Development and optimization of a clinical harmonic motion imaging system for breast tumor characterization and neoadjuvant chemotherapy response assessment

Niloufar Saharkhiz

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2023

© 2023

Niloufar Saharkhiz

All Rights Reserved

Abstract

Development and optimization of a clinical harmonic motion imaging system for breast tumor characterization and neoadjuvant chemotherapy response assessment

Niloufar Saharkhiz

Breast cancer is the most common cancer in women, accounting for almost one-third of new cancer diagnoses in the United States. The mortality rate has decreased by 42% since 1989 due to early diagnosis, improvements in imaging techniques and treatment regimens. Despite all the advances in imaging modalities, there is still a need for a non-invasive, non-ionizing, and low-cost diagnosis technique with high sensitivity and specificity to reduce the rate of invasive biopsies. For individuals diagnosed with locally advanced breast cancer and early-stage breast cancer, neoadjuvant chemotherapy (NACT) has become the standard of care. Pathologic complete response (pCR) is the ideal outcome of NACT, which is correlated with the prognosis and overall survival of the patients. The pCR is achieved in only about 15-20% of patients determined at the time of surgery; therefore, most patients receive a treatment that is not beneficial for them and has considerable side effects. Thus, early detection and monitoring of breast tumor response to NACT is critical for treatment planning and improving overall survival.

Ultrasound-based elasticity imaging techniques have gained interest in the clinic due to their potential to provide qualitative and/or quantitative information about tissue stiffness, which is presently not unachievable with standard ultrasonography. These techniques rely on the fact that a breast tumor's stiffness or Young's modulus is higher than that of the

surrounding normal tissues. In this dissertation, the clinical feasibility of a technique called harmonic motion imaging (HMI) for breast tumor classification, as well as for NACT response prediction and monitoring of solid tumors is investigated. HMI is an ultrasound-based elasticity imaging technique that evaluates the mechanical properties of the underlying tissues by inducing amplitude modulated (AM) displacements at a specific frequency.

First, we investigated whether HMI can characterize and differentiate human breast tumors based on their relative stiffness. We enrolled female patients with benign and malignant tumors and imaged them with a clinical HMI system. The malignant tumors were found to be associated with lower HMI displacements or higher stiffness than the benign tumors. Then, in order to verify our clinical findings, we estimated HMI displacements in the post-surgical breast specimens from the same subjects and compared them against the *in-vivo* estimations. Our findings indicated that HMI successfully differentiated tumors from the surrounding tissue in both *ex-vivo* and *in-vivo* conditions, with an excellent correlation between the results in the two different settings.

Second, we introduced and characterized a new HMI setup consisted of a multi-element focused ultrasound transducer (FUS) with electronic beam steering capability. Therefore, instead of mechanical translation of the HMI setup, the acoustic force could be electronically steered in the volumetric space to accelerate the data acquisition. A pulse sequence was developed to drive the HMI transducers assembly, the FUS and imaging transducer, using a single ultrasound data acquisition system to have a compact setup that is more applicable for clinical settings. The data acquisition was further improved by investigating the effect of AM frequencies on the quality of the HMI images and tumor detection. We found that higher AM frequencies are needed in order to improve the detection and characterization of small and stiff inclusions. On the contrary, soft and large inclusions are better resolved at lower AM frequencies.

Lastly, we investigated the feasibility of using HMI for early prediction of response to neoadjuvant chemotherapy in cancer mouse models and breast cancer patients. We acquired

longitudinal HMI images from pancreatic and breast cancer murine tumors during treatment with chemotherapeutic drugs and monitored the changes in the mechanical properties of the tumors. The tumors were found to soften when responsive to treatment, followed by the stiffness increase in the case of drug resistance. However, the untreated mice underwent steady stiffening of the tumors. Next, we imaged breast cancer patients at different time-points during their chemotherapy treatment. We found that tumors in the patients who achieved pCR had higher pre-treatment stiffness and higher softening from pre-treatment to a short-interval follow-up on treatment compared to the ones in patients with residual cancer cells at the completion of treatment. These findings indicate the promising potential of HMI in the early prediction of solid tumor response to chemotherapy interventions.

Table of Contents

Acronyms xix

Acknowledgments xix

Dedication xxiii

Chapter 1: Introduction 1

1.1 Motivation 1

1.2 Speci c aims 3

1.2.1 Speci c aim 1: Investigate the clinical feasibility of harmonic motion imaging (HMI) to characterize breast tumors in human subjects . . . 3

1.2.2 Speci c aim 2: Optimize and characterize the clinical HMI system . . 3

1.2.3 Speci c aim 3: Investigate the feasibility of HMI to assess response to neoadjuvant chemotherapy in solid tumors 4

1.3 Overview and Signi cance 4

Chapter 2: Breast Cancer Diagnosis and Treatment. 7

2.1 Breast Cancer 7

2.1.1 Types of Breast Cancer 8

2.2 Breast Cancer Screening 11

2.3 Breast Cancer Treatment 13

Chapter 3: Ultrasound Elasticity Imaging 17

3.1 Soft Tissue Biomechanics 17

3.2 Ultrasound Elasticity Imaging Techniques 19

3.3 Harmonic Motion Imaging 22

Chapter 4: Characterization of Breast Tumors in Human Subjects 26

4.1 Introduction 26

4.2 Materials and Methods 28

4.2.1 Experimental protocol 28

4.2.2 harmonic motion imaging (HMI) data acquisition 31

4.2.3 Data processing 32

4.2.4 Statistical analysis 34

4.2.5 Histopathology 35

4.3 Results 35

4.4 Discussion 40

4.5 Conclusion 45

Chapter 5: An E cient and Multi-focal Focused Ultrasound Technique for HMI . . . 47

5.1 Abstract 47

5.2 Introduction 48

5.3 Materials and Methods 49

5.3.1 Experimental setup 49

5.3.2 HMI Pulse Sequence 49

5.3.3 Electronic Beam Steering 51

5.3.4 harmonic motion imaging (HMI) Data Acquisition - Phantom Experiments 52

5.3.5 harmonic motion imaging (HMI) Data Acquisition - in-vivo Human Breast 54

5.3.6 harmonic motion imaging (HMI) Displacement Estimation 54

5.4 Results 57

5.4.1 Axial Focus Steering 57

5.4.2 Lateral Focus Steering 59

5.4.3 Multi-focal HMI 60

5.4.4 harmonic motion imaging (HMI) on Tissue-mimicking Phantom . . . 62

5.4.5 harmonic motion imaging (HMI) on anin-vivo Human Breast Tumor 63

5.5 Discussion 64

5.6 Conclusion 71

Chapter 6: Optimization of the amplitude modulation (AM) frequency 72

6.1 Abstract 72

6.2 Introduction 73

6.3 Materials and Methods 73

6.4 Results 80

6.5 Discussion 86

6.6 Conclusions 91

Chapter 7: Chemotherapy Response Monitoring in Cancer Mouse Models 93

7.1 Abstract 93

7.2 Introduction 93

7.3 Materials and Methods 96

7.3.1 Pancreatic Cancer Mouse Model and Treatment Protocol 96

7.3.2 Breast Cancer Mouse Model and Treatment Protocol 97

7.3.3 Animal Preparation 97

7.3.4 HMI Data Acquisition 98

7.3.5 fUS Data Acquisition 99

7.3.6 fUS Data Processing 101

7.4 Results 102

7.4.1 HMI detects chemotherapy-induced sti ness changes in pancreatic cancer 102

7.4.2 HMI detects chemotherapy-induced sti ness changes in breast cancer 103

7.5 Discussion 106

7.6 Conclusion 110

Chapter 8: Chemotherapy Response Monitoring in Breast Cancer Patients 111

8.1 Abstract 111

8.2 Introduction 111

8.3 Materials and Methods 112

8.3.1 Experimental Protocol 112

8.3.2 HMI Data Acquisition and Processing 114

8.3.3 Statistical analysis 115

8.4 Results 115

8.5 Discussion 119

8.6 Conclusion 122

Conclusion or Epilogue 124

8.7 Future directions 126

References. 129

Peer-reviewed Journals 148

Conference Proceedings, Presentations 150

Appendix A: Harmonic Motion Imaging in Human Subjects with Breast Masses. . . 155

Appendix B: Optimization of the amplitude modulation (AM) frequency 157

Appendix C: Longitudinal measurements of HMI displacement and tumor diameter in pancreatic cancer mice 159

List of Figures

2.1 Schematic of breast cancer.Source: https://alaskasurgicaloncology.com. 8

2.2 Prognosis and aggressivity of breast cancer molecular subtypes 11

2.3 Subsequent steps of tumorigenesis in breast cancer, from ductal hyperplasia to the formation of metastasis.Source: Transcriptional Factor Repertoire of Breast Cancer in 3D Cell Culture Models [38] 12

3.1 Ultrasound-based elasticity imaging methods can be categorized based on the source of tissue excitation, duration of excitation and location of the tracking beams. 21

3.2 Schematic of the harmonic motion imaging setup and harmonic motion imaging (HMI) displacement maps over time. Forty milliseconds corresponds to one cycle of oscillation at 25 Hz. 23

4.1 Schematic of the clinical harmonic motion imaging (HMI) setup. (a) Positioning of the patient and harmonic motion imaging (HMI) transducers. (b) Block diagramplitude modulation (AM) of harmonic motion imaging (HMI) data acquisition. 29

4.2 (a) Flowchart of harmonic motion imaging (HMI) data acquisition, processing, motion correction, and attenuation correction. (a) harmonic motion imaging (HMI) transducers were moved mechanically in a 1-D point-by-point raster scan regimen. (b) radio frequency (RF) data were acquired at each point. (c) radio frequency (RF) data were processed o ine according to the processing pipeline. (d) Large-scale motion artifacts were corrected by applying 2-D cross-correlation on the RF data in the lateral and axial directions (e) harmonic motion imaging (HMI) displacement values were co-registered based on the motion artifact correction. A 2-D displacement map was reconstructed accordingly. (f,g) Displacement values along the axial direction (red dashed line) were tted into two exponential curves to correct for acoustic force attenuation above and below the focused ultrasound (FUS) transducer focus. The blue and red lines show the displacement values along the axial direction before and after attenuation correction, respectively. (h) Overlaid harmonic motion imaging (HMI) displacement map on the B-mode image before attenuation correction. (i) Overlaid harmonic motion imaging (HMI) displacement map on the B-mode image after attenuation correction. 32

4.3 (a) Clinical B-mode image of a patient with a 0.9-cm invasive ductal carcinoma (IDC). (b) B-mode image of the mass acquired with the harmonic motion imaging (HMI) imaging transducer (2.5 MHz). (c) harmonic motion imaging (HMI) displacement map overlaid on the B-mode image (tumor contour is shown with white-dashed lines). (d-f) Hematoxylin and eosin (H&E) staining of the mass. The Red arrows show invasive carcinoma, the yellow arrows show brous normal breast tissue, and the blue arrow shows mature adipose tissue. 36

4.4 (a) Clinical B-mode image of a hypoechoic 1.4 cm solid breast mass. (b) B-mode image of the mass acquired with the harmonic motion imaging (HMI) imaging transducer (2.5 MHz). (c) Harmonic motion imaging (HMI) displacement map overlaid on the B-mode image (tumor contour is shown with white-dashed lines). (d-f) staining of the mass diagnosed brotic stroma with adenosis. The Yellow arrows show adenosis, and the blue arrow shows brous normal breast tissue and mature adipose tissue 37

4.5 (a) US image of a 2.2-cm microlobulated solid hypoechoic invasive ductal carcinoma. (b-c) Harmonic motion imaging (HMI) B-mode image and displacement map of the in-vivo tumor acquired before surgery (tumor contour is shown with white-dashed lines). (d-e) Harmonic motion imaging (HMI) B-mode image and displacement map of the tumor lumpectomy specimen scanned immediately after surgery. The contrast di erence between the ex-vivo displacement map and the corresponding in-vivo map shown in (c) might be due to the change in the boundary conditions and lack of physical constraints in the ex-vivo specimen. (f-g) Harmonic motion imaging (HMI) B-mode image and displacement map of the specimen at an imaging plane distant from the tumor. (h-j) Hematoxylin and eosin-stained section. The Red arrows show invasive carcinoma, the yellow arrows show brous normal breast tissue, and the blue arrow shows mature adipose tissue. 38

4.6 (a) A 4-cm invasive ductal carcinoma US image acquired using a clinical scanner (b-c) B-mode image and overlaid harmonic motion imaging (HMI) displacement map of the in-vivo tumor respectively before surgical resection (tumor contour is shown with white-dashed lines). (d-e) B-mode image and overlaid harmonic motion imaging (HMI) displacement map of the ex-vivo tumor, respectively, after surgical resection. (f-h) Hematoxylin and eosin-stained section. The red arrows show invasive carcinoma, the yellow arrows show brous normal breast tissue, and the blue arrow shows mature adipose tissue. 39

4.7 harmonic motion imaging (HMI) displacement estimated in (a) non-cancerous tissue and tumor in ve patients with malignant lesions, (b) surrounding tissue and tumor in ve patients with benign lesions, (c) malignant (n=5) and benign (n=5) tumors in vivo, (d) non-cancerous tissue and tumor in patients with malignant (n=4) and benign (n=1) masses, in vivo and ex vivo. *p < 0.05, **p < 0.01 and ***p < 0.001. 41

4.8 Estimated harmonic motion imaging (HMI) displacement in di erent size tumors. No correlation was found between the displacement and tumor size (Pearson r = -0.1588, P = 0.66). 42

5.1 Single system harmonic motion imaging (HMI) experimental setup and focused ultrasound (FUS) transducer beam pro le measured with a hydrophone. (a) The harmonic motion imaging (HMI) assembly is comprised of a 4.5 MHz 128-element focused ultrasound (FUS) transducer coaligned with a 7.8 MHz phased-array imaging transducer. The focused ultrasound (FUS) transducer was driven with the rst 128 channels of a 256 HIFU Verasonics system, while the imaging transducer was driven through the other 128 channels of the Vantage system. (b,c) Normalized hydrophone measured pressure pro le of the focused ultrasound (FUS) transducer in the axial plane and (d,e) transverse plane. The full width half maximum (FWHM) is denoted by red dashed lines. 50

5.2 harmonic motion imaging (HMI) pulse sequence. (a) Each cycle of the AM pulse (period: 20 ms) was divided into 40 segments. The relative width of each segment was proportional to $\frac{2}{c}$B8=$\frac{-1_1 \frac{92>BC^{3},11}{20}}{}$o at each segment,. (b) Hydrophone measured output of the focused ultrasound (FUS) transducer showing one cycle of the 50-Hz modulation. Therefore, 40 di erent transmit waveforms were synthesized, each with a period of 500 µs and relative pulse width as showen. (c) Magni cation of the region delineated with red dashed lines. Each segment is composed of a 175 µs pulse at the frequency of 4.5 MHz (35% duty cycle), interleaved (10 µs separation) by a 1.5 cycles imaging pulse. This pulse sequence resulted in an imaging frame rate of 2000 kHz. 51

5.3 Schematic of the two scenarios for imaging inclusions embedded within the tissue-mimicking phantom. (a) The transducer assembly was moved mechanically in a 2-D point-by-point raster scan regimen using the 3-D positioner, where the points were separated 2 mm axially and 1 mm laterally. The acoustic force was only generated at the geometric focus (gray ellipses). (b) The transducer assembly was moved mechanically using the 3-D positioner, where the points were separated 8 mm axially and 4 mm laterally. The acoustic force was generated at the geometric focus (gray ellipses). At each of these points, the focus was electronically moved in both directions (orange ellipses) with the step sizes of 2 mm axially and 1 mm laterally. 53

5.4 Data processing pipeline to generate 2-D harmonic motion imaging (HMI) displacement maps 55

5.5 (a) Normalized acoustic pressure pro les with respect to the maximum acoustic pressure at the geometric focus, measured with the bullet hydrophone for axial electronic steering distances of -5, -2, -1, 0, 1, 2 and 5 mm from the geometric focus. (b) Corresponding normalized 2-D harmonic motion imaging (HMI) displacement maps with respect to the maximum displacement amplitude at the geometric focus measured within the background of the tissue-mimicking phantom. The frames are shown at 10 ms after triggering a 20-ms harmonic motion imaging (HMI) signal (AM frequency: 50 Hz). 58

5.6 (a) Relative acoustic pressure (black) as a function of axial steering distance with respect to the pressure at the geometric focus measured with the bullet hydrophone in the free eld and relative maximum peak-to-peak displacement amplitude (blue) with respect to the maximum displacement at the geometric focus as a function of axial steering distance from displacement maps shown in Fig. 5.4b. (b) The location of the point with maximum acoustic pressure measured with the hydrophone (triangle) and location of the point with maximum peak-to-peak harmonic motion imaging (HMI) displacement amplitude (circle) versus the intended axial steering distance.(c) The axial length of the focus as a function of the axial steering distance measured with the hydrophone in the free eld. (d) The maximum intensity of the grating lobes as a function of axial steering distance. 59

5.7 (a) Normalized acoustic pressure pro les with respect to the maximum acoustic pressure at the geometric focus, measured with the bullet hydrophone for lateral steering distances of -2, -1, 0, 1and 2 mm from the geometric focus, (b) Corresponding normalized 2-D harmonic motion imaging (HMI) displacement maps with respect to the maximum displacement amplitude at the geometric focus, measured within the background of the tissue-mimicking phantom. The frames are shown at 10 ms after triggering a 20 ms harmonic motion imaging (HMI) signal (AM frequency: 50 Hz). 61

5.8 (a) Normalized maximum intensity level (black) as a function of lateral steering distance with respect to the intensity at the geometric focus measured with the bullet hydrophone in free eld and relative maximum peak-to-peak displacement amplitude (blue) with respect to the maximum displacement at the geometric focus as a function of lateral steering distance from displacement maps shown in Fig. 5.7b. (b) The location of the point with maximum acoustic pressure measured with the hydrophone (triangle) and location of the point with maximum peak-to-peak harmonic motion imaging (HMI) displacement amplitude (circle) versus the intended axial steering distance.(c) The axial length of the focus as a function of the axial steering distance measured with the hydrophone in free eld. (d) The relative maximum pressure of the grating lobes with respect to the one at the geometric focus as a function of axial steering distance. 62

5.9 Normalized acoustic pressure maps with respect to the maximum pressure at each map (rst row), normalized 2-D harmonic motion imaging (HMI) displacement maps with respect to the maximum displacement at each map (second row) and corresponding -6 dB displacement regions (third row) for (a, b, c) a single focus, (d, e, f) 2 foci separated by 1 mm in the lateral direction and (g,h,i) 2 foci separated by 2 mm in the lateral direction. 64

5.10 (a-d) B-mode ultrasound images, (c-h) 2-D harmonic motion imaging (HMI) displacement maps and (i,l) 2-D normalized displacement maps of inclusions where data was acquired through mechanical raster scanning, (m-p) 2-D harmonic motion imaging (HMI) displacement maps and (q-t) 2-D normalized displacement maps of inclusions where data was acquired through combined mechanical raster scanning and electronic beam steering. The inclusions are delineated using black dashed lines. 66

5.11 . (a) B-mode image acquired with a clinical ultrasound scanner, (b) B-mode image acquired with the 7.8 MHz imaging transducer and (c) normalized harmonic motion imaging (HMI) displacement map overlaid on the B-mode image of anin-vivo human breast tumor. The tumor was characterized as a 2.8-cm Fibroadenoma. The tumor is delineated with white dashed lines. . . . 68

6.1 Illustration of the experimental HMI setup 76

6.2 (a) Point-by-point mechanical movement of the HMI transducer assembly to generate localized ARF at each point and acquire the RF data. The inclusion is denoted with a white dashed line (b) Normalized pressure distribution at the focus of the FUS transducer in the transverse plane, (c) Normalized pressure distribution at the focus of the FUS transducer in the axial-elevational plane. The full-width half-max (FWHM) is denoted with blue dashed lines. (d) Signal processing pipeline for the data acquired at each point (e,f) The displacement maps generated at each point were used to reconstruct the nal 2-D HMI displacement map of the inclusion and its background. 78

6.3 (a) 2-D HMI displacement maps of a 44-kPa inclusion with the diameter of 4.1 mm embedded within a 5-kPa background material, imaged at di erent AM frequencies. The colorbar shows the peak-to-peak displacement amplitude in `m. (b) Corresponding normalized 2-D HMI displacement maps. The colorbar shows the relative displacement with respect to the normalizing pro le selected within the background. The inclusion boundaries are delineated with black dashed lines. The solid black rectangles represent the region of interest used for the calculation of image quality metrics. 80

6.4 (a) Peak-to-peak displacement amplitude, (b) particle velocity, (c) contrast, (d) CNR, and (e) lateral resolution estimated for inclusions with Young's modulus of 31 kPa and diameters of 2.4, 4.1, 6.5, and 10.4 mm. The inclusions were embedded within a 5-kPa background material. 82

6.5 (a) Peak-to-peak displacement amplitude, (b) particle velocity, (c) contrast, (d) CNR, and (e) lateral resolution estimated for inclusions with Young's modulus of 44 kPa and diameters of 2.4, 4.1, 6.5, and 10.4 mm. The inclusions were embedded within a 5-kPa background material. 83

6.6 (a) Peak-to-peak displacement amplitude, (b) particle velocity, (c) contrast, (d) CNR, and (e) lateral resolution estimated for inclusions with Young's modulus of 56 kPa and diameters of 2.4, 4.1, 6.5, and 10.4 mm. The inclusions were embedded within a 5-kPa background material. 84

6.7 Image contrast values for the inclusions with di erent Young's modulus of 31, 44, and 56 kPa with the diameters of a) 2.5 mm, b) 4.1 mm, c) 6.5 mm, and d) 10.4 mm 85

6.8 CNR values for the inclusions with di erent Young's modulus of 31, 44, and 56 kPa with the diameters of a) 2.5 mm, b) 4.1 mm, c) 6.5 mm, and d) 10.4 mm 86

6.9 Parametric space map of the AM frequencies at which (a) contrast and (b) CNR are maximized for each combination of inclusion size and Young's modulus explored in this study. 87

6.10 (a) 2-D peak-to-peak HMI displacement map, (b) normalized 2-D HMI displacement map of a post-surgical human breast specimen with a DCIS tumor. The tumor boundaries are delineated with white dashed lines. (c) B-mode image of the specimen. (d) Peak-to-peak displacement amplitude within the tumor and surrounding tissues, (e) particle velocity, (f) contrast, and (g) CNR as a function of the AM frequency. 89

7.1 (a) Experimental design for the therapeutic study of pancreatic cancer imaging study (HMI). Animals were treated with saline or Cisplatin once weekly for 3 weeks followed by one week of rest. (b) Experimental design for the therapeutic breast cancer imaging study (HMI and functional ultrasound (fUS)). Animals were treated with saline or Paclitaxel every day or with Doxorubicin every 7 days. 97

7.2 Illustration of the experimental setup 99

7.3 fUS was performed by emitting 7 planer ultrasonic waves tilted with di erent angles. The resulting images were summed up and fed into an SVD lter to remove the tissue signal. The remaining blood signal was used to generate a single power Doppler image. This process was repeated at di erent elevational planes to generate a stack of 2-D maximum intensity projections and reconstruct a 3-D tumor vasculature image. 100

7.4 Development of a pancreatic tumor in a KPC mouse (control mouse). (a) High-resolution B-mode images, (b) 2-D harmonic motion imaging (HMI) displacement maps overlaid on the B-mode images, (c) 2-D harmonic motion imaging (HMI) displacement maps, and (d) longitudinal changes of the tumor diameter and peak-to-peak harmonic motion imaging (HMI) displacement amplitude averaged within the tumor over a period of 26 days. 102

7.5 Longitudinal changes of a pancreatic tumor in% $_2$ mice treated with Cisplatin. (a) High-resolution B-mode images, (b) overlaid images, (c) 2D harmonic motion imaging (HMI) displacement maps, and (d) changes of peak-to-peak harmonic motion imaging (HMI) displacement amplitude averaged within the tumor and tumor diameter. The green shaded area corresponds to the period that the tumor was responding to the chemotherapy. 104

7.6 (a) harmonic motion imaging (HMI) displacement maps overlaid on the B-mode images of a control mouse. The tumors are delineated with dashed black lines. (b) Power Doppler images of the same planes superimposed on the B-mode images. The tumors are delineated with dashed white lines. (c) Maximum intensity projections of 3-D vascular images. (d) harmonic motion imaging (HMI) displacement maps overlaid on the B-mode images of a treated mouse. (e) Power Doppler images of the same planes superimposed on the B-mode images. (f) Maximum intensity projections of 3-D vascular images. (g-h) Longitudinal tumor volume and harmonic motion imaging (HMI) displacement measurements of the control and the treated mouse. 105

7.7 Longitudinal changes of the harmonic motion imaging (HMI) displacements and tumor volume measured for the (a-b) control group, (c-d) Paclitaxel group, and (e-f) Doxorubicin group. 107

7.8 (a) Relative changes of harmonic motion imaging (HMI) displacements within the tumors with respect to baseline (day1), (b) Relative changes of volume of the tumors with respect to the baseline. 108

8.1 (a-c) B-mode images of a 4.8-cm invasive ductal carcinoma (IDC) acquired with a commercial ultrasound scanner, (d-f) B-mode images of the tumor acquired with the 7.8-MHz HMI imaging transducer, (g-i) 2-D HMI displacement maps overlaid on the B-mode images, (j-l) Normalized HMI displacement maps overlaid on the B-mode images. The B-mode images and HMI maps were acquired at three time-points of baseline, three weeks on neoadjuvant chemotherapy, and at the completion of the treatment. histopathological analysis revealed residual cancer cells on the post-surgical breast specimen. . 116

8.2 (a-b) B-mode images of a 2.8-cm IDC (Patient 3) acquired with a commercial ultrasound scanner, (c,d) B-mode images of the tumor acquired with the 7.8-MHz HMI imaging transducer, (e,f) 2-D HMI displacement maps overlaid on the B-mode images, (g-h) Normalized HMI displacement maps overlaid on the B-mode images. The B-mode images and HMI maps were acquired at two time-points of baseline and three weeks on neoadjuvant chemotherapy. The tumor could not be localized on the B-mode images at the third time-point. This subject (Patient 1) achieved pCR according to the pathology reports. . 117

8.3 (a-b) B-mode images of a 3.2-cm IDC (Patient 5) acquired with a commercial ultrasound scanner, (c,d) B-mode images of the tumor acquired with the 7.8-MHz HMI imaging transducer, (e,f) 2-D HMI displacement maps overlaid on the B-mode images, (g-h) Normalized HMI displacement maps overlaid on the B-mode images. The B-mode images and HMI maps were acquired at two time-points of baseline and three weeks on neoadjuvant chemotherapy. The tumor could not be localized on the B-mode images at the third time-point. This patient achieved pCR, according to the pathology reports. 118

8.4 (a) Longitudinal ratio of HMI displacement within the tumors with respect to the surrounding tissues, (b) Tumor to surrounding tissues displacement at pre-treatment (time-point1) and short-interval follow-up on therapy (time-point2). **p < 0.01 120

8.5 (a) Change in normalized tumor HMI displacement from time-point 1 to time-point 2 and (b) Tumor HMI displacement with respect to the surrounding tissues ratio at the baseline for ve patients with npCR (n=3) and pCR (n=2) 121

8.6 (a) Ratio of the normalized HMI displacements within the tumor to those in the surrounding tissues at time-point 1 and time-point 2 for four patients. (b) Corresponding mean tumor diameter measured by a clinician. 122

A.1 An 89-year-old subject diagnosed with invasive lobular carcinoma (ILC) was imaged at a similar imaging plane before and after general anesthesia using the same acquisition setup and parameters to validate the reproducibility of the HMI estimations and the e ect of breathing. (a) Breast clinical US image of the patient. (b) B-mode image of the tumor captured using the 2.5-MHz imaging transducer before resection. (c) HMI displacement map overlaid on the B-mode. (d-e) B-mode image and overlaid HMI displacement map acquired after general anesthesia of the same patient (f-h) Hematoxylin and eosin stained section of the tumor. The Red arrows show invasive carcinoma, the yellow arrow shows brous normal breast tissue and the blue arrow shows mature adipose tissue. 155

A.2 Flowchart of all the participants in the study and exclusions. 156

B.1 (a) Total pressure (red) and deconvolved pressure (blue) for 20 cycles and (b) fast Fourier transform (FFT) of the deconvolved pressure of the FUS transducer (center frequency: 4 MHz) measured with a ber-optic hydrophone in free eld. 157

B.2 (a) HMI displacement pro les (orange) and ltered displacement pro les (blue) within the background (Young's modulus: 5 kPa), (b) magnitude spectrum of fast Fourier transform (FFT) of the displacement pro les (orange) and ltered displacement pro les (blue) within the background. (c) HMI displacement pro les (orange) and ltered displacement pro les (blue) within the inclusion (Young's modulus: 44 kPa), and (b) magnitude spectrum of FFT of the displacement pro les (orange) and ltered displacement pro les (blue) within the inclusion. The diameter of the inclusion was 4.1 mm. 158

List of Tables

2.1 TNM staging classi cation system for breast cancer.
Source: https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/stages-of-breast-cancer.html 10

4.1 Patient characteristics. 30

4.2 contrast-to-noise ratio (CNR), ratio of the harmonic motion imaging (HMI) displacement in the pathological tissue to that of the normal tissue¹) • ` (º and mean correlation coe cient values within the tumor¹) º and surrounding tissues¹ (º. 40

5.1 Raster scan parameters 56

8.1 Study calendar for patients receiving NACT 114

8.2 Demographics and clinical characteristics of 8 patients successfully completed the study 115

C.1 Summary of the diameter and HIM displacement change in % 2 • mice treated with Cisplatin 159

Acronyms

AM amplitude modulation

ANOVA analysis of variance

ARF acoustic radiation force

ARFI acoustic radiation force impulse

BI-RADS breast imaging reporting and data system

CNR contrast-to-noise ratio

CUSE comb-push ultrasound shear elastography

DCIS ductal carcinoma in situ

DWI-MRI di usion-weighted magnetic resonance imaging

ECM extracellular matrix

ER estrogen receptor

fUS functional ultrasound

FUS focused ultrasound

HER2 human epidermal growth factor receptor 2

HIFU high-intensity focused ultrasound

HMI harmonic motion imaging

HR hormone receptor

IDC invasive ductal carcinoma

ILC invasive lobular carcinoma

LCIS lobular carcinoma in situ

MRI magnetic resonance imaging

NACT neoadjuvant chemotherapy

PBS phosphate-bu ered saline

pCR pathologic complete response

PDA pancreatic ductal adenocarcinoma

PR progesterone receptor

RF radio frequency

ROE region of excitation

ROI region of interest

SDUV shear wave dispersion ultrasound vibrometry

SE strain elastography

SNR signal-to-noise ratio

SSI supersonic shear imaging

SVD singular value decomposition

SWEI shear wave elasticity imaging

TNBC triple-negative breast cancer

ULM ultrasound localization microscopy

US ultrasonography

Acknowledgements

First and foremost, I would like to thank my advisor Professor Elisa Konofagou for her continuous guidance and encouragement over these past years. Her insight and enthusiasm for science have always been a great inspiration for me. With all the research opportunities and necessary resources she provided, I could accomplish what I was looking for as a doctorate candidate.

I would also like to express my sincerest gratitude to my dissertation committee members, Professor Andrew Laine, Professor Elham Azizi, Professor Dawn Hershman, Professor Bret Taback, and Professor Richard Ha, for their expertise and insightful comments on my dissertation.

I am grateful to my clinical collaborators, Professor Bret Taback and Professor Richard Ha with whom I initiated the clinical studies and who have guided and supported my research throughout these years. I would also like to thank Professor Dawn Hershman, Professor Meghna Trivedi, Professor Katherine Crew, Professor Melissa Accordino, and Professor Neil Vasan for their support in extending my research to the oncology eld. A special thanks to Professor Julia McGuinness for every step of the collaboration, from drafting the study protocol and patient recruitment to interpreting the results. I would like to thank Professor Hanina Hibshoosh for his invaluable guidance with clinical and pre-clinical studies and for passionately teaching me all the pathology knowledge I have ever gained. Many thanks to Sandra Espinal, Jennifer Lebron Ruiz, oncology nurses, as well as radiology and surgery personnel for all their help with various clinical studies.

I would like to thank Professor Kenneth Olive, Carmine Palermo, and Stephen Sastra for their guidance and collaboration with the pancreatic cancer study. I would like to thank Professor Chandan Guha, Professor Indranil Basu and Dr. Saurabh Singh for providing the breast cancer mouse models and their insights into the pre-clinical studies.

I am grateful to Pablo Abreu, who helped with every step of the way and provided the tools and resources I needed. Thank you very much, Pablo, for all the assistance and support. I always enjoyed our casual chats and learning how to approach problems from your perspective.

I have also had the privilege to work with many brilliant and talented scientists in the UEIL lab, especially within the HMI team. Thomas Payen, familiarized me with the codes and protocols and taught me how to perform experiments and not be afraid of the mice. I am deeply indebted to Hermes Kamimura, who passionately helped me with various projects, provided invaluable feedback with results and papers, and though me how to be a better scientist. Also, thanks to Hermes for performing all my experiments during the time that I could not walk properly. I am grateful to Md Murad Hossain for sharing his in-depth ultrasound knowledge with me and for helpful discussions. I want to particularly thank Judy Xiaoyue Judy Li for four years of great help with various projects, being by my side during all the challenging clinical studies, her positive energy, and her smile during all the ups and downs we went through together. I am grateful to Rachel Weber for her signi cant role in clinical studies, as well as for bringing all the energy and positive vibe to the lab. I am thankful to Yangpei Liu, the newest addition to the team, for his assistance with the clinical projects over the past year and for taking my tea knowledge to another level. I want to take the opportunity to thank my other lab mates with who I had the pleasure to work at UEIL: Grigorios Karageorgos, Robin Ji, Alec Batts, Omid Youse an, Mark Burgess, Hairong Zhang, Nima Mobadersani, Nancy Kown, Maria Murillo, Antonios Pouliopoulos, Katherine Liu, Vincent Sayseng, Vittorio Ghatti, and Salah Mahmoudi.

I would like to thank my wonderful friendmily, who made my life full of joy and colorful

memories. Paul Kemper and Stephen Lee, I was fortunate to meet you rst day at the orientation. Thanks for all the support and chat whenever I was in need and for being there when I did not know many people in this city. Also, thanks Stephen for all the help and sharing your insights during various projects. Grace Sooyeon Lee and Melina Tourni, thanks for all the great memories. Lea Melki, Mirella Altoe, Elika Fallah and Marilena Karakatsani, I am grateful for having you in my life. I cannot express how thankful I am to Marziyeh Jadidi and Negin Moharrami, Niloofar Bayat, and Mehrnoush Sha ei, without whom life would be di cult away from family. You are truly the lifetime friends one would wish for. Many thanks to Sahand Seifnashri for his unconditional support and for making me believe in myself whenever I doubted my abilities.

Last but not least, I am grateful to my parents Hengameh and Mehdi. I owe all my strength, hope and courage to you. Thanks for believing in me and supporting me from thousands of kilometers away. This was a challenging journey without seeing you for more than six years and I would not be able to make it without the love and hope you gave me. Thanks to my best friend and number one supporter since the moment I was born, Yasaman. We have not been physically in the same location since I started my Ph.D., but you have been here virtually every second I needed you. I am the luckiest person to have a person like you as my sister.

Dedication

To all the strong women ghting with breast cancer.

Chapter 1: Introduction

1.1 Motivation

Breast cancer is the second most lethal cancer in women and it is believed that about 1 out of 8 American women develop breast cancer in their lifetime [1]. Early diagnosis of the breast cancer improves overall survival and prognosis [2]. Manual palpation and examination by the physician can detect breast tumors however, it is limited to the super cial tumors that are close to the skin.

Currently, the gold standard imaging techniques for breast cancer diagnosis are mammography, magnetic resonance imaging (MRI) or ultrasonography. Although mammography has signi cantly improved breast cancer diagnosis [3], radiation exposure risk and its limitations with breasts with high density impede the clinical applications of this imaging modality [4]. Although, MRI is recommended for high-risk patients, the low temporal resolution and high costs associated with MRI limit its applications in the clinical settings. An inexpensive and safe imaging option for patients who cannot undergo MRI or mammography is ultrasonography which itself has higher false positive rate than those two imaging modalities [5, 6]. Low speci city of the breast imaging technologies may also result in unnecessary invasive biopsies as only 20-40% of the biopsy results show cancer [7].

Once a patient is diagnosed with a locally advanced breast cancer, in ammatory breast cancer, or early stage breast cancer, the standard therapeutic strategy is neoadjuvant chemotherapy (NACT). The ideal outcome of NACT is pathologic complete response (pCR) which itself is associated with improved disease-free survival [8]. However, pCR is currently determined at the time of the surgery when the patient has received the whole cycle of the treatment. Early detection of non-responders to NACT could be used to halt ine ective treatment and

start a new chemotherapeutic regimen in order to achieve better tumor response and overall survival. Current techniques to monitor response to NACT based on tumor size changes include mammography, physical examination, ultrasonography and MRI [9, 10]. Clinically assessment of tumor size has been shown to be only moderately useful for chemotherapy response prediction in these patients because tumor shrinkage is a late marker of e ective treatment.

Ultrasound elasticity imaging techniques are complementary modalities to B-mode ultrasound images to improve tissue characterization by providing information on the viscoelastic properties of the underlying tissue. Mechanical properties of tissues have been shown to be correlated with the progression of the diseases such as cancer. In fact, the sti ness such as the Young's modulus of the pathological tissues are higher than that in normal tissues [11, 12]. In addition, the alteration of mechanical properties of tumors as a response of NACT can be detected using these ultrasound elasticity imaging techniques. However, limited studies have shown the feasibility of these techniques in prediction and monitoring the changes in the tumor hardness in response to NACT [13, 14, 15].

Harmonic motion imaging (HMI) is an ultrasound-based elasticity imaging technique that assesses tissue viscoelastic properties by inducing periodic oscillations [16, 17]. HMI uses a focused ultrasound (FUS) transducer to generate an amplitude modulated acoustic radiation force (ARF) at its focal zone. The resulting tissue oscillations are estimated using a confocally aligned imaging transducer, and the recorded radio frequency (RF) data is used to estimate the displacements.

In prior work by our group, HMI has shown to have promising potential in detection, characterization, and treatment assessment of ex-vivo pancreatic and breast tumors [18, 19]. However, the feasibility of HMI for tumor characterization and chemotherapeutic response monitoring of such tumors must be assessed in human patients to pave the way for application of HMI in clinical settings.

1.2 Speci c aims

The speci c aims of this dissertation and their associated objectives are listed as follows:

1.2.1 Speci c aim 1: Investigate the clinical feasibility of HMI to characterize breast tumors in human subjects

- Assessment of the clinical feasibility of HMI to di erentiate benign and malignant breast lesions in human subjects
- Comparison of HMI images between ex-vivo resected breast specimens and in-vivo breast tissue of the same patients in order to assess the e ects of di erent boundary conditions, breast geometries and motion artifacts

1.2.2 Speci c aim 2: Optimize and characterize the clinical HMI system

Sub-aim 2.1: Development and characterization of a single Vantage HMI system with electronic beam steering capability using a multi-element phased-array FUS transducer

- Develop and characterize a multi-element focused ultrasound transducer for electronic steering of the HMI force and evaluate the performance on tissue mimicking phantoms
- Develop and validate a pulse sequence to drive both HMI excitation and tracking pulses using a single Vantage system

Sub-aim 2.2: Frequency dependence of inclusion characterization in harmonic motion imaging

- Optimize the amplitude modulation frequency in HMI for improved contrast of inclusions with di erent geometries
- Optimize the amplitude modulation frequency in HMI for improved contrast of inclusions with di erent sti nesses

1.2.3 Specific aim 3: Investigate the feasibility of HMI to assess response to neoadjuvant chemotherapy in solid tumors

Sub-aim 3.1: Prediction and monitoring tumor response to neoadjuvant chemotherapy in mouse models

- Assess the potential of HMI displacement change as a biomarker for drug response and/or resistance in pancreatic tumor bearing mice
- Assess the capability of HMI for detecting and monitoring mechanical effects of chemotherapy on tumors in breast cancer bearing mice
- Evaluate the chemotherapy associated vascularization changes within the tumors using functional ultrasound (fUS)
- Investigate the correlation between vascularization and stiffness changes within the tumors

Sub-aim 3.2: Prediction and monitoring tumor response to neoadjuvant chemotherapy in patients with breast cancer

- Examine the clinical utility of HMI in monitoring breast cancer response to NACT and assessment of pCR in human patients with breast cancer
- Investigate whether HMI can assist in early detection of positive NACT response

1.3 Overview and Significance

This dissertation aims to demonstrate the clinical feasibility of HMI in tumor characterization and chemotherapy treatment response monitoring. The first step towards my goal is to evaluate whether HMI can characterize different breast masses based on their distinct mechanical properties. To achieve this goal in specific aim 1, I imaged a cohort of female patients with breast masses with the clinical HMI setup. Then, I compared the in vivo and

ex vivo HMI ndings, since the boundary conditions in the clinical settings are di erent from the ex-vivo masses shown in the previous studies. In order to achieve that goal, the post-surgical specimens of the patients who underwent surgery were imaged immediately after resection using a similar HMI system.

The next step in translating HMI to the clinic is to improve and optimize the current HMI system and overcome some of the limitations encountered in the rst clinical study. Thus, in speci c aim 2, I developed and characterized a new HMI system to improve data acquisition and shorten the procedures. In the rst part of this speci c aim (sub-aim 2.1), I focused on employing a multi-element FUS transducer with electronic beam steering capability. A customized sequence was implemented to drive the HMI transducers using a single Verasonics Vantage system, making the HMI system less complicated than the conventional system. The performance of the new compact and e cient system was evaluated on a tissue-mimicking phantom, as well as on an-vivo human breast tumor. Furthermore, the feasibility of simultaneous generation of multiple foci was demonstrated for applications where imaging or treatment of a larger region is needed. In the second part of this speci c aim (sub-aim 2.2), I evaluated the hypothesis that optimizing the AM frequency based on the tumor geometry and sti ness improves the tumor contrast and may assist in detecting and delineating small tumors. To evaluate my hypothesis, I used tissue-mimicking phantoms with embedded inclusions at di erent sizes and sti nesses.

In speci c aim 3, I examined the possibility of predicting and monitoring tumor response to chemotherapy treatment using HMI. First, genetically engineered mice with pancreatic ductal adenocarcinoma (PDA) receiving a chemotherapy agent were imaged longitudinally using our HMI technique to measure the relative sti ness change of the tumors over the course of several weeks. Following the success of HMI in detection and monitoring the chemotherapy-induced changes in the mechanical properties of the PDA tumors, I moved on to a di erent mouse model. Therefore, I assessed the chemotherapy-associated changes in sti ness and vasculature of the 4T1 mouse breast cancer tumors using HMI and FUS, respec-

tively. To achieve this goal, 4T1 xenograft mice were imaged using both imaging techniques while receiving chemotherapy. Finally, I investigated the feasibility of HMI in assessing chemotherapy-induced changes in the mechanical properties of breast tumors in human subjects and whether these assessments can predict the tumor response to the chemotherapy intervention. Towards that goal, I conducted a pilot study with female patients diagnosed with breast cancer and planed to receive NACT as part of their standard care. I imaged the tumors at three di erent time-points during their treatment and compare the changes in the measurements provided by HMI and pathological response rate, between baseline, during treatment, and end of the treatment.

The structure of this dissertation is as follows: Chapter 2 provides the basics of breast cancer, and discusses the techniques for breast cancer diagnosis and chemotherapeutic response monitoring assessment. Chapter 3 covers tissue mechanics, ultrasound-based elasticity imaging techniques, and their role in breast cancer diagnosis and treatment assessment, as well as introduces HMI and its previous applications. Chapter 4 describes the characterization of breast tumors in human subjects using HMI. Chapter 5 then presents developing and characterizing an e cient and multi-foci HMI system. Chapter 6 details optimizing the amplitude modulation frequency in HMI to improve image contrast and detection sensitivity. Chapters 7 and 8 describe the application of HMI in the prediction and chemotherapeutic response monitoring of tumors in mouse models and human subjects, respectively. Finally, the conclusion chapter summarizes this dissertations and provides future directions for HMI.

Chapter 2: Breast Cancer Diagnosis and Treatment

This chapter aims to provide an overview of breast cancer and the risks associated with breast cancer. We will review the di erent types of breast cancer and how the staging is determined. These factors will dictate the prognosis and treatment planning. Then, the current gold standard imaging techniques for breast cancer diagnosis will be presented. This will be followed by breast cancer treatment options, focusing on neoadjuvant chemotherapy and the methods currently used for treatment response monitoring.

2.1 Breast Cancer

Breast cancer is the most frequently diagnosed malignancy in women, excluding skin, with a lifetime risk of 12.4% in the United States. It is estimated that in 2022, approximately 287,850 new female cases will be diagnosed with breast cancer and there will be 43,250 deaths from it [20]. In general, breast cancer incidence increases with age [21]. Although the greatest incidence rate occurs in the middle-aged and older women (>60 y.o), young women have a higher risk of death and a worse prognosis, mainly due to late presentation and more aggressive tumor biology [22]. Breast cancer risk is 1.5 times greater for women with one a icted rst-degree female relative compared to those without a family history. However, the majority of women who have breast cancer don't have a family history of it. [23].

One of the most established biomarkers of breast cancer risk is breast density measured during a mammogram. The density of breast tissue re ects the proportion of glandular and connective tissue to fatty tissue within the total area of the breast. The American College of Radiology classi es breast tissue into four categories known as the breast imaging reporting and data system (BI-RADS): i) fatty; ii) scattered areas of broglandular tissue;

Figure 2.1: Schematic of breast cancer. Source: https://alaskasurgicaloncology.com

iii) heterogeneously dense; and iv) extremely dense. Women with breasts classi ed as C or D are referred to as having dense breasts". Approximately 36% of 40-74-year-old American women have heterogeneously dense breasts (BI-RADS C), and about 7% have highly dense breasts (BI-RADS D). High breast density has shown to increase the risk of developing breast cancer [24].

The death rate for breast cancer has decreased by 42% since 1989 as a result of improvements in detection and treatment methods [20]; therefore, early detection and diagnosis of breast cancer are of crucial signi cance in improving survival and prognosis [2].

2.1.1 Types of Breast Cancer

Based on the histological subtype of the tumor, breast cancer can be classi ed into in situ (preinvasive) and invasive. In-situ breast cancer cells are con ned to the ducts and do not in ltrate to the surrounding fatty and connective tissues. Lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS) are two subtypes of in situ breast cancer. LICS is characterized by an abnormal increase in the number of cells within the breast's milk glands (lobulus) [25]. LCIS is believed to be more of a marker of increased cancer risk than

a premalignant lesion [26]. DCIS is the most common type of in situ breast cancer and is considered a precursor to invasive breast cancer. According to the long-term research, about 20-53% of untreated DCIS are ultimately diagnosed with invasive breast cancer [27].

Most breast cancers (81%) are invasive in which the abnormal cells break through the ducts and lobular wall and grow into the surrounding tissues. The most common type of invasive breast cancer is invasive ductal carcinoma (IDC), which accounts for 80% of all diagnosed breast cancers. Invasive lobular carcinoma (ILC) is another invasive type of breast cancer, accounting for 10-15% of all cases. Less frequently occurring breast cancers include Medullary carcinoma, Mutinous carcinoma, Tubular carcinoma, in ammatory breast cancer, Paget's disease of the nipple, and Phylloides tumor [28].

Breast cancer can be further classi ed based on the molecular subtype. The cancer cells are examined for estrogen receptor (ER) and progesterone receptor (PR). Cells with these receptors depend on estrogen or progesterone to grow. The breast cancer subtypes are determined based on their hormone receptor (HR) (HR+ if test positive for ER or PR or both, and HR- otherwise) and expression of human epidermal growth factor receptor 2 (HER2), which is another protein that promotes cancer growth. The four main cancer subtypes are as follows [29]: 1) Luminal A (HR+/HER2-), which is the most common subtype with slower growing and less aggressive than the other subtypes. 2) Luminal B (HR+/HER2+), which has a higher grade than Luminal A and therefore, poorer treatment outcome. 3) HER2-enriched (HR-/HER2+), which are more aggressive and faster-growing than the Luminal subtypes and can have a worse prognosis 4) Basal-like (HR-/HER2-), which are associated with worse short-time prognosis. The majority (about 75%) of the triple-negative breast cancer (TNBC) are categorized as basal-like, which are among the most aggressive breast cancers (Fig. 2.2). Assessment of the breast cancer subtype is necessary for treatment planning and determination of the prognosis, which will be discussed later in this chapter.

In addition to the molecular subtype, the stage of the breast cancer is needed to determine the extent of the disease, management plan, and likely outcome of the treatment. The breast

Table 2.1: TNM staging classi cation system for breast cancer.
Source: https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/stages-of-breast-cancer.html

	Primary tumor (T)
Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T0	No evidence of primary tumour
T1	Tumour is less than 2cm in greatest dimension
T2	Tumor size is between 2 and 5 cm across
T3	Tumour is more than 5 cm across
T4	Tumour of any size with direct extension to skin or chest wall
	Lymph node stage (N)
Nx	Nearby lymph nodes cannot be assessed
N0	Cancer has not spread to nearby lymph nodes
N1	Cancer has spread to 1 to 3 axillary lymph node(s), and/or cancer is found in internal mammary lymph nodes on sentinel lymph node biopsy.
N2	Cancer has spread to 4 to 9 lymph nodes under the arm, or cancer has enlarged the internal mammary lymph nodes
N3	Cancer has spread to 10 or more axillary lymph nodes. Or it has spread to the lymph nodes located under the clavicle, or collarbone. It may have also spread to the internal mammary lymph nodes or to the lymph nodes above the clavicle
	Distant metastasis stage (M)
Mx	Distant metastasis Cannot be assessed
M0	No distant metastasis is found on imaging test or by physical exam
M1	Cancer has spread to distant organs as seen on imaging tests or by physical exam, and/or a biopsy of one of these areas proves cancer has spread and is larger than 0.2mm.

cancer staging system is called the TNM system overseen by the American Joint Committee on Cancer (AJCC) [30]. This system is based on the primary tumor (T), involvement of the lymph nodes (N), and presence of metastasis (M). The TNM staging classi cation system for breast cancer is summarized in Table 2.1. Accordingly, the stage of breast cancer can be determined as 0, I, II, III, or IV (Fig. 2.3).

Figure 2.2: Prognosis and aggressivity of breast cancer molecular subtypes

2.2 Breast Cancer Screening

Typically, the conventional diagnostic procedure for breast cancer consists of three steps. The rst step includes a medical history and physical examination. The clinician will manually palpate the breast tissue to detect any abnormality or a lump that is harder than normal tissue. The second stage is imaging the breast, followed by the third step, which is invasive needle biopsy if further evaluation is needed.

Mammography, MRI, or ultrasonography (US) are the imaging modalities now regarded as the gold standard breast imaging techniques. Mammography has been shown to reduce breast cancer mortality [31, 32]. Recent ndings from coordinated mammography programs in Europe and Canada suggest that mammography screening reduces the risk of mortality from breast cancer by more than 40% among women [33, 34, 35]. However, the sensitivity of the technique declines signi cantly in women with dense breasts [4, 36]. The presence of dense breasts raises the likelihood of getting breast cancer [24]. In addition, about 12% of digital mammography ndings are false positives that require unnecessary biopsies [37].

Contrast-enhanced MRI is recommended as a complement to mammography for high-risk individuals because MRI has the highest sensitivity among the other breast screening modalities [7, 39, 40]. It has been shown that when used as a supplement to mammography and ultrasound, MRI has discovered 14.7 additional instances of cancer per 1000 individuals

Figure 2.3: Subsequent steps of tumorigenesis in breast cancer, from ductal hyperplasia to the formation of metastasis. Source: Transcriptional Factor Repertoire of Breast Cancer in 3D Cell Culture Models [38]

[41]. However, the decreased speci city of MRI leads to a greater number of recalls and biopsies [7, 5]. In addition, contrast-enhanced MRI is expensive, in exible, involves intravenous gadolinium contrast administration, and is not widely available.

US is a screening method to mammography for individuals who cannot undergo MRI [42]. Shape irregularity, microlobulated or speculated margins, width-to-anteroposterior (AP) ratio, marked hypoechogenicity, and shadowing are characteristics of malignant tumors on ultrasound images [43, 44]. The use of hand-held ultrasound by a skilled technician or radiologist is e ective for nding tumors in dense breasts that are undetectable to mammography [45, 46, 47, 48, 49]. A large multi-institutional trial reported that combined ultrasound with mammography increased the cancer detection rate by 4.2 cancers per 1,000 women [50]. However, the US has a greater false-positive rate than mammography and MRI [6]. Recently, automated breast ultrasound (ABUS) has been demonstrated to reduce the amount of time needed for image acquisition and interpretation, as well as to be e ective in eliminating the operator's dependence on image quality and reproducibility. However, inadequate axillary breast tissue imaging and artifacts caused by the nipple are limitations associated with the ABUS systems [51, 52].

2.3 Breast Cancer Treatment

In early breast cancers, once the tumor is diagnosed (usually through core biopsy), the treatment plan is determined based on the molecular subtype, locoregional tumor load, and patients' wishes [53]. Only symptomatic patients or those at high risk for recurrence require staging and metastasis detection before nalizing the management plan. The treatment for early breast tumors is categorized into two groups of local therapy including surgery and radiotherapy; and systemic therapy including chemotherapy, hormone therapy, targeted therapy and immunotherapy. Typically, surgery followed by radiation or hormone treatment is used to treatin situ breast cancers. The majority of women with early-stage breast cancer will have surgery, which is often paired with various therapies such as radiation therapy,

chemotherapy, hormone therapy, and/or targeted therapy to lower the chance of recurrence [21].

When systemic treatment is administered to patients before surgery, it is referred to as neoadjuvant therapy. The objective of these treatments is to minimize tumor size and allow for less morbid nal breast procedures, such as breast-conserving surgery (also known as lumpectomy) as opposed to mastectomy (removal of the entire breast) and less extensive axillary lymph node dissection [54]. Systemic therapy is referred to as adjuvant therapy if it is administrated after surgery to destroy undetected tumor cells.

Neoadjuvant chemotherapy (NACT) has become the standard of care for locally advanced breast cancers, particularly those that are HER2-positive or TNBC. It results in similar outcomes as adjuvant therapy for women with operable breast cancer [55, 56]. This treatment modality also enables an in-vivo evaluation of tumor responsiveness to therapy and has become a valuable strategy for evaluating the biological e ects of novel cancer medicines. Furthermore, response to NACT is associated with long-term clinical outcomes. In a pooled analysis of 12 neoadjuvant trials and nearly 12,000, pathologic complete response (pCR), or the absence of residual invasive cancer in the breast or lymph nodes after NACT, was associated with improved event-free and overall survival across all breast cancer subtypes [8]. As a result, pCR has become a surrogate endpoint for many neoadjuvant trials in breast cancer.

Current approaches for assessing NACT response continue to be inadequate for predicting pCR. While subtypes such as TNBC and HR-negative, HER2-positive breast cancers have rates of pCR in the range of 30-50% [8], a biomarker of short-term response to neoadjuvant therapy could have clinical implications by identifying poor response to NACT and allowing for treatment changes to optimize clinical outcomes. Physical examination of the axilla and breast has been historically practiced to monitor the chemotherapeutic response. However, physical examination is di cult for tumors < 2 cm. The assessment could be inaccurate for tumors with poorly de ned margins or in the presence of residual brosis

and/or necrosis resulting from the chemotherapy itself [57]. Mammography and US are the most conventional imaging modalities for treatment assessment. Reduction in tumor size and density are the main indicators of treatment response on mammograms. However, the accuracy of mammography for predicting pathological outcome following NACT depends on the mammographic appearance of the tumor pre-treatment and the interpretation of the results are hindered by the calci cations and spicules. Indeed, studies have shown that the residual microcalci cations on mammography could be due to both the residual tumor and necrotic tissue resulting from treatment. Therefore, microcalci cations do not correlate with the extent of residual tumor burden after NACT [58, 59]. US has been shown to be more accurate than physical breast examination or mammography for tumor size assessment and monitoring pCR. Although some studies have shown that tumor echogenicity change after a few cycles of NACT correlates with pathological response [60], other studies concluded that the receptor status impacts the precision of US outcomes [61]. These techniques rely on macroscopic changes or morphological characteristics of tumors that are often late markers for pathologic response assessment. In a retrospective study of 141 women, Chagpar et al. reported that size estimations by palpation, US, and mammography were only marginally linked with residual tumor size following NACT (correlation coe cients 0.42, 0.42, and 0.41, respectively) [9].

Functional imaging techniques evaluate treatment response based on the tumors' vascular and metabolic changes that precede morphological alterations [62, 63]. These techniques have the advantage of detecting certain biologic tumor markers, evaluating therapeutic targets, assessing and predicting the early response to neoadjuvant chemotherapy, and customizing neoadjuvant chemotherapy for cancer therapy [63]. Dynamic contrast-enhanced MRI (DCE-MRI) is a functional imaging methodology that detects angiogenesis, changes in tumor microcirculation and uptake of contrast material due to the increased permeability of the new vessels in a progressing tumor [63]. A meta-analysis study including 2050 patients who underwent NACT reported a sensitivity of 83-87% and speci city of 54-83% for residual disease

detection using MRI [62]. Another methodology is di usion-weighted magnetic resonance imaging (DWI-MRI), which provides information on tumor cellularity and the integrity of cell membranes. Studies have shown that the association of DWI to DCE-MRI results in an increased MRI diagnostic accuracy [64]. Nevertheless, the accuracy of these techniques depends on the tumor subtype [65, 66, 67]. In addition to high costs, MRI is associated with some drawbacks, such as limitations for claustrophobic patients, patients with metal implants and those with allergic reactions to contrast agents. PET-CT has shown promise in predicting response to NACT based on both morphological and functional features of tumors; however, in addition to the high cost ($1000 per study) [68], the disadvantage of successive PET-CT is the exposure of patients to radioactive isotopes and ionizing radiation (30 millisieverts for a CT scan compared to 0.1 mSv for a chest X-ray). In addition, while change in tumor Ki67 proliferation index has been shown in pre-surgical studies to correlate with disease-free survival [69], application of this as a short-term biomarker of response to NACT would require repeat tumor biopsy during therapy, with associated potential harms of invasive procedures. There is therefore a clear unmet need for a sensitive, non-invasive, non-ionizing method of predicting response to NACT. More recently, using arti cial intelligence in di erent imaging methods such as ultrasound [70] and MRI [71, 72] has shown promising results and potential applications in prediction response to NACT.

Chapter 3: Ultrasound Elasticity Imaging

3.1 Soft Tissue Biomechanics

The breast has a heterogeneous structure with intrinsic mechanical properties. Glandular, adipose, and brous tissues have been shown to be the main contributing factor to the mechanical properties of the breast. Di erent factors such as age, menstrual cycle, pregnancy, lactation, hormone treatment, and menopause may change the structure and mechanical properties of the breast tissue during women's life [73]. In addition, these properties are altered as a result of pathological situations. Di erent diseases in the liver [74], thyroid [75], breast [76], prostate [77], and cardiovascular diseases [78] are accompanied by changes in the tissue mechanical properties.

The extracellular matrix (ECM) is a 3-D structure surrounding tissues [79]. Collagen is the primary structural component of the ECM that serve an essential role in preserving the biological and structural integrity of various tissues and organs [80]. Furthermore, collagen provides tensile strength and sti ness to the tissues [81] and has di erent density and orientation across di erent tissues. It has been shown that the sti ness of brous and glandular tissues are signi cantly higher than the adipose tissue [73]. Cancer is associated with deregulation of collagen metabolism, resulting in increased collagen expression, elevated deposition, and altered organization [82, 83]. This is the primary reason why the sti ness of cancerous breast tumors is higher than that of normal tissues [84, 11, 12]. A study on 169 fresh-ex-vivo breast tissue samples showed that the sti ness of brocystic disease and malignant tumors is 3-6 times higher than brogalndular tissue. The sti ness increases reaches to 13 fold for a high-grade IDC [12].

In general, elasticity and viscosity are the two components that constitute the mechanical

properties of tissues [85]. Soft tissues are anisotropic, nonlinear, and viscoelastic [85]. Under the assumption of small strains (under 10%) and neglecting the viscous forces, soft tissues can be considered elastic and linear materials. Therefore, under isotropic and homogeneous conditions, the relationship between stress and strain is described as:

$$f_{89} = \_n_{:} X_{89}, 2`n_{89} \tag{3.1}$$

where f is the Cauchy's stress tensor, n is the strain tensor, X is the Kronecker delta. $\_$ and $`$ are material coe cients known as Lamé constants [86].

There are three types of elastic moduli known as Young's modulus (E), Shear modulus (G), and Bulk's modulus (^). Young's modulus is an indicator of resistance of the material to uniaxial compression/tension and is de ned as:

$$= \frac{`^{1}3\_, 2`^{o}}{\_, `} \tag{3.2}$$

Poisson's ratio (a) represents the response of the material in the orthogonal directions to the uniaxial stress and is de ned as:

$$a = \frac{\_}{2^{1}\_, `^{o}} \tag{3.3}$$

The Shear modulus which is an indicator of the material's resistance to shear is determined as:

$$= \frac{}{2^{1}1, a^{o}} \tag{3.4}$$

Under the assumption of incomprehensibility, $a = 0{\bullet}5$, and therefore, the Shear modulus can be approximated as:

$$= \frac{}{3} \tag{3.5}$$

Lastly, the Bulk modulus is a measure of material's resistance to change in the volume when compression is applied from all the sides. The Bulk modulus is de ned as:

$$^{\wedge} = \_ \, , \, \frac{2}{3} \, ` \tag{3.6}$$

It is important to mention that shear modulus is more commonly used for elastography measurements than the bulk modulus. The reason is that magnitude of shear modulus may vary by two orders of magnitude based on tissue type, which provides tissue contrast. In contrast, the small di erence in bulk modulus of di erent tissues does not provide adequate tissue contrast for elastography [87].

3.2 Ultrasound Elasticity Imaging Techniques

As mentioned earlier in Chapter 2, the rst step in the routine physical examination is manual palpation. Manual palpation has been practiced for centuries to detect tissue abnormalities based on the fact that several pathological tissues are sti er than normal tissues. However, palpation is subjective, dependent on the examiner's experience, and limited to relatively large and shallow tumors. Although conventional imaging techniques provide valuable information on suspicious breast tumors, there is still a need for additional breast screening to reduce the false positive rate and subsequent unnecessary biopsies. An ideal breast screening tool must be non-invasive with high sensitivity and speci city and not be a ected by breast density. In addition, the cost is an essential factor in evaluating the likely extent of routine adoption.

Several elasticity imaging techniques have been developed to non-invasively estimate the mechanical properties of tissues with improved speci city and sensitivity than manual palpation. All these techniques involve mechanical excitation of tissue and assessment of the resulting tissue response. The tissue response can be detected using di erent medical imaging modalities based on ultrasound, magnetic resonance, or optics [88, 87, 89, 90].

In particular, ultrasound elasticity imaging techniques take advantage of ultrasound as an inexpensive, portable, and widely available modality that does not involve ionizing radiations. Ultrasound elastography was rst proposed in the 1990s for quantitative imaging of elastic properties of soft tissues [91]. Soft and hard tumors do not necessarily appear with di erent echogenicity on conventional B-mode US. Therefore, ultrasound elasticity imaging techniques are complementary modalities to B-mode US to improve tissue characterization. Since then several ultrasound-based elasticity imaging techniques have been developed [92, 93, 94, 87].

The tissue perturbation source can be applied externally, using a probe as in transient elastography [95], using an external dynamic vibrator [96], or manually by compressing a handheld ultrasound transducer, as in strain elastography (SE) [91]. However, external excitation methods are impacted by multiple factors such as the external boundaries, the coupling of the excitation with the targeted region, and the amount of stress applied by the operator [86, 97]. Furthermore, the image depth is limited by the attenuation and interaction of the signal with di erent tissue layers [97]. The tissue deformation can also be imaged in response to natural physiological motions [98, 99], or in responses to ARF that is internally applied.

Acoustic radiation force-based elasticity imaging techniques utilize a volumetric force resulting from momentum transfer from the ultrasound beam to the propagating medium due to acoustic absorption. ARF-based elasticity imaging techniques can be classi ed into two groups according to the location of the tracking beam: 1) within the region of excitation (ROE) or "on-axis" as shown in acoustic radiation force impulse (ARFI) imaging [100], Vibroacoustography [101] or HMI [16, 17], or 2) outside the ROE or "o -axis" as shown in shear wave elasticity imaging (SWEI) [102], supersonic shear imaging (SSI) [103], comb-push ultrasound shear elastography (CUSE) [104], and shear wave dispersion ultrasound vibrometry (SDUV) [105]. The tissue displacement has a greater amplitude at the ROE and decreases in amplitude outside that due to the spreading of the acoustic energy. Therefore, "on-axis" techniques are less limited by the depth and sti ness of the targeted tissue. However, these

Figure 3.1: Ultrasound-based elasticity imaging methods can be categorized based on the source of tissue excitation, duration of excitation and location of the tracking beams.

methods induce displacements dependent on the magnitude of the applied force, therefore providing a qualitative assessment of tissue mechanical properties. Although "o -axis" techniques provide quantitative measurements of the mechanical properties, they are impacted by the shear wave re ection, and distortion at interfaces in heterogeneous and small tissues [87, 106].

For the techniques in which imaging transducers generate the ARF, the depth of penetration and speci city may be limited due to the power limits of the imaging transducers and the attenuation of the vibration energy through di erent layers of tissue. It has been shown that lesion size, breast thickness, and lesion depth are among the factors that may lead to false ndings in ARFI shear wave imaging [107]. A comparison study between histopathology and ARFI elasticity imaging results has shown a 59.1% false positivity [108]. The source of ARF can also be a FUS transducer with a highly localized focus. Using a FUS transducer as a source of tissue perturbation can be advantageous for an ultrasound-based method for simultaneous monitoring and treatment during high-intensity focused ultrasound (HIFU) ablation. In Vibroacoustography, two confocal ultrasound elements of a spherically focused annular array generate a low-frequency oscillating force, and the resulting tissue displacements are detected using a hydrophone [101, 109]. Tissue sti ness estimation, however, is challenging since the hydrophone's signal is a ected by various factors, including the

mechanical and acoustical properties of the tissue [110].

3.3 Harmonic Motion Imaging

HMI is an ultrasound-based elasticity technique that assesses tissue viscoelastic properties by inducing periodic oscillations [16, 17]. HMI uses a FUS transducer to generate an amplitude-modulated ARF at its focal zone. The resulting tissue oscillations are tracked using a confocally aligned imaging transducer, and the recorded RF data is used to estimate the displacements. The amplitude of the displacements is inversely proportional to the sti ness of the underlying tissue under some assumptions such as uniformity of stress distribution and tissue homogeneity that are relatively valid for a small FUS focus ($1mm^3$). The underlying tissue oscillates at a speci c frequency, which makes HMI more robust to low-frequency respiratory, cardiac, or body movement artifacts than impulsive radiation force imaging techniques. The oscillation frequency of tissue is equal to twice the applied amplitude modulation (AM) frequency. Assuming plane wave propagation, the ARF in an attenuating homogeneous medium can be de ned as [111, 112]:

$$^{1}\text{Ȣ} = \frac{2U\ ^{1}\text{Ȣ}}{2} - \tag{3.7}$$

where Cis time, ^{1}Ȣ is volumetric force, U is the tissue absorption coe cient, I(t) is the average acoustic intensity, and c is the speed of sound. The time averaged intensity is de ned as [113]:

$$^{\sim 1}\text{Ȣ} = \frac{1}{)_2}\ ^{1}\ \text{Ç})_2\ \frac{?^{2\,1}g^{o}}{d2}3g - \tag{3.8}$$

where d is the density, $)_2$ is the period of the propagating wave, and $?^{1}$Ȣ is the acoustic pressure that can be expressed as:

$$?_{>}\ \ B8^{1}\!\!\!