



ULTRASOUND PARAMETRIC IMAGING AND
IMAGE ANALYSIS FOR BREAST CANCER
CHARACTERISATION AND TREATMENT
MONITORING

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This thesis is submitted to the Department of Engineering Science, University of Oxford, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The thesis is entirely my own work and, except where otherwise stated, describes my own research.

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Abstract

Breast cancer research based on medical ultrasound has traditionally focused on providing early diagnosis and breast cancer classification/characterisation. However, with the advances in cancer therapy treatments and innovations in cancer drug developments, developing methods for treatment monitoring is becoming ever more important. In particular fibrotic change in breast cancer is a common after-effect that accompanies successful breast cancer chemotherapy treatment where breast tumour cells are eradicated and replaced by fibrous tissue. As a result, the ability to monitor fibrotic changes can be used to indicate the effectiveness of chemotherapy treatments.

Ultrasound spectral parametric imaging is a method that looks at the information embedded in the frequency/spectral domain of ultrasound RF signals and can be used to characterise tissue ultrasound backscattering properties. In this thesis ultrasound spectral parametric imaging is first applied to characterise breast fibrosis and its efficacy for monitoring breast cancer neoadjuvant chemotherapy treatment is subsequently investigated in a pilot study. The pilot study suggests that an increase in ultrasound spectral intercept is able to indicate fibrotic

changes before and after treatments. These encouraging results suggest further work is considered to determine the suitability for monitoring intermediate changes.

As histopathology images are considered as the gold standard in breast cancer pathology, ultrasound parametric images need to be studied and compared against histopathology so information provided in ultrasound parametric images can be better understood. A new registration method is shown to improve the alignment of ultrasound parametric images and histopathology images that facilitates the comparison between the images. The registration method is based on the coherent point drift (CPD) algorithm and the thin plate spline (TPS) method.

All of the results show that ultrasound spectral parametric imaging is a promising tool for providing further understanding of breast cancer changes during therapy, which in turn will lead to improved breast cancer treatment monitoring and planning.

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Chapter 1: Introduction

1.1 Motivation

Ultrasound B-mode imaging is an important and widely used imaging modality for breast cancer diagnosis and treatment monitoring. It has the advantage of being more affordable than other imaging modalities such as magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET). In addition, it is non-invasive and does not bear the risk of radiation such as mammography, and has short scan times and is portable. Because of the variability in scanning and machine parameters, performing ultrasound scans and interpreting ultrasound B-mode images requires extensive training and experience.

Ultrasound B-mode imaging provides an image of relative echogenicity of the tissue by using only the amplitude of the raw backscattered ultrasound radiofrequency (RF) signal. However, additional information can be extracted from the RF signal in the frequency domain that provides useful information on the tissue. Ultrasound spectral parametric imaging is a technique that computes parameters in the frequency domain that characterise the underlying tissue scattering properties (Lizzi *et al.*, 1983 + other refs) and has been shown to

complement ultrasound B-mode imaging for diagnosis in a number of clinical applications (Feleppa *et al.*, 1988, 1996, Alam *et al.*, 2002).

When it comes to monitoring the effectiveness of breast chemotherapy treatments, ultrasound B-mode imaging is used to measure the approximate change in tumour size based on the appearance in B-mode as a rough guidance for response¹. However, effectiveness of treatments do not only manifest itself through the change in tumour size, cancer cell death and fibrotic changes can take place that may be measured by looking at the scattering properties of the tumour. If additional parameters other than the B-mode size of the tumour can give indications to the effectiveness of the treatment, it would improve treatment planning since patients typically receive a few courses of a particular type of drug treatment and if it is not effective, a different type of drug treatment is applied. Hence there is a great need to develop additional tools that can complement B-mode imaging for treatment monitoring.

In addition to developing new parametric imaging methods to complement B-mode, understanding information provided by the new methods is necessary.

¹ It is well known that boundaries in B-mode arise from differences in acoustic properties of the medium and is not the same as that in histopathology. Hence size measurements in B-mode may not accurately represent the physical size of the tumour.

Histopathology is regarded as the gold standard when it comes to breast cancer diagnosis and the evaluation of chemotherapy treatments. In order to understand the information provided by parametric images, they need to be compared against histopathology images. The first step requires the images to be aligned to facilitate the comparison. Registering ultrasound parametric images and histopathology images is a challenging task since ultrasound-based images typically lack distinct anatomical structures and tissues in histopathology images have undergone complex deformation during preparation. An image registration method for aligning ultrasound parametric images and histopathology images would therefore greatly benefit the comparison and understanding the information in ultrasound parametric images.

1.2 Aims & objectives

The primary aim of this thesis is to investigate the use of ultrasound spectral parametric imaging as a tool for providing additional information during breast neoadjuvant chemotherapy treatments. The initial investigation examines the efficacy of ultrasound spectral parameters for the characterisation of breast fibrosis with subsequent application to monitor fibrotic changes in the tumour. In addition, an effective image registration method to align ultrasound parametric images to histopathology images.

1.3 Contributions

This thesis presents several contributions to research in ultrasound parametric imaging and monitoring effectiveness of breast cancer neoadjuvant chemotherapy treatments:

- A method based on ultrasound for the characterisation of breast fibrosis (Chapter 3)
- A detailed framework and approach for monitoring changes in breast tumour throughout neoadjuvant chemotherapy treatments using ultrasound spectral parameters (Chapter 4)
- A novel image registration method for the registration of ultrasound parametric images to histopathology images (Chapter 5)

The image registration method (chapter 5) for aligning ultrasound parametric images and histopathology images and the preliminary results were published in the below:

Chuang, B *et al.*, “Interpreting ultrasound elastography: image registration of breast cancer ultra- sound elastography to histopathology images”, *Proceedings of the IEEE International Symposium on Biomedical Imaging 2010*.

1.4 Organization

This thesis consists of 6 chapters. Chapter 2 will provide the reader with the

relevant background information in both engineering and clinical terms to understand and appreciate the fundamental knowledge and the challenges in breast cancer research. Chapter 3 will present the work on using ultrasound spectral parametric imaging to characterise breast fibrosis. Chapter 4 extends the findings in chapter 3 and proposed a detailed framework to investigate extensively the use of ultrasound spectral parameters for monitoring breast neoadjuvant chemotherapy treatment. Chapter 5 presents the novel non-rigid image registration method based on the coherent point drift (CPD) algorithm (Myronenko and Song, 2009) and thin plate spline (TPS) to align ultrasound parametric images to histopathology images. A summary of the thesis and future work are presented in the final chapter.

Chapter 2: Background

2.1 Introduction

This chapter aims to provide the reader with background knowledge relevant to the rest of the thesis in breast cancer pathology and also imaging and image analysis techniques for breast cancer diagnosis and monitoring. Section 2.2 introduces the biology aspects of breast cancer and also breast cancer management and existing methods of monitoring breast chemotherapy treatments. Section 2.3 discusses the technical background of ultrasound imaging and section 2.4 gives examples of developments in ultrasound parametric imaging such as ultrasound elastography and ultrasound spectral imaging and other related work in the area. Section 2.4 provides background on image registration techniques and in particular the coherent point drift (CPD) algorithm and thin-plate spline technique that are used in chapter 3.

2.2 Breast Physiopathology

2.2.1 Breast anatomy and cancer

Breasts are modified sweat glands that produce milk in women, though also in rare cases, in men. A breast is made up of mainly glandular and adipose (fat) tissues in varying proportions. It can be seen from Figure 2 - 1 that several lobules

are linked by ductules, which combine to form the lactiferous ducts and open into the lactiferous sinuses and empty through the nipple. A breast consists of two layers of fibrous tissue – a superficial and a thick deep layer that are connected by Cooper's ligaments.

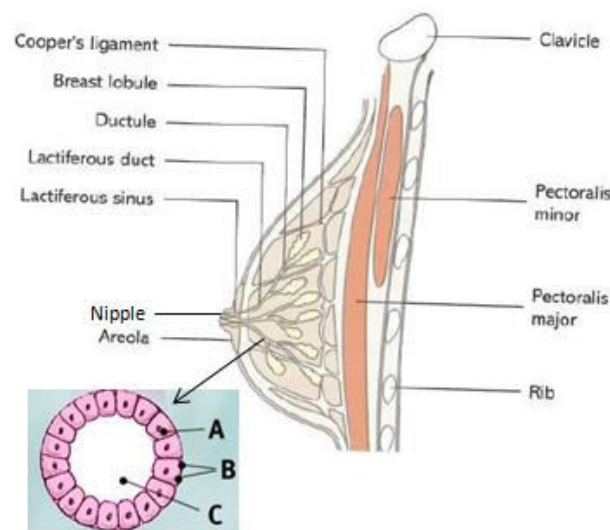


Figure 2 - 1. Anatomy of the breast. The large figure shows the macroscopic structures that form the breast including network of ducts that connect the lobules. The inset shows the structure of the ducts with A) the duct cells, B) the basement membrane, and C) the lumen (Baum and Schipper 2005)

Breast cancer is the second most common cause of cancer-related death for women in the UK accounting for more than 10,000 lives in 2010 (breast cancer research UK). Like other forms of cancer, breast cancers are a result of DNA mutation that leads to abnormal and uncontrollable growth of cells (Weinberg, 2007). The initiating cause of mutation can be due to many factors such as exposure to radiation and chemicals. Cancer cells have the potential to invade

nearby tissues and spread to distant parts of the body via blood vessels or the lymphatic system, a process termed metastasis.

Cancer cells can be classified as being invasive and non-invasive. In the case of breast cancer, cancer cells that remain within the basement membrane of ducts and lobules (Figure 2 - 1 inset B) without spreading into the surrounding breast tissues are classified as *in situ* or non-invasive. On the other hand, an invasive cancer is when cancer cells spread into surrounding tissues outside of the basement membrane of the ducts and lobules (Dixon, 2006). However, in the early stages of breast cancer development, most cancer cells are categorized as non-invasive until the “invasiveness” is observed. This means that a non-invasive cancer can develop into an invasive form, and it is the clinician’s task to monitor the tumour for any signs of invasiveness.

The two main forms of non-invasive breast cancer are ductal carcinoma in situ (DCIS), which is confined to the lactiferous ducts of the breast and lobular carcinoma in situ (LCIS), which is found in the lobules of the breast. The term carcinoma defines cancers cells that arise in the epithelium layer of the tissue. DCIS is the most common form of non-invasive breast cancer, accounting for 20-

25% of screen detected cancers; in contrast, LCIS is rare, accounting for only less than 1% of screen detected cancers (Dixon, 2006). Similarly, invasive breast cancers are categorised into invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC). Although evidence now suggests both IDC and ILC arise from the terminal duct lobular unit (Sims *et al.*, 2007), which renders the classification less appropriate, this classification is still widely used. A more appropriate method for classifying invasive breast cancers is based on features whereby tumours with distinct histopathology features are termed invasive carcinomas of special type and no special type otherwise (Table 2-1). In addition, breast cancers are categorised into four stages based on their invasiveness, with stage 0 describing non-invasive cancers, stage 4 representing cancers that have spread to other organs of the body and the intermediate stages (1,2 and 3) classified based on the appearance of the primary tumour and the involvement of the lymph nodes (Breastcancer.org).

It is common to find that the suspected lesions are non-cancerous; in fact, only about 20% of the lumps detected in clinical palpation are real cancers (Baum and Schipper, 2005). About one in five women develop some form of benign breast disease at some point in their life (breakthrough.org.uk). The most common

benign breast diseases are cysts and fibroadenomas. Cysts are fluid filled sacs and are usually lobular in shape. The fluid within the cavity can travel within the breast and hence the size of cysts can change over time. Fibroadenomas are the growth of fibrous scar tissues and possesses a rubbery texture, which can appear mobile during palpation.

Various other types of benign conditions and terminology are used in classifying benign breast conditions depending on the histopathology; but as chapter 3 of this thesis is concerned with breast fibrosis, some related benign conditions will be mentioned here. Breast fibrosis is characterised by thickening or increase in the density breast tissue and fibrous components. Causes of fibrosis can be due to hormone fluctuations and also as a response to injury and treatments. Fibroadenosis, also known as fibrocystic disease, is a term commonly used to describe cases that on the histopathology level consist of a combination of fibrosis and cystic components. Traumatic fat necrosis (TFN) is another condition related to fibrosis in which due to injury and the resulting inflammatory process, damaged fat tissue in breasts are replaced by fibrosis.

Special type	No special type
• Tubular • Muroid /mucinous • Cibriform • Papillary • Medullary • Classic lobular	• Commonly known as NST or NOS (not otherwise specified) • Useful prognostic information can be gained from grading such cancers

Table 2- 1. Classification of invasive breast cancers based on features (Baum and Schipper, 2005)

2.2.2 Breast cancer diagnosis

Since there are currently no approved methods for breast cancer prevention, early diagnosis becomes critical in reducing the mortality rate. Triple assessment, which combines clinical examination, medical imaging and pathological evaluation, is the current standard procedure for breast cancer diagnosis and is thought to provide confident diagnosis in 95% of cases (Baum and Schipper, 2005). The first step involves manual assessment of the lump (palpation) by a clinician that allows them to distinguish between a distinct lump and normal tissues. This is followed by either X-ray mammography or an ultrasound scan for patients under the age of 35, as breasts in younger women are too dense for X-ray mammography to be effective. In the UK, women who are under 50 and thought to be of high-risk (such as having family history in breast cancer), magnetic resonance imaging (MRI) scans are also available as an alternative to ultrasound imaging and X-ray mammography. If the diagnosis remains unclear, either the imaging step is repeated or a fine needle aspiration

cytology or core-cut biopsy is performed (NHSBSP, 2003).

However, despite the advances made in the area of medical imaging, in many cases, the ultimate decision-making step is down to the histopathology evaluation step, which requires some form of biopsy to extract samples of breast tissue from the area of interest. This holds for any stage of breast cancer, whether it is the initial diagnostic stage, post treatment stage or during therapy monitoring.

2.2.3 Breast cancer therapy and treatments

Most patients will undergo a combination of local treatments to control local disease and systemic treatment to combat any micro-metastatic disease, which is the undetectable spread of cancer outside of the breast and has yet created enough mass to be detected. (Baum and Schipper, 2005). Local treatments consist of surgery and radiotherapy. In the case of surgery, it can be a wide local excision (breast conservatory) or mastectomy (remove the whole breast), depending on breast size, number of lesions, aggressiveness of the cancer, and the willingness of the patient to accept higher rates of tumour recurrences after breast conservatory operation and radiation (Baum and Schipper, 2005).

Radiotherapy is given to patients after surgery to reduce the chance of reoccurrence.

Systemic treatment includes hormonal therapy, chemotherapy and also other methods. The treatment can be adjuvant (after local treatments) or neo-adjuvant (before local treatments) (Mauri *et al.*, 2005). In adjuvant therapy, the main aim is to reduce the risk of both local and systemic reoccurrence of cancer. Neoadjuvant therapy is of particular interest in cancer management because it aims to downstage large cancers and to reduce the need for mastectomy. Another aim is to provide patient-tailored treatments by studying the effectiveness of specific types of treatment on individual patients since the effectiveness in individual patients in adjuvant treatment cannot be assessed, as there is no advert disease to monitor. In addition, trials on the effects of adjuvant cases take years to produce results, whereas neo-adjuvant effect should be fairly immediate by observing the primary tumour.

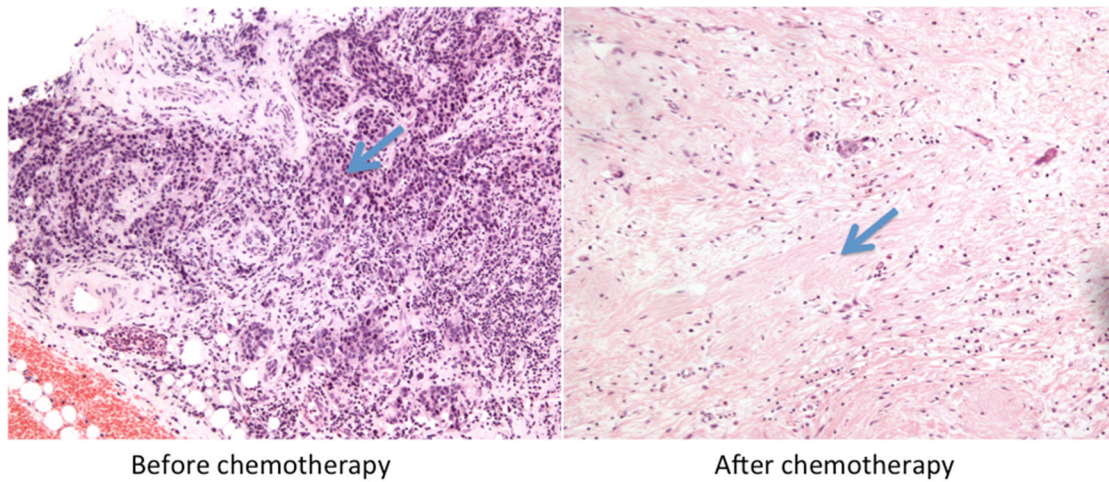


Figure 2 - 2. Histopathology images of a breast cancer patient (left) before and (right) after therapy treatment. The images illustrate the existence of islands of tumour cells before the therapy and the subsequent disappearance after the treatment and are replaced by fibrous tissue as pointed out by the blue arrows in the images.

From the histopathology point of view response to treatment is reflected in the physiological changes taking place at the cellular level whereby tumour cells are eradicated by treatments and usually replaced by fibrosis (Fan, 2009).

Figure 2 - 2 provides an example of histopathology images before and after a patient has undergone successful chemotherapy treatments. It can be seen from the figure that islands of cancer cells before the therapy have disappeared after the treatments and mostly left with necrotic cancer cells along with fibroblasts and their associated connective tissue. It is with this observation in mind that in chapter 3 and 4 we investigate methods that are able to characterise fibrosis as a proxy to monitor response to cancer treatments.

2.2.4 state of the art of medical imaging for treatment monitoring

The current state-of-the-art imaging techniques for monitoring tumour response

to treatments include dynamic contrast-enhanced Magnetic resonance imaging (DCE-MRI) and fluorodeoxyglucose positron emission tomography (FDG-PET). The principle behind these methods involve the use of contrast agents to measure tumour perfusion and also capillary leakage as tumour cells are well known for their ability to create their own blood supplies via a process called angiogenesis.

Both methods have been investigated for monitoring neoadjuvant chemotherapy and have shown that as a tumour responds to treatments, the uptake of the contrast agent used decreases (Pickles *et al.*, 2005; Drew *et al.*, 2001; Martincich *et al.*, 2004, Berriolo-Riedinger *et al.*, 2007; Schwartz *et al.*, 2005). Despite the initial success claimed by the studies, further investigations are required for instance, to formulate the threshold value needed to distinguish between response and no-response cases as the studies have so far designed the threshold values retrospectively. Other disadvantages of the methods include the requirement of contrast agent to be injected and as with most medications, contrast agents can have adverse effects such as nephropathy and allergies that would be a concern if frequent scanning is required during the treatment period. In comparison to ultrasound-based methods that are often very easy and quick to

carry out (typically within 10 minutes), DCE-MRI and FDG-PET require much longer scanning and preparation time, and in the case of FDG-PET 24 hour fasting is often required to obtain a stable blood sugar level. Furthermore, the costs of the scanners required, operating costs a patient throughput may limit the availability of the techniques. Nonetheless, these imaging methods provide in depth information of tumour metabolism and have shown promise in detecting therapy response at the early stages and will further develop as valuable research and potential clinical tools in the future in cancer and therapy monitoring.

More recently ultrasound based methods such as ultrasound elastography (Di Battista, 2013; Falou *et al.*, 2013; Hayashi *et al.*, 2012) have been investigated as non-invasive methods for monitoring breast neo-adjuvant chemotherapy treatments whereby the strain ratio between the core and the periphery of the tumour decreases as tumour respond to treatments. The work in chapter 4 follows the idea in developing an affordable and non-invasive technique based on ultrasound for monitoring breast neo-adjuvant chemotherapy treatments.

2.3 Ultrasound imaging

2.3.1 Imaging axes of ultrasound imaging

This section provides the reader with the terminology of the planes used in medical and ultrasound imaging. The left image in Figure 2 - 3 illustrates the sagittal plane, which is a vertical plane that runs between the left and the right hand side of the body, the transverse plane, which is a horizontal plane that runs between head and toes, the coronal plane, which is a vertical plane that runs between front and back of the body. The right image in Figure 2 - 3 shows the ultrasound scanning axes where the axial direction is the one that is perpendicular to the face of the probe, the lateral direction is the one that is along the face of the probe and the elevational direction is the one that is parallel to the face of the probe. It is important to note that a 2D probe only images the axial-lateral plane.

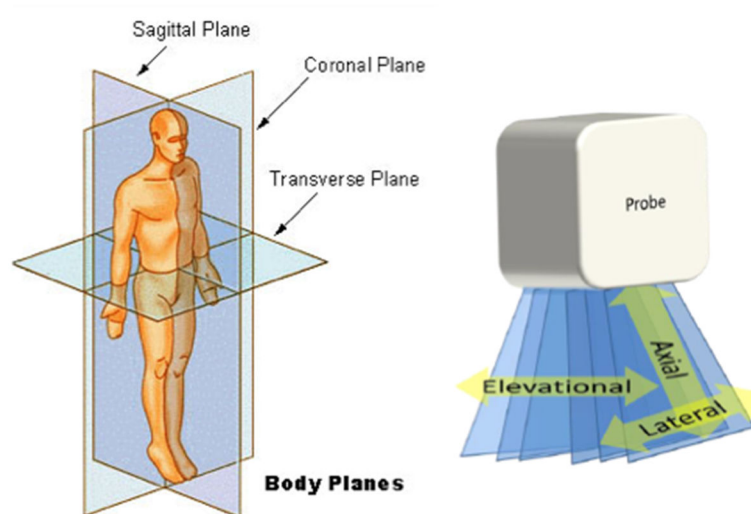


Figure 2 - 3. (Left) Body planes and (Right) ultrasound imaging axes

2.3.2 B-mode image formation

Ultrasound imaging is the most widely used modality today in medical diagnosis for applications such as echocardiography imaging (Nishimura *et al.*, 1985; Ommen *et al.*, 2000), breast imaging (Jackson, 1990; Kuhl *et al.*, 2005) and scanning for obstetrics and gynaecology (Steiner *et al.*, 1994; Stevenson *et al.*, 2010). The most common mode of ultrasound imaging is the Brightness-mode (B-mode), which is a spatial map of the echo intensity within the selected plane or volume and is produced by emitting sound waves at very high frequency (MHz range) and processing the received backscattered sound waves to produce a grey-level image in 2D or 3D (Halliwell, 2010; Wells, 2006).

To produce a B-mode image, the transducer probe transmits and receives ultrasound via an array of tiny piezo-electric elements that convert electrical signal into mechanical (vibration) signal and vice versa. A component called the beamformer works in both transmit and receive, in the transmit mode, it controls which group of elements in the array are excited and the appropriate focusing of the transmit wave is produced. In the receive mode, it acts as a spatial filter to focus on signals from a particular direction for analysis. It also corrects for the attenuation effect on ultrasound waves by applying a process called time gain

compensation (TGC), which basically gives weighting based on the time of arrival of the sound wave. The resulting collected signals for every axial line in the image are termed radio-frequency (RF) signals and the envelope/amplitude of the resulting signal is then extracted. Dynamic range compression is then applied to optimize the contrast in the grey-scale image to produce the B-mode image.

In ultrasound B-mode images, lesions usually appear as hypo-echoic regions (dark regions). However, it is a challenging task in breast cancer diagnosis as benign lesions such as cysts and fibroadenomas can also appear hypo-echoic in the B-mode. Figure 2 - 5 provides examples of the appearance in ultrasound B-mode and shows the lesions appear to have similar levels of echogenicity. Thus the interpretation of ultrasound B-mode images requires experienced clinicians and extensive training. Clinicians usually incorporate additional information that they learnt from experience such as the lesion shape and the texture within the lesion as guidelines for interpreting the images. However, the interpretation is still very much subjective and can vary between different clinicians and to make things more difficult, different image processing steps used to produce the final image in different machines and also differences in the specs and configurations used such as the ultrasound frequency and beamforming techniques affect the

quality and details of the image. It is with this in mind that parametric imaging techniques are developed and investigated with the aim to provide supplementary information that aim to be more quantitative and more objective.

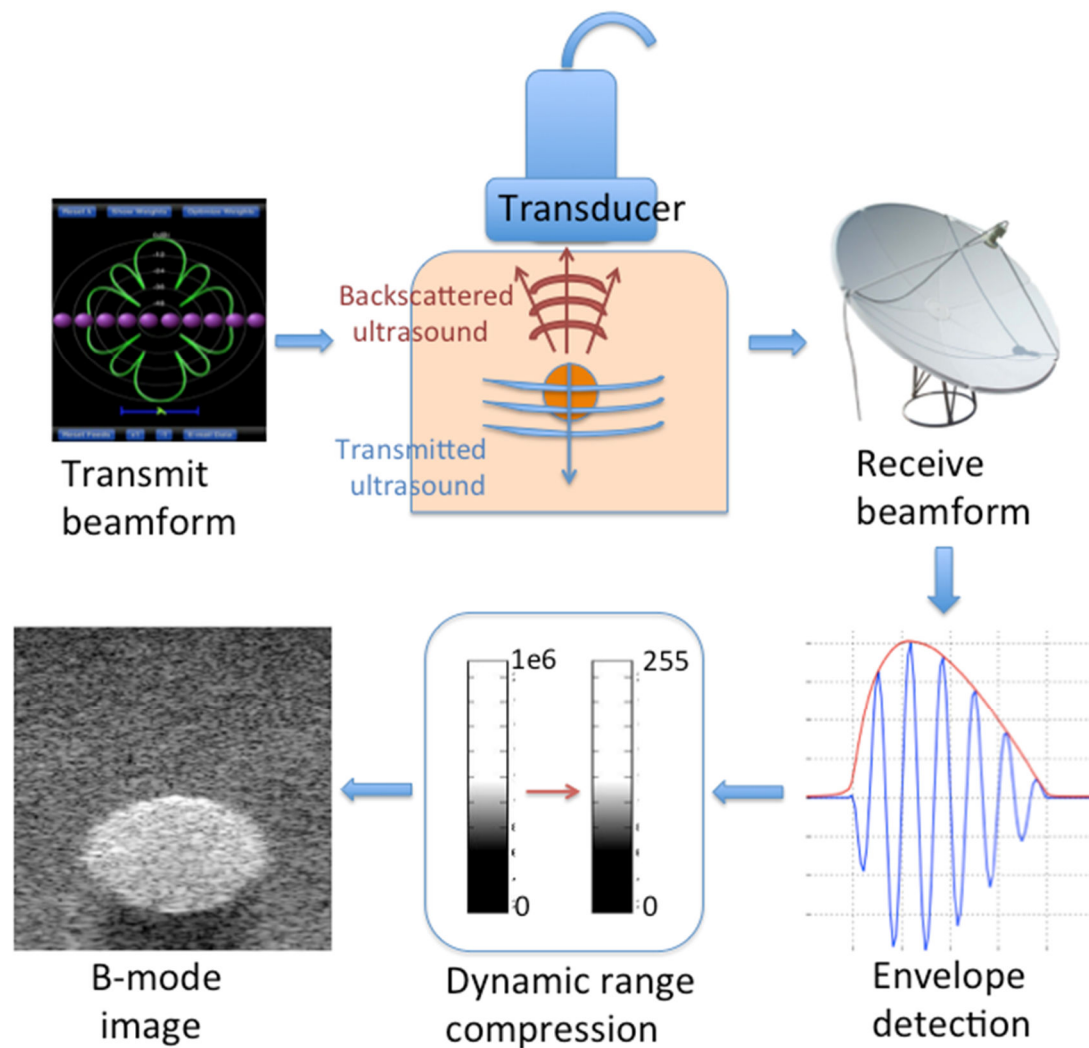


Figure 2 - 4. Ultrasound B-mode image formation

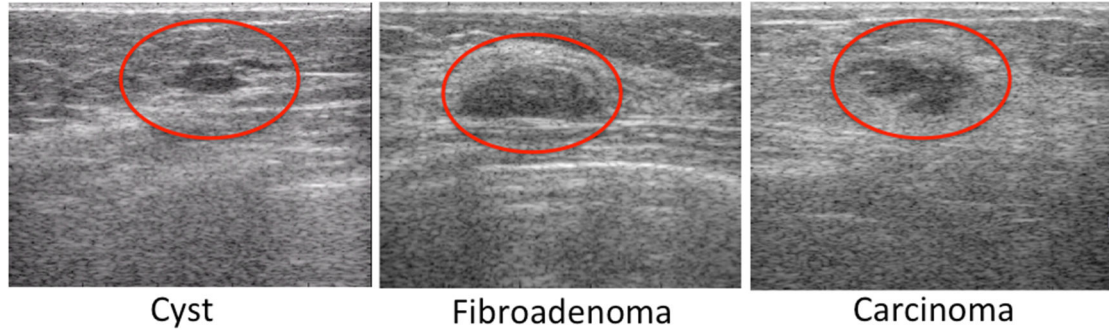


Figure 2 - 5. Examples of breast ultrasound B-mode images. The lesions are highlighted within the red circles and are (left) Cyst (middle) fibro-adenoma (right) Carcinoma.

2.3.3 IQ demodulation and envelope detection

The ultrasound machine (Zonare Z.one, ZONARE Medical Systems, Inc., Mountain View CA, USA) used in the fibrosis characterisation and the neoadjuvant treatment monitoring work in this thesis (chapter 3 and 4) uses a method called in-phase quadrature (IQ) demodulation for extracting the envelope of the signal. IQ demodulation also allows for down sampling of the RF signal while preserving the information in the signal for more efficient storage of data.

The real-value RF signal, $x(t)$, can be expressed by a general sinusoidal wave function.

$$x(t) = A(t) \cos(\omega t + \phi(t)) \quad (2.1)$$

$A(t)$ is the amplitude of the signal, ω is the angular frequency and $\phi(t)$ is the phase component . The signal is mixed with a complex sinusoidal, $e^{j\omega t}$, giving a complex signal $x'(t)$

$$x'(t) = A(t) \cos(\omega t + \phi(t)) e^{-j\omega t} \quad (2.2)$$

By using Euler's formula and trigonometric product as sums equations, equation 2.2 can be expressed as the sum of a real and imaginary part

$$x'_{REAL}(t) = \frac{A(t)}{2} ((\cos(2\omega t + \phi(t)) + \cos(\phi(t))) \quad (2.3)$$

$$x'_{IM}(t) = \frac{A(t)}{2} (\cos(2\omega t + \phi(t)) - \sin(\phi(t))) \quad (2.4)$$

After applying a low pass filter to remove the $2\omega t$ component, we have the real part as the in-phase (I) component and the imaginary part as the quadrature (Q) component.

$$I(t) = \frac{A(t)}{2} \cos(\phi(t)) \quad (2.5)$$

$$Q(t) = \frac{-A(t)}{2} \sin(\phi(t)) \quad (2.6)$$

Both signals are baseband and can hence be down-sampled and processed at much lower sampling rates. The amplitude and the phase information are preserved in the IQ demodulated signal and can be found by taking the absolute value and the argument of the complex signal after low pass filtering. The real value RF signal can also be reconstructed using the IQ signal. The ultrasound

machine used for the fibrosis characterisation and neo-adjuvant study provides a research package that allows for the access of the raw IQ demodulated signal.

2.4 Ultrasound parametric imaging and related developments

As mentioned in section 2.3.2, breast cancer diagnosis based on B-mode imaging is often subjective and can vary between clinicians, and although size change can be observed in ultrasound B-mode images for treatment monitoring, it is less clear whether any pathological changes take place (Figure 2 - 6). Ultrasound parametric imaging techniques have been studied to provide complementary tools to B-mode imaging. Most of the methods developed utilise the raw RF signal or the IQ demodulated signal as the source of analysis since they have not been post-processed unlike the B-mode image. In this section we introduce ultrasound elastography and ultrasound spectral parametric imaging as main examples since these two methods are the focus in this thesis.

2.4.1 Ultrasound elastography

Ultrasound elastography typically aims to estimate the stiffness of the imaged medium and is useful for imaging various types of cancers and diseases (Itoh *et al.*, 2006; Lyshchik *et al.*, 2005; Taylor *et al.*, 2005; Guibal *et al.*, 2012) because cancers are found to be stiffer than normal tissues (Wellman *et al.*, 1999; Samani

et al., 2007). It is this observation that is the key behind the breast palpation method for examining breast cancer for centuries. There are two main categories of ultrasound elastography: quasi-static (Ophir *et al.*, 1991; Kadour *et al.*, 2009; Di Battista and Noble, 2011) and dynamic methods (Nightingale *et al.*, 2003; Bercoff *et al.*, 2004).

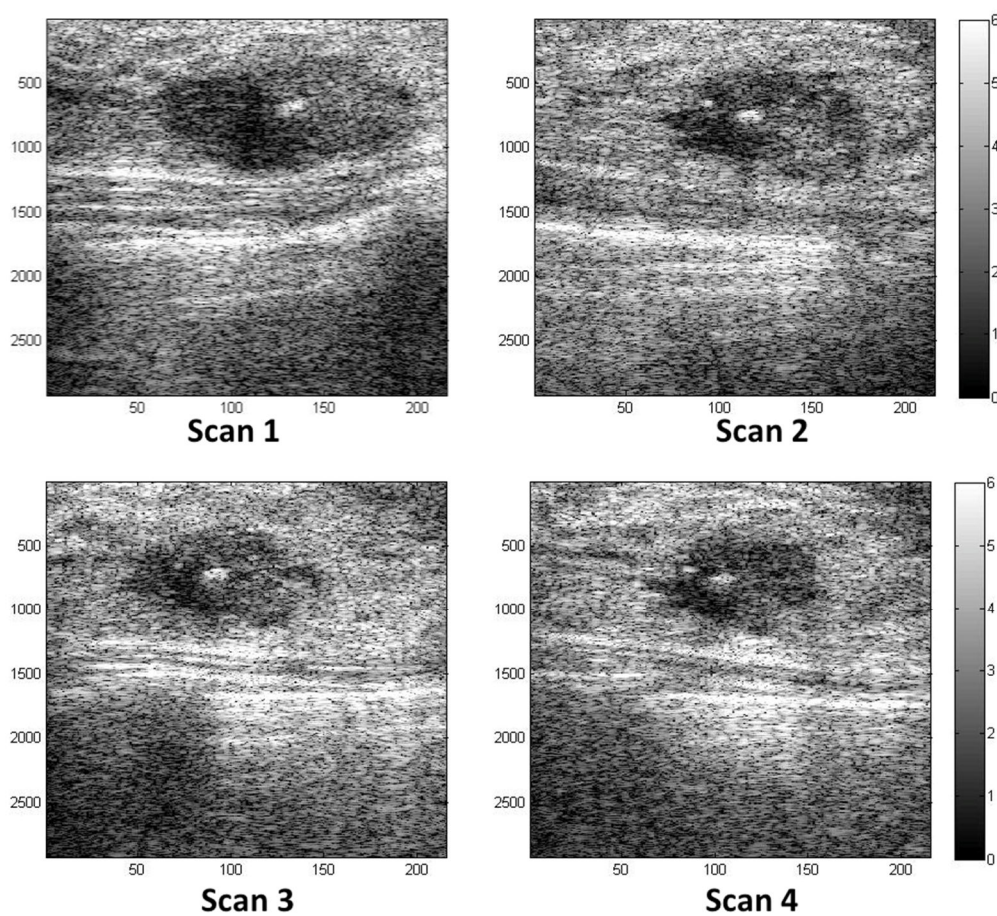


Figure 2 - 6. Example ultrasound B-mode images for the first 4 scans of a neoadjuvant chemotherapy study patient (P1 sagittal plane in chapter 5). It can be seen that although a clear change in size can be observed between the 1st and the 4th scan, it is more difficult to determine the intermediate size change between scan 2 and scan 3 for example. In addition, although the tumour reduces in size, it is unclear whether pathological changes occur within the tumour. Ultrasound parametric imaging is developed with the aim to provide additional information to that provided in the B-mode.

Quasi-static methods involve external compression of the tissue using an ultrasound probe and a strain image is produced by comparing the pre-compression and post-compression RF signals using either time-delay estimation (Cespedes *et al.*, 1995; Viola and Walker, 2003) or spectral methods (Hoyt *et al.*, 2005; Di Battista and Noble, 2011) to produce estimates of tissue displacement indicating relative tissue stiffness in the region of interest (ROI). The idea goes that the stiffer the medium, the less it will move and vice-versa. Dynamic methods use focused ultrasound, which requires additional configurations to remotely create a localised force within the tissue and estimate the speed of the shear wave propagation to model the tissue elasticity (Nightingale *et al.*, 2003; Bercoff *et al.*, 2004, McAleavey *et al.*, 2007). Assuming the model holds, dynamic methods provide quantitative measures of tissue elasticity whereas the quasi-static methods only provide a strain image and not an image of tissue stiffness. However, the accuracy of the so claimed quantitative measures would require further validation for *in-vivo* imaging as real-life tissues are very heterogeneous whereas the models assume homogeneous mediums.

Quasi-static methods have become a popular choice for clinical studies and are implemented in several commercial ultrasound diagnostic machines (Hitachi Hi-

RTE, Siemens eSie Touch™ and GE LOGIQ E9) due to its simplicity where no extra equipment setup is required. The efficacy for cancer detection and treatment monitoring has been evaluated in many clinical studies (Garra *et al.*, 1997; Itoh *et al.*, 2006; Zhi *et al.*, 2007, English *et al.*, 2011) and has been shown to reduce the number of unnecessary biopsies needed and improve cancer diagnosis and treatment monitoring when used together with conventional B-mode images. The elastography images used in the work in chapter 5 were acquired using the quasi-static method.

2.4.2 Ultrasound spectral parametric imaging

Ultrasound spectral parametric imaging is a method that analyses the distribution of power as a function of frequency from backscattered ultrasound RF signals. The power spectrum is usually calculated using short-time Fourier analysis (Welch, 1967) and is then normalised using a power spectrum obtained from a reference phantom using the same system settings as used to acquire the RF signal of the medium (Yao *et al.*, 1990). The assumption behind the calibration is that the spectrum of the ultrasound RF signal can be modelled as linear multiplications of all the transfer functions involved, such as the tissue backscattering and ultrasound system settings. The normalisation hence removes the contributions of the system settings and the ultrasound diffraction

terms as RF signals are usually acquired using different machine, probes and settings. Linear regression analysis is then performed on the logarithm of the calibrated spectrum to characterise the spectrum.

Two main parameters are extracted from linear regression analysis of the calibrated power distribution: the spectral gradient, which is the slope of the regression line, and the spectral intercept, which is the y-axis (dB) value at 0 MHz of the regression line. A third parameter called Mid-band fit (MBF) is defined as the interpolated y-axis dB value at the central frequency, and is often added as an extra parameter for analysis. The MBF is directly related to a well-known parameter in the literature called integrated backscatter (O'Donnell *et al.*, 1979, Lizzi *et al.*, 2003) that represents the total reflected ultrasound energy from a region.

The method has been studied extensively since the 1970s by Lizzi and colleagues (Lizzi *et al.*, 1983, 1987) and has been described in terms of a theoretical scattering framework (Lizzi *et al.*, 1983) to show that empirically- derived spectral parameters from linear regression analysis of the power distribution relate to the underlying tissue microstructure. Based on Lizzi's theoretical framework, the spectral gradient is dependent on the effective scatterer size of

the medium and frequency-dependent attenuation effect; and the spectral intercept is dependent on the effective scatterer size, acoustic impedance and the concentration of scatterers, and is unaffected by the attenuation effect, assuming the attenuation is linearly dependent of frequency (Lizzi *et al.*, 1987). Lizzi's model assumes the imaged medium being statistically homogeneous and its statistics exhibits wide-sense stationary property and hence stochastic tissue microstructure. The model also assumes the analysed segment is located in the focal zone of a moderately focused transducer, typically of most diagnostic ultrasound.

Many studies have examined the use of ultrasound spectral parameters in clinical applications such as ocular applications (Feleppa *et al.*, 1986, 1988), prostates (Feleppa *et al.*, 1996, 2001, 2011) and breasts (Alam *et al.*, 2002, Liu *et al.*, 2010). Most studies have focused on using the parameter as a tool for classification between cancerous and benign tumours and have concluded that the parameters are useful in providing additional information to B-mode ultrasound for diagnosis and are combined with other parameters in classifiers to provide better diagnostic results.

Based on Lizzi's framework, Insana *et al.*, (1990) extended the model by introducing Faran's scattering model and correlation functions term "form factors" to estimate scatterer size of the medium. Oelze *et al.*, (2002) later proposed a method based on the extended work by Insana *et al.*, (1990) to estimate the average scatterer diameter and the average scatterer concentration by comparing the power spectrum of the medium to a theoretical power spectrum. However, the methods make extensive modelling assumptions that are difficult to apply in real-life scanning such as that the scatterers in the medium following a Gaussian scattering model and also the medium exhibit constant attenuation property. In contrast, Lizzi's parameters based on linear regression of the calibrated log power spectrum although more simplistic, maybe a more direct and empirical measure and hence are the focused in chapter 3 and 4 of this thesis for the characterisation of breast fibrosis and monitoring neo-adjuvant treatment.

2.4.3 Other ultrasound parametric imaging techniques

Apart from ultrasound elastography and ultrasound spectral parametric imaging, other imaging techniques have been developed for tissue characterisation (Noble, 2010). Attenuation is a well-studied physical property in ultrasound physics whereby the intensity of ultrasound wave reduces through scattering and

absorption as the wave travels through mediums, and studies have found that ultrasound attenuation coefficients can be used to characterise tissues (Kuc and Schwartz, 1979; Landini and Sarnelli, 1986). Methods have been developed to estimate the attenuation coefficient of the imaged medium (Fink *et al.*, 1983; Kim and Varghese, 2007; Labyed and Bigelow, 2011) and most utilise the calibrated power spectrum for analysis by looking at the spectral changes to estimate attenuation. The methods work well for homogeneous mediums and phantoms but do not work in heterogeneous mediums and large window size is important to achieve accurate estimation of the attenuation coefficient (Labyed and Bigelow, 2011). As a result, the methods may still be suitable for studying large and fairly homogeneous organs such as the liver but will be difficult in applying in smaller and heterogeneous organs such as breasts.

Speckle statistics estimation is another field that has received much attention in parametric imaging. Speckle is an inherent characteristic of ultrasound imaging, which arises from the backscattering of random and coherent distributed scatterers in the tissue that is often seen as noise in ultrasound images and can be a problem for clinical diagnosis. However, because it carries information of the unresolvable part of the medium, it has been studied to provide a possible

method for tissue characterization. Several scattering models have been proposed with the use of statistical distributions such as the Rayleigh distribution (Burckhardt, 1978), the K-distribution (Shankar *et al.*, 2003) and more recently the Nakagami distribution (Shankar *et al.*, 2003 and Tsui *et al.*, 2008) to model the amplitude of the envelope signal. These methods have also been investigated for breast cancer diagnosis and have shown promise as additional tools for tissue characterisation (Shankar *et al.*, 2003; Tsui *et al.*, 2008; Larrue and Noble, 2012).

2.5 Image registration

2.5.1 Principles of image registration

Image registration is the process of aligning images through a geometric transformation from one coordinate system to another. The methods of registration can either be feature-based (Besl and McKay, 1992; Arun *et al.*, 1987; Chui and Rangarajan, 2003) or voxel-based (Ashburner and Friston, 2000) and the transformation used can either be rigid, affine or non-rigid (Maintz and Viergever, 1998). Most registration methods typically involve an optimisation procedure whereby the transformation is estimated and updated until a pre-defined cost function is minimised or a pre-set condition is reached, for example,

a certain number of iteration is reached. After determining the transformation, image interpolation is used to produce the aligned images. In voxel-based methods, the cost function is derived from the voxel-intensity such as sums of squared differences, and in feature-based methods, the features are pre-selected points or surfaces and the cost function is based on a distance measure. Feature based methods also require the determination of correspondence between points in the features.

In terms of the type of transformation used, a rigid transformation only allows for rotation and translation, hence features in the image stay “rigid” and preserve its original shape (4 degrees of freedom in 2D). In the non-rigid case, a transformation is often parameterised non-linearly and allows for many forms of local deformation that allow the image to behave like a fluid (numerous degrees of freedom). An extra category called affine transformation is a rigid transformation with additional shear and scaling parameters. The CPD-TPS method used in chapter 5 is an example of feature-based non-rigid registration.

2.5.2 Registration of histopathology with other medical images

Image registration of histopathology images with other modalities of medical images is a difficult task. The main challenges are due to complex tissue

deformations caused by the loss of surrounding organs to constrain the target organ and the loss of vascular pressurisation of intracellular fluids on the organ. In addition, further nonlinear deformations are introduced during the preparation of histopathology slides such as slicing and fixing and tissue shrinkage also occurs. Existing literature on the registration of histopathology and *in vivo* medical images are mainly of MRI images of brains and prostates due to anatomical structures that can be readily identified in MRI images (Jacobs *et al.* (1999); Kim *et al.* (1997); Breen *et al.* (2003); Pitiot *et al.* (2003), Meyer *et al.* (2006), Park *et al.* (2008)). To overcome problems posed by deformations in histopathology images, some methods firstly register the images rigidly, followed by non-rigid registration such as thin plate splines to warp the previously rigidly aligned histology contours to match those of the *in vivo* MRI (Jacobs *et al.* (1999), Breen *et al.* (2003)). Pitiot *et al.* (2003) proposed a piecewise affine registration method where the images were decomposed into pairs of sub-images after being firstly rigidly registered. The sub-images were then separately affine or rigidly registered before a hybrid affine or non-linear interpolation scheme was used to compose the registered images. Other methods developed have introduced intermediate images to facilitate the registration process where both the histopathology and *in vivo* images were registered onto some reference such as

ex vivo MRI images separately, and the resulting images were then registered to each other (Kim *et al.* (1997), Meyer *et al.* (2006), Park *et al.* (2008)).

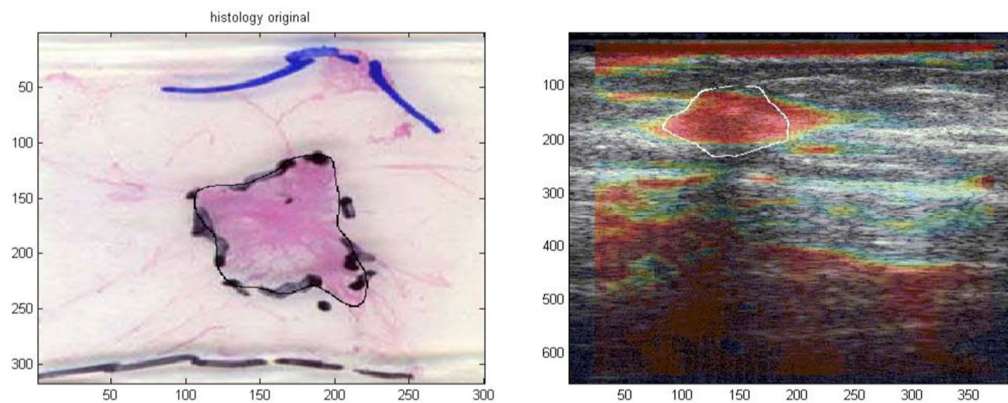


Figure 2 - 7. Example pair of (left) histopathology image and (right) ultrasound elastography image to be registered. The images are not to the same scale. It can be seen in the top left quadrant of the ultrasound elastography image, the tumour appears as a red/stiff area, however, the lack of a defined tumour boundary and clear anatomical structures in the ultrasound elastography image makes it difficult for image registration using methods developed for registering MR and histopathology. Moreover, complex deformation due to preparations in histopathology means the tumour shape and orientation is much different to the tumour in the ultrasound elastography (*in-vivo*), which is difficult to model with a rigid registration framework and requires non-rigid modelling methods.

There is limited literature on the registration of histopathology with ultrasound images (Moskalik *et al.* (1997), Taylor *et al.* (2004)) and none exists for registering ultrasound elastography images to histopathology images. One could try and apply the methods developed for registering MRI images to histopathology images, but this is not ideal as most MRI-based registration methods are intensity-based which is not workable for registering ultrasound images with other modalities. Hence in chapter 5 of this thesis a method is

proposed for the image registration of ultrasound elastography images and histopathology images. The task is considered as a point-set registration problem where corresponding features are identified manually in the histopathology and elastography images and the coherent point drift (CPD) algorithm developed by Myronenko and Song (2009) and thin-plate splines (TPS) (Bookstein, 1989) used to perform the registration.

2.6 Conclusion

This chapter has provided an overview of the background to the rest of the thesis. The importance of neo-adjuvant chemotherapy treatments in breast cancer management and the need to develop a non-invasive tool for monitoring treatments have been discussed. In addition, image registration and its role in facilitating the validation of medical images against histopathology images has also been reviewed. These motivations have helped to define the scope of this thesis and the motivations for the work in proceeding chapters.

Chapter 3: Application of Ultrasound Spectral Intercept for Breast Fibrosis Characterisation

3.1 Introduction

In this chapter we investigate the use of ultrasound spectral intercept as a parameter for breast tissue characterisation, more specifically, to identify breast fibrosis. We first describe the procedure for obtaining ultrasound spectral parameters from ultrasound radio-frequency (RF) signal, followed by the study.

3.2 Spectral parametric image computation

The method discussed in this section is used to compute the ultrasound spectral intercept parameter in this chapter and also chapter 4 (the neoadjuvant study).

The general method has been widely described in the literature (Lizzi *et al.*, 1987, 2003, Feleppa *et al.*, 2011, Kolis *et al.*, 2002) though details differ slightly in the use of the types of reference phantom and bandwidth of the spectrum used for the computation. The relevant literature review of ultrasound spectral parameter estimation has been discussed in chapter 2.

The method used in this thesis is as follows. Ultrasound RF data are acquired using a Zonare Z.one system with a L10-5 probe (8.5MHz with a footprint of 38.4

mm and adjustable axial depth, usually 40mm or 50mm in acquisition). All recording were done with default settings other than adjusting scanning depth for some scans and the settings for TGC and transmit power are fixed. Same depth settings were used to obtain the reference signal and the tissue signal. The research package from Zonare (Zonare Medical Systems, Inc., Mountain View CA, USA) provides access to raw ultrasound RF data. The steps below are then used to compute ultrasound spectral parametric images using MATLAB®:

1. A window of size 5mm x 5mm is used to extract N number of axial ultrasound RF signals, v_n , obtained from a sample medium such as the breast tissue. Spectral parameters are calculated with the window with the resulting values assigned to the centre pixel of the window.
2. From the N axial ultrasound RF signals, a 1D Fourier transform is used to obtain N Fourier spectrum and the Hamming window is used to reduce the effect of spectral leakage.

$$V_n(f) = \text{FFT}(v_n(t)H(t)) \quad (3.1)$$

where the subscript n represents the line n of N lines from the 5mm x 5mm window, $V_n(f)$ represents the Fourier spectrum of the RF line n, FFT is the Fourier transform, $H(t)$ is the Hamming window.

The power spectrum magnitude, $S_n(f)$, is obtained by the squared magnitudes of the resulting N Fourier spectra and then averaged to obtain the averaged power spectrum, $S(f)$.

$$S_n(f) = |V_n(f)|^2 \quad (3.2)$$

$$S(f) = \frac{\sum_n S_n(f)}{N} \quad (3.3)$$

3. Ultrasound RF signal is acquired from a reference breast phantom using the same system settings used to acquire the RF signal from the breast tissue. In our case we used a commercial ultrasound phantom model CIRS 059, as it was the best option available (CIRS, Virginia, USA) (Figure 3- 1). The phantom was originally designed for elastography and contains small inclusions with higher stiffness to the background material but the background material is homogeneous in terms of scatterer size, concentration and acoustic impedance, which suits our application. The reference RF data was collected using the help of elastography to avoid the inclusions (Figure 3- 2). An example of the reference data is shown in the left image of Figure 3- 3. The averaged power spectrum of this reference is then calculated using the previous steps

based on the same location extracted as that of in the sample signal (Figure 3-3).

4. A common frequency range is calculated between the –15dB bandwidth of the reference and the tissue power spectrum and the spectrum from this common frequency range is then extracted (Figure 3-4). The resulting tissue power spectrum is then divided by the reference power spectrum to remove contributions from the ultrasound system settings in the power spectrum.

$$S_{\text{calib}}(f) = \frac{S_{\text{sample}}(f)}{S_{\text{ref}}(f)} \quad (3.4)$$

and the resulting calibrated power spectrum is then converted to dB, which is a logarithmic unit (dB and r stands for decibel and relative) widely used to express power ratio over a reference level, in our case the reference phantom.

$$S_{\text{calib}} \text{ in dB}(f) = 10 \log_{10} (S_{\text{calib}}(f)) \quad (3.5)$$

5. Linear regression analysis is then used to obtain the spectral gradient and the spectral intercept parameters (Figure 3-5)

$$S_{\text{calib}} \text{ in dB}(f) = Mf + c \quad (3.6)$$

where M is the spectral gradient, f is frequency and c is the spectral intercept.

The spectral intercept is not affected by the attenuation effect assuming a linear relationship between attenuation and frequency (Lizzi *et al.*, 2003).

6. Steps 1-5 are repeated with one pixel overlap at a time across the whole RF data set from one scan/frame, both axially and laterally, to form a 2D spectral image. One pixel overlap is what's used in standard image processing as a starting point as we did not want to lose any potential information.



Figure 3- 1 CIRS 059 breast phantom used to obtain reference RF signals for calibration (model CIRS, Virginia, USA).

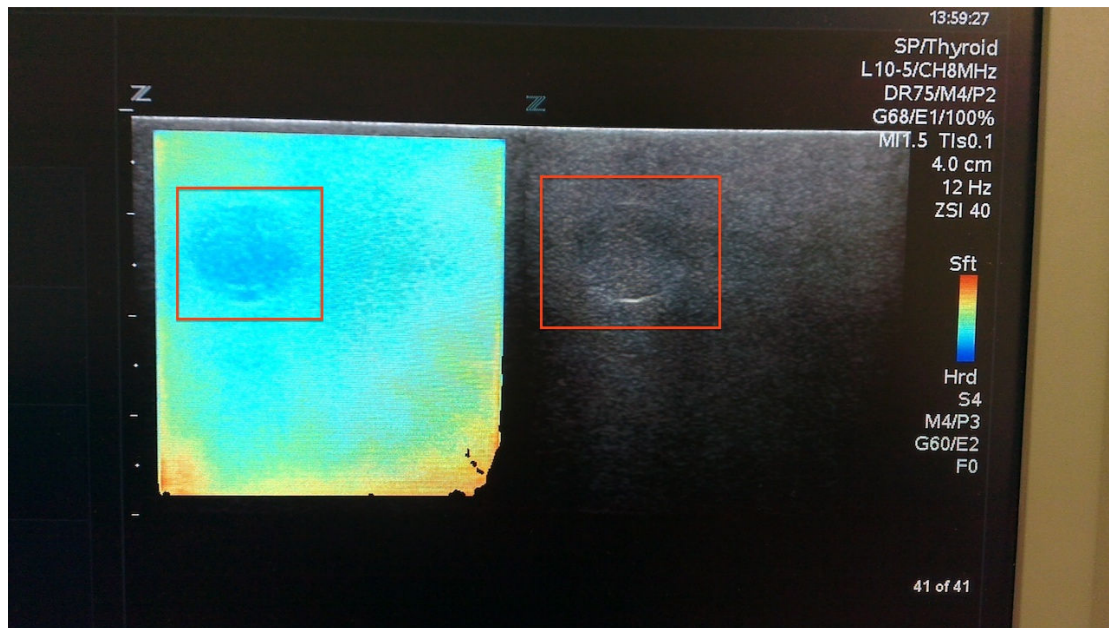


Figure 3- 2 Elastography (left, colour image) was used for guidance to avoid stiff inclusions and to obtain the reference RF data. This image illustrates the presence of a stiff inclusion (marked in the red squares) in the CIRS 059 ultrasound phantom. The image on the right is the ultrasound B-mode image.

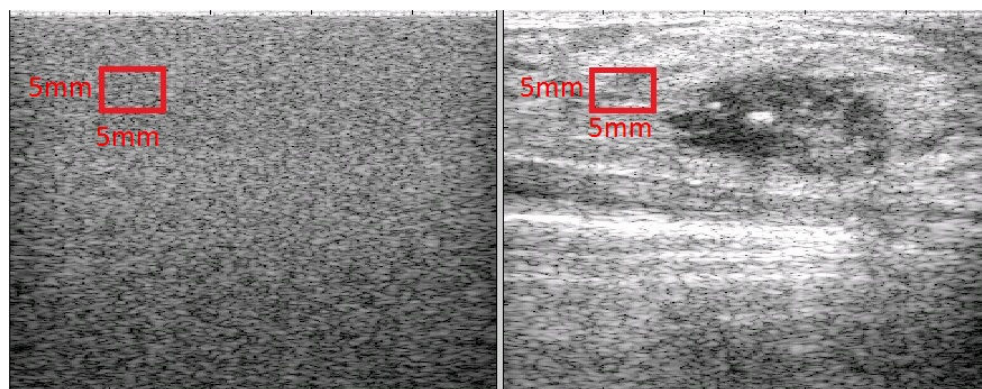


Figure 3- 3 Example B-mode images of (left) reference phantom and (right) breast tissue and cancer. The red ROIs are 5mm x 5mm and the same corresponding area from the reference and the sample are selected for RF data extraction and for spectral parameter computation.

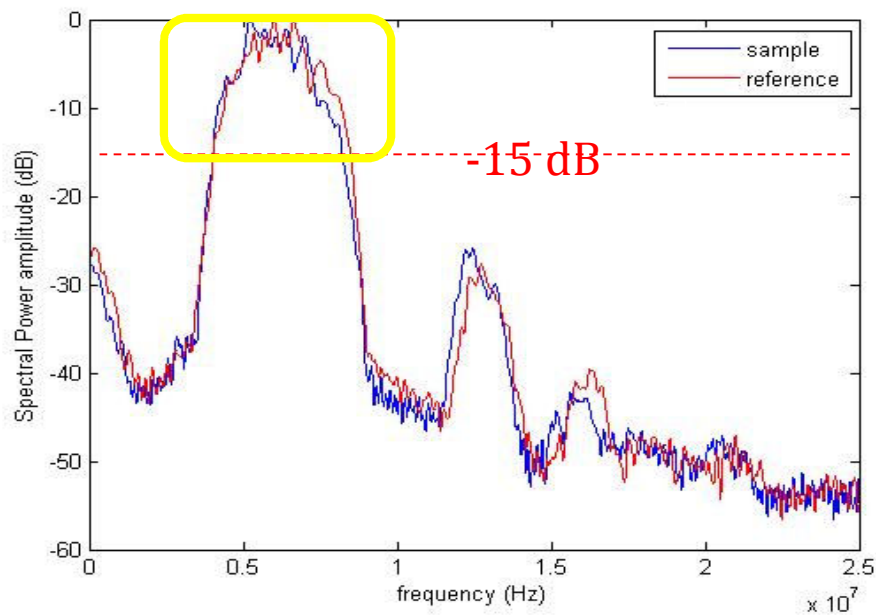


Figure 3- 4 Example power spectra of the tissue sample and the reference RF data. The figure shows the full spectrum computed by the FFT but only the spectra within the -15dB level (highlighted in the yellow box) are used for computing the spectral parameters

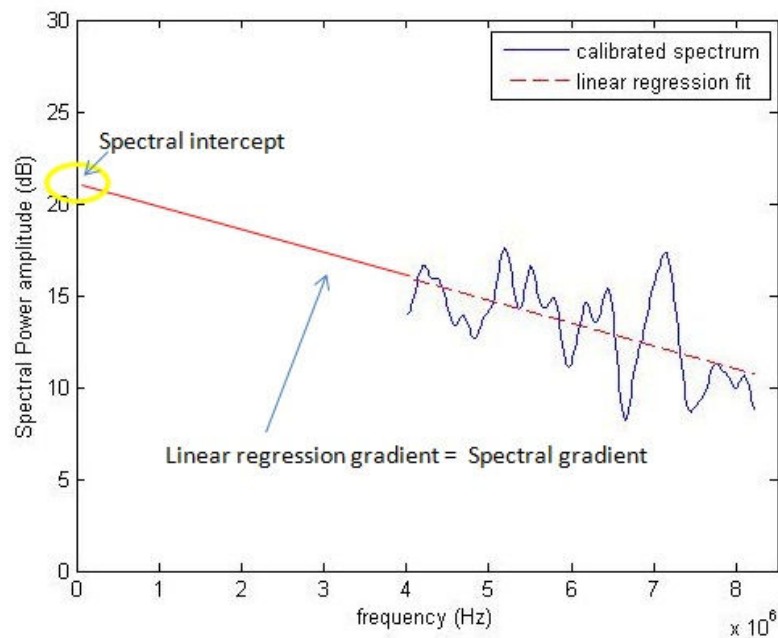


Figure 3- 5 Linear regression fit of the calibrated spectrum. The spectral intercept is taken as the amplitude value at which a regression line meets the axis at zero frequency. The gradient of the linear fit is the spectral gradient.

3.3 Choice of window size

In section 3.2 we mentioned that for every pixel in the computation of the spectral intercept, a 5mm x 5mm window is used to extract the RF data. The size of the window was selected based on an experiment using an ultrasound phantom. In that experiment, three window sizes were tested: 3mm x 3mm, 5mm x 5mm and 10mm x 10mm and the results of the spectral intercept image were visually compared against the B-mode image of the phantom. The results are shown in Figure 3- 6 and it can be seen that as the window size gets larger, the smoother the spectral images become but at the expense of the increase in the mismatch between the boundary of the inclusion in the phantom in the B-mode and the spectral intercept image. The window size of 5mm x 5mm was selected as the final parameter as the 3mm x 3mm result was noisier and the boundary mismatch was larger for 10mm x 10.

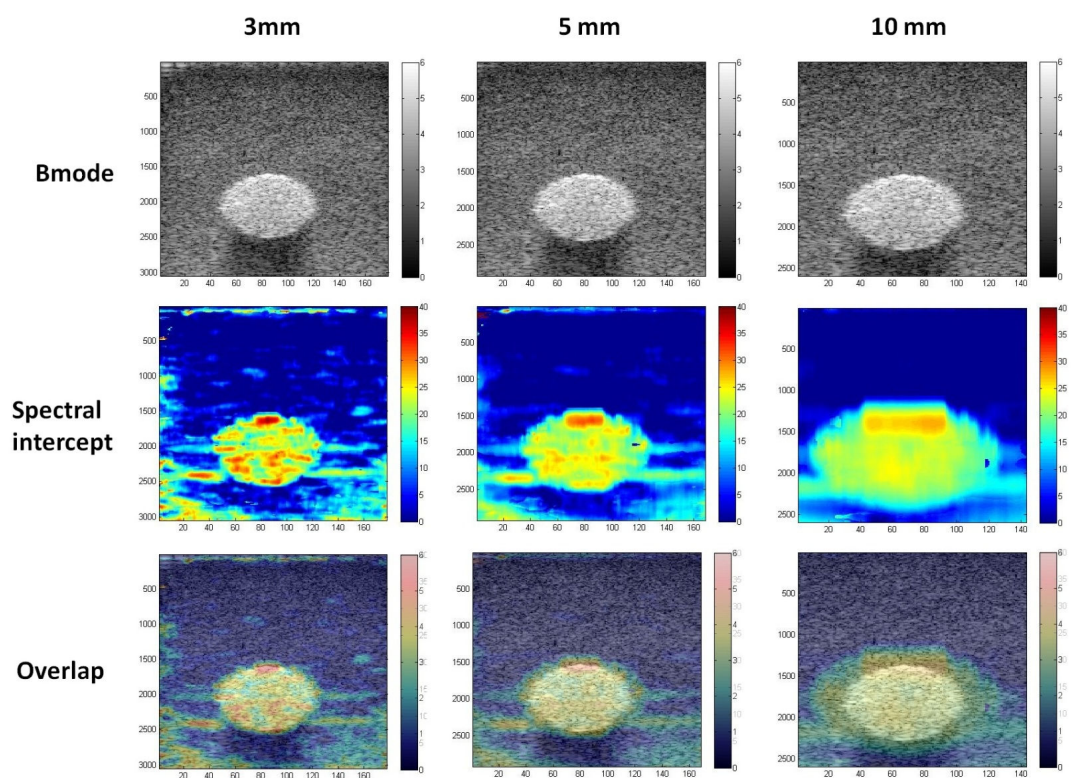


Figure 3- 6 (Top row) B-mode images (mid row) spectral intercept images and (bottom row) overlap of B-mode and spectral intercept images for (left column) 3 mm x 3mm (mid column) 5mmx5mm (right column) 10mmx10mm window. The spherical object in the images is an ultrasound phantom made with gelatin and scatterers, with the inclusion having a higher scatterer density. Note that the sizes of the inclusion in the 3 B-mode images are the same, but due to the window size used for image processing, appear to be different. This can be seen by the inclusion appears bigger for the 10mm window (top right) than the one for the 3mm window (top left).

3.4 Motivation on breast fibrosis characterisation

As discussed in chapter 2, fibrotic changes are a common after-effect to chemotherapy for many forms of cancer. Although our target is to characterise fibrosis, due to limited data availability we included data of fibrocystic and traumatic fat necrosis (TFN) cases (TFN is pathologically similar to fibrosis, see chapter 2) to look at characterising them as a group. For the rest of the chapter we will refer the fibrosis, TFN and fibrocystic cases as the fibrous group and the cancerous cases as the non-fibrous group. Our assumption is that by developing parametric imaging methods for characterising the fibrous group against the non-fibrous group, we may provide a useful tool for monitoring fibrotic changes for monitoring chemotherapy.

As mentioned in section 2.4.2, linear regression analysis of the spectrum also yields additional parameters such as the spectral gradient (equation 3.6) and the mid-band fit (MBF). These parameters have been studied in the literature but are excluded from our study because both the gradient and the MBF are affected by attenuation and require calibration (the intercept is not affected assuming a linear relationship between attenuation and frequency, which is in general an accepted assumption). In the literature, these values are typically corrected by using an attenuation coefficient of 0.5 dB/cm/MHz, which is a widely used value in ultrasound literature for soft tissue. However, because the breast tissue is very heterogeneous in nature, we believe using such generalised value would not be accurate, and as attenuation estimation *in-vivo* is a difficult topic by itself, we decided only to investigate spectral

intercept in this study.

Thus far in the literature, studies have investigated ultrasound spectral parameter estimation for identifying breast cancer from benign cases (Alam *et al.*, 2002) but to our knowledge, no studies have investigated ultrasound spectral parameterisation for the characterisation of breast fibrosis. The closest studies in the literature are by Zhou *et al.* (2008) and Yoshida *et al.* (2012) that explored the use of ultrasound spectral parameters for monitoring radiation-induced fibrotic changes by comparing against pre-radiated healthy subcutaneous breast tissue.

3.5 Methods for the characterisation study

All patient data used in this study have been acquired under full consent and ethical approval through the Oxford Breast Imaging Centre. The dataset used were part of the data acquired for a clinical study for ultrasound elastography on breast cancer (Di Battista *et al.*, 2012). In this study, 22 patients were included and the selection was based on the histology results. For cancerous cases only patients with a single type of cancer type, for example, only IDC were selected. In total there were 8 cases of fibrous group cases, 7 cases of IDC and 8 cases of various other forms of cancer. The summary is given in Table 3- 1. This study is concerned with characterising fibrosis from cancerous tissues in order to develop a tool for tracking fibrotic change in cancer tissues during treatments and hence we did not include other types of non-cancerous data. Normal breast tissue was not used as controls as we are trying to characterise fibrosis from cancerous tissue.

Ultrasound spectral intercept images were computed using the method outlined in section 3.2. The boundaries of the lesions were delineated in the B-mode images by

Dr. Ruth English at the Oxford University hospitals trust NHS, (referred to as the clinician hereafter) based on the appearance of the lesion in B-mode ultrasound together with additional information from the histology and mammography images. The corresponding areas in the ultrasound spectral images within the tumour boundary were then extracted for analysis.

Fibrous group

Tissue type	Patient numbers
Fibrosis (F)	P349, P350, P388, P418
Fibrocystic (FC)	P295, P333
Traumatic fat necrosis (TFN)	P371

Non-fibrous group

Tissue type	Patient numbers
Invasive ductal carcinoma (IDC)	P299, P341, P354, P357, P391, P395, P399
Mucinous carcinoma (MC)	P389
Invasive tubular carcinoma (ITC)	P336
Invasive basal carcinoma (IBC)	P302
Invasive micropapillary carcinoma (IMC)	P408
Invasive adenoid cystic carcinoma (IACC)	P412
Invasive lobular carcinoma (ILC)	P318, P359
Ductal carcinoma in situ (DCIS)	P417

Table 3- 1 Summary of the cases and tissue types used for the fibrosis characterisation study.

3.6 Results

In this section we present the results of average intercept values and also show examples of spectral intercept images together with B-mode images and the areas marked by the clinician. The full set of images can be found in Appendix A and It is important to note that spectral intercept images, including areas other than the lesion, are shown for completeness of the study and visualisation purposes. The main focus of the study is on the lesions and the average intercept values within the lesions. Overall, the average spectral intercept in the fibrous group is found to be

larger than the non-fibrous group, with the fibrous group having an average intercept of 21.57 +/- 7.45 dB and 15.06 +/- 11.09 dB for the non-fibrous group. The results are summarised in Table 3-2.

The range of values used for the colour map to plot the images are based on the mean of all values within an image +/- 2 standard deviations and hence the same spectral intercept value can appear as a different colour in different images. The B-mode images are shown in standard grey scale colour map and the darker the region the more hypo-echoic the tissue. The spectral intercept images are shown in colour using an inverse 'jet' colour map in MATLAB[®]. The inverse jet colour map assigns larger values with a bluer colour and was used so that the colour of lesions appear redder in the image. In each visualisation, the bluer the region in the spectral intercept image, the higher the spectral intercept values. The left column in each figure shows the B-mode image on the top and B-mode image with the area marked by the clinician in green on the bottom. The right column shows the spectral intercept image on the top and the spectral intercept image overlay on the B-mode image on the bottom.

Figure 3- 7 is an example of a non-fibrous case (P399, IDC) where corresponding tumour areas are marked by white rectangles. It can be seen that the tumour area in the B-mode image appears as a hypo-echoic region and in the spectral intercept image appears as a red region, indicating low spectral intercept value relative to the mean value of the image. The average intercept value was measured to be 7.72 +/-

8.31 dB within the tumour area marked by the clinician. The subcutaneous fat tissue that is marked by green rectangles also appears red in the spectral image.

Non fibrous group				Fibrous group			
Patient number	Pixels within lesion	Mean intercept (dB)	S.D. intercept (dB)	Patient number	Pixels within lesion	Mean intercept (dB)	S.D. intercept (dB)
P399 IDC	42468	7.72	8.31	P418 F	1788	16.53	4.23
P395 IDC	48717	1.81	9.13	P388 F	8706	19.68	4.78
P391 IDC	17567	1.67	7.63	P371 TFN	6921	30.58	6.58
P357 IDC	1280	27.09	7.72	P350 F	22566	8.26	9.75
P354 IDC	27888	9.92	6.72	P349 F	29168	24.34	9.74
P341 IDC	20960	17.99	7.25	P333 FC	29053	26.81	7.59
P299 IDC	3946	37.84	6.53	P295 FC	7839	24.80	10.94
P302 IBC	128055	7.29	8.35	Average		21.57	7.45
P318 ILC	14666	12.99	9.80				
P336 ITC	6318	36.22	3.28				
P359 ILC	11921	20.67	6.99				
P389 MC	66167	12.30	6.52				
P408 IMC	9180	8.55	6.58				
P412 IACC	91381	11.76	6.33				
P417 DCIS	7741	12.04	6.37				
Average		15.06	11.09				

Table 3- 2 Mean and standard deviation (SD) of the measured spectral intercept for (left) the non-fibrous group and (right) the fibrous group. All mean and SD values for individual patients are calculated based on the values within the lesion in each patient and the mean and SD average of the non-fibrous category is calculated using the mean intercept values of all patients.

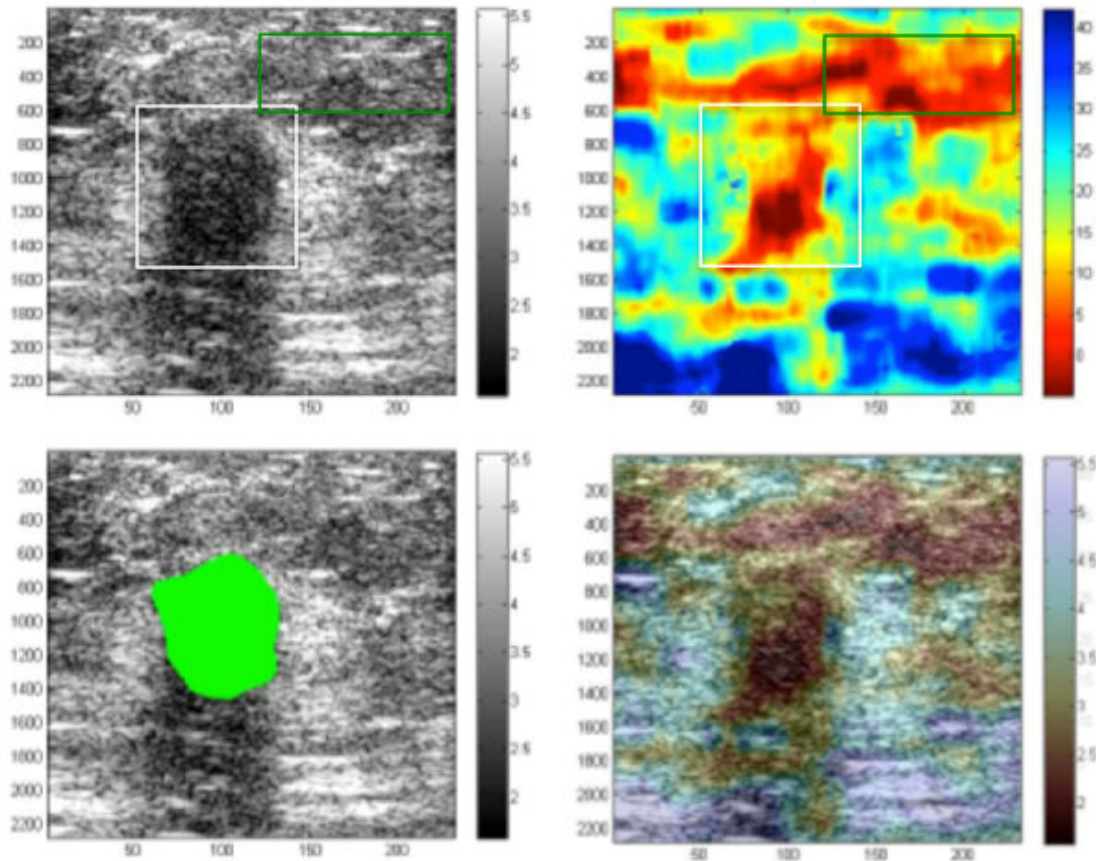


Figure 3- 7 Images for P399, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image. The white rectangles in the top row mark the corresponding tumour areas and the green rectangles mark the subcutaneous fat tissue.

Figure 3- 8 is an example of a fibrous case (P349, fibrosis) where corresponding tumour areas and subcutaneous fat tissues are again marked by white and green rectangles respectively. It can be seen that the tumour area in the B-mode image appears as a relatively hypo-echoic region in comparison to its surroundings and in the spectral intercept image appears as a mixture of red and yellow region, indicating low to medium spectral intercept values relative to the average in the image. The average intercept was measured to be 24.34 ± 9.74 dB within the tumour area marked by the clinician. The subcutaneous fat tissue in this case also exhibits similar low spectral intercept values as observed in **Figure 3- 7**, and this observation is consistent in other cases in the case study.

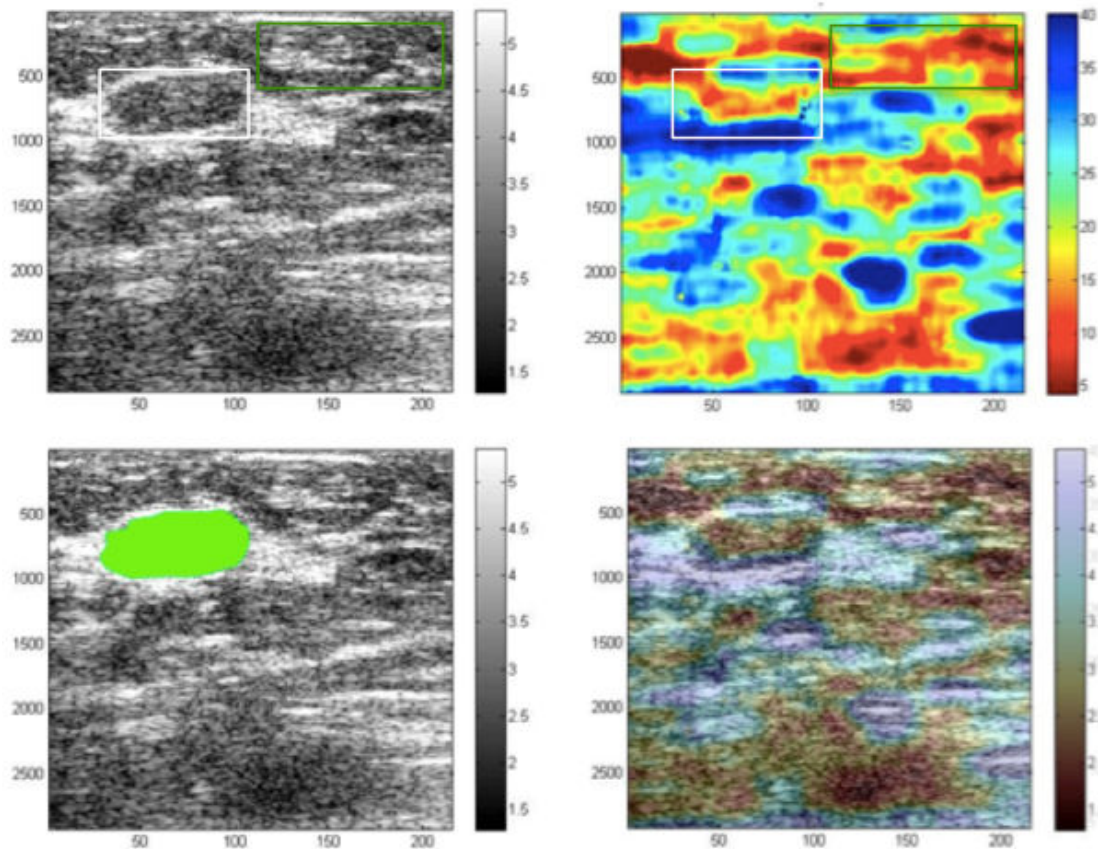


Figure 3- 8 Images for P349, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image. The white rectangles in the top row mark the corresponding tumour areas and the green rectangles mark the subcutaneous fat tissue.

Apart from results that suggest fibrous tissues exhibit higher spectral intercept values, there are also a couple of outlier results that are shown in Figure 3- 9 and Figure 3- 10. Figure 3- 9 shows a fibrous case (P350) where the lesion appears as a hypo-echoic area in the B-mode and a red region in the spectral intercept image, indicating low spectral intercept value compare to the average in the image. The average intercept value within the marked tumour was measured to be 8.26 ± 9.75 dB. This is noticeably lower than other cases in the fibrous group (Table 3-2) and the average of the group.

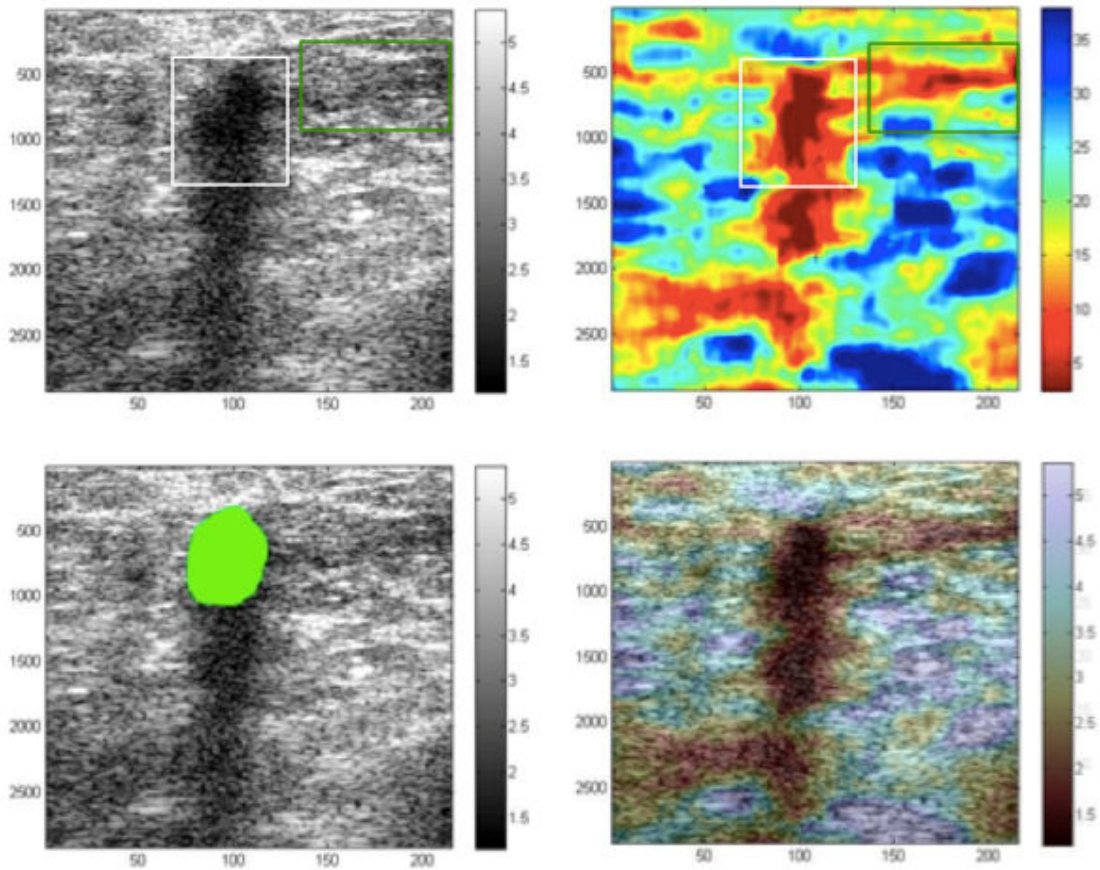


Figure 3- 9 Images for P350, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image. The white rectangles in the top row mark the corresponding tumour areas and the green rectangles mark the subcutaneous fat tissue

Figure 3- 10 shows a non-fibrous case (P336) where the lesion appears as a hyper-echoic area in B-mode and a yellow and green region in the spectral intercept image, indicating medium to high spectral intercept value compare to the average in the image. The average intercept value within the marked tumour was measured to be 36.22 ± 3.28 dB. This is noticeably higher than other cases in the non- fibrous group (Table 3-2) and the average of the group.

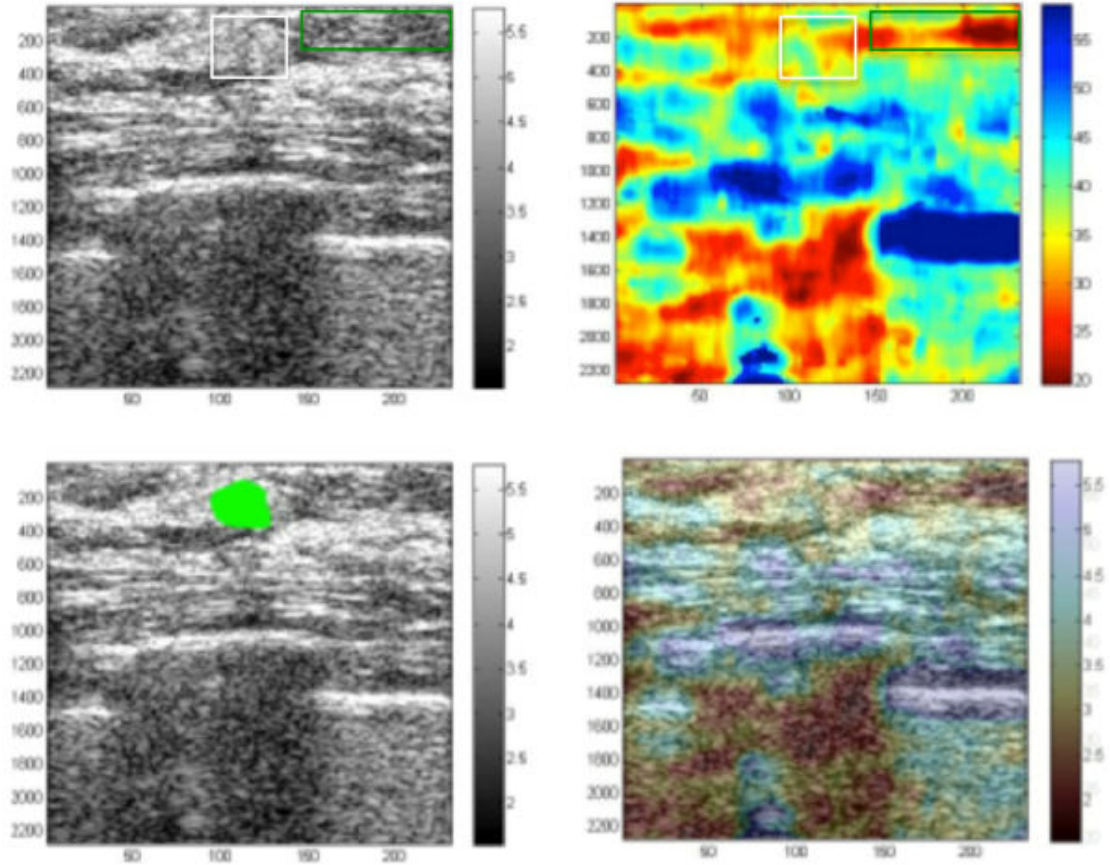


Figure 3- 10 Images for P336, ITC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image. The white rectangles in the top row mark the corresponding tumour areas and the green rectangles mark the subcutaneous fat tissue.

3.7 Uncertainty level and noise

For this study, the uncertainty level is given in the standard deviation of the results. It is difficult, if not impossible, to determine the uncertainty level for the clinician's input and hence we did not investigate. In this study, the clinician utilised full information available including mammography images, histology information and ultrasound images when performing the marking. The only possible method is to have input from multiple clinicians when determining the lesion area to reduce uncertainty.

The spectral intercept was not evaluated against ground truth as the parameters are not determined simply by a single physical parameter such as the tissue stiffness or scatterer concentration and hence we believe it would not provide additional insight

and value to the clinical application.

3.8 Analysis and Discussion

Upon closer inspection of the patient data, there are patients with very small lesion size, and as mentioned in section 3.3, because a 5mm x 5mm window (325 x 25 pixels/data points in rows and columns) was used to calculate the spectral parameters, the average parameters calculated from these cases may be biased by the estimation window size. In order to minimise bias due to estimation window size, we applied a simple threshold to filter out cases where the number of pixels within the lesions are less than half the total number of pixels within the estimation window

Non fibrous group				Fibrous group			
Patient number	Pixels within lesion	Mean intercept (dB)	S.D. intercept (dB)	Patient number	Pixels within lesion	Mean intercept (dB)	S.D. intercept (dB)
P399 IDC	42468	7.72	8.31	P388 F	8706	19.68	4.78
P395 IDC	48717	1.81	9.13	P371 TFN	6921	30.58	6.58
P391 IDC	17567	1.67	7.63	P350 F	22566	8.26	9.75
P354 IDC	27888	9.92	6.72	P349 F	29168	24.34	9.74
P341 IDC	20960	17.99	7.25	P333 FC	29053	26.81	7.59
P302 IBC	128055	7.29	8.35	P295 FC	7839	24.80	10.94
P318 ILC	14666	12.99	9.80	Average		22.41	7.79
P336 ITC	6318	36.22	3.28				
P359 ILC	11921	20.67	6.99				
P389 MC	66167	12.30	6.52				
P408 IMC	9180	8.55	6.58				
P412 IACC	91381	11.76	6.33				
P417 DCIS	7741	12.04	6.37				
Average		12.38	8.96				

Table 3- 3 Mean and standard deviation (SD) of the spectral intercept for (left) non-fibrous cases and (right) fibrous cases after the threshold is applied. All mean and SD values for individual patients are calculated based on the values within the lesion in each patient and the mean and SD average of the non-fibrous category is calculated using the mean values of all patients.

(325 x 25 pixels), which is around 4000 pixels. Based on the threshold we filtered out one case from the fibrous group, P418, and two non-fibrous cases, P299 and P357. **Table 3- 3** shows the results of the filtered cases. After thresholding, fibrous group have an average spectral intercept of 22.41 +/- 7.79 dB and 12.38 +/- 8.96 dB for the fibrous and non-fibrous group respectively.

The mean values of spectral intercept appear to show differences between the fibrous group and the non-fibrous group cases, however, in order to show this difference is not by chance, a statistical test is required to test the significance of the data observed. For statistical significance testing, first the Kolmogorov-Smirnov (KS) test was used to determine whether the data is from a Normal distribution. The KS statistic compares the cumulative distribution function (CDF) of a given data with that of the Normal distribution by computing

$$D = \max(|F(x) - G(x)|) \quad (3.7)$$

where $F(x)$ is the CDF of the data and $G(x)$ is the CDF of the normal distribution and D measures the biggest difference in the CDF of the data to that of the Normal distribution. The null hypothesis is that the data, $F(x)$, follows a Normal distribution and if the difference is bigger than a certain value (depending on the significance level which is usually 5%) then the null hypothesis is rejected and the measured data is said to not follow the Normal distribution.

If we fail to reject the null hypothesis (i.e. data follows a Normal distribution), the Student's T-test is performed to see if the fibrous group is significantly different to

that of the non-fibrous cases but if the null hypothesis is rejected, then the Wilcoxon Rank-sum test is performed to see if the fibrous group is significantly different to that of the non-fibrous cases (see Figure 3- 11 for the flow diagram).

For the T-test, because the fibrous and non-fibrous data are not of the same size, and we cannot assume they have equal variance, a two sample T-test for unequal sample size and variance was selected. The T-test statistic in this case is given as

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (3.9)$$

where $\bar{X}_1$ and $\bar{X}_2$ are the mean of the two data sets, S_1 and S_2 are the standard deviation of the two data sets and n_1 and n_2 are the number of samples in each data. Once the T value is calculated, a P-value can then be calculated using a standard table of values from Student's t-distribution. If the resulting P value is below the threshold for a particular statistical significance level (usually 5%) then the null hypothesis is rejected meaning that the difference between two data is most likely not due to chance.

For the Rank-sum test, a two-sided test is used since we are interested if the two data sets are different rather than if one is significantly greater or smaller than the other. The two data are merged and then ranked in ascending order with the smallest value of the two data ranked as 1 and the biggest is ranked N, where N is the total number of samples of the merged data. The ranks for the first set of data are summed and compared with a standard probability table to obtain a P-value. Again,

if the P-value is below a particular threshold such as a 5% significance level, the null hypothesis is rejected.

Following the procedure outlined in Figure 3- 11, the KS test showed that the mean spectral intercept of the fibrous ($p = 8.88\text{e-}16$) and the non-fibrous cases ($p = 1.07\text{e-}11$) do not follow a Normal distribution and hence the Rank-sum test was performed to determine if the measured spectral intercept values from the thresholded fibrous cases are significantly different to that of the thresholded non-fibrous cases. The result of the Ranksum test showed that there are significant differences between the intercept values of the fibrous and non-fibrous cases ($p = 0.0365$).

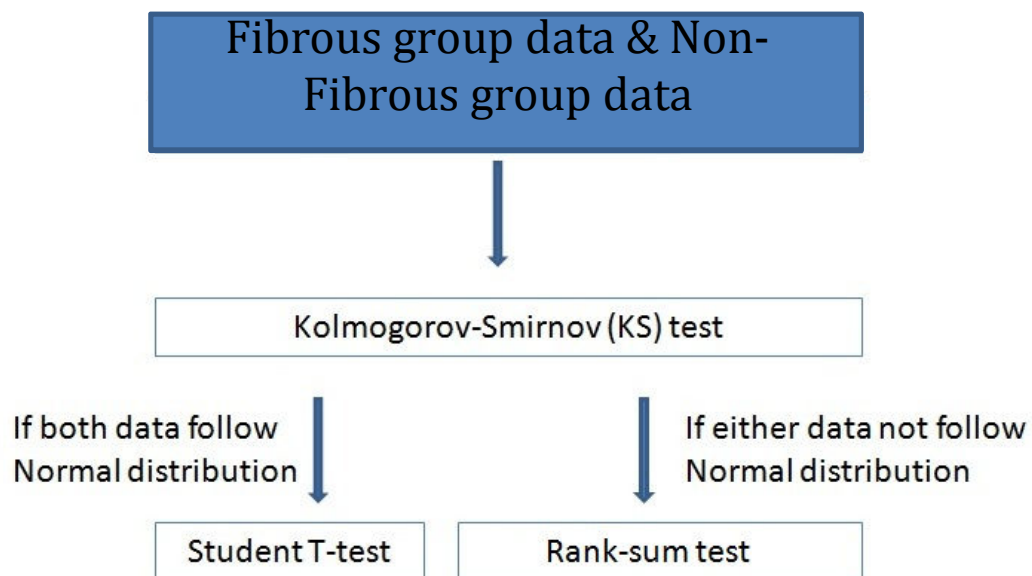


Figure 3- 11 Flow chart of statistical significance testing

3.9 Conclusion

In this chapter we have investigated the use of the spectral intercept to characterise breast fibrosis against cancerous cases. This is a novel contribution to the field as no other studies have shown the use of ultrasound spectral intercept to distinguish between fibrotic tissues against cancerous tissues. Thresholding was introduced as a method to remove effects due to the estimation window size, and although only a relatively small data set was used for the study (7 fibrous and 15 non-fibrous before thresholding, 6 fibrous and 13 non-fibrous cases after thresholding), using the Wilcoxon Rank-sum test, the results showed that there are significant (5% significance level) differences between fibrous and non-fibrous cases. In the next chapter we will apply our findings to investigate the efficacy of ultrasound spectral parameters for monitoring neoadjuvant chemotherapy treatments.

Chapter 4: Monitoring Neoadjuvant Breast Chemotherapy Using Ultrasound Spectral intercept

4.1 Introduction

This chapter investigates the use of ultrasound spectral intercept for monitoring tumour responses to breast neoadjuvant chemotherapy.

4.1.1 Motivation

The need for developing a tool for monitoring chemotherapy has been discussed in Chapter 2 and results in Chapter 3 suggest that ultrasound spectral intercept can segregate fibrous lesions from non-fibrous lesions. In this chapter, we have carried out a pilot study in applying measures based on ultrasound spectral intercept to evaluate results on data acquired from patients who have undergone breast neoadjuvant chemotherapy at Oxford. The aim is to provide future studies with an example framework to which they can compare and build upon. The eventual goal is to develop a clinical tool based on ultrasound spectral parameters that can provide additional diagnostic information to current methods for assessing a patient's therapy and also provides guidance for treatment planning.

At the time of writing this chapter, Sadeghi-Naini *et al.*, (2013) published an independent study of the same application. However, although the methods for estimating the spectral parameters are similar, the methodologies of analysis are different. The main differences worth noting are that in the work by Sadeghi-Naini *et al.*, (2013), no specific details were given in how ultrasound data were acquired from

the same plane, whereas in our work landmarks were used to ensure data acquired were from the closest plane possible in comparison to the baseline scan. This is crucial in making accurate comparisons between scans at different time points as breast tissue is heterogeneous and the scans were acquired in 2D. Another key difference is the local approach taken in this work whereby because tumours do not react to treatments in a uniform fashion and because breast tissue is heterogeneous, we ensured that we compared the closest local regions with the help of an experienced clinician and the imaging landmarks. In contrast the work by Sadeghi-Naini *et al.*, (2013) only looked at the tumour as a whole. Given these differences, it is clear that the results presented in this chapter are unique and provides a different perspective to that of Sadeghi-Naini *et al.*, (2013).

4.2 Patients & Methods

4.2.1 Overview

Patients with primary breast cancer undergoing neoadjuvant chemotherapy were scanned periodically over the course of the treatment. B-mode ultrasound images were acquired and analysed, and the RF signal were stored to allow the processing of spectral intercept images after. For B-mode, conventional analysis such as observing changes in tumour size as a result of treatment were noted and were used for drawing comparisons to the spectral intercept image.

Some limited pathology information was available to correlate to the findings in the B-mode and spectral intercept images. These included the result of a core biopsy

taken before the start of the course of treatments and the biopsy/surgical excision results following the end of the course of treatments. In addition, MRI scans were also used to observe any residual disease present after treatments. Together, the MRI and pathology data provided the gold standard information on the type of response the patient had in response to the chemotherapy and also the type of cancer that was present before and after treatments. The information is summarised in Table 4.1.

4.2.2 Patient recruitment

The cases used in this study were recruited with full consent and ethical approval through the NHS Oxford Cancer Imaging Centre. The data were also used to study the efficacy of elastography in monitoring neoadjuvant chemotherapy (Di Battista, 2012). A total of seven patients participated in the study (Table 4.1.)

Of the seven patients, patient 6 and 7 left the study to continue treatments at other hospitals and for patient 2 and 4 only lymph nodes could be imaged. Patient 3 had a very fast response whereby the tumour had disappeared by the third scan which meant not enough data could be obtained to study any intermediate changes as a result of the treatment. At the end, only two patients (P1 and P5) had a complete set of data with sufficient scans to observe any intermediate changes during chemotherapy. These two patients were used in the pilot study. Of the two patients used in our final study, P1 had complete response to chemotherapy, which means no cancer cells can be detected after the therapy; P5 on the other hand had a partial

response, which means that the tumour has shrunk but not all cancer cells were killed, and some were still detected after the treatment.

Patients were scanned in both the transverse and sagittal plane just prior to receiving their chemotherapy treatment that took place approximately once every 3 weeks. To ensure consistent monitoring of the same region of interest (ROI) over the course of treatments, most patients were fitted with acoustically visible medical-grade coils (MREye®, Cook Medical) to serve as imaging landmarks. In this case the exception was patient 1 (P1) where the patient had micro-calcifications, the main micro-calcification was used as the landmark and medical coils were not used (Figure 4- 1). The landmark system was the best method that is clinically available (and ethically acceptable) and was designed/selected after planning with the clinician. Although the system is not perfect, we accept the limitation but it was agreed to be the best option to ensure repeatability throughout a longitudinal study.

Patient	Histology	No. scans	Coil fitted?	Clinical response	Histology Responses
1	Poorly Differentiated IDC	8	NO	Complete response	N/A
2	Moderately Differentiated IDC	3	YES	N/A	N/A
3	Poorly Differentiated IDC	5	YES	Complete response	N/A
4	Moderately Differentiated IDC	6	YES	Unchanged	Grade 2 ILC 100mm diameter + all lymph nodes pos.
5	Poorly Differentiated IDC	7	YES	Partial Response	50mm high grade DCIS low invasive +all lymph nodes neg.
6	Poorly Differentiated IDC	3	YES	Partial Response	35mm grade 3 IDC, 3 pos. lymph nodes
7	IDC	3	YES	N/A	N/A

Table 4. 1 Summary of patients who participated in the study.

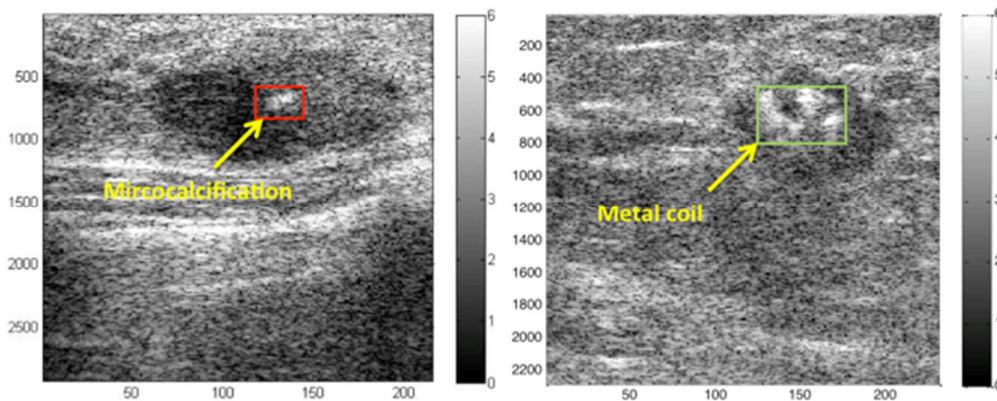


Figure 4- 1. (Left) Example of microcalcification in ultrasound B-mode image (Right) Example of metal coil inserted as landmark for imaging.

4.2.3 Tumour boundary and area selection for analysis

All the tumour boundaries and areas selected in this study for analysis were

performed by Dr. Ruth English at the Oxford University Hospital NHS Trust, hereafter referred to as the clinician.

4.3 Results and discussion

4.3.1 Spectral intercept images

The focus of our ultrasound spectral parametric analysis is to measure quantitatively changes in the average spectral properties of the tumour to provide additional diagnostic information to B-mode images. The spectral parametric images are presented and discussed here for completeness of the study and to give the reader a more extensive picture of the measure that we are using.

In ultrasound B-mode imaging, the brighter the area the more hyperechoic the region is, and tumours usually appear to be more hypoechoic. In spectral intercept images, the redder the area, the lower the spectral intercept values and the bluer the area, the higher spectral intercept values. **Figure 4- 2** and **Figure 4- 3** show the B-mode, the spectral intercept and the overlap of the two images for patient 1 in the sagittal and transverse plane over 8 scans. The yellow boxes in the B-mode images highlight the tumour regions and white boxes in the intercept images. In both planes, it can be clearly seen that the tumour reduced in size significantly from the 5th to the 6th scan in the B-mode and this is reflected in the spectral intercept images where the dark red areas associated with the tumour disappeared after the 5th scan. It should be noted that in the spectral intercept image, landmarks such as the micro-calcification or the inserted metal coil exhibit high intercept values and hence appear

blue.

Figure 4- 4 and **Figure 4- 5** show the B-mode, the spectral intercept and the overlapped images for patient 5 in the sagittal and the transverse plane over 7 scans. Based on size of the tumour in B-mode, the tumour reduced significantly in size between the 5th and the 6th scan in the sagittal plane and showed gradual reduction in size in the transverse plane. For the spectral intercept images, in the sagittal plane, the tumour area increased in intercept values similar to that in patient 1, with red areas associated with low intercept values turning bluer towards the end of the treatments. However, in the transverse plane the intercept values increase initially and then drop off towards the end of the treatments.

The spectral intercept images in **Figure 4- 2** to **Figure 4- 5** provide a qualitative visualisation but offer limited quantitative measures to compare between scans and observe intermediate changes due to chemotherapy. Finding an accurate quantitative measure of change due to treatments is the ultimate goal of treatment monitoring and this is what we set out to do in the remainder of this chapter.

4.3.2 Tumour sizing

Change in tumour size in ultrasound B-mode is frequently used as a measure of effectiveness of breast cancer chemotherapy treatments and was used in this study as a reference comparison. The clinician determined tumour sizes in the B-mode by measuring maximum depths and lengths (sagittal measure) in the sagittal plane and

maximum widths (transverse measure) in the transverse plane (Figure 4- 6). The measured tumour sizes in P1 and P5 are given in Table 4. 2 and Table 4. 3 and are shown in Figure 4- 7 and Figure 4- 8.

For both patient 1 and 5 the tumours showed consistent reduction in size in both the sagittal and the transverse plane. For patient 1, the changes between the 1st and the final scan were 1.2 cm, 1.74 cm and 0.51 cm for the sagittal, transverse and depth measurements respectively. For patient 5, the changes between the 1st and the final scan were 2.01 cm, 2.52 cm and 1.13 cm for the sagittal, transverse and depth measurements respectively. However, despite the measured reduction in the size of the tumour, histology results in Table 4.1 shows that only patient 1 had a complete response to the treatment and patient 5 had a partial response. This suggests that although size is a good indication to treatment response, additional information and measures would be needed to monitor chemotherapy.

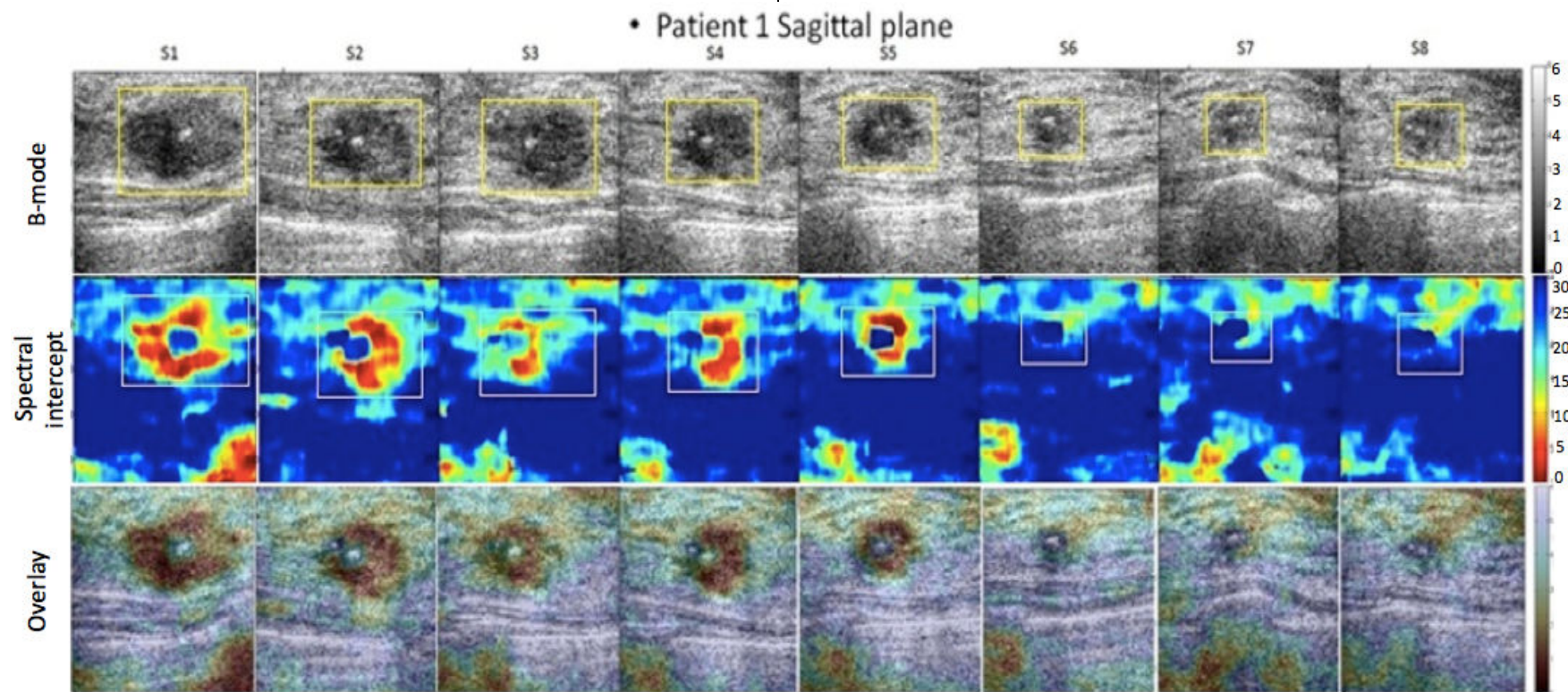


Figure 4- 2. Patient 1 data over 8 scans in the sagittal plane. (Top) B-mode image (middle) spectral intercept image (bottom) overlap of the B-mode and spectral images. The tumour areas are marked in the yellow box in the B-mode and marked in white in the intercept images. As the tumour reduce in size in the B-mode, the tumour in the spectral intercept images increase in intercept value, changing from red to blue and a big change can be observed between the 5th and 6th scan in both image modalities. The images all have the same depths of 40mm and widths of 38.4mm and the same colour range/mapping within each image modality. It must be noted that in the spectral intercept image, the micro-calcification that is used as the landmark exhibits high intercept values and hence appear blue inside the tumour that appears red.

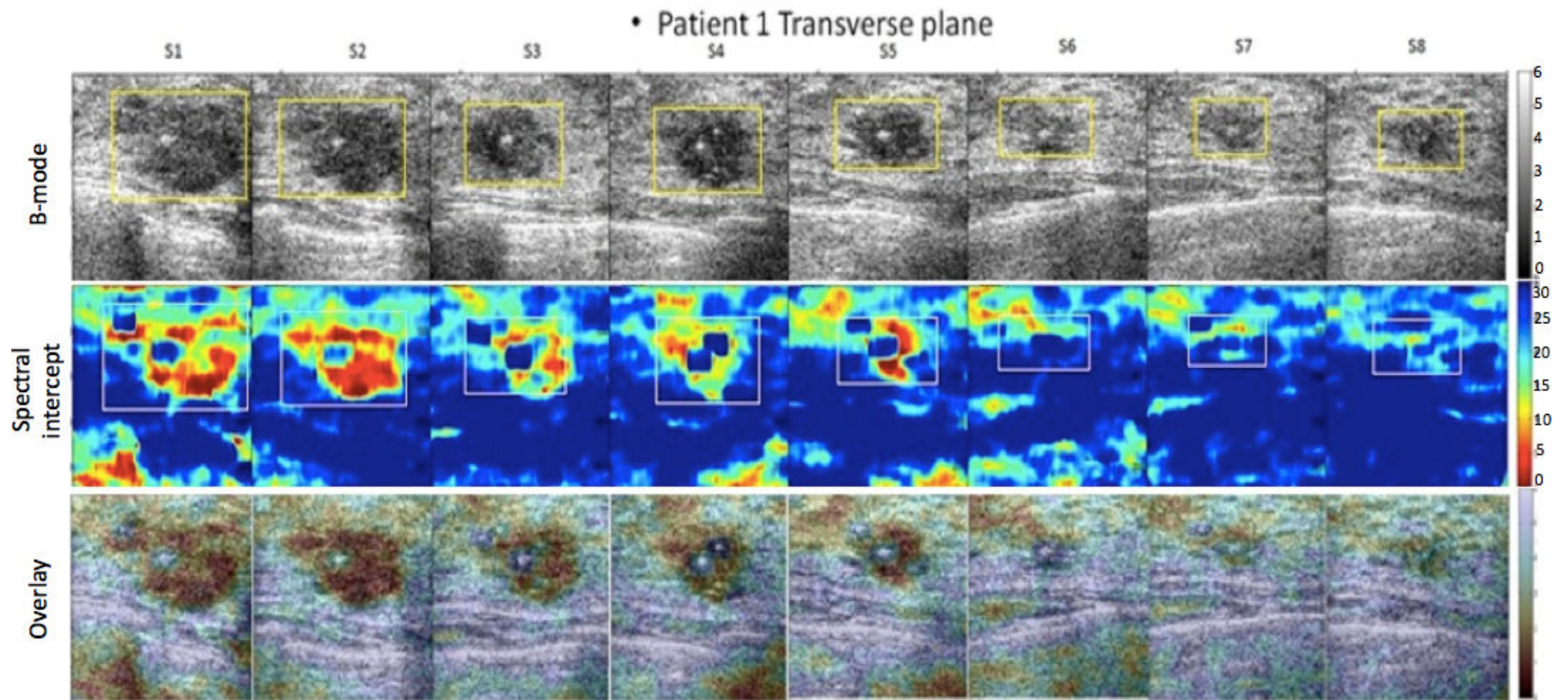


Figure 4- 3. Patient 1 data over 8 scans in the transverse plane. (Top) B-mode image (middle) spectral intercept image (bottom) overlap of the B-mode and spectral images. The tumour areas are marked in the yellow box in the B-mode and marked in white in the intercept images. As the tumour reduce in size in the B-mode, the tumour in the spectral intercept images increase in intercept value, changing from red to blue and a big change can be observed between the 5th and 6th scan in both image modalities. The images all have the same depths of 40mm and widths of 38.4mm and the same colour range/mapping within each image modality. It must be noted that in the spectral intercept image, the micro-calcification that is used as the landmark exhibits high intercept values and hence appear blue inside the tumour that appears red.

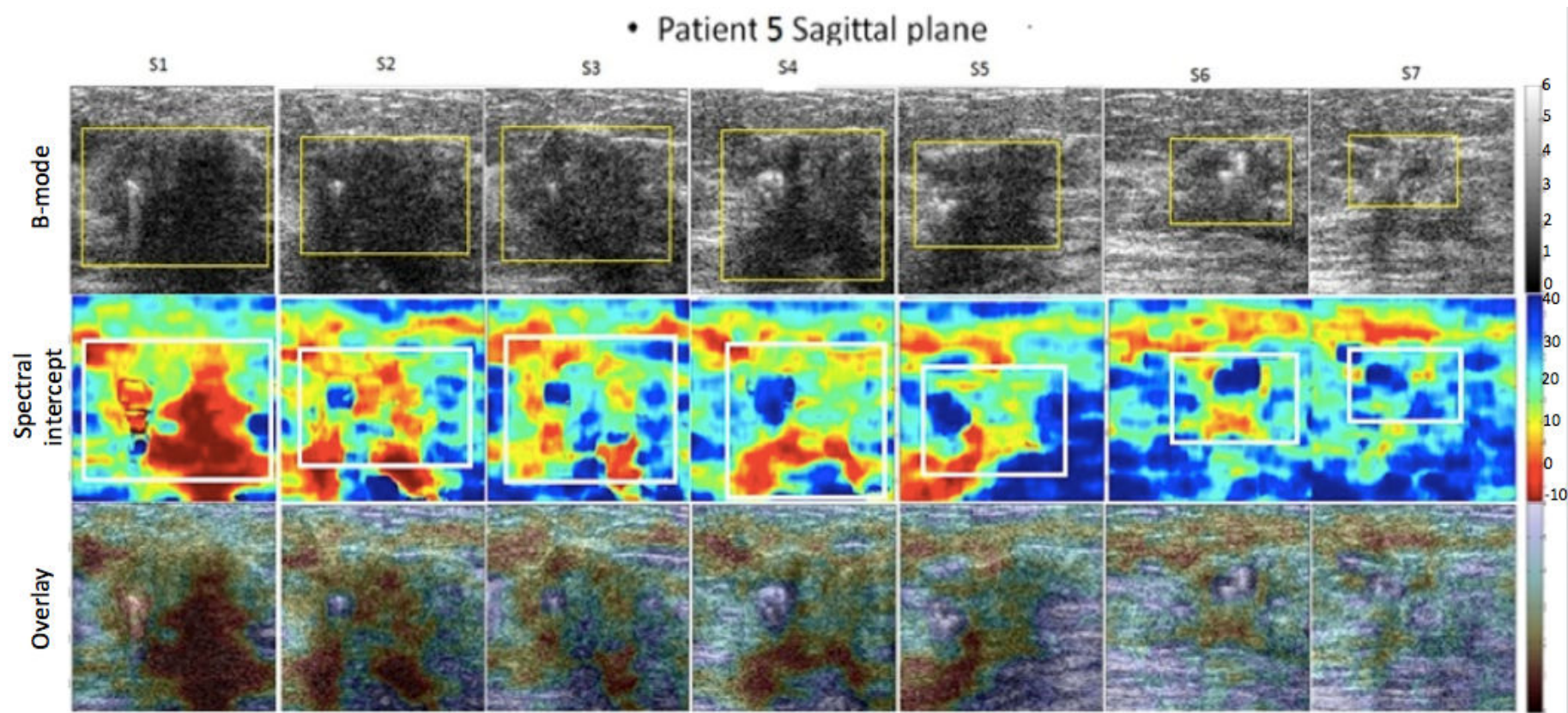


Figure 4- 4. Patient 5 dataset in the sagittal plane. (Top) B-mode image (middle) spectral intercept image (bottom) overlap of the B-mode and spectral images. The tumour areas are marked in the yellow box in the B-mode and marked in white in the intercept images. In B-mode, the tumour show large change in size between the 5th and 6th image. In the spectral intercept images, the tumour area increased in intercept value between the 1st and the final scan, changing from red to blue. The images all have the same depths of 40mm and widths of 38.4mm and the same colour range/mapping within each image modality. It must be noted that in the spectral intercept image, the micro-calcification that is used as the landmark exhibits high intercept values and hence appear blue inside the tumour that appears red.

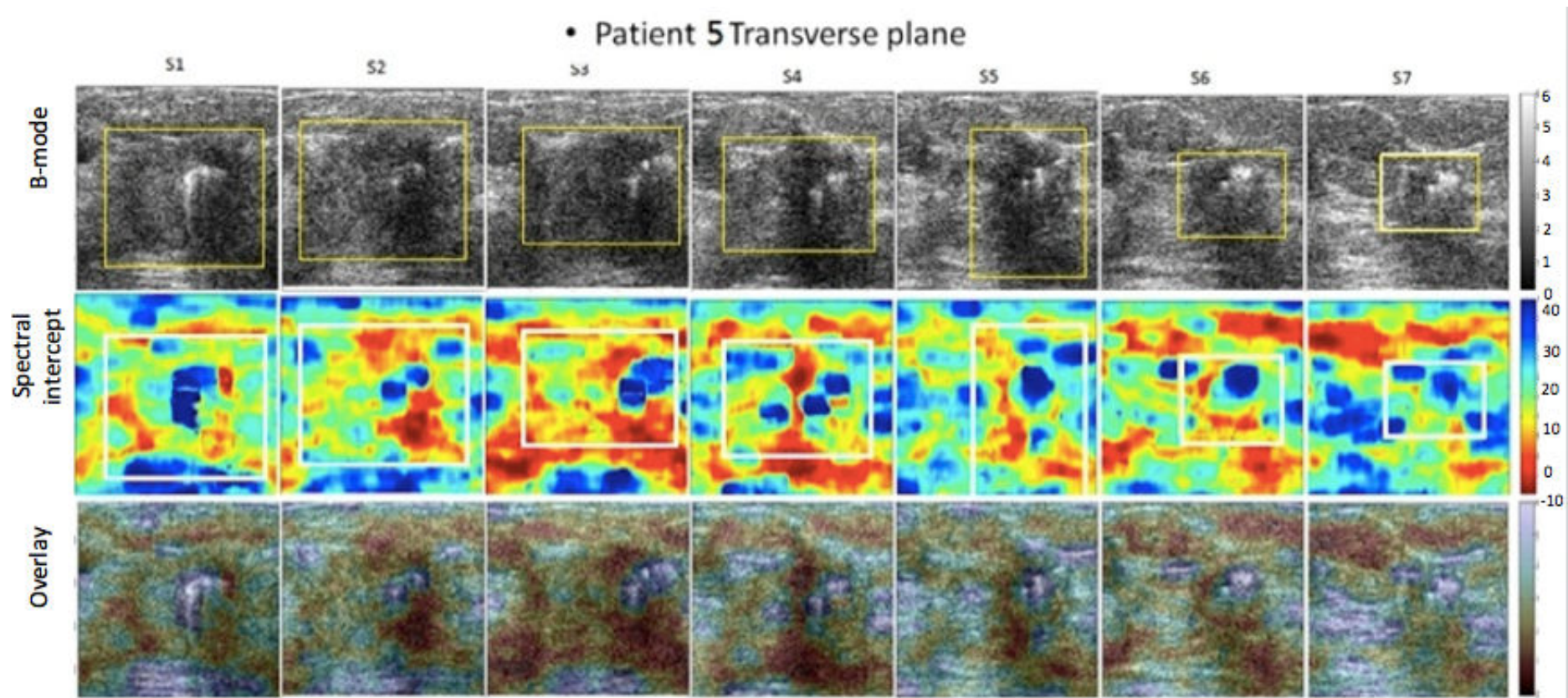


Figure 4- 5. Patient 5 data in the transverse plane. (Top) B-mode image (middle) spectral intercept image (bottom) overlap of the B-mode and spectral images. The tumour areas are marked in the yellow box in the B-mode and marked in white in the intercept images. In B-mode, the tumour show gradual change in size but in spectral intercept images, the corresponding tumour area increased in intercept value, changing from red to blue. The images all have the same depths of 40mm and widths of 38.4mm and the same colour range/mapping within each image modality. It must be noted that in the spectral intercept image, the micro-calcification that is used as the landmark exhibits high intercept values and hence appear blue inside the tumour that appears red.

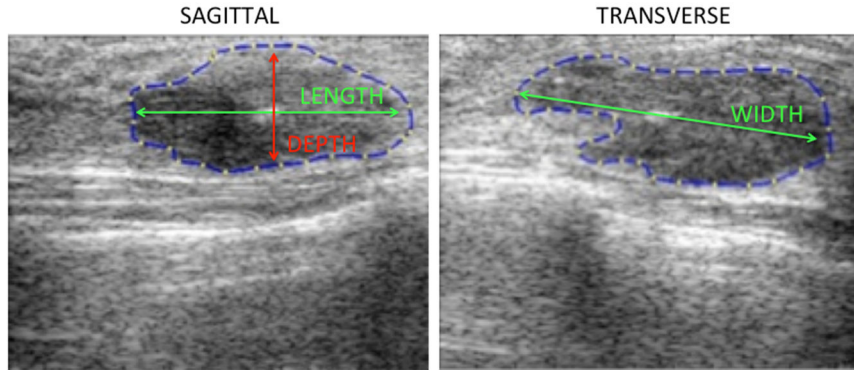


Figure 4- 6. Measurement of tumour size in ultrasound B-mode. The length and depth are measured in the sagittal plane (left) and the width is measured in the transverse plane (right).

Scan number	Sagittal (cm)	Transverse (cm)	Depth (cm)
Scan 1	2.10	2.40	1.20
Scan 2	2.18	2.20	1.30
Scan 3	1.55	2.00	1.09
Scan 4	1.48	1.79	1.05
Scan 5	1.37	1.59	0.87
Scan 6	0.75	1.28	0.69
Scan 7	0.88	1.09	0.74
Scan 8	0.90	0.66	0.69
Change between 1 st and final scan	1.20	1.74	0.51

Table 4. 2. Measurements of tumour size in ultrasound B-mode in patient 1.

Scan number	Sagittal (cm)	Transverse (cm)	Depth (cm)
Scan 1	3.37	3.39	1.95
Scan 2	3.09	2.89	2.09
Scan 3	2.82	3.01	2.82
Scan 4	2.36	2.60	1.74
Scan 5	2.26	1.43	1.39
Scan 6	2.34	1.25	1.04
Scan 7	1.36	0.87	0.82
Change between 1 st and final scan	2.01	2.52	1.13

Table 4. 3. Measurements of tumour size in ultrasound B-mode in patient 5.

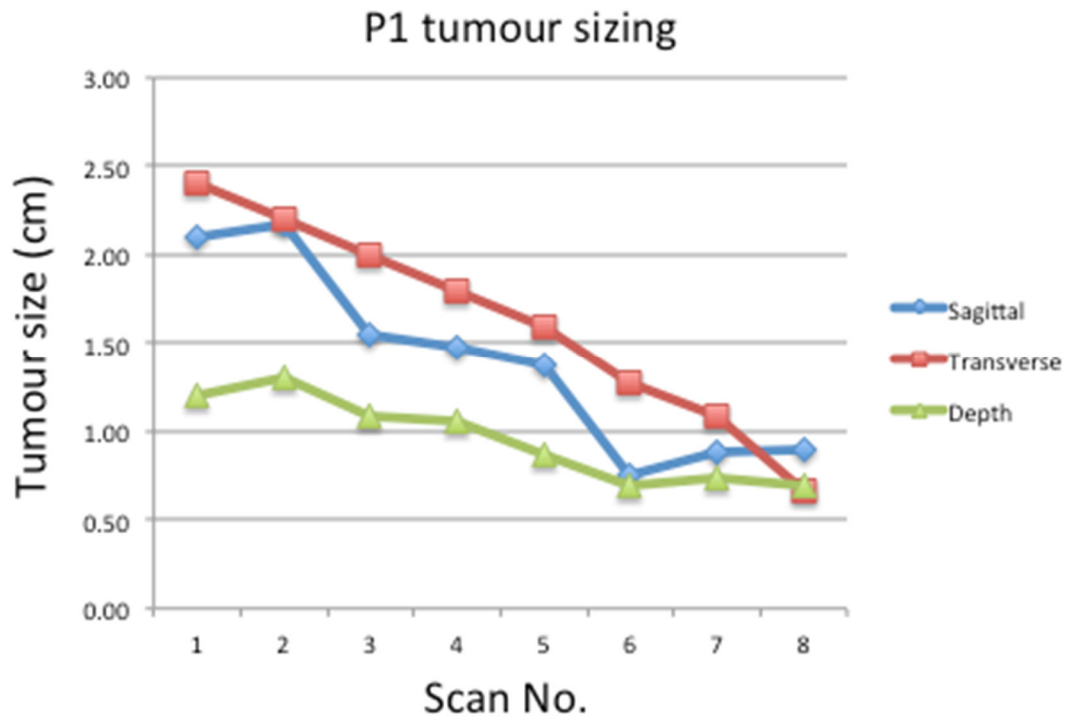


Figure 4- 7. Measured tumour size in ultrasound B-mode in patient 1

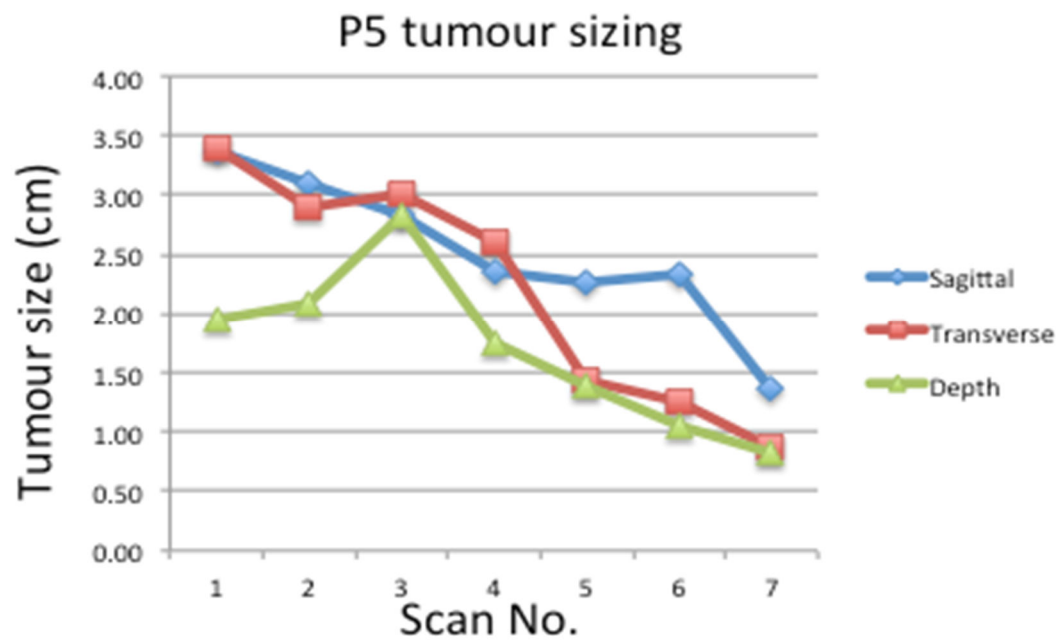


Figure 4- 8. Measured tumour size in ultrasound B-mode in patient 5.

4.3.3 Measuring changes in tumour spectral intercept

In order to measure changes in spectral intercept due to treatment, we proposed two methods whereby one tries to measure the local changes within the tumour, which we call the area of best match (ABM) analysis; the second method tries to give a more global measure in change by tracking the same four ROIs around the landmark and the ROIs are not restricted by the tumour boundary.

Imaging landmarks were initially used as a means to ensure repeatability in scanning the same plane and tumour region and to facilitate treatment monitoring in B-mode ultrasound (see **Figure 4- 1**) for the longitudinal study. However, as previously mentioned, they also give rise to high spectral intercept values that are uncharacteristic of the tumour and affects the results when included in the analysis. The high intercept values can be seen in the spectral intercept images in **Figure 4- 2** to **Figure 4- 5**, whereby a dark blue region appears within the tumour corresponding to the position of the landmark used. Because of this reason, we did not measure the overall average of spectral intercept within the tumour.

The methods proposed in this section avoid the landmarks whereby the landmark in each ultrasound B-mode image was first boxed out by a rectangular ROI (the reference ROI) centred on the landmark. The reference ROI needs to be big enough to include the whole landmark based on the appearance on ultrasound B-mode images. The reason that reference ROIs are not of the same size is because the metal coil can change shape with time due to tumour shrinkage and physiological changes can happen which affects the size of micro-calcification. Scanning in 2-D ultrasound also meant that planes scanned were not identical each time.

4.3.3.1 Area of best match (ABM) analysis

For the ABM analysis the clinician was asked to select regions within the tumour in ultrasound B-mode images, away from the landmark, as areas for comparison. Based on the clinician's best judgement, the clinician first selected areas from the first scan, and then used these as a reference for choosing what they believe were the same areas in subsequent scans. The areas were not of the same size since tumours change shape and size through time and treatments. Average spectral intercept values and standard deviation were then calculated from the selected areas.

To illustrate the ABM method, **Figure 4- 9** and **Figure 4- 10** show the areas selected by the clinician in the sagittal and the transverse plane respectively for patient 1 and the results of average intercept and standard deviation from these areas are shown in **Table 4. 5** and is plotted in **Figure 4- 11** and **Figure 4- 12** together with the tumour sizing. Overall spectral intercept values increased in all areas in both planes between the first and the final scan (**Table 4. 4**). In the sagittal plane, a big increase in intercept values can be observed between the 5th and the 6th scan in **Figure 4- 11** (increase of 21 dB, 17.7 dB, 12.9 dB and 13.3 dB in the left, bottom, right and top ROI respectively), which also corresponds to the disappearance of the red area within the tumour in the spectral intercept image **Figure 4- 2**. In the transverse plane (**Figure 4- 12**), the changes in most areas were more gradual, though a large increase can again be observed in the bottom middle and the top right ROI between the 5th and the 6th scan (an increase of 24 dB and 21.7 dB in the bottom middle and the top right ROI as opposed to a decrease of 6.9 dB in the left ROI and an increase of 3.9 and 9.2 dB in

the top middle and bottom right ROI). Outliers and fluctuation in the average intercept values can be observed especially in the transverse plane (**Figure 4- 12**) but in general there were increases in the spectral intercept value between the first and the final scan (Table 4. 4).

ABM			
Patient 1			
Sagittal (dB)		Transverse (dB)	
P1_sagittal_L	32.4	P1_trans_L	11.7
P1_sagittal_B	22.3	P1_trans_TM	11.4
P1_sagittal_R	13.7	P1_trans_BM	15.8
P1_sagittal_T	7.1	P1_trans_TR	10.5
		P1_trans_BR	17.4
Patient 5			
Sagittal (dB)		Transverse (dB)	
P5_sagittal_LT	3.8	P5_trans_LT	6.2
P5_sagittal_LM	6.1	P5_trans_LB	-24.2
P5_sagittal_LB	30.4	P5_trans_RT	-3.8
P5_sagittal_R	28.1	P5_trans_RB	-12.9

Table 4. 4 Differences in ABM spectral intercept values between the first and the final scan for patient 1 and 5. T, B, L, R,M stand for top, bottom, left, right and middle

Figure 4- 13 and **Figure 4- 14** show the areas selected by the clinician in the sagittal and the transverse plane respectively for patient 5 and the results of average intercept and standard deviation from these areas are shown in **Figure 4- 15** and **Figure 4- 16**. The average intercept values increased between the first and the final scan in all ROIs of the sagittal plane but showed a mixture of a small increase and a large decrease in the transverse plane (Table 4. 4). However, it is to be noted that intercept values from the 1st scan in the transverse plane for P5 appear to be very high in comparison to values of other initial scans measured in P1 and also in the sagittal plane in P5 (see **Figure 4- 16**). Nevertheless, upon inspecting the standard deviation of the measured values, they were found to be similar to that of other

scans.

Although there were only 2 cases in this study, based on the differences between the first and final scan intercept values (Table 4. 4), patient 1 who had complete response to chemotherapy showed bigger increases in both planes in comparison to patient 5 who only had partial response to treatments. There were consistent increases for all ROIs measured in both imaging planes for patient 1 but more mixed results, with increases in the sagittal plane and a mixture of small increases and decreases in the transverse plane for patient 5. The mixed results in patient 5 maybe due to the intercept measure picking up the tumour shrinkage which corresponds to the increase in intercept in the sagittal plane, while the decrease and relatively little change reflects partial response to treatments. The standard deviation of average intercept values did not seem to be affected by the size of the ROI but possibly more affected by the position selected by the clinician and the boundary effect of the parameter estimation when ROIs were selected near the boundary.

The ABM method proposed here was designed in an attempt to capture potential changes within the tumour as it responds to treatment and avoids interference caused by the landmark. However, it suffers from some limitations. First, the ROIs were selected based on the clinician's best judgement and the placement and size of these can vary from person to person. Secondly, even accommodating this variation, the ROIs were not fixed in size and as the tumour became smaller, so did the selected ROIs selected chosen to compare against the earlier scans. These limitations are addressed in the next section.

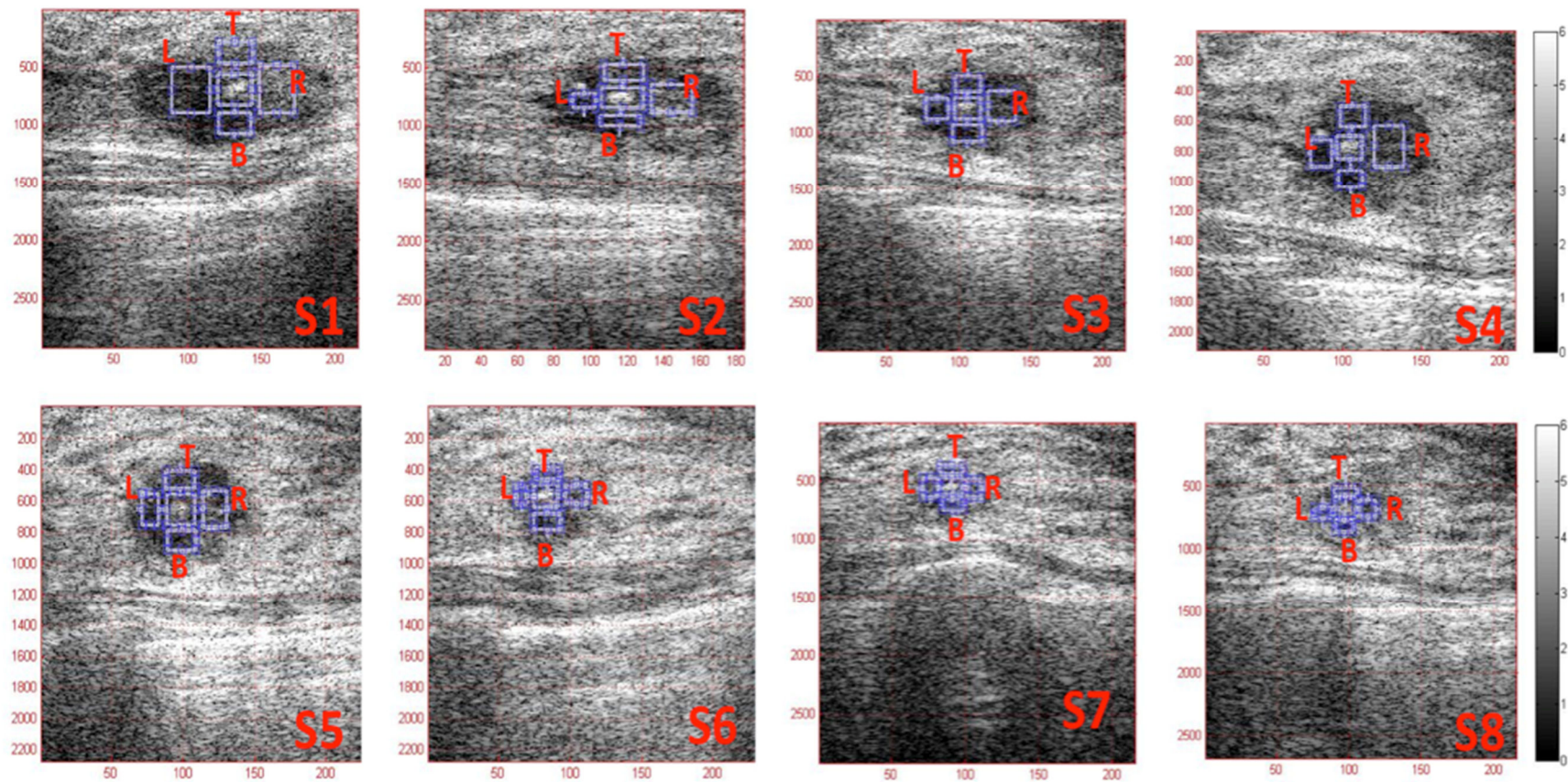


Figure 4- 9. Areas selected for ABM analysis for patient 1 in the sagittal plane for scan 1 (S1) to scan 8 (S8). The centre ROI is the landmark. Average spectral intercept values are calculated from the other ROIs and compared. Note that the images are of different depth. L, R, B and T represent the left, right, bottom and top ROIs.

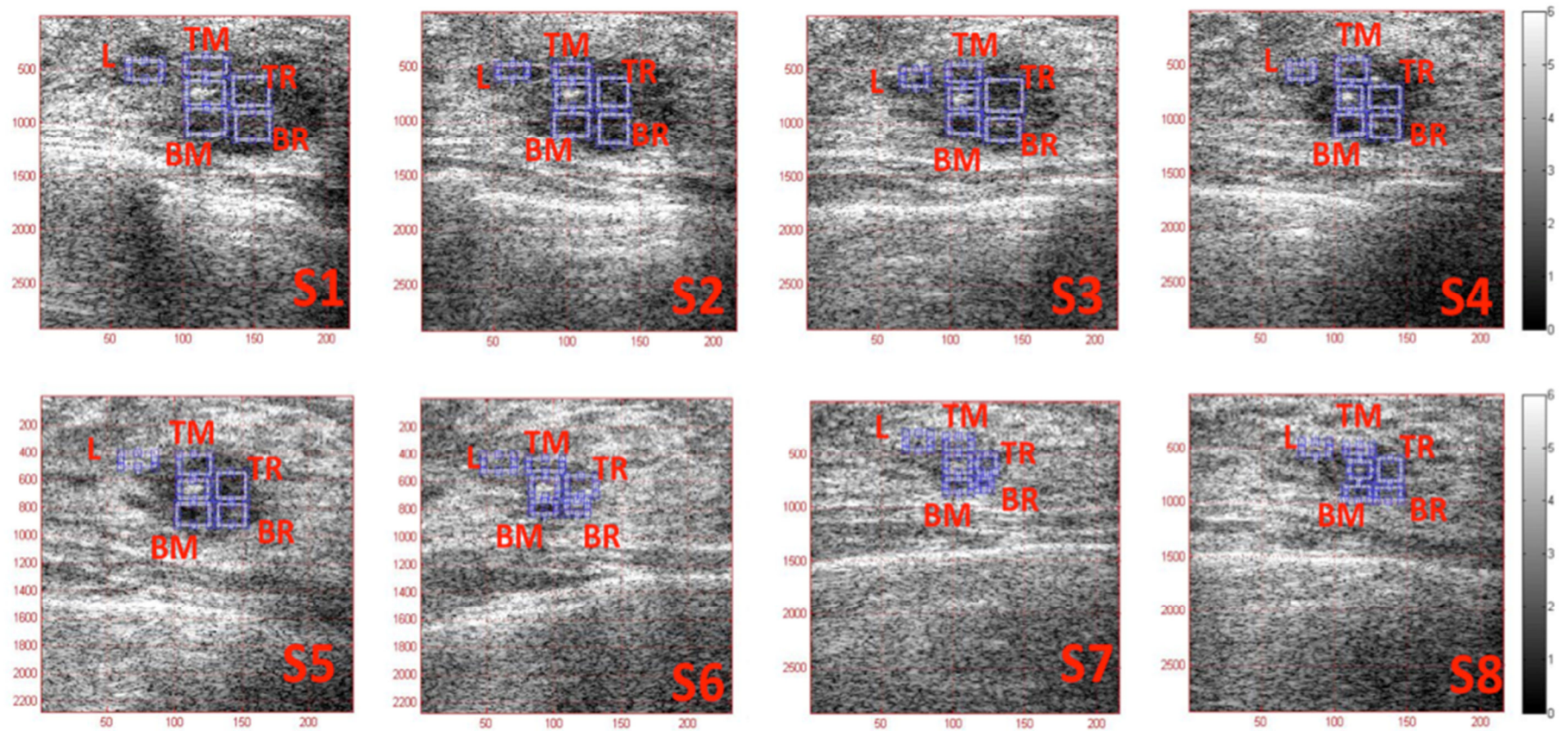


Figure 4- 10. Areas selected for ABM analysis for patient 1 in the transverse plane for scan 1 (S1) to scan 8 (S8). The centre ROI is the landmark. Average spectral intercept values are calculated from the other ROIs and compared. Note that the images are of different depth. L, TM, TR, BM and BR represent the left, top-mid, top-right, bottom-mid and bottom-right ROIs.

			ABM (dB)					
Scan #	1	2	3	4	5	6	7	8
p1_sagittal_L	6.9+/-5.0	17.8+/-7.2	19.2+/-4.4	22.4+/-4.5	12.0+/-3.7	33.0+/-4.8	35.4+/-8.7	39.3+/-2.9
p1_sagittal_B	3.5+/-3.7	5.0+/-4.7	5.3+/-3.1	4.8+/-2.0	9.2+/-4.6	26.9+/-4.8	23.1+/-7.6	25.8+/-2.6
p1_sagittal_R	11.1+/-3.9	7.0+/-3.2	10.6+/-2.5	6.1+/-2.7	7.3+/-5.7	20.2+/-10.1	24.3+/-6.7	24.8+/-8.2
p1_sagittal_T	6.8+/-4.3	6.3+/-3.3	7.8+/-2.6	4.5+/-3.9	3.3+/-3.1	16.6+/-2.0	19.7+/-3.1	13.9+/-2.7
Scan #	1	2	3	4	5	6	7	8
p1_trans_L	11.2+/-11.7	6.3+/-2.4	18.9+/-8.6	10.5+/-2.3	24.3+/-5.8	17.4+/-2.1	26.1+/-4.9	22.9+/-3.8
p1_trans_TM	9.7+/-4.5	2.7+/-2.8	9.9+/-3.1	19.6+/-13.1	10.4+/-5.5	14.3+/-2.9	16.2+/-4.4	21.1+/-3.2
p1_trans_BM	7.4+/-3.7	5.4+/-3.6	11.8+/-4.0	14.0+/-3.4	6.8+/-8.5	30.8+/-3.6	20.4+/-5.7	23.2+/-2.2
p1_trans_TR	12.3+/-2.4	7.4+/-3.3	13.0+/-6.0	20.3+/-12.5	6.9+/-4.4	28.6+/-4.4	22.9+/-5.8	22.8+/-6.2
p1_trans_BR	3.4+/-5.1	1.8+/-3.6	15.7+/-4.9	18.1+/-6.6	21.1+/-7.3	30.3+/-3.4	23.8+/-2.6	20.8+/-2.1

Table 4. 5 Results of ABM analysis for P1 for the sagittal and the transverse plane. The units are in dB and the values are the average spectral intercept +/- standard deviation. L, B, R, T, M stand for left, bottom, right, top and middle respectively

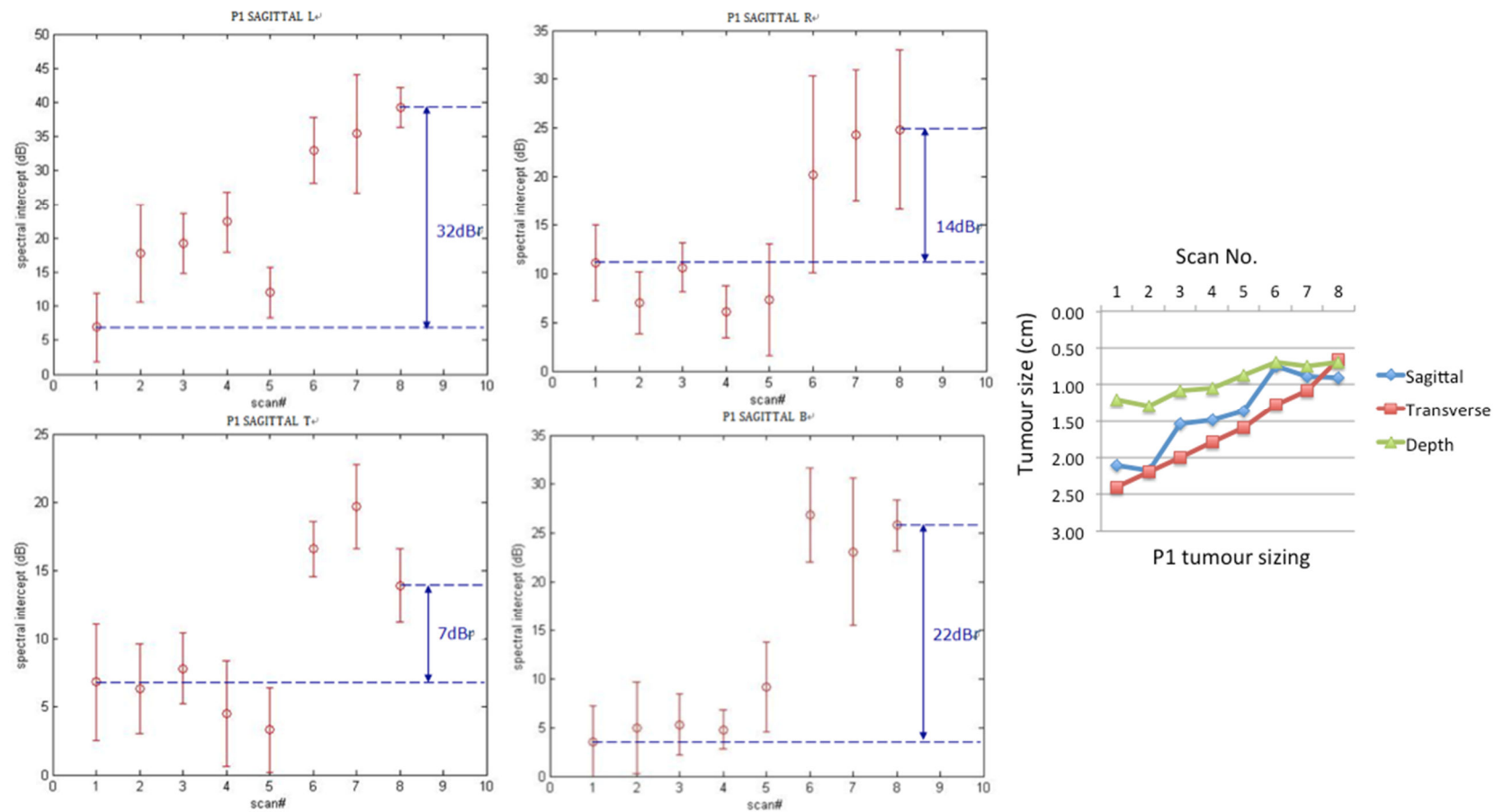


Figure 4- 11. Results of ABM analysis of patient 1 in the sagittal plane. (Top left) L, (Top middle) R, (Bottom left) T and (Bottom middle) B represent results from the left, right, top and bottom ROIs from Figure 4- 9. The circles in the figures are the means and the bars represent +/- one standard deviation of spectral intercept values of the ROIs. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. (Right) P1 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

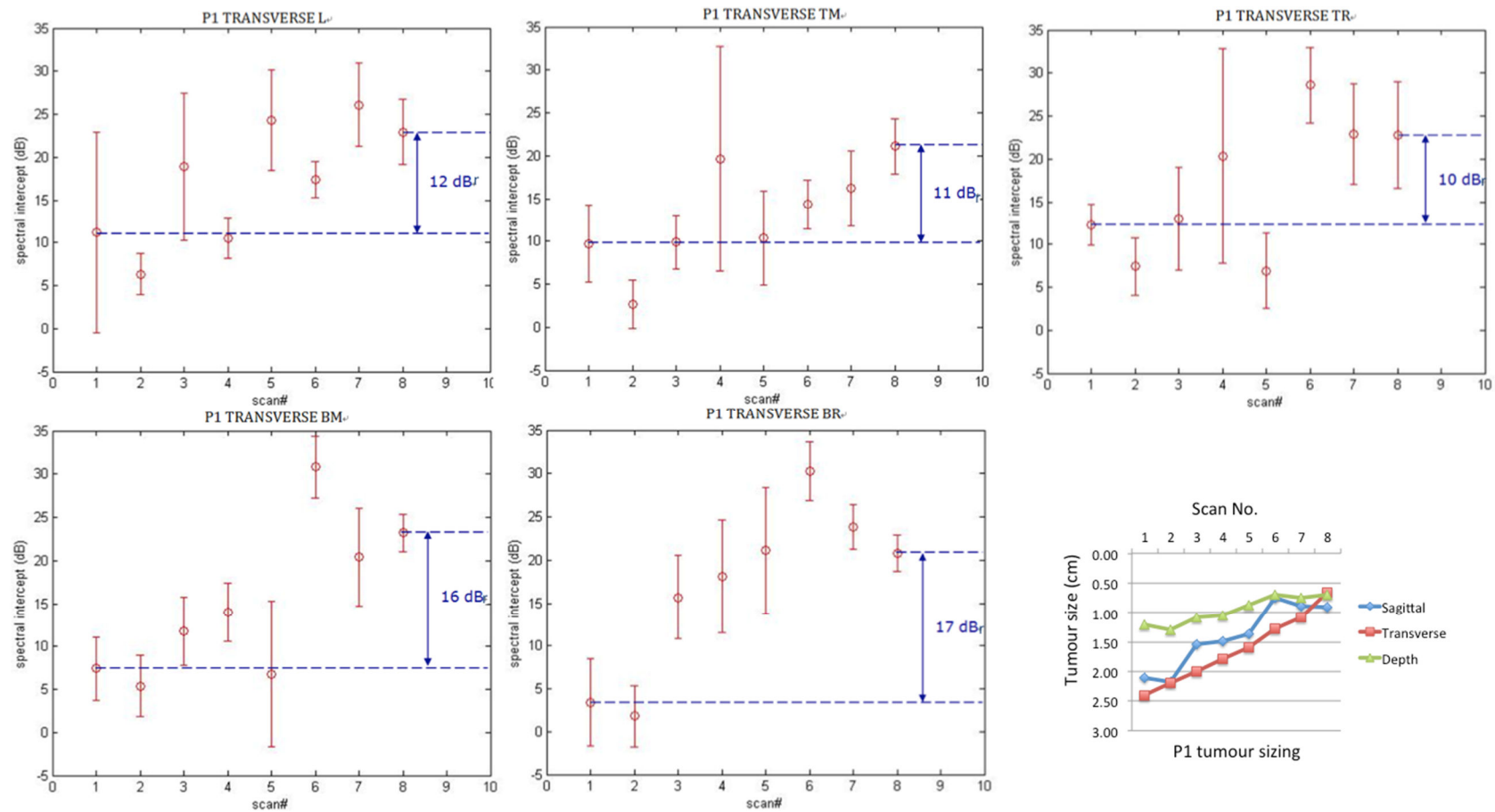


Figure 4- 12. Results of ABM analysis of patient 1 in the transverse plane. (Top left) L, (Top Middle) TM, (Top right) TR, (Bottom left) BM and (Bottom middle) BR represent results from the left, top middle, top right, bottom middle and bottom right ROIs from the Figure 4- 10. The circles in the figures are the means and the bars present $\pm$ one standard deviation of spectral intercept values of the ROIs. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. (Bottom right) P1 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

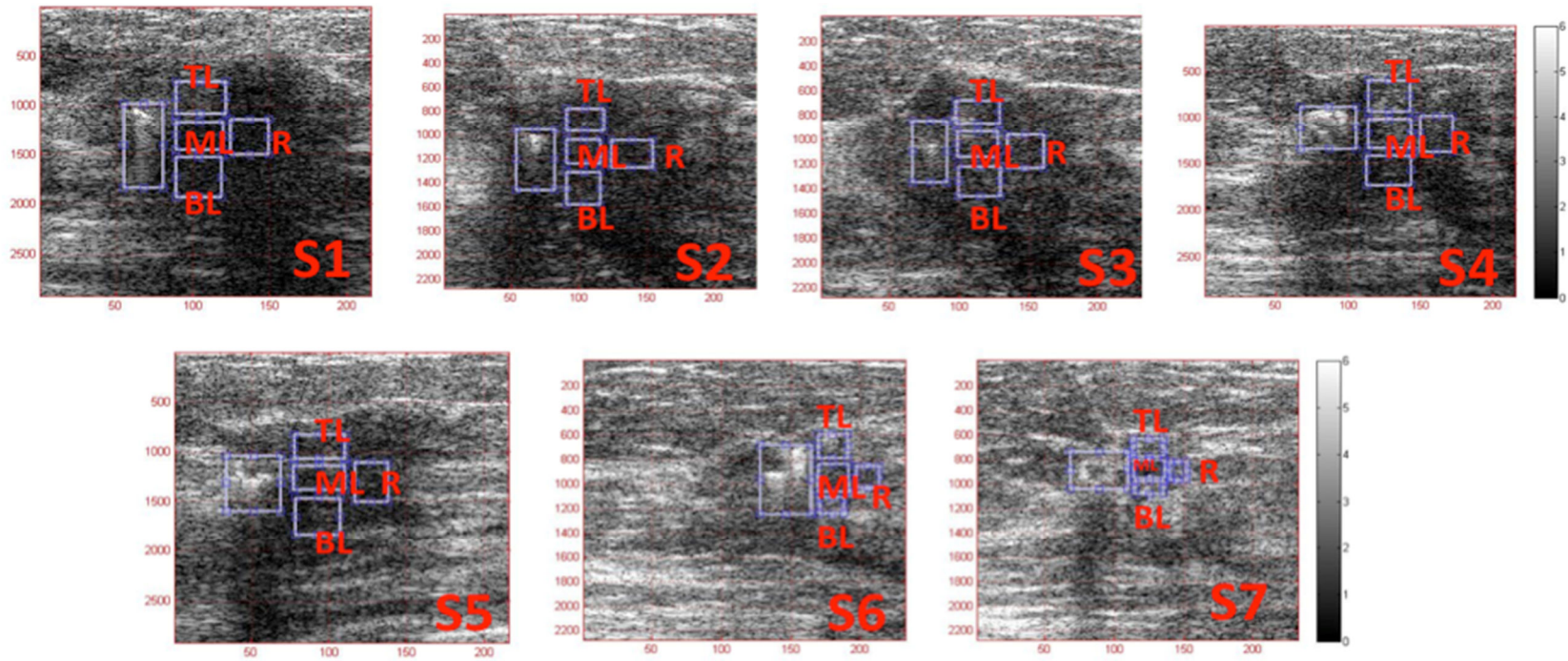


Figure 4- 13. Areas selected for the ABM analysis for patient 5 in the sagittal plane for scan 1 (S1) to scan 7 (S7). The furthest left ROI is the landmark in each image. Average spectral intercept values were calculated from the other ROIs and compared. Note that the images are of different depth. TL, ML, BL and R represent the top-left, mid-left, bottom-left and right ROIs.

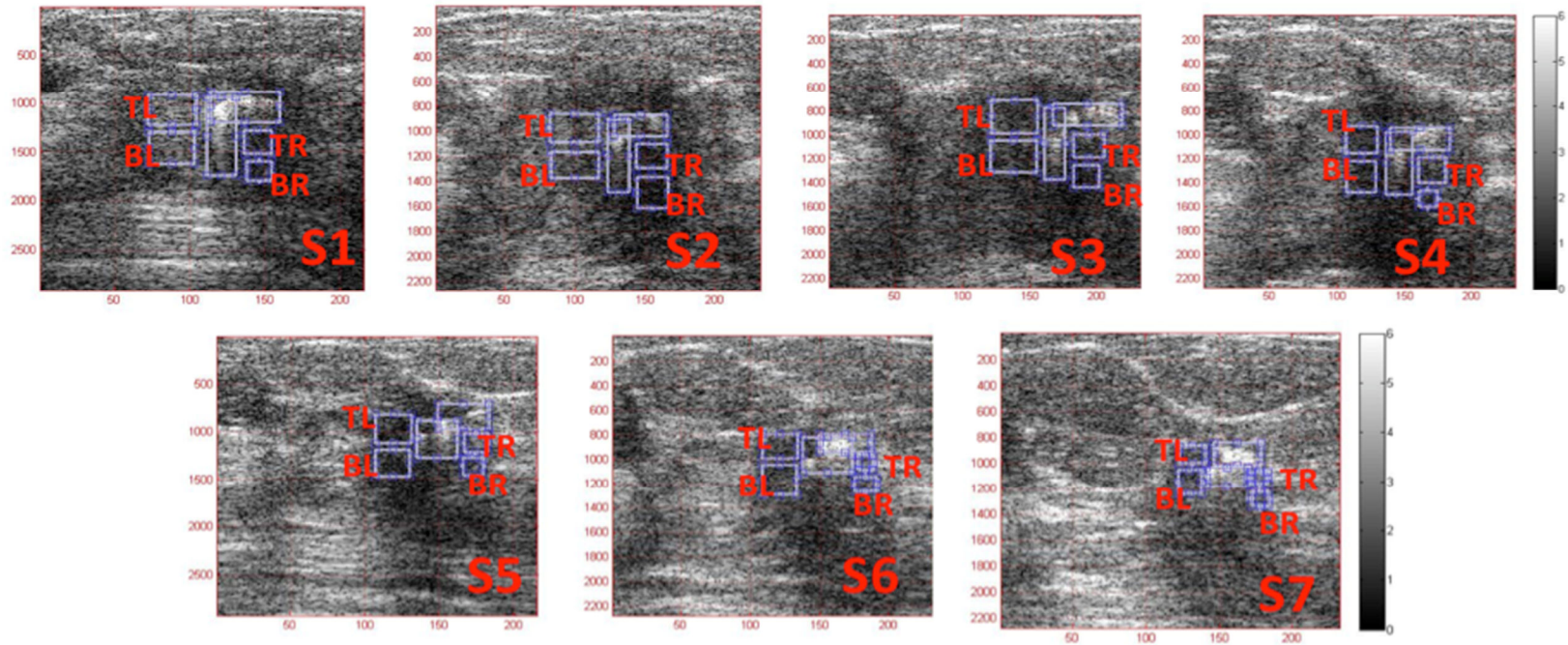


Figure 4- 14. Areas selected for the ABM analysis for patient 5 in the transverse plane for scan 1 (S1) to scan 7 (S7). The centre inverted L-shaped ROI is the landmark. Average spectral intercept values are calculated from the other ROIs and compared. Note that the images are of different depth. TL, BL, TR and BR represent the top-left, bottom-left, top-right and bottom-right ROIs.

		ABM (dB)					
Scan #	1	2	3	4	5	6	7
P5_sagittal_LT	13.8+/-2.2	5.7+/-3.4	15.2+/-5.9	15.4+/-3.7	15.6+/-4.3	22.1+/-6.5	17.6+/-4.1
P5_sagittal_LM	3.6+/-9.6	3.7+/-3.4	20.6+/-2.2	15.7+/-5.1	16.4+/-4.7	15.9+/-10.3	9.7+/-4.4
P5_sagittal_LB	-(6.7)+/-5.4	8.3+/-6.3	26.4+/-4.6	5.8+/-5.7	4.7+/-6.5	23.9+/-5.0	23.7+/-4.1
P5_sagittal_R	-(9.3)+/-2.2	6.8+/-4.5	9.1+/-3.4	15.7+/-2.6	13.0+/-3.2	18.0+/-4.3	18.8+/-3.8
Scan #	1	2	3	4	5	6	7
P5_trans_LT	12.8+/-3.0	12.7+/-5.7	2.5+/-4.3	3.7+/-10.7	9.4+/-6.7	6.9+/-8.1	19.0+/-7.0
P5_trans_LB	38.2+/-4.9	12.2+/-3.9	11.0+/-5.6	11.6+/-13.1	6.3+/-5.2	9.5+/-6.7	14.0+/-4.4
P5_trans_RT	26.9+/-3.7	3.6+/-8.3	23.2+/-10.9	11.6+/-7.1	14.7+/-6.3	18.5+/-5.9	23.1+/-3.1
P5_trans_RB	30.8+/-6.7	-(8.4)+/-2.3	0.7+/-1.7	11.0+/-2.5	11.0+/-5.8	11.4+/-3.1	17.9+/-4.4

Table 4. 6 Results of ABM analysis for P5 for the sagittal and the transverse plane. The units are in dB and the values are the average spectral intercept +/- standard deviation. L, B, R, T, M stand for left, bottom, right, top and middle respectively

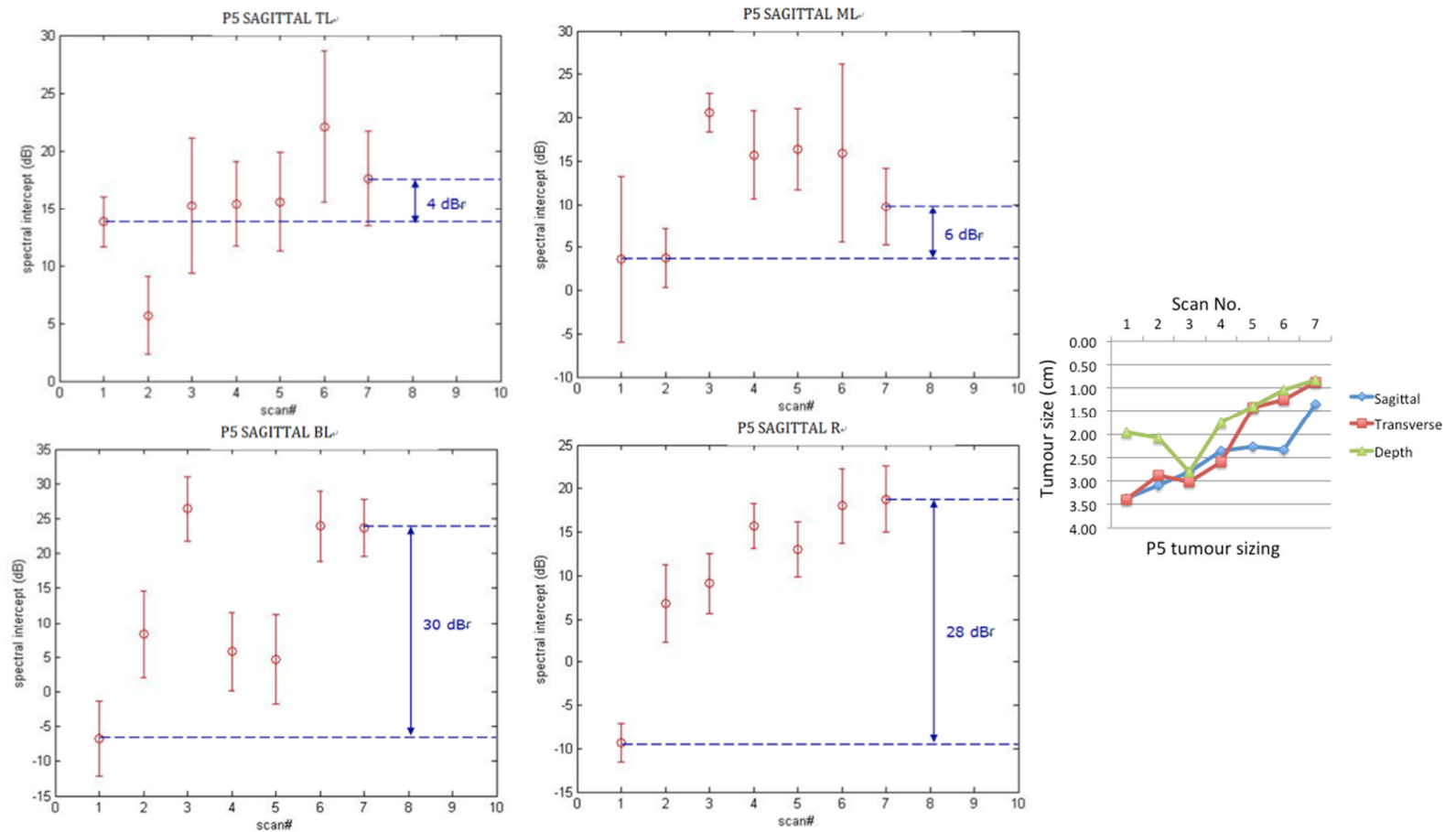


Figure 4- 15. Results of the ABM analysis of patient 5 in the sagittal plane. (Top left) TL, (Top middle) ML, (Bottom left) BL and (Bottom middle) R represent the top-left, mid-left, bottom-left and right ROIs. The circles in the figures are the means and the bars present +/- one standard deviation of spectral intercept values of the ROIs. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. (Right) P5 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

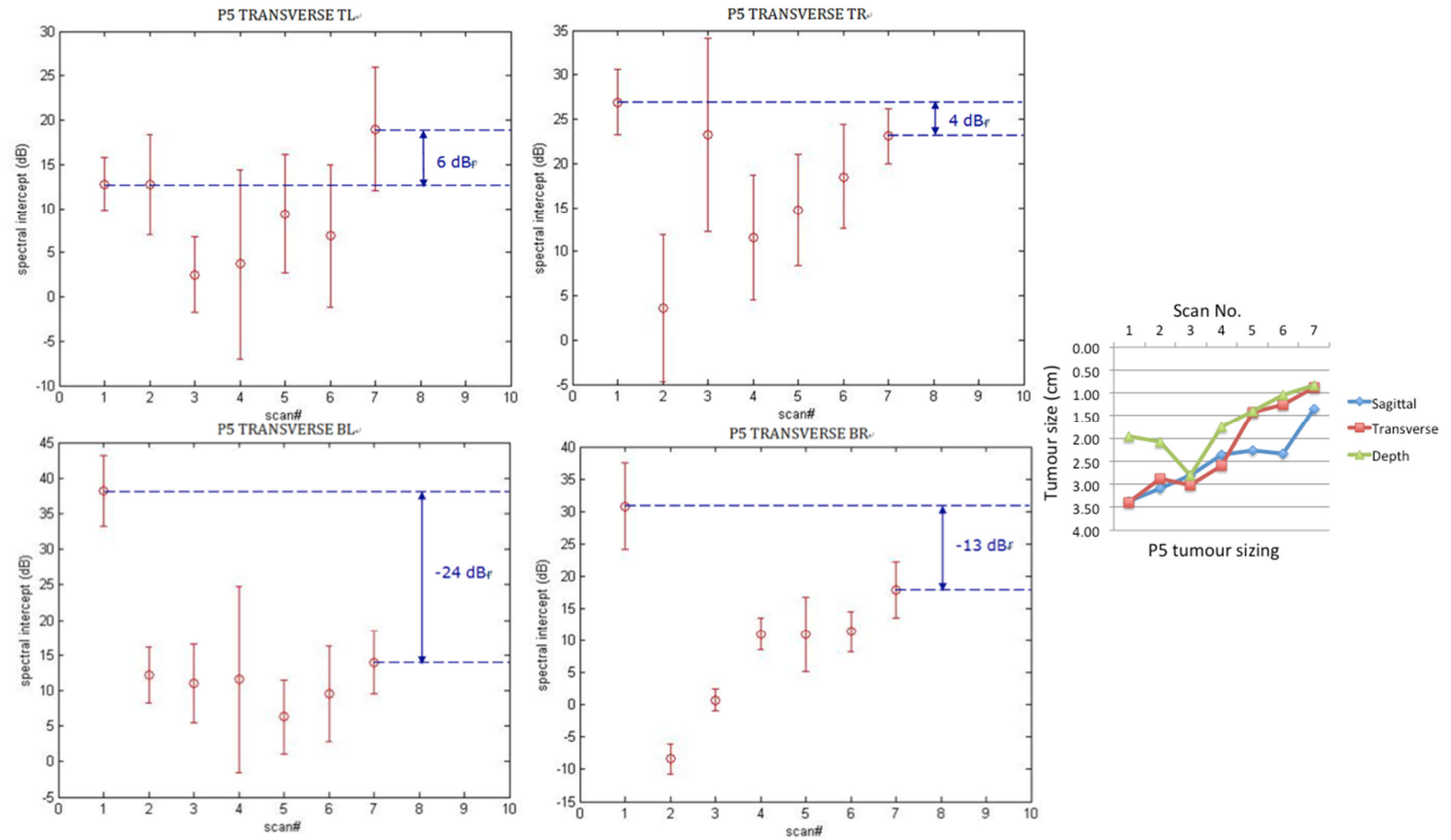


Figure 4- 16. Results of the ABM analysis of patient 5 in the transverse plane. (Top left) TL, (Bottom left) BL, (Top middle) TR and (Bottom middle) BR represent the top-left, bottom-left, top-right and bottom-right ROIs. The circles in the figures are the means and the bars present +/- one standard deviation of spectral intercept values of the ROIs. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. The vertical axes are spectral intercept values in dB and the horizontal axes are the scan number. (Right) P5 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

4.3.3.2 Fixed position analysis (FPA)

In the FPA method, the landmarks are again avoided using the same method as in the ABM method, but instead of selecting corresponding areas based on the clinician's judgement, four rectangular ROIs (300x30 pixels, approximately 5mm x 5mm) were placed at fixed distances in the top-left, top-right, bottom-left and bottom right quadrant relative to that of the landmark ROI. These quadrants were selected instead of top, bottom, left and right because the tumours tend to be ellipsoid in shape and we chose these quadrants to maximise the areas within the tumours. Average intercept values were then calculated from these areas. This is done on every scan and for each patient the selected areas were compared to observe changes in parametric values across the treatment. This approach is clearly one that would be more applicable to automation and the purpose was to see if using this approach significantly affected interpretation.

Figure 4- 17 and **Figure 4- 18** show the areas that were selected from patient 1 for the FPA in the sagittal and transverse plane respectively. **Figure 4- 19** and **Figure 4- 20** show the averaged intercept value results in the sagittal and the transverse plane for patient 1 from the analysis and also the tumour size measured. Overall there were gradual increases in the intercept values in all four quadrants in both planes, and the average intercept values increased throughout the chemotherapy in both planes but outliers and fluctuation in the average intercept values were observed such as scan number 4 in top-right quadrant in the transverse plane (**Figure 4- 20**). The results are given in **Table 4. 8** and overall there were more than 10 dB increase in the spectral intercept except the top-right ROI in the sagittal plane and the top-left ROI in the

transverse plane.

FPA			
Patient 1			
Sagittal (dB)		Transverse (dB)	
P1_sagittal_TL	14.6	P1trans_TL	7.9
P1_sagittal_BL	23.7	P1trans_BL	14.2
P1_sagittal_TR	2.9	P1trans_TR	10.9
P1_sagittal_BR	17.2	P1trans_BR	17.4
Patient 5			
Sagittal (dB)		Transverse (dB)	
P5_sagittal_TL	14.3	P5_transverse_TL	10.9
P5_sagittal_BL	12.4	P5_transverse_BL	2
P5_sagittal_TR	4.5	P5_transverse_TR	-2.6
P5_sagittal_BR	15.3	P5_transverse_BR	11.7

Table 4. 7 Differences in ABM spectral intercept values between the first and the final scan for patient 1 and 5 . T, B, L, R stand for top, bottom, left and right

Figure 4- 21 and **Figure 4- 22** show the areas that were selected in patient 5 for the FPA in the sagittal and the transverse plane respectively. **Figure 4- 23** and **Figure 4- 24** show the averaged intercept value results in the sagittal and the transverse plane of patient 5 from the analysis and the measured tumour size. In general the intercept values increased between the first and the final scanning session, with the sagittal plane showing more noticeable changes. However, the values did not increase monotonically in time but fluctuated. The results are similar to the results of the ABM analysis, bigger increase in the spectral intercept can be observed in the sagittal plane in comparison to the transverse plane.

Based on these results, again, patient 1 who had complete response to treatment showed higher changes in the average intercept values in both planes in comparison to patient 5 who had only a partial response. Whereas the ABM analysis discussed in

the previous section does not pick out changes across the boundary of the tumour as one of the criteria for selecting areas by the clinician was that the areas need to be within the tumour, the FPA analysis does pick out the boundary change due to the fact that the ROIs were fixed in size and were more sensitive to overall changes to the tumour induced by the treatments. However, there was still fluctuation and outliers within the results (for example the TR window in **Figure 4- 20**).

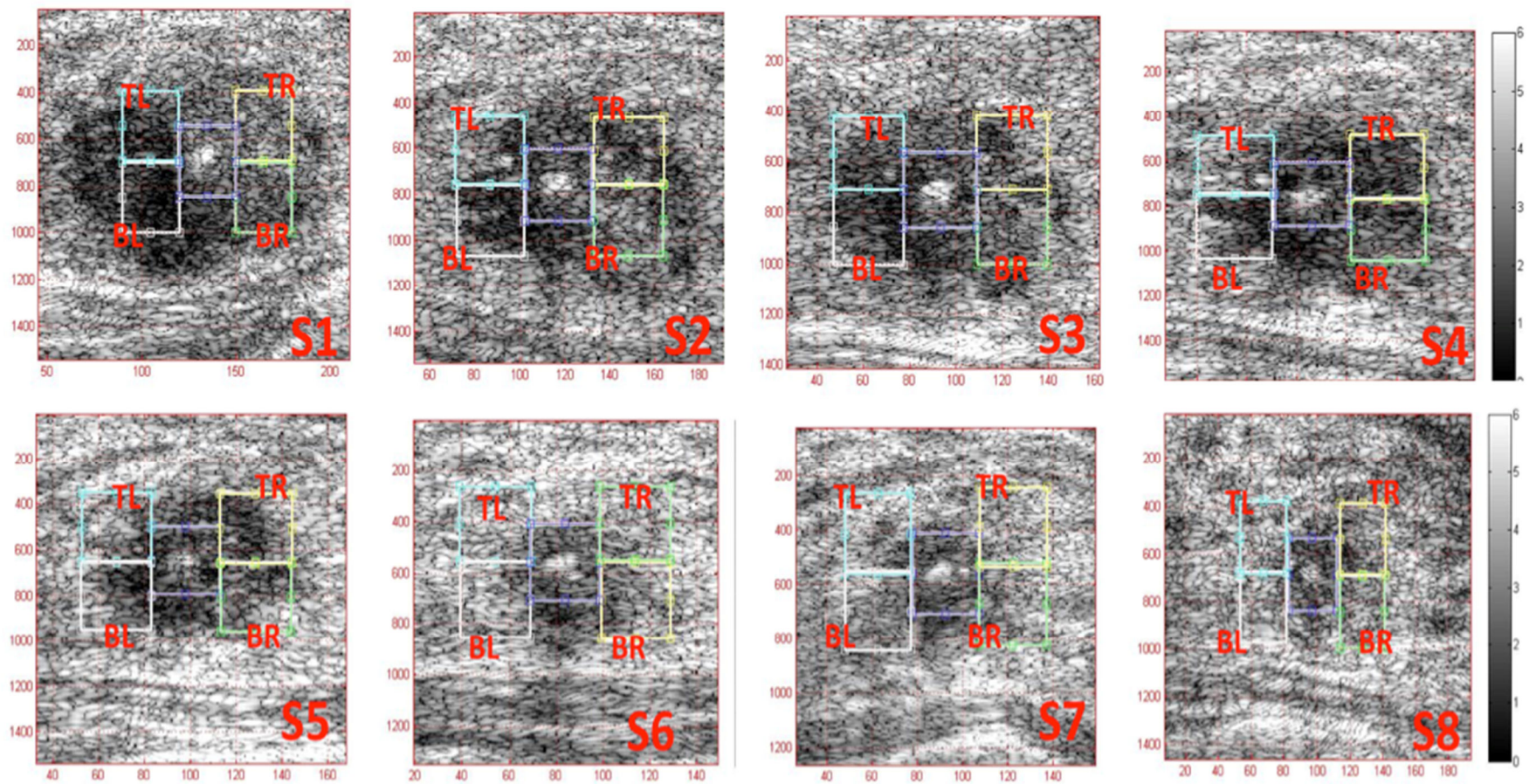


Figure 4- 17. Areas selected for FPA for patient 1 in the sagittal plane. The centre dark blue ROI is the landmark. Average spectral intercept values were calculated from the other four ROIs and compared. Note that the widths and depths are not the same for all images. TL, BL, TR and BR represent the top-left, bottom-left, top-right and bottom-right ROIs.

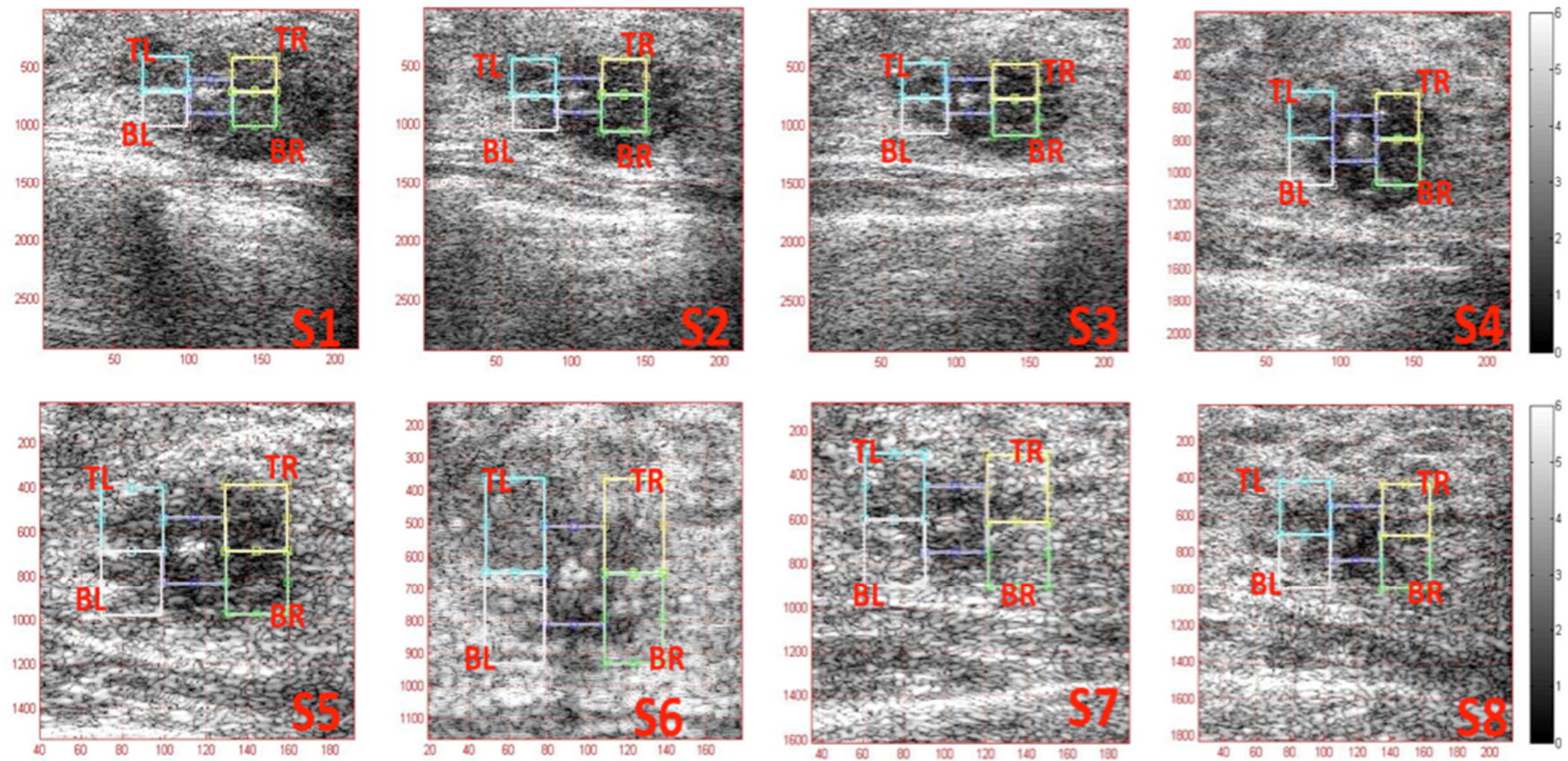


Figure 4- 18. Areas selected for FPA for patient 1 in the transverse plane. The centre dark blue ROI is the landmark. Average spectral intercept values are calculated from the other four ROIs and compared. Note that the depths are not the same for all images. TL, BL, TR and BR represent the top-left, bottom-left, top-right and bottom-right ROIs.

			FPA (dB)					
Scan #	1	2	3	4	5	6	7	8
p1_sagittal_TL	11.3+/-6.5	24.1+/-10.1	16.8+/-5.0	23.2+/-5.1	17.9 +/-4.9	23.3+/-4.4	22.7+/-4.8	25.9+/-5.4
p1_sagittal_BL	7.2+/-6.0	16.9+/-5.6	16.9+/-2.2	22.7+/-3.9	27.56+/-7.4	35.7+/-5.7	32.1+/-3.9	30.9+/-4.2
p1_sagittal_TR	13.1+/-2.6	5.6+/-4.1	14.5+/-4.4	9.0+/-4.2	9.7+/-7.3	16.3+/-2.7	16.1+/-3.6	16.0+/-4.8
p1_sagittal_BR	9.9+/-4.5	8.9+/-2.3	14.8+/-5.5	8.1+/-4.5	16.8+/-8.4	25.9+/-4.5	24.6+/-5.4	27.1+/-4.7
Scan #	1	2	3	4	5	6	7	8
p1trans_TL	14.1+/-8.0	10.5+/-3.7	18.7+/-8.1	10.1+/-4.9	23.0+/-6.9	17.6+/-4.4	22.4+/-6.8	22.0+/-5.6
p1trans_BL	16.2+/-7.3	18.2+/-7.0	23.1+/-6.1	21.1+/-6.5	27.7+/-8.8	32.9+/-4.9	29.2+/-3.4	30.4+/-6.2
p1trans_TR	10.6+/-4.4	7.7+/-3.7	14.1+/-5.2	28.1+/-16.1	12.1+/-5.5	21.2+/-5.8	20.3+/-4.1	21.5+/-2.5
p1trans_BR	9.4+/-4.3	4.2+/-1.8	11.8+/-5.5	16.7+/-4.8	16.7+/-9.2	30.5+/-4.2	30.6+/-4.3	26.8+/-5.0

Table 4. 8 Results of FPA for P1 for the sagittal and the transverse plane. The units are in dB and the values are the average spectral intercept +/- standard deviation. L, B, R, T stand for left, bottom, right and top respectively

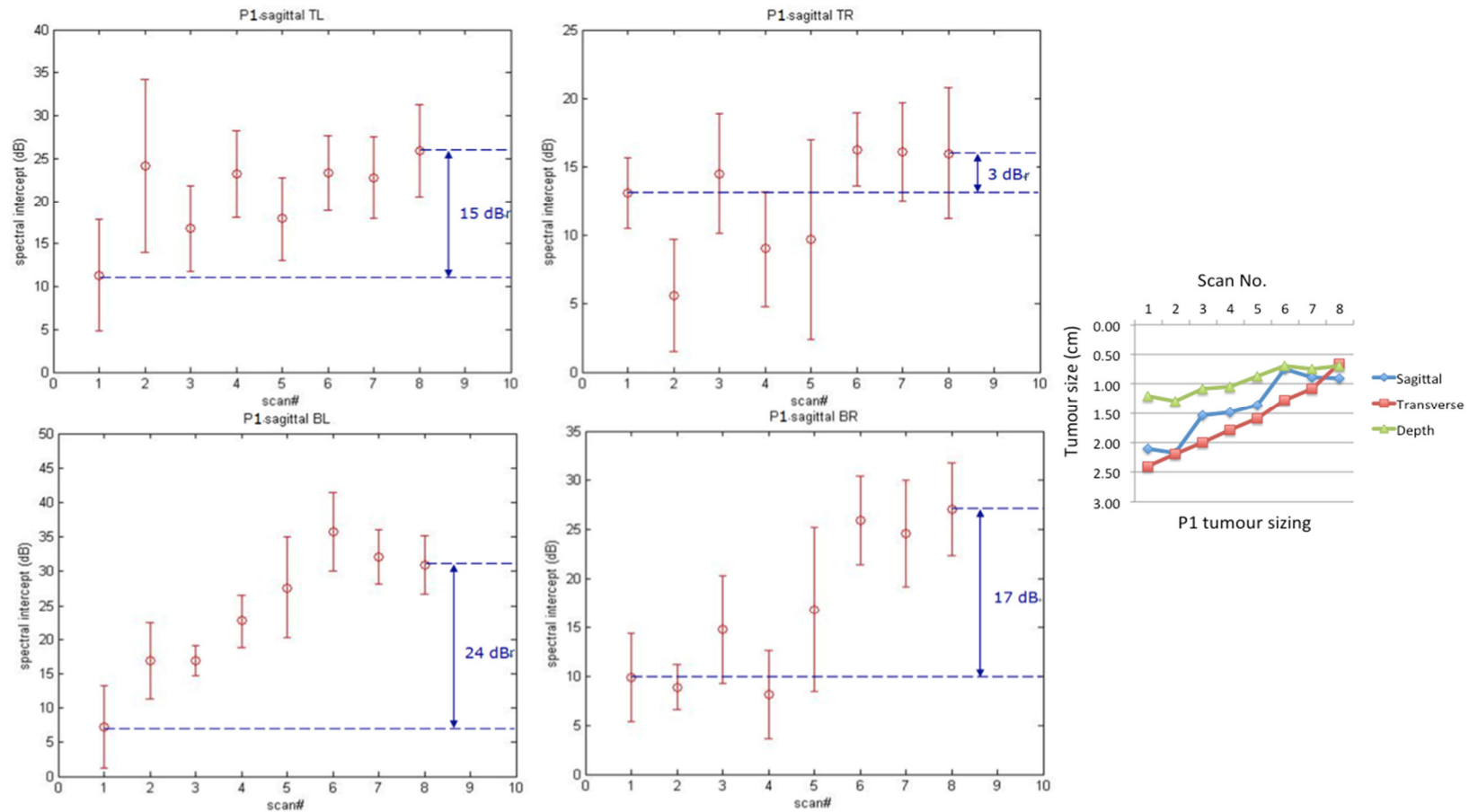


Figure 4- 19. Results of FPA of patient 1 in the sagittal plane. (Top left) TL, (Top middle) TR, (Bottom left) BL and (Bottom middle) BR represent results from the top left, top right, bottom left and bottom right ROIs from Figure 4- 17. The circles in the figures are the means and the bars present +/- one standard deviation of spectral intercept values of the ROIs. (Right) P1 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

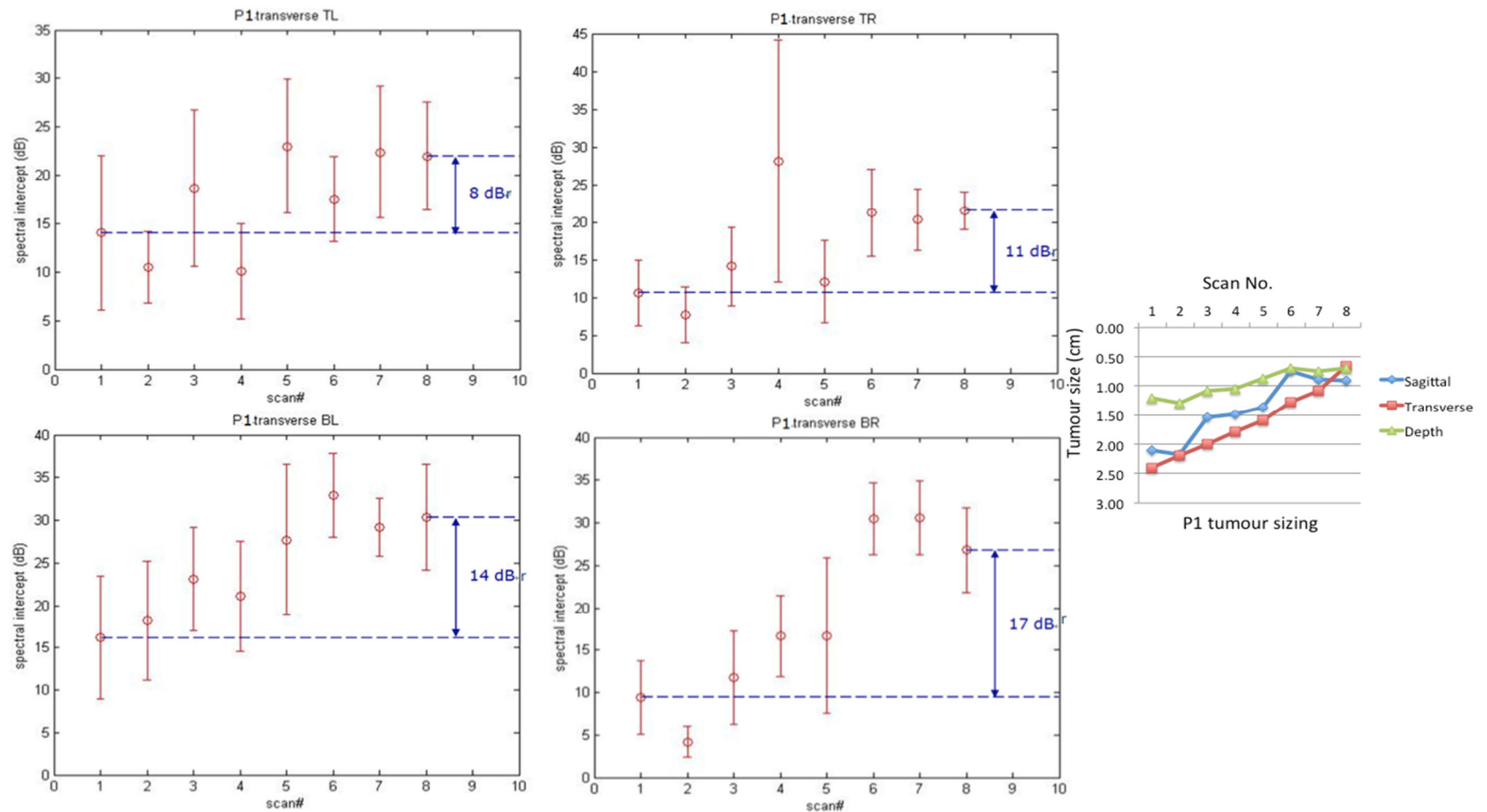


Figure 4- 20. Results of FPA of patient 1 in the transverse plane. (Top left) TL, (Top middle) TR, (Bottom left) BL and (Bottom middle) BR represent results from the top left, top right, bottom left and bottom right ROIs from Figure 4- 18. The circles in the figures are the means and the bars present +/- one standard deviation of spectral intercept values of the ROIs. (Right) P1 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

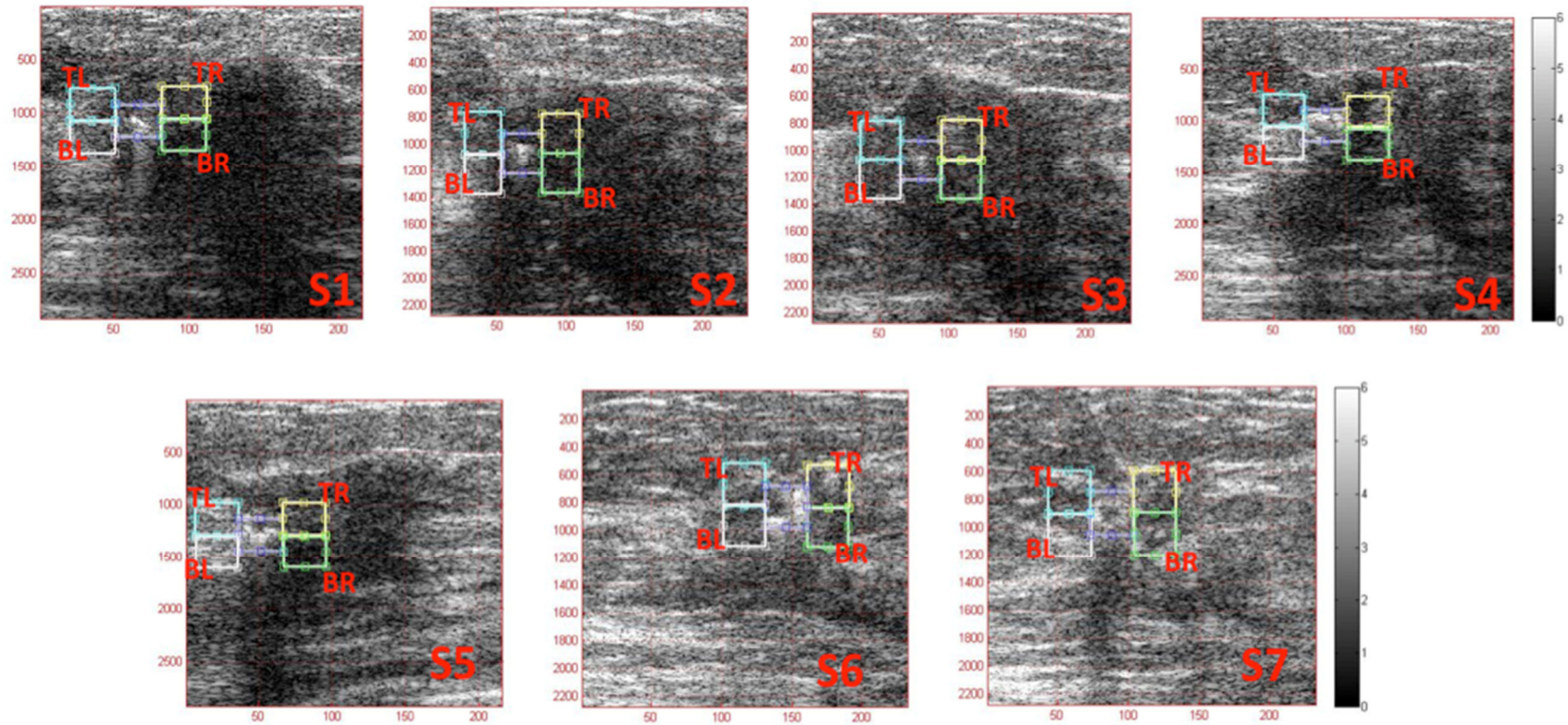


Figure 4- 21. Areas selected for FPA for patient 5 in the sagittal plane. The centre dark blue ROI is the landmark. Average spectral intercept values are calculated from the other four ROIs and compared. TL, TR, BL and BR represent results from the top left, top right, bottom left and bottom right ROIs. Note that the widths and depths are not the same for all images.

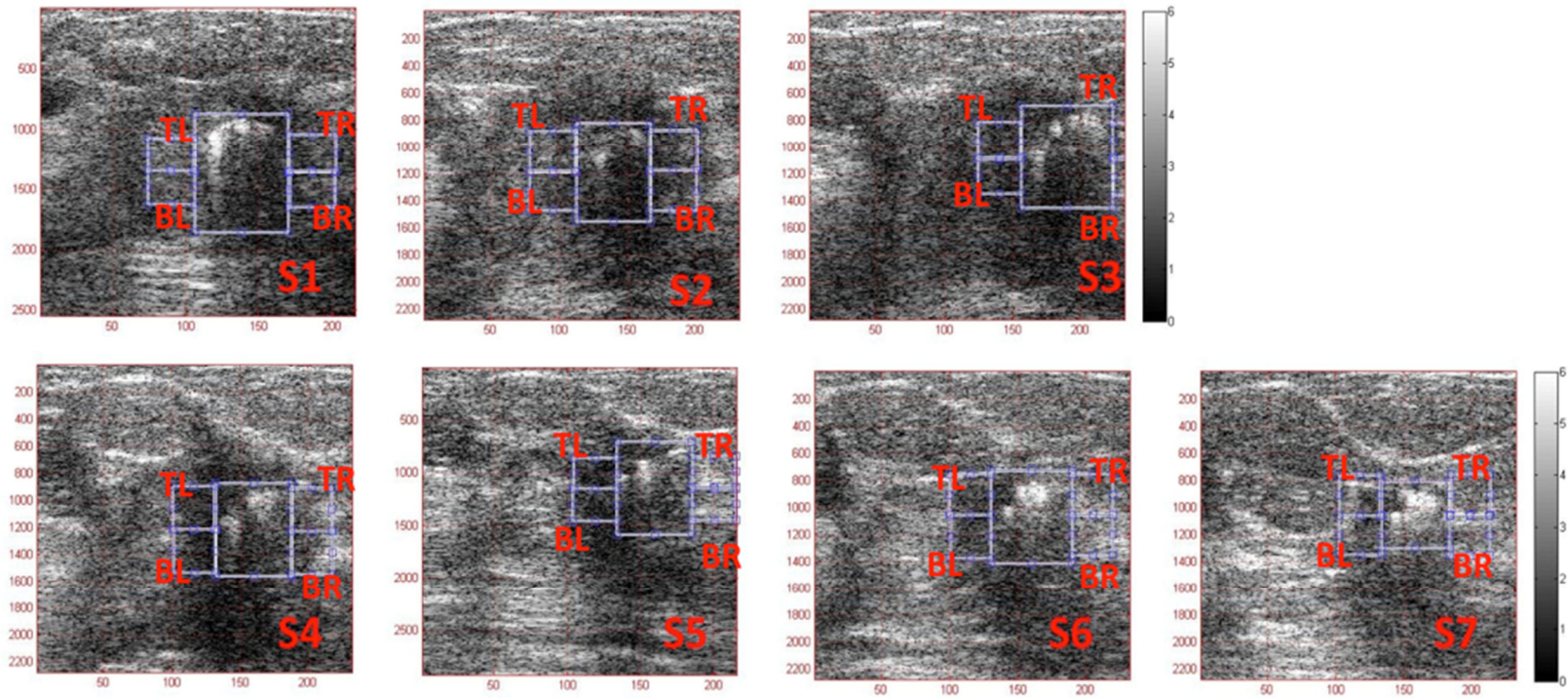


Figure 4- 22. Areas selected for FPA for patient 5 in the transverse plane. The centre dark blue ROI is the landmark. Average spectral intercept values are calculated from the other four ROIs and compared. TL, TR, BL and BR represent results from the top left, top right, bottom left and bottom right ROIs. Note that the widths and depths are not the same for all images.

		FPA					
Scan #	1	2	3	4	5	6	7
P5_sagittal_TL	11.6+/-6.0	11.1+/-4.2	9.2+/-3.1	17.3+/-5.0	27.3+/-5.1	11.1+/-6.7	25.9+/-13.9
P5_sagittal_BL	12.9+/-6.9	12.4+/-5.0	8.4+/-5.4	23.8+/-3.1	21.5+/-2.8	18.7+/-10.3	25.3+/-7.1
P5_sagittal_TR	13.4+/-2.7	5.8+/-3.7	19.4+/-3.4	21.5+/-10.4	19.2+/-6.0	25.3+/-10.5	17.9+/-6.7
P5_sagittal_BR	9.1+/-8.9	6.2+/-5.5	23.4+/-5.8	18.3+/-9.4	8.9+/-11.5	21.9+/-14.9	24.4+/-6.7
Scan #	1	2	3	4	5	6	7
P5_transverse_TL	12.0+/-5.9	14.3+/-4.4	6.9+/-5.9	7.3+/-11.0	10.0+/-6.7	10.2+/-11.4	22.9+/-11.0
P5_transverse_BL	13.6+/-10.2	12.7+/-3.0	6.1+/-6.1	15.2+/-15.4	7.2+/-5.3	8.4+/-5.4	15.6+/-4.6
P5_transverse_TR	17.8+/-3.5	11.8+/-8.2	16.3+/-4.7	21.2+/-8.3	21.4+/-2.6	14.8+/-2.7	15.2+/-5.0
P5_transverse_BR	12.0+/-4.3	5.5+/-7.3	27.2+/-11.2	26.4+/-10.7	23.5+/-5.3	21.1+/-4.8	23.7+/-5.7

Table 4. 9 Results of FPA for P1 for the sagittal and the transverse plane. The units are in dB and the values are the average spectral intercept +/- standard deviation. L, B, R, T stand for left, bottom, right and top respectively

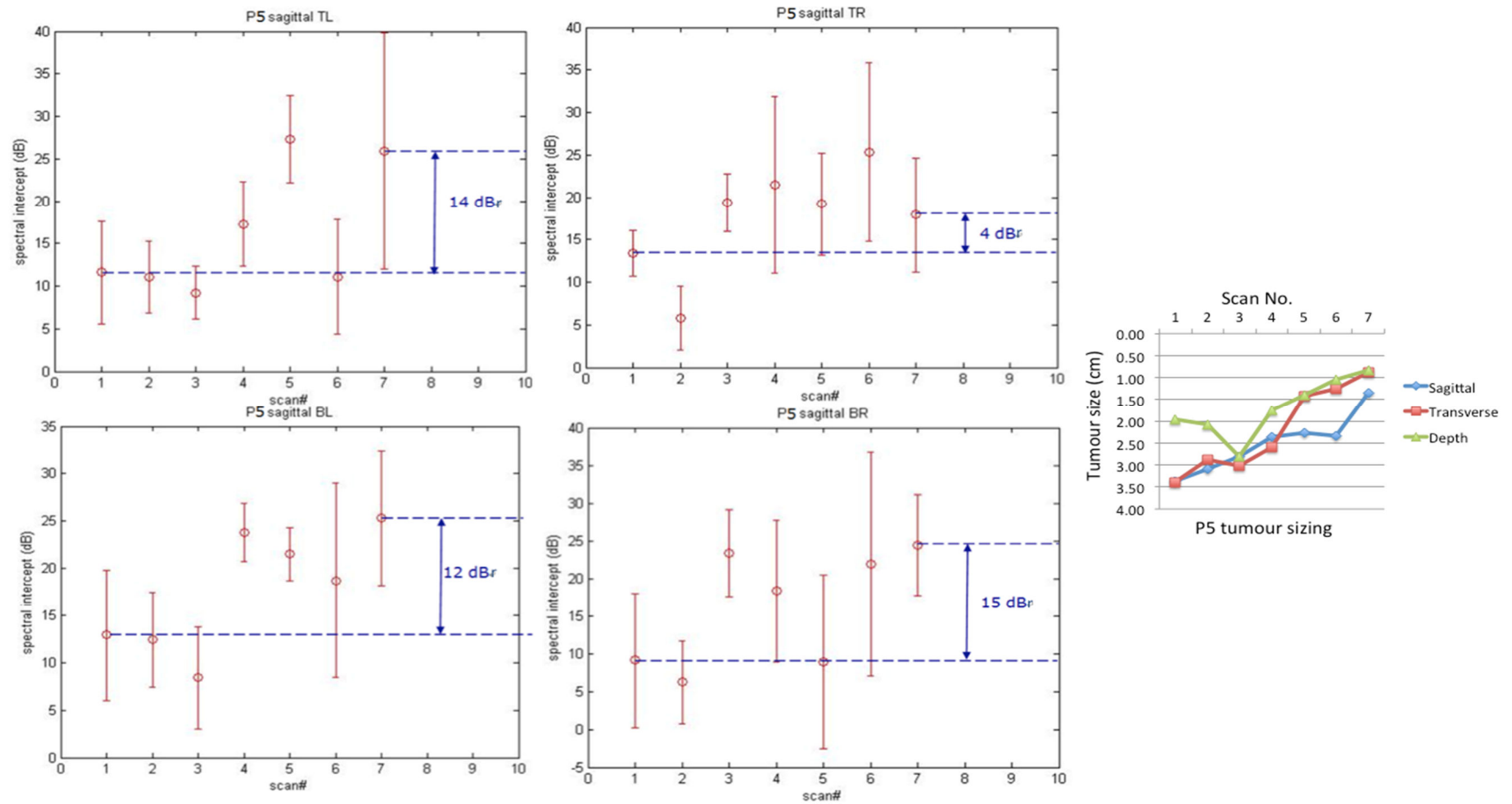


Figure 4- 23. Results of FPA of patient 5 in the sagittal plane. (Top left) TL, (Top middle) TR, (Bottom left) BL and (Bottom middle) BR represent results from the top left, top right, bottom left and bottom right ROIs from Figure 4- 21. The circles in the figures are the means and the bars present $\pm$ one standard deviation of spectral intercept values of the ROIs. (Right) P5 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

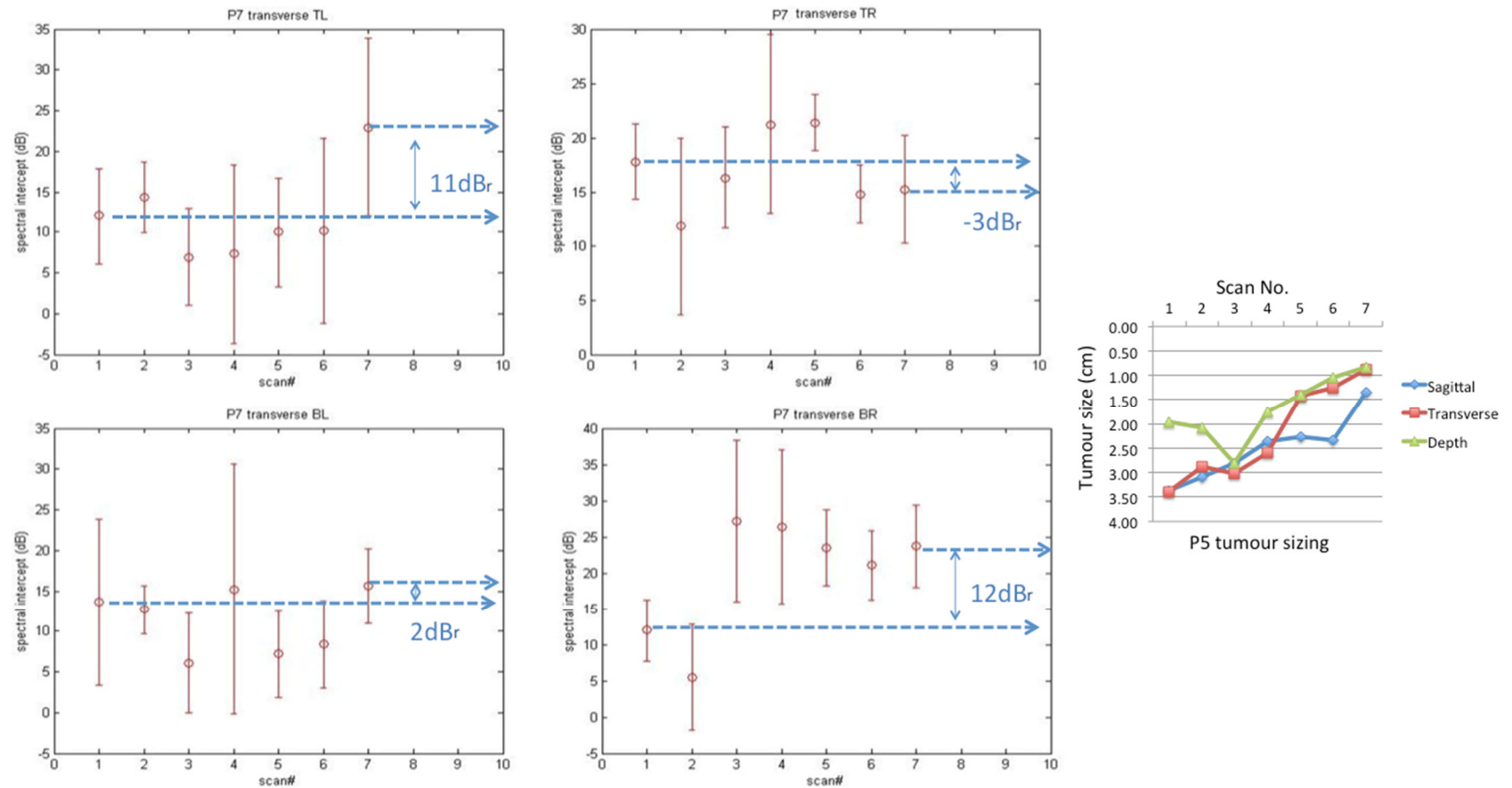


Figure 4- 24. Results of FPA of patient 5 in the transverse plane. (Top left) TL, (Top middle) TR, (Bottom left) BL and (Bottom middle) BR represent results from the top left, top right, bottom left and bottom right ROIs from Figure 4- 22. The circles in the figures are the means and the bars present +/- one standard deviation of spectral intercept values of the ROIs. (Right) P5 tumour sizing with the sagittal (blue), transverse (red) and depth (green) measurements.

4.4 Conclusion

In this chapter we have presented detailed methods for investigating the use of spectral intercept as a parameter for monitoring breast neoadjuvant chemotherapy and have proposed and explored novel methods for quantifying changes that occurred as tumour respond to chemotherapy. All methods were investigated in a way to provide consistency in measurement across all scans and cases.

Measures based on spectral intercept showed smaller increase in the partial response case (P5) in comparison to the complete response case (P1), whereas tumour sizing show significant reduction in sizing for both cases. Overall despite the intermediate fluctuations and outliers observed and also the low number of reported cases, the results in this chapter show that changes in measures based on the spectral intercept offers additional information to tumour sizing.

Chapter 5 : Image Registration of Ultrasound Parametric and Clinical Histopathology images

5.1 Introduction

In this chapter, a feature based image registration method is proposed for the registration of ultrasound parametric images and histopathology images. The technique was evaluated using ultrasound elastography images with histopathology images and was also used to provide examples of registering ultrasound spectral intercept images from previous chapters to histopathology images. An early version of this work was presented at the 2010 IEEE ISBI conference (Chuang *et al.*, 2010).

5.1.1 Motivation

Developments in ultrasound parametric imaging such as ultrasound elastography (Di Battista *et al.*, 2011, Bercoff *et al.*, 2004, Nightingale *et al.*, 2002, Ophir *et al.*, 1991) and ultrasound spectral imaging (Lizzi *et al.*, 1983, Feleppa *et al.*, 2011) complement conventional ultrasound B-mode imaging and mammography in cancer diagnosis and provide additional diagnostic information. However, the interpretation of ultrasound parametric images is by no means straightforward, and in “real-life scans”, there are many factors that lead to artefacts and provide potentially misleading information to the diagnosis. This can be exemplified by the ultrasound elastography image in Figure 5 - 1 where the area within the red circles correspond to the tumour and appears as a hypo-echoic region in the B-mode image (left) and is indicated in the elastography

image (right) as a red area, which represents stiffer areas in elastography. However, no corresponding features can be found in the B-mode image (left) that correlate to the stiff area within the red rectangle (right) in the elastography image.

In order to understand and evaluate the information that ultrasound parametric images provide, the images need to be studied alongside with histopathology images that are regarded as the ground truth in clinical studies. To achieve this goal, the images need to be registered to facilitate comparisons of the images. The main challenges in registering medical images to histopathology images are the complex tissue deformations introduced during the preparation of the histopathology slides and the differences that arise between *in-vivo* acquisition of the medical images and the *in-vitro* acquisition of the histopathology images. Thus a method of image registration that models the deformation in histopathology images and the differences due to *in-vivo* and *in-vitro* imaging will facilitate the study comparison of the images and is the focus of this chapter.

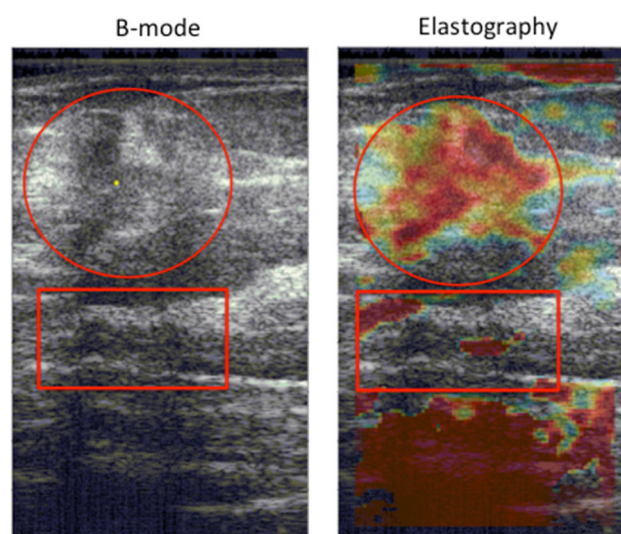


Figure 5 - 1. (Left) B-mode image (Right) with elastography overlaid where the stiffer the region the redder the area.

5.2 Method

5.2.1 Overall Procedure

A total of 14 pairs of ultrasound elastography and histopathology images were used in our study. The images were selected from a clinical study on breast cancer, which used the assisted freehand ultrasound (AFUSON) system (Kadour and Noble, 2009) to acquire B-mode and elastography images, the cases used in our study were recruited with full consent and ethical approval through the Oxford Breast Imaging Centre. The histopathology slides were prepared from the preserved wide local excision specimens and were digitised and analysed. The ultrasound elastography image from each patient was paired up with a histopathology image that corresponds best to the elastography image by the clinician. The planes were recorded when performing ultrasound imaging and also when preparing histology slicing. The clinician then goes through all the histology slices to manually match the image that best match the ultrasound image. The histology preparation is a standard exercise for clinical studies and is not possible to validate/evaluate the accuracy of slicing, as it is a limitation when performing the slicing.

Since the tumours are stiffer than the surrounding fatty tissues, their shapes are less affected by deformations introduced in the preparation of histopathology images and hence corresponding boundaries of tumours were selected as features to be aligned. The task was considered as a non-rigid point-set (feature-based) registration problem to model the complex deformation in histopathology images and corresponding features in the elastography and histopathology images were manually marked by the radiologist using the Wacom Cintiq 20WSX

interactive pen-display system (Figure 5 - 2). The features were marked retrospectively using information available in the B-mode, elastography and histopathology images.

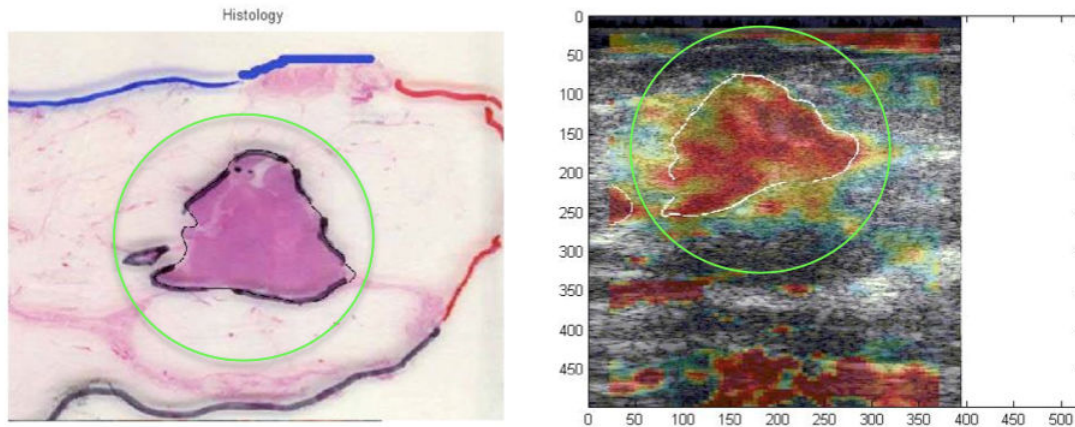


Figure 5 - 2. The feature/tumour boundaries are marked in green circles in (left) the histopathology image and (right) the ultrasound elastography image. The other colour markings in the histopathology image represent anatomical positions with superficial in blue, deep in black and inferior in red.

The histopathology point-set was used as the reference for the elastography point-set to align to. The Coherent Point Drift (CPD) algorithm (Myronenko and Song, 2009) was used to align the two point-sets and to obtain a post-registered elastography point-set. The thin-plate spline (TPS) method (Bookstein, 1989) was then used to estimate the transformation between the pre-and post-registered elastography point-set. The post-registered elastography image was then formed based on the transformation obtained from the TPS and image interpolation.

The CPD method is particularly well suited for tackling the problem of complex deformations introduced to the histopathology due to it being able to allow for local alignments and does not require equal number of points in the reference

and the floating point-set. Furthermore, no assignment is required for the correspondence between the points in the point-sets.

5.2.2 CPD algorithm

The CPD point-set registration code used for this work is the implementation in MATLAB[®] by Andriy Myronenko (see link in reference). The algorithm considers the alignment of two point-sets as a probability density estimation problem using Gaussian mixtures models (GMM). The floating point-set is modelled as GMM centroids and is transformed by a set of transformation, T , with parameters, θ , to fit onto the reference point-set, which is modelled as data points generated by the GMM centroids. In our case, the reference point-set is the histopathology point-set and the floating set is the elastography point-set.

Consider point-set Y that acts as GMM centroids and contains M number of points and point-set X that act as the data points generated by the GMM centroids with N number of points. The GMM probability density function is modelled as:

$$p(x) = \frac{w}{N} + \frac{(1-w)}{M} \sum_{m=1}^M p(x|m) \quad (5.1)$$

$$p(x|m) = \frac{1}{(2\pi\sigma^2)^{D/2}} e^{-\frac{\|x-y_m\|^2}{2\sigma^2}} \quad (5.2)$$

The term w is bounded between 0 and 1 and accounts for the weighting of the noise and outliers in the point set that is modelled as a uniform distribution $1/N$

in the model (equation 5.1). The model also considers equal contribution of each point, m , of the GMM centroids. The covariance of the Gaussians, σ^2 , is assumed isotropic and the dimension of the point sets is D , in our case D equals 2. The GMM centroid locations are parameterised by a set of transformation parameters, θ , and the aim is to estimate the parameters that best transform the GMM centroids onto the data points. The parameters are estimated by maximising the likelihood, which can be done by minimising the negative log-likelihood function

$$E(\theta, \sigma^2) = - \sum_{n=1}^N \log \sum_{m=1}^{M+1} P(m)p(x|m) \quad (5.3)$$

where $P(m)$ is the prior probability and $p(x|m)$ is the likelihood. The $M+1$ in the second summation term is an additional uniform distribution term, $p(x|M+1) = 1/N$, added to the mixture model to represent noise and outliers. The correspondence probability between any two points in Y and X is defined as the posterior probability of the GMM centroid given the data

$$p(m|x_n) = \frac{P(m)p(x_n|m)}{p(x_n)} = \frac{1}{M} \frac{p(x_n|m)}{p(x_n)} \quad (5.4)$$

To estimate the optimal parameters, a standard expectation-maximisation (EM) algorithm with GMM is used (Bishop, 1995). The EM algorithm first guesses some values for the parameters (E-step) that are then used to compute the Bayesian posterior probability, $p^{\text{old}}(m|x_n)$, using equation 5.4. The new parameters are then estimated by minimising the complete negative log-

likelihood (M-step), Q , where

$$Q = - \sum_{n=1}^N \sum_{m=1}^{M+1} p^{\text{old}}(m|x_n) \log(P^{\text{new}}(m) p^{\text{new}}(x_n|m)) \quad (5.5)$$

The algorithm iterates between the E and M steps until convergence or a pre-specified number of iterations. Ignoring the constants independent of θ and σ^2 , equation 5.5 can be written as

$$Q(\theta, \sigma^2) = \frac{1}{2\sigma^2} \sum_{n=1}^N \sum_{m=1}^M p^{\text{old}}(m|x_n) \|x_n - T(y_m, \theta)\|^2 + \frac{N_p D}{2} \log(\sigma^2) \quad (5.6)$$

where

$$N_p = \sum_{n=1}^N \sum_{m=1}^M p^{\text{old}}(m|x_n) \quad (5.6.1)$$

$$p^{\text{old}}(m|x_n) = \frac{\exp(-\frac{1}{2} \left\| \frac{x_n - T(y_m, \theta^{\text{old}})}{\sigma^{\text{old}}} \right\|^2)}{\sum_{k=1}^M \exp(-\frac{1}{2} \left\| \frac{x_n - T(y_k, \theta^{\text{old}})}{\sigma^{\text{old}}} \right\|^2) + c} \quad (5.6.2)$$

$$c = (2\pi\sigma^2)^{D/2} \frac{w}{1-w} \frac{M}{N} \quad (5.6.3)$$

The final estimated parameters are the optimal parameters for the transformation. In the case of non-rigid registration, the transform function is defined as

$$T(Y, v) = Y + v(Y) \quad (5.7)$$

where v is a displacement function. In order to enforce smoothness of the

displacement function, a regularizing term is introduced to the norm of v . This in term imposes a constraint on the negative log-likelihood term given in equation 5.3.

$$f(v, \sigma^2) = E(v, \sigma^2) + \frac{\lambda}{2} \phi(v) \quad (5.8)$$

where E is the negative log-likelihood term in equation 5.3, $\phi(v)$ is the regularization term, λ is a trade-off parameter for the smoothness of the regularization used (i.e. higher the λ , the smoother). $\phi(v)$ is given as (Myronenko and Song, 2009)

$$\phi(v) = \int_{\mathbb{R}^D} \frac{|v(s)|}{G(s)} ds \quad (5.9)$$

where G is a Gaussian and s is the path/curve. By using calculus of variation and further derivation (Myronenko and Song, 2009), equation 5.7 can be written as

$$T(Y, v) = Y + GW \quad (5.10)$$

where W is a matrix of coefficients with dimension $M \times D$ and G is a kernel matrix with dimension $M \times M$ and elements

$$g_{ij} = G(y_i, y_j) = \exp\left(-\frac{1}{2} \left\| \frac{(y_i - y_j)}{\beta} \right\|^2\right) \quad (5.11)$$

where $\beta > 0$ and is a parameter that reflects the amount of smoothness in the regularisation. The parameters w , λ , and β throughout the studies in this chapter were set to the default values of 0.1, 3 and 2 respectively.

5.2.3 Thin-plate splines

The thin-plate spline method computes the spatial deformations at every point in the 2D plane or 3D volume specified by a set of source and target points. The method models the bending of a thin plate surface $z(x,y)$ where the bending energy at a point in 2D is

$$E_b = \iint_{R^2} \left(\left(\frac{\partial^2 z}{\partial x^2} \right)^2 + 2 \left(\frac{\partial^2 z}{\partial x \partial y} \right)^2 + \left(\frac{\partial^2 z}{\partial y^2} \right)^2 \right) dx dy \quad (5.12)$$

x, y are the Cartesian coordinates and the closed form solution that minimise the bending energy is

$$z(x, y) = U(r) = -r^2 \log(r^2) \quad (5.13)$$

where r is the distance $\sqrt{x^2 + y^2}$ from the Cartesian origin. In 2D, the function $f(x,y)$ that describes the transformation on every point in the image is given by

$$f(x, y) = a_1 + a_2 x + a_3 y + \sum_{i=1}^n w_i U(|P_i - (x, y)|) \quad (5.14)$$

The first three terms in equation 5.14 give the linear part of the transformation while the final term gives the non-rigid terms. The U term is the radial basis function given in equation 5.13 and P is the Cartesian coordinates of the source point set, and hence the term $|P_i - (x, y)|$ is the radial distance between each point in the source point set and every point in the plane.

5.2.4 Rigid registration v.s. non-rigid registration

The CPD algorithm allows for both rigid and non-rigid registration of the feature point-sets selected from the images but since the assumption is that the boundaries selected in the images correspond to each other, a method that best align the boundaries is required. In this regard, the non-rigid method provides higher degrees of freedom than the rigid method and hence is selected in this study. Figure 5 - 3 illustrates the difference in results between the rigid and non-rigid registration method and clearly shows the non-rigid method aligns the point-sets better. Figure 5 - 4 shows the ultrasound elastography images before and after rigid and non-rigid registration.

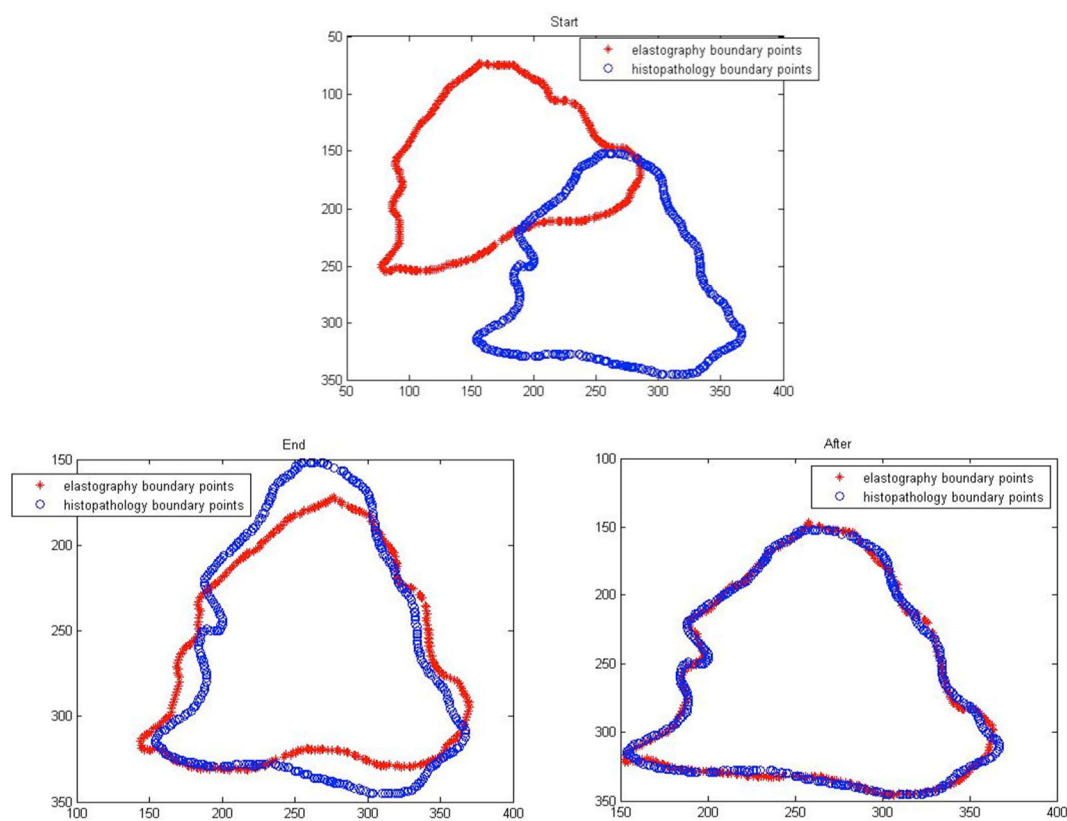


Figure 5 - 3. Comparison of CPD rigid and non-rigid registration results. Ultrasound features are in red and histopathology features are in blue (Top) Before registration (Bottom left) features after rigid registration (Bottom right) features after non-rigid registration.

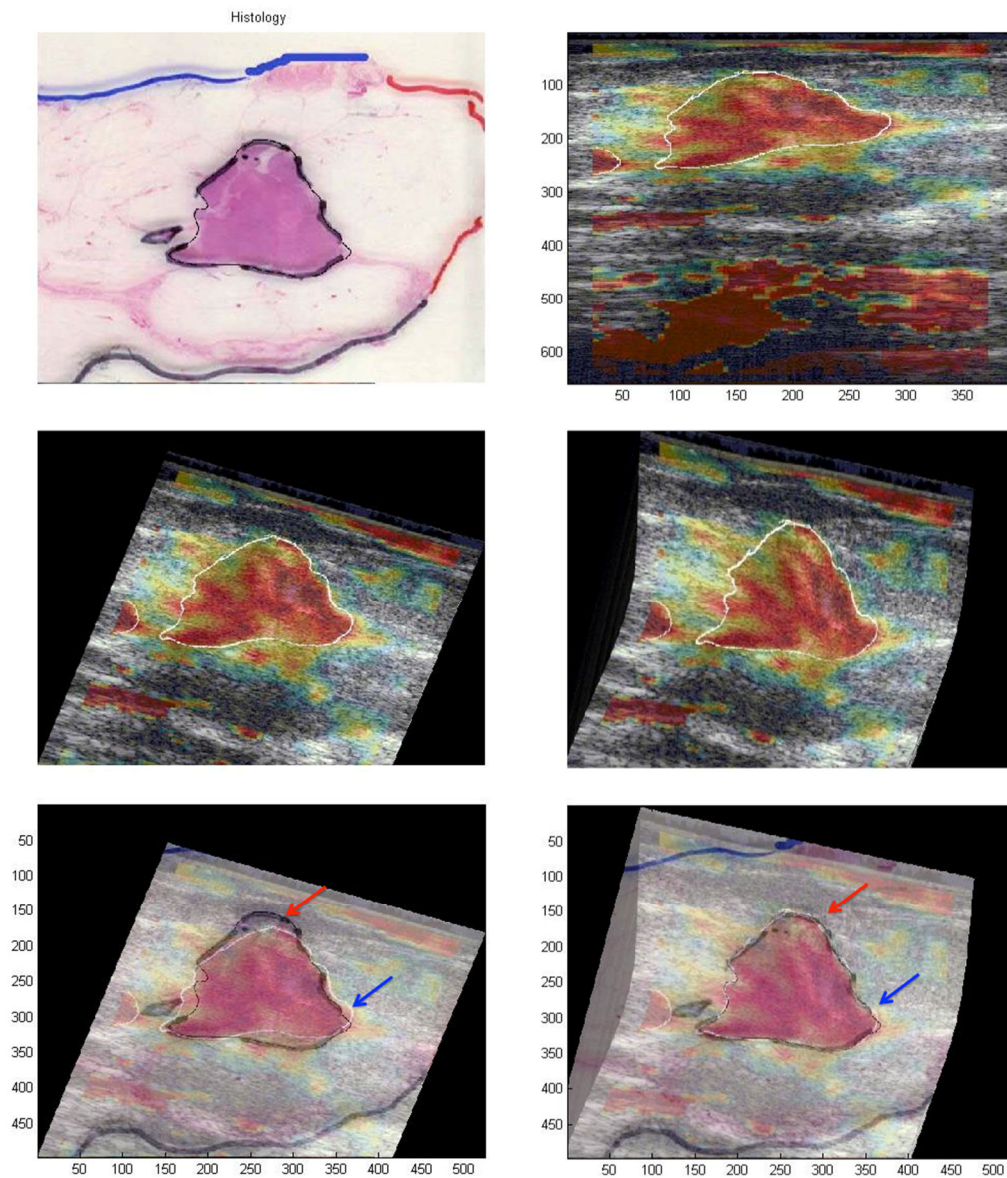


Figure 5 - 4. Comparison of CPD rigid and non-rigid registration results. (Top left) Histopathology image (Top right) Ultrasound Elastography image (Mid left) rigid-registered B-mode-elastography image (Mid right) non-rigid-registered B-mode-elastography image (Bottom left) Rigidly- registered B-mode-elastography overlay on histopathology image. (Bottom right) Non-rigid-registered B-mode-elastography overlay on histopathology image. In the bottom row, the white (blue arrow) and black (red arrow) boundaries represent the tumour boundaries in the elastography and the histopathology image respectively.

5.3 Results and discussion

5.3.1 Registration of ultrasound elastography and histopathology images

The results of image registration between ultrasound elastography and histopathology images are presented in this section (Figure 5 - 5 to Figure 5 - 18). The figures present the histopathology images at the top; the middle rows show the ultrasound elastography images before registration on the left and after registration on the right. The superposition of the histopathology image and the ultrasound elastography images are shown in the bottom rows, again, with pre-registration on the left and post-registration on the right. There were in total 14 cases of registration performed and all features in histopathology images and ultrasound elastography images were marked in black and white respectively. The ultrasound elastography images were displayed on the same scale and size as the histopathology images to facilitate comparison. B-mode images

The results of P207 are given in Figure 5 - 5. The registration improved the alignment of the boundaries slightly and the image warping effect is more noticeable in the left half of the elastography image, in a way introducing a folding effect on the image. Figure 5 - 6 shows the results of P211 and from the bottom row images it can be seen that the shape of the boundary in the elastography matches closer to that in the histopathology image after registration. The images warping effect in this case is more noticeable in the right half of the elastography image and the transformation introduced some shearing towards right in the elastography image.

There were two regions selected as features in P214 and is shown in Figure 5 - 7. The registration introduced noticeably a translation in the elastography image and improves the matching of the feature boundaries before and after registration. Figure 5 - 8 shows the results for P218 and the registration introduced anti-clockwise rotation and some shearing effect in the elastography image together with some local deformation particularly in the area below the tumour. The registration greatly improved the alignment of the boundaries in both position and shape.

The results of P239 are given in Figure 5 - 9 where the features in elastography and histopathology differed in shape before registration as can be seen in the bottom left image where there is a mismatch between the black and white boundaries. The registration improved the alignment by introducing clockwise rotation and also local shearing effects. Figure 5 - 10 presents the results of P244 where a slight translation was introduced together with local deformations to align the boundaries.

Figure 5 - 11 shows the results of P246 whereby three features were outlined in this case representing the tumours. The registration improved the alignments of the features but image degradation can be seen in the post-registered elastography image, which is shown in the mid-right image in Figure 5 - 11 by the red ellipse. The blurring artefact is most likely due the interpolation process required to produce the transformed image based on the transformation estimated between the pre- and post-registered point-sets.

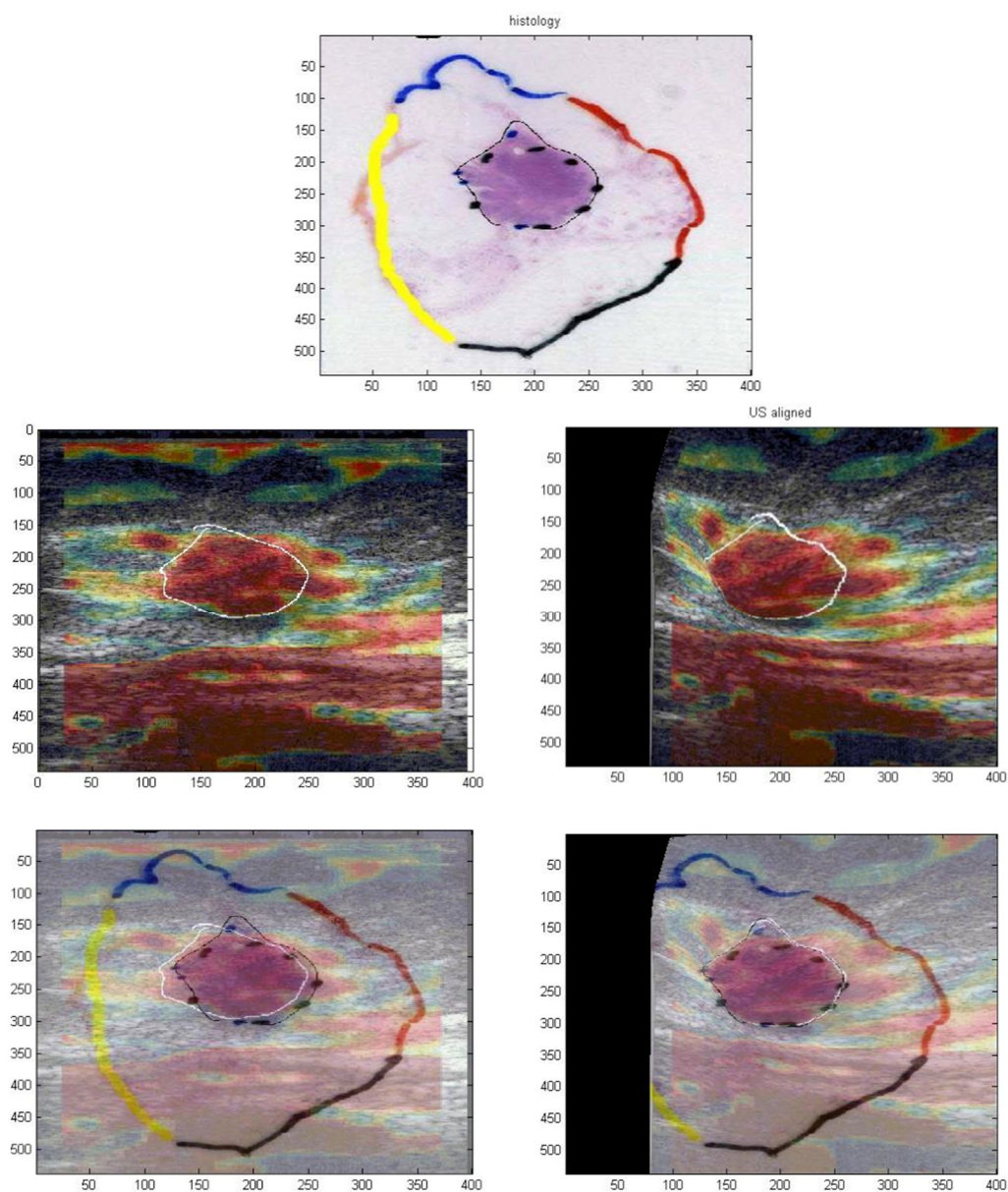
The results of P253 and P255 are shown in Figure 5 - 12 and Figure 5 - 13 respectively. In both cases the features selected are both fairly circular in shape and the registration improved the alignment of the boundaries in both cases as can be seen from the bottom row of the figures.

Figure 5 - 14 shows the result of P256 and is a case worth pointing out where in the histopathology image the entire fibrous structure near the tumour can be clearly identified in the ultrasound elastography image (in the underlying B-mode image). In this case the registration improves not only the alignment of the boundaries but also nearby features that were not included in the registration. This alignment of the fibrous structure is illustrated in Figure 5 - 19.

Figure 5 - 15 and Figure 5 - 16 show the registration results of P261 and P263 and both showed improved alignment between the boundaries after registration.

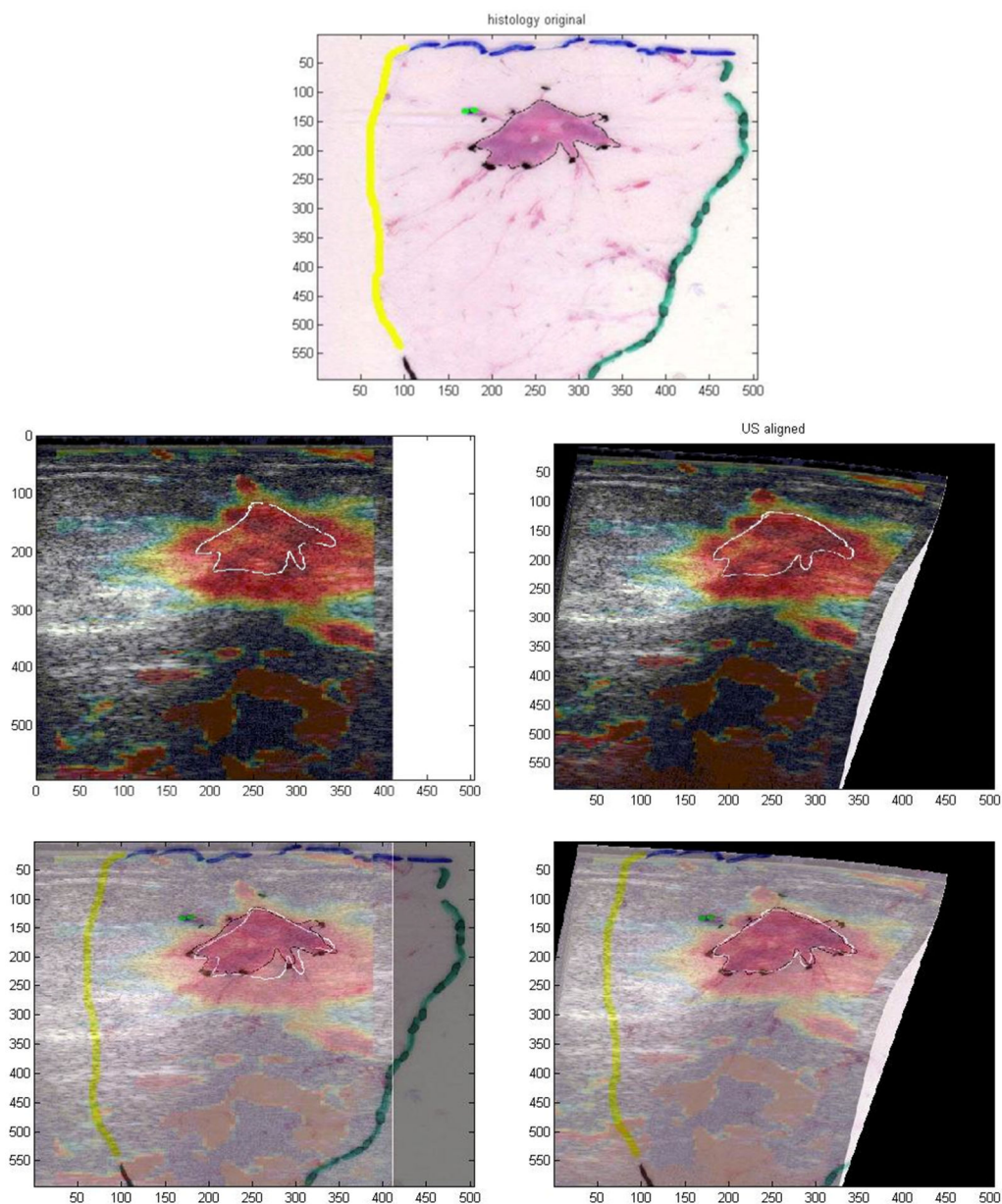
Figure 5 - 17 shows the results of P265 where again like the case in P246, blurring artefact can be observed at the end of the post-registered elastography image.

Figure 5 - 18 presents P266 and showed good alignment between the boundaries after registration and no image artefact is observed.



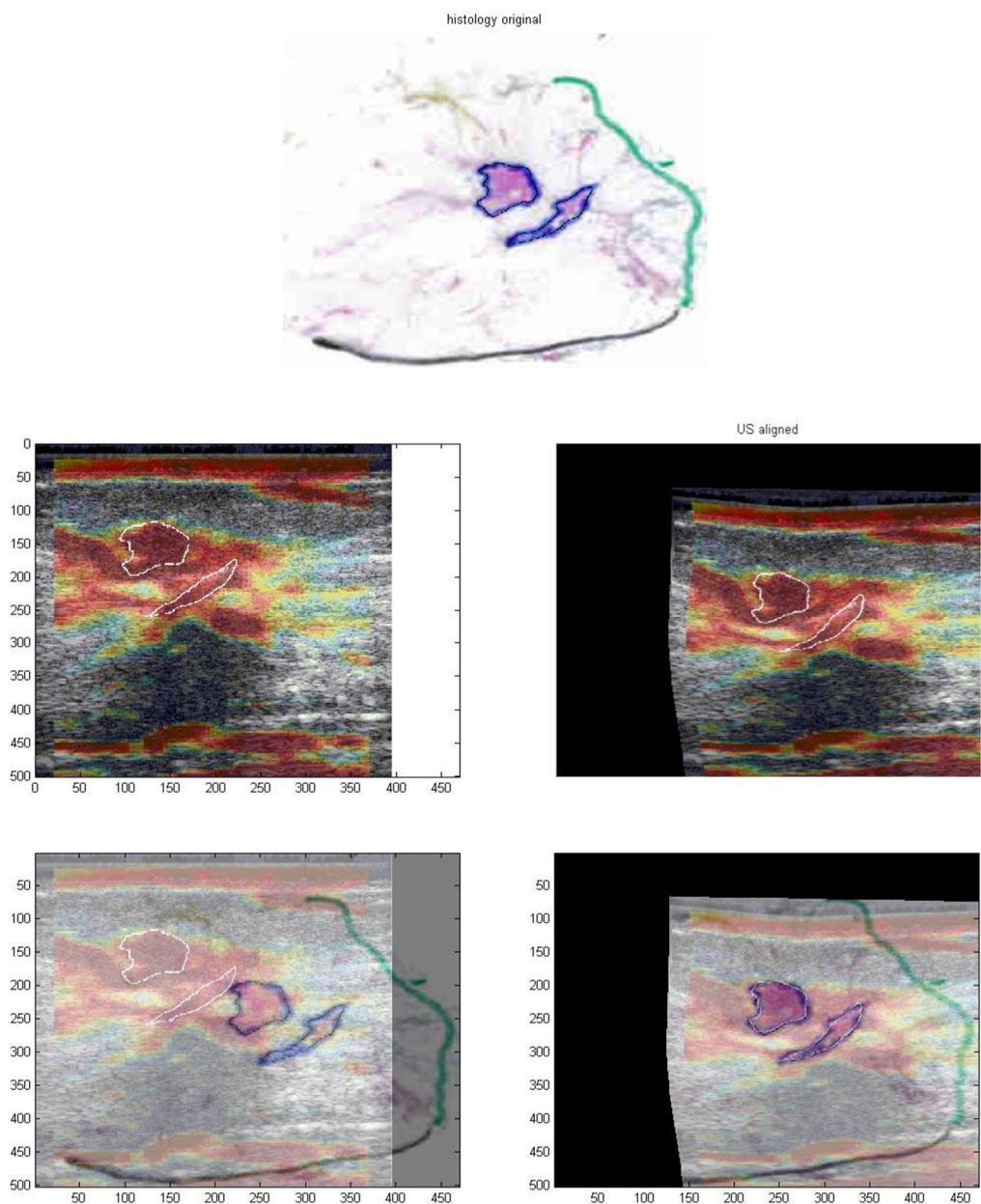
P207

Figure 5 - 5. Image registration results of P207 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



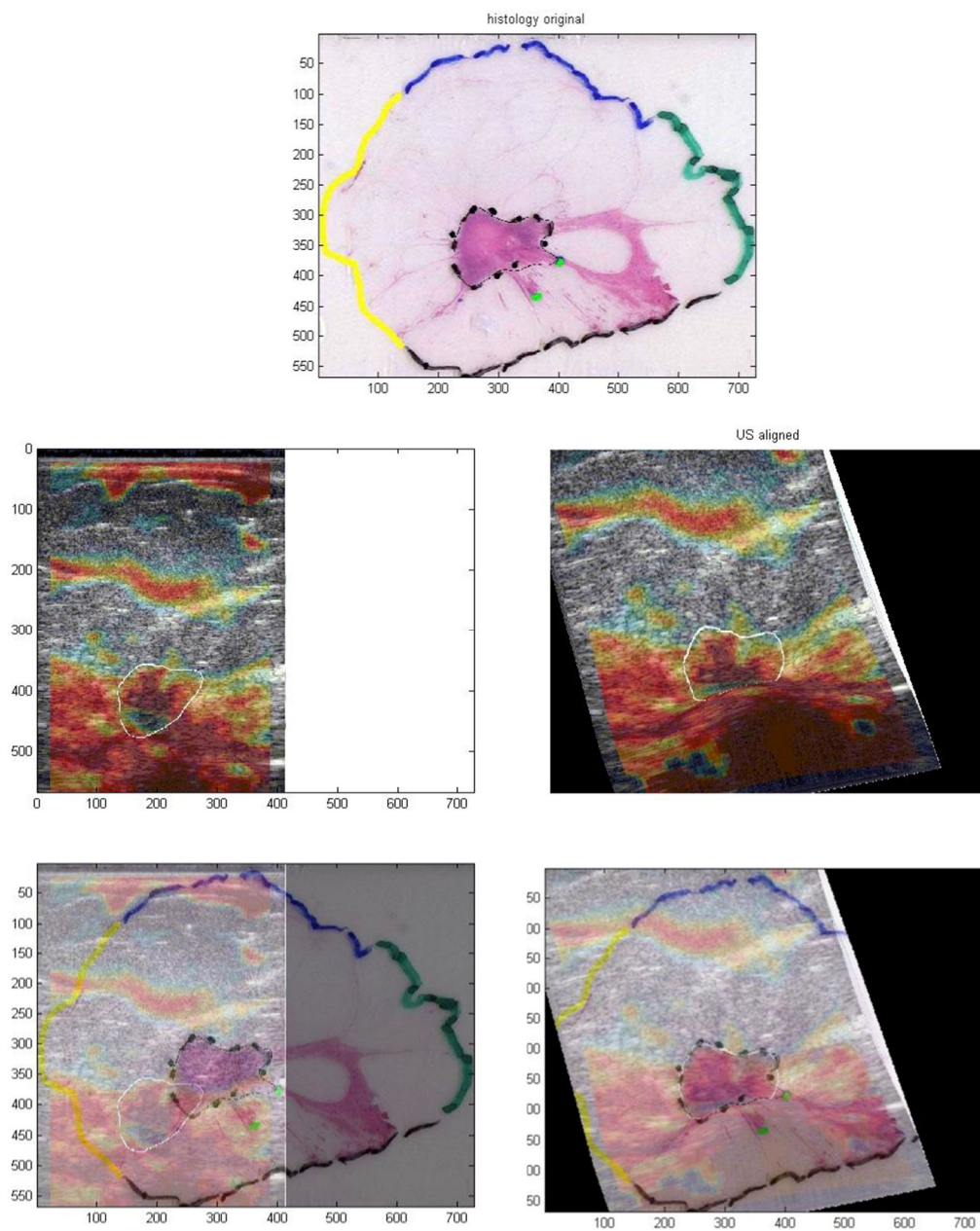
P211

Figure 5 - 6. Image registration results of P211 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



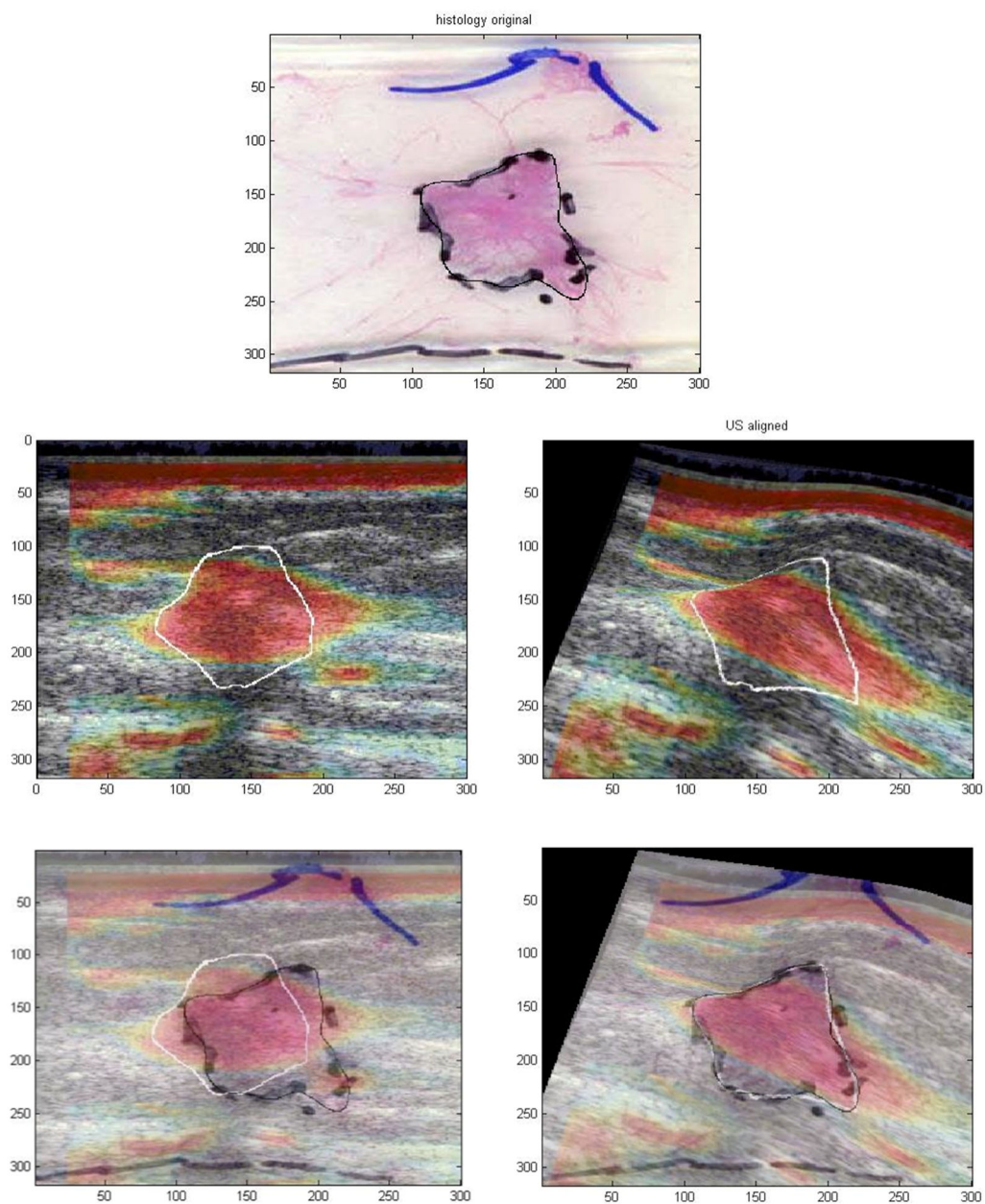
P214

Figure 5 - 7. Image registration results of P214 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



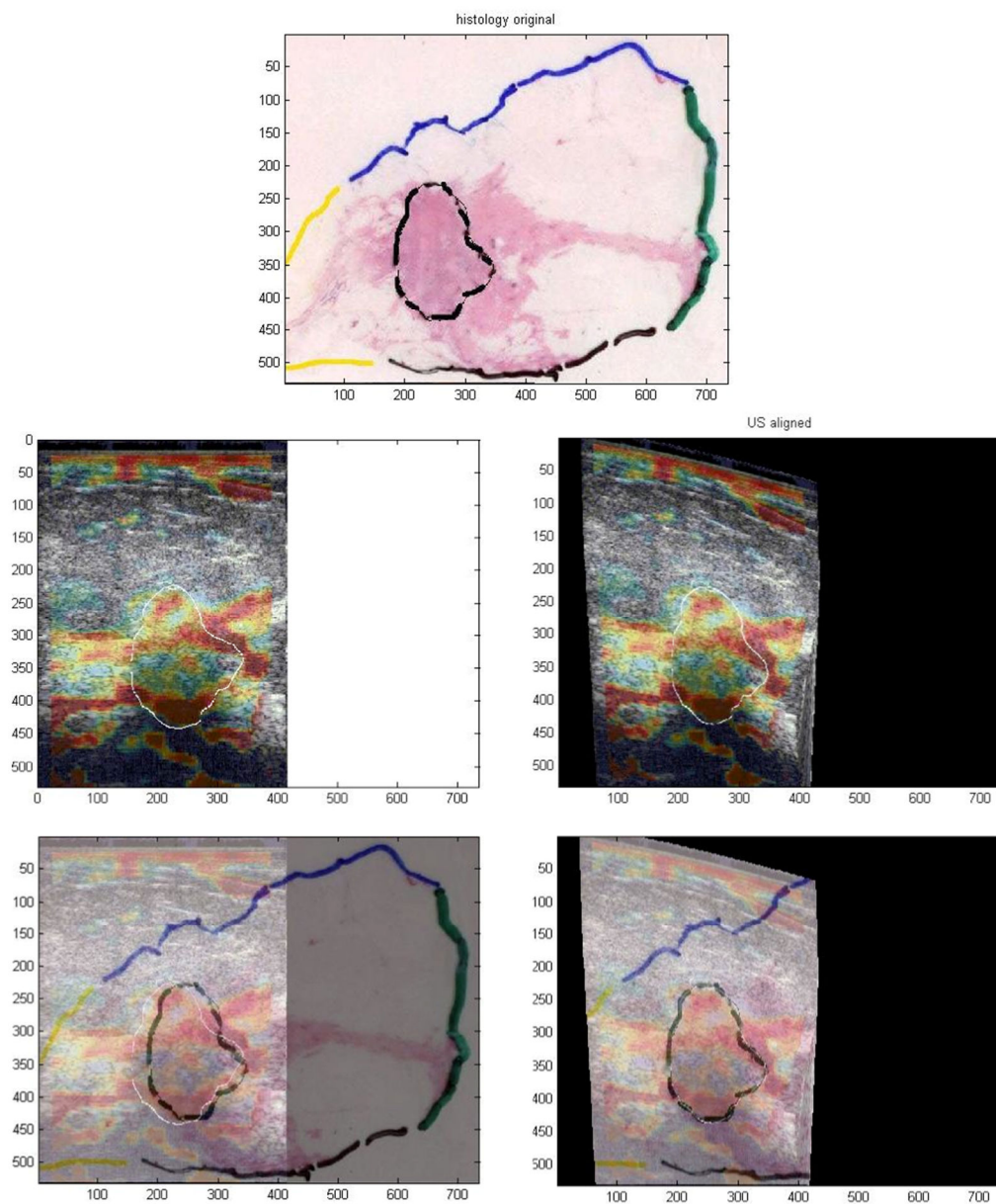
P218

Figure 5 - 8. Image registration results of P218 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



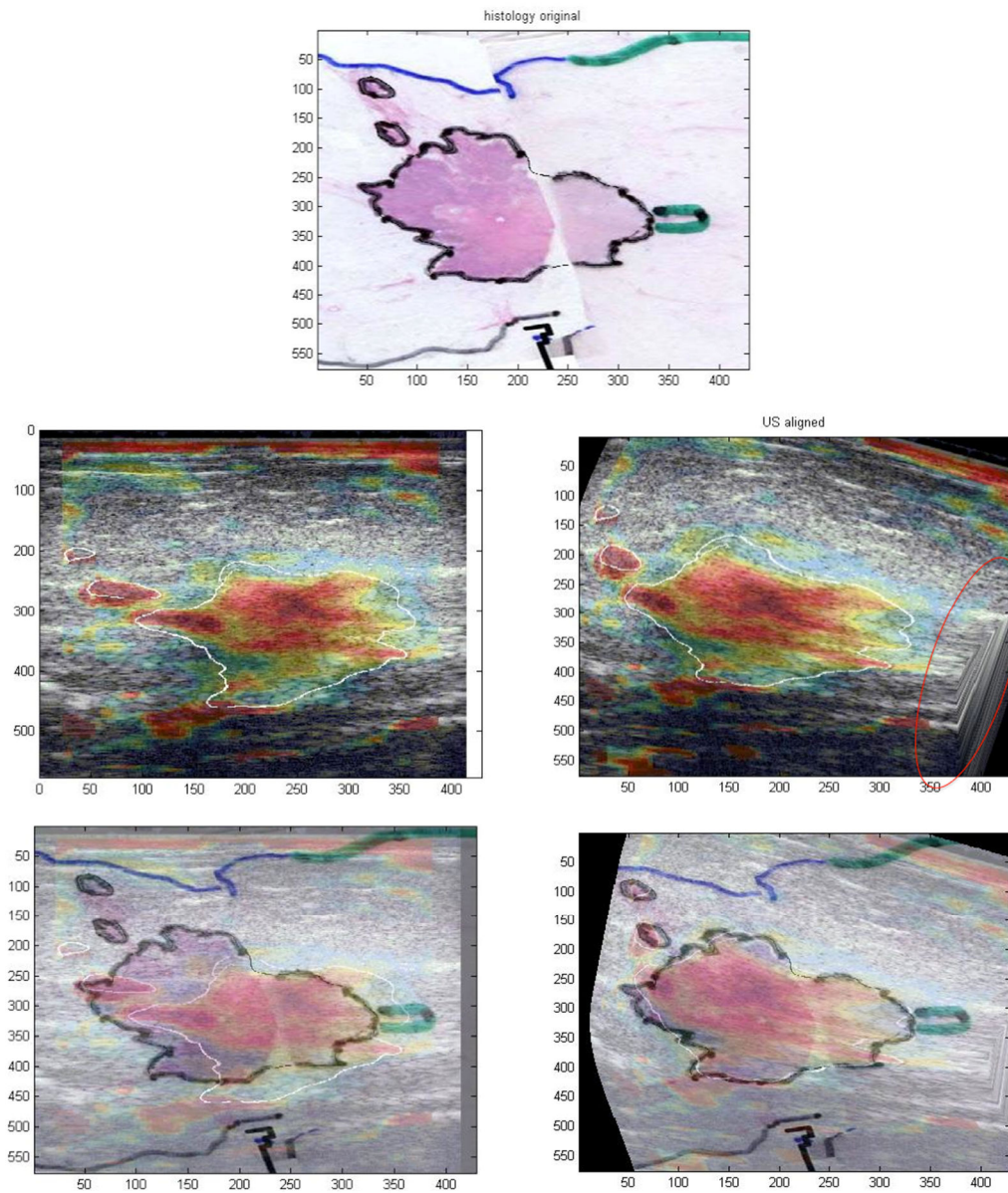
P239

Figure 5 - 9. Image registration results of P239 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



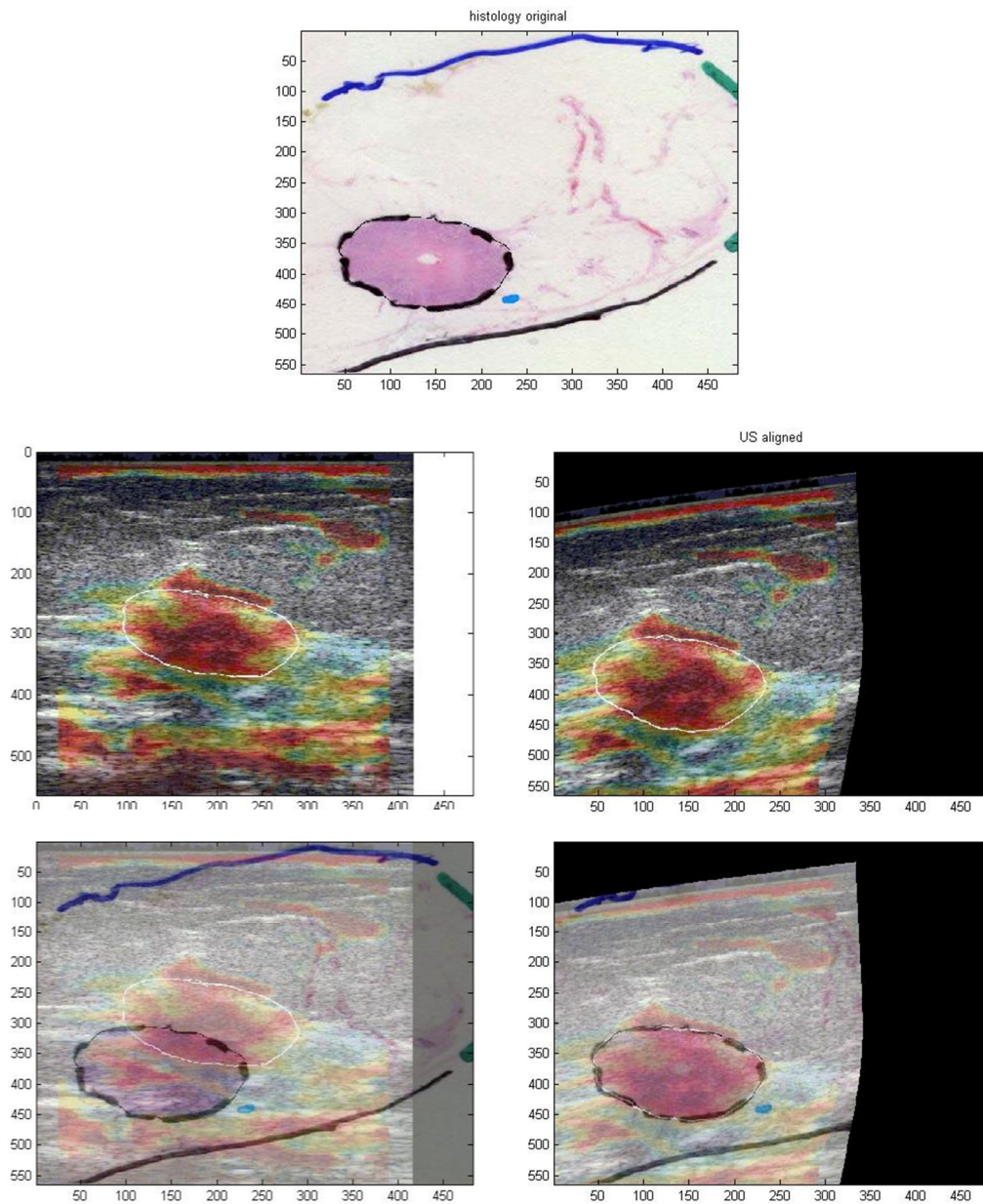
P244

Figure 5 - 10. Image registration results of P244 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



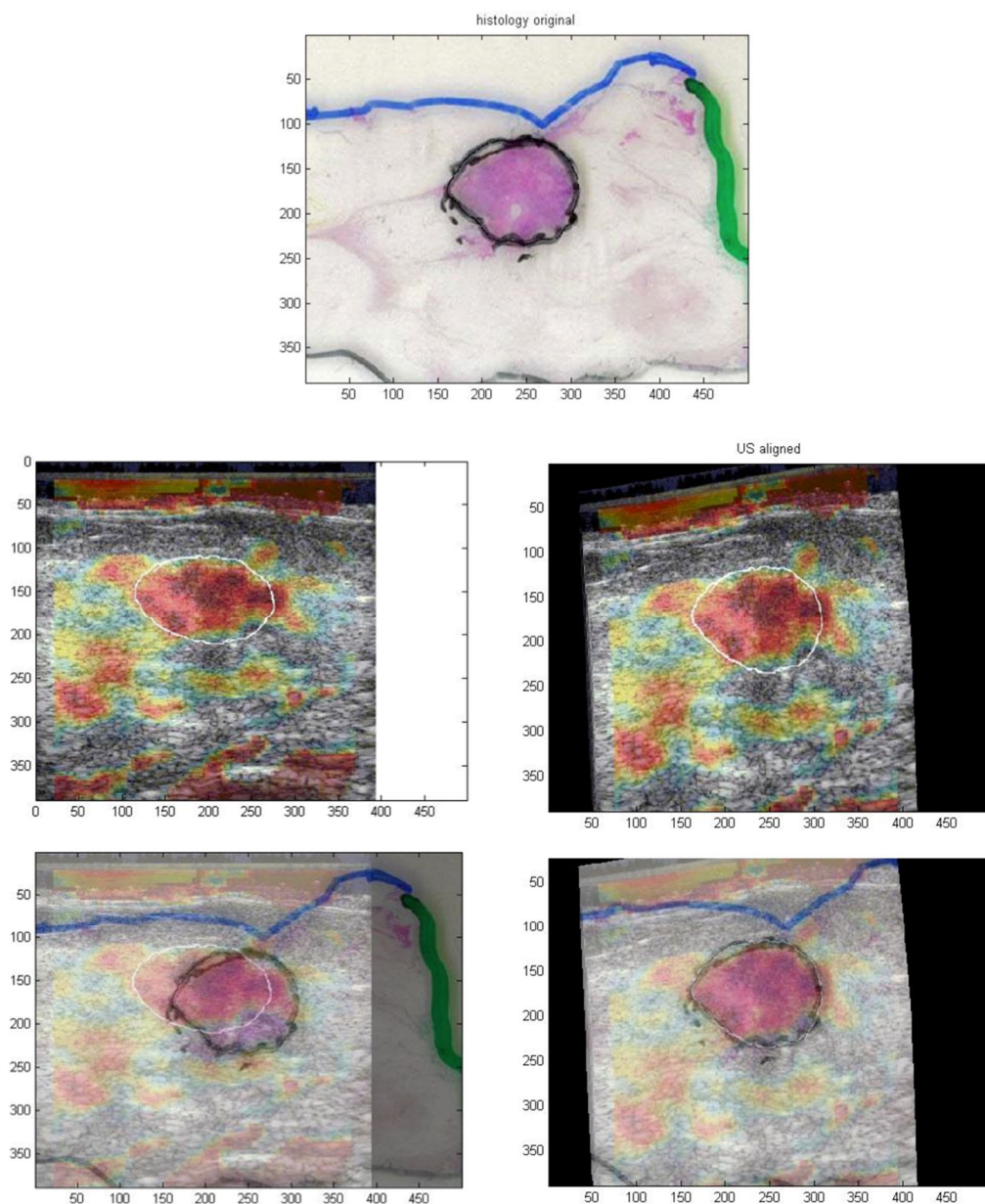
P246

Figure 5 - 11. Image registration results of P246 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration. The red ellipse outlines the blurred area introduced by the interpolation process in forming the transformed image (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



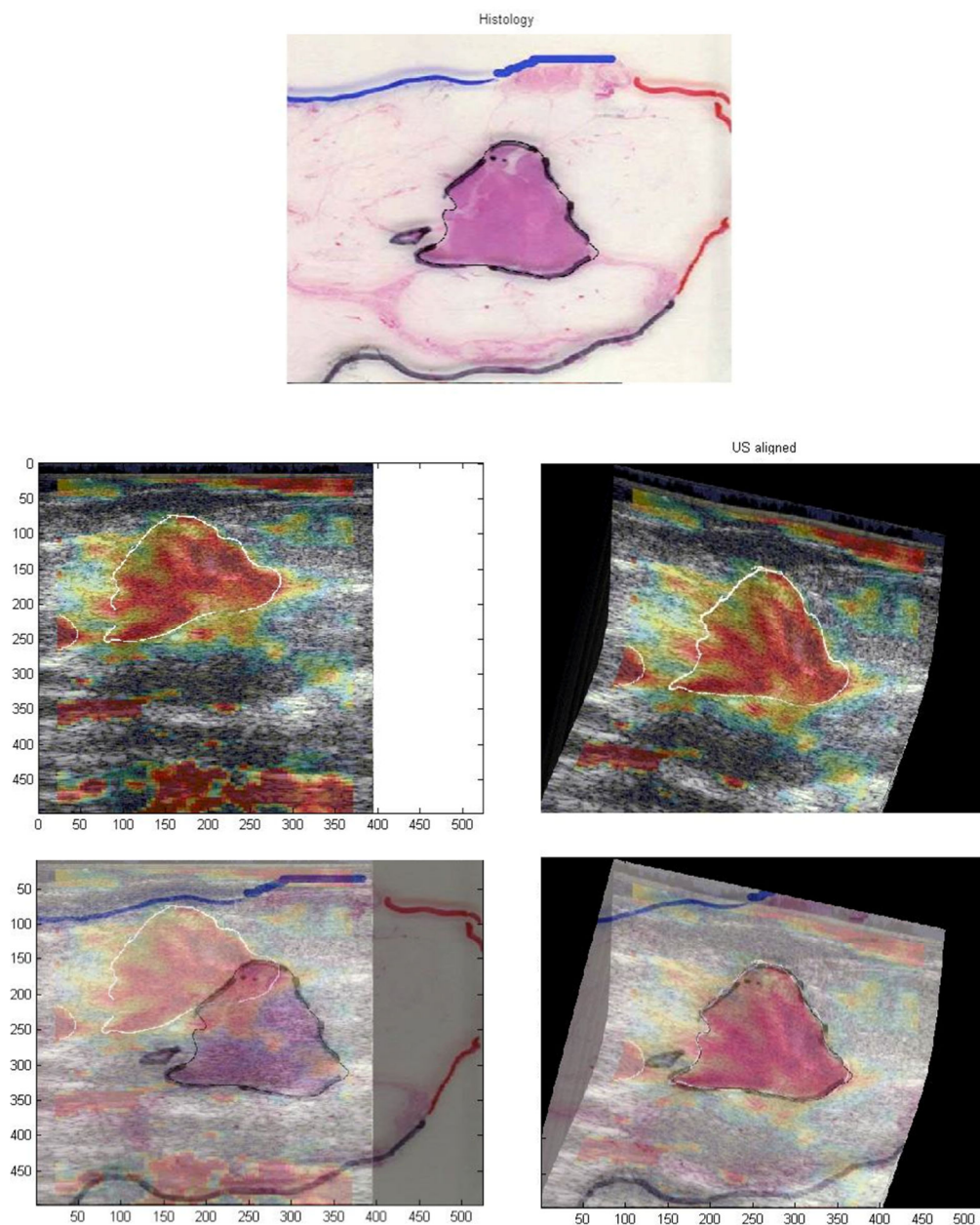
P253

Figure 5 - 12. Image registration results of P253 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



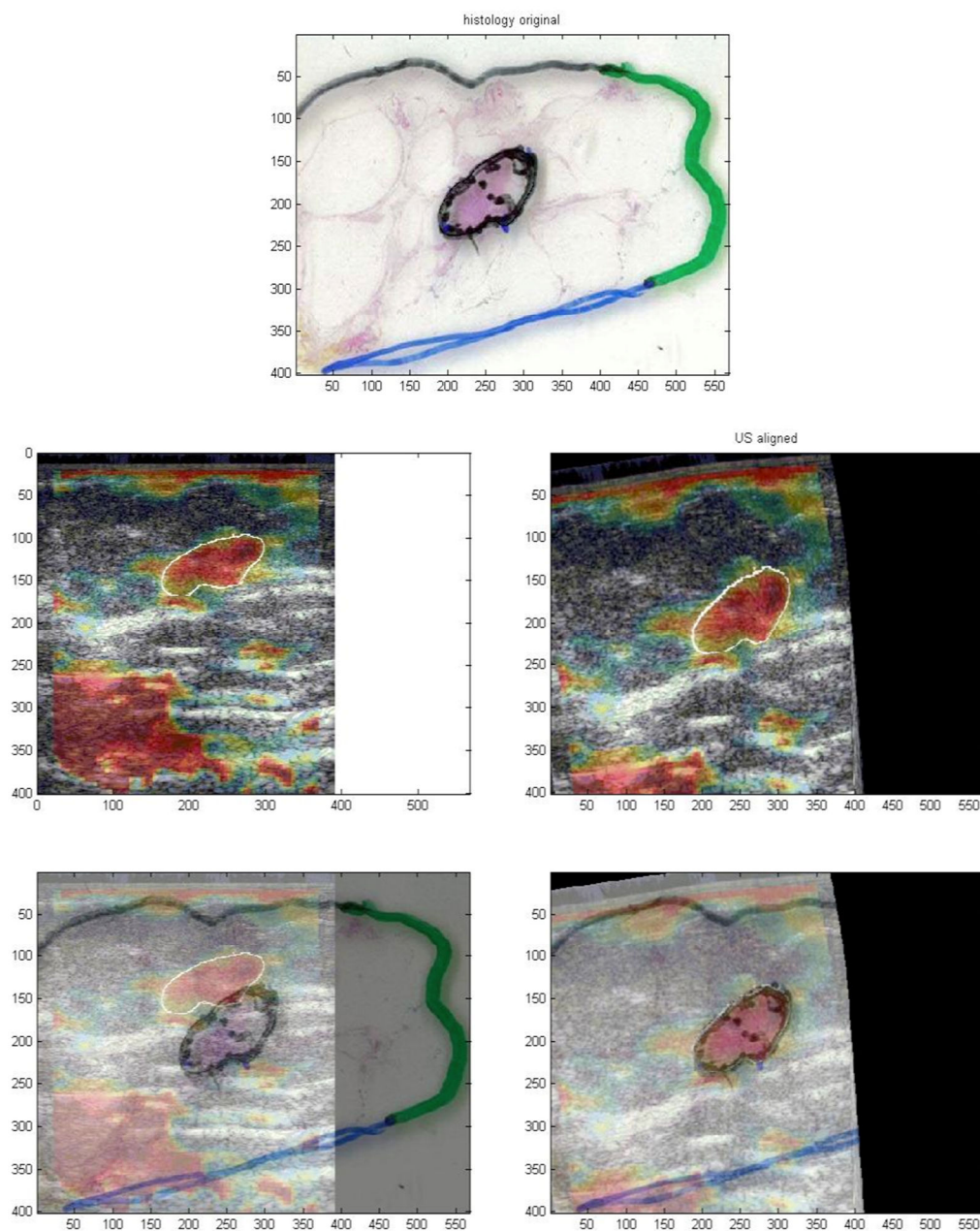
P255

Figure 5 - 13. Image registration results of P255 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



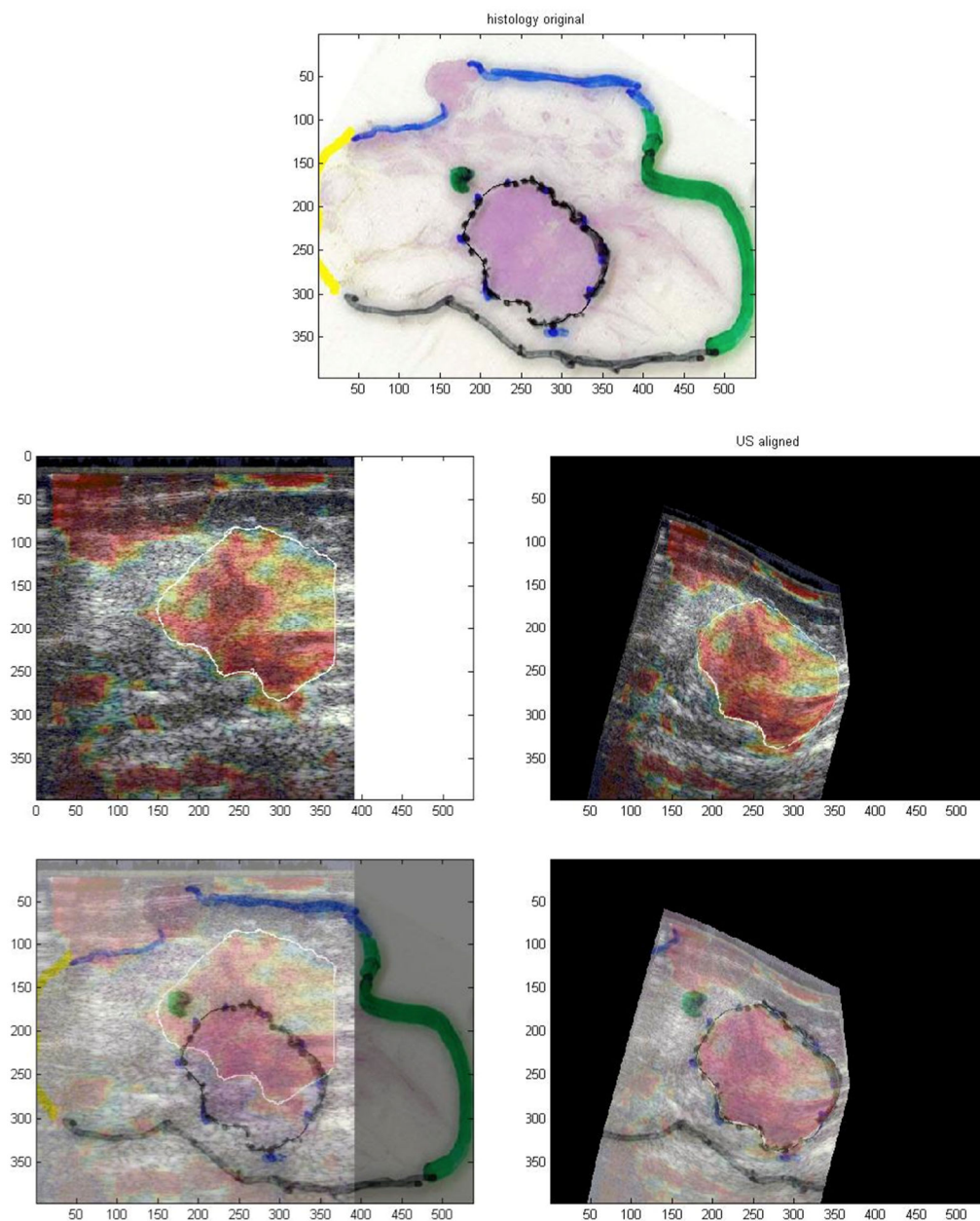
P256

Figure 5 - 14. Image registration results of P256 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



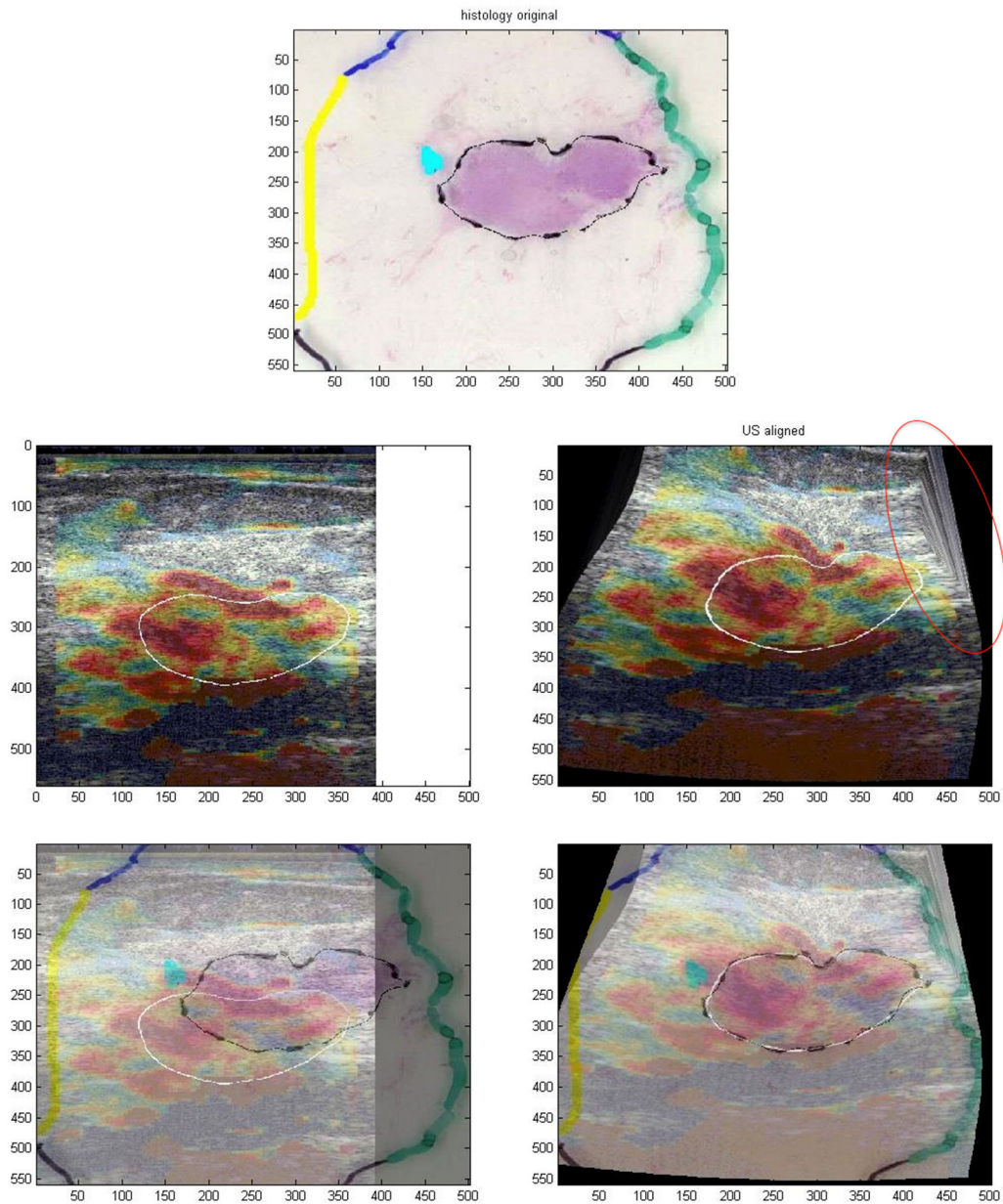
P261

Figure 5 - 15. Image registration results of P261 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



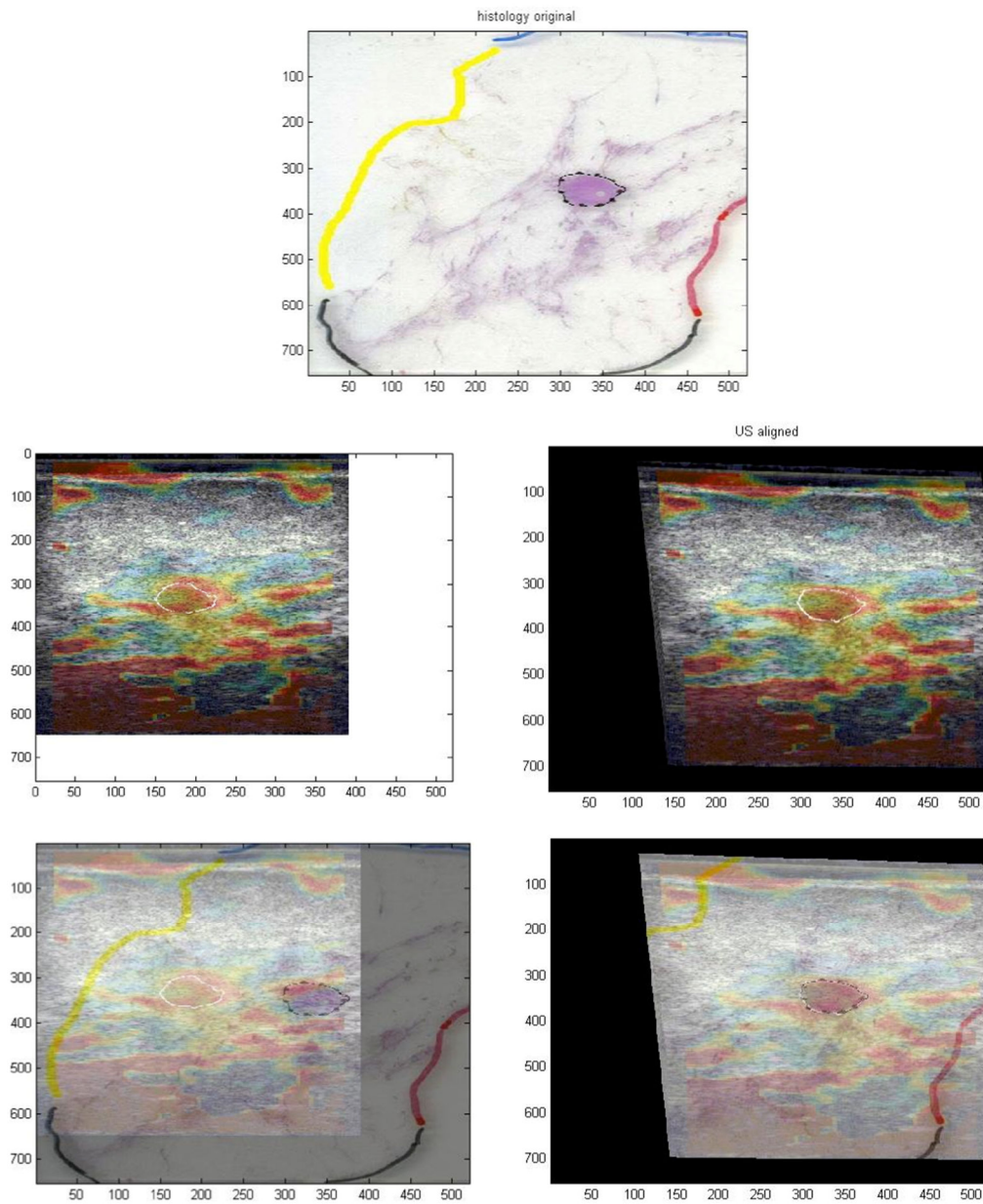
P263

Figure 5 - 16. Image registration results of P263 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



P265

Figure 5 - 17. Image registration results of P265 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration. The red ellipse outlines the blurred area introduced by the interpolation process in forming the transformed image (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.



P266

Figure 5 - 18. Image registration results of P266 (Top) histopathology image (Mid-left) Elastography image before registration (Mid-right) Elastography image after image registration (Bottom-left) elastography image overlay on the histopathology image before registration (Bottom right) elastography image overlay on the histopathology image after registration.

5.3.2 Evaluation of registration effectiveness

The registration results presented in the previous section showed improved alignments between the features used for the registration visually in all cases but the alignment between the point-sets selected for registration does not guarantee alignment of other features in the image that were not used in the point-set registration. In order to validate and evaluate the registration, additional corresponding features in the images that were not used in the registration were identified and delineated by the clinician (example given in Figure 5 - 19). Pre- and post-registration target registration error (TRE) was used for comparison. Because features selected from the images are point-sets rather than point landmarks, simple distance measure such as the Euclidean distance is difficult to apply as the corresponding point-sets can have different number of points and also it is not possible to assign corresponding points in one point-set to the other to compute a simple Euclidean distance measure which is normally used for computing the TRE. As a result, the Hausdorff distance was selected as a distance measure between the point-sets.

The Hausdorff distance ($h(A,B)$) is a distance measure which is defined as the longest distance required to get from any of the points from one point-set to the other set:

$$H(A, B) = \max\{h(A, B), h(B, A)\} \quad (5.14)$$

$$h(A, B) = \max_{a \in A} \{ \min_{b \in B} \{ d(a, b) \} \} \quad (5.15)$$

where A and B are point-sets with points a and b respectively, $d(a, b)$ is the Euclidean distance between the points a and b . It should be noted that the Hausdorff distance, $h(A, B)$ is usually non-symmetric and hence $h(A, B)$ does not equal $h(B, A)$, and $H(A, B)$ is taken as the final Hausdorff distance, and the shorter the Hausdorff distance, the closer the two point-sets are.

The clinician was asked to select corresponding features other than the ones used for registration to the best of their ability from the 14 sets of images. In total, 34 corresponding features were selected from 10 of the patients, as it was difficult to select features from the other four cases (P211, P263, P265 and P266). Table 5- 1 summarises the results of the change in Hausdorff distance between all features selected, a positive number in change shows a reduction in the Hausdorff distance, meaning a closer alignment due to the registration. Based on the change in Hausdorff distance, it can be seen that the registration worked better in some cases such as P207, P214, P218, P239, P256 and P261 where there were consistent decreases in the Hausdorff distance measured for nearly all features (except feature one in P214), and in some cases such as P218 and P256, there were large changes. P244 showed relatively small decrease in Hausdorff distance of the features selected, and P246 and P253 both had a mixture of large increases and decreases of Hausdorff distance within the set of features selected. P255 is the only case where the features selected showed consistent increase in the Hausdorff distance.

Based on the 34 corresponding features used for the validation, the average change in Hausdorff distance before and after registration between corresponding features in the elastography and histopathology images was a decrease of 30.0 pixels. Figure 5 - 20 shows the decrease in Hausdorff distance as a boxplot with a median value of 23.6 pixels and an upper and lower quartile of 48.7 and 3.3 pixels respectively. The results suggest that overall the registration does mostly improve the alignment of features that were not used in the registration process in addition to the features used for the point-set registration. However, the unit of Hausdorff distance in this case is in pixel and is not very precise since the axial and lateral resolution per pixel is not equal in the elastography image but is equal in the histopathology image. Nevertheless, it is difficult to measure real distances after the image is non-rigidly transformed and hence the unit used here provides a simple and effective measure for the effectiveness of the registration.

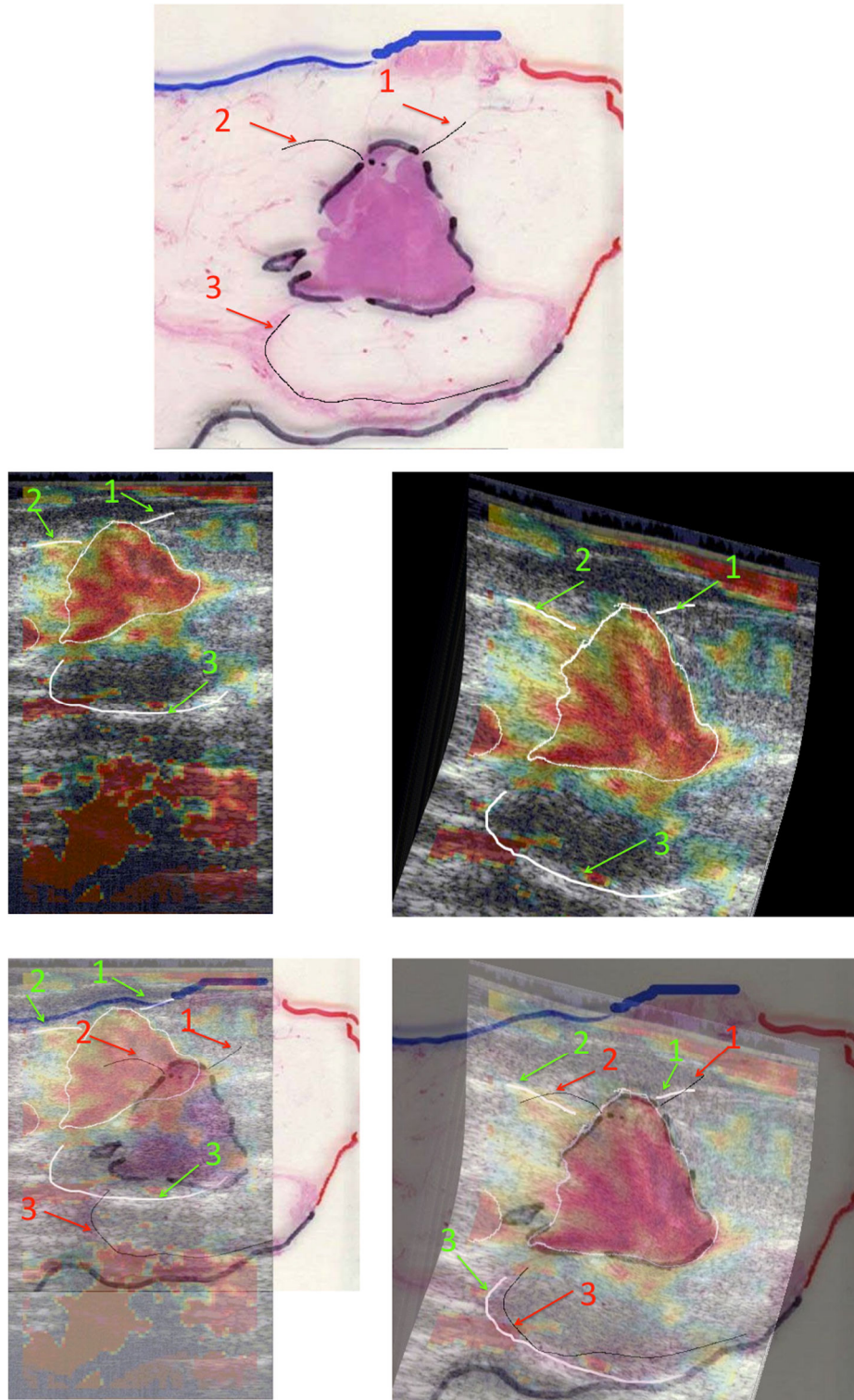


Figure 5 - 19. Additional features used for the validation of registration were marked in the elastography image in white and labelled in green; and were marked in the histopathology image in black and labelled in red. The numbering used provides matching between features in the images. Delineation of features was performed by a radiologist and was done manually by highlighting the boundaries in Microsoft Paint and using the Wacom Cintiq 20 WSX interactive pen-display.

P207	BEFORE	AFTER	DIFFERENCE	P246	BEFORE	AFTER	DIFFERENCE
Feature 1	133	108	25	Feature 1	101	44	58
Feature 2	126	22	104	Feature 2	24	55	-31
Feature 3	71	47	23	Feature 3	21	67	-45
				Feature 4	41	78	-37
AVERAGE			51	Feature 5	74	106	-32
P214	BEFORE	AFTER	DIFFERENCE	AVERAGE			-17
Feature 1	71	98	-27	P253	BEFORE	AFTER	DIFFERENCE
Feature 2	132	59	73	Feature 1	88	44	44
Feature 3	103	53	50	Feature 2	92	37	55
Feature 4	136	54	82	Feature 3	104	89	15
Feature 5	112	39	73	Feature 4	62	136	-73
				Feature 5	114	187	-73
AVERAGE			50	AVERAGE			-7
P218	BEFORE	AFTER	DIFFERENCE	P255	BEFORE	AFTER	DIFFERENCE
Feature 1	178	56	121	Feature 1	46	84	-38
Feature 2	122	32	90	Feature 2	95	104	-8
				AVERAGE			-23
AVERAGE			106	P256	BEFORE	AFTER	DIFFERENCE
P239	BEFORE	AFTER	DIFFERENCE	Feature 1	142	71	71
Feature 1	43	28	15	Feature 2	132	71	61
Feature 2	57	18	39	Feature 3	131	9	122
				AVERAGE			85
AVERAGE			27	P261	BEFORE	AFTER	DIFFERENCE
P244	BEFORE	AFTER	DIFFERENCE	Feature 1	119	70	49
Feature 1	63	54	9	Feature 2	75	50	26
Feature 2	128	100	28	Feature 3	64	35	29
Feature 3	168	155	13				
Feature 4	52	60	-9				
AVERAGE			10				
(UNIT: PIXELS)				AVERAGE			35

Table 5- 1. Summary of the results of the Hausdorff distance measure for evaluating the target registration error. The units are in pixels

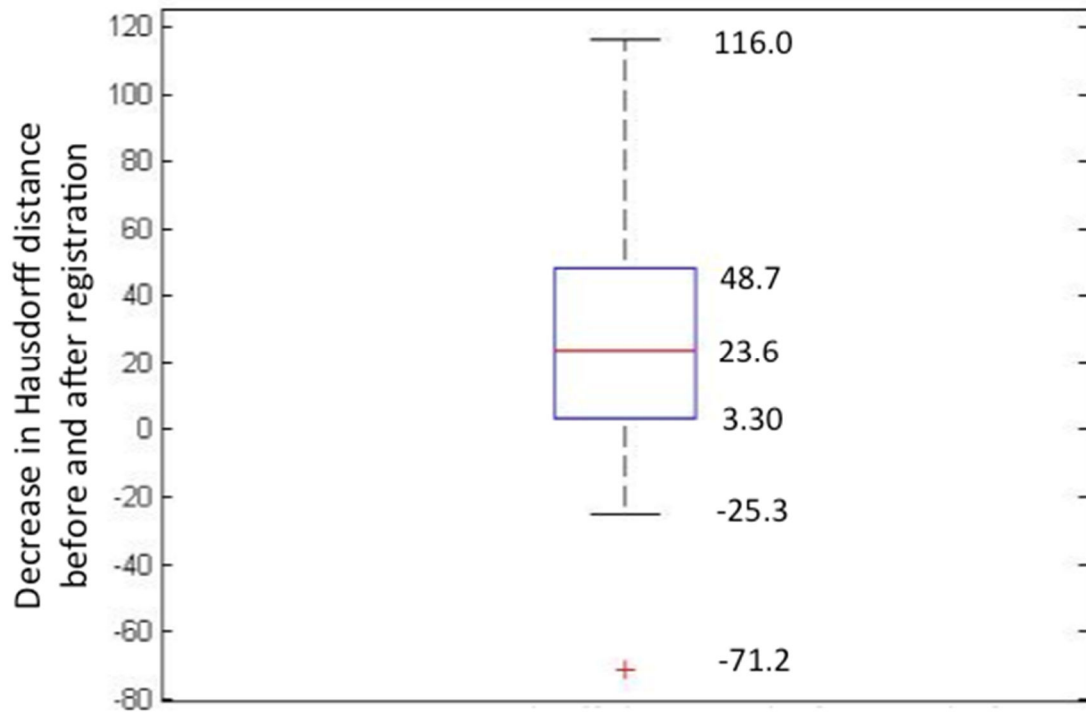


Figure 5 - 20. Boxplot of the decrease in Hausdorff distance before and after registration between features selected in elastography images and histopathology images for validation. The red line is the median change of 23.6; the blue boxes are the upper and lower quartiles of 48.7 and 3.3. The whiskers at the ends are the maximum and minimum values of 116.0 and -25.3 that were not considered as outliers. Outliers are showed as red crosses, in this case there is only one outlier (-71.2). The units are in pixels and a positive value represents a decrease in distance between the features.

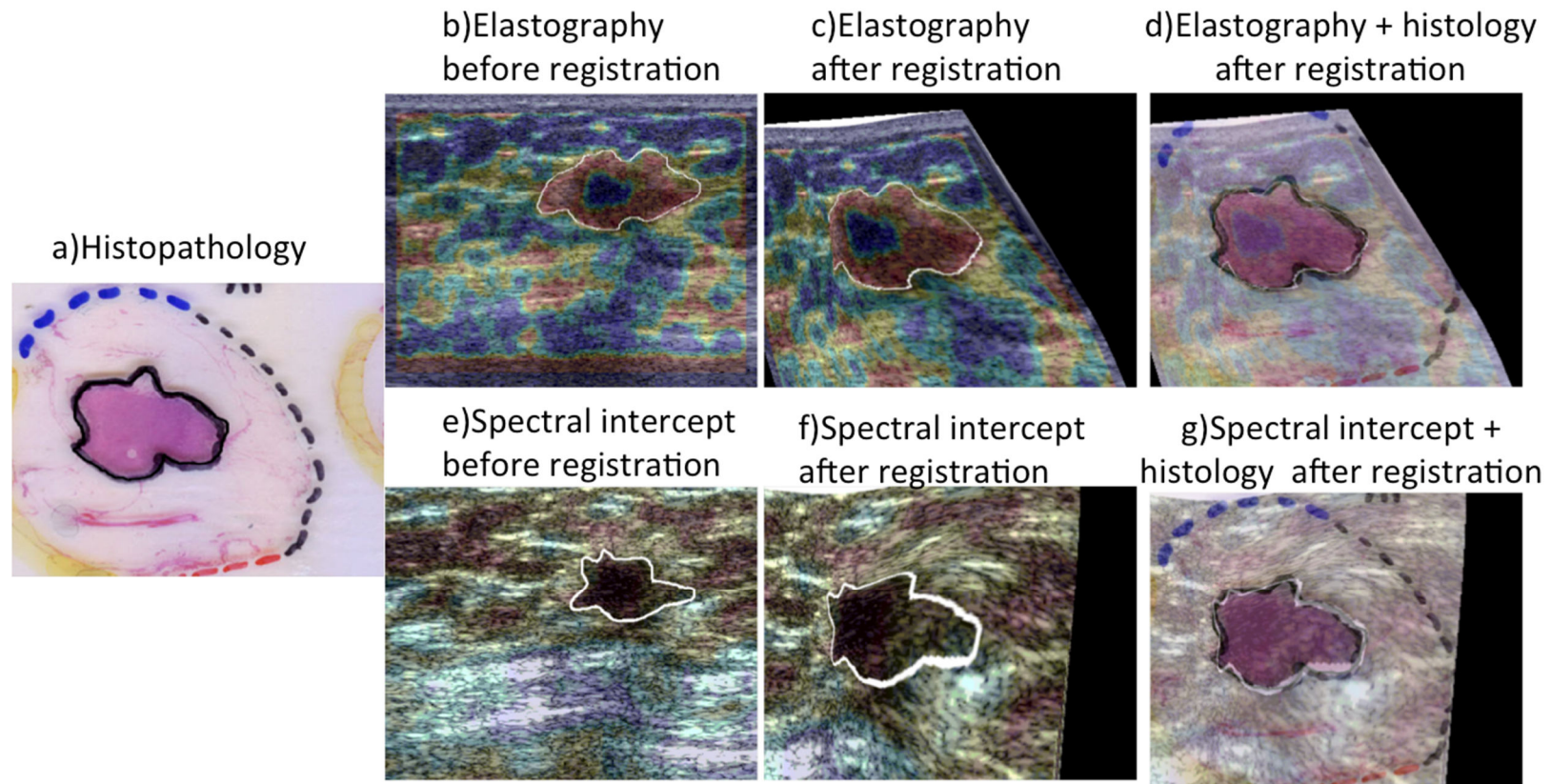
5.3.3 Registration of ultrasound spectral intercept image and ultrasound elastography image to histopathology image

In previous chapters ultrasound spectral intercept was investigated for the use of tissue characterisation of breast fibrosis and also for monitoring neo-adjuvant chemotherapy treatment in breast cancer. In this section the image registration method proposed in this chapter is applied to both ultrasound elastography and ultrasound spectral intercept images to two IDC cases (P391 and P395) from chapter 3 where the histopathology images are available.

The results of the registration are given in Figure 5 - 21 and Figure 5 - 22 with the histopathology image displayed on the far left and the ultrasound elastography

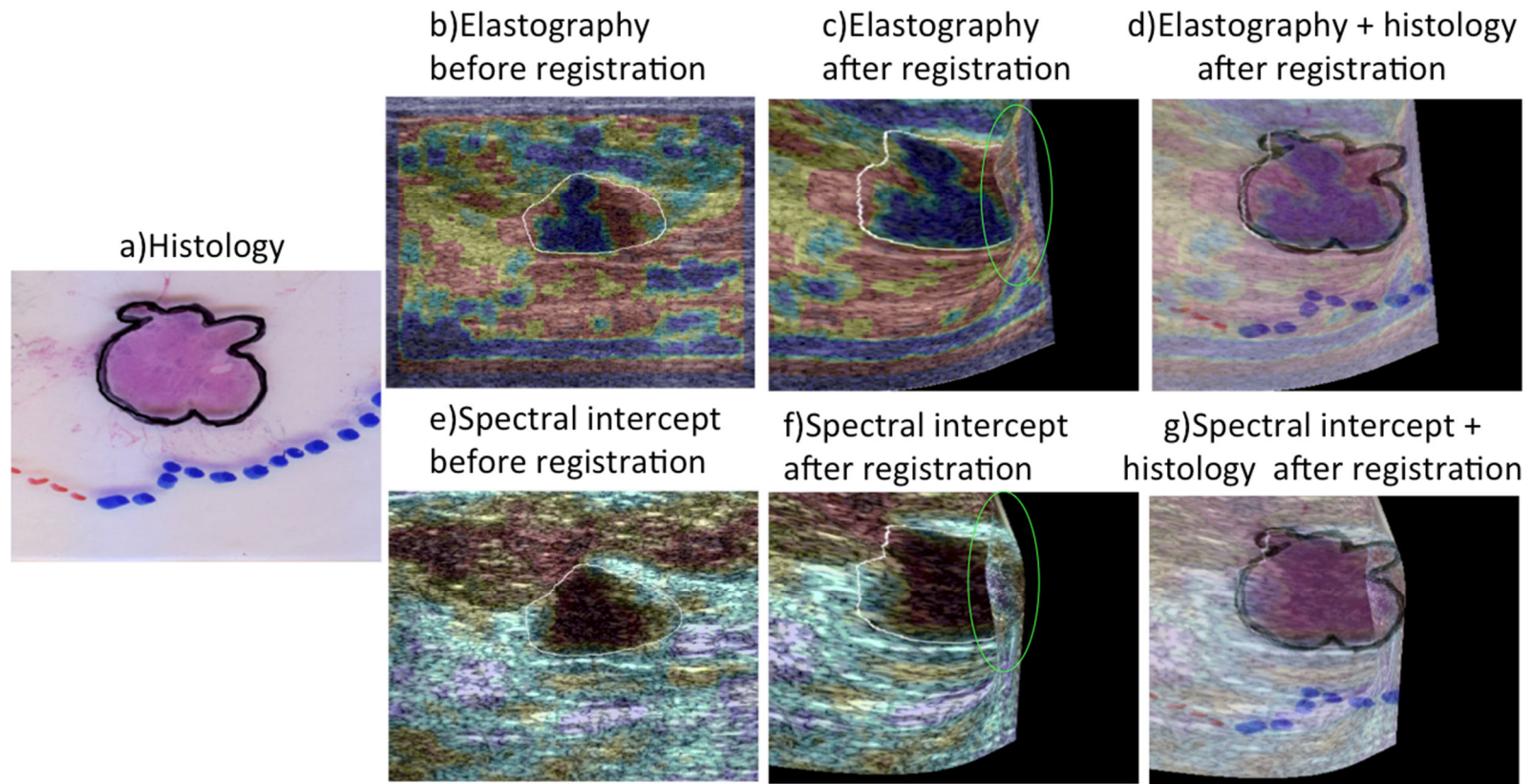
images displayed on the top row and the spectral intercept images displayed on the bottom row. Dr. Andrew Di Battista provided the ultrasound elastography images in these cases. The same clinician marked the tumour boundaries in the images, however, unlike the 14 cases shown in section 5.3.1 where the boundaries in ultrasound elastography were marked with the knowledge of the histopathology image, the tumour boundaries in ultrasound elastography and ultrasound spectral intercept images were marked separately without the knowledge of the histopathology images.

Overall, both cases showed much improved alignments in the features after registration, although in the case of P395 blurring artefact due to image interpolation can be observed similar to that in P246 (Figure 5 - 11) and P265 (Figure 5 - 17). The proposed registration method hence facilitates the analysis and comparing histopathology images with ultrasound elastography and ultrasound intercept images and also between the two parametric image modalities.



P391

Figure 5 - 21. Image registration results of P391 a) histopathology image, ultrasound elastography image b) before and c) after registration, d) registered ultrasound elastography image superimposed with the histopathology image, spectral intercept image e) before and f) after registration and g) spectral intercept image superimposed on histopathology image after registration.



P395

Figure 5 - 22. Image registration results of P395 a) histopathology image, ultrasound elastography image b) before and c) after registration, d) registered ultrasound elastography image superimposed with the histopathology image, spectral intercept image e) before and f) after registration and g) spectral intercept image superimposed on histopathology image after registration. The green circles in c) and f) highlight the artefacts due to image interpolation.

5.4 Selection of parameters and features

As mentioned in section 5.2.1 the CPD algorithm has three parameters w , λ , and β that can be varied, where w controls the weighting of the noise/outlier term in the GMM model and reflects the user's prior belief in how noisy the point sets selected for registration are. Since an experienced clinician marked the boundaries used in this chapter, the default value of 0.1 was used for w . By keeping λ and β constant ($\lambda = 3$ and $\beta = 2$) and varying w to be 0.1, 0.5 and 0.9, Figure 5 - 24 illustrates the results of varying w . It can clearly be seen from the green circles marked in Figure 5 - 24 that the histopathology points in the area are being regarded as outliers as w increases.

The parameter λ (equation 5.8) and β (equation 5.10) influence the smoothness of the regularization term whereby λ acts as a trade off factor between the goodness maximum likelihood fit and regularization and β controls the width of the Gaussian filter of the regularization (Myronenko and Song, 2009). The effect of these two parameters on the point-set registration is illustrated in Figure 5 - 24 and Figure 5 - 25 based on the point sets selected for P211 (Figure 5 - 6). It can be seen by comparing the top and bottom right figure in Figure 5 - 24 and Figure 5 - 25 that the lower the λ or β , a closer match in the overall shape is obtained between the histopathology feature (in blue) and the elastography feature (in red) but less smoothness is imposed (see the red feature circled in green in the top right of Figure 5 - 24 and Figure 5 - 25). Throughout this chapter the default values of 3 and 2 are used for λ and β respectively in order to achieve a balance in fit and smoothness.

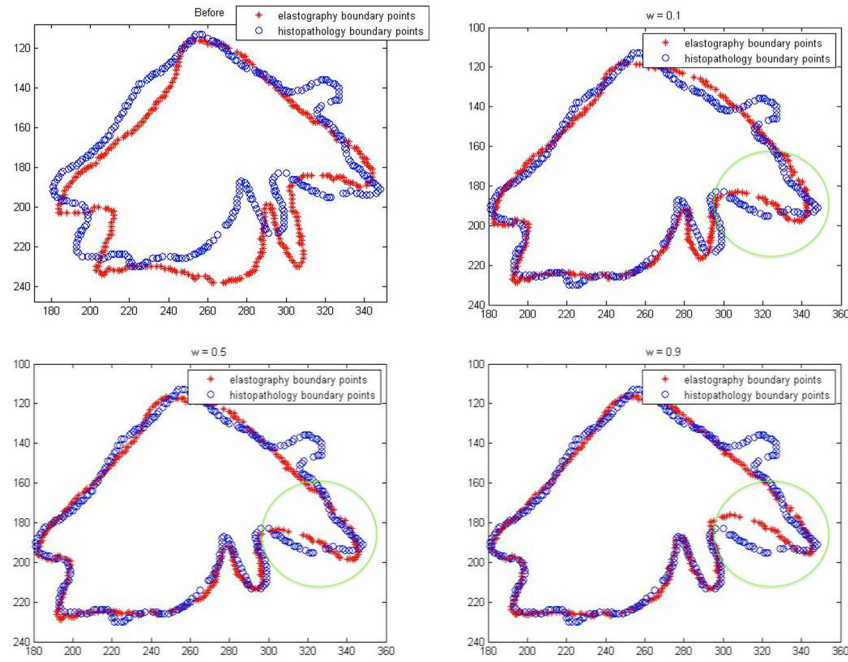


Figure 5 - 23. Effect of the outlier weighting parameter on the point set registration of the features selected in P211. The main differences are highlighted in the green circle. The histopathology feature is in blue and the elastography feature is in red. The other parameters were set as default (Top left) Original boundaries before registration (Top right) post-registration using $w = 0.1$ (Bottom left) post-registration using $w = 0.5$ (Bottom right) post-registration using $w = 0$.

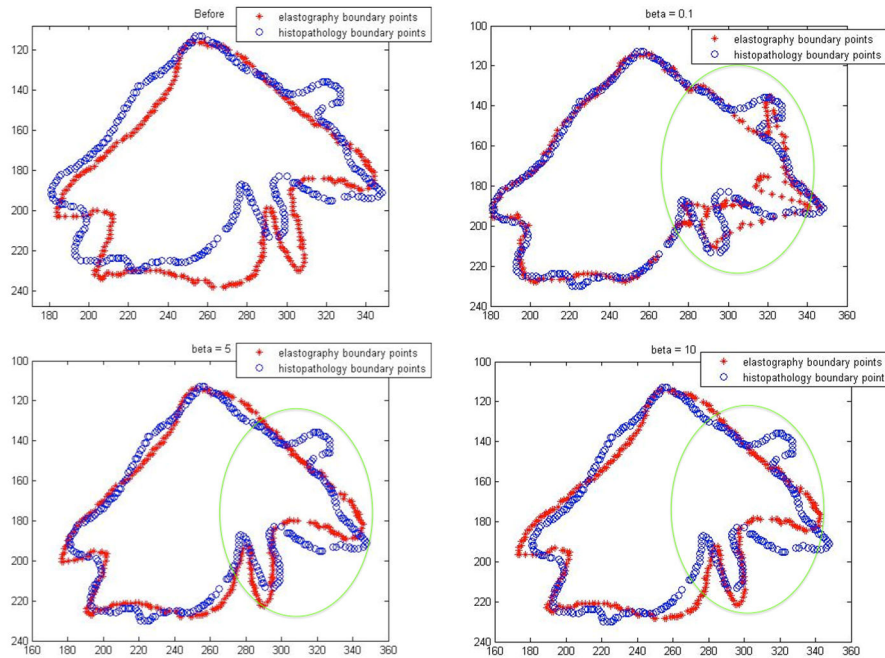


Figure 5 - 24. Effect of the β parameter on the point set registration of the features selected in P211. The main differences are highlighted in the green circle. The histopathology feature is in blue and the elastography feature is in red. The other parameters were set as default (Top left) Original boundaries before registration (Top right) post-registration using $\beta = 0.1$ (Bottom left) post-registration using $\beta = 5$ (Bottom right) post-registration using $\beta = 10$.

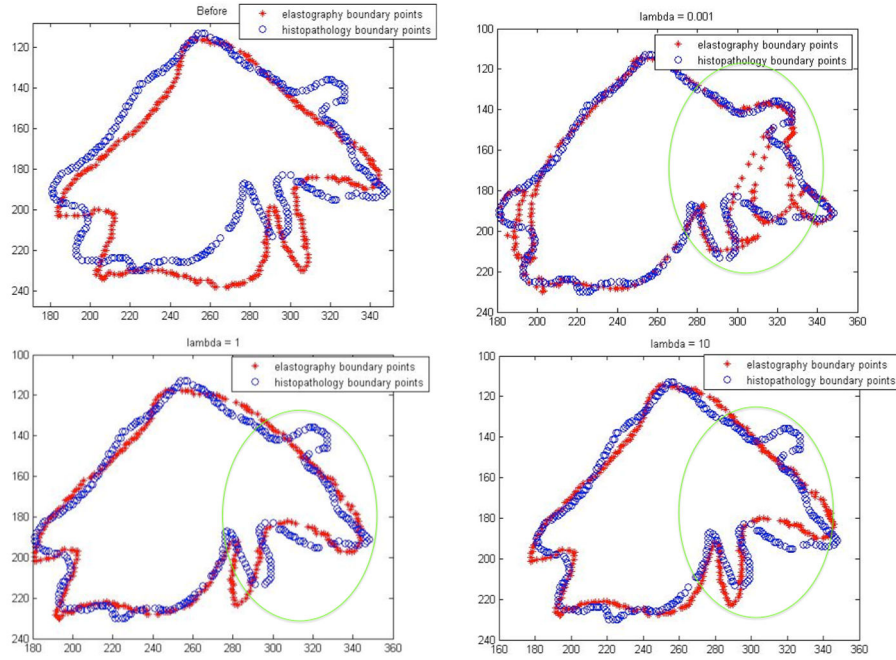


Figure 5 - 25. Effect of the λ parameter on the point set registration of the features selected in P211. The main differences are highlighted in the green circle. The histopathology feature is in blue and the elastography feature is in red. The other parameters were set as default (Top left) Original boundaries before registration (Top right) post-registration using $\lambda = 0.001$ (Bottom left) post-registration using $\lambda = 0.1$ (Bottom right) post-registration using $\lambda = 10$.

In terms of feature selection, in general, the more features that can be confidently selected to be used in the point-set registration the better the registration result for the overall image as it imposes more constraints. This is illustrated in Figure 5 - 26, whereby the registration result with three features achieves better alignment than the single feature scenario as would be expected. However, as corresponding features may be difficult to identify for inter-modality registration, and also difficult to accurately delineate boundaries in ultrasound-based images, radiologist's expert knowledge to confidently identify and select corresponding features in the images is key to improve the confidence in the registration result.

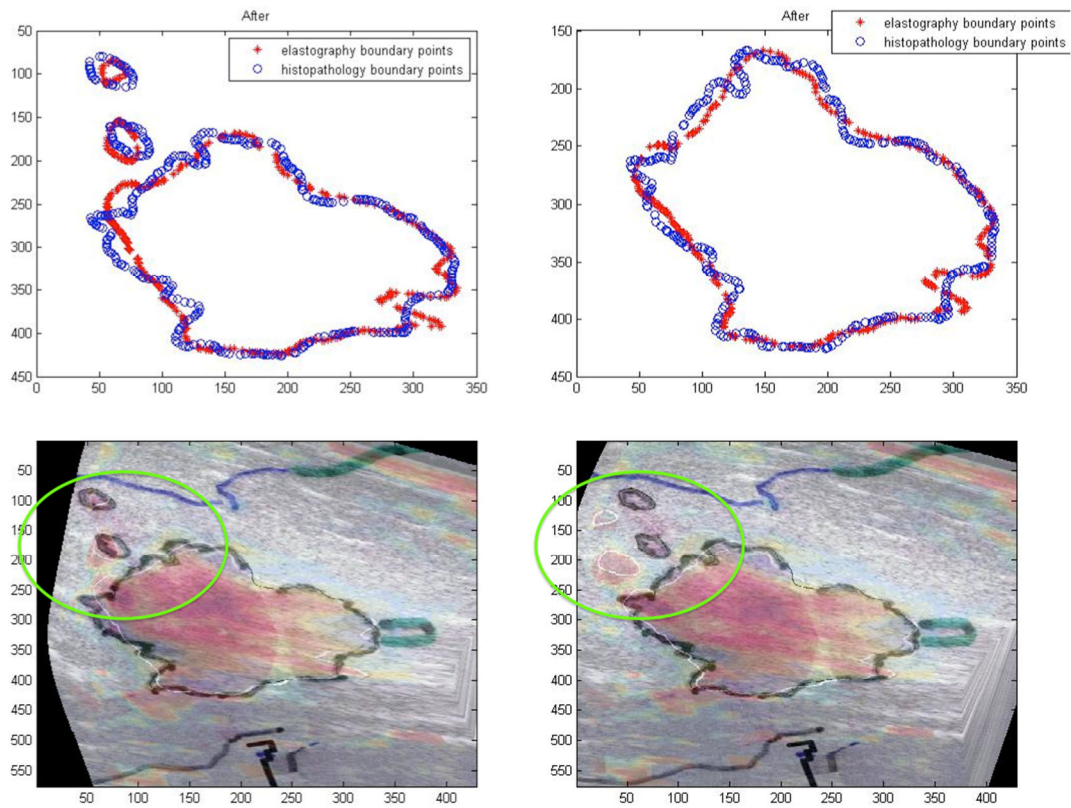


Figure 5 - 26. Registration of P246 where in the left column are the results based on three features used for registration and the right column are the results of a single feature registration. The green circles highlights the differences in registration results where the three feature registration achieve better alignment.

5.5 Conclusion

This chapter proposed and evaluated a novel method for the registration of ultrasound parametric images to histopathology and designed to overcome the complex deformation in histopathology images, which has not been done before. Based on the Hausdorff distance measure, most cases showed improved alignments in features that were not used for the registration. The method not only facilitates the study and comparisons between ultrasound parametric images to histopathology images, it can also be applied to other imaging modalities such as MR and CT as long as corresponding features can be identified in both images and hence provides a useful tool that supports future research in the field of medical imaging analysis.

Chapter 6: Summary and Future Work

6.1 Summary

This thesis provides a novel method based on ultrasound spectral intercept for the characterisation of breast fibrosis (chapter 3) and for monitoring tumour response to neoadjuvant chemotherapy treatments (chapter 4). It also proposes a novel registration method (chapter 5) for the registration of ultrasound parametric images to histopathology images that are regarded as ground truths in clinical studies.

Results from chapter 3 suggest that ultrasound spectral intercept can be used to characterise fibrous tissues, which has not been shown before. The pilot study on neoadjuvant therapy monitoring in chapter 4 showed promising initial results in that spectral intercept provides additional information to tumour sizing in B-mode ultrasound, which is commonly used as a measure to indicate response to chemotherapy treatments. Results in chapter 4 showed mixed and smaller changes in spectral intercept measures for the patient with partial response to treatment and larger and more consistent changes for the patient with complete response to treatment whereas B-mode tumour sizing showed large reduction for both partial and complete response. The pilot study provides insights in the use of ultrasound spectral intercept for monitoring neoadjuvant therapy and will contribute to future studies as a useful reference for comparison, with the ultimate goal of providing a complementary tool to ultrasound B-mode imaging for treatment monitoring.

The registration method proposed in chapter 5 provides an image analysis tool to facilitate the comparison between ultrasound parametric images and also to provide insights to understanding ultrasound parametric images. The results show improved alignments between the images after registration and facilitate comparisons between the images. No method has been proposed before for registering ultrasound parametric images to histopathology images and tackling /modelling the complex deformation in histopathology images.

6.2 Future work

6.2.1 Ultrasound spectral parameters for fibrosis characterisation and Neoadjuvant therapy monitoring

The methods and results presented in this thesis for monitoring neoadjuvant therapy contribute greatly to future studies in the area by providing detailed frameworks and results that can be compared upon despite the limitations that the methods currently suffer. The major challenges that need to be overcome include having more complete histology results to allow us to know more about the status of the tumour at intermediate stages of the treatment to further support the spectral intercept findings. Furthermore, because the landmarks used for tracking tumour throughout therapy lead to artefacts in the spectral intercept results and need to be excluded in the analysis, a less user-dependent method for excluding the landmarks can be proposed such as using the wavelet decomposition proposed by Gergiou and Cohen (2001). In addition, the effect of the size of the areas selected in the FPA analysis on the results may need to be investigated to ensure no huge disparity arises.

For the fibrosis characterisation study, we currently only investigated the use of the mean and standard deviation of spectral intercept. Additional statistical measures such as the normalised entropy value could be investigated in future work to study if there are any significant differences in heterogeneity between fibrous and non-fibrous cases.

We introduced in chapter 3 a simple empirical threshold of half the estimation window size to filter out cases where the tumour size was too small and affected the analysis. Future work can investigate the relationship between the statistics of tumour spectral parameter values and the size of the estimation window to design a smarter threshold.

Both the fibrosis characterisation and the neoadjuvant treatment monitoring work will benefit from more targeted patients as currently only 22 patients were used for the fibrosis characterisation study (19 after thresholding) and only 2 patients in the neoadjuvant treatment monitoring study had full data set for analysis. For the fibrosis study, due to limited data availability we included fibroadenosis and traumatic fat necrosis (TFN) in the fibrous group. Given more data available in the future, future work can test whether the assumption of including fibroadenosis and TFN made in this thesis is correct. Further insights will be learnt as future studies include more patients and can be used to back the findings presented in this thesis. Moreover, analyses in this thesis were performed on 2D images and although we tried to measure the same plane consistently to the best of our ability (with an experienced clinician), future studies with ultrasound 3D imaging will greatly provide further insights. At the

time of conducting the research, suitable 3D probes were not available.

6.2.2 Registration

Currently our registration method requires manual segmentation of tumour boundaries to provide features for registration and segmentation of tumours in ultrasound B-mode is by no means easy even for experienced clinicians. To facilitate clinicians, future work can incorporate segmentation algorithms (Noble 2006) to extract boundaries and provide automated features or guidance of features (semi-automated) to clinicians, making the evaluation process easier.

We've briefly discussed the effects of parameters w , λ , and β in the CPD algorithm where w determines the weight of the outlier and λ and β determine the smoothness/regularisation term in the registration. In this thesis, we've used the default values for our work but future work could investigate deriving the optimal set of parameters to use based on feature properties such as shape, orientation and similarity between the corresponding features selected.

The registration method proposed in this thesis provides a powerful and useful tool for studying and analysing medical images and is not only confined to applications in this thesis. In section 5.3.3 we have applied the method to register ultrasound elastography and ultrasound spectral intercept images to histopathology images, which shows the method would benefit future clinical studies that involve multiple imaging modalities (Yeh *et al.*, 2005).

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Appendix A

This section provides the images that were used in the analysis of the fibrosis characterisation study in chapter 3. Images of the fibrous group are shown first followed by the non-fibrous group. For each images, the ultrasound B-mode image is shown in the top left, the spectral intercept image is given in the top right, the bottom left shows the ultrasound B-mode image with the lesion selected by the clinician for analysis marked in green, the bottom right shows the overlap of the B-mode and the spectral intercept image. The units are in dB.

Fibrous group

P295 Fibrocystic

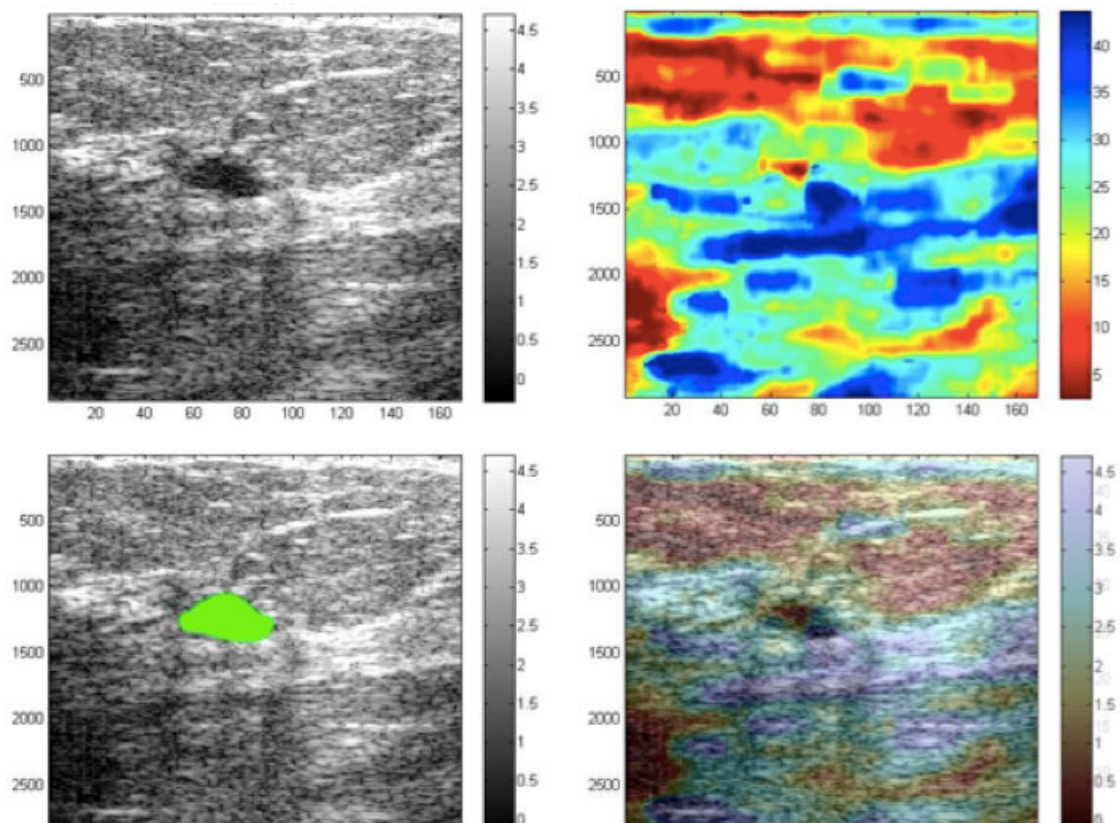


Figure A- 1 Images for P295, fibrocystic case. . (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image.

P333 Fibrocystic

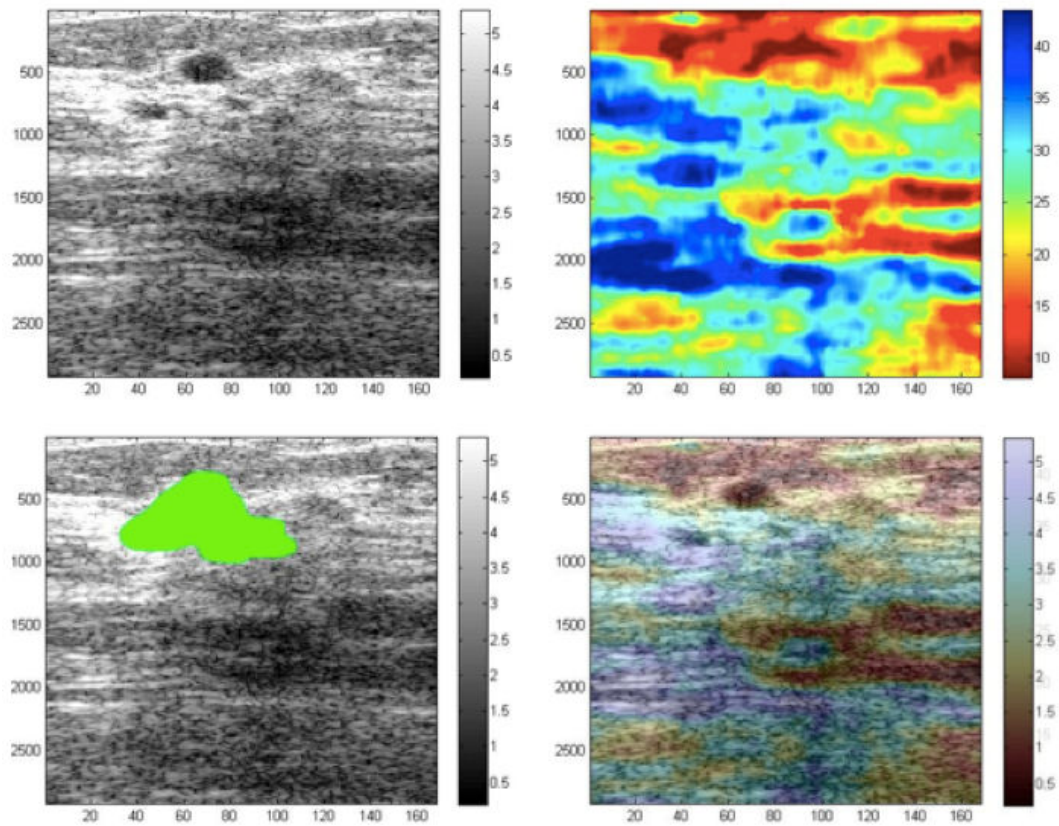


Figure A- 2_Images for P333, fibrocystic case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image.

P349 Fibrosis

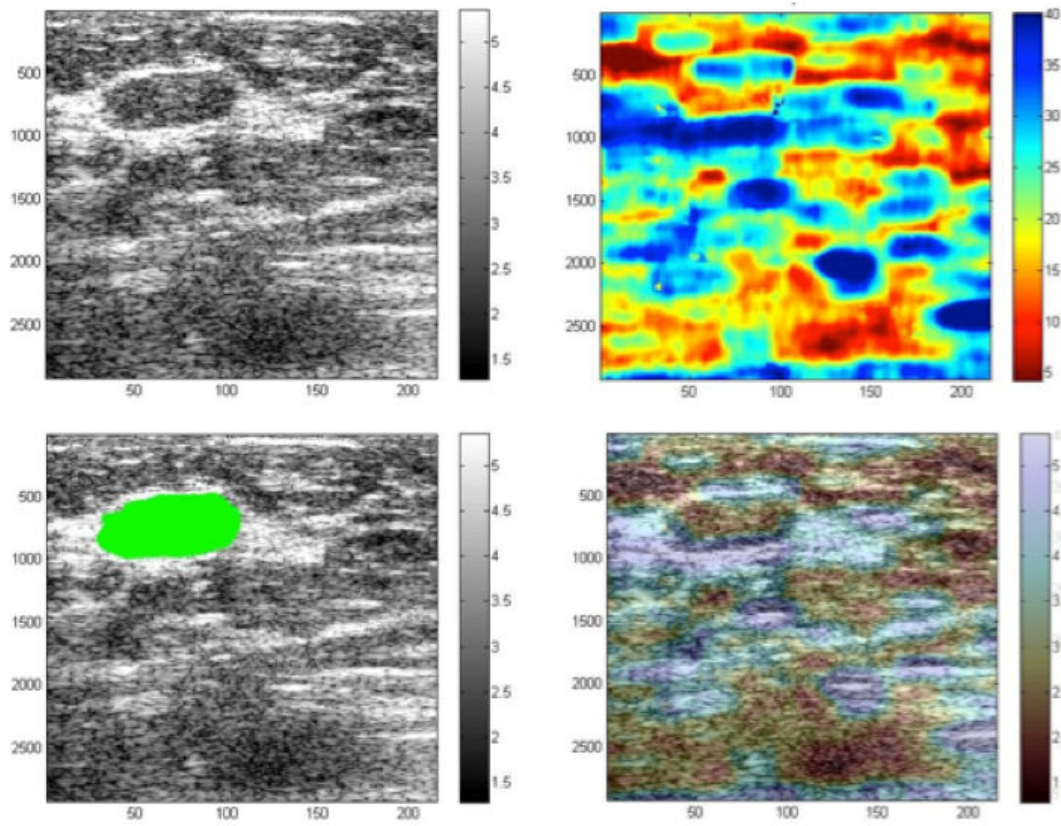


Figure A- 3_Images for P349, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image.

P350 Fibrosis

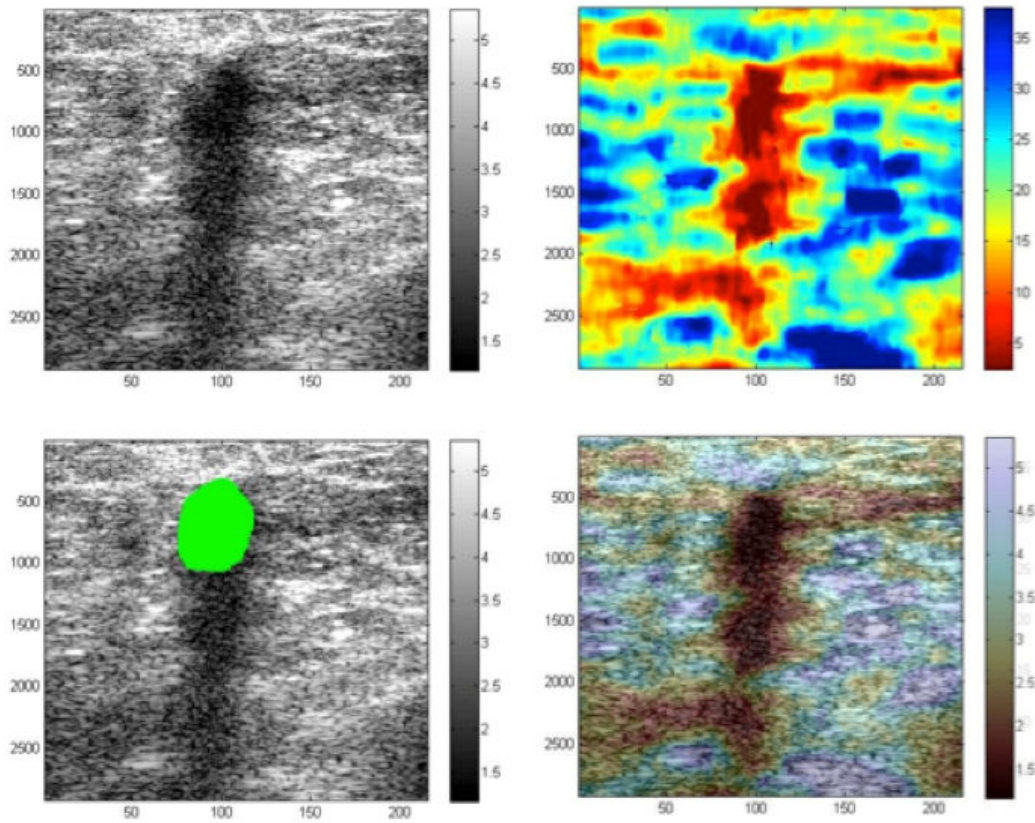


Figure A- 4_Images for P350, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image .

P371 TFN

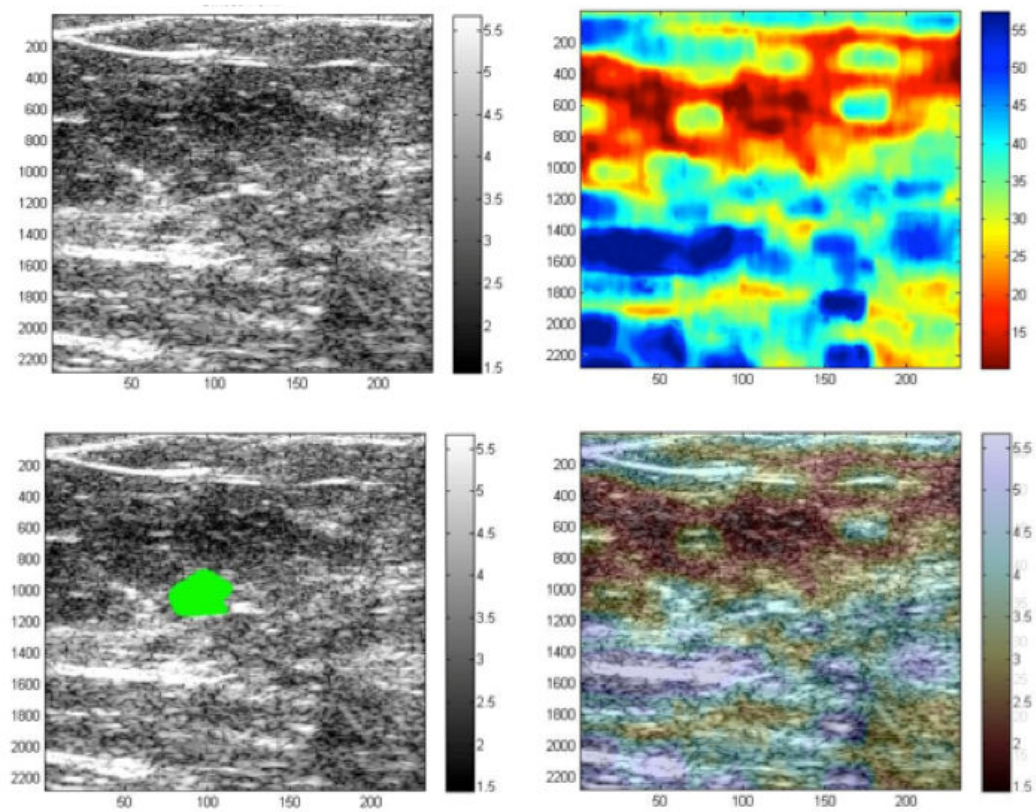


Figure A- 5_ Images for P371, TFN case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P388 Fibrosis

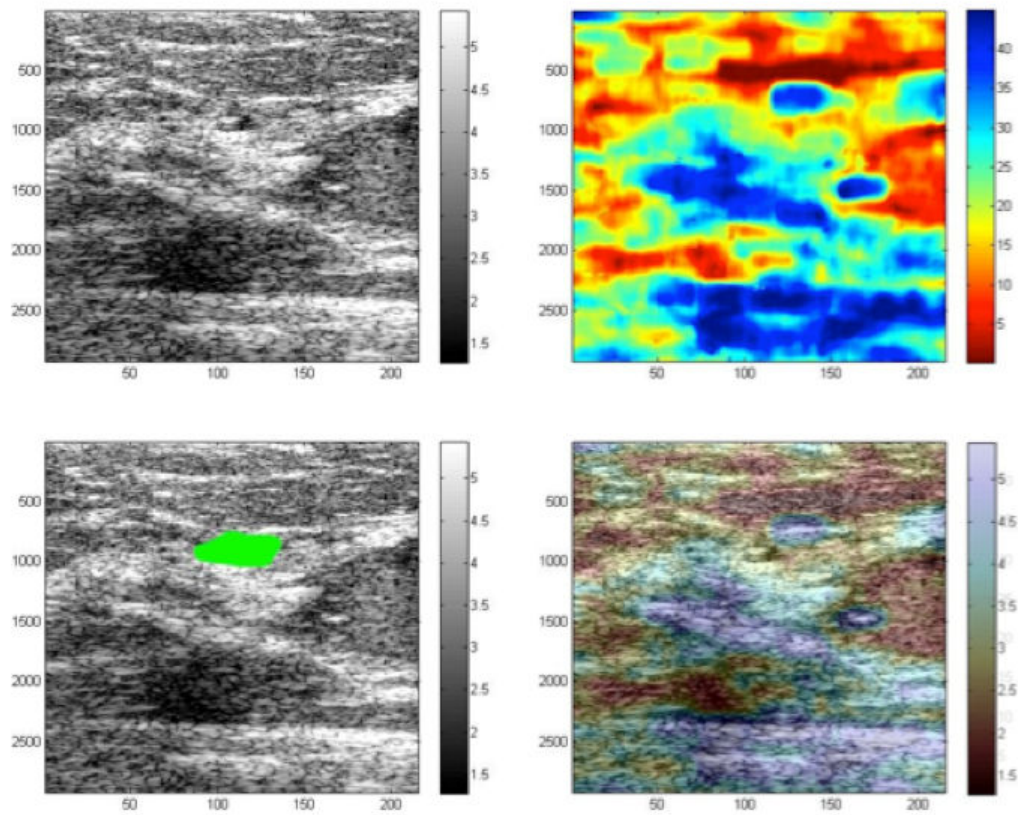


Figure A- 6_ Images for P388, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image .

P418 Fibrosis

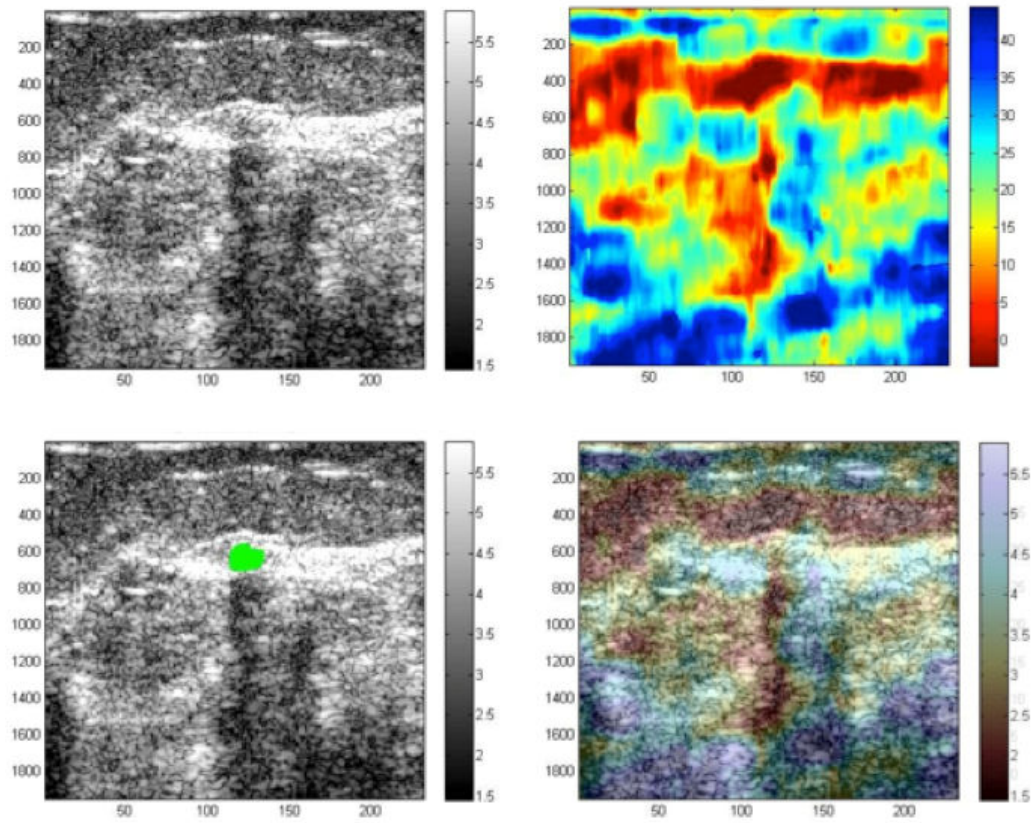


Figure A- 7_ Images for P418, fibrosis case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

Non-fibrous cases

P299 IDC

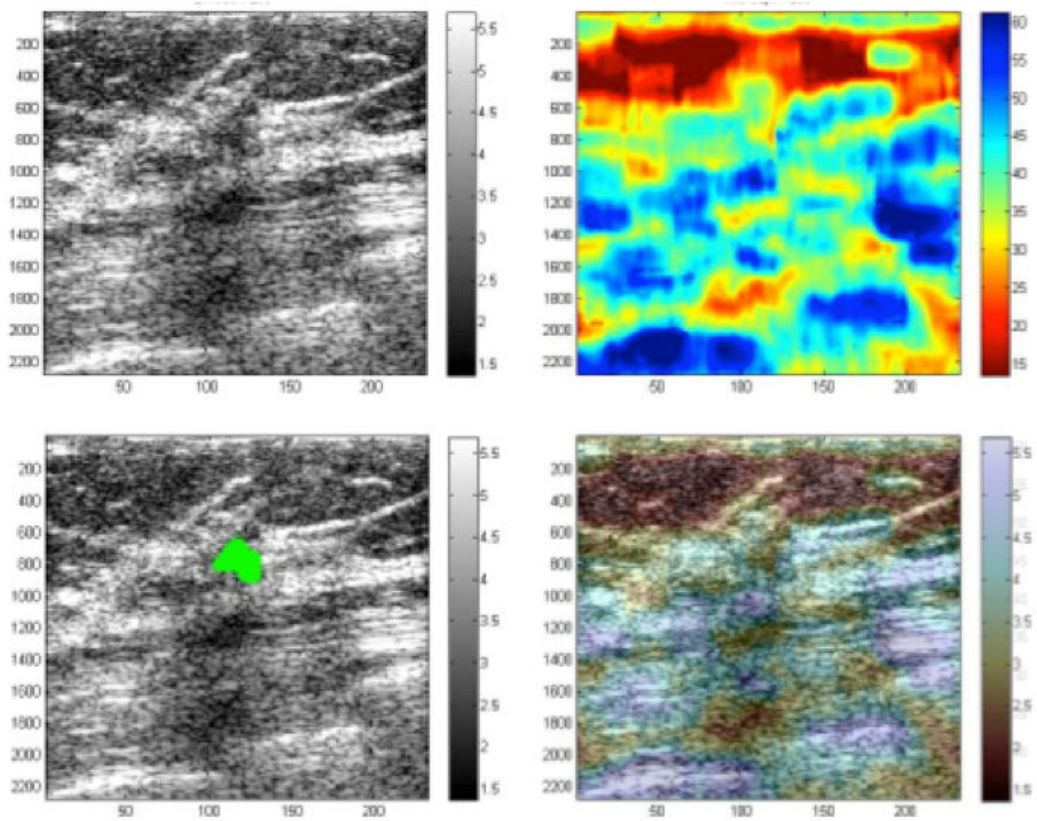


Figure A- 8_ Images for P299, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P341 IDC

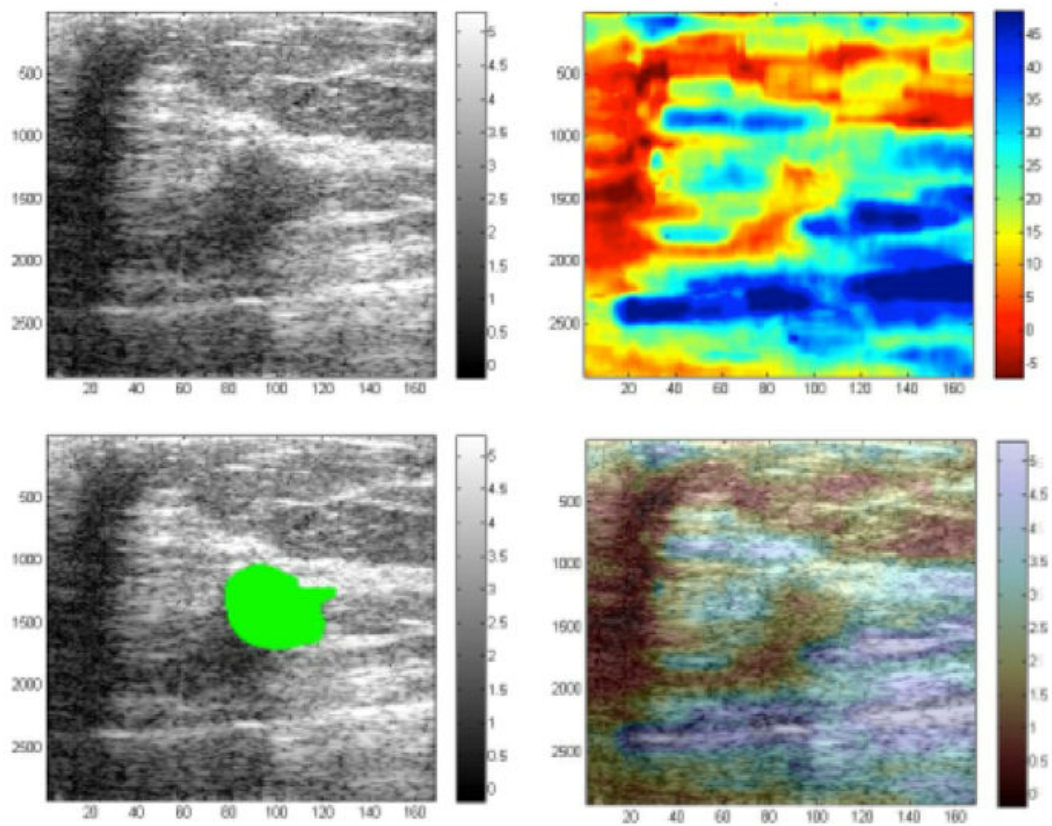


Figure A- 9_ Images for P341, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top mid) spectral intercept image (bottom mid) overlay of the B-mode with the spectral intercept image

P354 IDC

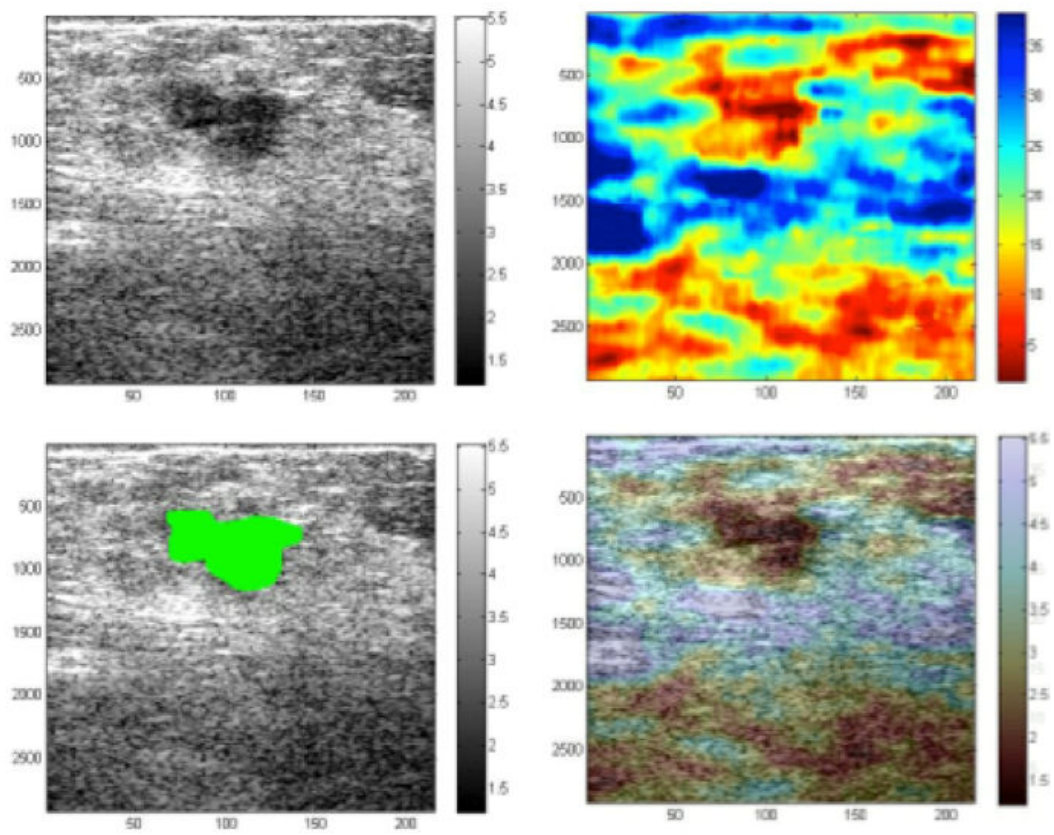


Figure A- 10_ Images for P354, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P357 IDC

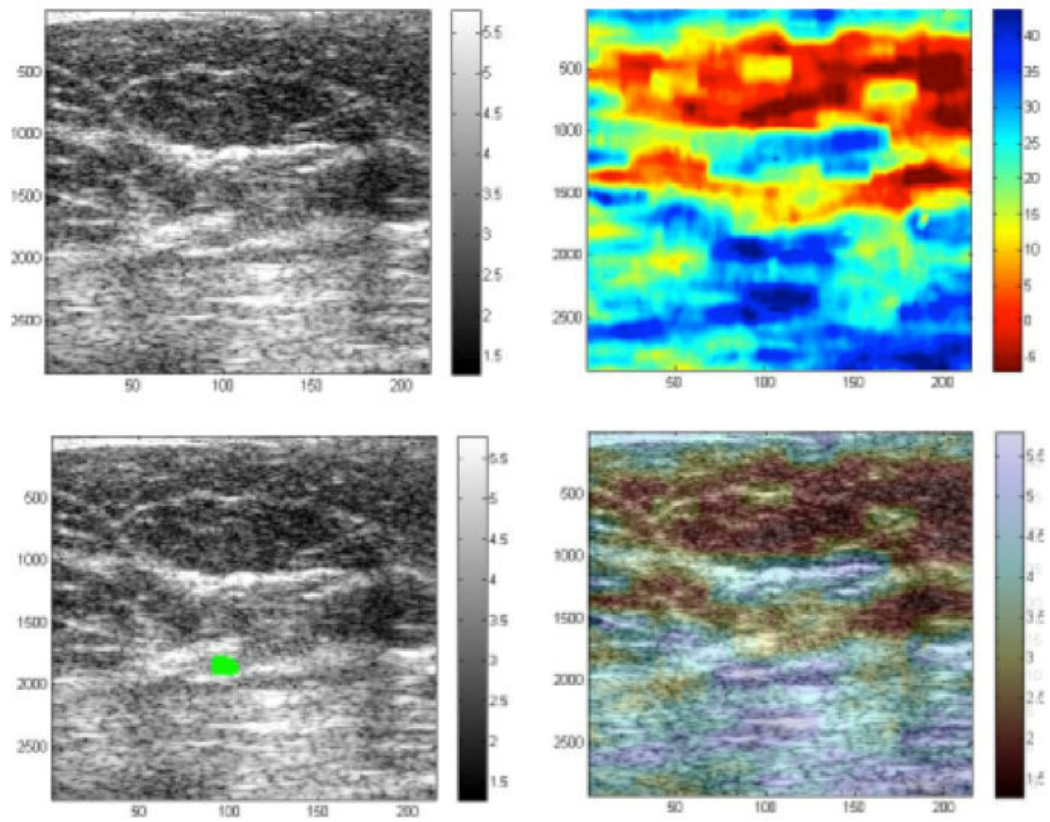


Figure A- 11_ Images for P357, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P391 IDC

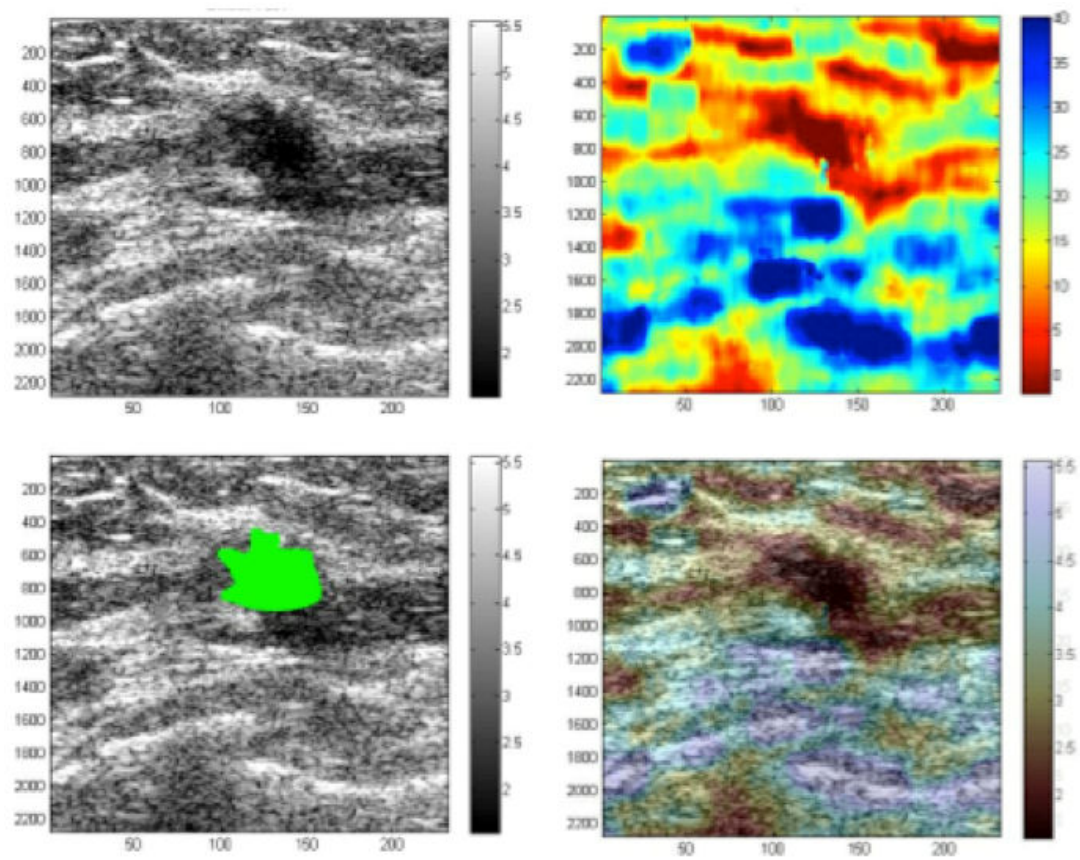


Figure A- 12_ Images for P391, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P395 IDC

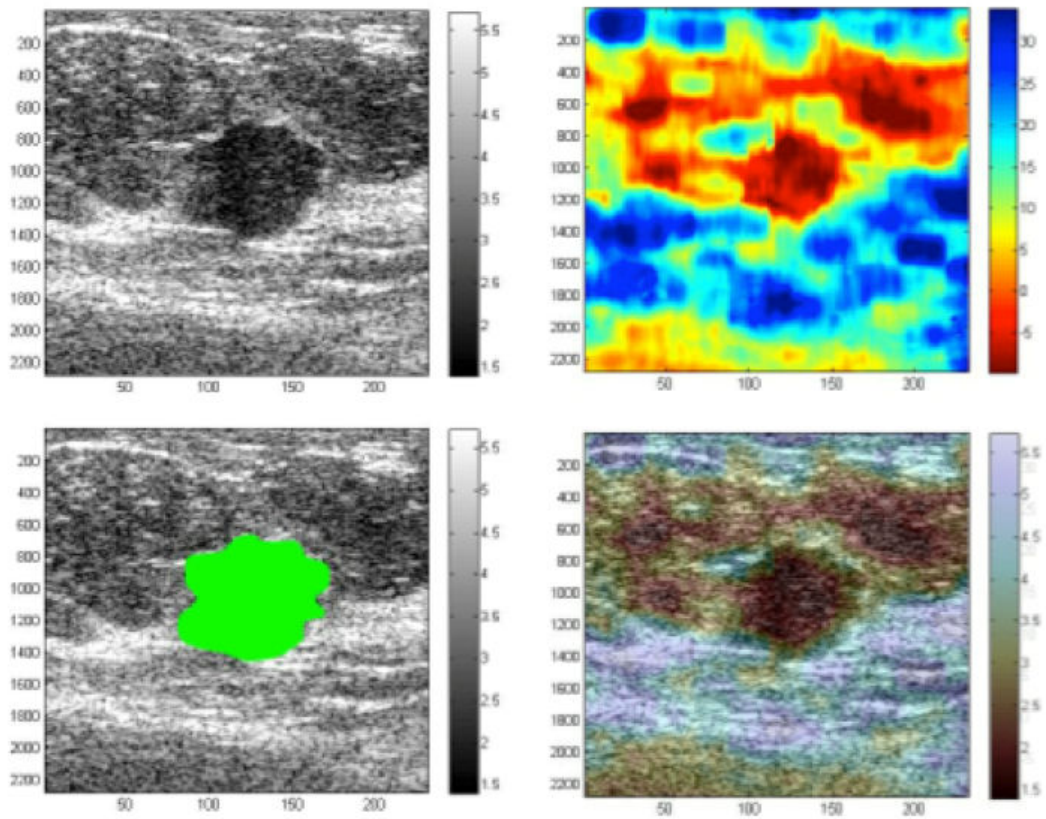


Figure A- 13_ Images for P395, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P399 IDC

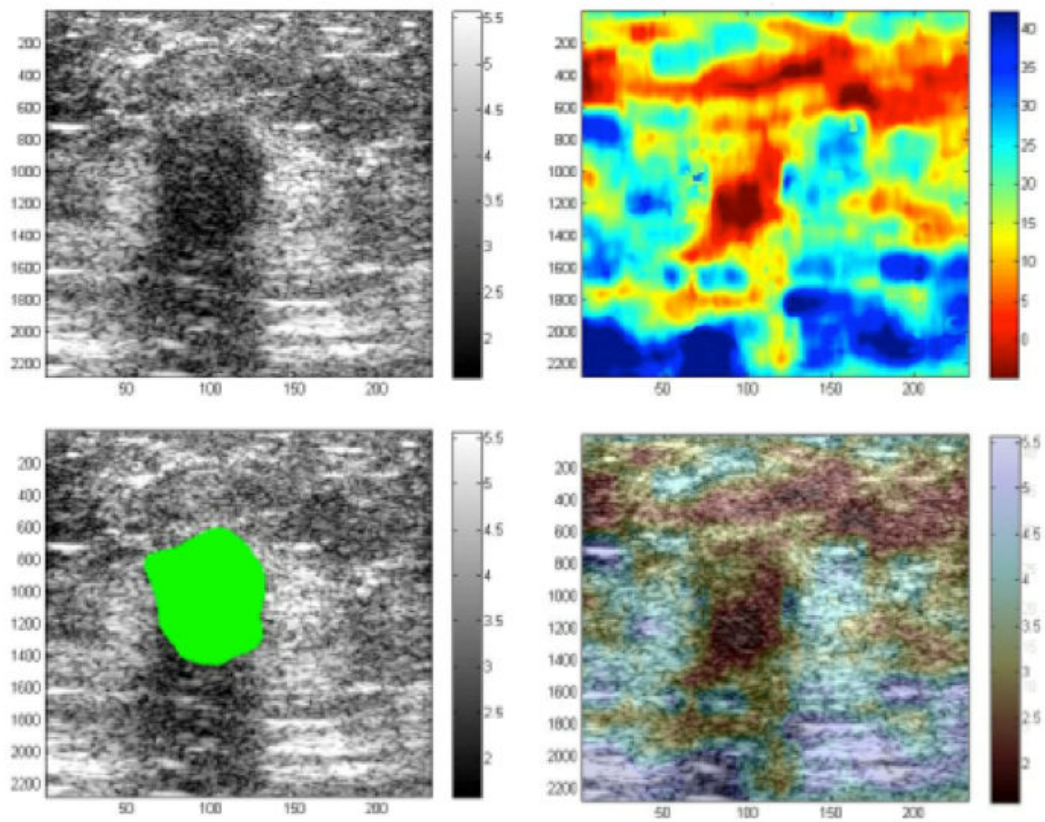


Figure A- 14_ Images for P399, IDC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image.

P302 Invasive Basal Carcinoma

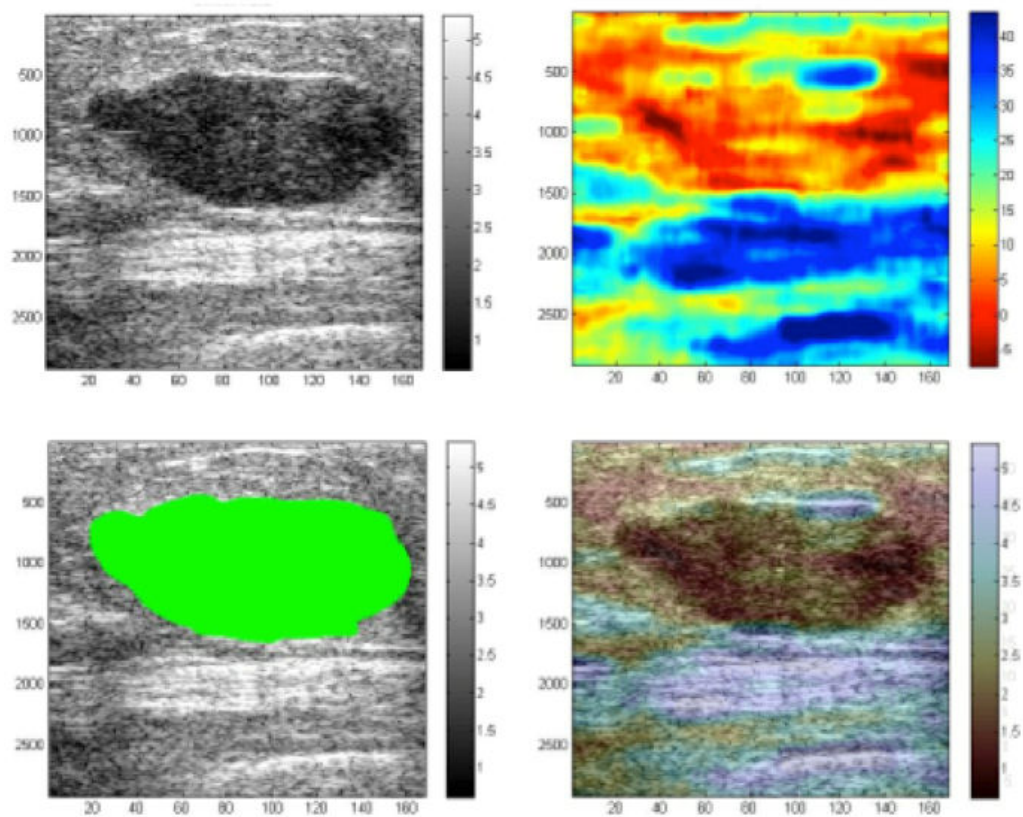


Figure A- 15_ Images for P302, IBC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P318 Invasive Lobular Carcinoma

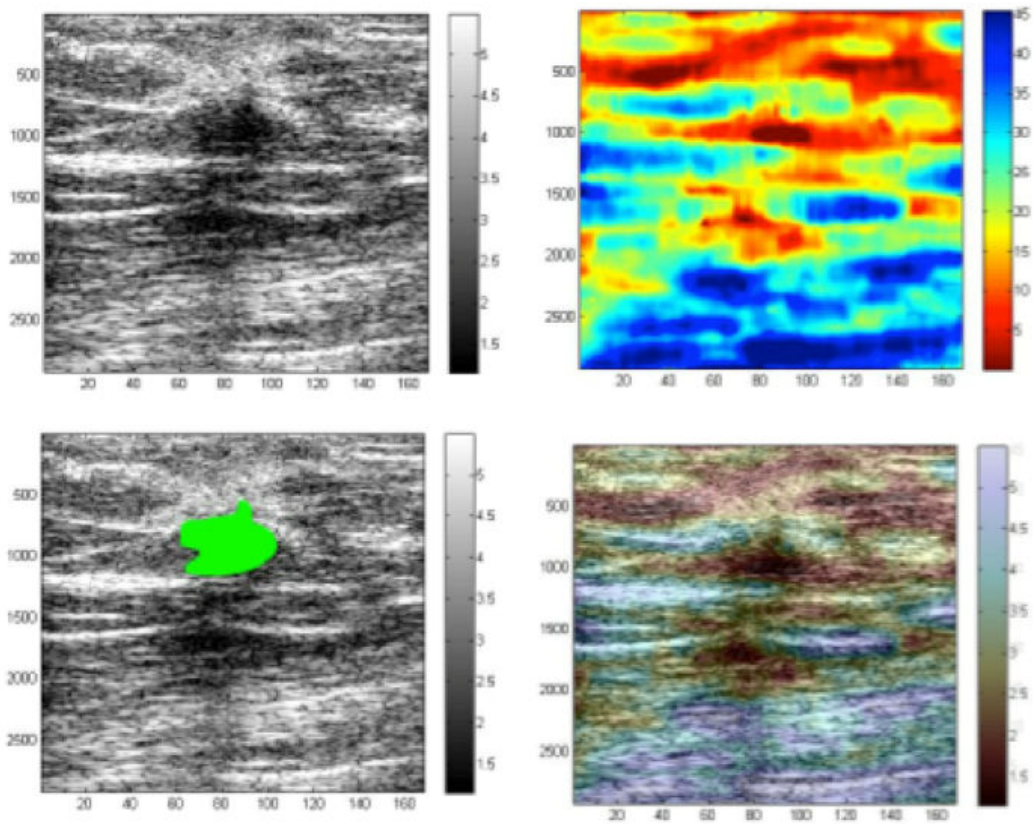


Figure A- 16_ Images for P318, ILC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P359 Invasive Lobular Carcinoma

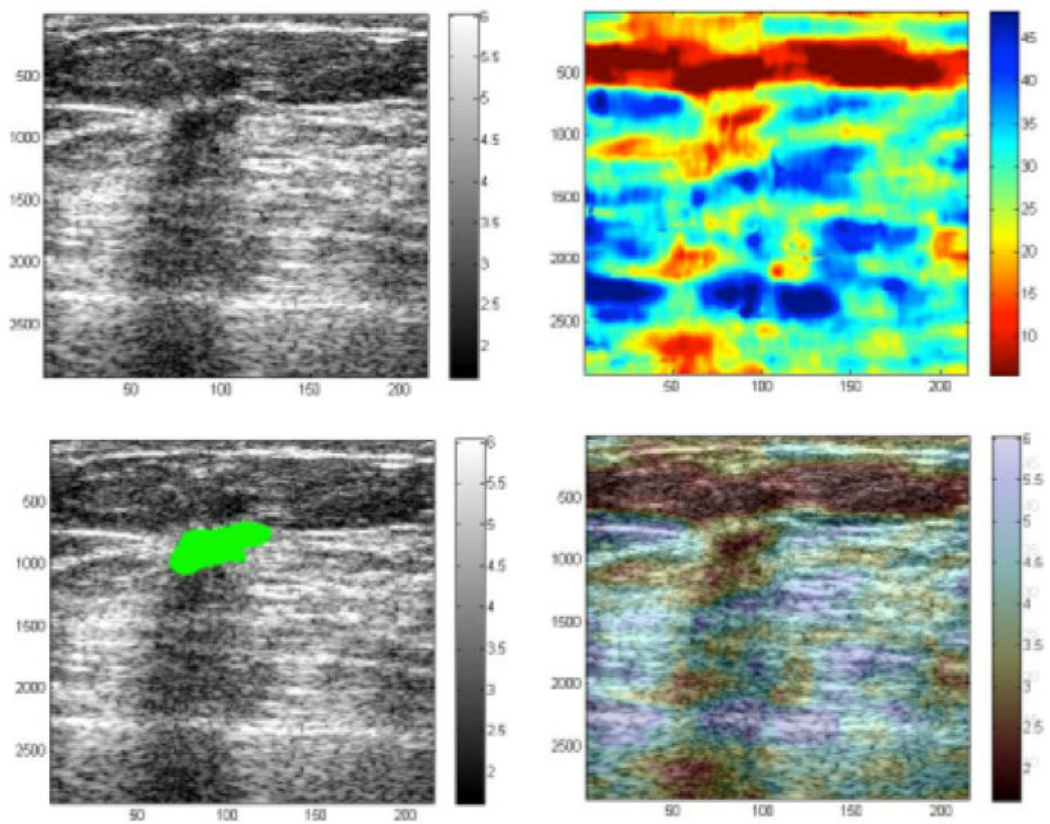


Figure A- 17_ Images for P359, ILC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P336 Invasive Tubular Carcinoma

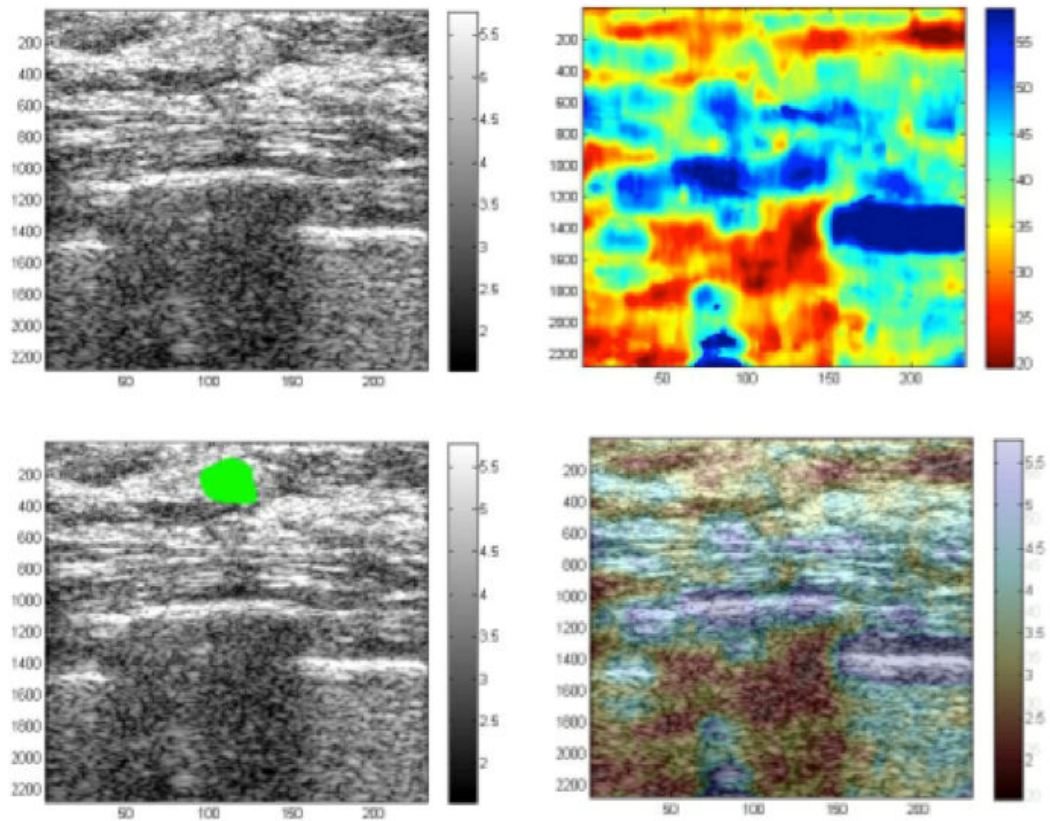


Figure A- 18_ Images for P336, ITC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P389 Mucinous Carcinoma

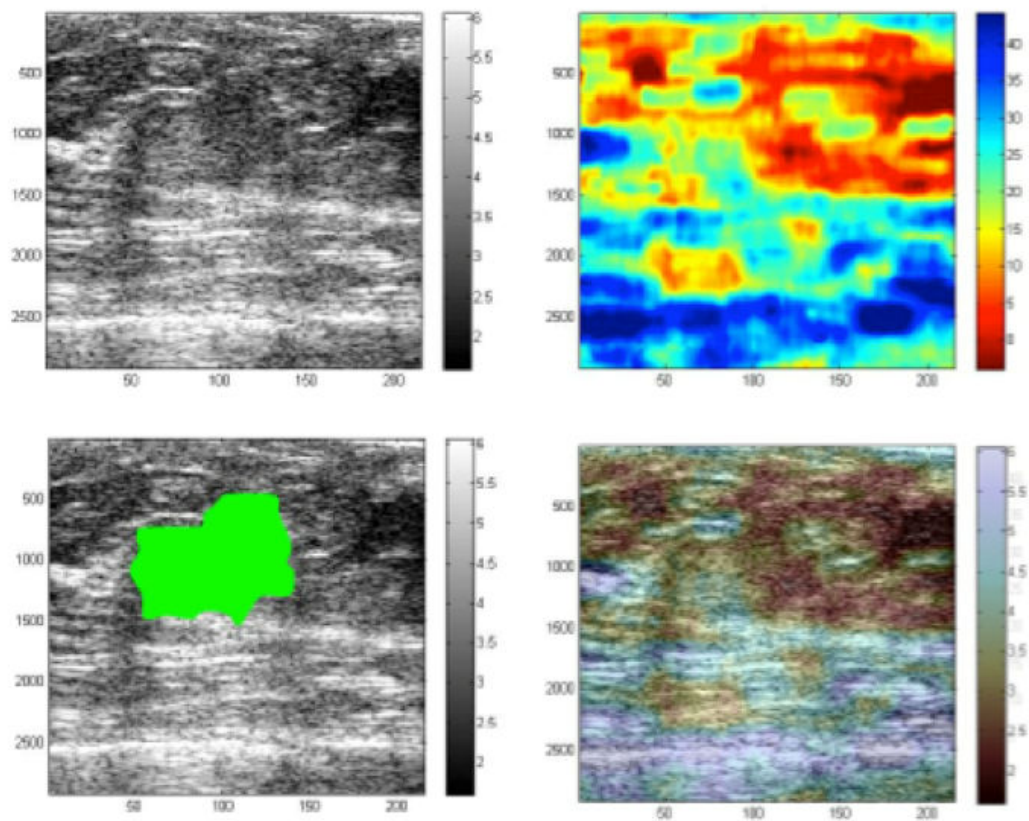


Figure A- 19_ Images for P389, Mucinous Carcinoma case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P408 Invasive Micropapillary Carcinoma

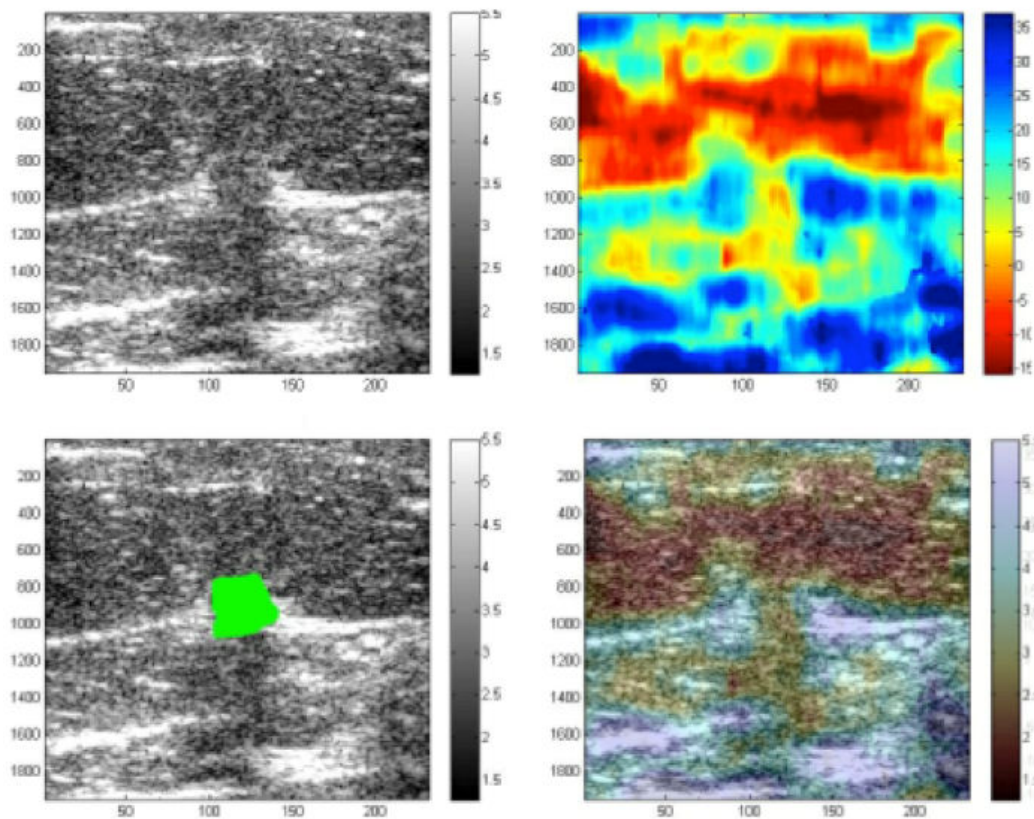


Figure A- 20_ Images for P408, IMC case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P412 Invasive Adenoid Cystic Carcinoma

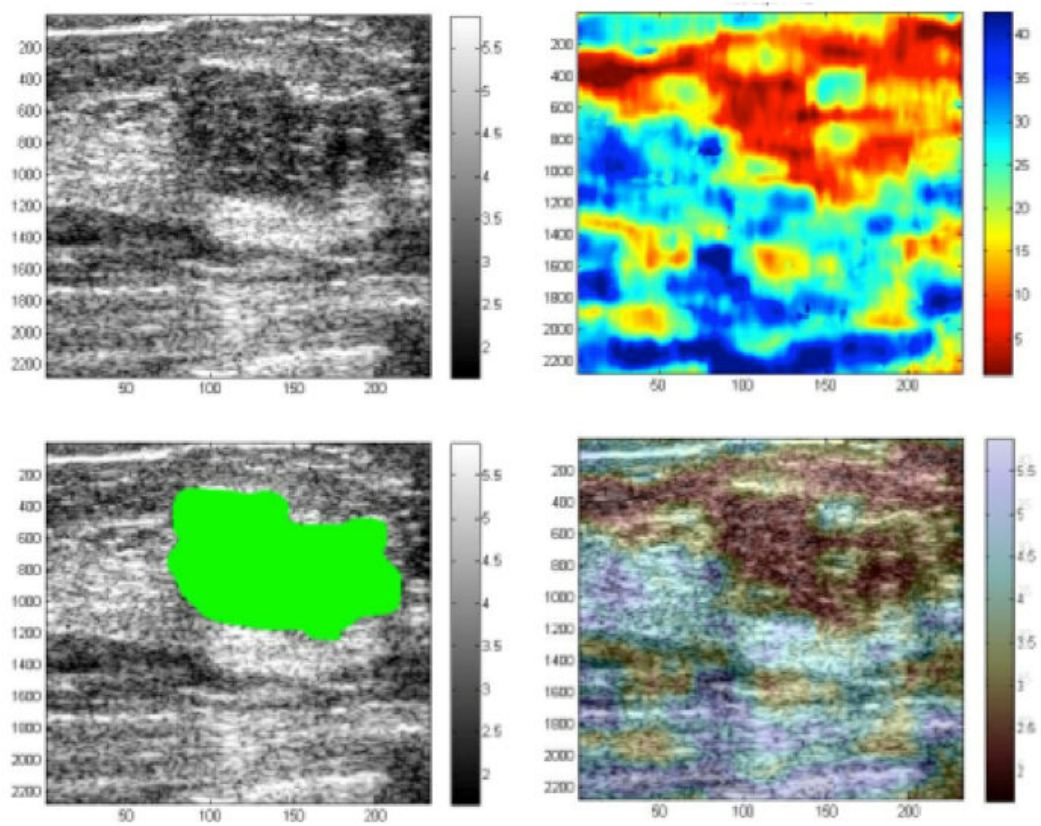


Figure A- 21_ Images for P412, Invasive Adenoid Cystic Carcinoma case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

P417 DCIS

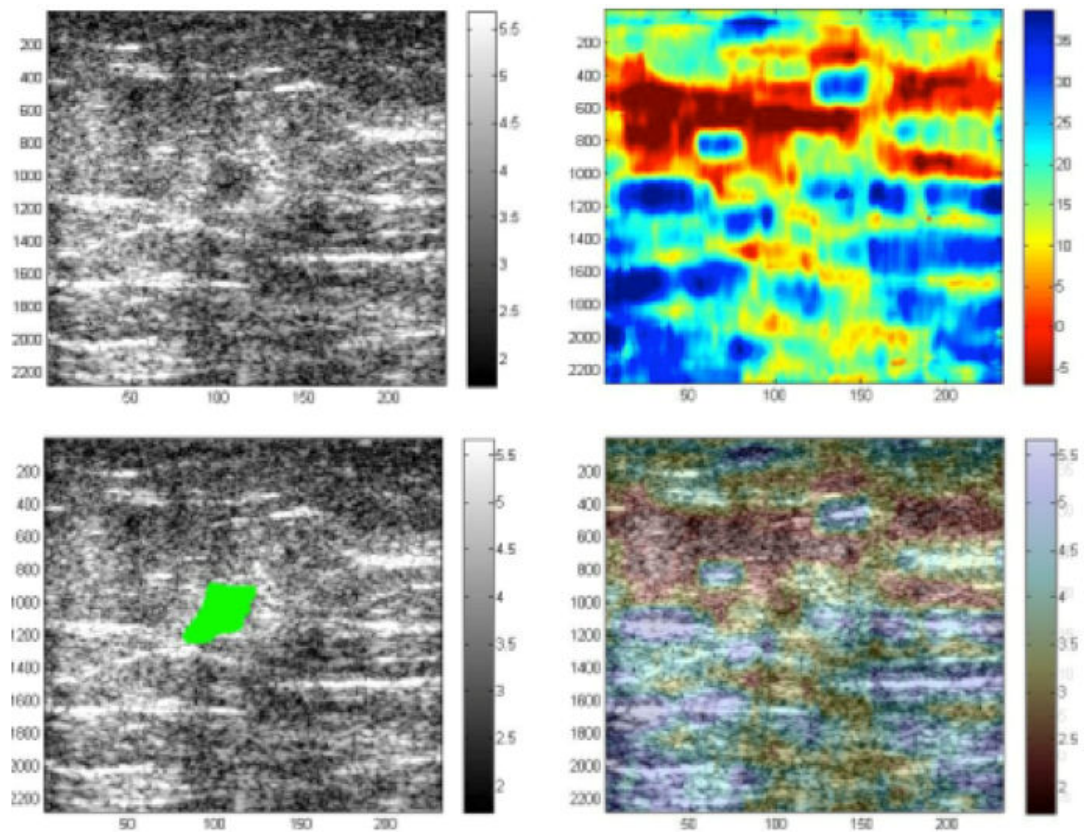
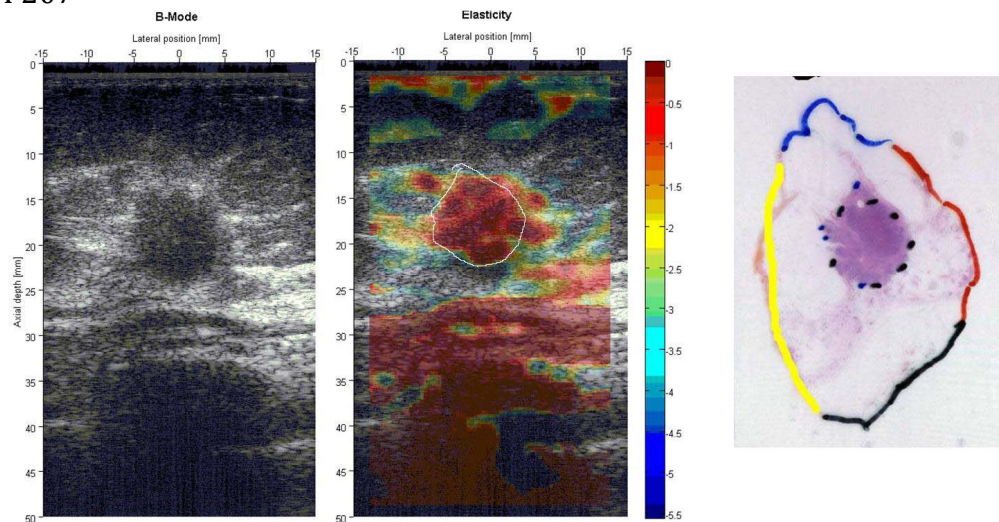


Figure A- 22_Images for P417, DCIS case. (Top left) B-mode image (bottom left) B-mode image with the lesion marked by the clinician in green (top right) spectral intercept image (bottom right) overlay of the B-mode with the spectral intercept image

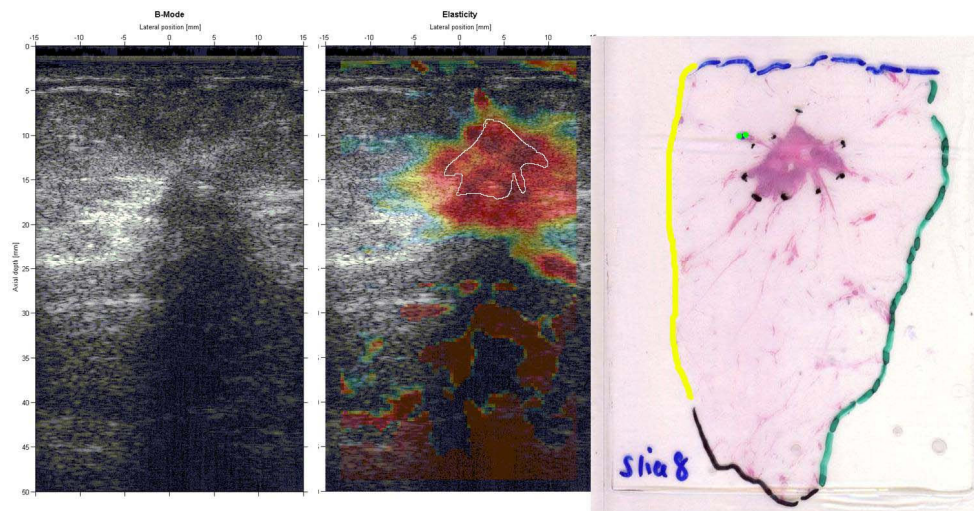
Appendix B

This section shows the images used for the registration work in chapter 5. In these images, ultrasound B-mode image is given on the left, the overlay of elastography images on ultrasound B-mode image is in the middle and the elastography image is on the right. The boundaries used for registration are marked white in the middle image and marked black in the histopathology image.

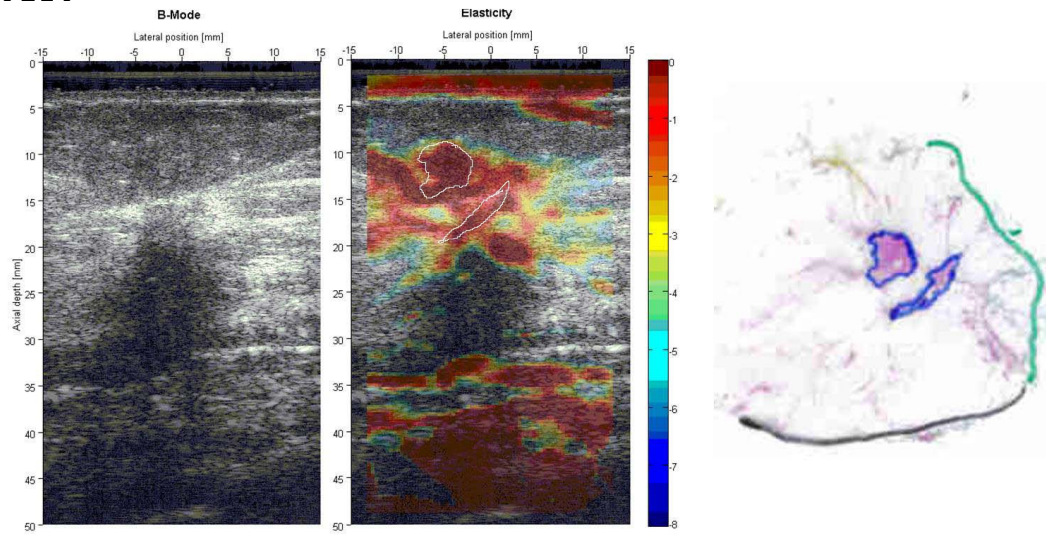
P207



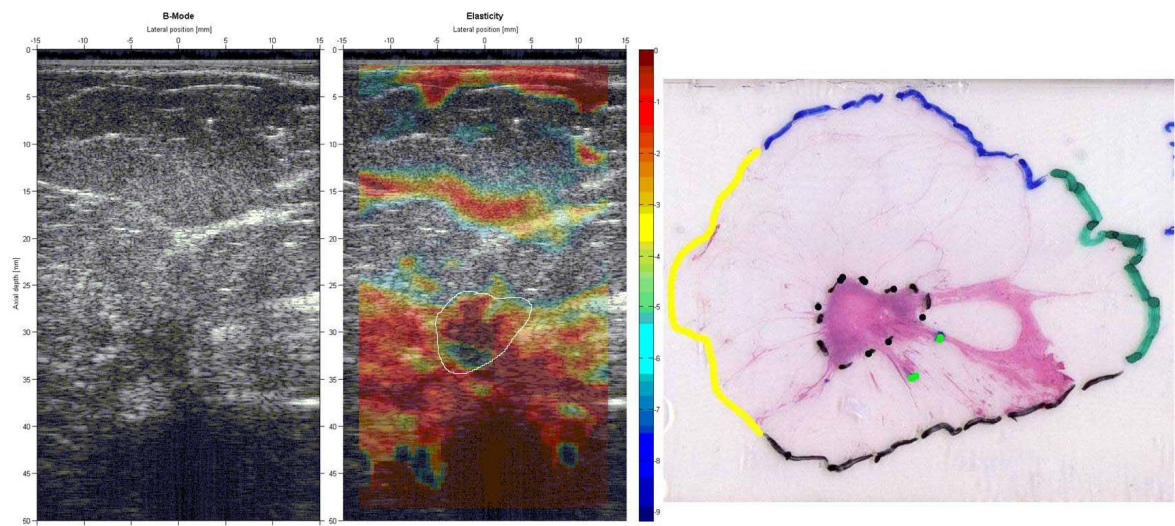
P211



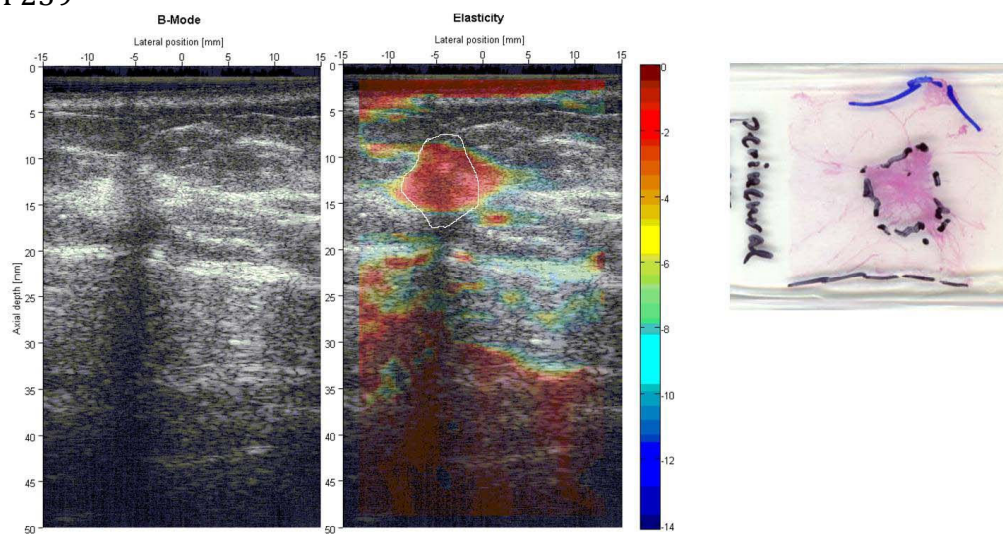
P214



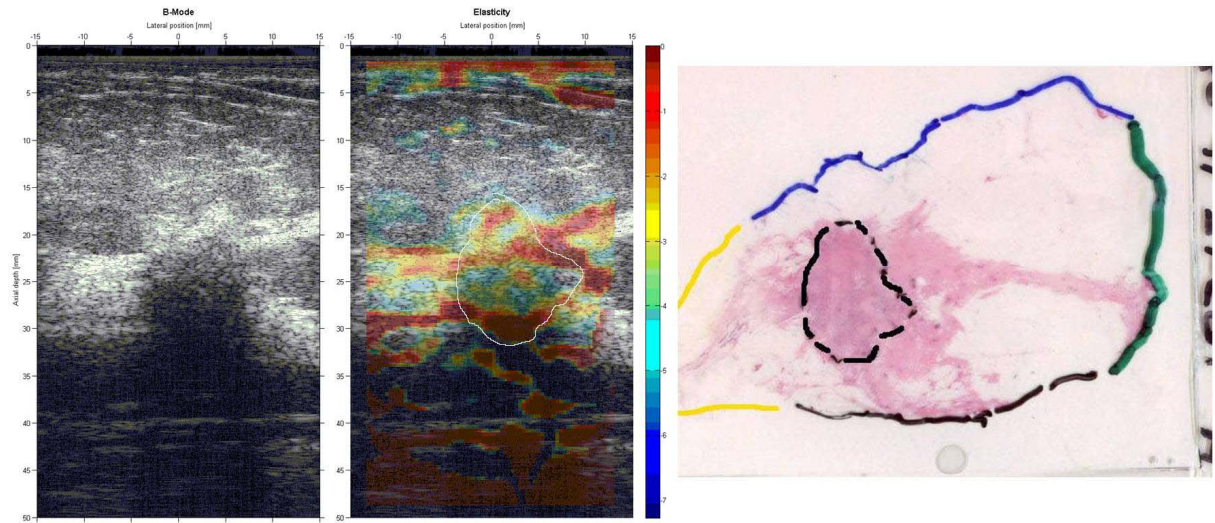
P218



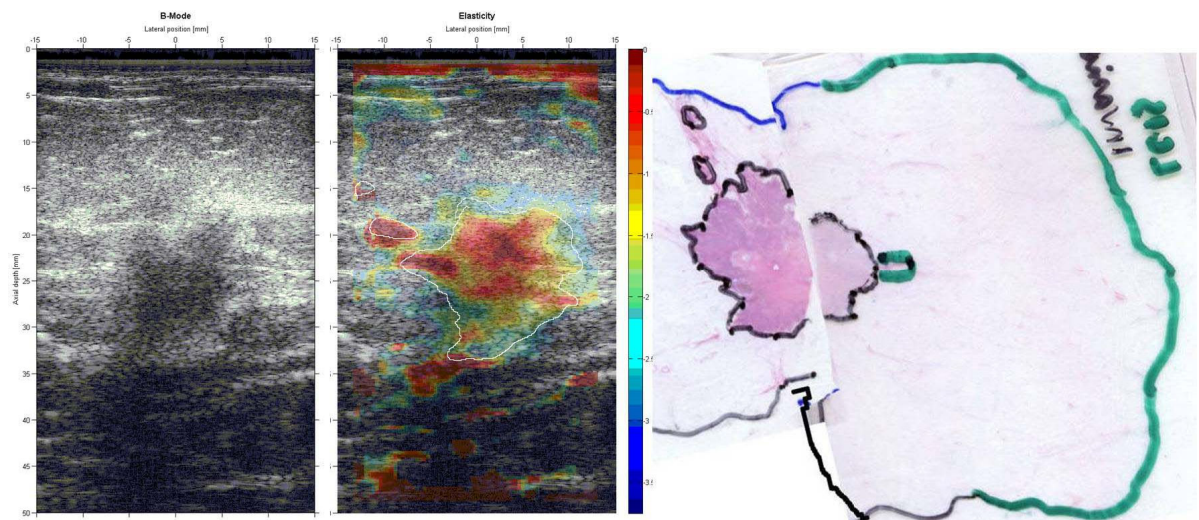
P239



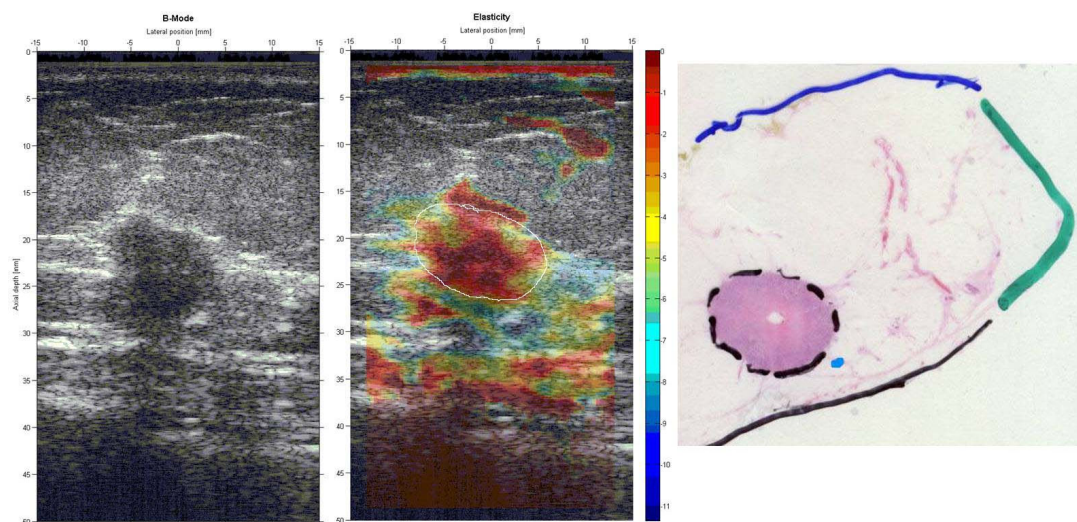
P244



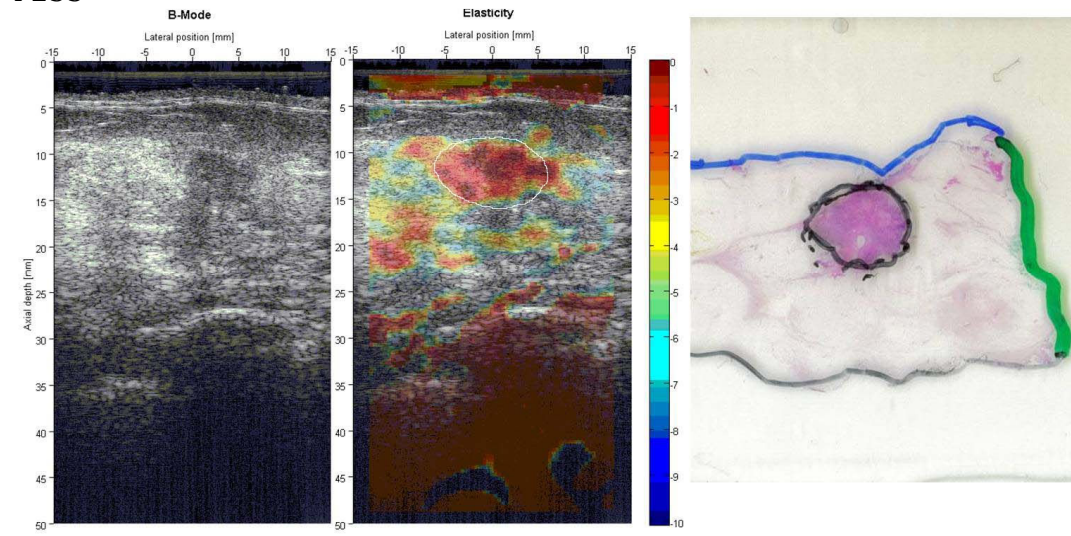
P246



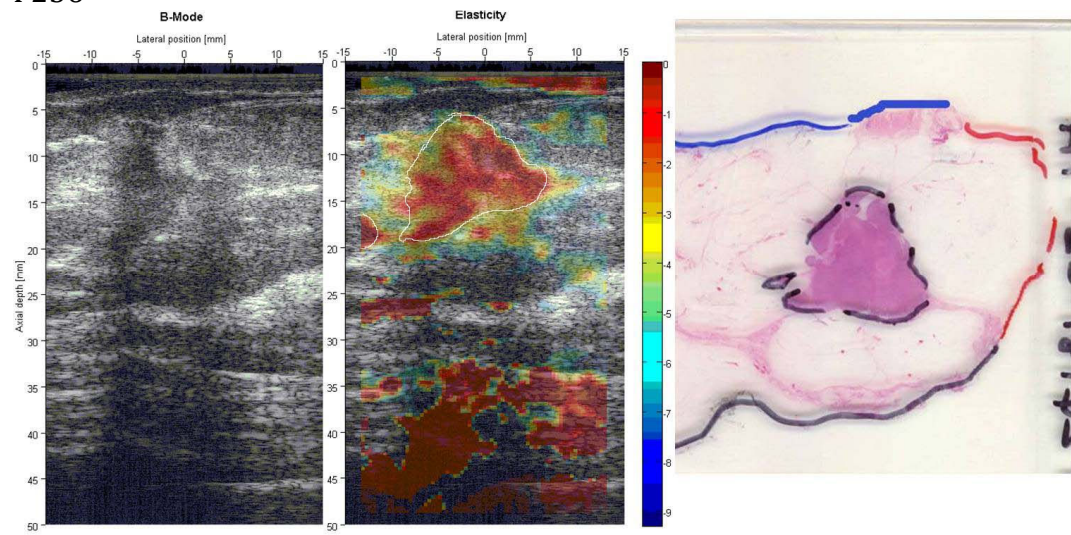
P253



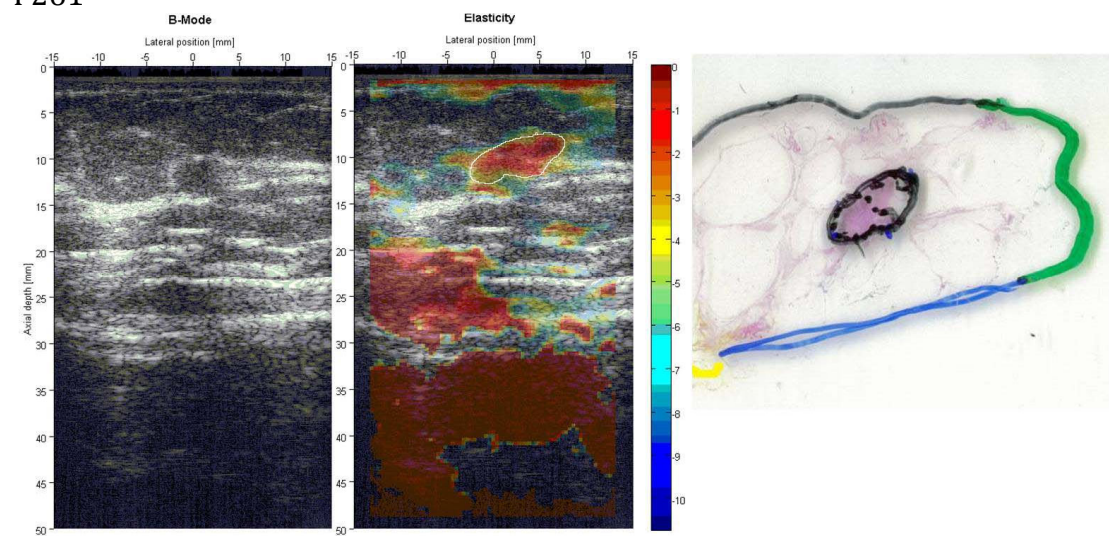
P255



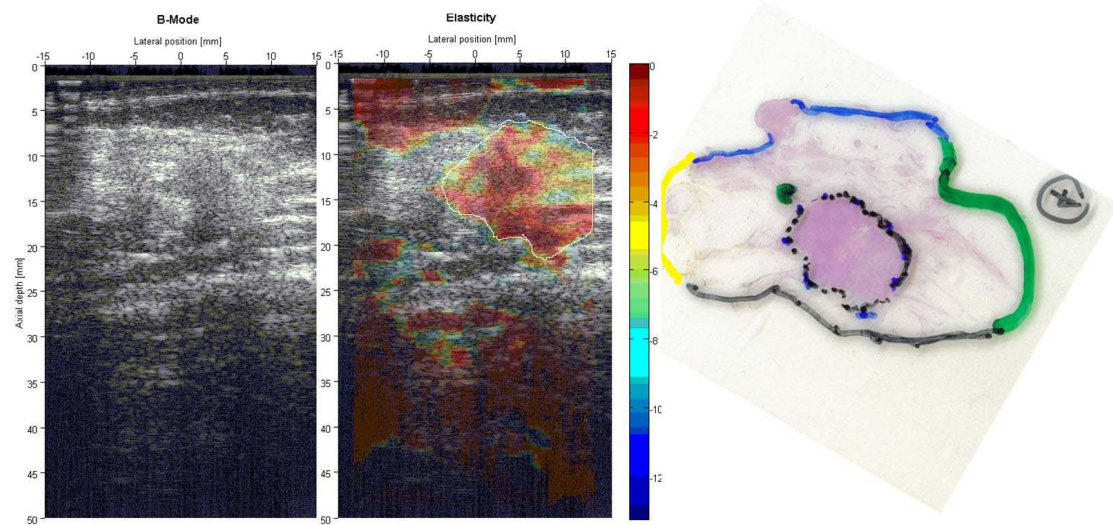
P256



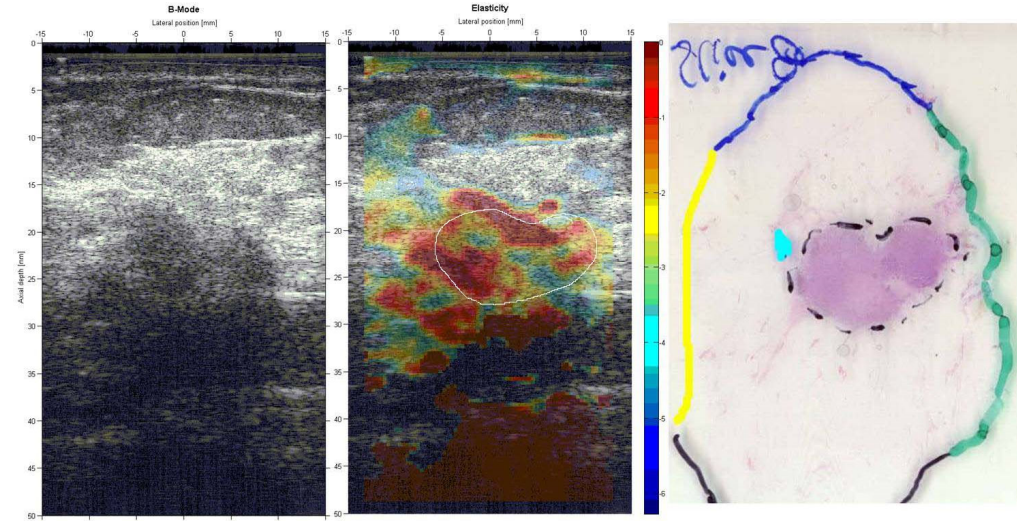
P261



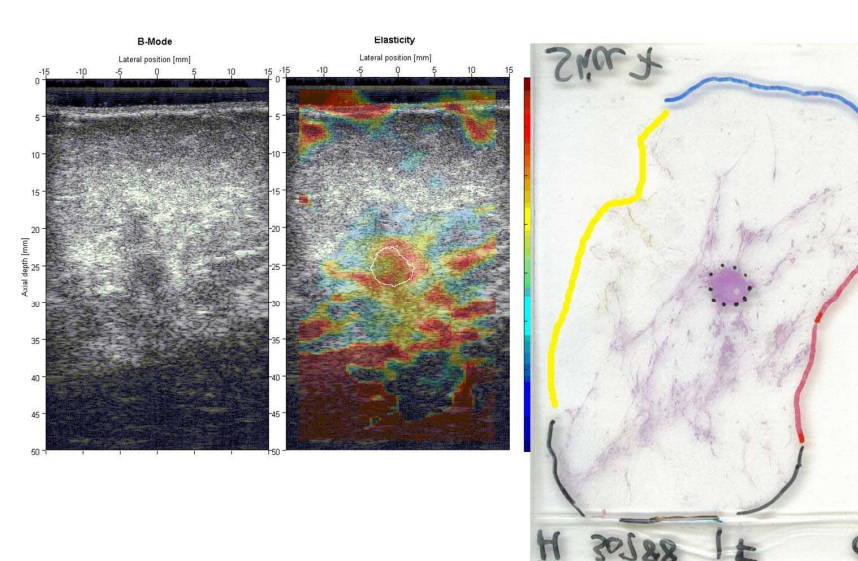
P263



P265



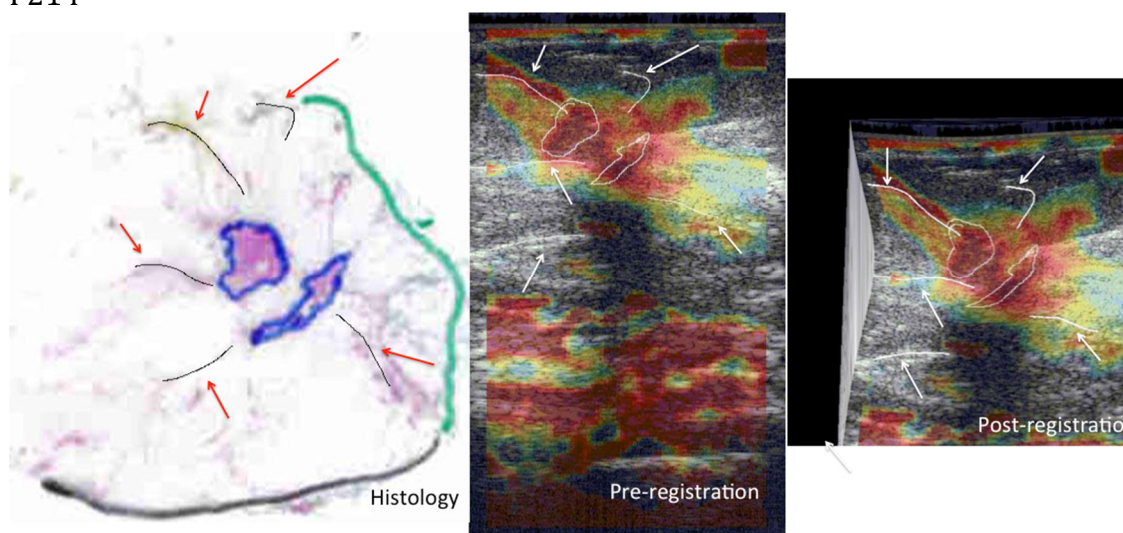
P266



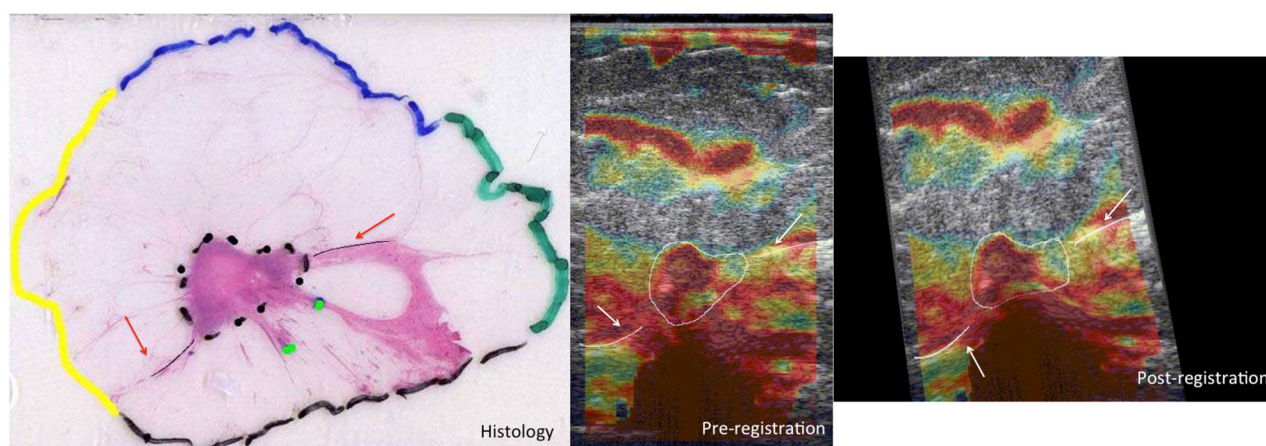
Appendix C

This section shows the features that were selected for the validation of registration in chapter 5 (section 5.3.2). In the images, the histology is given on the left, the pre-registered elastography image is given in the middle and the post-registered elastography image is given on the right. The corresponding features are marked in black (pointed by the red arrows) in the histology images and marked white in the elastography images (pointed by the white arrows).

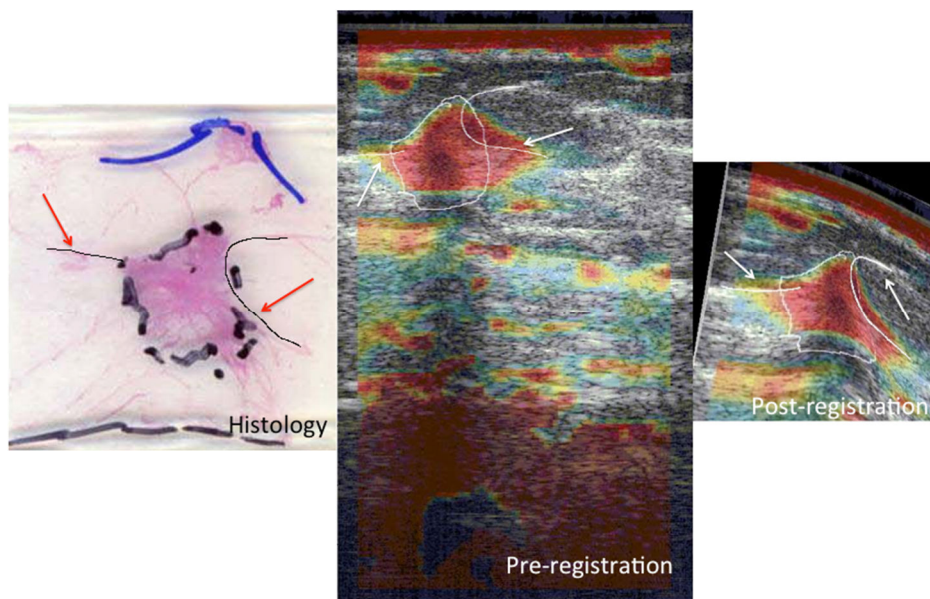
P214



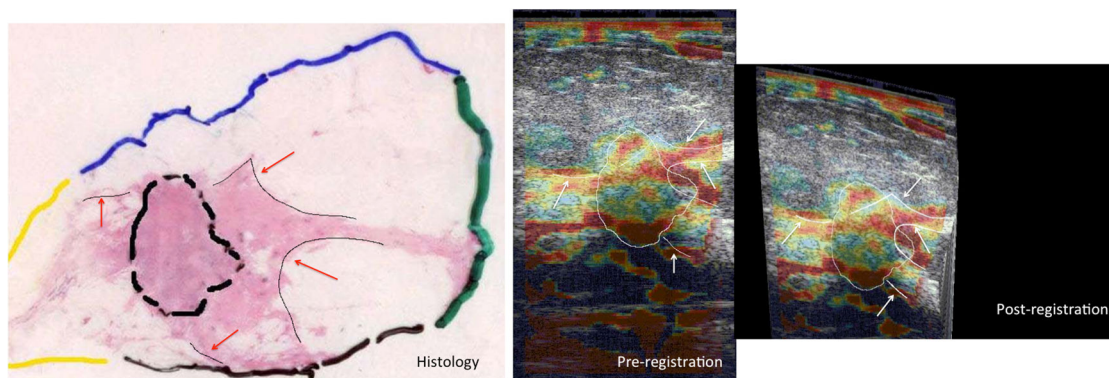
P218



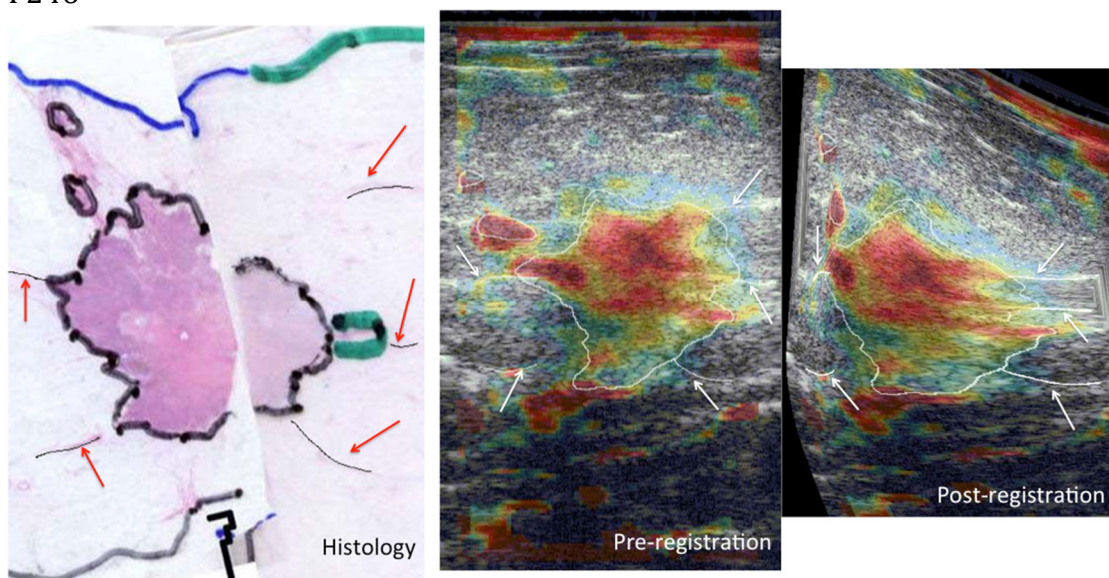
P239



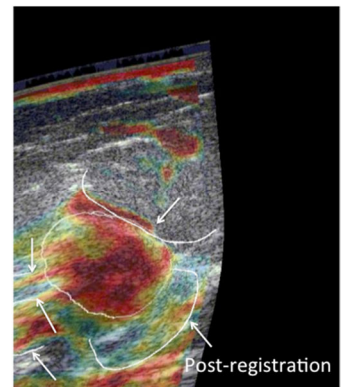
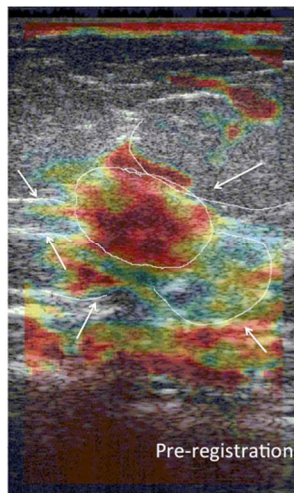
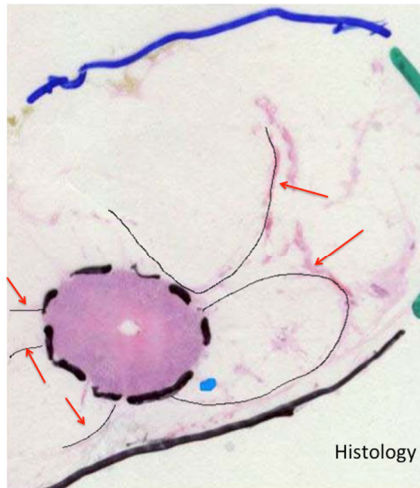
P244



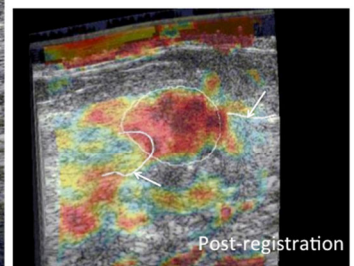
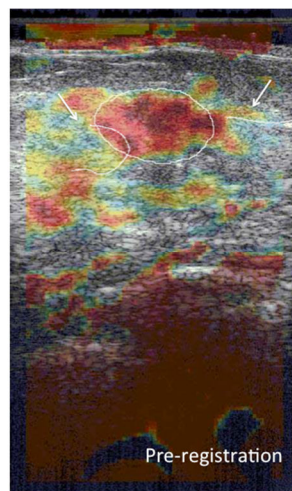
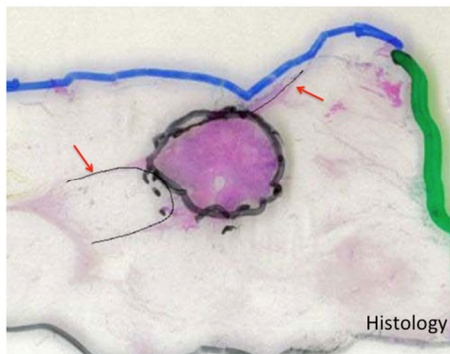
P246



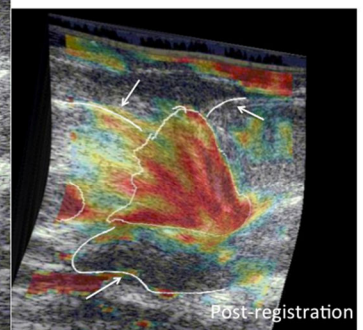
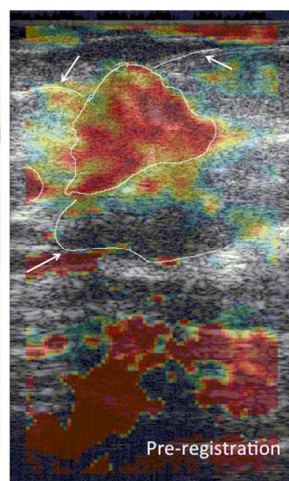
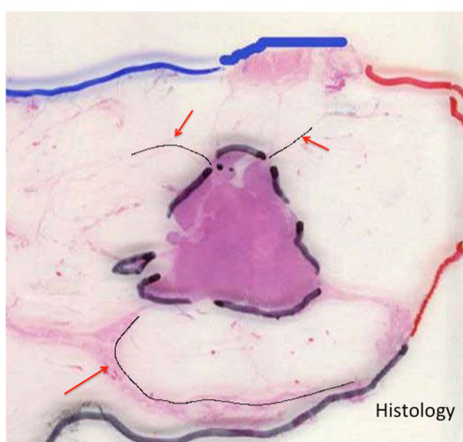
P253



P255



P256



P261

