In Vivo Detection and Thermal Treatment Monitoring of Breast Tumors Using Harmonic Motion Imaging (HMI)

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ABSTRACT

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Palpation is a standard screening procedure for the detection of several superficial cancers including breast, thyroid, prostate, and liver tumors through both self and clinical examination. This is because solid masses typically have distinct stiffnesses compared to their surrounding normal tissue. Conventional imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and mammography can provide morphological characteristics; however, these modalities do not provide the information elicited upon palpation. Accurate depiction of tissue elasticity for accurately and efficiently detecting tumors is therefore needed.

The work presented in this dissertation investigates a novel elasticity imaging technique, namely Harmonic Motion Imaging (HMI). HMI is an ultrasound based technique for tumor detection and classification. It uses a focused ultrasound (FUS) transducer to generate an oscillatory acoustic radiation force for an internal,
non-contact palpation to internally estimate relative tissue hardness. HMI is unique among other elasticity imaging techniques in the sense that it provides a spatially localized distribution of an oscillatory acoustic radiation force that can be applied across a wide range of frequencies. The dynamic tissue response at the interrogated region can be related to the underlying tissue mechanical properties, which may differentiate healthy from diseased tissue.

The mechanical response of soft tissue to the oscillatory acoustic radiation force is investigated using a finite-element model (FEM) of elastic, homogenous gels and stiff inclusion gels. The HMI experiments in gelatin and polyacrylamide tissue-mimicking gels were performed to validate mechanical responses in FEM. FEM and gel HMI studies demonstrate that the tissue dynamics, in response to an oscillatory force occur at the same frequency, and that the size and stiffness of the inclusions affect the dynamic tissue response. HMI has also been demonstrated to accurately map 17 post-surgical breast specimens (i.e., normal, benign, and malignant tissues) and reliably differentiate benign from malignant human breast tumors \( p = 0.008 \) based on their distinct oscillatory displacements.

Since HMI uses a highly focused beam, it can be easily integrated with ultrasound thermal therapy for the monitoring of the latter, aimed at regionally and thermally treating the detected tumors. The resulting integration of imaging and therapy into the HMI for Focused Ultrasound (HMIFU) system can be used as
an image guidance tool for visualization of the targeted tissue (e.g. tumor), and generation and monitoring of the relative tissue stiffness changes during heating through the successful detection of the ablation onset. HMIFU is applied in 7 in vitro and 5 ex vivo liver tissues as well as 11 transgenic mice of breast cancer in vivo. The results show that the HMIFU system could follow the tissue stiffness change during heating and indicate the onset of coagulation necrosis so that the treatment procedure can be performed in a time-efficient manner.

In conclusion, the dissertation study presented herein demonstrates that HMI can combine imaging and therapeutics for cancer treatment into a single, all-ultrasound, fully-integrated system. HMI may thus constitute a non-ionizing, a cost-efficient, and a reliable alternative method for detection of tumor and real-time monitoring of tumor thermal treatment.
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For my fiancé, Christopher Ganis, who has offered me unconditional love, guidance and encouragement throughout my doctoral research.
Chapter 1

INTRODUCTION

Breast cancer is the second leading cause of cancer death in women and is the most common cancer among women. According to the World Health Organization, more than 1 million women will be diagnosed with breast cancer each year worldwide and over 500,000 will die from the disease \(^1\). In the United States, breast cancer is the most common cancer and the second most common cause of cancer death (after lung cancer). The Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute estimated that 192,370 women will be diagnosed with and 40,170 women will die of breast cancer in 2009 \(^2\).

The statistical values are estimated based on population averages. The risk of each woman to have breast cancer can be higher or lower, depending upon several factors, including family history, genetics, age of menstruation, and other
factors that have not yet been identified. While breast cancer is less common at a young age (i.e., in their thirties), younger women tend to have more aggressive breast cancers than older women, which may explain why survival rates are lower among younger women. The vast majority of breast lumps found by self-examination are determined to be non-cancerous (benign). Early diagnosis and treatment would reduce breast cancer-related mortality.

Mammography, sonography and MRI are currently the most sensitive modalities for detecting breast tumors. There are still many women who have to undergo biopsy due to suspicious masses, and 70% to 90% of the breast biopsies performed were actually benign. Unnecessary biopsies may lead to patient discomfort, anxiety, risk of infection, and additional medical expenses. Therefore, there is a need for the development of additional reliable methods to complement the existing diagnostic procedures.

Elasticity imaging has been developed to estimate tissue mechanical response and properties, i.e., displacement, stiffness or viscoelastic parameters that could help differentiate benign from malignant. Tissue deformations can be induced externally, such as elastography, magnetic resonance elastography (MRE), transient elastography (TE), or internally using an acoustic radiation force, such as acoustic radiation force impulse (ARFI), supersonic shear imaging (SSI)
shear wave elasticity imaging (SWEI) and Ultrasound-Stimulated Vibro-Acoustography (USVA), have been evaluated to characterize tissue mechanical properties, and thus indicate tissue pathology.

In this dissertation, an acoustic radiation-force-based technique, known as Harmonic Motion Imaging (HMI) is described. HMI can provide tissue mechanical properties for tumor characterization and thermal tumor treatment. Developing this new approach may significantly improve the ability to detect breast tumors and may also provide data that is needed to better understand and improve diagnosis.

The objective of this study is to optimize and assess the performance of HMI, which 1) is entirely noninvasive (non-contact), 2) is simple to implement, 3) offers real-time monitoring of thermal treatment, and 4) provides quantitative measurement of viscoelastic parameters.

In Chapter 5, a theoretical framework using a finite-element model (FEM) with tissue-mimicking gel validation in order to assess the sensitivity and specificity of the performance of the HMI technique is presented. The theoretical framework consists of 1) an established three-dimensional (3D) (axisymmetric), FEM of gels with spherical inclusions using Comsol Multiphysics finite-element software (Comsol Inc., Burlington, MA, USA), 2) an acoustic pressure field simulation
(FIELD II) of a focused ultrasound (FUS) transducer, 3) a 2D ultrasonic image formation model, and 4) a 1D axial displacement estimation method (Chapter 4 details the motion estimation). First, the resulting tissue motion in simulations was compared to experiments. Second, the HMI displacements were compared to the Young’s moduli. The Young’s moduli of the tissue-mimicking gels were separately measured using mechanical testing (dynamic indentation test). Hence, this aims at evaluating the obtained displacement in relation to stiffness qualitatively.

A FEM study to validate the quantitative measurement of viscoelastic parameters using HMI has been performed in our laboratory. A series of HMI experiments has been validated in tissue-mimicking gels and rheometry. The preliminary results from a normal and a malignant breast specimen are presented in Chapter 6.

The HMI is used to image 17 post-surgical breast specimens, i.e., 3 normal, 5 benign and 9 malignant tissues, against histology findings in Chapter 6. The accuracy of HMI displacement images within several locations in the tumors was evaluated. An unpaired Student’s t-test was performed to quantify whether different pathologies could be differentiated based on the HMI displacement. The HMI method is proven to be capable to 1) qualitatively differentiate pathological
from normal tissue based on stiffness and 2) quantitatively measure the
viscoelastic behavior of soft tissue induced by HMI.

Currently, minimally invasive image-guided tumor ablation techniques
include cryoablation, radio-frequency (RF) ablation, laser ablation, and focused
ultrasound (FUS) ablation \(^{23,24}\). However, FUS ablation is the only non-invasive
procedure because it requires no probe insertion and employs acoustic energy to
coagulate tissue. Various imaging modalities, mainly in the field of ultrasound
(US) and magnetic resonance imaging (MRI), are used to guide and monitor the
therapeutic procedure and assess treatment response.

The Harmonic Motion Imaging for Focused Ultrasound (HMIFU) technique
for real-time monitoring and thermal treatment is demonstrated in 7 \textit{in vitro}
bovine liver and 5 \textit{ex vivo} porcine liver specimens (Chapter 7) \(^{25}\). The temperature
rise is recorded during heating and coagulation. Displacement images of the
relative mechanical properties can be followed throughout the heating process and
are used to determine the optimal parameters, such as the vibration frequency,
force amplitude and treatment time. Histopathology is used to confirm the extent
of the lesion size and location.

The HMIFU technique is then used to monitor the thermal treatment in a
transgenic breast cancer murine model \textit{in vivo} (Chapter 8) \(^{26,27}\). The FUS transducer
is used to ablate the tumor. HMI images before and after ablation (from 11 mice) are compared to histopathology results to assess whether HMI measurements correctly detect, characterize and depict the ablated tumor margins, i.e., the tumor cell necrosis within the treated region.

In conclusion, HMIFU offers the unique capability of detecting the presence of tumors, generating and simultaneously monitoring lesion formation during (and after) thermal ablation without interrupting the treatment of the targeted tumors with a precise and optimal treatment time (thermal dose) and controlled lesion size. Last, but not least, since HMIFU is an all-ultrasound-based technique, it is less costly and more portable (compared to MRI), non-ionizing (unlike mammography) and simple to implement in conjunction with other imaging or therapy modalities.
Chapter 2

BREAST DIAGNOSIS AND TREATMENT

2.1 Introduction

The female breasts or mammary glands are accessory organs of the female reproductive system. The adult female breast is normally hemispherical in shape and protrudes from the anterior chest wall overlaying the second to sixth ribs bilaterally (Figure 2.1).

Although breast tissue is mostly composed of fat and connective tissue, the breast also consists of milk ducts, blood vessels, lymph node, lobes and lobules. Each breast is made of 12 to 20 lobules that branch out from the nipple as seen in Figure 2.1. The nipple is in the center of a dark area of skin called ‘areola’. Each lobule contains groups of tiny mammary glands that can produce milk. The lobules are connected by a network of thin tubes (ducts). The spaces around the
lobules and ducts are ligaments, fatty tissue and connective tissue (stroma) that determine breast size, shape and composition. Oxygen and nutrients could travel to the breast through the blood in the arteries and capillaries. Breast also contains lymph vessel. The lymphatic system is a network of blood vessels, lymph nodes and lymph ducts that helps fight infection, trap bacteria and other harmful substances.

Figure 2.1: Breast profile adapted from www.breastcancer.org (A) ducts, (B) lobules, (C) dilated section of duct to hold milk, (D) nipple, (E) fat, (F) pectoralis major muscle, (G) chest wall/rib cage (2nd to 6th).

Enlargement: (A) normal duct cells, (B) basement membrane, and (C) lumen (center of duct).
Cancer begins at the cellular level. A few cells, for reasons not yet understood, start to accumulate genetic errors that cause them to grow abnormally. From a medical perspective, tumors are classified into two important groups, benign and malignant. These two groups exhibit different behavior with regard to their rate and manner of proliferation.

2.2 Benign

Benign tumors are not cancer. Benign tumor cells are usually uniform and well-defined. They grow slowly within one location, and remain localized, neither invading the adjacent tissue nor growing into secondary tumors. Benign tumor cells are condensed to form a fibrous capsule around the tumor and the surrounding tissue is more resistant. There are several types of benign tumors, and only the most commonly diagnosed are described below:

Cyst

Breast cysts result from a duct obstruction and localized fluid accumulation. They are common and occur in between 20% and 50% of women, usually in premenopausal women aged 40 to 50 years.
Fibrocystic disease

Fibrocystic disease relates to hormonally stimulated mammary glands and ducts that create fibrous lumps, i.e., a small or multiple cysts (fluid-filled sacs)\(^28\). The fibrocystic disease may occur in the breast as a result of age-related degeneration of glandular breast tissue and perimenopausal hormonal imbalances. This disease occurs in approximately 50% to 60% of women\(^30\).

Intraductal Papilloma

Intraductal papilloma is a benign tumor that grows in the lining of the mammary ducts\(^30\).

Fibroadenoma

Fibroadenoma is a solid lump of fibrous and glandular tissue. It is the most common benign solid breast tumor and the most commonly diagnosed breast tumor in women between the ages of 18 and 35\(^30\).

2.3 Malignant

Malignant tumors tend to grow rapidly, their shape and size varies, and they invade the nearby tissue, spreading to other parts of the body where secondary
tumors may develop. This spread of cancer is termed as *metastasis*. The type of malignant breast conditions includes:

**Ductal carcinoma in situ (DCIS)**

Ductal carcinoma in situ (DCIS) entails abnormal cells (cancer cells) found inside the lining of a duct but not yet spread into the surrounding breast tissue (in situ or non-invasive) as seen in Figure 2.2. DCIS is the most common non-invasive breast malignancy. DCIS is treated aggressively because there is a risk of developing invasive breast cancer.

![Figure 2.2: Normal breast with non-invasive ductal carcinoma in situ (DCIS) adapted from www.breastcancer.org. Breast profile: (A) ducts, (B) lobules, (C) dilated section of duct to hold milk, (D) nipple, (E) fat, (F) pectoralis major muscle, and (G) chest wall/rib cage. Enlargement: (A) normal duct cells, (B) ductal cancer cells, (C) basement membrane, and (D) lumen (center of duct).](image)
Lobular carcinoma in situ (LCIS)

Lobular carcinoma in situ (LCIS) denotes abnormal cells arising in the lining of mammary lobules that have not spread into surrounding breast tissue (Figure 2.3). LCIS is more commonly found in younger women and seldom becomes invasive.

Figure 2.3: Normal breast with lobular carcinoma in situ (LCIS) adapted from www.breastcancer.org. Breast profile: (A) ducts, (B) lobules, (C) dilated section of duct to hold milk, (D) nipple, (E) fat, (F) pectoralis major muscle, and (G) chest wall/rib cage.

Enlargement: (A) normal lobular cells, (B) lobular cancer cells, and (C) basement membrane.
Invasive ductal carcinoma (IDC)

Invasive or infiltrating ductal carcinoma (IDC) indicates the abnormal cells (cancer cells) that have spread outside the ducts and start to invade nearby breast tissue as seen in Figure 2.4. Cancer cells may also have spread to lymph nodes within the breast area or other areas of the body. Approximately 75% of the invasive breast cancer is diagnosed as IDC.

Figure 2.4: Normal breast with invasive ductal carcinoma (IDC) adapted from www.breastcancer.org. Breast profile: (A) ducts, (B) lobules, (C) dilated section of duct to hold milk, (D) nipple, (E) fat, (F) pectoralis major muscle, and (G) chest wall/rib cage.

Enlargement: (A) normal duct cells, (B) ductal cancer cells breaking through the basement membrane, and (C) basement membrane. 30
Invasive lobular carcinoma (ILC)

Invasive or infiltrating lobular carcinoma (ILC) is the second most common type of invasive breast cancer, accounting for 12% to 15% of breast cancer in women. In this case, the cancer cells have spread outside the lobules and to nearby breast tissues (Figure 2.5). ILC can be difficult to diagnose because the tumor does not generally form a solid mass.

Figure 2.5: Normal breast with invasive lobular carcinoma (ILC) adapted from www.breastcancer.org. Breast profile: (A) ducts, (B) lobules, (C) dilated section of duct to hold milk, (D) nipple, (E) fat, (F) pectoralis major muscle, and (G) chest wall/rib cage.

Enlargement: (A) normal cells, (B) lobular cancer cells breaking through the basement membrane, and (C) basement membrane.
2.4 Breast cancer detection

Breast self-examination is recommended by physicians, because it may help identify breast diseases. Breast exam was once thought essential for early breast cancer detection; however, now it is considered optional. Breast cancer typically shows no symptoms when the tumor size is small. Therefore, finding a breast abnormality early (or, in a small size) increases the chances for a cure, if the mass is determined to be malignant. A smaller tumor may help the patient to have less invasive surgical procedure and other treatment options, compared with a tumor when it is found in larger size\(^3\).

However, breast self-examination or clinical breast examination may not be sufficient to detect tumor in breast tissue. American Cancer Society guidelines recommend, depending on a woman’s age, annual screening using mammography and MRI for women at high lifetime risk of the disease. Currently, imaging modalities used for tumor detection in breast are mammography, MRI and sonography\(^3\). The following sections will discuss each imaging modality, their advantages and disadvantages.
2.4.1 Mammography

Mammography uses a low-dose X-ray that allows visualization of the internal structure of the breast. Mammography has a sensitivity range of 68% to 77% and specificity from 82% to 98% \(^{32}\). Mammography does not detect a small percentage of breast cancer, typically in women with high breast density or inadequate positioning of the breast.

2.4.2 MRI

Magnetic Resonance Imaging (MRI) has a higher sensitivity as compared to mammography, and could identify invasive breast cancer in high-risk patients \(^{33}\). Breast MRI could provide information about the tissue vascularity that is not available in mammography. The injection of an intravenous contrast agent (Gadolinium) could enhance the neovascularity, i.e., abnormal or excessive formation of blood vessels, in tumor. However, MRI is costly and prohibited for patients with pacemakers, aneurysm clips, or claustrophobia.
2.4.3 Sonography

Sonography has a higher sensitivity in women with dense breasts (contrary to mammography). Sonography has a sensitivity from 65.9% to 74.6% and a specificity from 80% to 96%. The ultrasound system is portable device, therefore, easily accessible. It is readily available and the cost of the system is lower compared to MRI. Ultrasound imaging is a non-ionizing technique (in contrast to mammography). However, ultrasound imaging is typically operator-dependent, in other words, a breast ultrasound examination is dependent upon the knowledge and skill of the person performing the scan \(^{28}\).

2.5 Breast cancer treatment

Mastectomy is a surgery procedure to remove the entire breast tissue with or without the incision of the pectoral muscle in order to treat breast cancer. This treatment has increased survival rate in women with breast cancer. Breast-conserving surgery, or lumpectomy, is a surgery to remove only the tumor and peripheral edges around the tumor. Based on a 20-year follow-up study of different breast cancer treatment (i.e., mastectomy, lumpectomy, and lumpectomy
with radiotherapy), Fisher et al. 34 demonstrated that lumpectomy combined with radiotherapy is as equally effective as total mastectomy. This further facilitated the implementation of lumpectomy in clinical practice. Although lumpectomy has a relatively low morbidity rate, other complications such as bleeding (2% to 10%) and infections (1% to 20%) could occur 23. In the following sections, currently available clinical treatments for breast cancer are described.

2.5.1 Systemic therapy

Systemic therapy uses substances, e.g., anti-cancer drugs, that are injected into a vein or taken orally 3. These substances travel through the bloodstream, reaching and affecting cells all over the body. Systemic therapy includes biologic therapy, chemotherapy, and hormone therapy. Some patients may be treated by systemic therapy before surgery (i.e., neoadjuvant therapy) in order to shrink the tumor 35. Thus, less extensive surgery (e.g., breast-conserving surgery) can be performed instead of mastectomy. Neoadjuvant therapy has been found to be as effective as therapy given after surgery in terms of survival rate, disease progression, and distant recurrence 35.
Adjuvant therapy is given to patients after surgery in order to destroy any undetected cancer cells that may have migrated to nearby organs or other parts of the body. Physicians decide whether or not to perform adjuvant therapy based on tumor size, biopsy, histology, and the presence of cancer in axillary nodes. Systemic therapy may be used in treating patient with metastasis breast cancer.

2.5.2 Chemotherapy

Chemotherapy is another clinically-used breast cancer treatment method that requires oral intake or intravenous injection of a drug. These drugs can kill cancer cells, but may also damage normal cells leading to several patient side-effects, such as fatigue, nausea and vomiting, loss of appetite, hair loss, mouth sores, changes in menstrual cycle, etc. Permanent side-effects can include early menopause and infertility. The total treatment cycle usually lasts for 3 to 6 months and is typically administered to patients with metastatic breast cancer. Chemotherapy has also been shown effective when administered prior to lumpectomy or mastectomy in order to minimize the possibility of metastatic responses of certain tumors as a result of resection.
2.5.3 Radiation therapy

Radiation therapy is a clinically-used breast cancer treatment method that applies high-energy X-rays to kill cancer cells. The radiation beam can be applied externally or internally (using radioactive seeds). Treatment is usually administered five days a week over a period of about 6 or 7 weeks. Each treatment lasts only a few minutes and is usually painless. Common side-effects of radiation therapy are swelling and heaviness in the breast, skin burns in the treated area and fatigue.

Technology advances over the last decade have increased interest in less invasive treatment of patients with localized breast cancer. Currently available minimally invasive image-guided tumor ablation techniques include cryoablation, laser ablation, radio-frequency (RF) ablation, and high-intensity focused ultrasound (HIFU) or, focused ultrasound (FUS) ablation are discussed below.

2.5.4 Cryoablation

Cryoablation is the process of using freezing temperatures to destroy cancer cells. A cryoprobe circulating liquid nitrogen is inserted into the tumor under
ultrasound guidance, causing the cells to freeze. The tumor is frozen, thawed, and refrozen until the tumor cells are completely destroyed.

2.5.5 Laser ablation

Interstitial laser thermotherapy is a minimally invasive technique for treating small cancer. Needle probes are placed percutaneously under ultrasound or magnetic-resonance imaging (MRI) guidance, to deliver laser energy into a tumor to slowly heat and destroy the tumor cells.

2.5.6 RF ablation

Radio-frequency (RF) ablation is a rapidly emerging technology as a minimally invasive alternative to lumpectomy. For this treatment, a needle-like or star-burst electrode is injected around the tumor tissue and the tip of the electrode is positioned at the center of the tumor. The tip causes frictional heat generated by intracellular ions moving in response to an alternating current. The electrode is connected to a function generator and the electrical current flows and raises the local temperature up to 95°C maintaining it for about 15 minutes. Since the first
clinical feasibility study in 1999\textsuperscript{38}, several clinical studies have followed indicating that the treated tissue shows that malignant cells can be completely destroyed by RF ablation\textsuperscript{39-43}.

2.5.7 HIFU or FUS ablation

High-Intensity Focused Ultrasound (HIFU) or Focused ultrasound (FUS) ablation is a non-invasive procedure and uses a high level of acoustic energy that is converted to heat in order to coagulate tissue (thermal lesions), while the surrounding tissues remain relatively unheated\textsuperscript{44}. The rapid temperature rise at the transducer focus that causes tissue coagulation is at least 20°C and results in immediate protein denaturation and coagulative necrosis. The extent of the tissue damaged is determined both by the temperature elevation and the treatment time. The treatment time for a breast tumor with several cm in diameter is rather long and ranges from 45 minutes to 2.5 hours\textsuperscript{23}. FUS ablation constitutes a promising method for non-invasive treatment of benign or malignant breast tumors. Currently, image guidance modalities for FUS ablation are mainly in the field of MRI and ultrasound.
2.6 Summary

In this chapter the breast anatomy, types of breast tumors, imaging modalities for early detection of tumors, and tumor treatment were briefly presented. If the breast tumor is detected early, minimally invasive tumor treatment could be performed and thus improve the survival rate.

Minimally invasive or non-invasive treatment, such as cryoablation, laser ablation, RF ablation, and FUS ablation, are alternative methods to breast-conserving surgery. These treatments could efficiently and completely destroy the tumor locally; however, they require accurate visual localization of the tumor within the breast. HMI, as an elasticity imaging method to monitor thermal ablation (FUS ablation), could offer a promising method for local tumor treatment. The application of HMI in vitro and ex vivo, as well as in vivo will be presented in Chapters 7 and 8, respectively.
Chapter 3

TISSUE ELASTICITY IMAGING

3.1 Introduction

Palpation is a standard screening procedure for the detection of tissue abnormalities. It is performed based on the assumption that diseased tissue is different in its geometry and stiffness relative to adjacent tissues. However, palpated diseased tissue may be undetected by X-ray, conventional ultrasound imaging, or Magnetic Resonance Imaging (MRI). This is because most of available imaging modalities do not provide the information elicited by palpation.

Elasticity imaging has been developed to estimate tissue mechanical properties from various forms of tissue perturbation for the detection of stiffer masses. Tissue deformations induced by an externally applied mechanical source, or an internal source using an ultrasound stimulus, have been evaluated to characterize tissue
elasticity. In this chapter, the basic theory of elasticity is discussed, followed by a literature review on different perturbation methods, i.e., static and dynamic.

3.2 Theory of elasticity

3.2.1 Linear elastic

Let us consider a square-shaped element of a homogeneous, isotropic material that is compressed with a load ($\sigma$) within a cross-sectional area ($A$) as seen in Figure 3.1. The material is then deformed and its height changes from $L$ to $L - \Delta L$ (Figure 3.1).

![Figure 3.1: Illustration of the applied load ($\sigma$) on a square material within a cross-sectional area ($A$). The height of the material changes from $L$ to $L - \Delta L$.](image)
In order to represent the material behavior independently of its size, we define the strain \((\varepsilon)\) as a dimensionless ratio of elongation with respect to the original length, i.e., \(\varepsilon = \Delta L/L\).

Thus, in that case, under the assumption of linear elasticity, the applied stress \((\sigma)\) and corresponding strain \((\varepsilon)\) are related by \(\sigma = E\varepsilon\), where \(E\) is the Young’s modulus, a parameter that describes the elastic behavior of the material. This was a 1D example of a uniaxial deformation.

In a continuous 3D medium, the linear elasticity is derived from Hooke’s Law, under the assumption that the medium is homogenous, elastic, isotropic, and subject to small deformations. Hooke’s Law, in this case, in terms of the Lamé coefficients \((\lambda\) and \(\mu)\) is written as

\[
\sigma_{ij} = \lambda \delta_{ij} \varepsilon_{kk} + 2\mu \varepsilon_{ij}
\] (3.1)

where \(\varepsilon_{ij}\) denotes the strain \((i, j = 1, 2, 3)\) and \(\varepsilon_{kk} = \varepsilon_{11} + \varepsilon_{22} + \varepsilon_{33}\), \(\alpha_{ij}\) is the stress \((i, j = 1, 2, 3)\), and \(\delta_{ij}\) is the Kronecker delta \((\delta = 1 \text{ for } i = j \text{ and } \delta = 0 \text{ for } i \neq j)\). This equation represents tissue elastic behavior. Eq. 3.1 can be inverted to express the strain \((\varepsilon)\) in terms of stress \((\sigma)\) as

\[
\varepsilon_{ij} = \frac{-\lambda}{2\mu(3\lambda + 2\mu)} \delta_{ij} \sigma_{kk} + \frac{1}{2\mu} \sigma_{ij}.
\] (3.2)

where \(\sigma_{ij}\) is stress \((i, j = 1, 2, 3)\) and \(\sigma_{kk} = \sigma_{11} + \sigma_{22} + \sigma_{33}\).
For a simple uniaxial stress in the x₁ direction as seen in Figure 3.1, a different set of elastic parameters, namely, Young’s modulus (E) and Poisson’s ratio (ν), can be introduced so that the strain (ε) can be written as

\[ \varepsilon_{ij} = \frac{1+\nu}{E} \sigma_{ij} - \frac{\nu}{E} \delta_{ij} \sigma_{kk} \]  

(3.3)

where the relationships between E and ν to λ and μ are

\[ \lambda = \frac{E\nu}{(1+\nu)(1-2\nu)} \quad \text{and} \quad \mu = \frac{E}{2(1+\nu)}. \]  

(3.4)

Soft tissue is assumed to be nearly incompressible, i.e., with a Poisson’s ratio (ν) of 0.5. In that case, the shear modulus (μ) (or, commonly written as G) can be written as

\[ \mu = \frac{E}{2(1+\nu)} = \frac{E}{3}, \quad \text{or} \quad E = 3G, \]  

(3.5)

thus, the Young’s modulus (E) is linearly proportional to the shear modulus (G).

This relationship indicates that for soft tissue, under the linearity, isotropy, and incompressibility assumptions, the same information can be inferred from both compressive and shearing mechanical excitations.
3.3 Static methods

In static elasticity imaging, a slow rate of compressive stress is imposed on tissue (similar to uniaxial stress illustrated in Figure 3.1), and the resulting tissue response is monitored using an imaging system. This response is related to the tissue mechanical properties and boundary conditions.

Ophir et al. \(^8,45\) developed the method of elastography that applied a small external static compression (on the order of 1%) and used cross-correlation techniques on radio-frequency (RF) signals in order to estimate tissue strains resulting from the external compression. This method has been proven to produce good quality strain images (or, elastograms) in several tissues, especially in the breast and muscle \(\text{in vivo}\) \(^46-55,56\).

The quality of strain images is mainly dependent on the precision of the estimated displacement, because the strain is calculated from the derivative of the estimated displacement. O’Donnell et al. \(^57\) and Skovoroda et al. \(^58\) proposed a method that applied multiple small compressions to the tissue, and the total applied strain level is within 5%, in order to improve the signal to noise ratio (SNR) in the displacement and strain images.
A temporal stretching method can be applied on the post-compression RF signals to reduce the strain noise by restoring coherence between the signals (i.e., pre- and post-compression) \(^{29,59,60}\). One-dimensional cross-correlation was then applied to estimate the displacement \(^{29,59,60}\). Similar to temporal stretching, Chaturvedi et al. \(^{61}\) used a 2D companding method to improve the strain images. In this method, signal scaling was applied on the pre-compression RF signals in order to improve the coherence between the data before and after compression. The scaled RF signal was then compared with the post-compression RF signals using sum of absolute differences (SAD) method. The results show that the displacements were preserved while their variances were reduced, thus the strain images were improved. Konofagou and Ophir \(^{49}\) also developed a recorrelation method for 2D strain estimation.

Similar approaches have been proposed by using Magnetic Resonance Imaging (MRI) to track displacement in gels and breast tumors \textit{in vivo} before and after a quasi static compression \(^{62}\).
3.4 Dynamic methods

In dynamic elasticity imaging, a dynamic stress is applied that can be in the form of transient or oscillatory stresses. The resulting tissue response could be estimated based on the shear wave propagation, or tissue displacement at the excited region. The response is related to tissue mechanical properties within tissues.

3.4.1 External excitation

Krouskop et al. imposed an external vibration and estimated the tissue elastic modulus by measuring the resulting Doppler shift. Parker et al. measured the tissue response to external mechanical vibrations at low frequency (100 to 300 Hz) for 'sonoelasticity imaging' or 'sonoelastography' for detection of hard lesions, as well as for the study of tissue viscoelastic properties. They estimated the amplitude and/or phase of the periodic movement of tissues by estimating the Doppler resulting shift. A theoretical framework has been shown for the detection of inhomogeneities in a vibrating medium and the application of sonoelasticity has also been applied on healthy human skeletal muscle.
femoris and biceps brachii) in vivo, liver, prostate, breast, and other organs.

The Transient Elastography (TE) method uses an ultrasound transducer mounted on a vibrating piston to produce a shear wave at low frequency (50 to 500 Hz). The ultrasound RF signals are then processed to obtain shear wave propagation by measuring its velocity that is related to the tissue stiffness.

In the MR Elastography technique (MRE), tissue mechanical properties (e.g., shear modulus) are mapped based on the observed phase shift of the MR signal in response to an external mechanical vibration. MRE provides tissue displacement maps incurred from a low-frequency shear wave induced by an external excitation. The shear wave velocity, which is measured using its wavelength, allows the direct estimation of the local mechanical properties of the tissue. Using an adequate inversion approach, MRE used the propagating shear wave to reconstruct the local shear modulus of the medium. However, modulus reconstruction using shear wave methods especially those that use external excitation are highly dependent on the boundary conditions, heterogeneity, pre-compression strain levels, out-of-plane motion assumptions, shear wave reflections, and mode conversion.
3.4.2 Internal excitation

Another approach to measuring tissue mechanical properties is the use of a radiation force to generate an internal vibration. Sugimoto et al. introduced the use of a focused ultrasound transducer to produce an impulse radiation force that caused a localized compression deep inside the tissue to evaluate localized tissue stiffness \(^86\). The tissue deformation was measured as a function of time by using a pulse-echo method.

Other research groups have also used the impulse radiation force to induce brief mechanical excitations locally and follow the resulting tissue response while RF data were collected during tissue relaxation (Acoustic Radiation Force Impulse; ARFI) \(^11,12\) or shear wave propagation, e.g., Supersonic Shear Imaging (SSI) \(^13\), and Ultrasound-Stimulated Vibro-Acoustography (USVA) \(^15,16\).

Nightingale et al. proposed an Acoustic Radiation Force Impulse (ARFI) imaging method. ARFI induces a localized radiation force and images the tissue response immediately after force application, using a modified linear array transducer \(^11\). The tissue displacements are estimated using a speckle tracking technique. Supersonic Shear Imaging (SSI) induces shear waves using an acoustic radiation force (generated by a modified 1D array transducer) at different locations.
in the tissue and images the resulting wave propagation at high frame rates (up to 5000 images/s) \(^{87}\).

The Shear Wave Elasticity Imaging (SWEI) \(^{14}\) method employs a focused ultrasound transducer and an ultrasound imaging transducer or low frequency acoustic detector. An amplitude-modulated focused beam is used to generate a time-varying acoustic radiation force. The modulation frequency is typically on the order of few kHz. The shear wave resulting from the radiation force is detected by an ultrasound imaging transducer or a surface detector which is used to characterize the viscoelastic properties of the targeted medium.

In SDUV \(^{88}\), a burst signal was used to drive a focused transducer in order to generate a vibration, and the resulting oscillation was tracked by a separate ultrasound beam and/or laser vibrometer. The phase of the shear wave between two different locations is used to calculate the shear wave propagation speed at different frequencies. An inverse approach is used to estimate the tissue viscosity and elasticity.

Orescanin \textit{et al.} \(^{89}\) used a single-element focused transducer to transmit a continuous-wave burst to deform a hydrogel. Doppler pulses were used to simultaneously track the incurred motion. The aim of this technique is to estimate the viscoelastic properties of the gel.
In Ultrasound-Stimulated Vibro-Acoustography (USVA)\textsuperscript{15,90}, two confocal ultrasound transducers and a hydrophone are typically used. The interference between the beams of the two confocal ultrasound transducers at slightly different frequencies (low kHz range) causes a vibration at the focus. The amplitude and the phase of the shear wave is recorded by a hydrophone and used to form an image.

Michishita \textit{et al.}\textsuperscript{91} employed a similar technique to USVA, but a pulse-echo transducer was used instead of a hydrophone. A cross-correlation technique was used to estimate small cyclical displacements of the silicone rubber in order to locally measure its complex elastic modulus. The intended goal of this technique was to measure the complex elastic modulus of the skin. The ultrasonic transducer used low acoustic intensities (≤ 1W/cm\textsuperscript{2}) for force generation that was removed while the displacement was measured. Thus, the measurement of the silicone rubber motion was not obtained during force activation.

The Localized Harmonic Motion (LHM) technique uses a series of quasi-static excitations at a specific rate. Heikkila \textit{et al.}\textsuperscript{92-94} have developed a LHM simulation framework to test the performance of LHM for lesion detection. Two configurations were simulated involving either a 1D linear phased array transducer, or two confocal single-element transducers, for both sonication and imaging. A burst waveform with several repetition frequencies (e.g., 50, 100, and
150 Hz) was then used to induce dynamic excitation inside a medium. Their simulation findings indicated good agreement with the in vivo LHM experimental results in the rabbit muscle\textsuperscript{92,95}.

Harmonic Motion Imaging (HMI)\textsuperscript{96,97} utilized the same transducer configuration as that used for USVA but used an imaging transducer and RF tracking to acquire the echoes during oscillation of the tissue and estimate the corresponding displacements. The advantage of this technique is that it does not depend as highly on the acoustic properties during signal acquisition, as the USVA technique may be.

The Harmonic Motion Imaging (HMI) technique has two different designs; 1) using two focused ultrasound (FUS) transducers with an ultrasound imaging transducer \textsuperscript{17}, or 2) using one (FUS) transducer with an ultrasound imaging transducer \textsuperscript{18}. The imaging and the FUS transducers are confocal and concentric.

In the two-FUS-transducer configuration, the interference between the two focused beams produced an acoustic radiation force that continuously moved across the focal region (details in Chapter 4) \textsuperscript{18}. On the other hand, in the single FUS-transducer configuration, the amplitude-modulated (AM) FUS beam (on the order of Hz) generated a time-varying radiation force at the focal region (i.e., the force did not move spatially) (details in Chapter 4). The resulting oscillatory
motion at the focus was detected during the force application using an imaging transducer. The amplitude of the induced motion was estimated using cross-correlation on the acquired RF signals to estimate the resulting tissue displacement at and around the FUS focus. Since the induced motion is highly localized, the response of the tissue is mainly related to the underlying tissue mechanical properties.

HMI is a radiation-force-based technique that can probe and track tissue internally with a sinusoidal stress/force. HMI is thus suitable as a non-contact indentation method that is similar to the dynamic indentation testing for modulus measurement that, in comparison with external compression technique, does not require a large sample, medium homogeneity, or specific boundary conditions. HMI also offers a wider range of loading frequencies and depths of application. This method could measure displacement during the stress/force application, thus any changes in tissue structure throughout testing can be observed. The HMI method is capable of monitoring thermal ablation and assessing tissue stiffness changes before, during, and after treatment. The results of HMI application in thermal treatment monitoring will be presented in Chapters 7 and 8.

In this dissertation, HMI displacement is used as a qualitative measurement to estimate the relative tissue stiffness based on the induced motion. Since the
applied stress/force (FUS beam) is localized and the focal region is assumed to be homogenous, the estimated displacement at that region can be related to the tissue stiffness, i.e., Young’s modulus ($E$).

Since soft tissues typically behave as viscoelastic media, a linear elasticity approximation may not be sufficient to correctly quantify tissue mechanical properties. Viscoelastic parameters may carry additional information of the tissue structure that can be used to differentiate healthy from diseased tissues compared to elasticity alone.\textsuperscript{20,98}

### 3.5 Viscoelasticity

Let us consider a material subjected to shear deformation. In that case, we can write the relationship between shear stress ($\sigma$) and shear strain ($\varepsilon$) as $\sigma = G\varepsilon$, where $G$ is the shear modulus. If we assume that such quantities depend on time, a small change in stress ($d\sigma$) due to a change in strain ($d\varepsilon$) can be written as:\textsuperscript{99}

$$d\sigma = Gd\varepsilon$$ \hspace{1cm} (3.6)

that can be written as:

$$d\sigma = G\frac{d\varepsilon}{dt} dt \quad \text{or} \quad d\sigma = G\dot{\varepsilon} dt$$ \hspace{1cm} (3.7)
where $\dot{\varepsilon} = \frac{d\varepsilon}{dt}$ represents the strain rate.

By integrating Eq. 3.7, the stress function becomes

$$
\sigma(t) = \int_0^t G(t - t') \dot{\varepsilon}(t') \, dt'
$$

(3.8)

where $\sigma(t)$ is the shear stress and $\varepsilon(t)$ is the imposed shear strain. The time-dependent shear modulus, $G(t)$, is related to the viscoelastic properties of the material. Eq. 3.8 is the 1D constitutive model for temporal linear viscoelastic behavior (time domain approach).

Viscoelastic properties can also be defined in the frequency domain, which is more suitable for HMI technique, because we impose a sinusoidal (oscillatory) stress (i.e., one frequency component). Assuming linear viscoelasticity, the frequency-dependent shear modulus, $G(\omega)$, can be written in the frequency domain as:

$$
G(\omega) = G'(\omega) + iG''(\omega)
$$

(3.9)

where $i^2 = -1$. $G'(\omega)$ is the shear storage modulus and is related to the energy stored under deformation, i.e., it represents the elasticity of the material. $G''(\omega)$ is the shear loss modulus that is related to the energy loss under deformation, i.e., it represents the viscosity.
Our laboratory has developed a method using HMI to quantitatively measure the viscoelastic parameters using an inverse method \(^{20}\) (Appendix A). This method has been validated in gels \(^{20}\), and the preliminary results of measured viscoelastic parameters based on post-surgical breast specimens \(^{22}\) are provided in Chapter 6.

### 3.6 Summary

The focus of this dissertation is to study the feasibility of amplitude-modulated (AM) Harmonic Motion Imaging (HMI) technique (i.e., one FUS transducer configuration) for the detection and diagnosis of breast tumors, tumor thermal treatment, and tissue assessment after treatment. The theory of HMI is provided in next chapter.
Chapter 4

THE HARMONIC MOTION IMAGING (HMI)
TECHNIQUE

4.1 Introduction

In the previous chapter, the advantages of amplitude-modulated (AM) Harmonic Motion Imaging (HMI) technique were presented. The HMI technique produces an internal vibration that is spatially invariant. This internal vibration is used to interrogate the tissue and measure its mechanical properties. The HMI technique provides displacement images, or displacement maps (i.e., HMI image), of the interrogated region. These HMI images could be used as complementary information for diagnosis of diseased tissue, which is usually stiffer compared to normal tissues.
The combination of imaging and therapy using HMI into an integrated system is named Harmonic Motion Imaging for Focused Ultrasound (HMIFU). The HMIFU system is capable of providing real-time information on the tissue mechanical properties during the application of the acoustic radiation force.

This chapter details different aspects of the HMI systems that are important for a good understanding throughout this dissertation. First, the theory of HMI is given. Then, the HMI instrumentation, experimental setups, and data acquisition are presented. Lastly, the method of the resulting tissue motion estimation is provided.

4.2 Theory

An acoustic radiation force is caused by the change in momentum of the acoustic wave as it propagates through a medium. In the case of a single-element focused ultrasound (FUS) transducer, the radiation force is localized mainly in its focal region. In an attenuating homogenous medium and assuming plane wave propagation, the radiation force at the focus can be expressed as follows\(^{101-103}\)

\[
F(t) = \frac{2\alpha I(t)}{c},
\]

(4.1)
where \( t \) is time, \( F(t) \) is a volumic radiation force \([\text{N/m}^3]\), \( \alpha \) is the tissue absorption coefficient \([1/\text{m}]\), \( I(t) \) is the average acoustic intensity \([\text{W/m}^2]\), and \( c \) is the sound speed \([\text{m/s}]\).

When an AM waveform is used to drive the FUS transducer, the radiation force has a temporal profile oscillating at the modulation frequency \((\omega_m)\). The acoustic pressure \( p(t) \) of the AM waveform generated at the focus can be given by

\[
p(t) = p_0 \cdot \cos(\omega_m t) \cdot \cos(\omega_c t),
\]

(4.2)

where \( p_0 \) denotes the maximum instantaneous acoustic pressure and \( \omega_c \) is the carrier frequency (i.e., the center frequency of the single-element FUS transducer). Figure 4.1 illustrates the resulting acoustic pressure \( p(t) \) from the AM waveform.

\[\text{(a)}\]
\[\cos(\omega_m t) \times \cos(\omega_c t) = \]
\[\text{(b)}\]
\[\cos(\omega_m t) \cdot \cos(\omega_c t)\]

**Figure 4.1:** (a) a sinusoidal waveform with a modulation frequency \((\omega_m)\) is multiplied by a sinusoidal waveform with a carrier frequency \((\omega_c)\). The modulation frequency \((\omega_m)\) determines the frequency of the oscillatory pressure. The carrier frequency is the center frequency of the single-element FUS transducer. (b) multiplication in (a) results in an AM waveform.
The acoustic intensity is the rate, at which the ultrasound energy is applied to a specific tissue location. It is the quantity that must be considered with respect to biological effects and safety. Currently, the acoustic measurement used to describe the acoustic exposure can be expressed as $I_{spta}$ and $I_{ppu}$. The spatial-peak, temporal average intensity ($I_{spta}$) relates to the ability of the transmitted wave to heat tissue and potentially cause a thermally induced bioeffect. The spatial-peak, pulse-average intensity ($I_{ppu}$) describes the intensity of the transmitted pulse waveform. Figure 4.2 shows the ultrasonic pulses with a certain duration ($t$), and this pulses are repeated at every $T$. The $I_{spta}$ is obtained by multiplying the $I_{ppu}$ by the pulse duration ($t$) and dividing it by the pulse repetition time ($T$), i.e.,

$$I_{spta} = I_{ppu} \times \frac{t}{T}.$$  

(4.3)

![Figure 4.2: Illustration of acoustic intensity profile. $I_{ppu}$ is related to $I_{spta}$ through $t/T$, i.e., $I_{spta} = I_{ppu}(t/T)$](image)
In the AM case, the average acoustic intensity, \( I(t) \), is characterized by the spatial peak-pulse average \( (I_{ppa}) \), which is calculated as the mean intensity over the pulse duration at the position of the maximum intensity in the field (i.e., at the focus of the FUS transducer) \(^{19} \). The \( I_{ppa} \) can be calculated by simply integrating \( p^2(t) \) over time (the time in which the pressure is applied), i.e., calculating the area under the waveform, (the shaded area shown in Figure 4.2),

\[
I(t) = \int_0^t \frac{p^2(\tau)}{\rho c} d\tau, \tag{4.4}
\]

\[
I(t) = \frac{p^2}{\rho c} \int_0^t \left\{ \cos(\omega_m \tau) \cdot \cos(\omega_c \tau) \right\}^2 d\tau, \tag{4.5}
\]

where \( \tau \) is the variable of integration, \( t \) is the insonation time, \( \rho \) is the density, and \( c \) is the sound speed. By solving the integral in Eq. 4.5, we obtain \(^{19} \)

\[
I(t) = \frac{p^2}{\rho c} \left\{ \frac{1}{16} \left[ 4t + \frac{2\sin(2\omega_m \tau)}{\omega_m} + \frac{\sin(2(\omega_m - \omega_c) \tau)}{\omega_m - \omega_c} + \frac{2\sin(2\omega_c \tau)}{\omega_c} + \frac{\sin(2(\omega_m + \omega_c) \tau)}{\omega_m + \omega_c} \right] \right\} \bigg|_0^t \tag{4.6}
\]

and the solution for \( I(t) \) can then be written as follows:

\[
I(t) = \frac{p^2}{16\rho c} \left\{ 4t + \frac{2\sin(2\omega_m t)}{\omega_m} + \frac{\sin(2(\omega_m - \omega_c) t)}{(\omega_m - \omega_c)} + \frac{2\sin(2\omega_c t)}{\omega_c} + \frac{\sin(2(\omega_m + \omega_c) t)}{(\omega_m + \omega_c)} \right\}. \tag{4.7}
\]

In this study, the assumed density \( (\rho) \) is 1000 kg/m\(^3\) and sound speed \( (c) \) is 1540 m/s to simulate soft tissues. The sinusoidal function with a carrier frequency
component \( (\omega_c) \) is assumed to be negligible, due to the larger denominator \( \omega_c \) being in the MHz range compared to modulation frequency, \( \omega_m \) in the Hz range. In other words, the intensity is mainly dominated by the modulation frequency \( 2\omega_m \), i.e.,

\[
I(t) \approx \frac{p_o^2 \sin(2\omega_m t)}{16 \rho c \omega_m t}.
\] (4.8)

Both the radiation force (Eq. 4.1) and resulting motion oscillate at frequency equal to twice the modulation frequency \( (2\omega_m) \) (Eq. 4.8). This relationship has also been confirmed through experimental analysis. For instance, when a 15 Hz modulation frequency was used, the resulting displacement oscillated at a frequency equal to 30 Hz \(^9\). The motion resulting from this acoustic radiation force was detected through 1D cross-correlation method on the radio-frequency (RF) signals, which were acquired using an imaging transducer. The estimated motion was then used to characterize the medium being studied (section 4.4).

### 4.3 Instrumentations

The HMI system is an all-ultrasound based system for both imaging and monitoring of thermal ablation. It consists of a single-element FUS transducer and
an ultrasound imaging transducer. The FUS transducer (Imasonic, Voray sur l’Ognon, France) was a single-element, air-backed with a center frequency of 4.5 MHz and a fractional bandwidth of 10%. A 0.2-mm needle hydrophone (Precision Acoustics LTD, Dorchester, Dorset, UK) was used to measure the dimensions of the FUS beam (Figure 4.3b). The needle hydrophone was mechanically moved in 2D, i.e., 20 mm in the axial and 20 mm in the lateral direction, at a step size of 1 mm. Since the needle hydrophone was very sensitive, additional time delays between steps were applied to avoid ambient vibration due to mechanical movement.

Figure 4.3: (a) DC Coupler (Precision Acoustics LTD, Dorchester, Dorset, UK) that provides dc power to the submersible preamplifier and a coupling between the preamplifier and the signal measurement instrumentation such as oscilloscope. (b) A 0.2-mm needle hydrophone (Precision Acoustics LTD, Dorchester, Dorset, UK) that was used to measure the dimensions of the FUS beam.
The measured acoustic pressure of the FUS beam is shown in Figure 4.4. The beam has an ellipsoidal shape with a major axis (axial) of 2 ± 0.5 mm and an orthogonal minor axis (lateral) of 1 ± 0.5 mm (Figure 4.4 in the focal zone). The FUS transducer was driven by an AM waveform with the modulation frequency being typically on the order of a few Hz. An AM focused beam was used to generate a time-varying acoustic radiation force. The resulting oscillatory motion at the focus was detected during the force application using an imaging transducer. The imaging transducer is a single-element pulse-echo transducer (HMI-1, Chapter 4.3.1), or a phased-array transducer (HMI-2, Chapter 4.3.2).

Ultrasound imaging is based on the pulse-echo principle. The transducer transmits pulses and subsequently receives the reflected waves (‘echoes’) from the tissue. Therefore, adequate time must be allowed for all echoes to return before the transducer is pulsed again. A transducer at high center frequency emits shorter wavelengths and thus correspondingly shorter pulses, which decay more rapidly inside the tissue (lower penetration), but provides higher axial resolution. Greater wavelengths or lower frequencies, generally penetrate much further into the tissue and result in lower absorption; consequently, the axial resolution is poorer. Depending on the type of application, the frequencies and transducer types need
to be adapted to optimize the tradeoff between resolution and depth of penetration.

Figure 4.4: 2D image of the beam profile with the 4.5 MHz FUS transducer. The transducer is at the top of the image. The scale bar denotes the normalized acoustic pressure in dB relative to its peak at the focus (red or 0 dB).

Two HMI systems (i.e., HMI-1 and HMI-2) are presented in this dissertation. A 7.5-MHz, pulse-echo transducer was implemented in the HMI-1 system, because 1) it provided high axial resolution, which in turn improved the quality of axial displacement estimation, 2) its imaging frequency range did not overlap with the FUS frequency and its harmonics, 3) it could fit through the central opening of the
FUS transducer, and 4) it could be integrated with our data acquisition (DAQ) and computer-controlled positioner systems. HMI-1 has been used for tissue mapping and diagnosis of diseased tissue in post-surgical breast specimens.

The HMI system was further improved by utilizing a phased-array imaging transducer (i.e., HMI-2). Compared to the single-element imaging transducer used in HMI-1, the phased-array imaging transducer had a lower center frequency, and thus a lower axial resolution; however, it provided a 2D view of the targeted region. The advantage of the HMI-2 system includes 1) the capability to acquire RF frames throughout the entire ablation process (up to 6 s), 2) visualization of both the targeted region and the position of the FUS beam, and 3) mapping of the propagating shear wave, which can be used to estimate the viscoelastic parameters of the targeted tissue (Appendix A)\textsuperscript{20}.

Both HMI-1 and HMI-2 systems have been used interchangeably depending on the aims of the experiment and tissue sample sizes. For instance, in order to map tissue specimens, HMI-1 is used because it is integrated with our data acquisition systems and positioner, thus the mapping process is fast and efficient; or, for the quantitative measurement of viscoelastic properties of tissues, HMI-2 is used to image the tissue samples because it can provide 2D maps of the shear wave propagation, which can be used to quantify the tissue viscoelastic properties. A
summary of the two HMI systems is shown in Table 4.1, and the details are provided in the following sections.

When the HMI technique is used for simultaneous imaging and ablation during the thermal ablation, or thermal treatment, the integrated system is named HMIFU for Harmonic Motion Imaging for Focused Ultrasound. Therefore, there are two integrated systems in HMI, i.e., HMIFU-1 and HMIFU-2. The HMIFU-1 and HMIFU-2 systems have been applied for monitoring of thermal treatment and tumor mapping for pre- and post-treatment assessments (Chapter 7 and 8)\textsuperscript{25,27}. 
Table 4.1- Summary of the HMI systems

<table>
<thead>
<tr>
<th></th>
<th>HMI-1</th>
<th>HMI-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUS transducer</td>
<td>4.5 MHz single-element FUS transducer (Imasonic)</td>
<td>4.5 MHz single-element FUS transducer (Imasonic)</td>
</tr>
<tr>
<td>Imaging transducer</td>
<td>Single-element pulse-echo transducer (Panametrics)</td>
<td>Phased-array transducer (64 element) (Ultrasonix)</td>
</tr>
<tr>
<td>Parameters:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Center frequency</td>
<td>7.5 MHz</td>
<td>3.3 MHz</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Sampling frequency</td>
<td>80 MHz</td>
<td>40 MHz</td>
</tr>
<tr>
<td>PRF*/FR**</td>
<td>PRF = 5.4 kHz</td>
<td>FR = 200 - 400 frames/s</td>
</tr>
<tr>
<td>Filter process</td>
<td>Analog bandpass filter</td>
<td>Digital lowpass filter</td>
</tr>
<tr>
<td>Advantages</td>
<td>• Higher axial resolution</td>
<td>• Simultaneous B-mode</td>
</tr>
<tr>
<td></td>
<td>• Higher temporal resolution</td>
<td>• 2D view of the targeted region</td>
</tr>
<tr>
<td></td>
<td>• Integrated with current DAQ and computer control positioner</td>
<td>• Image deeper into the tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Data acquisition up to 6 s</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>• No 2D view of the targeted region</td>
<td>• Lower axial resolution</td>
</tr>
<tr>
<td></td>
<td>• Data acquisition is limited up to 1 s</td>
<td>• Lower temporal resolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Suboptimal data synchronization</td>
</tr>
<tr>
<td>Objectives</td>
<td>• Monitoring of thermal treatment</td>
<td>• Monitoring of thermal treatment</td>
</tr>
<tr>
<td></td>
<td>• Tumor mapping for pre- and post-treatment assessment.</td>
<td>• Tumor mapping for pre- and post-treatment assessment.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Quantitative measurement of viscoelastic parameters***</td>
</tr>
</tbody>
</table>

*PRF = pulse repetition frequency
***Appendix A
**FR = frame rate
4.3.1 HMI-1

The HMI-1 system comprises of a single-element FUS transducer and a single-element pulse-echo transducer (Figure 4.5). A 7.5-MHz pulse-echo transducer (Panametrics, Waltham, MA, USA) with a diameter of 12 mm was placed through the center of the FUS transducer, with the two beams were aligned. A pulser/receiver (Panametrics 5051PR, Waltham, MA, USA) was used to drive the pulse-echo transducer at a pulse repetition frequency (PRF) of 5.4 kHz.

Figure 4.5: (a) the 4.5-MHz single-element, FUS transducer and (b) 7.5 MHz single-element, pulse-echo transducer. A silicone mold (in blue) keeps the pulse-echo transducer aligned with the FUS one.
An analog bandpass filter, i.e., 7th order type II Chebyshev band-pass filter, (Reactel, Inc., Gaithersburg, Maryland, USA) with cutoff frequencies of \( f_{c1} = 5.84 \) MHz and \( f_{c2} = 8.66 \) MHz (at -60 dB), was used to filter out the fundamental frequency of the FUS beam and its harmonics from the pulse-echo spectrum (Figure 4.6)\(^\text{104}\). The filtered RF signals were sampled at 80 MHz and 14-bit digitization (CS14200, Gage Applied Technologies, Lachine, Canada) and were then used to estimate tissue displacement using cross-correlation technique (Chapter 4.4).

Figure 4.6: The spectra of the acquired RF signal. (a) Before bandpass filtering. The filtered RF signal is indicated by blue in (b) overlaid on the unfiltered RF signal shown in gray.
4.3.1.1 Experimental setup

The HMI-1 experiment setup is shown in Figure 4.7. A coupling cone (Figure 4.7c), filled with degassed distilled water, provides a transmission path through a thin membrane at its tip. Two frequency generators (Agilent (HP) 33120A, Palo Alto, CA, USA) were used to produce a carrier frequency of 4.5 MHz and a low modulation frequency within the range of 10 to 30 Hz. A linear chirp test between 10 and 30 Hz was used to investigate the optimal vibration frequency for each targeted object.

The limitation regarding the range of the modulation frequency lies in the accuracy of the displacement estimation and data storage capabilities of the system. A fast data acquisition, i.e., approximately 0.5 s for each dataset, is required to scan the entire tissue efficiently and is an important aspect in in vivo studies or future clinical applications. For instance, when the modulation frequency of 5 Hz was applied, the data acquisition time was 5 s for each dataset and the total scanning time was approximately 2 hours, which cannot be practically used in a clinical setting. When the modulation frequency exceeds 30 Hz, the tissue may not spontaneously respond to the applied radiation force. This in turn may result in an increased uncertainty in the displacement estimation. For example, if the modulation frequency of 50 Hz were used in the experiment, the
acquired RF signals would have a low Signal-to-Noise Ratio (SNR), and thus it would be difficult to estimate displacement accurately.

The resulting AM waveform was amplified by 50 dB using an RF power amplifier (E&I, Rochester, NY, USA). The amplification was necessary in order for the FUS transducer to transmit enough energy to vibrate the tissue (at least 2 MPa is needed in order to induce tissue motion). The setup for the targeted object shown in Figure 4.7e can be adapted according to the type (i.e., in vitro, ex vivo, or in vivo) and objective (i.e., tumor mapping and tumor ablation) of the study.

![Figure 4.7: A block diagram of the experimental setup for HMI-1. (a) the 7.5 MHz single-element pulse-echo transducer (shaded region). (b) the 4.5 MHz FUS transducer, (c) a coupling cone filled with degassed distilled water, (d) computer-controlled 3D positioner, and (e) targeted objects, such as tissue mimicking gels, in vitro, ex vivo specimens, and in vivo mice. The shaded area is the characteristic of the HMIFU-1 system.](image-url)
4.3.1.2 Data acquisition

RF signals acquired throughout the tissue depth were collected and displayed in time, i.e., is an M-mode format. Similarly, estimated displacements along the tissue depth displayed in time are termed as M-mode displacement. Each M-mode acquisition had duration of

\[ \tau = \frac{N}{PRF}, \]

where \( N \) is the number of RF signals (typically between 600 and 900) and \( PRF \) is the pulse repetition frequency (typically \( PRF = 5.4 \) kHz) (Figure 4.8).

**Figure 4.8:** RF signals acquired by a pulse-echo transducer (a) over time, i.e., \( T_1, T_2, \ldots, T_N \).
4.3.2 HMI-2

As mentioned in the previous section, a phased-array imaging transducer was implemented in the HMI-2 system. A phased-array imaging transducer (Figure 4.9b; Ultrasonix Medical Corporation, Richmond, Canada) with a center frequency of 3.3 MHz and a fractional bandwidth of 60% was inserted through a central opening of the single-element FUS transducer. The beams of the two transducers were aligned (Figure 4.9c,d). The consecutive RF frames were acquired at a sampling frequency of 40 MHz and a frame rate of 200 to 400 frames/s.

Figure 4.9: (a) an example of a B-mode image and (b) the console of the Sonix RP system used. The HMI-2 transducer consists of (c) a 4.5 MHz single-element 4.5 MHz transducer and (d) a 3.3 MHz phased-array imaging transducer.
Since there is no analog anti-aliasing filter built in the sonix system, a digital low-pass filter i.e., 100th-order, equiripple low-pass filter, with a cutoff frequency of 4.2 MHz was applied on the acquired RF signals in order to filter out the FUS beam, prior to displacement estimation. The processing time using the digital filter was longer compared to the analog filter. Digital filter required additional signal processing, whereas in the analog filter could be integrated into the hardware, and thus filter signals in real time.

Figure 4.10a depicts an unfiltered RF frame, where the bright region at the center of the B-mode image is the beam interference between the imaging and the FUS beams. After filtering, the FUS beam and its interference with the phased array beams are removed (Figure 4.10b).

Figure 4.10: A B-mode image of liver tissue placed on the top of gel. (a) unfiltered RF frame and (b) filtered RF frame. Note that the FUS beam was successfully removed in (b).
4.3.2.1 Experimental setup

The HMI-2 experimental setup is similar to the HMI-1 system. The only difference is that the data is stored within the ultrasound system as indicated by a shaded area in Figure 4.11. The diagnostic ultrasound system and the FUS transducer are controlled separately. The same setup (Figure 4.11e) can be adapted to suite our gel, \textit{in vitro}, \textit{ex vivo}, or \textit{in vivo} studies.

![Figure 4.11: A block diagram of the experimental setup for HMI-2.](image)

(a) the 3.3 MHz phased-array imaging transducer, (b) the 4.5 MHz single-element FUS transducer, (c) a coupling cone filled with degassed distilled water, (d) computer-controlled 3D positioner, and (e) targeted objects such as, tissue mimicking gels, \textit{in vitro}, \textit{ex vivo} specimens, or \textit{in vivo} mice. The shaded area is the characteristic of the HMIFU-2 system.
4.3.2.2 Data acquisition

For HMI-2, the region of interest is the tissue motion along the symmetry axis of the B-mode image (yellow dashed line in Figure 4.12a) over consecutive frames in time (M-mode). The collected RF frames in time during the radiation force application had duration of

\[ \tau = \frac{N}{FR}, \]  (4.10)

where \( N \) is the number of frames and \( FR \) is the frame rate [frames/s] (typically between 200 and 400 frames/s) (Figure 4.12a).

Figure 4.12: (a) RF signals along the symmetry axis of the B-mode image (dashed yellow line) over consecutive frames in time, i.e., \( T_1, T_2, \ldots T_N \).
4.4 HMI motion estimation

Assuming uniform application of the radiation force, the resulting tissue motion (or, oscillatory displacement) can be related to the underlying tissue stiffness. In order to monitor the resulting tissue motion due to the applied force, time-domain RF signals are acquired during the force application. The similarity between RF signals is determined through the 1D normalized cross-correlation, which in turn can be related to tissue motion based on the sampling frequency and assumed sound speed. The 1D normalized cross-correlation can be written as

\[
R = \frac{\sum_{i=1}^{N} (A_i - \bar{A})(B_i - \bar{B})}{\sqrt{\sum_{i=1}^{N} (A_i - \bar{A})^2 \sum_{i=1}^{N} (B_i - \bar{B})^2}}
\]

(4.11)

where \( A \) and \( B \) are the reference and the consecutive RF signals, respectively; \( \bar{A} \) is the mean of \( A \), \( \bar{B} \) is the mean of \( B \), and \( N \) indicates the window length. Correlation coefficient, \( R \), is calculated at each search position within a pre-defined search region, and the position where the correlation coefficient is maximum corresponds to the new scatterer position. The axial shift (\( \Delta s \)) between
the two positions indicates the amount of the tissue motion along the axial direction.

For instance, in Figure 4.13b, the estimated axial shift is $\Delta s_1$ samples, and in Figure 4.13c is $\Delta s_2$ samples. The negative values indicate that the tissues move away from the transducer. Cosine interpolation was applied around the peak of the cross-correlation function in order to improve the precision of the displacement estimation. The cross-correlation function was performed throughout the entire depth of the targeted object, with a window size equal to 1 mm and a 85% overlap. However, the window size and overlap were adapted according to the quality of the acquired RF signals, i.e., signal-to-noise ratio (SNR).

Cumulative displacement was estimated by 1D cross-correlation on the consecutive RF signals to the reference signal, which was chosen to be the RF signal when the input force was at zero. The magnitude of cumulative displacement denoted the incurred motion. Thus, when the radiation force was applied, the tissue moved away from the transducer indicated by higher displacement amplitude. When the radiation force decreased, the tissue relaxed, as indicated by lower displacement amplitude. The negative displacement value denoted the motion away from the transducer. Both calculated correlation
coefficients and cumulative displacements were estimated throughout the entire tissue depth and were displayed over time, which were termed as correlation coefficient M-mode and displacement M-mode images, respectively.

Figure 4.13: A is the reference RF signal and B is the consecutive RF signals. (d) variation of correlation coefficient, $R$, within the correlation window. $R$ is calculated at each search position within a pre-defined search region (n), and the position where the correlation coefficient is at maximum corresponds to the new scatterer position. The axial shift ($\Delta s_1$ or $\Delta s_2$) between the two positions indicates the amount of tissue motion. For instance, in (b) the estimated axial shift is $\Delta s_1$ sample, and in (c) is $\Delta s_2$ sample.
Figure 4.14 a and b show examples of the calculated correlation coefficient M-mode and displacement M-mode images in a homogeneous, tissue-mimicking gel. The oscillatory motion was visible through the variation in displacement in Figure 4.14b. Negative displacement values denote the motion away from the transducer. The oscillatory displacement at the focus (from Figure 4.14c) shown in Figure 4.15 was used to illustrate the tissue motion during the force application. The arrow in Figure 4.15a illustrates the reference signal when the input force is at zero. This reference was used to calculate the cumulative displacements.

Figure 4.14: A homogenous tissue mimicking gel: (a) M-mode correlation coefficients (within the range of 0.85 to 1) and (b) M-mode displacement. Negative displacement values denoted the motion away from the transducer. (c) Focus location. The modulation frequency used was equal to 15 Hz.
Figures 4.15 b,c show the estimated displacement both in samples and in microns. When the force application started ($t = 0$ ms) and the tissue began to move away from the transducer indicated by large displacement amplitudes (Figure 4.15 b,c). When the force decreased ($t = 35$ ms), the tissue relaxed and moved towards the transducer, as indicated by the small displacement amplitude. The motion continuously progressed during the force application. The modulation frequency in this experiment was 15 Hz, so the input force and displacement (Figure 4.15 a, b, and c) oscillated at 30 Hz, twice the modulation frequency (Eq. 4.8). The phase shift ($\phi$) between the input force and the estimated displacement has been shown to relate to the viscosity of the tissues $^{106,107}$, this phase shift is used to estimate tissue viscosity in HMI (Appendix A) $^{20}$.

Although the tissue motion can follow the modulation wave (Figure 4.15), it does not directly provide any information on the tissue mechanical properties. Instead, the peak-to-peak displacement amplitude (i.e., $D_{HMI}$ in Figure 4.15) from the displacement M-mode can directly indicate the relative tissue stiffness at the interrogated region. Therefore, the peak-to-peak displacement amplitude is calculated from displacement M-mode and used as a relative measure of tissue stiffness.
Figure 4.15: (a) normalized input force. (b, c) oscillatory displacements shown in samples and microns, respectively. Data in (a, b, and c) oscillate at 30 Hz. $D_{HMI}$ is the peak-to-peak displacement amplitude.

The peak-to-peak displacement amplitude was termed as HMI displacement ($D_{HMI}$) as indicated in Figure 4.15. This amplitude was estimated at all depths by using a fast-Fourier transform (FFT) method. Based on the FFT method, the highest spectral peak was centered on a low-frequency excitation, as seen in
Figure 4.16a,b for input force and displacement. The highest peak of the spectrum was located at twice the modulation frequency (30 Hz) (Eq. 4.8).

\[ D_{\text{HMI}} = \frac{c \cdot P_{\text{FFT}}}{f_s \cdot \bar{N}_d} \cdot \frac{1}{2} \]  

(4.12)
where $c$ is the sound speed $1540.10^6 \text{[\mu m/s]}$, $f_s$ is the sampling frequency [MHz], $P_{FFT}$ is the peak of the displacement spectra (Figure 4.16c), and $N_d$ is the displacement data points $^{108}$. 

Note that in HMI-2 system, the input signal was not recorded during insonation due to instrumentation limitations. Thus, the first frame (i.e., when the acquisition started) was used as the reference frame for displacement estimation. However, this is not a concern in HMI since the measured peak-to-peak displacement amplitude ($D_{HMI}$) is independent of this offset.

In the next chapters, simulation (Chapter 5), in vitro and ex vivo (Chapter 6 and 7), and in vivo (Chapter 8) studies using the HMI system will be presented.
Chapter 5

THE ASSESSMENT OF HMI PERFORMANCE USING FINITE-ELEMENT MODELS AND GEL EXPERIMENTS

5.1 Introduction

In this chapter, the potential of the HMI technique in assessing different sizes/stiffnesses of a stiff inclusion embedded in a soft medium is presented. A finite-element model (FEM) is used to evaluate the dependence of the estimated displacement on the acoustic parameters and mechanical properties of tissue, such as the acoustic pressure amplitude and the tissue Young’s modulus.

The chapter is organized as follows: first, the simulation study including the acoustic pressure simulation, the FEM, and the image formation model is
described. Second, experimental validation is performed in homogenous tissue-mimicking gels and stiff inclusions gels. Finally, the results of the simulation and experiments are compared and discussed.

5.2 FEM for linear elastic model

Figure 5.1 shows the schematic of the simulation process to study the performance of the HMI technique. The process is as follows: 1) The 2D pressure field of the FUS transducer is simulated in Field II \(^{109,110}\). 2) The 2D pressure field is then used as a loading condition in the FEM study. 3) The FEM solution yields temporal displacement (oscillatory displacement) that is used to move the scatterers in the simulated gel. 4) The image formation model was employed to simulate RF data. 5) One-dimensional cross-correlation technique was applied on the RF signals obtained from the simulated gel image to estimate the motion. 6) The peak-to-peak displacements \(D_{HMI}\) from the FEM solution and 1D cross-correlation techniques were compared to validate the HMI motion estimation technique. The following sections details every step used in this simulation study.
Figure 5.1: Schematic of the simulation process. The left diagrams with white arrows are FEM solution and the right diagrams with black arrows are the estimated displacement from simulated gel. The comparison shows the quality of the HMI displacement.
5.2.1 Acoustic pressure field simulation

In order to first identify the advantages of using one (FUS) transducer \(^ {18}\) versus the two-transducer configuration \(^ {17}\), simulations of the pressure field were performed. The pressure fields were calculated using the Field II \(^ {109}\). Two different types of transducers were designed. The first one was a single-element transducer operating at 1 MHz central frequency, with a 70 mm diameter and a focal length of 100 mm. An amplitude-modulated wave at 50 Hz was simulated for this transducer.

The second transducer was a confocal and concentric transducer. It had two separate elements: the first element had a diameter of 50 mm and a focal length of 100 mm. It was surrounded by the second annular element that had an inner diameter of 50 mm, an outer diameter of 70 mm and a focal length of 100 mm. A 1-MHz continuous wave was simulated for the first element, and a 1.00005 MHz continuous wave was simulated for the second element, i.e., the difference frequency is 50 Hz. Both transducer types had the same total surface. For both simulations, the sampling frequency was 100 MHz, and the pressure field was calculated in a region around the focus of 40 mm (lateral) x 60 mm (axial) with a
pitch of 0.1 mm. The bandwidth for both AM and continuous waves was approximately 100%.

Figure 5.2 shows the acoustic intensity emitted by the two types of transducer configurations over one period of oscillation \((t_1, t_2, \ldots, t_5)\). Since the acoustic radiation force is linearly related to the acoustic intensity, these results show that, in the two-beam configuration, the overlapping, FUS beams produced an acoustic radiation force field continuously moving across the focal region at the difference frequency as indicated by white arrows in Figure 5.2a. On the other hand, the AM beam offered the advantage of sustaining the application of the radiation force at the same stable focus within the tissue through the entire excitation time (Figure 5.2b).

The acoustic pressure field for a 4.5 MHz single-element FUS transducer was simulated in Field II \(^{109,110}\). This framework employs a linear acoustic model to calculate the pressure field corresponding to specific transducer geometry and parameters. The single-element concave (FUS) transducer (Figure 5.3a) with a circular opening in its center for the placement of an imaging transducer (Figure 5.3b) was modeled and simulated with identical parameters used in the experiments.
Figure 5.2: (a) simulated intensity map of the acoustic radiation force produced by two overlapping focused ultrasound (FUS) beams at two different frequencies, $f_1 = 1$ MHz and $f_2 = 1.00005$ MHz. The force field is shown every 4 ms (top to bottom, i.e., $t_1$, $t_2$, ... $t_5$). The beam continuously moves across the focal region as indicated by white arrows. (b) acoustic intensity at 50 Hz produced by one FUS transducer. The beam intensity level changes in time but it does not move spatially.
The concave transducer was assumed to have a center frequency of 4.5 MHz, a focal length of 40 mm, and inner and outer diameters of 30 mm and 70 mm, respectively. The aperture was divided into 1256 rectangular elements 111, and the area of each element was equal to 1 mm².

The apodization technique was applied for each element to represent active and non-active regions. The center opening had a diameter of 30 mm and the apodization in this region was set to zero, i.e., non-active elements, as indicated by the blue-shaded area in Figure 5.3b. The apodization of the active area (an annular region) was set equal to one (active elements) as denoted by the pink-shaded area in Figure 5.3b. This method was used to simulate the FUS transducer with an AM waveform, i.e., a combination of a carrier frequency at 4.5 MHz and a modulation frequency at 15 Hz, was used to drive the transducer.

The 2D pressure field, \( p(x,z) \), was sampled at 80 MHz and calculated for a 10 mm (axial) by 10 mm (lateral) region at a resolution of 0.1 mm, and the attenuation coefficient (\( \alpha \)) of 0.3 dB/cm to simulate soft tissue 112. The resulting pressure field had a focal spot size equal to \( 0.8 \times 0.8 \times 1.6 \) mm³ (lateral x elevational x axial). The acoustic intensity levels (\( I_{\text{pta}} \)) were varied between 26.39 and 986.97 W/cm² to verify the linear relationship between the acoustic intensity and the estimated displacement.
This particular acoustic intensity range was selected to match the previously used experimental parameters for imaging and therapy purposes (details in Chapter 6 and 7) \cite{18,25}. The resulting 2D pressure field was then used as a loading condition in the FE-model.

Figure 5.3: A concave transducer with diameter of 70 mm and a center opening with a diameter of 30 mm, simulated in Field II. Colorbar indicates the apodization value, i.e., zero means non-active element (hole) and one means active element. (a) side view and (b) top view of the simulated FUS transducer.
5.2.2 The FE model

An axisymmetric cylindrical gel was constructed in commercial FE software (Comsol Multiphysics™, Comsol Inc., Burlington, MA, USA) with a radius of 18.5 mm and a height of 20 mm. A hard spherical inclusion was defined inside the simulated gel, with a distance between the background and the inclusion surfaces equal to 10 mm. The background Young’s modulus ($E$) was equal to 10 kPa. The diameter and stiffness of the inclusions were varied according to Table 5.1.

<table>
<thead>
<tr>
<th></th>
<th>Background Young’s modulus (kPa)</th>
<th>Inclusion diameter (mm)</th>
<th>Inclusion Young’s modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>10</td>
<td>3</td>
<td>25 and 50</td>
</tr>
<tr>
<td>Type 2</td>
<td>10</td>
<td>5</td>
<td>25 and 50</td>
</tr>
<tr>
<td>Type 3</td>
<td>10</td>
<td>10</td>
<td>25 and 50</td>
</tr>
</tbody>
</table>

The model was assumed to be nearly incompressible (Poisson’s ratio of 0.4999999), a density of 1000 kg/m³, and have zero viscosity. The FUS transducer was moved axially with a step size of 1 mm to cover the entire gel depth (Figure 5.4). The harmonic radiation force was applied sequentially at $n$ preselected locations ($p_1, p_2, ..., p_n$) in and around the inclusion (Figure 5.4).
The bottom boundary of the cylindrical model was constrained in the axial direction and free in the lateral direction, while the remaining boundaries were free to move in both the axial and lateral directions. In total, the number of nodes and triangular elements generated was equal to approximately 3000 and 7000, respectively. This included the refined triangular elements that were specifically selected around and inside the inclusion, allowing thus sufficient resolution for the spatial variation of the corresponding tissue displacement within the transducer focus.

Figure 5.4: (Left) One-dimensional raster-scan diagram along the axial direction at different location, e.g., p₁, p₂, ... pₙ. d₁, ..., d₅, ... dₙ represents the HMI displacement amplitude for each location. (Right) Graph representation of HMI displacement versus axial depth.
The FEM solution of the temporal displacement (oscillatory displacement) at different axial depth (Figure 5.4 - right) was used to move the scatterers in the simulated gel. The image formation model was then employed to simulate RF data from the simulated gel.

5.2.3 Image formation and displacement estimation

The RF signals were simulated in Matlab 7.2 (Mathworks, Natick, MA, USA) using a linear convolutional scattering model. The scatterer was randomly distributed. The linear array had 64 elements, a center frequency of 7.5 MHz, a frame rate of 124 frames/s, beamwidth of 2 mm, and 60% bandwidth. In this simulation, the speckle pattern was simulated as a Rayleigh distribution, and thus the tissue motion incurred by the applied force could be estimated. The RF signals were sampled at 40 MHz. A 1D cross-correlation technique was applied on consecutively generated RF signals, with the RF signal at zero pressure as the reference, at a window length equal to 1 mm and an 85% window overlap. For further details of the displacement estimation, the reader is referred to chapter 4.
5.2.4 Contrast-transfer efficiency (CTE)

In HMI, the applied force/stress is highly localized at the focus and thus the resulting displacement is related to the relative tissue modulus (stiffness) within the focal region, typically on the order of 1 to 2 mm, i.e., the focal spot size of the FUS. The difference between the displacement in the inclusion and background regions can be used to detect inclusions by their stiffness difference.

The contrast-transfer efficiency (CTE) parameter \(^{117-119}\) (Eq. 5.1) was used to quantitatively evaluate the performance of HMI. In this dissertation, CTE was defined as the ratio of the average displacement contrast \((C_d)\) to the elasticity contrast \((C_e)\). When CTE is close to 1, it means that the displacement ratio could perfectly predict the contrast of inclusion stiffness relative to the background.

CTE represents the percentage, at which displacement profiles depict the underlying elasticity distribution in the medium given by

\[
CTE = \frac{C_d}{C_e} = \frac{\bar{d}_{\text{background}}/ar{d}_{\text{inclusion}}}{E_{\text{inclusion}}/E_{\text{background}}}. \tag{5.1}
\]

The average displacement contrast \((C_d)\) was calculated as the ratio between the average HMI displacement of the background \((\bar{d}_{\text{background}})\) to the average HMI displacement of the inclusion \((\bar{d}_{\text{inclusion}})\).
The elasticity contrast \((C_r)\) was defined as the ratio of the inclusion Young’s modulus \((E_{\text{inclusion}})\) to the background Young’s modulus \((E_{\text{background}})\). The Young’s moduli (inclusion and background) for the simulated gels were shown in Table 5.1. The Young’s moduli for tissue-mimicking gels were measured using mechanical testing (Section 5.3.3). In this dissertation, we hypothesize that the displacement contrast can be used to closely represent the relative stiffness of the inclusion as long as the CTE remains relatively high.

5.3 HMI validation against mechanical testing

5.3.1 Preparation of tissue-mimicking gel

Two types of tissue-mimicking gels were used in this study. The first type of gels was made based on polyacrylamide. These gels are fragile. Polyacrylamide gels behave as linear solids, thus they are selected to match the assumptions made in the FEM, i.e., linear elasticity. The second type of gels is made from gelatin. Gels based on gelatin behave as nonlinear solids, thus they are suitable to represent soft tissues. Gelatin was used to test the capability of the HMI technique to locate tumor.
Four uniform and six spherical inclusion-embedded polyacrylamide gels with distinct stiffnesses were used for both mechanical testing and HMI experiments. Polyacrylamide gels were prepared using the following guidelines: pre-mixed 40% liquid acrylamide (19:1 acrylamide:bis-acrylamide ratio) (Thermo Fisher Scientific, Waltham, MA) was diluted in deionized water to produce a range of acrylamide concentration from 25% (weight/volume) to 40% (weight/volume) at 5% increments. The percentage of acrylamide in the mixture determines the stiffness of the gel after it is polymerized. The resulting solution was dissolved (1.75 ml per total ml) in 1M trishydroxymethylaminomethane (TRIS, 1.0 ml per total ml) with deionized water (7.16 ml per total ml). 10% ammonium persulfate (APS, 8.4 μl per total ml) and N,N,N′,N′-tetramethylethylenediamine (TEMED, Sigma-Aldrich, St. Louis, MO, 0.5 μl per total ml) subsequently added. Agar power (Acros Organics, Geel, Belgium) was added to the mixture to create speckle. The amount of agar powder added was equal to 2% of the mixture volume. The mixture was allowed to polymerize at room temperature for approximately 15 minutes prior to use.

In order to independently measure the Young’s modulus of all four homogeneous gels, a dynamic indentation test was performed. The description of the indentation test setup is provided in section 5.3.3. Based on the test results (Table 5.2), 25% acrylamide concentration was used to generate the soft
background \((E = 13 \text{ kPa})\), similar to the Young's modulus of the soft background in the simulation \((E = 10 \text{ kPa})\). The acrylamide concentrations of 30\% and 40\% were respectively used for the hard inclusions of 25 kPa and 50 kPa. These values were chosen for the quantitative comparison between the simulated and polyacrylamide gels. The three inclusions used were 3 mm, 5 mm, and 10 mm in diameter to simulate growing tumor dimensions.

Table 5.2 - Young's moduli of polyacrylamide gels with different acrylamide concentrations and loading conditions.

<table>
<thead>
<tr>
<th>Acrylamide concentration (%)</th>
<th>Young's Moduli (kPa)</th>
<th>20% pre-compression (5% dynamic loading)</th>
<th>5% pre-compression (2% dynamic loading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 Hz</td>
<td>1 Hz</td>
<td>0.1 Hz</td>
</tr>
<tr>
<td>25</td>
<td>13.3</td>
<td>13.4</td>
<td>13.9</td>
</tr>
<tr>
<td>30</td>
<td>24.2</td>
<td>29.1</td>
<td>24.7</td>
</tr>
<tr>
<td>35</td>
<td>36.2</td>
<td>37.1</td>
<td>37.4</td>
</tr>
<tr>
<td>40</td>
<td>49.9</td>
<td>48.4</td>
<td>51.4</td>
</tr>
</tbody>
</table>

The gelatin material (Gelatin 50 bloom, MP Biomedicals, Irvine, CA, USA) was used to make three stiff inclusion gels. The first model was two gelatin gels (20 kPa) with embedded cylindrical or spherical inclusions (40 kPa). The inclusions were colored by dark blue dye and they represented tumor masses. In the second model, an olive was placed inside the 20 kPa gel approximately 20 mm away from the surface. The olive was on average 17-mm long with a cavity diameter of 7 mm.
Gel preparation was completed using the following steps: degassed, de-ionized water and gelatin powder were mixed in a 500 ml solution. The amount of gelatin powder was calculated according to

\[ E_{\text{gelatin}} = 0.003C^{2.09}, \]  

(5.2)

where \( E_{\text{gelatin}} \) denotes the Young’s modulus of the gelatin in kPa and \( C \) denotes the concentration of gelatin powder in g/L. The concentration used was 63.6 g/L and 88.6 g/L in order to obtain stiffnesses of 20 kPa and 40 kPa (Eq. 5.2). The mixture was constantly stirred and heated until the temperature reached 50°C. The gelatin powder was assumed to have been uniformly dissolved at this state. The mixture was then placed into a water bath for cooling until the temperature decreased to 35°C. Isopropanol and agar powder were then added to the mixture. Isopropanol was added to increase cross-linking and thus increase the melting point of the gelatin while agar powder was added to create speckle. Note that agar only bloomed at temperature above 80°C, thus it did not contribute significantly to the increase of the gel stiffness.

The amount of agar powder (Acros Organics, Geel, Belgium) was equal to 10% of the total gelatin powder added. The stirrer was removed from the mixture when the temperature reached 30°C. The solution was covered with plastic wrap
to minimize dehydration and was placed in a refrigerator for approximately 12 hours.

5.3.2 HMI for homogeneous gels

The HMI experimental setup is shown in Figure 5.5. The HMI-1 system was used to generate an oscillatory acoustic radiation force at 15 Hz, because it was determined to be the optimized oscillatory displacement throughout the stiffness range used in the study. The gel was cast in a glass container with a silicone rubber embedded inside. A silicone rubber/absorber (McMaster-Carr, Dayton, NJ, USA) was placed at 45° under the gel to further reduce the specular reflection from the bottom of the glass container. The FUS transducer was moved axially and downward by using a computer-controlled positioner with a step size of 1 mm to cover the entire gel depth (Figure 5.4). A 1D cross-correlation method on the acquired RF signals was used to estimate displacement with a window length equal to 1 mm and 85% window overlap.
5.3.3 Mechanical testing – dynamic indentation test

A dynamic indentation test was used to assess the Young’s modulus of the polyacrylamide gels. The system consisted of an indentation probe (indenter) that vertically applied a small sinusoidal deformation on the gel surface (Figure 5.6).
The indenter had a flat and rough surface to avoid possible slippage during testing. The diameter of the indenter was equal to 6.33 mm.

The loading site was selected by placing the indenter at the center of the gels. The gels were placed on a rigid cylindrical plate with a thickness of $14.3 \pm 0.56$ mm and a diameter of 87.1 mm, which was four times larger than that of the indenter so that the dimensionality assumptions of the theoretical model (Eq. 5.3) were satisfied. A small amount of deionized water was added sparingly to keep the gels moisturized. The temperature was kept within the range of 25.5 to 25.6 °C. The applied dynamic displacement was controlled by an Instron Microtester (Instron, Inc., Norwood, MA) by using a 10 N load cell (Figure 5.6).

The precision of the load cell was 0.5% of its total capacity (10 N). Two different tests were performed on each gel. The first test entailed a 20% pre-compression (i.e., $2.56 \pm 0.12$ mm) followed by an oscillation at a magnitude of 5% apparent strain (displacement divided by initial thickness, i.e., $0.715 \pm 0.028$ mm); and the second test entailed a 5% pre-compression (i.e., $0.715 \pm 0.028$ mm) followed by an oscillation magnitude of 2% apparent strain (i.e., $0.286 \pm 0.0112$ mm) (Figure 5.7). To test reliability, each indentation test was performed and compared at two different loading frequencies of 0.1 Hz and 1 Hz.
Figure 5.6: Mechanical testing machine, Instron Microtester (Instron, Inc., Norwood, MA). (a) the indenter was loaded by a 10 N load cell with a precision of 0.5% of the total capacity (10 N). (b) tissue-mimicking gels was placed beneath the indenter.

The temporally varying force was measured during the entire indentation process, and these data were used to calculate the Young’s modulus ($E$) of the gel samples using the following equation\textsuperscript{121}:

$$E = \frac{2 \left(1 - v^2\right) q a}{w}, \quad (5.3)$$

where $v$ is the Poisson’s ratio (assumed to be nearly incompressible material, $v = 0.49999$), $q$ is the loading pressure (force per unit area), $a$ is the radius of the indenter tip, and $w$ is the applied displacement amplitude.
Figure 5.7: Graph representation of the dynamic mechanical testing. The pre-compression (i.e., 20% and 5%) was imposed, followed by an oscillation of apparent strain, i.e., 5% and 2% (displacement divided by initial thickness). Both loading protocols were tested at the frequencies of 0.1 Hz and 1 Hz.

5.3.4 Results

The mechanical testing results show that the measured Young’s modulus increases with the concentration of acrylamide (Table 5.2). The calculated Young’s moduli ($E$) for 25%, 30%, 35%, and 40% acrylamide concentrations are $13.42 \pm 0.32$, $25.4 \pm 2.46$, $37.38 \pm 1.08$, and $50.2 \pm 1.43$ kPa (mean $\pm$ SE), respectively. The Young’s moduli of different gels did not change with the loading frequencies at different applied strain levels. This demonstrates that the polyacrylamide gels behave as elastic materials at that loading frequencies. These results were used as a guideline
to generate a hard inclusion in the gel with stiffness similar to the simulated gels. The acrylamide concentration of 25% was used to model the soft background with a Young’s modulus of $E = 13$ kPa (Table 5.2).

Acrylamide concentrations of 30% and 40% were selected to match the inclusion Young moduli ($E$) in the simulated gel, which are equal to 25 kPa and 50 kPa, respectively (Table 5.2). The estimated Young’s moduli obtained from mechanical testing and the inverse of the estimated HMI displacement from gel experiments exhibit a linear relationship ($r^2 = 0.97$) (Figure 5.8). This further confirms that the HMI displacement can be directly related to the underlying tissue stiffness.

![Figure 5.8: A linear relationship between the inverse of the HMI displacement from experiment and Young’s Moduli from mechanical testing in homogenous gels.](image)
5.4 HMI validation against FEM

5.4.1 FEM results

To study the performance of HMI, the cross-correlation method and parameters used were tested regarding their capability of estimating the entire resulting displacement range. The comparison between the FEM and the HMI displacements in a homogeneous medium with a Young’s modulus of 10 kPa is shown in Figure 5.9.

Figure 5.9: Displacement fields (for every RF signals in the image) resulting from the acoustic force distribution in a homogenous gel. The highest displacement occurs at a depth of 40 mm (FUS focus). (a) FEM solution and (b) estimated displacement.
The highest displacement is located at the center of the focal zone (depth of 40 mm), radially spanning approximately ± 3 mm from the symmetry axis, as expected, because a linear assumption was used for both acoustic model and elastic model.

Figure 5.10 depicts the relationship between the displacements and inclusion size/stiffness of simulated gels. The Young’s moduli of stiff inclusions were 25 kPa and 50 kPa and their diameters were equal to 3, 5, and 10 mm. The peak positive acoustic pressure amplitude was equal to 1.98 MPa, the same value as that used in the experimental gels. The diameter of the inclusion can be estimated based on the displacement profiles. For example, in Figure 5.10a, the HMI displacement decreases at the axial depth of 9 mm and then increases at the axial depth of 12 mm; thus, the 3-mm-diameter inclusion is correctly depicted with a diameter of 3 mm.

Figures 5.10 b and c show the estimated inclusion sizes at 5 mm and 10 mm, respectively. As the focus of the FUS beam moves toward the center of the inclusion, the resulting displacement amplitude steadily decreases, correctly representing thus the relative stiffness of the inclusion. For instance, the displacement amplitude close to the center of the hard inclusion, i.e., between 10 and 12 mm, is the lowest (Figure 5.10 b,c). The displacement at the depth of 20 mm
is approximately zero, because the bottom surface of the simulated gel was constrained.

5.4.2 HMI for stiff inclusion gels

In the HMI experiments, the oscillatory acoustic radiation force was axially moved across the inclusion, as seen in Figure 5.4. The RF frames were acquired for each gel in four independent iterations to test the reproducibility of the HMI technique in reliably imaging inclusion sizes. The mean and standard error (SE) of the HMI displacement profiles among the four iterations for each gel are shown in Figure 5.11. The small inclusions, i.e., 3 and 5 mm in diameter, are both detectable (Figure 5.11a,b).

The assessment of the estimated displacement contrast that is related to the underlying elasticity distribution in the heterogeneous medium, is evaluated by calculating the CTE (Eq. 5.1). The CTE values from the simulated gels (triangle) and polyacrylamide gels (circle) are shown in Figure 5.12. Here, the CTE is calculated for three inclusion diameters (3, 5 and 10 mm) with two different Young’s moduli (25 kPa and 50 kPa).
Figure 5.10: Normalized HMI displacement from simulated gel along the central axis of the FUS transducer for various inclusion sizes (a = 3 mm, b = 5 mm and c = 10 mm) with two different Young’s moduli (-□- 25 kPa and -◊- 50 kPa). The background Young’s modulus was equal to 10 kPa. The dotted lines indicate the diameter of the inclusions.
Figure 5.11: HMI displacement from experiments for various inclusion sizes (a = 3 mm, b = 5 mm, and c = 10 mm) and two different stiffnesses (−□− 30% acrylamide ≈ 25 kPa and −◊− 40% acrylamide ≈ 50 kPa). The mean and SD based on four iterations completed for each inclusion size. The background stiffness was 25% acrylamide (≈ 13 kPa). The dotted lines indicate the diameter of the inclusions.
Figure 5.12: Contrast-transfer efficiency (CTE) for heterogeneous gel with inclusion (3, 5 and 10 mm in diameter). Triangle denotes result from simulated gels and circle denotes experimental results from tissue-mimicking gels. Solid circle/triangle represents results for 25 kPa-inclusion. Clear circle/triangle represents results for 50 kPa-inclusion.

In this experiment, the displacement contrast is close to the elasticity contrast for larger and stiffer inclusions (CTE > 0.7). The CTE is below 0.5 for the 3-mm inclusion, even though the inclusion/background interface is clearly depicted on the HMI displacement profile (Figure 5.11a).
5.4.3 HMI mapping of stiff inclusion gels

The HMI-1 system was used to probe and analyze gel properties in a point by point. At each point, a 1D cross correlation method was applied on the RF signal to estimate the HMI displacement. The HMI displacement for every point was then used to reconstruct the HMI image. Therefore, each pixel in HMI images represents the relative tissue stiffness at that probed region, i.e., at one point.

HMI-1 system was used to image a gelatin gel (20 kPa, denoted by yellow region) with embedded cylindrical inclusion (40 kPa) (Figure 5.13a). The inclusion was colored by dark blue dye and it represented a tumor mass (Figure 5.13a). The two-dimensional map of the HMI displacement, i.e., HMI image, is shown in Figure 5.13b. In the inclusion, the average displacement is 3.5 μm (Figure 5.13b), while in the surrounding gel the displacement is 6.1 μm (Figure 5.13b).

The average displacement amplitude in the yellow region is approximately 1.7 times higher than the average displacement amplitude in the dark blue region. This result is consistent with the inverse relationship between the displacement and the gel stiffness (i.e., gel concentration) during harmonic excitation as shown in Figure 5.13. The cylindrical inclusion is accurately mapped by HMI technique, it has a diameter approximately 10 mm as seen in Figure 5.13a.
Figure 5.13: Result of the tissue-mimicking gel experiment on a stiff inclusion. (a) photograph of the gelatin gel and (b) HMI image. Concentration of background gel was 63.6 g/L (~20 kPa) and concentration of inclusion was 88.6 g/L (~40 kPa) which was colored by dark-blue dye.

The sonogram and HMI images were compared to test whether HMI technique could correctly locate the stiff inclusions. A 7-MHz linear array transducer (Terason Ultrasound, Teratech Corporation, Massachusetts, USA) with sampling frequency of 30 MHz was used to image two stiff inclusion gels. Figure 5.14a shows a tissue-mimicking gel with a stiff spherical inclusion. Figure 5.14b displays an HMI image overlaid on the corresponding B-mode image. The HMI-image is co-registered with the B-mode image. The inclusion appears hyperechoic in the B-mode image (Figure 5.14a), but it is not as clear as in HMI image. The inclusion is a stiffer region (i.e., smaller displacement indicted by blue/cyan) surrounded by a
slightly softer region (i.e., larger mean displacement indicated by red) in the HMI image.

Figure 5.14: (a) images of tissue mimicking gel (~ 20 kPa) with embedded spherical inclusion (~ 40 kPa), (b) overlay of HMI image on the corresponding co-registered with B-mode image in (a).

Figure 5.15a shows a gel with a pitless olive was placed inside, approximately 20 mm away from the gel surface. The olive was on average 17-mm long with a cavity diameter of 7 mm. The goal was to identify the olive and its stiffness in HMI image. The olive appeared hyperechoic, whereas the cavity (i.e., filled with a red-
pepper) appeared hypoechoic. Figure 5.15a shows an acoustic shadowing of the target mass that is visualized as a low contrast hypoechoic structure.

In this experiment, the gel was palpated in order to estimate the location and the size of the olive. After palpation, the HMI-1 system was placed at the suspicious lesion, which was used as a starting position for scanning process. The HMI-1 system was then moved in point by point to cover a 30 by 30 mm$^2$ region at step-size of 1 mm. The HMI image was then co-registered with B-mode image to validate the olive size and location. The mass appeared slightly hyperechoic with shadowing artifact in the B-mode image, and in the HMI image as a yellow/orange region ($< 13 \mu m$) of low displacement. In this model, HMI image indicates that the stiffness of the olive and the red-pepper inside the cavity were similar, despite different echogeneity.
Figure 5.15: (a) B-mode image of an olive placed inside a tissue-mimicking gel. (b) HMI image of the same olive-gel overlaid on b-mode image. The olive was used to represent tumor (a stiffer inclusion). The size of the olive is approximately 17 mm in diameter from both images.

5.5 Discussion

The aim of this study was to investigate the potential of the HMI technique as a non-contact method for mapping and quantifying relative tissue stiffness. Owing to the AM application, the oscillatory radiation force was sustained at the same location at a frequency twice the modulation frequency (Eq. 4.7). Since the FUS
focal spot was small (approximately 0.8 x 0.8 x 1.6 mm³), the force was highly localized and the probed tissue within the focal region could be directly associated with the underlying tissue modulus. For imaging, the radiation force could be applied in a 2D fashion using a raster-scan technique and a 3D HMI displacement image could be obtained by combining multiple 2D planes at variable depths \(^{122,123}\).

In the FE analysis, the acoustic pressure level of 1.98 MPa was applied at the center axis and moved downward with a step size of 1 mm. Since the model was linearly elastic, the behavior at higher acoustic pressure levels could be extrapolated in order to predict the resulting displacements. In this experiment, the applied pressure was approximately 1.96 MPa for 400 ms with an expected peak temperature rise of 0.9 °C. This temperature rise was considered low (\(\Delta T < 1°C\)), because it would not pose a risk to the patient during ultrasound exposure \(^{124,125}\). For future clinical applications, the duration of the force in HMI will be even further reduced to 100 ms with the estimated temperature rise equal to 0.24 °C, which is considered safe and acceptable for ultrasound imaging. The effects of inclusion size and stiffness on the displacement profile were demonstrated in simulations (Figures 5.10) and experiments (Figures 5.11). Three different hard inclusion diameters (3, 5, and 10 mm) were used to investigate the capability of the HMI technique in small inclusion detection. This indicates that HMI can be used
for early tumor detection because it can detect or map a smaller tumor (early tumor growth). In the case of the 10 mm inclusion (Figure 5.11c), the boundary of the inclusion is not distinct. Thus, if the background medium is larger than the inclusion, its boundary would be more well-defined.

The CTE value represented how well the HMI displacement related to the underlying elasticity distribution in the heterogeneous medium. For the experimental results, the CTE was relatively lower than in the simulations for most inclusion cases. Two possible explanations are provided, i.e., 1) different gel geometries were used, and 2) the conditions of the two techniques used to measure displacement contrast by HMI and elasticity contrast by mechanical testing were different. Here, the displacement was measured by HMI where the localized force was applied deep inside the heterogeneous gels, whereas the elastic modulus was measured by mechanical testing, where the force was applied on the surface of the uniform gels.

The HMI and mechanical testing could not be tested within the same frequency range due to fundamental differences. In HMI, the data storage capability has been optimized for AM frequency range above 10 Hz. The instrumentation is currently being upgraded in order to test a lower frequency range (< 10 Hz). Vappou et al. \textsuperscript{20} (Appendix A) showed that polyacrylamide gels mainly exhibit an elastic behavior,
and the estimated Young’s moduli remained relatively constant within the frequency range of 0.1 to 40 Hz. Therefore, the stiffness of the polyacrylamide gels were assumed to be remained the same when measured at 0.1 Hz or 15 Hz \(^{20}\). In mechanical testing, when the loading frequency exceeded 1 Hz, the strain does not immediately follow the applied stress, finally causing a gap (~ 1 mm) between the indenter and the gel. Moreover, in the mechanical testing, the slippage might occur between the indenter and the gel during testing. The two techniques thus require different testing conditions, and therefore have different boundary conditions and imposed stress levels. Nevertheless, this study demonstrates that the HMI displacement contrast is nearly equivalent to the elasticity contrast of a stiffer inclusion embedded in a soft background. Displacement contrast could be used to represent the relative inclusion stiffness, which in turn can be used for tumor diagnosis and characterization.

When the CTE value is lower, the contrast of displacement underestimates the stiffness contrast; however, as shown in Figure 5.11a, HMI could clearly detect the 3-mm inclusion despite the relatively lower CTE. The HMI sensitivity might thus be beneficial to detect early tumor growth. The results presented herein confirm two important findings: first, HMI technique has the potential of being successfully applied in the clinical setting for early tumor detection (for tumor size
> 3 mm in diameter) and diagnosis. Second, HMI could serve as an alternative non-contact indentation method that mimics the dynamic indentation testing for modulus measurement but without requiring direct contact, gel homogeneity, or specific boundary conditions, with the advantage of a wider range of loading frequencies.

The capability of HMI for measuring both the modulus and viscosity and their roles in the HMI displacement in a viscoelastic medium has been investigated by our laboratory (Appendix A)²⁰. Since tissue elastic properties are typically frequency dependent and the elastic moduli measurements are dependent on the excitation frequency, the modulation (or, AM) frequency can be varied to determine the degree of frequency dependence. This is one of the advantages of the HMI technique, i.e., it is capable of inducing radiation force within a wide range of frequencies by varying the AM frequency and adapting to the tissue type and the boundary conditions encountered.
5.6 Conclusions

The linear relationship between the inverse of the HMI displacement and the tumor/inclusion Young’s moduli were validated through mechanical testing on polyacrylamide gels. HMI image could show the relative stiffness map for an inclusion-containing media. The 2D HMI image could also detect stiff inclusions, which was consistent with sonography findings. Most importantly, good agreement between HMI imaging and sonography in both the inclusion shape and size was shown. The feasibility of the HMI technique for mapping and diagnosis of breast tumor in post-surgical breast specimens will be presented in Chapter 6.
Chapter 6

HMI OF HUMAN BREAST TISSUES EX VIVO

6.1 Background

For years, breast cancer diagnosis has been based on information from clinical examinations combined with anatomical imaging, such as mammography and sonography. Mammography and sonography are currently the most sensitive modalities for detecting breast tumors. However, the sensitivity of mammography declines significantly with increasing breast density, particularly for older women with dense breasts. Dense breasts have a high proportion of glandular tissue that renders tumor detection difficult in mammography. The sensitivity of mammography has been reported to vary between 68% and 77%, and its specificity from 82% to 98%.
In contrast, sonography has a higher sensitivity in women with dense breasts than mammography. It has been reposted to have a sensitivity from 65.9% to 74.6% and a specificity from 80% to 96%. A standardized system to quantify breast ultrasound imaging parameters that can help differentiate benign from malignant masses was reported by the American College of Radiology (ACR) in 2003. In practice, the diagnostic value of the examination is unavoidably dependent upon the knowledge and skill of the person performing the scan. Different interpretations of breast sonograms that define features of malignant and benign masses often result in inconsistent diagnosis. While the approach to suspicious masses is often a surgical biopsy, 70% to 90% of the breast biopsies performed turn out to be benign. Unnecessary biopsies lead to the patient’s discomfort, anxiety, risk of infection, and additional medical expenses. Therefore, there is a need for the development of additional reliable methods to complement the existing diagnostic procedures.

Since tumors are in general harder than the surrounding tissues, self and clinical breast examinations using palpation are commonly used to detect the presence of abnormalities that could indicate pathologies. Several imaging techniques other than mammography, mainly ultrasound and MRI, have been
developed to estimate tissue stiffness and thus detect tumors, with various forms of tissue perturbation for the detection of stiffer masses.

In the field of ultrasound, Krouskop et al. applied dynamic indentation at 0.1 Hz, 1 Hz and 4 Hz, and at different pre-compression strain levels (i.e., 5% and 20%) to measure ex vivo breast tissue elastic moduli (Figure 6.1). They showed that the elastic moduli of breast tissues do not change with the frequency of the applied displacement. This demonstrates that the breast tissue samples behave as an elastic material, i.e., viscous component is insignificant with the parameters used. Figure 6.1 shows the elastic moduli of normal breast tissue, which consists of mostly fat and glandular tissue, and cancerous breast tissue, i.e., are ductal carcinoma in situ (DCIS) and invasive (or, infiltrating) ductal carcinoma (IDC) 121.

Tissue elastic moduli of fat and glandular breast tissues are independent of the pre-compression stress level. On the other hand, disparities between the measured elastic moduli of fibrous, DCIS, and IDC indicate that different pre-compression strain levels are needed to differentiate normal from cancerous tissue. Thus, this technique relies on the pre-compression strain levels and boundary conditions of the sample.
Figure 6.1: Elastic moduli of different components of breast tissue, obtained at a loading frequency of 1 Hz for pre-compression strain levels of 5% (blue) and 20% (purple) \(^{121}\). Note that normal fat \((n = 8)\), normal glandular tissue \((n = 31)\), fibrous tissue \((n = 18)\), DCIS = ductal carcinoma \textit{in situ} \((n = 23)\), and IDC = invasive or infiltrating ductal carcinoma \((n = 32)\).

'Sonoelasticity imaging' or 'sonoelastography' has been proposed for the detection of lesions in a vibrating medium (Chapter 3.4.1) \(^{64-68,69}\). They estimated the amplitude (and/or phase) of the periodic movement of tissues by estimating the Doppler resulting shift \(^{66,71,72}\). Sonoelastography has been applied on \textit{ex vivo} breast tissues \(^{126}\) and have started clinical trials for non-palpable breast lesion detection \(^{80-85}\). Ophir \textit{et al.} developed the method of elastography that applied a small external static compression (on the order of 1\%) and used cross-correlation techniques on radio frequency (RF) signals in order to estimate tissue strains.
resulting from the external compression. This method has been proven to produce good quality strain images (or, elastograms) in the breast in vivo. Several clinical trials have been initiated to establish elastography in the clinical setting, and the method is currently being tested regarding its potential to enhance breast cancer detection and diagnosis.

Sarvazyan et al. implemented the elastography method in the design of a hand held scanning device that combined a transducer probe and a 2D pressure sensor array. The probe is used to slightly compress soft tissue, in other words, the probe acted similar to palpation. This method provides a real-time 2D pressure pattern and elasticity map, and is termed as Tactile Imaging (TI), or ‘stress imaging’, or ‘mechanical imaging (MI)’.

Quasi-static elastographic techniques have also been demonstrated as feasible methods for the detection of breast cancer in vitro using magnetic resonance imaging (MRI). In the MR Elastography technique (MRE), tissue mechanical properties (e.g., shear modulus) are mapped based on the observed phase shift of the MR signal in response to an external mechanical vibration. MRE could provide a tissue displacement map incurred by the low-frequency shear wave induced by the external excitation. The shear wave velocity, which was measured using the wavelength, was governed by the local mechanical properties of the
tissue. MRE used the propagating shear wave to reconstruct the local shear modulus of the medium.

MRE has been applied in the imaging of breast tumors and various breast tissues, with the aim to explore a new parameter for the diagnosis of breast lesions \[4,98,131-135\]. The *in vivo* measurement of the shear modulus using MRE has shown that there was good separation between malignant and benign (i.e., fibroadenoma and mastopathy) tumors based on their shear moduli \[134\]. However, the measured shear viscosity did not show separation between benign and malignant, which might be related to different static pre-compression strain levels applied on tissues \[98\]. Thus, modulus reconstruction using the external deformation method was dependent on the boundary conditions, internal structure, pre-compression strain levels, and out-of-plane motion assumptions.

Transient Elastography (TE) (Chapter 3.4.1) has also been tested and validated as a quantitative technique to provide soft tissue viscoelastic properties \[10\]. Bercoff *et al.* applied this technique clinically for breast tumor detection \[136\]. Experiments were conducted in 15 subjects who had palpable breast lesions. The resulting tissue elasticity map showed that the lesions had a different echogenicity than the surrounding tissues, which were validated by standard ultrasound imaging.
Apart from the aforementioned external excitation techniques, more recently, ultrasound-based internal perturbation methods have emerged that can produce a concentrated force in a targeted region, deep inside the tissue and can be used for probing and analyzing tissue properties within the targeted region. Other research groups have also applied the impulse radiation force to induce brief mechanical excitations locally and imaged the resulting tissue response while RF data were collected during tissue relaxation (Acoustic Radiation Force Impulse; ARFI)\textsuperscript{11,12}, or shear wave propagation, i.e., Supersonic Shear Imaging (SSI)\textsuperscript{13,87}, and Shear Wave Elasticity Imaging (SWEI)\textsuperscript{14}.

ARFI imaging applies a short-duration acoustic radiation force to generate localized displacements in tissues, and the resulting displacements are tracked during relaxation using cross-correlation based methods. The tissue response is monitored both spatially and temporally. ARFI has been applied to detect a breast lesion \textit{in vivo}\textsuperscript{125} and the resulting ARFI image showed a stiffer inclusion which was determined to be an infected lymph node by core biopsy.

Initial clinical evaluation of \textit{in vivo} elastography for breast lesion imaging using the concept of supersonic shear imaging (SSI) was reported by Tanter \textit{et al.}\textsuperscript{137}. The SSI technique uses a modified 1D array transducer to generate a radiation force that results in transient shear wave propagation. An ultrafast imaging sequence
was performed to acquire successive radiofrequency (RF) signals at high frame rates (up to 2000 frames/s). The results demonstrated that the SSI elasticity maps could detect breast masses and provide quantitative information on their stiffness. A summary on the clinical data on benign-malignant breast lesion differentiation by the aforementioned elasticity imaging methods is provided in Table 6.1. This table is adapted from Sarvazyan et al. (2008) and has been updated for the purpose of this dissertation.

In Ultrasound-Stimulated Vibro-Acoustography (USVA), two confocal ultrasound transducers and a hydrophone are used. The interference of two confocal ultrasound transducers at slightly different frequencies (low kHz range) causes a vibration at the focus. The amplitude or phase of the shear wave is recorded by a hydrophone and used to form an image. USVA has been applied on the human breast in vivo and shown capable of detecting microcalcifications in the breast regardless of the breast density.

In this chapter, the application of the HMI system for human tumor detection and mapping of ex vivo is presented. The HMI technique may significantly improve the ability to detect breast tumors and may also provide data that is needed to better understand and improve diagnosis. The advantages of using HMI include; 1) it is a non-contact method to measure relative tissue stiffness, 2) it
provides quantitative measurement of viscoelasticity parameters using an inversion method, 3) measurements are repeatable regardless of the material size, and 4) it facilitates the excitation over a wide range of frequencies.

6.2 Methods

6.2.1 Experimental protocol

Specimen collection and handling of post-surgical breast tissues were approved by the Institutional Review Board (IRB) board of Columbia University and informed consent was obtained from all enrolled patients. HMI imaging was performed in 17 breast masses from 17 human subjects (with mean age of 51 years, median age of 52 years, and age range from 30 to 80 years). Candidates were recruited from the Department of Surgery, Irving Pavilion of the Columbia Presbyterian Medical Center, with the following categories; 1) the candidates had lesions that were palpable or visible on the sonogram and/or mammogram and warranted biopsy. 2) The candidates would undergo lumpectomy, i.e., removal of the tumor (the “lump”) and some perilesional tissue.
Table 6.1- Summary of clinical data on benign-malignant breast lesion differentiation by elasticity imaging techniques.

<table>
<thead>
<tr>
<th>No.</th>
<th>Method</th>
<th>Number of analyzed lesions</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Citation</th>
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<td>TE*</td>
<td>15 total</td>
<td>55.5</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>USE*</td>
<td>52 Malignant 59 Benign</td>
<td>88.5</td>
<td>89.8</td>
<td>51</td>
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<tr>
<td>3</td>
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<td>95.0</td>
<td>141</td>
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<tr>
<td>4</td>
<td>USE</td>
<td>49 malignant 59 benign</td>
<td>91.8</td>
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<td>127</td>
</tr>
<tr>
<td>5</td>
<td>MRE*</td>
<td>38 malignant 30 benign</td>
<td>95.0</td>
<td>80.0</td>
<td>142</td>
</tr>
<tr>
<td>6</td>
<td>USE</td>
<td>88 total</td>
<td>96.0</td>
<td>61.0</td>
<td>143</td>
</tr>
<tr>
<td>7</td>
<td>TI*</td>
<td>34 malignant 76 benign</td>
<td>94.4</td>
<td>-</td>
<td>144</td>
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<tr>
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<td>96.0</td>
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<td>90.0</td>
<td>-</td>
<td>146</td>
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<td>97.5</td>
<td>48.0</td>
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<td>MRE</td>
<td>39 malignant 29 benign</td>
<td>100</td>
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<tr>
<td>15</td>
<td>SSI*</td>
<td>4 malignant 11 benign</td>
<td>-</td>
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</table>

TE* - Transient Elastography, USE* - Ultrasound Elastography, MRE* - Magnetic Resonance Elastography, TI* - Tactile Imaging, and SSI* - Supersonic Shear Imaging.
Seventeen post-surgical breast specimens (5 benign, 9 malignant and 3 normal (i.e., mostly fat, glandular and parenchyma) tissues were collected immediately after surgery. The tumor and its perilesional tissue were obtained approximately 30 minutes after excision and prior to histopathology. In the case of lumpectomy with needle localization procedure, the specimens were imaged following surgery by sonography or mammography prior to HMI. Immediately upon excision, these tissues were placed in a sterilized container and submerged in degassed phosphate buffered saline (PBS) solution for the HMI experiments. The specimens were carefully handled and placed within a gelatin matrix to protect and contain them, while simulating the surrounding breast.

The specimens were palpated to verify the tumor location (target). The HMI-1 system (Chapter 4) was then placed over the specimen as a reference position prior to scanning. All specimens were scanned using the HMI technique and the findings were compared to mammography or sonography, and then histopathology, findings. None of the breast specimens were fixed by formalin until after imaging. Orientation of the specimen was provided by the surgeon. The specimens were then returned to pathology for diagnosis. The histology image of the specimen was not available due to the patient confidentiality agreement and the Institutional Review Board (IRB) of Columbia University regulation.
6.2.2 Experimental setup

Figure 6.2 displays the experimental setup used for imaging post-surgical breast tissue specimens. The HMI-1 system was used to generate an oscillatory radiation force. For tissue mapping, the transducers were moved in a 2D raster-scanning fashion at a step size equal to 1 mm in the lateral and elevational directions using a computer-controlled positioner (Velmex Inc., Bloomfield, NY, USA). The scan planes were adapted based on the specimen’s size and the acoustic intensity \( I_{\text{ppp}} \) was equal to 31.4 W/cm\(^2\) with a duration of 0.2 s at each point. In order to determine the optimal AM frequency, a linear chirp test within the range of 10 to 30 Hz was applied to investigate the optimal vibration frequency for the highest mechanical contrast between the tumor and the surrounding regions.

The tissue motion was estimated using a 1D cross-correlation technique on the acquired RF signals. Chapter 4 details the displacement estimation used in this experiment. The tissue stiffness was qualitatively estimated according to the HMI displacement, and the HMI image of the relative stiffness could accurately detect the tumor contained in the breast mass.
Figure 6.2: A block diagram of the experimental setup for tissue mapping using HMI-1 system. (a) pulse-echo transducer, (b) FUS transducer attached to (d) the 3D positioner and (c) a coupling cone filled with degassed distilled water. (g) the specimen rested between (f) 2 gels and was submerged in (e) degassed PBS. In order to reduce possible reflections, i.e., specular reflection, (h) an absorber was placed on the bottom of the glass beaker.
6.2.3 Evaluation of HMI safety

It is essential to ensure that the HMI system remains safe and produces no adverse effects when used for imaging. The physical effects of ultrasound can be generally categorized as either mechanical or thermal.

Mechanical effects are radiation forces leading to streaming in fluids and stress at tissue interfaces (cavitation). Cavitation denotes the formation of gas bubbles at high negative pressures. The Mechanical Index (MI) is used as an indicator to predict such mechanical effects. The MI is a quantity related to the potential for tissue damage based on mechanical effects during an ultrasound examination and is defined by \(^\text{(6.1)}\),

\[
MI = \frac{p_-}{\sqrt{f}}.
\]

where \(p_-\) is the peak negative pressure and \(f\) is the center frequency of the imaging transducer. The US Food and Drug Administration (FDA) regulate that for ultrasonic imaging \(^\text{149}\) the MI must be under 1.9, so that the patient risk is minimized.

The acoustic pressure of HMI was measured using a 0.2-mm needle hydrophone (Precision Acoustics LTD, Dorchester, Dorset, UK). The positive and negative peak pressures at the focus had the same absolute amplitude of 3.15 MPa
due to the applied sinusoidal force. Also, both pressures were within the linear region. The acoustic pressure at the focus was estimated using a derating factor of 0.3 dB/cm/MHz at 4.5 MHz frequency and 1.5 cm depth to account for the attenuation through the gel. The calculated acoustic pressure at the focus was then equal to 1.98 MPa and the mechanical index (MI), after taking into account the derating factor of 0.3 (MI\(_{0.3}\)), was equal to 0.93, i.e., below the FDA limit of 1.9.

The thermal effect of HMI implies heating of tissue upon the tissue’s absorption of the ultrasonic wave. Heat can also be produced at the transducer surface. The thermal effect measurement is termed as thermal index (TI). TI is defined as the ratio of the power used to the power required to cause a maximum temperature increase of 1°C. A thermal index of 1 indicates a power causing a temperature increase of 1°C.

The potential temperature rise (\(\Delta T\)) during the HMI application can be estimated by solving the bioheat transfer equation. Here, the bioheat transfer equation is assumed to have a linear distribution of thermal sources without the convection and conduction effects:\(^{125,150,151}\)

\[
\Delta T = \frac{2 \alpha I}{\gamma_v} t, \tag{6.2}
\]

where \(\Delta T\) is the temperature rise in °C, \(I\) is the acoustic intensity (\(I_{\text{ppa}}\)), \(\gamma_v\) is the volume specific heat for tissue (4.2 J/cm\(^3\)/°C), \(\alpha\) is the absorption coefficient of
tissue (0.16 Np/cm, i.e., 0.3 dB/cm/MHz at 4.5 MHz), and $t$ is the duration of the oscillatory force (approximately 400 ms).

The spatial peak-pulse average intensity ($I_{ppa}$) of HMI was calculated numerically using Eq. 4.7 with an instantaneous acoustic pressure ($p_0$) of 1.98 MPa, the gel density ($\rho$) was assumed to be 1000 kg/m$^3$, $c$ of 1540 m/s and $t$ of 400 ms. The calculated acoustic intensity ($I_{ppa}$) was equal to 31.4 W/cm$^2$, which is also below the FDA $I_{ppa}$ limit of 190 W/cm$^2$. For the applied oscillatory force in a single location, the anticipated peak temperature rise ($\Delta T$) was estimated to be up to 0.96°C. The reader should note that the reported temperature rise is the maximum possible value since the heat convection and blood perfusion were not taken into account.

6.3 Results

Figure 6.3i displays the HMI image in the benign tumor case, where the tumor was more spherical and the margins of the tumor were better defined as indicated in red (high displacement in the perilesional tissue). For the malignant tumor, the shape was irregular (Figure 6.3ii) and surrounded by glandular tissue and fat. In both cases, the tumor masses were palpable, but only the fibroadenoma, i.e., the
benign tumor (Figure 6.3i), was detected on both the mammogram and the sonogram. The specimen size was equal to approximately 10 mm x 10 mm x 25 mm. Figure 6.3i shows the high uniformity of the benign tumor (darker, middle region) surrounded by normal tissue (lighter region).

Figure 6.3: Ex vivo 3D HMI imaging: i) Benign and ii) IDC with (a) carcinoma (red) and (c) fat (yellow) surrounded by (b) glandular (orange) tissue.

An invasive ductal carcinoma (IDC) specimen was obtained after mastectomy. The 3D HMI image of the IDC specimen (Figure 6.3ii) spans over a larger area, as the specimen provided was much larger than in the benign case (Figure 6.3i). The HMI image shows multiple regions of lower displacement (in red) relative to the
glandular (orange) and fat (yellow) tissue, indicating several regions of lower stiffness (i.e., carcinoma). All of these regions combined into a large area of approximately 40 mm x 40 mm x 30 mm.

Figure 6.4a shows a sonogram that was performed on a 31-year-old female to follow up the previously obtained biopsy results. The patient had no history of breast cancer and was diagnosed to have a benign fibroadenomas. The tumor mass is visible as a hypoechoic region and measured to approximately 6.7 mm x 4 mm x 12 mm in Figure 6.4a. A needle localization was performed to guide the surgeon during surgery. After the tumor mass was removed, it was imaged by ultrasound (Figure 6.4b) and the size of the specimen is approximately 8.7 mm in length. The hyperechoic line denotes the needle. The HMI image indicates that the size of the suspected tumor (Figure 6.4c – blue, $D_{HMI}$ below 20 μm) was equal to about 12 mm in length and 4 mm in width surrounded by fat and glandular breast tissues (Figure 6.4c – yellow and red, $D_{HMI}$ above 30 μm ). On the HMI image (Figure 6.4c), the tumor is shown in blue because it is stiffer compared to the surrounding tissue.
Figure 6.4: (a,b) B-mode image of the breast tissue before and after the surgery, respectively. ‘N’ denotes needle used to guide the surgeon during the surgery. (c) HMI image plane (30 x 30 mm²), cyan (≈20 μm) indicated a stiffer region that denoted fibroadenoma (indicated by a dashed black lines). Yellow-red (> 35 μm) denoted a softer region, breast tissue.
Figures 6.5a displays a mammogram of a 58-year-old female with family history of breast cancer. The biopsy confirmed an invasive ductal carcinoma (IDC). The tumor was palpable, but could not be distinguished from normal breast tissue on the mammogram. The breast was heterogeneously dense, which may lower the sensitivity of mammography, so that the tumor mass was difficult to interpret in Figure 6.5c.

However, the tumor was visible on the B-mode image after it was excised, with an acoustic shadowing as indicated in Figure 6.5b. A hyperechoic region indicated the location of the biopsy needle. A biopsy needle was placed prior to surgery as a guidance tool for the surgeon to identify the lesion and confirm its successful removal (denoted by ‘N’ in Figure 6.5c). The tumor size was 3.7 mm in diameter. HMI image of breast tumor is shown in Figure 6.5d. The specimens consisted of a piece of pale yellow breast tissue measuring approximately 20 mm x 20 mm x 16 mm.

A sharp stiffness contrast between the tumor and the surrounding breast tissues was obtained (Figure 6.5d). The tumor (stiffer region) is indicated in light blue with an area of approximately 10 mm by 10 mm on the HMI image. The surrounding tissue is softer (indicated by yellow/red).
Figure 6.5: (a) Mammogram of the breast tissue before surgery. (b) B-mode image and (c) X-ray of the post-surgical breast tissue with ‘N’ indicates a biopsy needle. (d) HMI image plane (20 x 20 mm²), cyan (≈ 20 μm) indicated a stiffer region that denoted the invasive ductal carcinoma (IDC) (delineated by a dashed black line). Yellow-red (> 35 μm) denoted a softer region, breast tissue.
Figure 6.6a shows a sonogram of a 49-year-old female diagnosed with IDC. The lesion was palpable and was denoted as a hypoechoic region on the B-mode image. The diagnosis was confirmed with biopsy, and lumpectomy was performed to remove the lesion without needle localization. The size of the tumor was measured to have an approximate area of 20.2 mm x 13.1 mm.

Figure 6.6b displays a B-mode image of an invasive ductal carcinoma (IDC) after it was removed. The tumor diameter was approximately 16.7 mm. The HMI image of the same specimen in Figure 6.6c displays the breast tumor (IDC) delineated by two dashed black lines and is stiffer (indicated as blue) compared to the surrounding tissue (denoted as yellow/red). The stiffer region is approximately 14 mm x 22 mm.
Figure 6.6: (a) B-mode of the pre-surgical breast specimen. The size of the tumor was 20.2 mm x 13.1 mm. (b) B-mode of the post-surgical breast specimen. (c) HMI image, breast tumor (invasive ductal carcinoma, IDC) is delineated by dashed line, and is stiffer (blue) compared to surround tissue (yellow/red).
Figure 6.7a shows a follow up sonogram of a 70-year-old female after she received chemotherapy for 7 months. The patient had a 60 mm mass prior to chemotherapy but was no longer evident after the treatment. The tumor size was reduced to 12.5 mm x 0.38 mm as shown in Figure 6.7b. The mammogram was not presented here because it did not show enough contrast between the tumor and the surrounding breast tissue because of the high density of the breast. An ultrasound-guided biopsy was performed to diagnose the lesion, and the result was found to be IDC. A lumpectomy with needle localization was performed to remove the remaining lesion.

The HMI image (Figure 6.7b) indicated the possible location of the lesion (blue, $D_{HMI}$ is below 25 μm) surrounded by breast tissues (yellow and red, $D_{HMI}$ is above 45 μm). The size of the lesion in HMI was measured to approximately 12 mm by 4 mm, which is in good agreement with the B-mode image before the surgery, i.e., 12.5 mm by 3.8 mm (Figure 6.7a).
Figure 6.7: (a) B-mode image of the breast tissue before surgery. (b) HMI image plane (60 x 60 mm$^2$), cyan (≈20 μm) indicated a stiffer region that denoted the invasive ductal carcinoma (IDC) (delineated by a dashed black line). Yellow-red (>35 μm) denoted a softer region, breast tissue.
Figure 6.8a displays a mammogram of a 64-year-old female diagnosed with invasive lobular carcinoma (ILC). There is a cluster of calcifications in the right central breast indicated by a white line, i.e., 22.24 mm, in Figure 6.8a. The placement of the clip was confirmed with post-biopsy mammograms. A lumpectomy procedure was performed and a clip was used to guide the surgery to confirm the removal of the tumor. Figure 6.8b shows the X-ray of the specimen after surgery. A clip was attached to the lesion (tumor) as shown in Figure 6.8b.

Figure 6.8c shows the HMI image of the post-surgical breast tissue shown in Figure 6.8b. The HMI image plane is 30 mm by 40 mm and it is indicated by a dashed white box in Figure 6.8b. The yellow/red region (i.e., $D_{HMI}$ was above 40 μm) indicates soft breast tissue. The blue region (i.e., $D_{HMI}$ was below 20 μm) indicates a stiffer region, i.e., tumor (or, denoted by two dashed black lines in Figure 6.8c). The tumor size in HMI was found to be equal to approximately 16 mm by 10 mm that is smaller than the one measured in mammogram. The HMI image shows good contrast between the tumor and breast tissues as seen using mammography.
Figure 6.8: (a) mammogram of the breast tissue before surgery. (b) X-ray of the breast specimen. ‘N’ is the needle biopsy attached to the tumor. (c) HMI image with blue (stiffer region), denotes breast tumor (invasive lobular carcinoma, ILC) and yellow/red denotes normal breast tissue (softer).
Figure 6.9 displays the summary of the HMI displacement of post-surgical breast specimens, i.e., fibroadenomas, invasive lobular carcinoma (ILC), invasive ductal carcinoma (IDC), ductal carcinoma *in situ* (DCIS), and normal breast tissues. The normal breast tissues (9 specimens in total) were obtained from mastoplasty cases (n=3) and perilesional tissues (n=6). Each bar represents mean ± SD, calculated based on 3 x 3 x 3 mm³ volume, and n indicates number of the patients for each type.

![Graph showing displacement amplitude](image)

**Figure 6.9:** Comparison between each tumor types and normal tissues. Each bar represents mean ± SD, calculated based on 3 x 3 x 3 mm³ volume.
Figure 6.10 shows the comparison between benign, malignant and normal breast tissues. Malignant tumors include ILC, IDC and DCIS cases. The significance is determined by an unpaired Student’s t-test with a confidence interval of 90%. There are significant differences between benign and normal (p-value < 0.005). There is also a significant difference between malignant and normal (p-value = 0.008). It means that about 0.8% chance of obtaining the same HMI displacement for both benign and malignant tumors (Figure 6.10).

Figure 6.10: Comparison between general categories, i.e., benign, malignant and normal tissue. Each bar represents mean ± SD, calculated based on 3 x 3 x 3 mm³ volume. Significance was determined using an unpaired Student’s t-test with confidence interval of 90%.
Other mechanical parameters, such as shear modulus and viscosity that is related to the tissue mechanical properties, may provide additional measures for noninvasive classification of breast tumors as benign or malignant. Our laboratory has developed a method (Appendix A) to quantify viscoelastic parameters of soft tissues, and this method has been validated in tissue mimicking gels 20).

The HMI-2 system was used in this experiment instead of the HMI-1 system, because the 3.3-MHz phased-array transducer could provide 2D images that better visualize of the probed tissue and also image the shear wave propagation. A 4.5-MHz FUS transducer was used to generate an oscillatory radiation force at low frequencies (from 5 to 40 Hz at 5 Hz increment). The preliminary results from the two specimens are shown in Figure 6.11.

The HMI measurements of $G'(\omega)$ and $G''(\omega)$ (storage modulus and loss modulus, respectively, details in Chapter 3 and Appendix A) for invasive ductal carcinoma (IDC) and normal tissue were compared in Figures 6.11 and 6.12. The specimens’ size was approximately 50 mm in diameter. The HMI data provided a good estimate of the magnitude of the storage modulus ($G'$) and relative viscosity ($G'/G''$) over a wide range of frequencies. Significant differences in $G'$ between normal tissue and IDC by almost two orders of magnitude indicates that IDC is
stiffer than normal tissue. This behavior is consistent throughout the frequency range used in this experiment.

![Graph showing storage modulus (G') for normal tissue and invasive ductal carcinoma (IDC) over a wide frequency range (10 to 80 Hz).](image)

Figure 6.11: Measured storage modulus (G') for normal tissue and invasive ductal carcinoma (IDC) over a wide frequency range (10 to 80 Hz). IDC has significantly stiffer than normal tissue 22.

The measured relative viscosity (G''/G') is shown in Figure 6.12. The normal tissue increased at high frequencies (this behavior indicated that the normal breast tissue was viscous). Figure 6.12 shows that the IDC is less viscous than normal breast tissue. In conclusion, from this preliminary results, IDC is found to be stiffer and less viscous than normal tissue, i.e., softer and more viscous. However, additional breast samples are needed to validate this result.
Figure 6.12: Measured relative viscosity ($G''/G'$) for normal tissue and invasive ductal carcinoma (IDC) for a frequency range from 10 to 80 Hz. Normal tissue is more viscous than IDC.

6.4 Discussion

In this study, the potential of the HMI technique as a non-contact method of mapping and quantifying tissue stiffness was demonstrated. An unpaired Student t-test with 90% confidence interval was performed to quantify whether different pathologies could be differentiated based on the HMI images. The normal breast tissue (mostly fat, glandular and parenchyma) can be differentiated from the
benign and malignant at high statistical significance (p < 0.005). The benign tumors experienced a lower average displacement (i.e., stiffer) than the malignant tumors at lower significance (p = 0.008). Thus, the HMI image could categorize tumor types based on their relative stiffness.

These findings are not in agreement with Krouskop et al. 121, where benign tumors were found to be softer than malignant tumors. Three possible explanations can be provided as follows; 1) different specimen geometries and handling were used. 2) The boundary conditions of the two techniques used to measure displacement by HMI and elastic modulus by mechanical testing are significantly different. In mechanical testing, difficulties were encountered in holding the specimens effectively to avoid slippage, because of the low friction between the material of the indenter and wet soft specimens. One of the solutions was to apply large pre-compression; however, the specimens were then deformed and rapidly damaged. Although friction could be minimized by using a different type of indenter with a higher friction, large compression remains necessary and this result in damage of the tissue structure. On the other hand, in HMI, the displacement was measured at the localize region where force was applied deep inside the specimens at no pre-compression.
Figures 6.11 and 6.12 show that HMI-2 system could provide a quantitative measurement, e.g., shear and elastic moduli, of tumor and normal breast tissues. This method has been developed in our laboratory \(^{20}\) (Appendix A), and could potentially be used to enhance differentiation of the breast tumor type in the future.

The results presented in this chapter show that HMI could map and differentiate abnormal tissues, such as breast tumors, particularly in the case where lesions may not be detectable using conventional imaging methods. The clinical objective of this technique is to provide an alternative method to detect small tumor masses (~3 mm in diameter) and help distinguish benign from malignant so that reduce the number of biopsy. In the next chapter, the feasibility of HMI as a method for visualization of the targeted region and monitoring of the thermal ablation will be presented.
Chapter 7

IN VITRO AND EX VIVO SONICATION AND MONITORING OF THERMAL ABLATION USING HMIFU

7.1 Background

In practice, surgery aims at resecting solid tumors with adequate surrounding normal tissues. Surgery itself may pose significant risks to patients. Some of the known risks associated with the surgical removal of solid tumors include complications from anesthesia, infections, immune suppression, and long recovery periods. Therefore, if a minimally invasive technique can properly treat the same tissue volume, the outcome of disease-free survival should be at least equivalent. The development of minimally invasive methods as alternatives to surgery for
localized tumor destruction has been of interest in the field of tumor treatment. Such techniques include radio-frequency ablation (RFA), cryoablation and laser ablation that are described in chapter 2. In this chapter, the technique of High-Intensity Focused Ultrasound (HIFU) is described.

Treatment with HIFU is often considered to be a promising technology within minimal, or non-invasive, therapy segments of medical research. Recently, the potential of HIFU as a non-invasive surgical tool has been demonstrated in several clinical applications for tumor treatment of the prostate, liver, kidney, pancreas, breast, and uterine fibroids \textsuperscript{152-154}. Magnetic Resonance Imaging (MRI) and ultrasound imaging-based methods have been tailored for HIFU treatment guidance in order to accurately identify the target volume. The following sections describe the HIFU method along with its available image guidance techniques. Lastly, the potential of the HMIFU system proposed in this dissertation work as a non-invasive thermal treatment is presented.
7.2 Overview of HIFU

Over the past 60 years, researchers have investigated the potential of utilizing HIFU (or, focused ultrasound (FUS)) as a non-invasive tumor treatment modality. HIFU generates acoustic waves propagating through soft tissues and depositing high levels of acoustic energy mainly at the localized focus of the transducer. High levels of acoustic energy at the localized focus can cause temperature elevation that is sufficient to initiate coagulative necrosis in tissues (thermal lesions), while the surrounding tissues remain relatively intact. The damaged cells are then removed only by the body’s natural mechanisms (phagocytosis).

The HIFU capability of causing irreversible damage in tissues has received attention from researchers as a technique for non-invasive tumor treatment. Lynn et al., and Lynn and Putnam introduced the first application of HIFU for local modification of brain function in live animals, e.g., cats and dogs. Fry et al., and Lele continued the development of HIFU therapy and produced lesions deep in the brain tissues of cats and monkeys. Fry et al., applied this technique in an attempt to treat patients with Parkinson’s disease after removing parts of their skulls to avoid aberration and attenuation of the acoustic wave through the skull. Despite the initial success, early enthusiasm for HIFU was hindered, because
an effective drug L-dopa, was introduced for Parkinson’s patients at the same time.

Coleman and Lizzi demonstrated the use of HIFU for ophthalmology application, such as treatment of glaucoma, retina, capsular tears, and certain ocular cancers. A large-clinical study was performed to treat glaucoma using HIFU in 1982. There were 880 patients treated, and the result showed that 79.3% of the patients had a sustained lowered intra-ocular pressure after 1 year. Although HIFU had received Food and Drug Administration (FDA) approval for ophthalmological applications, the HIFU application in this field was displaced by laser-based alternative. Laser surgery was introduced concurrently, and it was perceived as being simpler to use.

The application of HIFU was later introduced for tumor treatment in 1956. HIFU has continued to be an area of intense research, well into the present day in a number of other therapeutic applications, such as lithotripsy, thrombolysis, acoustic hemostasis, and tissue erosion. The objective in the HIFU treatment of tumors is to deliver the energy required to necrose the target tissue by means of both thermal and mechanical mechanisms. The thermal mechanism entails the bulk heating of tissue due to its viscous absorption of acoustic energy. Mechanical effects include acoustic cavitation, or radiation pressure.
delivery of heat can be based on focusing the acoustic wave through a spherically-curved transducer, lens, or phased array transducer. HIFU has been shown capable of inducing complete tumor necrosis for the treatment of disease, such as human prostate cancer \textit{in vivo} \textsuperscript{180-184} and uterine fibroids \textsuperscript{185-188}. Despite apparent success in the field of HIFU, its application as a non-invasive surgical tool poses many challenges. For instance, the precise control of the HIFU beam location into the target tissue and visualization of the relevant physical effects, such as quantification of the extent of tissue damage, and accurate measurement of thermal dose to achieve desired tissue destruction over the required tissue volume, can be very difficult.

The work presented in this dissertation is mainly focused on the application of HIFU as a thermal therapy in tumor necrosis. Hence, it is important that a method to be developed for the measurement of temperature rise as a function of time is compared between multiple exposures. The area of thermal coagulation observed during heating was modeled by Sarapeto and Dewey \textsuperscript{189} that distinguish between different temperature effects on tissues. The thermal dose was used as a measure for predicting the extent of tissue damage caused by heating exposure. It was derived from \textit{in vitro} cell survival studies during hyperthermia, when the cells were exposed to temperatures from 43°C to 50°C \textsuperscript{190}. Damianou \textit{et al.} (1994) also
reported that a lethal dose was found at and beyond 43°C. The effect of heating and the equivalent heating time at 43°C in hyperthermia can be described by the following empirical model, i.e.,

\[ t_{43} = \sum_{t=0}^{t_{\text{final}}} k^{(43-T_t)} \Delta t, \]  

(7.1)

where \( t_{43} \) is the equivalent time at 43°C [min], \( T_t \) is the average temperature during heating \( (\Delta t) \) [°C], and \( k = 0.5 \) above 43°C for many tissues and \( k = 0.25 \) below 42°C.

The temperature rise corresponds to the transformation of the acoustic energy into thermal energy as the ultrasound waves are absorbed by the tissue. Thus, the extent of resulting tissue damage depends on the exposure time and temperature rise. The information relating the effectiveness of treatment to the onset of adverse effects in tissue is essential, especially in clinical cases.

The thermal dose model (Eq. 7.1) was used in section 7.4.1.4 to verify the onset of coagulative necrosis during ablation. The comparison between the calculated threshold \( (t_{43}) \) and the recorded temperature values, along with the estimated HMI displacements at different sonication durations are presented in section 7.4.1.4. Currently, several imaging modalities have been proposed to guide and monitor HIFU treatments as described in the next section.
7.2.1 Imaging modalities for monitoring HIFU

Two major limitations of HIFU surgery include the difficulty of monitoring the changes in temperature and tissue mechanical properties, and the lack of ability to optimally control precise thermal exposure upon lesion formation. Several non-invasive temperature monitoring methods for HIFU therapy have been proposed for estimating thermal dose, such as Magnetic Resonance Imaging (MRI), MR thermometry, MR elastography (MRE), conventional ultrasound, ultrasound thermometry, ultrasound elastography, and acoustic radiation force methods.

Magnetic Resonance Imaging (MRI) has been used clinically for noninvasive guidance and monitoring of HIFU therapies because it provides quantitative spatial maps of the induced temperature elevation at high spatial resolution \(^{195-197}\). This technique is known as MR-guided Focused Ultrasound Surgery (MRgFUS) \(^{186,195,198-203}\). The focused ultrasound transducer is positioned above the cancerous tissue so that its beam can ablate the latter, while magnetic resonance imaging (MRI) is used to detect the tumor, monitor the treatment, and assess the lesion site. MRgFUS has been used clinically to treat breast neoplasia and uterine fibroids \(^{188,204}\), and the treatment has been shown successful with only few complications, e.g., minor skin burn, residual pain, and longer recovery period.
In the Magnetic Resonance Elastography (MRE) technique, tissue mechanical properties (e.g., shear modulus) are mapped based on the observed phase shift of the MR signal, in response to an external mechanical vibration. Real-time imaging and treatment monitoring using the MRE technique was applied on ex vivo bovine muscle. Wu et al. showed that the MRE was capable of quantitatively estimating the mechanical properties (shear modulus) during heating and ablation. Furthermore, the MRE results indicated that the ablated tissues were stiffer than the normal tissues. Despite these initial successes of HIFU-surgery monitoring using the MRI, its high-cost and low temporal resolution may result in the confinement of this promising treatment to large research centers worldwide. More precisely, the HIFU technique is in itself a low-cost treatment technique but currently requires a high-cost monitoring device, i.e., an MRI system. Ultrasound imaging, on the other hand, is inexpensive, extensively available, and capable of real-time imaging and capturing of blood flow as well as the movement of the body’s internal organs such as respiration.

Conventional B-mode ultrasound imaging has been widely used to monitor the progress of thermal therapy. However, this imaging technique is not optimal to identify coagulative tissue because the tissue echogenicity does not change
significantly during thermal therapy. The coagulative tissues can only be detected when cavitation, or boiling, occurs due to the high bubble concentrations that cause hyperechoic appearance of the treated area. However, bubble occurrence is usually unpredictable and therefore unreliable for HIFU treatment monitoring.

Ultrasound elastography has been shown capable of monitoring tumor ablations in rabbit paraspinal skeletal muscle in vitro, bovine liver in vitro, and human prostate in vivo due to its sensitivity to stiffness changes. Souchon et al. showed a 40% to 50% decrease in average strain at the treated region of human prostates in vivo after HIFU application. However, the required external tissue compression may complicate positioning and reference during treatment planning and guidance.

Non-invasive ultrasound thermometry is based on the fact that the sound speed in non-fatty tissues increases with temperature. The focused ultrasound (FUS) transducer generates localized heating at the focus, producing changes in the tissue characteristics. These changes are related to the sound speed and tissue thermal expansion that cause echo shifts in the backscattered ultrasonic signal. These findings have been validated theoretically and experimentally in non-invasive thermal therapy.
Several recent studies used the acoustic radiation force for monitoring HIFU ablation. The acoustic radiation force was used to induce brief, mechanical excitations locally, and the resulting tissue response was followed while RF data were collected during tissue relaxation (Acoustic Radiation Force Impulse; ARFI) or shear wave propagation, e.g., in ARFI, and Supersonic Shear Imaging.

Nightingale et al. proposed an Acoustic Radiation Force Impulse (ARFI) imaging method. ARFI induces a localized radiation force and images the tissue response immediately after force application, using a modified linear array transducer. The tissue displacements are estimated using a speckle tracking technique. Lizzi et al., and Fahey et al., applied the ARFI method to monitor the formation of lesions during HIFU therapy in vitro and ex vivo. After HIFU treatment, the results showed that the displacement was much smaller in the coagulated tissue than in the normal tissue.

Bercoff et al. utilized the supersonic shear imaging (SSI) technique to monitor HIFU therapy. SSI was used to induce shear waves using an acoustic radiation force at different locations in the tissue and image the resulting wave propagation at high frame rates (up to 5000 images/s). A separate FUS transducer was used to generate lesions in ex vivo tissue. The HIFU and imaging sequences were interleaved, and a set of wave propagation data was performed during ablation.
Thus the lesion stiffness changes could be detected. One limitation of the aforementioned techniques for HIFU therapy monitoring is that, currently, they cannot monitor tissue displacement during force application due to the use of pushing and tracking beams at different times and locations.

7.3 HMIFU system for monitoring its thermal treatment

HMIFU uses amplitude modulated (AM) wave to generate an oscillatory force (i.e., vibration) deep inside tissue. This vibration acts like a palpation to sense the tissue geometry and mechanics (such as size, shape, stiffness, or location) through tissue deformation. Different from other radiation force techniques, in HMIFU, the oscillatory force is applied for simultaneous ablation and monitoring treatment. The HMIFU system, thus, has a real-time feedback of the associated variation in tissue stiffness change during the application of the force.

The HMIFU system has two separate applications. The first application is to assess tissue stiffness changes before (pre-) and after (post-) thermal ablation. The FUS transducer is driven by AM pulses at low acoustic powers, but sufficient to
induce adequate motion with an associated negligible temperature rise i.e., within FDA limit. Chapter 6 details the HMIFU safety when used for tissue assessment (or, diagnosis). In its second application, a higher acoustic power was applied for generating and monitoring thermal ablation. The resulting synchronous monitoring system has the ability to follow and identify the areas of necrosis indicated by tissue stiffness change.

The fact that speed of sound changes as a function of temperature has been reported to induce an artifactual shift of the backscatter ultrasonic signal or, a temperature-induced displacement. The estimated displacement at the focal region is proportional to its temperature rise. In order to study the effect of sound speed changes during heating, a type T thermocouple (MT-29, Physitemp Instruments, Inc., Clifton, New Jersey, USA) with a diameter of 0.33 mm was inserted into the ex vivo porcine liver to independently monitor temperature changes before, during and after heating. The FUS transducer was moved by a computer-controlled positioner (Velmex Inc., Bloomfield, NY, USA) and it was driven at a low acoustic power level. Once the thermocouple was inserted into the tissue, the positioner was used to locate the tip of the thermocouple, which was indicated by both a rapid temperature increase from the thermocouple reading and high amplitude signals acquired by the confocal pulse-echo transducer. Thus,
the FUS beam was approximately aligned with the tip of the thermocouple. A digital thermocouple reader (HH506A, Omega Engineering, Stamford, CT, USA) was used to record the temperature at 1 s intervals.

As shown in Figure 7.1, the HMIFU system could follow the changes in the sound speed when an AM wave was applied for heating, which was simply indicated by a linear trend (slope = 0.63 mm/s) in the displacement (dotted line in Figure 7.1a). This linear trend was successfully separated from the oscillatory HMI displacement (Figure 7.1b). The displacement and sound speed are linearly proportional and the sound speed is known to vary linearly with temperature. An advantage of the HMIFU method is thus the filtering of the oscillatory displacement from the linear speed-of-sound-dependent (artifact) displacement for potential separation and measurements of both sound speed and stiffness effects. Although HIFU damage mechanism is related both thermal and mechanical effect, such as cavitation, cavitation effect is beyond the scope of this dissertation.
Figure 7.1: (a) oscillatory displacement with a sound speed effect during heating and (dotted line) is a linear fitting. (b) Oscillatory displacement after the speed of sound effect is removed and low-pass filtering.

The application of this technique for simultaneous tumor localization as well as real-time monitoring of its ablation based on the associated tissue stiffness changes 7 \textit{in vitro} bovine livers and 5 \textit{ex vivo} porcine livers are discussed in the following section. In this study, liver tissue is first selected to represent breast tissue because of their similarity in tissue properties (attenuation/absorption) and structure (firmness). In addition, the lesion formed in the liver can simply be observed and confirmed by its color and stiffness change.
7.4 *In vitro* and *ex vivo* study

There are two types of HMIFU systems used in this experiment, i.e., HMIFU-1 and HMIFU-2 (Chapter 4). The imaging transducer in the HMIFU-1 system is a single-element pulse-echo transducer, and a phased-array imaging transducer in the HMIFU-2 system. Chapter 4 details the advantages and disadvantages together with data acquisition and signal processing methods for both systems.

7.4.1 HMIFU-1 experimental procedure

7.4.1.1 Tissue sample preparation

Experiments were performed in seven (n = 7) *in vitro* bovine liver specimens with three (m = 3) different locations in each liver, i.e., 21 locations in total. Sample tissues were degassed for at least 30 minutes in phosphate buffered saline (PBS) solution prior to each experiment. The average size of all seven bovine liver specimens used was 120 x 50 x 12 mm$^3$ (L x W x D). Each specimen was then placed into a glass beaker and submerged in PBS. A hot plate/stirrer (Corning PC-420, Corning, NY, USA) was positioned underneath the glass beaker to maintain a
uniform temperature of 37°C throughout the entire tissue specimen to simulate human body temperature.

7.4.1.2 Experimental setup

For this study, a continuous AM wave was used during heating, whereas a pulsed AM signal was applied without heating, i.e., before and after heating. A pulsed AM was used to avoid any significant temperature rise during HMIFU monitoring. Temperature effects between a pulsed AM and a continuous AM wave were tested on a homogeneous gelatin \(^{18,120}\). The temperatures were measured at various positions near the focus for a 40-s exposure at the low acoustic intensity \((I_{\text{split}})\) of 231 W/cm\(^2\) (Figure 7.2).

This experiment was conducted using the same setup as seen in Figure 7.3, with the gel having a stiffness of 40 kPa \(^{120}\) placed inside the container (Figure 7.3g). The application of a pulsed AM (10 cycles with AM frequency at 25 Hz and a 36 % duty cycle) shows a relatively constant temperature \((\Delta T_{\text{max}} \approx 1.2^\circ\text{C})\) (Figure 7.2b) compared to that induced by the continuous AM wave (Figure 7.2a).
Figure 7.2: Comparison of temperature rise between two types of AM waves, a continuous AM wave (a) and a pulsed AM wave (b), both generated at the same low acoustic intensity ($I_{pta}$) of 231 W/cm². Temperatures were measured near the focus of the FUS beam for duration of 40 s. The application of a pulsed AM shows a relatively constant, low temperature ($\Delta T \sim 1.2^\circ$C) compared to that induced by the continuous AM wave.

This result is expected because the acoustic energy generated from a continuous AM is constantly absorbed by the gel and transformed into heat. On the other hand, by using a pulsed AM, the gel is allowed to cool down for 0.71 s between pulses. Thus, a pulsed AM is more suitable for tissue assessment before and after heating.
The experimental setup is shown in Figure 7.3. A 4.68 MHz FUS transducer (Riverside Research Institute, New York, NY, USA) was first used while the 4.5 MHz FUS transducer was still being manufactured. The focal region of the 4.68 MHz FUS transducer had an ellipsoidal shape with the long axis parallel to the ultrasound beam. The -6 dB dimensions were 4.0 mm in the axial direction and 0.5 mm in the lateral direction. The 4.68 MHz FUS transducer was used to generate the acoustic radiation force using a low-frequency (AM) wave at 25 Hz.

A 7.5 MHz pulse-echo transducer (Panametrics, Waltham, MA, USA) with a diameter of 12 mm was placed through the void center of the FUS transducer, with the beams of the two transducers being confocal. A pulser/receiver (Panametrics 5051PR, Waltham, MA, USA) was used to drive the pulse-echo transducer at a PRF of 5.4 kHz. An analog bandpass filter (Reactel, Inc., Gaithersburg, Maryland, USA) with cutoff frequencies of $f_{c1} = 5.84$ MHz and $f_{c2} = 8.66$ MHz was used to filter out the high-frequency focused beam and its harmonics on the acquired RF signals (Chapter 4).
Figure 7.3: A block diagram of the experimental setup for therapy. (a) pulse-echo transducer, (b) FUS transducer attached to (d) the 3D positioner and (c) a coupling cone filled with degassed distilled water. (f) the specimen rested on (g) a membrane and was submerged in (e) degassed PBS. In order to reduce possible reflections, i.e., specular reflection, (h) an absorber was placed on the bottom of the glass beaker. (i) A hot plate/stirrer maintained the surrounding temperature at 37°C. Two thermocouples, t₁ (thermocouple probe with a diameter of 1.5 mm) and t₂ (a needle thermocouple with a diameter of 0.33 mm), were used to measure the external and internal specimen temperatures, respectively.

A silicone rubber/absorber (McMaster-Carr, Dayton, NJ, USA) was placed under the specimen to further reduce the specular reflection from the bottom of the glass container. The filtered RF signals were captured at 80 MHz at a 14-bit digitization rate (CS14200, Gage Applied Technologies, Lachine, Canada). RF
signals were acquired continuously before, during and after heating at approximately 1.11 s intervals. The estimated HMI displacements ($D_{HMI}$) before and after heating could follow the relative stiffness change during heating. In this experiment, the heating duration was selected within the range of 5 s to 30 s.

### 7.4.1.3 Data acquisition and signal processing

Sequences of M-mode frames were acquired before, during and after heating (Figure 7.4). Each M-mode frame (Figure 7.4c) contained 600 RF signals and had a duration of $\tau = N/PRF = 111 \text{ ms}$, where $N$ was the number of RF signals ($N = 600$) and $PRF$ was 5.4 kHz; thus, each frame spanned over two periods of AM oscillations (> 5 Hz) (Figure 7.5). The time interval ($\Delta \tau$) between two consecutive M-mode frames was approximately 0.71 s. The effective frame rate was equal to 1.4 frame/s. The HMI displacement ($D_{HMI}$) was estimated using 1D cross-correlation on the acquired RF signals with a window length of 0.47 mm and 90% window overlap (Chapter 4 for details). The HMI displacement was estimated at all depths and plotted with respect to depth.
These steps were applied for every M-mode frame acquired at different times and the resulting depth-dependent HMI displacements were plotted against time. The time needed to generate the entire M-mode frames was approximately 30 s. The variation in displacements can be clearly visualized in the M-mode maps, which were used to detect the tissue coagulation (Chapter 7.4.1.4). The HMI

Figure 7.4: (a) the HMIFU technique consists of two types of AM waves: 1) a continuous AM for heating (th) and 2) a pulsed AM for before (tb) and after (tf) heating. The acoustic pressures for A1 and A2 were 5.3 MPa, and 2.6 MPa respectively. (b) detailed representation of the dashed rectangle in (a). Enlargement of (c) is shown in Figure 7.5.
displacements were then normalized by the pressure amplitude (A₁ or A₂; Figure 7.4).

Figure 7.5: (a) AM wave was applied throughout imaging and sonication process. (b) M-mode frames were acquired at 1.11 s intervals, i.e., the frame rate is equal to 1.4 frame/s. (c) A M-mode frame (τₙ for n = 1, 2, ..., j, ..., M; M = total treatment time), has a period of 111 ms so that each frame has at least 2 periods of AM oscillations. (d) M-mode displacement.
7.4.1.4 Results

Figure 7.6 depicts the HMI displacement (obtained from 1 sample) over time with blue, red and green lines indicating the baseline, heating and cooling periods, respectively. The baseline period duration was 5 s in all cases. The heating time was varied from 10 s to 30 s (Figure 7.6 a to c), and a minimum of 10 s heating was selected to make sure that a lesion was formed. The cooling period was 10 s in all cases. The liver tissue was ablated using an AM beam that induced both an oscillatory motion and heating at the same time. This oscillatory displacement increased at the beginning during heating, indicating tissue softening. When the heating was sustained, the displacement decreased, which indicated that the tissue had become stiffer; in other words, a lesion had been formed. This pattern of tissue displacement change reflects heat-induced structural changes in the tissue. This was verified during cooling, when the displacements were slightly lower compared to the baseline (before heating) period. These results showed that, beyond a 10-s heating, the lesion size was large enough to display sufficient changes in the estimated displacement to clearly indicate tissue stiffening.

The transition of the HMI displacement from the end of heating to cooling, was indicated by the grey region in Figure 7.6 (a and c). These regions indicate that at the end of heating, the temperature rapidly decreased from 50°C, during which
tissue was still coagulating, and therefore displacements further decreased from 18 to 8 μm.

Figure 7.7 shows the corresponding M-mode images of the HMI displacement and the gross pathology after lesions were formed for the 10-s, 20-s and 30-s exposures. The tissue depth is displayed along the vertical axis, and the observation time is displayed along the horizontal axis. The HMI displacements increased immediately after thermal treatment started, reached a peak and gradually decreased over time, indicating lesion formation.
Figure 7.6: HMI displacement ($D_{HMI}$) versus time. Blue (---), red (→) and green (...) lines indicate the baseline (5 s), and heating i.e., (a) 10 s, (b) 20 s, and (c) 30 s and cooling periods (10 s).
Figure 7.7: HMI displacement ($D_{HMI}$) M-mode (a, c and e) and the corresponding gross pathology images of liver tissues (b, d and f) for 10 s, 20 s, and 30 s heating.
Figure 7.8a portrays HMI displacements at the focal region for a 50-s heating. A lesion was formed approximately 4 mm from the liver surface as seen in Figure 7.8b. Each line in Figure 7.8a represents HMI displacement at a specific location in the lesion. The distance between neighboring lines was 0.5 mm. The HMIFU system was shown capable of following lesion formation during prolonged heating. Tissue softening occurred within the first 15 s (from 27 to 38 μm), followed by a rapid decrease in displacement indicating coagulation onset (~18 μm) at t = 18 s, and tissue hardening beyond 30 s. The heating was stopped after 50 s and the liver was sectioned to verify the lesion formation (Figure 7.8b).

Figure 7.8: (a) HMI displacements at the focal region versus 50-s heating time. Each line represents one location in the lesion region that is approximately 4 mm from the liver surface (b). The distance between each line was 0.5 mm. (b) gross pathology image of liver tissue after ablation.
Figure 7.9 was derived from data shown in Figure 7.7. The average HMI displacements at the focal region during thermal exposure were plotted as a function of temperature (lower axis) and thermal dose (upper axis). These graphs indicate that the total displacement increase was at least 10 μm during the initial heating phase (up to 45°C).

A linear least squares method was applied on the HMI displacement and temperature data, and the estimated slope of the temperature-displacement graph. The slope in the initial heating phase was approximately 0.8 μm/°C, and reversed upon and during lesion formation (~ -0.8 μm/°C), as shown in Table 7.1.

<table>
<thead>
<tr>
<th>Sonication duration (s)</th>
<th>Initial heating phase (μm/°C)</th>
<th>During lesion formation (μm/°C)</th>
<th>Lesion size</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.87 ± 0.082</td>
<td>-0.88 ± 0.080</td>
<td>2.0 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.4 ± 1.14</td>
</tr>
<tr>
<td>20</td>
<td>0.85 ± 0.12</td>
<td>-0.71 ± 0.16</td>
<td>3.4 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.1 ± 1.15</td>
</tr>
<tr>
<td>30</td>
<td>0.80 ± 0.14</td>
<td>-0.79 ± 0.19</td>
<td>4.7 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.4 ± 0.39</td>
</tr>
</tbody>
</table>

*Average and SD for 7 locations (n = 7) for each sonication.
Figure 7.9: The relationship between temperature (or thermal dose, $t_{43}$, in min) vs. displacements for (a) 5 s, (b) 10 s, (c) 20 s, and (d) 30 s heating. Tissues started to soften at the initial heating (less than 47°C, a-d) and became stiffer (b-d) at the temperatures above 47°C or $t_{43} = 1$ min.
Figure 7.10b shows an example of the temperature and HMI displacement profiles for 40 s heating. A similar profile has been reported by 207, i.e., the displacement change in Figure 7.10b from higher (region I) to lower (region II) values with increasing temperature. The arrow shows the time sequence of the tissue displacement changes during heating and cooling.

This effect can be understood as an indication that the tissue mechanical properties have changed during sustained heating. The peak and reversal of the slope could be detected by HMI during thermal ablation. This is important, especially since each region, or type of tissue/organ, has a different thermal absorption coefficient, and thus changes in the slope may occur at different temperatures and heating times.
Figure 7.10: (a) temperature vs. time during, 5 s (◊), 40 s heating (○), and 30 s cooling (●) in an in vitro liver sample. (b) HMI displacement vs. temperatures. (c) HMI displacement vs. time.
7.4.2 HMIFU-2 experimental procedure

7.4.2.1 Experimental setup

A 4.5 MHz FUS transducer (Imasonic, Voray sur l'Ognon, France) was used to generate the acoustic radiation force using a low-frequency AM wave at 15 Hz. The acoustic intensity level was the same as that used in the previous HMIFU-1 experiments. In this study, an 8-s exposure time was applied on 5 \textit{ex vivo} porcine livers.

Figure 7.11: A block diagram of the experimental setup for therapy: (a) phased-array imaging transducer, (b) FUS transducer attached to (d) the 3D positioner and (c) a coupling cone filled with degassed distilled water. (f) the specimen rested on a (g) membrane and was submerged in (e) degassed PBS. In order to reduce possible reflections, i.e., specular reflection, (h) an absorber was placed at the bottom of the glass beaker. (i) a hot plate/stirrer maintained the surrounding temperature at 37°C.
A 3.3-MHz phased array imaging transducer (Ultrasonix Medical Corporation, Richmond, Canada) was inserted into the FUS transducer; hence, the beams of the two transducers were confocal. The consecutive RF frames were acquired at a sampling frequency of 40 MHz and a frame rate of 288 frames/s. A digital lowpass filter cutoff frequencies of 4.2 MHz was used to filter out the FUS beam.

Figure 7.12a shows an envelope-detected RF frame before ablation. The liver specimen was placed on top of a gel, so the force was positioned in the middle of the liver at a depth of 50 mm. Figure 7.12b depicts an unfiltered RF frame during heating, where the bright region at the center of the B-mode image corresponds to the beam interference between the imaging and the FUS beams. After filtering, the hyperechoic region located inside the specimen at a depth of 50 mm was clearly seen (Figure 7.12c). Since the bright region was not present before heating, as shown in Figure 7.12a, this indicates that a lesion was formed during heating.
Figure 7.12: (a) RF frame before ablation, (b) unfiltered RF frame (during ablation) and (c) filtered RF frame. Note that the FUS beam was successfully removed in (c). (i) a liver specimen with a thickness of approximately 12 mm and (ii) a gel used to elevate the *ex vivo* liver.

7.4.2.2 Results

Figure 7.13 shows the oscillatory displacement during the entire heating procedure; the peak-to-peak displacement amplitudes decreased after 3 s due to lesion formation (as indicated by arrow in Figure 7.13).
Figure 7.13: Oscillatory displacement throughout the entire heating. Note that the peak-to-peak displacement amplitude decreased as a lesion was formed (a), and the lesion was stiff, indicated by lower displacement in (b). The treatment was completed as indicated by lower signals in (c).

If we enlarged the region at the initial heating, between 1.75 and 2.25 s, the peak-to-peak displacement amplitude was estimated for approximately 30 μm (Figure 7.14a). The peak-to-peak displacement amplitude decreased to about 5 microns because the tissue became stiffer shown in Figure 7.14b (between 4.75 and 5.25 s). This shows that a lesion was formed, which was confirmed by a gross pathology image (Figure 7.15).
Figure 7.14: Oscillatory displacement (a) at the beginning of heating and (b) towards the end of the heating process. The peak-to-peak displacement amplitude decreased by 25% due to lesion formation.

Figure 7.15: Gross pathology image shows evidence that the lesion was formed deep inside the liver.
7.5 Discussion

In this section, the potential of HMIFU for thermal ablation monitoring was demonstrated. The experimental results presented herein indicate the feasibility of using displacements induced by a radiation force for real-time monitoring of thermal lesion formation. The HMIFU system was used with two objectives in mind. The first objective was to obtain a baseline of the HMI displacement before and after thermal ablation. In this case, a low level of acoustic power (i.e., lower than the threshold of thermal ablation) was used to drive the FUS transducer, sufficient to induce adequate motion without significant heating (Figure 7.4 - ‘t_b’ and ‘t_f’). The second objective entailed a FUS beam applied to induce thermal ablation (Figure 7.4 - ‘t_h’).

At the beginning of heating, high temperature elevation caused the HMI displacement increase gradually (slope ≈ 0.8 μm/°C), reached a peak and sometimes a plateau (depending on the duration of heating), followed by a gradual decrease (slope ≈ -0.8 μm/°C), which indicated that lesions were formed. The slope of the displacement variation with temperature in the initial heating phase (0.8±0.11 μm/°C) was reversed upon and during lesion formation (-0.79±0.14 μm/°C) as shown in Table 7.1; this effect could be used as an indication
that the tissue mechanical properties change during heating and ablation. The peak and the changes in slope could be detected by the HMI during thermal ablation. This is important, especially since each region or type of tissue/organ has distinct thermal properties, and thus the changes in the slope may occur at different temperatures and heating times.

7.6 Conclusion

The tissue stiffness changes caused by heating were dictated by the induced temperature rise and duration exposure. In other words, when the thermal dose exceeded a certain threshold, irreversible mechanical tissue changes occur and a lesion is formed. The attenuation increase beyond coagulation \(^{228}\) was ignored here. However, it did not interfere with the results reported since increased attenuation leads to smaller radiation force, and thus displacement decrease beyond coagulation.

Wu et al. \(^{207}\) have shown that the tissue stiffness initially decreases with temperature. When coagulative necrosis occurs, e.g., at a temperature beyond 50°C, the tissue stiffness increases (i.e., smaller displacement amplitude) \(^{224,229}\). Experimental results presented in this dissertation have shown a similar pattern of
displacement (or, relative tissue stiffness) variation with temperature during heating. In tissues, heat-absorption changes typically occur beyond 50°C ($t_{43} = 2 \text{ min;}$ Eq. 7.1) $^{228}$.

The HMIFU system has a simple transducer design that produces a steady oscillatory force at the focus within the tissue for planning and monitoring of its HIFU treatment. This technique has the ability of simultaneous monitoring temperature-induced variation in tissue mechanical properties in a fully integrated system in real-time. By monitoring the tissue response in real time, the relative tissue stiffness changes during HIFU treatment and the onset of adverse effects in tissues can be reliably detected. The in vivo feasibility of HMIFU is presented in the next chapter.
Chapter 8

IN VIVO FEASIBILITY OF HMIFU

8.1 Introduction

The feasibility of the HMIFU technique for monitoring thermal treatment, and tissue assessment before and after ablation has been presented in Chapter 7. In this chapter, the validation of two HMIFU systems for monitoring changes in the tissue mechanical properties during thermal therapy is demonstrated in a transgenic breast cancer murine model in vivo. The first application is to assess tissue stiffness changes before and after thermal ablation. In its second application, HMIFU is used to simultaneously generate and monitor thermal ablation. The resulting synchronous monitoring system has the ability to follow and identify the areas of necrosis. The results presented in this chapter show that the HMIFU technique has the ability to monitor tissue stiffness changes during heating while simultaneously
probing and forming lesions. Pathology and histology assessment are given to confirm the outcome of the treatment. In the *in vivo* study, a transgenic breast cancer murine model was chosen because it was readily available through our collaborators and could be used to study the effect of HIFU treatment on tumors *in vivo*.

### 8.2 *In vivo* murine study

The transgenic mouse model of breast cancer was provided by our collaborator, Dr. Thomas Ludwig of Department of Clinical Pathology and Cell Biology at the Columbia University. These mice were treated by HIFU using HMIFU system. Histopathology was performed to verifying the ablation with the HMIFU treatment on the tissue. *In vivo* experiments were performed in 15 mice with mammary tumors and 11 out of 15 mice were successfully treated using the HMIFU systems (Table 8.1 for detailed summary).
Table 8.1- Summary of HMIFU experiments *in vivo*

<table>
<thead>
<tr>
<th>System</th>
<th>Treatment</th>
<th>Sacrifice time</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMIFU-1†</td>
<td>11 mice*</td>
<td>5th or 6th day</td>
</tr>
<tr>
<td>HMIFU-2‡</td>
<td>4 mice*</td>
<td>within 1 h</td>
</tr>
</tbody>
</table>

* 2 died during experiment
† Refer to section 8.2.3 for results
‡ Refer to section 8.2.4 for results

8.2.1 Transgenic murine model of breast cancer

Transgenic mice with mammary tumors (female, average weight of 32 ± 2 g), carrying conditional alleles for Brca1, Brca2, Bard1, and p53 were treated with HMIFU. More than one mammary tumor mass could develop in each mouse. The mammary tumors were invasive adenocarcinomas that formed bulky, round, solid regions, invading the surrounding fat and underlying pectoral muscle. These mammary tumors can have high metastatic potential and typically grow up to 3 to 10 mm in diameter. The reader is prompted to Ludwig *et al.* 2001 and Shakya *et al.* 2008 for further details in the development of the transgenic mouse model used in this study.
8.2.2 Histopathology

Pathological and histological analysis was performed to observe coagulation necrosis, i.e., cell death and viable cells, in the tumor region after HIFU treatment. Pathology and histology procedures are provided in the next paragraph.

For pathology, a 30 ml fresh 1% of 2,3,5-Triphenyltetrazolium Chloride (TTC) was prepared. The TTC solution is sensitive to light, so it has to be placed in a protected glass container and has to be kept at 4°C. The treated mice were euthanized, and the mammary tumors were subsequently excised and sectioned throughout the entire axial depth with thickness of 1 mm (Figure 8.1). The sectioned tissue were completely covered in the TTC solution (note that this process was performed in a dark room) and kept at 37°C for approximately 15-20 minutes. The TTC was carefully aspirated. TTC staining resulted in viable tissue stained in “brick-red” as the tetrazolium salts react with the dehydrogenases in the viable cell; while the necrotic cells were shown in pale color. The stained tissue slices were photographed.
The sectioned tissue was then kept in 10% phosphate-buffered formalin (pH = 7) at low temperature (4°C) for 24 hours. The specimen was then washed in distilled water for two minutes, kept in 70% isopropanol, and immediately submitted to histology. The specimens were embedded in paraffin, sectioned in 5-μm thick slices (with 50 μm space in between each sections) and stained with hematoxylin and eosin (H&E). Tissue blocks were sampled from the central and peripheral edges of the treated region and surrounding untreated breast tumor for assessing the effect of FUS ablation.
8.2.3 HMIFU-1 experimental procedure

All animal experimental procedures were approved by the Columbia University Institutional Animal Care and Use Committee (IACUC). A timeline of the experimental procedure was presented in Figure 8.2, and detailed as follows: 1) the mice were anesthetized with isofluorane (1-5% mixture with 100% oxygen) and their pectoral areas were depilated; 2) The mice were placed on a heating platform (THM100, Indus Instruments, Houston, TX, USA) to maintain constant body temperature (Figure 8.3h,i); 3) the FUS transducer was positioned over the tumor without requiring the animal to be submerged underwater. A water chamber was placed over the animal’s chest in order to provide an acoustic wave propagation path to the body, maintain a normal body temperature and prevent thermal effects at the skin level (Figure 8.3f).
Figure 8.2 Timeline of the HMIFU-1 study. HMI imaging and thermal treatment can be repeated in phase (A)

The water chamber was supported by a circular clamp, which was attached to a ring stand in order to keep the clamp stable and avoid any chest compression. There was approximately $10 \pm 2$ mm at the interface of the water chamber and the skin. Degassed ultrasound coupling gel was placed to ensure acoustic wave transmission (Figure 8.3g). The water chamber did not obstruct the normal respiratory patterns of the mice. The water chamber was kept at standard room temperature, i.e., between $20^\circ$C and $25^\circ$C; 4) the HMIFU-1 system was moved using a raster-scanning method (shown in Figure 8.4) at a step size of 0.5 mm before and after treatment with the acoustic intensity ($I_{spta}$) of 231 W/cm$^2$ and an insonation duration of 0.16 s. The same raster-scanning method (as shown in Figure 8.18e) with a step-size of 0.5 mm was used to ablate the tumor with an acoustic intensity ($I_{spta}$) equal to 1086 W/cm$^2$. 
Figure 8.3: A block diagram and schematic of the experimental HMIFU setup. (a) the 7.5 MHz pulse-echo transducer, (b) the 4.5 MHz FUS transducer was mounted on (d) the computer controlled positioner, (c, f) degassed water and (g) degassed ultrasound gel. (e) a ring stand was used to hold the water chamber. (i) the mouse was placed on (h) a heating platform.

The total treatment lasted approximately 90 min and was dependent on the tumor volume. Bupivacaine, a local anesthetic drug combined with epinephrine (i.e., 0.25% Bupivacaine and 1:100,000 epinephrine) was diluted 1:10 with saline. The mixture was injected subcutaneously to the treatment sites for 0.004 ml/g. Bupivacaine was co-administered with epinephrine to prolong the duration of its
effect. This combination drug was expected to reduce any possible pain for up to 24 hours after treatment.

Figure 8.4: (a) side view and (b) top view of (c) mammary tumor. (d) an imaging plane for before and after ablation. (e) a smaller ablation region was placed in the middle in order to observe necrotic region within the same tumor mass. (g) top view of (e) the ablated mammary tumor. (f) an example of a lesion.

8.2.3.1 Results

Figure 8.5 shows HMI images using the HMIFU-1 system. The tumor mass was detected within a region of 10 x 10 mm². In order to compare to the untreated region within the same tumor mass, in this experiment, only a 4 x 4 mm² region was treated.
Figure 8.5: HMI displacement imaged before and after treatment. The tumor region is delineated by dotted black line, T. The treated region (ROI) is approximately 4 x 4 mm².

Figure 8.5 indicates the high uniformity of the tumor (cyan-blue region) surrounded by normal tissue (yellow-red region) 26. The approximate dimension of the tumor mass on the HMI image was 9 mm in diameter (Figure 8.5; delineated by dotted black line, T). The treated region size was equal to about 4 mm in diameter (Figure 8.5, ROI). The treatment region was stiffer, indicated by a decrease in the displacement amplitude (dark blue).
Figure 8.6: Each bar represents mean ± SD from 9 mice (n = 9), calculated based on the ablated region, before (mean = 23.16 μm, SD = 4.75) and after lesion (mean = 12.72 μm, SD = 4.67) was formed. Significance was determined using an unpaired Student's t-test. P-value = 0.0122. The mice were sacrificed 5 (or 6) days after treatment for histopathology.

Comparison of the treated region from untreated region using an unpaired Student’s t-test showed statistical significance (p-value = 0.0122) (Figure 8.6). This finding is also confirmed by gross pathology and histology shown in Figure. 8.7 and 8.8, respectively. After treatment, the mice were observed to exhibit normal behavior with normal eating habits, and minor skin burns. The mice were euthanized 5 or 6 days after treatment.
Figure 8.7 depicts the gross pathology of the treated mammary tumor. The white-pale regions denote a necrotic region, while the surrounding tissue (pink region) indicates viable cells.

![Figure 8.7: (a) Gross pathology of the treated mammary tumor. The Triphenyl Tetrazolium Chloride (TTC) staining was used to indicate viable cells. Tetrazolium salts react with the dehydrogenases in the viable cell indicated by “red-pink” while (c and d) the necrotic cells were unstained and appeared in pale color. (c) the necrotic region in gross pathology image was in good agreement with HMI image (b).](image)

This finding is also confirmed by H&E staining, as seen in Figure 8.8. The dark purple regions indicate dead cells as denoted as ‘D’ in Figure 8.8, which are surrounded by viable cells (pink region, ‘T’ in Figure 8.8). The HMI images have shown good agreement with the size of the ablated region from gross pathology...
and H&E. HMIFU could thus map the mammary tumor and correctly ablate the targeted region with a desired dimension.

![Figure 8.8: H&E histology images with 12.5x magnification. Scale bar = 500 μm. Ablated regions are indicated as dark purple (D) in (a) and (b). Non-ablated regions (or, viable cells) are shown in pink (T).](image)

8.2.4 HMIFU-2 experimental procedure

The HMIFU-2 system was used to treat two mice with large tumors (approximately 15 mm in diameter). The 3.3-MHz phased-array transducer implemented in HMIFU-2 system could provide 2D tumor imaging. In addition, the HMIFU-2 system has the capability of acquiring RF data continuously during
the HIFU treatment (< 6 s). The acoustic intensity ($I_{top}$) at the focus during treatment was equal to 1086 W/cm². The total exposure time for each lesion was selected to be 13.5 s in order to generate a larger lesion. There were eight lesions generated in each mouse, i.e., a total of 16 lesions for the two tumors studied. The experimental setup is shown in Figure 8.9.

Figure 8.9: A block diagram and schematic therapy. (a) the 3.3 MHz phased-array imaging transducer, (b) the 4.5 MHz FUS transducer, (c,f) degassed water and (g) degassed ultrasound gel. The HMIFU-2 system was mounted on (d) the computer controlled positioner. (i) the mouse was placed on (h) a heating platform. (e) a ring stand was used to hold the water chamber.
8.2.4.1 Results

Figure 8.10 shows the HMI displacement overlaid onto a B-mode image during heating. The targeted tumor region is shown in Figure 8.10b. The focal region of the FUS transducer is located at the center (0 mm) and at a depth of 45 ± 2 mm. The average HMI displacement at the focal zone was approximately equal to 20 μm (Figure 8.10; in cyan-blue) move away from the transducer.

Figure 8.10: Grayscale B-mode images of a mammary tumor during heating *in vivo*. The colorbar denotes HMI displacement in μm. (a) the water chamber membrane and (b) the targeted region (mammary tumor). The HMI displacements were averaged over a 3 x 3 mm² region (black square). Dashed white line denotes a central RF line.
Figure 8.11 shows an example of the M-mode images in the tumor region of approximately 8 mm in diameter with overlaid HMI displacement. The oscillatory displacement was estimated with respect to the initial frame. Tissue motion during heating is visible through the variation in displacement amplitude, which is denoted by the alternating orange and blue in Figure 8.11a, or light green and blue in Figure 8.11b. The motion away from the transducer is represented in orange (or, light green) and the motion away from the transducer is denoted in blue.

Figure 8.11: Grayscale M-mode image of the tumor region with overlaid color-coded HMI displacements during heating. Colorbars denote displacement amplitude in μm. (a) at initial heating phase (between 0.5 and 0.9 s) and (b) after lesion formation (between 10.5 and 10.9 s).
Figure 8.11a shows that, at the focal region (a depth of 45 ± 2 mm) the tumor tissue was initially displaced by at least twice the motion in Figure 8.11a (HMI displacement is approximately 30 μm) than that after lesion formation (HMI displacement is approximately 20 μm in Figure 8.11b). Note that the size of the displaced tissue at the focal region is approximately 2 mm in length, which is well correlated with the axial focal spot size of the FUS beam (2 ± 0.5 mm).

In order to estimate the relative tissue stiffness change during heating, the resulting displacement around the focal region was then averaged over a 3 x 3 mm² region (indicated as a black square in Figure 8.10) and plotted in time (Figure 8.12) 27. This respiratory motion mainly remained along the axial direction since the animal was in the supine position and was filtered out.

Figure 8.12: Oscillatory displacement variation during heating in vivo. The duration of exposure was approximately 13.5 s.
The oscillatory displacements when heating started (Figure 8.13a) and after the lesion was formed (Figure 8.13b) were plotted side by side to assess the qualitative tissue stiffness change during heating. After the lesion was formed, the relative tissue stiffening was identified by a 30% decrease in the peak-to-peak displacement amplitude. The change in the peak-to-peak displacement amplitude during heating implied that the tissue coagulated 25.

![Graphs](a) ![Graphs](b)

**Figure 8.13:** Oscillatory displacement within the selections in Figure 8.12, i.e., at 0 to 0.3 s and 10.5 to 10.8 s of heating, respectively.

The average HMI displacements from 16 lesions before and after lesion formation were equal to 27.34 ± 1.34 μm and 20.98 ± 1.82 μm, respectively (Figure 8.14). Comparison of HMI displacement from the two cases using an unpaired
Student’s t-test showed a statistically significant difference in HMI displacement before and after lesion formation (p < 0.001).

![Graph showing HMI displacement before and after treatment](image)

**Figure 8.14:** HMI displacement from in two mice, i.e., 16 lesions total, before lesion formation (between 0 and 6.5 s); mean = 27.34 μm, SD = 1.34, and after lesion formation (between 6.6 and 13.5 s); mean = 20.98 μm, SD = 1.82. There is a statistically significant difference in HMI displacement before and after lesions were formed (p < 0.001).

The two mice were euthanized immediately after HIFU treatment. The H&E histology images of the first mouse are shown in Figure 8.15. The solid tumor nodules were separated by stroma, and each contained densely packed tumor cells (blue-purple) with the white arrows denoting erythrocytes (red blood cells). However, the histology image (H&E stain, original magnification ×100) of the
Ablated mammary tumor shows that the cells became damaged and necrotic as indicated by hemorrhage (as denoted by yellow arrows in Figure 8.15b) and fine fibrous strands were outlined by adipose tissue (white lobes, ‘L’).

Figure 8.15: Histology images with 100x magnification. Scale bar = 100 µm. (a) non-ablated tumor cells (=C); white arrows denote erythrocytes (red blood cells). (b) ablated mammary tumors; damaged and necrotic cells indicated by hemorrhagic regions (yellow arrows) and fine fibrous strands outlined by adipose tissue (white lobes, L).
8.3 Discussion

In this in vivo study, an acoustic intensity \((I_{puls})\) of 1086 W/cm² and duration of exposure of 13.5 s for each location were used to treat two large tumors. The overlaid HMI displacement images (Figure 8.10 and 8.11) offer important, complementary information to the B-mode or M-mode images during heating and can be used as feedback information to adjust the thermal dose when implemented in real time. Figure 8.15 shows that the tumor cells were damaged by the appearance of hemorrhage and necrotic cells in the ablated region in contrast with the non-ablated tumor cells.

A limitation of this study lies in the absence of temperature measurement during thermal treatment. The placement of a thermocouple within the tumor will generate additional heat accumulation due to the beam interference with the thermocouple. This would influence the associated displacement estimation. Hence, simultaneous temperature measurement within the tumor during treatment was excluded in this study. However, results from in vitro experiment (Chapter 7) have shown good agreement between the HMI displacement profiles and the temperature variation at various exposure durations. The ex vivo experimental results also showed that HMIFU could detect the onset of
coagulation necrosis indicated by increased in HMI displacement (tissue softening) when the heating was initially applied (Chapter 7). In this in vivo study, the HMI displacement did not increase at the exact time the heating started, i.e., tissue softening was not observed. One possible explanation is the effect of heating on tumor cells and the tumor vasculature. Since the tumor is highly vascularized, the cooling effect from perfusion (blood flow) could influence the heat distribution.

8.4 Conclusion

The HMIFU system (i.e., HMIFU-1 and HMIFU-2) could generate constant oscillatory force at the focus deep inside the tissue. This technique has the capability of real-time synchronous monitoring of temperature-induced variation in tissue mechanical properties in a fully integrated system. By monitoring the HMI response in real time, the relative tissue stiffness changes during thermal treatment can be reliably detected.

Statistical results (from a total of 11 mice) show significant increase in tissue stiffness after lesion formation (Figure 8.6). The HMIFU systems can thus be used
as a guidance tool for visualization of the targeted region and monitoring of the relative tissue stiffness changes during heating in real time so that the treatment procedure can be performed in a time-efficient manner. HMIFU could successfully 1) monitor the lesion formation, 2) control the lesion size and 3) timely stop the treatment upon lesion formation. HMIFU may thus constitute a cost-efficient and reliable alternative method for real-time monitoring of thermal ablation. However, the technique is not limited to ultrasound therapy and can be applied in conjunction with other thermal treatments, such as RF ablation. The tissue stiffness changes near the RF catheter tip (i.e., the treated tissue) could be monitored during the treatment.
Chapter 9

CONCLUSIONS AND FUTURE WORK

9.1 Conclusions

9.1.1 HMI for imaging

The potential of the amplitude-modulated (AM) harmonic motion imaging (HMI) technique as a non-contact method of mapping and quantifying tissue stiffness was presented in this dissertation. The advantages lie in the fact that HMI can; 1) image deeper-seated organs, such as liver, 2) provide better targeting because the tissue structure can be visualized (through HMIFU-2 system), 3) provide the relative stiffness contrast (based on displacement ratio) between the tumor and the surrounding normal tissues, and 4) quantify tissue viscoelastic parameters.
9.1.2 HMIFU for therapy

In this dissertation, the HMIFU was shown capable of monitoring and identifying the onset of the protein-denatured lesion formation based on the variation of the HMI displacements \textit{in vitro}, \textit{ex vivo}, and \textit{in vivo}. The overlaid HMI displacement offers important, complementary information to B-mode or M-mode images during heating, and can be used as feedback information to adjust the thermal dose once implemented in real time. This method could, therefore, be applied for real-time monitoring of temperature-related stiffness changes of tissues during HIFU or other thermal therapies so that the treatment procedure can be performed in a time-efficient and cost-efficient manner.

HMIFU as a non-invasive ablative therapy for small breast cancer or benign tumor could be a successful next step in breast conserving surgery. This treatment may come with great benefits with respect to patient comfort and cosmetic results. Moreover, HMIFU could monitor the treatment, and therefore could determine the completeness of the tumor destruction, while HMI imaging could be used to display changes in tissue before, during and after therapy.
9.2 Future work

Future HMI studies in cancer diagnosis would include 1) FEM models of tissue heating and thermocouple measurements to determine the thermal safety and accurate thermal dose delivery, 2) a large number of specimens to classify benign and malignant tumors based on quantitative measurements, i.e., displacement, for indirect measurement to indicate tissue stiffness and direct measurement for viscoelastic properties, as well as breast composition, density, tumor size, and depth.

Future studies in cancer treatment could focus on 1) the analysis of accuracy and precision of HMI lesion and heating patterns by a temperature simulation in a finite-element model that provides temperature distribution, e.g., a thermal dose contour, for tissue with various mechanical parameters, 2) the assessment of the FUS ablation on transgenic murine model of breast cancer. In the in vivo mice experiments, the displacement changes during tissue heating, which can be used as a feedback to control the thermal dose, will be further quantified. A respiration sensor will be used for respiratory-gated thermal therapy in order to target the tumors more accurately. A follow-up protocol will be designed for; 1) survival study, for instance the measurement of the tumor size using HMI images and
sonograms after treatment to determine if the tumor size is reduced or more treatments are needed to ablate the residual tumor tissue. 2) A combination of angiogenesis inhibitor and thermal ablation to treat the tumor. Angiogenesis inhibitor is used to prevent tumor growth by targeting and inhibiting the function of a natural protein called vascular endothelial growth factor (VEGF). 3) Observation of the post-treatment animal behavior (e.g., normal appetite, physical changes). 4) Sonogram and histology images will be used to further assess the success of the HMIFU treatment.

Inertial or stable cavitation might occur at subharmonic and/or higher harmonic frequencies during heating. These subharmonics and harmonics may have been removed after the filtering process. However, the possibility of stable or inertial cavitation occurring during heating should be studied.

For AM-HMI system improvements, such as: 1) implementation of a faster signal processing (using C++ program), so that the HMI measurements can be viewed together with the sonograms, 2) the use of a higher frequency imaging transducer for a better image resolution, 3) improvement of the ultrasound system firmware to provide higher frame rate, thus increase the sensitivity of viscoelasticity estimation that could be used as a complimentary screening process to differentiate benign and malignant. Incorporation of real-time feedback can be
used to control 1) the exposure levels during tissue diagnosis in the clinical setting and 2) sonication time during tumor ablation in vivo.

Finally, the future clinical application of HMI is to have one ultrasound system for detect the tumor in organs such as the breast, liver and pancreas. After the tumor is found, then the same device can be used to locally treat the tumor by generating, and simultaneously monitoring lesion formation over the targeted region with a precise and optimal treatment time (thermal dose) and controlled lesion size (Figure 9.1).

Figure 9.1: (a) detection of the area to be ablated (e.g., tumor) using the HMIFU technique. (b) ablation and simultaneous monitoring in order to map the response of the tumor overlapped on the B-mode for display together with displacement M-mode.
APPENDIX A

QUANTITATIVE MEASUREMENT OF VISCOELASTICITY USING HMI

Theory of viscoelasticity for HMI

In Chapter 4, we showed that the HMI displacement can successfully be related to the underlying Young’s modulus in a purely elastic material. However, soft tissues are typically viscoelastic and therefore a more complex analysis of the HMI technique is warranted. In this appendix, we present such a novel method that has been developed in our laboratory \(^{20}\) for measuring the linear viscoelastic properties of soft tissue over a wide range of frequencies. This hybrid method uses two different equations to solve for the elasticity and viscosity parameters. First, the shear wave propagation equation relates shear modulus \((G)\) to the wavenumber \((k)\) as follows:
where \( \rho \) indicates the tissue density and \( \omega_0 \) indicates the excitation frequency.

Second, the viscoelastic tissue can be described in terms of a complex shear modulus having both real and imaginary parts, i.e.,

\[
G = G' + iG'', \quad \text{and} \quad k = k' + ik''.
\]  

The shear storage modulus \( (G') \), the real part of \( G \), represents the elastic component, and the loss modulus \( (G'') \), the imaginary part of \( G \), represents the viscous component. \( k' \) and \( k'' \) represent the real and imaginary parts of the wavenumber \( (k) \). By equating both real and imaginary parts from Eq. (A.1) and Eq. (A.2),

\[
G' = \rho \omega^2 \frac{k'^2 - k''^2}{(k'^2 + k''^2)^2},
\]

\[
G'' = -2 \rho \omega^2 \frac{k'k''}{(k'^2 + k''^2)^2}.
\]

In order to solve for the two parameters of \( G \), i.e., \( G' \) and \( G'' \), two parameters HMI parameters are used, namely the wavenumber \( k \) and the ratio of \( G'' \) to \( G' \). The ratio \( (R) \) of \( G'' \) to \( G' \) is given by

\[
R = \frac{G''}{G'} = -\frac{2k'k''}{k'^2 - k''^2}.
\]
Solving for $k''$ by quadratic formula, the positive and negative solutions are as follows:

\[
k'' = k'(R + \sqrt{1 + R^2}) \quad \text{and} \quad (A.6)
\]
\[
k'' = k'(R - \sqrt{1 + R^2}), \quad (A.7)
\]

respectively. We have shown that the response of the probed tissue undergoes wave attenuation due to its viscous properties, so negative $k''$ is chosen to satisfy this physical condition. If we are able to estimate $k'$ and $R$ from experimental data, $G''$ and $G'$ can be calculated based on Eq. (A.5) and Eq. (A.7), respectively. The following sections explain how $k'$ and the ratio $(R)$ of $G''$ to $G'$ can be estimated from HMI displacement measurements.
Estimating the real part of wavenumber $k'$

Figure A.1(a) shows an incremental axial displacement map $u(x,z,t)$ during excitation. The excitation source is located at the center of the displacement map, and the shear wave propagates along the $x$ axis. Let $u(x,t)$ represent sinusoidal displacement over time,

$$\ddot{u}(x,t) = u_0 e^{i(\omega_0 + k' x)z},$$ \hspace{1cm} (A.8)

where $\omega_0$ and $k$ indicate the angular frequency and wavenumber, respectively, and assuming that the displacement is along the $z$ axis (axial displacement). We can replace wavenumber ($k$) in Eq. (A.8) with complex wavenumber ($k'$) in Eq. (A.2) so that $\ddot{u}(x,t)$ becomes,

$$\ddot{u}(x,t) = u_0 e^{i(\omega_0 - k' x)z} e^{k' x z}$$ \hspace{1cm} (A.9)

The temporal Fourier Transform $U(x,\omega)$ of Eq. (A.9) yields:

$$U(x,\omega) = 2\pi \delta(\omega - \omega_0) e^{-j k' x} e^{k' x}$$ \hspace{1cm} (A.10)

The phase $\Phi(x,\omega = \omega_0)$ at the excitation frequency ($\omega_0$) can be expressed as,

$$\phi(x,\omega = \omega_0) = -k' x.$$ \hspace{1cm} (A.11)

Finally, the real part ($k'$) of wavenumber, $k$, can be estimated by averaging the gradient of the phase $\phi(x,\omega = \omega_0)$ within the region of interest (ROI) close to the excitation region (Figure A.1(c)).
Figure A.1: (a) incremental displacement maps over time, (b) location of the excitation, and (c) map of $k'$.

Estimating the ratio ($R$) of $G''$ to $G'$

Dynamic Mechanical Analysis (DMA) measures the mechanical properties of materials while they are subjected to a periodic stress, usually applied sinusoidally. In DMA, we measure the stress ($\sigma$) versus time. The shear modulus $G(\omega)$ corresponds to the transfer function between stress and strain. From the amplitude and phase of the transfer function, both the shear storage $G'(\omega)$ and the shear loss $G''(\omega)$ moduli can be calculated. Let us consider the expression of the induced sinusoidal stress in one location, as $\sigma(\omega) = \sigma \, e^{i\omega \text{t}}$. The resulting strain will oscillate at the same frequency with delay $\Phi$, so that $\varepsilon(\omega) = \varepsilon \, e^{i\omega \text{t} - \Phi}$. The ratio between stress and strain therefore can be expressed by
\[
\frac{\sigma(\omega)}{\varepsilon(\omega)} = \frac{\sigma_o}{\varepsilon_o} e^{i\phi} = \frac{\sigma_o}{\varepsilon_o} \cos(\phi) + i \frac{\sigma_o}{\varepsilon_o} \sin(\phi) = G' + i G'' \tag{A.12}
\]

Thus,

\[
G' = \frac{\sigma_o}{\varepsilon_o} \cos(\phi) \tag{A.13}
\]

\[
G'' = \frac{\sigma_o}{\varepsilon_o} \sin(\phi) \tag{A.14}
\]

The ratio \(R\) of \(G''\) to \(G'\) can be expressed as,

\[
\frac{G''}{G'} = \tan(\phi) \tag{A.15}
\]

In the HMI experiment, the stress is related to the induced force, which is acquired and stored with the RF frames simultaneously. The shear strain, \(\varepsilon_{zs}\), is the derivative of displacement \(u(z)\) with respect to \(x\). A small ROI close to the focus is selected for each frame. The calculated shear strain for each ROI is averaged and plotted over time (Figure A.2(b)). The phase shift between the stress (input force (Figure A.2(c))) and the shear strain can thus be estimated.
Figure A.2: (a) shear strain image, where the ROI is chosen close to the excitation region, (b) averaged strain during excitation, (c) input force (stress), and (d) phase shift between averaged strain and input force.


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