncentre.nhs.uk
Or to both for joint applications to both banks

For BTNW or WRTB use only:

Date Application Received	30/7/14
Application Number	
Project Title	Diagnostic Innovation in Cancer
Date sent to BTNW/WRTB Review Panel	31/7/14
Names of Reviewers	Mr A Ray, Dr T Warr, Dr M Morris

Decision of BTNW/WRTB Committees	Approve/~~Reject~~ Date: 21/08/14

9.7 *Poster presented at Spec Summer School 2015.*

Diagnostic Innovation In Cancer

D Bury[1], Katherine Ashton[2], Timothy P. Dawson[2], Robert W. Lea[3], Matthew J. Baker[1,4*]

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[2]Department of Pathology, Lancashire Teaching Hospitals NHS Trust, Royal Preston Hospital, Sharoe Green Lane , Preston, Lancashire, PR2 9HT, UK
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[4]WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL

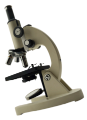
*email: matthew.baker@strath.ac.uk

Goal Deliver innovation into the diagnostic process and enable patient benefit through the reduction of mortality, morbidity and economic burden

Aims
1. Determine Public and Clinician preferences surrounding cancer diagnostics
2. Establish whether Raman or FTIR gives superior results on frozen brain tissue
3. Move into clinical environment using fresh brain tissue sent for intraoperative diagnosis comparing spectroscopy to gold standard of histopathology

Introduction

- Currently 1 in 2 people in the UK will get cancer in their lifetime[1]
- With 9400 people/year diagnosed with brain tumours[2]
- Cancer diagnostics is a complex area with a variety of new techniques competing to enter the clinical arena and attempt to revolutionise care.
- Current diagnostic methods can be invasive and therefore declined by patients
- Histopathology has long been the gold standard for diagnosis. In order to improve the current NHS targets for cancer diagnostics alternative methods may provide earlier and faster diagnostics without the need for invasive biopsies

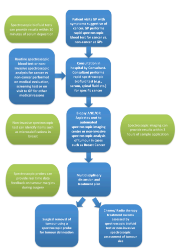


- Vibrational spectroscopy has been shown to be able to differentiate a variety of tumour types based on serum, blood and tissue
- Pathology input is required to train the spectral metrics, however kappa scores for pathology have been shown to be lower than that for Raman, 0.76 compared to 0.89[3]

PhD Plan

	0–6 mths	7–12 mths	13-18 mths	19-24 mths	25-30 mths	31-36 mths	37-42 mths	43-48 mths	49-54 mths	55-60 mths	61-66 mths	67-72 mths
Theme 1	M1	M2	M2&5	M2&5	M3&4							
Theme 2			M6&8	M6	M6&8	M6&7	M7&8	M7	M7&8	M7	M7&8	M7
Theme 3	M9	M9	M9	M9&10	M9	M9	M9	M9&10	M9	M9	M9	M9&10

Milestones
Theme 1
M1 – Questionnaire format decided and ethical approval received
M2 – Questionnaire released to patients and clinicians via online hosting, interviews and post
M3 – Questionnaire time period closes and data analysis
M4 – Report and peer-reviewed publication of patient and clinician preference for innovation in diagnosis
M5 – Investigation of the clinical environment to identify any barriers that may bar the uptake of new technology
Theme 2
M6 – Combination of exiting spectral datasets from the Baker and Martin Labs for quick win publications
M7 – Proof of Principle projects to deliver preferential innovation depending on the outcome of Theme 1
M8 – Impact events that will focus on two way engagement via focussed clinical breakfasts, patient engagement workshops and evening seminars. Opportunities to communicate this research to wider audiences will also be developed
Theme 3 – Full project management (Budget Management, Health and Safety etc.)
M9 – Interdisciplinary team meetings
M10 – Industry Engagement Meetings to technological developments are progressing with research findings



Theme 1 - Public Engagement

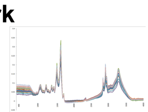
- The Kings Report highlighted the need to understand patient preference to allow informed choice, with Public Patient Involvement (PPI) felt to be crucial[4]

To do this:
- A questionnaire has been designed to look at Public and Clinician preferences surrounding cancer diagnosis and where they think improvements could be made
- The questionnaire was launched via smartsurvey.com
- Focus groups have been planned held at Royal Preston Hospital and UCLan to discuss in an open format Clinician and Public opinions, including any barriers they perceive to new technology
- Ethical approval for both was obtained (13/WA/0411)

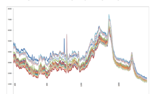
So far:
- 100 patient and 50 Clinician responses to the questionnaire
- 2 focus groups for both patients and Clinicians have been held

Theme 2 - Laboratory Work

- Permission granted by BTNW for use of frozen tissue samples
- 99 cases were cut onto Raman grade calcium fluoride slides
- Samples were run on both Raman and FTIR, with 20 spectra obtained on each from different areas



Normal brain sample run on FTIR and Raman



- Raman: Horiba Jobin-Yvon LabRAM HR800

400-1800cm^{-1}
785nm laser, x50 obj
300gr/mm, filter 100%
Acc: 30s x 2

- FTIR: Agilent 660

4000-400cm^{-1}
Res 4cm^{-1}
32 co-added scans

Acknowledgements
The authors would like to acknowledge the support of the Rosemere Cancer Foundation and Brain Tumour North West

rosemere CANCER FOUNDATION Reg. Charity No: 1131583

BRAIN TUMOUR NORTH WEST

References
1. http://www.cancerresearchuk.org/about-cancer/what-is-cancer
2. http://www.cancerresearchuk.org/about-cancer/type/brain-tumour/about/brain-tumour-risks-and-causes
3. Kendall, C., et al., *Raman spectroscopy, a potential tool for the objective identification and classification of neoplasia in Barrett's oesophagus.* J Pathol, 2003. **200**(5): p. 602-9.
4. Mulley A, Trimble C, Elwyn G, *Patients' Preference Matter – Stop the silent misdiagnosis*, **The Kings Fund**, 2012, ISBN 978 1 85717 637 7

Integration into a clinical setting of a handheld Raman with fibre optic probe to allow rapid diagnosis and sub-categorisation of brain tumours

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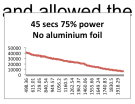
[2]Department of Neuropathology, Lancashire Teaching Hospitals NHS Trust, Royal Preston Hospital, Sharoe Green Lane , Preston, PR2 9HT, UK

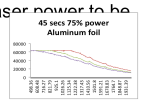
Introduction:

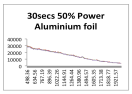
- Rapid diagnosis and sub-categorisation of brain tumours intra- and post-operatively is critical.
- Intraoperative assessment of brain tissue via a smear preparation is frequently used to determine the nature of the tumour.
- This can be used to dictate the surgery required.
- In order to determine if Raman spectroscopy can be used to aid diagnosis during the intraoperative phase a B&Wtek handheld Raman system with 785 nm laser and flexible fibre optic probe has been placed within the histopathology department.
- Spectra will be taken on fresh brain tissue in conjunction with smear preparation and results compared to the current gold standard of histopathology.
- In order for this to happen preliminary studies are being conducted to investigate laser power and acquisition time to avoid destruction of the fragile tissue.

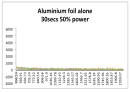
Preliminary work:

- Post-operative brain tissue is placed on a slide in accordance with routine clinical practice and prior to being smeared to allow for staining and histology, a probe with trigger feature is used to acquire fingerprint spectra.
- This is performed in a category 3 set-up within a bespoke-made black box, with both plain glass slides and slides covered with aluminium foil.
- Acquisition times of 30 and 45 seconds allowed identifiable spectra to be obtained, with those at 30 seconds more reproducible.
- Use of aluminium foil reduced the need for longer acquisition and allowed the laser power to be decreased.

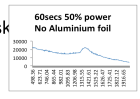








- Reducing the laser power reduces risk tissue.
- We found acquisition times of 60 seconds led to burning of the tissue.



Set up:

- Prior to obtaining spectra a bespoke box was created to allow spectral acquisition in darkness within the laboratory environment.
- This is done to reduce background noise.
- The box was designed to allow for slide stabilization during spectral acquisition and allow movement of the slide both vertically and horizontally to enable location of the tissue under the laser beam.

Moving forward:

- Future studies will interrogate brain tumour fingerprint spectra including glioma, meningioma and metastases in comparison to benign tissue.
- We will evaluate this approach to determine its applicability towards rapid diagnosis and sub-categorisation in predicting treatment regimen or potential for disease re-emergence. Implementation of such a rapid screening approach in a clinical setting potentially allows more rapid identification of tumour tissue margins (critical in brain surgery) and more targeted triaging of patients in neuro-oncology.

Acknowledgements
The authors would like to acknowledge the support of the Rosemere Cancer Foundation and Brain Tumour North West

References:
Cui, L.; Butler, H. J.; Martin-Hirsch, P. L.; Martin, F. L. Aluminium foil as a potential substrate for ATR-FTIR transflection FTIR or Raman spectrochemical analysis of biological specimens. Analytical Methods. 2016. 8;481-487.

9.9 Abstract accepted by the American Society of Clinical Oncology

Spectrochemical analysis highlights chemical constituent similarities in adenocarcinomas irrespective of primary tissue origins

INTRODUCTION: Identification of primary tissue origin of brain metastases can be histopathologically challenging. Initial diagnosis of malignancy can be provided on intraoperative tissue smears; however, it requires formal histopathological examination along with immunohistochemistry to reach a final diagnosis. On occasion this will still fail to determine primary origin. This study aims to demonstrate if Raman spectroscopy is able to determine primary tissue origin of brain metastasis of three common primary tumours.

METHOD: Formalin fixed paraffin embedded tissue from twenty brain metastasis comprising colorectal adenocarcinomas (*n*=7), lung adenocarcinomas (*n*=7) and melanomas (*n*=6) were obtained from the Brain Tumour NorthWest tissue bank. Sections (10-µm thick) were placed onto glass slides covered in aluminium foil and de-waxed prior to spectral acquisition. 25 spectra per section were collected at random, using a 785 nm laser at 1200 g mm^{-1} grating with an acquisition time of 30 sec. Computational analysis within a MatLab environment was then conducted.

RESULTS: Following PCA-LDC analysis, classification accuracy of the three groups was colorectal adenocarcinoma 71.3%, lung adenocarcinoma 71% and melanoma 70%. A one-way ANOVA showed statistically significant differences between the three groups, $p=0.0014$. On combining adenocarcinoma groups accuracy increased to 87.8%, whilst melanoma fell to 68.9%. A student's t-test confirmed statistical significance between the two groups, $p<0.0001$.

CONCLUSION: Raman spectroscopy can classify different tumours by type, though sensitivity and specificity is diminished if used to classify primary tissue origin of adenocarcinomas. Therefore for clinical use, such new tools may aid the clinician to determine tumour type intra-operatively but classic histopathology is still required for tissue origin confirmation. Such technologies may provide a useful adjunct to more conventional approaches; for instance, decreasing the number of immunohistochemical stains required to determine tissue origin. These results also suggest strong similarities between adenocarcinomas of different primary origins.

9.10 Standard Operating Procedure and Risk Assessment for Hand Held Raman Probe

TITLE			
FILE NAME		Thesis_Introduction.docx	
VERSION		Q PULSE DOCUMENT NUMBER	
REPLACES VERSION		New document	
DATE OF ISSUE		02/03/2017	
REVIEW INTERVAL		2 Yearly	
AUTHORISED BY		Kate Ashton	
OWNER		Bury Danielle (BFWH)	
COPY		No. 1	
LOCATION OF COPIES		Neuro Cut-up room 112 Research Lab room 10A	

Review date	Page no.	Amendment details	Signature
		Add any changes that have been made to this version	

1. Introduction

Raman spectroscopy is to be compared with light microscopic H&E smear examination for the intra-operative diagnosis of brain tumours.

2. Specimen Requirements

Fresh brain tissue sample

3. Principle of the Test

The Raman spectra produced from shining a laser through a piece of tissue is unique to the tissue. The position of the peaks can be used to differentiate between tumour and non-tumour tissue and between different types and grades of tumour.

4. Health and Safety

CAUTION - Laser can cause blindness and burning to the skin and clothing

Never look directly into the laser beam path or scattered laser light from any reflective surface.

Never look directly into the laser source.

Maintain a low beam level when performing experimental setup to prevent inadvertent beam-eye contact.

As a precaution against accidental exposure to the laser beam or its reflection, users should always wear laser safety glasses with sufficient attenuation for the laser.

Never point laser directly at skin or clothing

Standard laboratory Health and safety precautions must be adopted at all times. For full details see, 'Safe working and Prevention of infection in Clinical Laboratories and similar facilities' or **S DI SPECHANDLING**

5. Equipment and Materials

Portable i-Raman spectroscopy machine and probe

PC with BWspec software installed
Laser protection glasses
Glass slides covered with tinfoil

BioPure™ 20nm Gold Nanoparticles

Phosphate buffer

Pipetters and tips

Vortexer

6. Method

If using nanoparticles, vortex BioPure™ 20nm Gold Nanoparticles solution immediately before dilution

Dilute 2 µL of vortexed nanoparticles with 200 µL phosphate buffer

Turn on computer and login

Turn on laser at back of machine (interlock plug and key in desk top drawer in Neuro main lab)

On/Off power switch to On position

Put interlock plug into plug port

Insert key and turn to On position

Put tissue on slide coated with aluminium foil.

If using nanoparticles, cover tissue with 100 µL of freshly vortexed, diluted nanoparticle solution.

Allow tissue to absorb solution for a few minutes.

Open software on PC – BWspec

'Online' appears at the bottom left corner of the BWSpec main screen, indicating that the communication between the i-Raman and the computer has been established.

Put on laser protection glasses

Remove protective sleeve from end of probe

Secure probe in black box using clamp

Perform 'Dark Scan'

Close manual shutter on probe

In BWspec change milliseconds field to 30,000

Set laser power to 75

Set Y axis type to 'Dark'

Click 'Dark Scan' button

After scan has completed set Y axis type to 'Dark Subtracted'

Insert slide into black box, centre laser on tissue using platform screws

Slide manual laser shutter to open

Check probe is over the top of the tissue, approx. 6mm above tissue and laser is shining through the tissue

Check milliseconds and laser power settings on BWspec haven't changed

Click on 'Acquire Overlay' play button to record spectrum

Repeat nine times more to give 10 spectra on graph. Move tissue slightly between scans.

Right click in 'Spectrum List Panel'
Save All
Or File
Save All Spectra As – save each spectrum individually
Close

Save as txt and csv file
Filename use N17.xxx

Fix fresh tissue analysed with i-Raman with the remaining intra-operative sample

Slide manual laser shutter to closed

Switch off laser

Turn key to Off position

Remove interlock plug

On/Off power switch to Off position

Remove laser from black box by releasing clamp

Replace protective sleeve on end of probe

Close BW spec software

Log off, turn off computer if no further intra-operative specimens expected

Replace key and interlock plug to drawer, glasses to case

7. References

290020077-G i-Raman Manual T:\Pathology-RPH\Neuropathology - RPH\Data\Research\RTB Brain CNS\Applications for tissue\Approved\1407 Danielle Bury\290020077-G i-Raman Manual.pdf

290020175-E-BAC100 BAC102 Lab Grade Raman Probe User Manual T:\Pathology-RPH\Neuropathology - RPH\Data\Research\RTB Brain CNS\Applications for tissue\Approved\1407 Danielle Bury\290020175-E-BAC100 BAC102 Lab Grade Raman Probe User Manual.pdf

BioPure™ Gold Nanoparticles Safety Data Sheet https://cdn.shopify.com/s/files/1/0257/8237/files/NCX.MSDS-BioPure.Gold.Citrate.pdf?25143

HEALTH AND SAFETY

RISK ASSESSMENT FORM

Locations – Ward/Department Pathology

Personnel Involved In The Assessment Biomedical scientists and pathologists.

1 *Date of initial assessment 02/03/2017*

Date of re-assessment

Date of next review 02/03/2018

Assessor Kate Ashton **Signature**

Hazard and effect of the hazard.

Biohazard – Fresh tissue

All unfixed tissue, intra-operative specimens for smears or frozen sections, that are sent to the department for processing pose a risk of infection that may not be known.

Electrical hazard – Portable Raman machine and PC are plugged in to the mains power

Physical hazard – sharps – glass slides and scalpel blade

Scalpel blades and other dissection equipment are sharp and pose a risk of laceration if not handled carefully

Laser hazard – Laser can cause blindness and burning to the skin and clothing

Who may be harmed and how.

List groups of people who are specifically at risk from the significant hazards identified. Specify numbers

- All grades of BMS staff - 4
- Pathologists - 4

Is the risk adequately controlled?

List existing control measures here or note where the information can be found. E.g. existing policies, procedures, work instructions etc.

Biohazard - Wear appropriate PPE (lab coat and/or protective apron and nitrile gloves) when handling fresh tissue. Handle inside the category 2 safety cabinet. Clean all equipment with 1% Distel and/or 70% alcohol before removing from cabinet.

Electrical - All Electrical equipment is CE marked and PAT tested for safety as per the Trust policy.

Electrical equipment must be isolated from the supply if internal maintenance is to be performed during servicing or repair.

Physical – Handle glass slides with care, dispose in a sharps bin if any edges or corners are broken and when no longer required. Scalpel blades should be handled with care and removed from handle using special box provided in safety cabinet.

Laser – The system has two safety features: a key-activated laser switch and an interlock. These are designed to prevent the user from accidentally turning on the laser. To turn on the laser, insert the interlock plug and insert the supplied key into the key-switch. Turn the key 90 degrees clockwise to turn on the laser. The laser will turn on after a delay of 5 – 10 seconds. The key cannot be removed when it is in the ON position.

When the safety interlock plug is removed from the system, all electrical power to the laser will be turned off. The safety interlock MUST be inserted before the laser can be turned on. Laser emission will stop if the interlock is removed while the laser is on. Laser safety glasses should always be worn when the laser is in use.

Training – Biomedical scientists and pathologists are trained to handle fresh tissue and sharps safely and do so daily.

2. Residual risk rating after controls

Biohazard

P = Probability 1

C = Consequence 2

R = Risk Rating PxC 2

Electrical

P = Probability 1

C = Consequence 1

R = Risk Rating PxC 1

Physical

P = Probability 1

C = Consequence 2

R = Risk Rating PxC 2

Laser

P = Probability 1

C = Consequence 4

R = Risk Rating PxC 4

High (>15)	Significant (8 to 14)	Moderate (4 to 7)	Low (1 to 3)

What further action is necessary to control the risk? List the risks which are not adequately controlled and the actions you will take to mitigate the risks in so far as is reasonable practicable. None	Date to be Taken	Action By

C. Potential Consequence of incident/hazard/estimated financial loss			**P. Probability**
Death or any incident where there may be grounds for taking legal action	Long term sickness	Major financial loss/ National media attention	5. Almost Certain
4. Long term disability or impairment of health (physical or mental)	more than 3 weeks absence.	Major financial loss/ Local media attention	4. Likely
Short term disability or impairment of health (physical or mental)	more than 3 days absence	Minimal financial loss/ Loss of public confidence	3. Possibl

			e
2. Inconvenience/minor injury requiring substantial treatment	less than 3 days absence	Minimal financial loss/ Loss of confidence to those involved	2. Unlikely
No actual harm, no corrective treatment necessary	no absence	No financial loss/ No loss of confidence	1. Rare

1.11.1.2Key		Low Risk		Moderate Risk		Significant Risk		High Risk

Probability	**1**	**2**	**3**	**4**	**5**
Descriptor	**Rare**	**Unlikely**	**Possible**	**Likely**	**Certain**
Frequency Probability	Not expected to occur for years =<5% Chance	Expected to occur at least annually	Expected to occur at least monthly 20-49% Chan	Expected to occur at least weekly 50-	Expected to occur at least daily =>8

	Will only occur in exceptional circumstance.	6-19% Chance Unlikely to occur	ce Reasonable chance of occurring	79% Chance Likely to occur	0% Chance More likely to occur than not

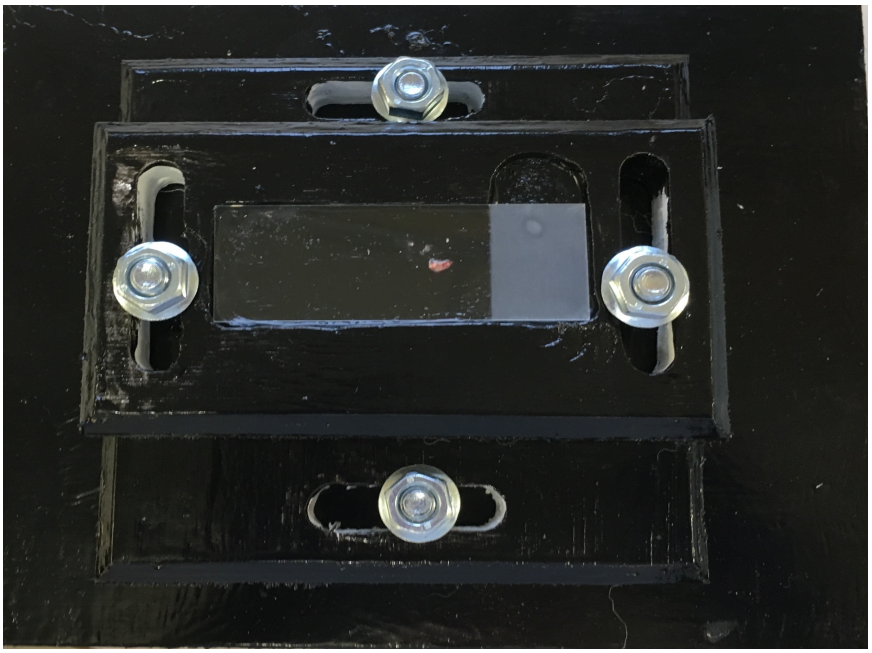
CONSEQUENCES	*1.11.1.3PROBABILITY*				
	1.11.1.4*Rare* 1	*1.11.1.5Unlikely* 2	*1.11.1.6Possible* 3	*1.11.1.7Likely* 4	*1.11.1.8Certain* 5
Negligable – 1	1	2	3	4	5
Minor –2	2	4	6	8	10
Moderate – 3	3	6	9	12	15

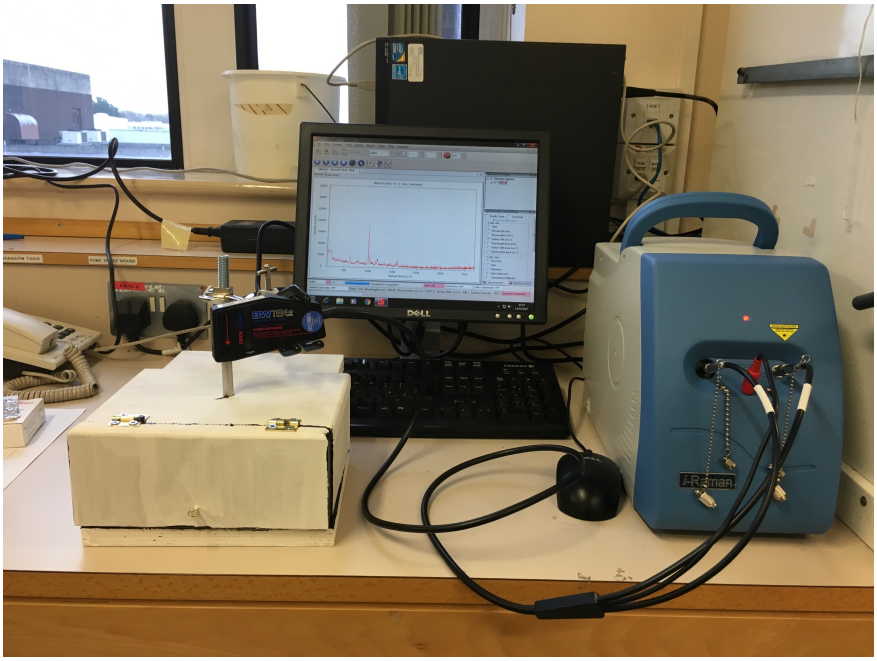
Major –4	4	8	12	16	20
Viability under threat - 5	5	10	15	20	25

9.11 Set up documents for the Hand Held Raman Probe

Prior to using the hand held Raman machine a custom built box was required to ensure darkness when analysing the tissue. As this was being placed into a working laboratory it would not be possible to work in darkness and it would also need to fit into a category 2 fume hood for work with fresh tissue. With this is mind a box was custom engineering (see figure 1) using plywood. A stage was built within to allow the slide to be moved in the x and y planes with custom cut out area for the slide to be held securely. This was to allow the tissue to be accurately positioned under the probe. A clamp was then secured to the box to allow the probe to be moved in the z plane to allow it to be positioned at the correct height above the tissue.


scheppach
scheppach





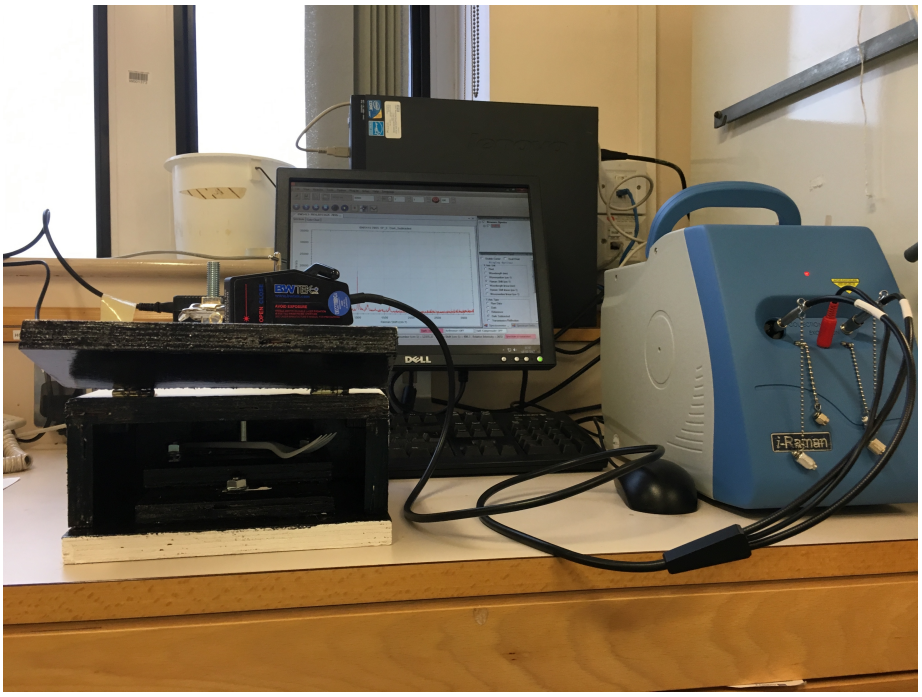
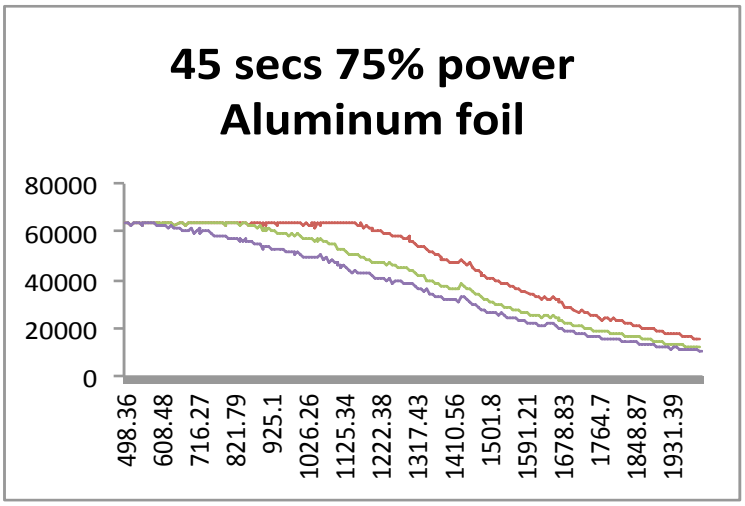
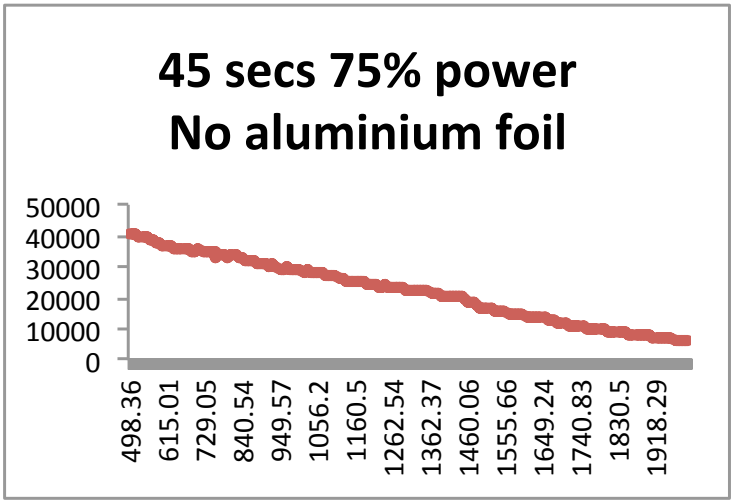


Figure 9.1: Pictures showing the production of the box; from initially cutting the wood, to routing the slide shape, painting to the finished product in situ in the neuropathology department at Royal Preston Hospital.

The box was painted with black paint inside to minimise reflection of any light entering it. It also enabled it to be wiped clean if required.

Initially frozen tissue from a high-grade glial tumour was selected from the BTNW brain bank. This was to ensure no fresh diagnostic material was damaged during the initial work up phase. This also allowed comparison of spectra from the tumour at different settings on the hand held Raman machine. Different laser power and time periods were tested. Raman analysis was performed over a range of exposure times (25-60seconds) and laser power (25-100%). The tissue was thawed prior to use and an amount similar to that used for a smear (less than 5mm in maximum diameter) was placed onto a glass side and compared with a glass slide covered with aluminium foil. This was placed into the Raman box for analysis. Spectra were analysed and tissue processed into a smear and a separate FFPE block to examine for any diathermy or other tissue damage.


45 secs 75% power
Aluminum foil
80000
60000
40000
20000
0
498.36
608.48
716.27
821.79
925.1
1026.26
1125.34
1222.38
1317.43
1410.56
1501.8
1591.21
1678.83
1764.7
1848.87
1931.39


45 secs 75% power
No aluminium foil
50000
40000
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0
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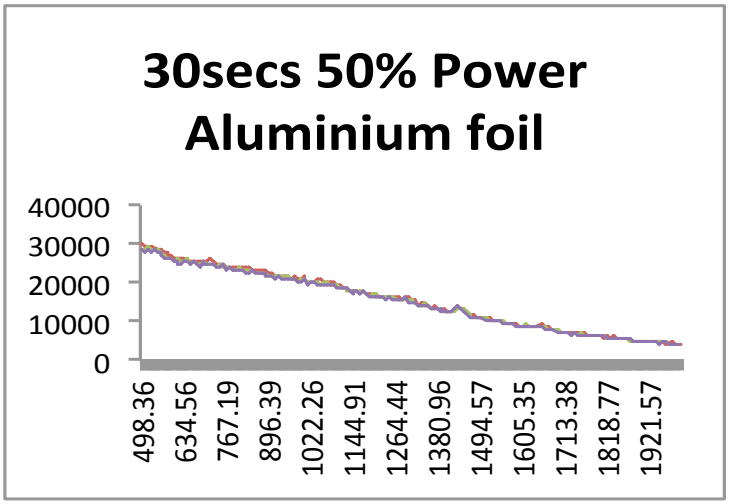
949.57
1056.2
1160.5
1262.54
1362.37
1460.06
1555.66
1649.24
1740.83
1830.5
1918.29


30secs 50% Power
Aluminium foil
40000
30000
20000
10000
0
498.36
634.56
767.19
896.39
1022.26
1144.91
1264.44
1380.96
1494.57
1605.35
1713.38
1818.77
1921.57

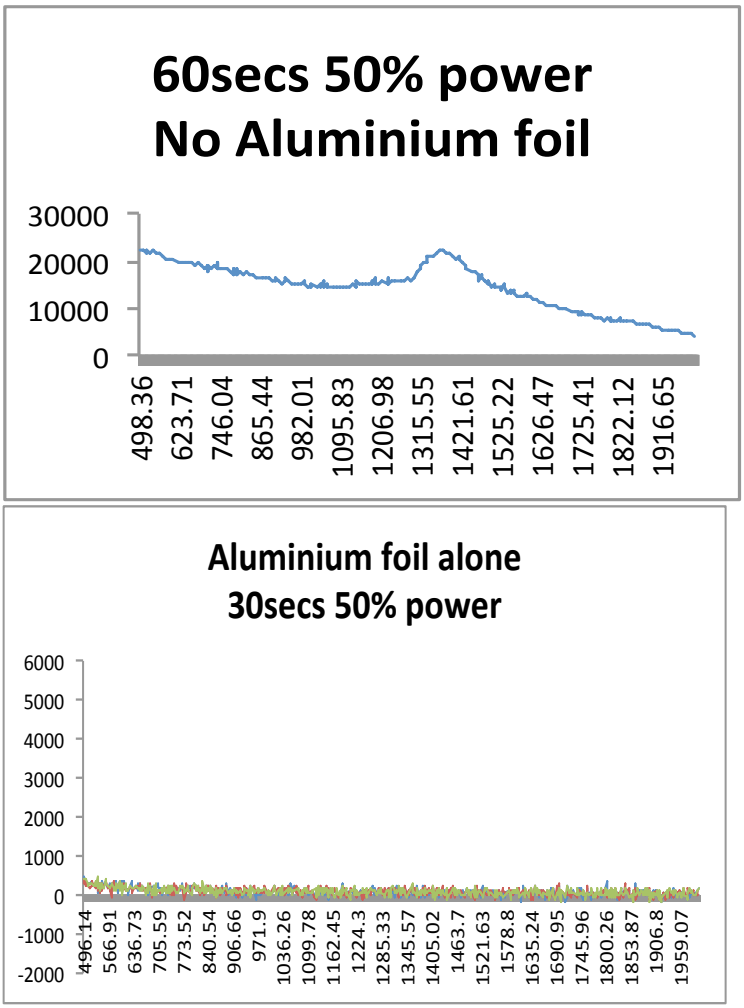


Figure 9.2: Graphs demonstrated a variety of laser power and time using fresh brain tissue.

This demonstrated that the aluminium foil being present improved the quality of the spectra and also that 30 seconds was the optimum time as the graph did not show saturation (straight line seen on 45 second graphs). The power was increased to 75% as this did not damage the tissue. At 60 seconds the graph showed marked distortion though the tissue was undamaged. A graph comprising just aluminium foil was also taken to show this did not interfere with the results. Ten spectra per case were obtained, nanoparticles were then added to enhance spectral quality.

The lack of structural tissue damage was also assessed by a consultant neuropathologist (Prof. T Dawson), to ensure he was happy with the tissue post analysis. Once the settings were determined a standard operating procedure (SOP) document for the use of the Raman machine was produced (above) along with a risk assessment. These were required for it to be used within the laboratory with samples.