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ABSTRACT

A model based on the microstructure-accounting mechanical field theory (doublet/nano mechanics) was developed, in combination with the use of a characterization-mode ultrasound testing method, for the reconstruction of otherwise inaccessible information on the physical properties of biological tissue samples. The change in the physical properties is well recognized to associate with disease inception. Accurate quantitative characterization on these properties, however, has been proved elusive. This study investigated a novel theoretical model, in comparison with that built upon conventional mechanical theories, to test the hypothesis that a discrete representation that takes into account the complex but intrinsic micro-architecture of biological tissue, could offer beneficial implications for separating normal tissue from the abnormal based on the differences of their physical properties.

In this study, surgical remnant, snap-frozen breast tissue from multiple subjects were obtained and prepared as very thin sections embedded between two pieces of glass. Both normal and malignant tissue was procured from the same subject. The sandwich structures were tested on a novel ultrasonic non-destructive evaluation system that is capable of accurate measurement on the mechanical responses of the inspected samples through their reflection coefficients within a broad range of frequencies. One broadband
non-focal ultrasound transducer (with central frequency 10MHz) was employed to
generate ultrasonic pulses that interact with the sandwich structure; and another
transducer with same characteristics was used to receive the reflections. The reflection
spectra were obtained through standard Fast Fourier Transformation (FFT) from the
gated pulse signals. Theoretical models that depict plane, elastic wave propagation in
microstructured, multi-layered media were constructed to predict the reflection responses
of the thin tissue layer. The quantitative information on the micro-level mechanical and
structural properties was made available through an inverse algorithm that searches in the
parameter space to generate the set of values that allows the optical agreement between
the model prediction and the experimental data. A comparative study was carried out to
evaluate the results from the doublet and continuum mechanics models. The experimental
data showed that the spectra from normal and malignant tissue were appreciably and
consistently different. The statistic analysis on the quantitative reconstruction of tissue
parameters presented significant difference on certain properties. This study has
demonstrated that the ultrasonic system developed in this study was sensitive in detecting
the differences in the physical properties of normal and malignant tissue. The
microstructure accounting mechanical model may provide an advantageous approach for
quantitative analysis of tissue physical properties towards the goal of cancer detection
and diagnosis.
To my family, with love
ACKNOWLEDGMENTS

I first thank God, who has shown His amazing grace to me throughout the years of my graduate studies at the United States. He has blessed me a wonderful family, an inspiring advisor, an exciting project and many supportive professors, coworkers and friends. Thank God for His extravagant love! He has protected me; He has showered His mercies and goodness on me; and He has faithfully led me to His paths.

I thank my advisor, Professor Mauro Ferrari, who is artistic in developing his students into independent and creative thinkers with vision. He himself has been such a role model! I deeply appreciate the liberty he has generously granted me during the development of my research projects. It is the trust that has brought out the best of a novice researcher. I also thank him for his contagious passion for teaching and mentoring; thank him for the patience in correcting my academic writings; thank him for the insightful guidance at moments of doubts; above all, I thank him for being a wonderful friend who sincerely cares about my career, my well being, and my family.

I thank my dissertation committee member, Professor Stanislav Rokhlin, who has kindly provided the ultrasonic equipments to obtain the experimental data in this project. His expert guidance on sample preparation, data acquisition and general experimental

vi
design was very helpful and instructive. Thank him also for his continuous
encouragement and his exemplary devotion to research. I thank Professor Nicanor
Modolvan for important and constructive critiques on the research design while it was
just a sketchy proposal for my candidacy exam. Thanks to him for teaching me the
concepts of building rigorous logic into experimental design. My gratitude also goes to
Professor Tom Rosol, Dr. Manju Prasad and Dr. Jennifer Lewis for their enthusiastic
assistance with obtaining animal and human tissue.

I want to thank the students at the Nondestructive Evaluation lab: Bin Xie, Lugeng
Wang, Vadim Iakovlev, who kindly shared their knowledge and experience in ultrasonic
systems, and also their labs with me. Thanks to the ladies at the Histology Core
Facility—Mary Marin and Karen Williams, who were extremely supportive and
cooperative to meet my special needs for tissue cutting. Thank Shawn Coontz for
generously donating the glass and providing it in a timely fashion. Thank the people at
the Tissue Procurement Office for coordinating tissue acquisition. I also want to thank Ed
Herderick, who provided invaluable discussion on the statistic analysis, and Vlad
Marukhlenko for general computer technical support. Thank the wonderful ladies at
BME, Kirsten Gibbons, Melanie Senitko and Anita Bratcher, for their support and care.

I am truly grateful for my dear friends Jeanie Pee and Chana Ulm. Their
friendship has been my extraordinary comfort during these years. I treasure the warm
memories of hanging out with them, chatting about our lives and praying for each other
with genuine love. Their encouragement and support have helped me persevere.
To my darling little girl, Anrei, I want to say thank you for allowing mommy this specially challenging and fulfilling experience of being a parent-student. The project started when she was just born. She brought a fresh new page to my life. Thank her for all the laughers and cuddling; thank her for reminding me to enjoy life’s simplest fun and excitement; thank her for greatly reducing the time that I would have otherwise spent in the project! I also want to express my deep gratitude to my parents and parents-in-law—they came from thousands of miles away to help out at times of need and to give their love as they always do.

Finally I thank my dearest husband, my best friend, Xueliang Pan. So many times he has patiently listened to me, sharing my joys and sorrows in my work and studies. He is always quick to understand and eager to help with his wisdom and creativity. His trusting heart has blessed me with the courage to face the Goliaths in my life. I can’t thank God enough for him!

I gratefully acknowledge the State of Ohio for funding this project.
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CHAPTER 1
INTRODUCTION

1.1 Background: Tissue Analysis

Tissue analysis refers to make a diagnosis based on the characteristics of tissue. The analysis can be made while the tissue is still intact within the body (in vivo methods), but routinely it is done after the tissue has been removed out through surgical procedures.

The current practice of tissue analysis typically starts from the clinicians' request for a biopsy on the region of concern. Surgical pathologists will then conduct careful gross examination of excised tissue, first with the naked eye or with the help of a dissecting microscope. The tissue will then be processed, usually fixation and paraffin embedding, before sectioning. Frozen tissue is sometimes ordered for intraoperative diagnosis. After these processes, technician specialties prepare 3-5 micron thick tissue sections by using microtomes, and stain them with hematoxylin and eosin (H & E) and occasionally other dyes to prepare microscopic slides. Pathologists will then perform a detailed examination on the tissue sections in the compound light microscope.

Information on cellular architecture (the spatial relationship of cells), shape and size of cells, frequency of mitosis, cellular abnormalities or invasion, the degree of
cellular differentiation may all be relevant in the pathologic assessment, especially for tumor diagnosis. For example, there are more than ten types of proliferative breast diseases [Cotran et al, 1999]. The diagnostic differentiation has to be carefully made based on the microscopic characteristics of the tissue.

It is however recognized that visual examination sometimes is not sufficient. Seen on a slide with malignant tissue, the shapes of the cells or even the spatial distribution may be exactly the same as that with normal tissue [Cotran et al, 1999]. Molecular pathology takes one-step further to analyze expression of specific genes or gene products through immunohistochemistry (IHC) [Dabbs, 2002]. Besides going down to molecular level, pathologists also resort the reports from imaging technologies to make final decisions. What imaging technologies essentially provide is the information on certain physical properties of the tissue, for instance, density in X-ray and acoustical impedance in ultrasound. It is clear that useful information on cancer characteristics resides in different scales. Molecular biochemistry, cellular morphology, and tissue physical properties offer complimentary evidence for comprehensive diagnosis.

Although visual examination on tissue slides by well-trained pathologists is the gold standard for tissue diagnosis, and will justifiably continue to be so, there is room for improvement—it remains a time-consuming, subjective and non-quantitative practice to date. Time-consuming and labor-intensive translate directly into high cost. Subjectivity is manifested in the concerning inter-observer differences, especially in the practice of IHC for breast cancer staging [Thomson et al, 2001]. Quantification on the visual information
has been attempted to remedy the situation, but the results were not satisfactory [Morrison et al, 2002].

In light of these needs, an ultrasound characterization method was proposed to investigate tissue physical properties towards the goal of objective and quantitative tissue diagnosis. The underlying hypothesis is that pathological conditions could be identified through variations in the physical properties of tissue. Given that quantitative information on these properties of the tested biological domains is difficult to obtain by conventional imaging modalities, an engineering non-destructive evaluation (NDE) method was therefore employed.

A major difficulty in the adaptation of this potentially beneficial engineering approach to biomedicine is the fact that it demands appropriate theoretical models to allow for the reconstruction of the appropriate physical properties from the measured ultrasound propagation data. The conventional mechanics of solids, including biological domains, is based on a continuum representation [Fung, 1994], which is a mathematical idealization that regards matter as infinitely divisible and completely microstructure-free. This extraordinarily successful modeling strategy, however, is not so justified for domains such as seen in biological soft tissue that are apparently microstructure-rich at the scale of the wavelength of medical ultrasound. Previous attempts have been made to address these concerns by representing the interested domains as composites with continuum matrices and inclusions [Mura, 1982]. These theoretical frameworks, collectively known as micromechanics, still lack the capability to incorporate the discrete micro-architecture that might be critical in dictating the mechanical responses of such
domains. In this study, a novel microstructure-accounting mechanical framework, doublet mechanics [Ferrari et al, 1997], which permits the representation of matter as discrete nodes with an arbitrary micro-architecture, was employed for the interpretation of the mechanical responses of biological soft tissue. The theory is also denoted as nanomechanics to first distinguish itself from micromechanics, and second to highlight its multi-scale nature, which allows it to model discrete nodes at finite distances, as small as nanometer range, if desired.

1.2 Research Objectives

The objective of this research is to develop the fundamental technology of a quantitative method for microstructure-accounting analysis of the physical properties of biological tissue towards the goal of cancer detection and diagnosis. The focus of the current study is on the ex vivo breast tissue that is surgical remnants resembling biopsy specimens. Further development for in vivo scenarios is envisioned and discussed in chapter eight.

The specific aims in this study are as follows:

Aim 1: Develop sample preparation protocol that is suitable for measurement of physical properties of ex vivo breast tissue through non-destructive ultrasound techniques;

Aim 2: Construct ultrasonic measurement system for acquisition of wave propagation data in tissue media;
Aim 3: Design data analysis software based on microstructure-accounting doublet (nano)mechanics model for biological tissue;

Aim 4: Evaluate doublet (nano)mechanics approach in comparison with conventional continuum mechanics approach.

1.3 Significance and Outline

SIGNIFICANCE

The main element of innovation in the project is the application of a microstructure-accounting mechanical field theory (doublet/nano mechanics) in combination with the use of characterization-mode ultrasound testing, for the deconvolution of otherwise inaccessible information from the tissue samples. Such information includes the architecture of the microstructures, as well as a new set of physical parameters. This project serves as the fundamental studies for the establishment of a technological platform with significant short-term, and potentially long-term beneficial implications.

The direct significance of the proposed technological platform is in the development of a novel modeling approach for biological tissue. It is the first time that a discrete-based doublet mechanics model was built for analyzing plane elastic wave propagation in tissue. This project explored the potential advantages of such a representation against the common practice of continuum simplification. Meanwhile, the
project investigated the feasibility of transferring the well-developed NDE technologies from analyzing engineering structures to biological domains.

The long-term significance may be the generation of a method/device for rapid, automated, and quantitative ultrasound-based identification of malignancy on tissue slides. This "slide reader" could dramatically reduce pathological assessment time by quickly preprocessing/screening a large number of tissue slides so that pathologists could focus their energy on the suspicious ones. It could also potentially suffice a rapid sampling method for a big trunk of tissue such as obtained through mastectomy, which contains a mixture of normal, benign, malignant and metastatic malignant tissue. Currently there is no method available to perform such a task in a thorough and timely fashion. Quantification on the properties of tissue may also increase interobserver and interlaboratory agreement, leading to the opportunity for the greater standardization of diagnostics, staging, and therapeutic practices.

The long-term impact of this technology is not restricted to ex vivo tissue analysis. The development of non- or minimally- invasive in vivo applications for tumor early detection, diagnosis and therapy is envisioned. This study serves as a basic research platform towards this goal in the sense that we gained knowledge in the following aspects: 1) The extent of the difference in the mechanical responses between pathological and normal tissue, which dictates the feasibility of ultrasound characterization method; 2) The microstructural mechanisms for determining the characteristics of high frequency elastic wave propagation, which would not be
retrievable otherwise but potentially enhance the differentiating capabilities of the quantitative method.

OUTLINE

The motivation and the objective of this study are introduced in the first chapter. Chapter 2 contains information of the relevant literature that spans across at least three fields including tissue elasticity, NDE models and doublet mechanics. Chapter 3 and chapter 4 are detailed description on the methodology of the ultrasonic measurement and the microstructural accounting analysis, respectively. The experimental data and analysis results are presented in chapter 5. Chapter 6 is a discussion on the microstructure-accounting model, the resulting property reconstruction in comparison with conventional models, and the limitations of the current setup. Chapter 7 gives the conclusions drawn from the study. Future potentials and directions are charted in chapter 8.
CHAPTER 2
LITERATURE REVIEW

2.1 Measurement of the mechanical properties of tissue

2.1.1. Introduction:

Measurement of the mechanical properties of biological soft tissue has been proved particularly elusive because it is not well-behaved material in terms of being easily modeled or described mechanically. Soft tissue is inhomogeneous, anisotropic, non-linear, viscoelastic and physiologically active (if not fixed). Its mechanical properties are also time, moisture and load dependent. Given this complexity, previous attempts on the measurement or analysis of tissue mechanical properties inevitably resort to idealization or simplification on the characteristics of the medium. For example, soft tissue is usually modeled as an elastic, linear, isotropic, homogenous and continuous material. To further reduce the necessary parameters for characterization, incompressibility is also assumed so that the measurement or analysis on one single parameter (usually Young’s modulus) is sufficient.
2.1.2 *Previous Studies:*

Tissue mechanical properties are either measured directly on *ex vivo* samples or indirectly through *in vivo* methods.

*Ex vivo* methods:

Direct methods on *ex vivo* tissue involve the adaptation of engineering approaches for mechanical testing.

Burke et al. presented a preliminary study on the measurement of the shear wave speed of sound and attenuation coefficients from *ex vivo* specimens of human breast tissue [Burke et al, 1990]. Mono-frequency (1.5 MHz) sinusoidal plane shear wave was applied normally from a silicon substrate to the tissue sample. The reflections from the interface were used to compute the wave speed. It was found that shear wave speed was in the range between 20 and 900 m/s, and dependent on the composition of the tissue and the time post excision.

Krouskop et. al. measured the elastic moduli of breast and prostate tissue under compression [Krouskop et al, 1998]. Displacement loading with desired frequency (0.1, 1.0 or 4.0 Hz) was used by a hydraulic servo Instron testing machine. A model of uniform load acting over the partial boundary of a semi-infinite elastic solid was employed to extract modulus data from the experimental measurement. The results showed that breast fibrous tissue was 1 or 2 orders of magnitude stiffer than fat tissue at a fairly high strain level (20%); while invasive ductal carcinoma were much stiffer than any other types of breast tissue. The Young’s modulus for normal fat breast tissue was in
the range of 18-22 kPa for 5% precompression and 20-24 kPa for 20% precompression; while for invasive ductal carcinoma 93-112 kPa for 5% precompression and 460-558 kPa for 20% precompression.

A system that was capable of measuring small tissue samples was developed by [Erkamp et al, 1998]. It applied a known deformation to the tissue sample and simultaneously measured the resulting force. The modulus of the tissue was obtained using calibration of the system with plastisol samples of known Young’s modulus. Measurements on canine kidney samples showed that the system had good reproducibility, low elastic modulus variability for similar tissue, and reasonable sensitivity for changes associated with malignancies.

*In vivo methods:*

Acoustic, ultrasonic, tactile and MRI (Magnetic Resonance Imaging) methods are among the indirect approaches to measure tissue elasticity. They are usually coupled with representation of the measurement results in a pictorial format—images—that are essentially spatial mappings of the measured properties. The general field of elasticity imaging is called “elastography” [Gao et al, 1996], and the acquired images are called Elastograms.

The acoustic methods involve the application of low-frequency vibration energy to the tissue or organ and the simultaneous monitor of the mechanical response from the interested region. These methods required the construction of theoretical models on the wave propagation in the medium of tissue. The elasticity was interpreted from the
experimental measurement in combination with the models. In Sonoelasticity imaging [Krouskop et al, 1987; Parker et al, 1990; Yamkoshi et al, 1990], a low-frequency vibration (20-1000 Hz) was externally applied to excite the internal tissue. Doppler ultrasound was used to detect the perturbation in the ultrasonic wave propagation caused by the vibrations. The elastic modulus was calculated from local wavelength of the shear waves, or the amplitude and phase of the vibration [Yamkoshi et al, 1990]. In ultrasound-stimulated vibro-acoustic spectrography [Fatemi et al, 1998], interfering focused ultrasound was applied to the tissue to produce vibrations whose energy was received and recorded by an external hydrophone. The region of interest, i.e., the focal point of the two ultrasound beams, was controlled by raster scanning. The phase and amplitude of the acoustic emission contained information on the mechanical properties of the region under inspection.

Ultrasonic methods involve the application of static loads to the tissue with the mechanical response monitored by ultrasound imaging [Ophir et al, 1999]. Small compression loads were applied in stepwise increment. Ultrasound imaging device was used to monitor the local displacement of different points in the tissue medium. The A-line signals acquired before and after the compression were compared piecewise using cross-correlating techniques. The distribution of axial displacement or strain was thus estimated directly from the comparison. It is noted that the estimation of axial strain/displacement can be directly imaged to serve for diagnostic purposes, or the mechanical properties can be inversed as solutions of the forward mechanical models.
Tactile/mechanical imaging [Wellman 1999; Sarvazyan, 1998] involves the application of mechanical sensors on the surface of an organ to evaluate the “hardness” of the underlying tissue. The pressure or stress was directly measured by the mechanical sensors while they were used to compress the tissue. Mechanical properties of the tissue were estimated from the solution to an inverse problem using the data of the stress pattern recorded by the sensors.

The MRI method [Muthupillai et al, 1995] involves the application of the MRI imaging to monitor the physical response of tissue to harmonic mechanical excitation. Acoustic strain waves (50-1000 Hz) were generated through the interaction between the alternating flux of an electrical coil and the main magnetic field that was applied to the tissue. MRI was used to observe and record the displacement pattern from which strain or other mechanical characteristics related to the wave propagation could be computed.

2.1.3 Discussion:

Tissue elasticity has shown significant potential as useful diagnostic information that cannot be obtained through traditional medical imaging. The measurement and corresponding imaging techniques of tissue physical/mechanical properties still require further development to become useful modalities for clinical situations. A number of issues remain unanswered and call for further study. First, a sufficient and reliable database of tissue elasticity data at normal and pathological conditions still needs to be constructed for the evaluation of the performance of the overall diagnostic system. The unavailability of sufficiently sized biological tissue has posed a difficult constraint on the
attempt to achieve this goal through efficient engineering methods. In other words, the standard material testing equipment is often ill suited for testing soft tissues. Secondly, the available data on soft tissue such as breast is not only scarce, but also quite widespread according to [Aglyamov et al, 2000]. Possible reasons for that include the uncontrollability of certain measurement conditions and the variation of the interpretation methods on the experimental data. Thirdly, the current methods for tissue characterization usually suffer from a lack of "golden standard"—an objective reference that can be used for evaluation of the measurement. Fourth, the adequacy of the models for tissue is still a subject of debate in view of the complicated nature of biological tissue. Cellular composition and microstructures/organization [Fung, 1993] are believed to be important determinants of tissue elasticity, however, their quantitative mechanisms are elusive goals based on the simplified models widely used so far.

2.2 Quantitative ultrasound with NDE models for material testing

2.2.1 Introduction:

The field of non-destructive evaluation of materials has existed for a few decades. Its primary concern is the detection of defects and characterization of material properties non-destructively. An important recent extension of the field is the development of NDE methods with models that can be used to predict the measurement system's response in relationship with the physical properties of the material or structure under of interest. It is recognized that numerical analysis based on the models is essential in the objective
interpretation of the experimental data. The models are also indispensable for the construction of inverse techniques that generate quantitative information for material characterization.

2.2.2 Previous Studies:

For extensive literature on the subject of advances in the quantitative NDE models, the reader is referred to the most recent review article by [Achenbach, 2002]. A brief review on ultrasonic NDE for characterization of thin layer is presented here due to its particular relevance to this study. Thin layer characterization has been widely applied in engineering realms including the evaluation of adhesive bonds, thin films deposited on material surfaces, laminates of composites and so on. Studies of the propagation of elastic waves in layered media were reported decades ago as in the books by Ewing et al [Ewing et al, 1957] and Brekhovskikh [Brekhovskikh, 1966]. Nayfeh [Nayfeh, 1991] gave an exact treatment to the interaction of harmonic elastic waves with $n$-layered anisotropic plates, where a global transfer matrix was constructed to satisfy the continuity conditions at the interlayer interfaces. Kinra et al [Kinra et al, 1995] devised a general method to determine one of the four acoustical properties of a thin plate (thickness, wave-speed, density or attenuation) assuming the other three were known. The thin plate was immersed in water while plane longitudinal waves were applied normally upon it. The properties of the thin plate were determined by solving the inverse problem, which utilized a secant method in conjunction with the method of least squares. Lavrentyev et al [Lavrentyev et al, 1997] designed a two-angle ultrasonic spectroscopy method to
simultaneously determine the elastic moduli, attenuation, and thickness of an adhesive layer embedded between two known substrates. A normal incident and an oblique incident wave were applied upon the three-layer structure. The reflection spectra from the thin layer were used to inverse all the properties through six non-dimensional parameters.

Discussion:

Ultrasonic NDE methods have been proven to be successful in characterizing material properties for specific configuration and problems. To our best knowledge, the methods have not been substantially explored on biological tissues.

2.3 Doublet (Nano)mechanics

2.3.1 Introduction:

The theory of doublet mechanics was originally developed to address the modeling difficulty for physical domains that are too irregular to be treated as continuum and yet too large for atomic analysis. It bridges continuum mechanics and lattice dynamics with contradiction to neither—its unique multi-scale nature permits it to recover both continuum mechanics and lattice dynamics at the appropriate limits.

Under the theoretical framework of doublet mechanics, physical domains are composed of discrete entities or nodes, which are geometrical points relating to each other through finite distances and specific orientations.
Each lattice node is assumed endowed with a rotation and translation vector with increment vectors that may be expanded in a convergent Taylor series about the lattice nodal point. The order $M$ at which the series is truncated defines the degree of approximation employed. Most importantly, the fundamental equations relating microstrains to the displacement vectors contain the lattice geometry/micro-architecture and internodal distances. For more description on the fundamental governing equations in doublet mechanics, please refer to Appendix.

2.3.2 Previous Studies:

In Granik and Ferrari [Granik et al, 1993], the system of equations was supplemented by linear elastic, homogenous constitutive equations relating the microstresses to the microstrains. A solution to the celebrated Flamant’s problem was obtained with predictions consistent with abundant experimental results. That was not achievable from continuum mechanics modeling. In the field of viscoelasticity, Maddalena and Ferrari [Maddalena et al, 1995] employed Doublet Mechanics to the Maxwell and Kelvin-Voigt type viscoelastic modeling. It was determined that materials that are Kelvin-Voigt type at the micro-mechanical level also presented a continuum-level Kelvin-Voigt constitutive behavior, while strong conditions on the geometry of the underlying lattice would assure the micro-mechanical level Maxwell material would be of Maxwell type at the continuum level. The other area that was investigated by Ferrari and Granik [Ferrari et al, 1994; Ferrari et al, 1995] was the derivation of continuum-level failure criteria starting from microstructural considerations.
In direct relevance to the proposed work, the free-boundary effects on multi-scale elastic waves in micro-mechanical domains were analyzed by Zhang and Ferrari [Ferrari et al, 1997]. Seminal findings were obtained regarding the conditions of mode conversion in relationship to microstructural parameters such as internodal distance.

2.3.3 Discussion:

Doublet Mechanics not only stands out when compared to the conventional continuum mechanics, but also distinguishes itself from other micromechanics. For an extensive analysis on the salient features and the recommended application domains, please refer to the fourth chapter in [Ferrari et al, 1997].

It is noted that biological tissue is an example of such physical domains that are suitable to be analyzed by Doublet Mechanics. Tissue has distinct microstructures that could be easily observed under microscope. It is composed of structures such as cells and extracellular matrices. A microstructural-accounting theory such as Doublet mechanics is especially needed for the analysis on tissue that is examined through high frequency mechanical waves such as ultrasound. The characteristic dimensions of the microstructures may reach the level of the wavelengths, and therefore play a non-negligible role in determining the mechanical response of the tissue.
CHAPTER 3
ULTRASOUND MEASUREMENT SYSTEM FOR BIOLOGICAL TISSUE:
DESIGN AND PROTOCOL

The overall design of the experimental system employed in this study was a modification on one type of the general ultrasound evaluation systems: an immersion-testing with planar interfaces. Specifically, the tissue samples (embedded between substrates with known physical properties) were immersed in water. This setup avoided the necessity of direct contact between transducers and bio-hazardous material such as surgical remnant biological tissue. The interfaces that the ultrasound waves encountered were all planar (as ensured by the geometry of the substrates). Accordingly, the wave propagation problem was simplified to planar reflections and transmissions without the complexity introduced by irregular interfaces.

3.1 Sample Preparation Protocol

To quantitatively examine biological tissues by ultrasound, a three-layered structure (see Figure 3.1) was constructed by placing a very thin layer of biological tissue between two, relatively thick, glass layers. This structure is referred to as a sandwich
structure in this study. The sandwich structure was designed for the following reasons: first, the interface between the glass and the tissue enhanced the reflection from tissue specimen due to the acoustic impedance mismatch between glass and tissue. If a piece of tissue were put directly in water and ultrasound waves were applied perpendicular to the tissue surface, there would be reflections from the tissue-water interface; nevertheless, the reflections would be too small to be accurately analyzed, because the acoustical impedances of water and tissue are just slightly different. Secondly, the multi-layered structure created a geometric possibility for acoustical resonance to happen. Multiple reflections at the top and bottom interfaces between tissue and glass made it possible for multiple waves with a phase-lag propagating within the thin-layer. The constructive and destructive summations of these waves gave rise to characteristic reflection spectra that could be measured within a certain range of frequencies. This further enhanced the measurement sensitivity of the reflections from the thin layer.

Glass was chosen to be the substrate because it had substantially different acoustic properties compared to most biological soft tissues. Furthermore, its mechanical properties were homogenous and frequency-independent as tested by experiments. It was also readily available and fairly cheap. To focus on examining the properties of the biological tissue thin layer, the physical properties (density, Lame’s constants) were experimentally measured for the glass used in this study. The density of the glass was calculated from the direct measurement of its mass and volume. The longitudinal and shear ultrasonic wave speeds in the glass were measured using standard time-of-flight method [Rose, 1999].
Human breast tissue was obtained from the Tissue Procurement Office at The Ohio State University (protocol approved by Biomedical Sciences Institutional Review Board, The Ohio State University, March 2000). Both the malignant tumor and its surrounding normal tissue were obtained as blocks preferably with 1 cm X 1 cm surface area. The blocks were snap-frozen and stored at ultra-low temperatures (-80°C) right after surgical removal. Either fixation and paraffin embedding or fresh-freezing, was necessary to prepare/preserve the tissue prior to cutting. Previous studies on liver tissue showed that changes in the acoustical properties that were introduced by freeze/thaw cycles were negligible compared to paraffin embedding cycles [Van Der Steen et al, 1991]. Therefore, the method of fresh-freezing was chosen for this study to obtain measurement of the parameters close to those under in vivo settings. Before cutting, the blocks were
transferred to a cryostat microtome whose temperature was maintained at -21°C (Figure 3.2). The tissue was then cut to 150μm thin sections. Biological tissue is largely heterogeneous, but a thin layer of 150μm could be approximately assumed to be homogenous through the thickness. The cellular integrity was also preserved at this thickness (cell dimension is usually at the order of 10μm). The heterogeneity on the plane could be addressed by using transducers with very small beam size to scan the whole plane.

Figure 3.2 The cryostat microtome for tissue cutting (Courtesy Histology Core Facility, the Ohio State University)

The tissue was first mounted on the center of one piece of glass. Another piece of glass was carefully put on top. Efforts were made to avoid trapping air bubbles between
the tissue and the glass. It was observed during some experiments that air bubbles affected the reflected signals significantly. To ensure the exact thickness of the tissue layer (soft tissue is compressible), paper spacers of specific thickness were inserted at the two ends before clamping the two pieces of glass together. Because measurements were taken while the sandwich structure was immersed in water, the edges were sealed with epoxy glue so that water would not enter the space between the two layers of glass. Otherwise, the presence of water could significantly interfere with the measurement. The epoxy glue was allowed to dry for one hour or two before measurements.

3.2 Ultrasound Measurement System

The elements of a basic ultrasound nondestructive evaluation system are shown in the sketch (Figure 3.3). The energy-provider of the system is the pulser section of a pulser-receiver. The pulser usually generates very short (0.1 μsec in duration), repetitive (approximately 1 msec apart) electrical pulses. The electrical pulses are of very high voltage (on the order of several hundred volts) and they drive a transducer (typically piezoelectric material) to produce mechanical vibrations, which then transmit as a beam of ultrasound in media.

If the ultrasound beam hits a material discontinuity while propagating in a medium, a portion of the energy will be reflected/scattered and transmitted along another direction of wave propagation. The reflected/scattered or transmitted signals can be
detected by a second receiving transducer, which transforms mechanical pulses into electrical pulses.

The electrical energy transferred from ultrasound vibrations is usually small (on the order of 0.001 volt). It is amplified to the order of 1 volt through an amplifier. The waves of the electrical pulses can be displayed as a voltage versus time trace on an oscilloscope or the screen of a computer.

For further process and quantitative evaluation on the received signals, it is necessary to capture and store them. This is achieved through digital oscilloscope or an external digitizer with high sampling rate (above 100MHz) to preserve all the details in the signals. Once in the digital form, the signals can be readily stored in the computer for analysis.

![Diagram of Ultrasonic Nondestructive Evaluation Measurement System](image)

**Figure 3.3:** Elements of an ultrasonic Nondestructive Evaluation measurement system.
The ultrasound measurement system that was adopted in this study was basically an immersion-type test with angle beam incidence on planar surfaces. The examined samples were immersed in water, which served as coupling agent for mechanical waves to propagate from transducers to samples. The incident ultrasonic waves came from a non-zero angle with respect to the perpendicular direction of the sample surfaces.

The incident ultrasound beam could theoretically take any angle less than 90 degrees. According to previous studies, it should be between the two mode-conversion angles of the water-glass interface to optimize measurement sensitivity [Lavrentyev et al., 1997]. Theoretical analysis (simulations) showed that all the angles between the two mode-conversion angles had similar sensitivity. The oblique angles that the available transducer holders took were 17 degrees in the current laboratory (Nondestructive Evaluation Lab, the Ohio State University); and it also happened to be between the two mode-conversion angles of the water-glass interface. Thus, 17 degree was chosen as the incident angle in this study.

The instrumental setup is shown in Figure 3.4. An externally triggered pulser (Panametrics, 5052PR) generated electrical pulses and sent them to an unfocused broadband transducer (Panametrics, central frequency 10 MHz, diameter \( \frac{1}{2} \) inch). The transducer transferred electrical pulses into mechanical vibrations—ultrasonic pulses that propagated through the water and interacted with the sandwich structure. The interaction caused multiple reflections from the sandwich structure that were distinguishable in time domain: reflection from the upper surface of the glass, reflection of the whole thin layer (lumped together), reflection from bottom surface of the second glass and so on (Figure
3.5). These reflections were all received by a receiver transducer (Panametrics, central frequency 10 MHz, diameter $\frac{1}{2}$ inch) that transferred mechanical vibrations to electrical signals. The electrical signals were then amplified by the receiver (Panametrics, 5052R) that was used at a fixed gain of +20 dB. The signals were digitized by PDA500M waveform digitizer (SignaTec) at a sampling rate of 125 MHz. The digitizer was interfaced with a computer (700Mhz Dell Pentium III PC) in which the data were stored and further processed. The computer also controlled an XYZ translation system (Velmax 86) in which the transducer and sample were mounted.
Figure 3.4: Instrumental Setup (Courtesy Non-destructive Evaluation Lab, the Ohio State University)
Figure 3.5 Multiple reflections that are detected by receiver transducer: left, the time domain signal of normal incidence observed at the receiver transducer; right, the corresponding reflections at different interfaces due to wave propagation in the multi-layered sample.
3.3 The Measurement Model

A generic input-output system could be described by the schematic shown in Figure 3.6, where the system takes input $i(t)$ to produce an output $o(t)$. Both $i(t)$ and $o(t)$ are functions of time $t$. The ultrasonic measurement system for biological tissue samples designed in this study had many components that were themselves complex electromechanical systems. To model such a measurement process from a system point of view is a challenging task. One important and useful technique of simplification is to treat each of the components and therefore the overall system as Linear Time-Shift Invariant (LTI) systems, where

$$o(t) = L[i(t)] \quad (3.1)$$

![Figure 3.6 General input-output system](image)

The linearity of the system requires

$$o(t) = L(c_1 i_1(t) + c_2 i_2(t)) = c_1 L[i_1(t)] + c_2 L[i_2(t)] \quad (3.2)$$

where $i_1$ and $i_2$ are two arbitrary inputs and $c_1$ and $c_2$ are two arbitrary constants.

The time-shift invariant property of LTI system requires

$$o(t - t_0) = L[i(t - t_0)] \quad (3.3)$$
which means that a delay in the input signal produces an identical delay in the output.

For a system comprised of multiple LTI systems (Figure 3.7), the overall response in time-domain is determined by the convolution of the impulse response of each component:

\[ o(t) = g_1(t) \ast g_2(t) \ast \cdots \ast g_n(t) \ast i(t) \]  
(3.4)

where \( g_k(t) \) is the impulse response of the \( k \)th component.

\[ i(t) \rightarrow g_1(t) \rightarrow g_2(t) \rightarrow \cdots \rightarrow g_n(t) \rightarrow o(t) \]

**Figure 3.7** A LTI system with multiple LTI components

Convolution in time-domain is equivalent to product in frequency domain. Another useful technique in the analysis of such a system is Fourier Transformation that describes the responses in terms of the decomposition of a pulse (time domain signal) into a distribution of sinusoids with different magnitudes at different frequencies. Through Fourier transformation, the response of the overall system can be obtained from products of the component responses rather than from complicated multiple convolution integrals in time domain.
\[ O(\omega) = G_1(\omega)G_2(\omega) \cdots G_n(\omega)I(\omega) \quad (3.5) \]

**Figure 3.8** An ultrasound measurement system for biological tissue samples

Figure 3.8 shows the components of the ultrasound measurement system for biological tissue samples. The multiple physical processes occurred during the transformation from the input voltage to the output voltage can be assumed to be linear...
and time-shift invariant [Schmerr, 1998]. Therefore the overall system response in frequency domain is as follows:

$$V_{os}(\omega) = V_i(\omega)B(\omega)D(\omega)W_1(\omega)S(\omega)W_2(\omega)C(\omega)R(\omega)$$  \hspace{1cm} (3.6)

The definitions of each term are shown in Figure 3.9. Except $S(\omega)$, all the other terms are irrelevant to the unknown properties of the sample. Nevertheless, to obtain each of the functions is not practical because there are a large number of combinations of pulse/receiver settings and transducer choices. The key concept of measuring a reference signal was introduced. This practice allows the grouping of those unknown, difficult-to-obtain terms together and the measurement of this bulk factor through a well-designed reference signal so that the measurement on real samples will be only dependent on the sample properties.

If the sandwich structure sample is replaced by a piece of glass with identical thickness but free surfaces, the system input-output relationship could be described as follows:

$$V_{og}(\omega) = V_i(\omega)B(\omega)D(\omega)W_1(\omega)G(\omega)W_2(\omega)C(\omega)R(\omega)$$  \hspace{1cm} (3.7)

where only one term is different from (3.6). The term $G(\omega)$ can be calculated if the physical properties of the substrate (glass) and water are known. In fact, this function is a frequency independent constant fully determined by the acoustical impedances of water and glass, and the incident angle.

$$G(\omega) = K$$  \hspace{1cm} (3.8)
Figure 3.9 The ultrasonic measurement system as a series of LTI systems
From equations (3.6) and (3.7), we can obtain the true response from the tissue sample as below:

\[ S(\omega) = \frac{V_{os}(\omega)}{V_{oc}(\omega)} K \]  

Therefore, the reflection from a single piece of glass placed in water was adopted as the reference signal in this study. This piece of glass had the exact thickness and the same properties as the top glass in the sandwich structure. Therefore the first signal (that from the top surface of the glass) would be exactly the same in the reference reflection signals and later measured sample reflection signals (illustrated in Figure 3.10). The later measured reflections from the sandwich structure could all be deconvoluted against this reference signal to decouple the effects of transducers and other unknown system factors such as ultrasound beam effects. The frequency components of the reflected signals (the reflection spectra) from the tissue samples were obtained by diving the magnitude of the measured output frequency components against reference frequency components, and then multiplying with constant \( K \).

For the glass used in this study, constant \( K \) was explicitly calculated.

\( K=0.76, \) for incident angle 17 degrees (oblique incidence))

\( K=0.8154, \) for incident angle 0 degree (normal incidence)
3.4 Data Acquisition

The sandwich-structured sample was put on a small glass table mounted on the bottom of the watertank (Figure 3.11). Another transducer that was placed at the center of the two oblique transducers generated ultrasonic beams that were adjusted to be perpendicular to the top surface of the sample. The height and the orientation of the transducers were manually adjusted to ensure that the transducers and the sample were aligned as illustrated in Figure 3.12.
The data acquisition procedure is as follows. First, a reference signal was recorded by measuring the reflections from the interface of the single piece of glass and water. After measuring the reference signal, the glass was removed and a sandwich sample was put on the glass table to measure the reflections from the tissue layer. The alignment of the transducers was readjusted for each measurement.

Figure 3.11 Sample mounting and positioning
Figure 3.12: Spatial configuration of transducers and sandwich-structured tissue sample. For a fixed oblique incident angle (17° in this study, the range of the incident angle is determined by the two mode-conversion angles for glass-water interface, specific angle was chosen empirically), the positions and orientation of the transducers have to be finely tuned to fulfill the geometrical requirements shown in the figure, namely: the normal incident beam should be perpendicular to the sample surface, the focal point of the oblique incident beam should be at the center of sample both vertically and horizontally.
The digitized time domain data were stored in computer and further processed for the purposes of this study. First, the time domain signals were averaged over 100 repetitive signals to reduce system noise factor. The pulses were then gated manually by utilizing a user-friendly interface (Figure 3.13). Four signals were gated for each measurement: the normal reflection from top glass surface, the normal reflection from the thin layer, the oblique reflection from the top surface and the oblique reflection from the thin layers. These gated time domain signals were transferred to frequency domain, therefore became “spectra”, by using a standard Fast Fourier Transformation (FFT). Both the time domain signals and the spectra of the four reflections were stored separately for future analysis.

For every batch of experiments (conducted on the same day), six to eight sandwich structures were constructed and tested. These samples included three-four normal sections and three-four malignant sections from one subject. Multiple (>2) independent measurements were conducted on each sample to reduce operator error.
Figure 3.13 User interface and gating technology
CHAPTER 4
QUANTITATIVE ULTRASOUND WITH MODELS FOR PROPERTY RECONSTRUCTION

The approach adopted in this study is a non-destructive method with a theoretical model that quantitatively correlates the mechanical responses to possible contributing factors such as the physical properties of the examined material or structure. Inversion algorithms, combining the experimental measurement and the model predication, are used to infer the quantitative information on the properties of the specimen under inspection. This approach, namely quantitative ultrasound with models is advantageous due to a number of practical reasons. First, it is generally more sensitive than ultrasound measurements without models. The reason is that other system factors such as transducer choices and settings may well interfere the absolute responses of the system. That could make the response less sensitive to the changes in certain parameters under examination. It is possible to carefully choose calibration specimens that closely mimic the material and conditions of the actual setup, and then carefully specify test procedures to minimize variations due to settings, however it is normally an expensive and tedious task. With a model-based approach, it is possible to develop calibration procedures (for example, the measurement of reference signals in this study) that are independent of a particular
measurement setup. Secondly, the quantitative information on the material properties inversed from models makes it practical to compare the results across different materials and geometries.

4.1 System Description and Assumptions

Two models, one based on conventional continuum mechanics, the other employing Doublet mechanics, were constructed to depict the wave propagation in the ultrasonic system. The models were to solve for the quantitative relationship between the mechanical responses of the tissue (e.g., the reflection spectra) and its physical properties.

The relevant mechanical events were the interactions between the ultrasound waves and the sandwich structure. Wave propagation in the other parts of the system is not of concern in the model because that could be dealt with by using the reference signal. There are NDE models that could be employed to study the detailed cause-effect in all parts of the system; however, they are not of the interest of this study.

The transmitting ultrasound pulses were high frequency, elastic, time-harmonic plane waves. The medium in which the ultrasound beams propagated was composed of three layers, i.e., the upper glass, the thin tissue layer and the lower glass. The interfaces between adjacent layers were assumed to be planar and parallel. These assumptions not only accounted for the real system with reasonable accuracy, but also simplified the modeling problem to the well-studied plane wave propagation in multi-layered media, and in this system only wave reflection and transmission, but not scattering was considered.
The models considered a medium that was configured as two continuous, infinite subspaces with a thin layer embedded in between. The subspaces were assumed to be isotropic continua characterized by material parameters of density \( \rho_i \) and Lame's constants: \( \lambda_i \) and \( \mu_i \). The thin tissue layer was assumed to be an elastic continuum in the continuum mechanics model, which is identified by density \( \rho_2 \) and Lame's constants: \( \lambda_2 \) and \( \mu_2 \). Conversely, in the doublet mechanics model the thin tissue layer was discrete-structured, and identified by the material parameters \( \rho_2 \) (density), \( A_{\alpha\beta} \) (micromoduli), \( \tau_{\alpha\beta} \) (node orientation vector), and \( \eta \) (internodal distance, or particle size/diameter). A more detailed description on the micro-architecture and its associated parameters is given in section 4.3.

The governing equations under doublet mechanics are multi-scale in nature, with the order of the scale varying in accordance with the problem at study. One of the objectives of this study is to compare the two frameworks and to establish non-continuum response features that may be of significance in the development of innovative ultrasound-based medical diagnostics. This objective may be reached by limiting our considerations to the approximation degree \( M=2 \).

The stress and displacement fields in the glass and the tissue layers were solved for according to equilibrium conditions. Continuity conditions were employed to solve for the unknown reflections and transmissions. A least square minimization method was used to reconstruct the properties of the tissue layered combining the prediction of the theoretical model and the experimental data.
4.2 Wave Propagation in three-layered Medium

Figure 4.1 illustrates the wave interactions that exist in the sandwich structure upon ultrasonic plane wave application at an oblique angle. There were mode conversions at the two interfaces between the glass and the tissue, resulting in eight reflection and transmission waves in this three-layered system.

The waves in this system are: the incident wave (0) (shear or longitudinal), the reflected longitudinal wave (1), the reflected shear wave (2), the transmitted longitudinal wave in the layer (3), the transmitted shear wave in the layer (4), the reflected longitudinal wave in the layer (5), the reflected shear wave in the layer (6) and the transmitted longitudinal wave (7) and shear waves (8) in the bottom subspace (see Figure 4.1).
Upon application of plan waves at an arbitrary angle, the incident energy is portioned into several different waves. They are the reflections back to the upper
substrate and the transmissions into the thin layer and further the bottom substrate. There are also multiple reflections within the thin layer.

Regardless of the type of the incident wave (be it longitudinal or shear), mode conversion at the interface caused split of the waves into both longitudinal and shear types that travel at different wave velocity and propagation angle. The angles of reflection and transmission at the interface are dictated by Snell’s law, which is that the ratio of the wave number and the sine of the propagation angle should remain constant at each interface. For example, at the upper interface, the following relationships hold:

\[
\frac{k_0}{\sin \theta_0} = \frac{k_1}{\sin \theta_1} = \frac{k_2}{\sin \theta_2} = \frac{k_3}{\sin \theta_3} = \frac{k_4}{\sin \theta_4} = \frac{k_5}{\sin \theta_5} = \frac{k_6}{\sin \theta_6}
\] (4-1)

where \( k_i \) is the wave number of the \( i \)th wave and \( \theta_i \) is the propagation angle of the \( i \)th wave as specified in Figure.

The wave propagation equations in the substrates (assumed infinite) were obtained through equilibrium conditions. The result is the so called Navier’s equations:

\[
(\lambda + \mu)u_{j,ji} + \mu u_{i,ji} + \rho f_i = \rho \dot{u}_i
\] (4-2)

where \( u \) is the vector of displacement. \( \lambda \) and \( \mu \) are Lame’s constants of the medium, \( \rho \) is the density per unit volume of the medium, \( f_i \) is the body force per unit mass of the material. The operator comma denotes partial differentiation with respect to position variables

\[
u_{j,ji} = \frac{\partial^2 u_j}{\partial x_j \partial x_i}
\] (4-3)
The solution of plane waves propagating in infinite continuous media is as follows (indicidal form):

\[ u_i = A_i f(n_k x_k - ct) \] (4-4)

where \( A_i \) is the amplitude (constant) of the wave, \( f(x) \) is arbitrary function that is determined by the source of the disturbance, \( n_k x_k \) denotes the summation of the product of the position variable with the unit vector of wave propagation, \( t \) is the time variable and \( c \) is the wave speed with which plane waves travel in an infinite continuous medium. There are two possible wave speeds in this case, and both are fully determined by the material properties of the medium.

\[ c_1 = \sqrt{\frac{\lambda + 2\mu}{\rho}} \] (4-5)

\[ c_2 = \sqrt{\frac{\mu}{\rho}} \] (4-6)

The governing equations in the thin layer were derived under the framework of continuum mechanics and Doublet mechanics respectively. The continuum mechanics analysis for the thin tissue layer is exactly the same as that for the substrates. The Doublet mechanics counterparts are described herein.

A simplified version of the governing equations at the scale \( M=2 \) were obtained with the following assumption: the particle interactions were assumed to be longitudinal (central), so that the shear and torsional microstresses vanished everywhere.

The micro-constitutive relationship corresponding to the assumption is:
\[ p_a = \sum_{\beta} A_{ab} \varepsilon_\beta \]  

(4-7)

where \( p_a \) is the overall microstress in the \( \alpha \) doublet, and \( \varepsilon_\beta \) is the axial microstrain associated with doublet \( \beta \), \( A_{ab} \) is the micromodulus between \( \alpha \) and \( \beta \).

For \( M=2 \), the micro-level kinematical relationship is:

\[
\varepsilon_\alpha = \tau_{a_1}^2 \frac{\partial u_1}{\partial x_1} + \tau_{a_1} \tau_{a_2} \left( \frac{\partial u_1}{\partial x_2} + \frac{\partial u_2}{\partial x_1} \right) + \tau_{a_2}^2 \frac{\partial u_2}{\partial x_2} + \eta_\alpha \left( \tau_{a_1} \frac{\partial^2 u_1}{\partial x_1^2} + 2 \tau_{a_1} \tau_{a_2} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} + \tau_{a_2}^2 \frac{\partial^2 u_1}{\partial x_2^2} \right) \\
+ \tau_{a_1} \tau_{a_2} \frac{\partial^2 u_2}{\partial x_1^2} + 2 \tau_{a_2} \tau_{a_1} \frac{\partial^2 u_2}{\partial x_1 \partial x_2} + \tau_{a_2}^2 \frac{\partial^2 u_2}{\partial x_2^2} \right)
\]

(4-8)

where \( \varepsilon_\alpha \) is the micro-strain associated with node \( \alpha \), \( \tau_\alpha \)'s are the direction cosines of the unit vectors connecting two nodes, \( u_1 \) is the displacement at \( x_1 \) direction, \( u_2 \) is the displacement at \( x_2 \) direction, and \( \eta_\alpha \) is the internal distance associated with node \( \alpha \) (assuming all doublets share the same internodal distance).

In the Doublet mechanics model, the continuum stresses in the tissue at the interface needed to be solved for in order to apply the continuity conditions (please see Section 4.4). Due to a transition relationship derived in Doublet mechanics, the continuum stresses were not calculated from stress tensors, as done in conventional mechanics, but directly from micro level physical and geometrical parameters such as \( A_{ab} \), \( \tau_\alpha \)'s and \( \eta_\alpha \).

The transition from micro to macro/continuum stresses was achieved through imposing natural boundary conditions. The resulting equation is as follows:

\[
\sigma_{ij} = \sum_{\alpha=1}^{n} \left( \tau_{a_i} \tau_{a_j} p_\alpha - \frac{\eta_\alpha}{2} \tau_{a_i} \tau_{a_j} \tau_{a_k} \frac{\partial \eta_\alpha}{\partial x_k} \right)
\]

(4-9)
where $\sigma_{ij}$ is the continuum stress.

The governing equations of wave propagation and the formula for the wave speeds in the Doublet mechanics model are derived as follows.

First the conservation of linear momentum holds:

$$
\tau_{\alpha} \sum_{\alpha=1}^{n} \sum_{\chi=1}^{M} (-1)^{\chi-1} \frac{(-\eta_{\alpha})^{\chi-1}}{\chi!} \tau_{\alpha k} \cdots \tau_{\alpha r} \frac{\partial^x \rho}{\partial x_{\kappa} \cdots \partial x_{r}} = \rho \frac{\partial^2 u_j}{\partial t^2} \tag{4-10}
$$

The conservation of linear momentum in terms of micro level strain and displacements was obtained by inserting the micro level constitutive relationships into the above:

$$
\tau_{\alpha} \sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \sum_{\chi=1}^{M} (-1)^{\chi-1} \frac{(-\eta_{\alpha})^{\chi-1}}{\chi!} \tau_{\alpha k} \cdots \tau_{\alpha r} \frac{\partial^x \varepsilon_{\beta}}{\partial x_{\kappa} \cdots \partial x_{r}} = \rho \frac{\partial^2 u_j}{\partial t^2} \tag{4-11}
$$

The relationship of micro level strain and displacement is defined as follows:

$$
\varepsilon_{\beta} = \tau_{\beta} \sum_{\alpha=1}^{n} \frac{(-\eta_{\alpha})^{\chi-1}}{\chi!} \tau_{\beta k} \cdots \tau_{\beta r} \frac{\partial^x u_j}{\partial x_{\kappa} \cdots \partial x_{r}} \tag{4-12}
$$

Combining equations (4-11) and (4-12), the governing equations for wave propagation in Doublet mechanics were obtained:

For $M=1$,

$$
\sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \tau_{\alpha} \tau_{\beta} \tau_{\alpha k} \tau_{\beta} \frac{\partial^2 u_k}{\partial x_{\alpha} \partial x_{\beta}} = \rho \frac{\partial^2 u_j}{\partial t^2} \tag{4-13}
$$

For $M=2$,

$$
\sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \left[ \tau_{\alpha} \tau_{\beta} \tau_{\beta k} \tau_{\alpha h} \frac{\partial^2 u_j}{\partial x_{\alpha} \partial x_{\beta} \partial x_{\alpha} \partial x_{\beta}} - \frac{(\eta_{\alpha})^2}{4} \tau_{\alpha} \tau_{\alpha k} \tau_{\alpha k} \tau_{\alpha k} \tau_{\beta} \tau_{\beta h} \tau_{\beta h} \tau_{\beta h} \frac{\partial^4 u_j}{\partial x_{\alpha} \partial x_{\alpha} \partial x_{\alpha} \partial x_{\alpha} \partial x_{\beta} \partial x_{\beta} \partial x_{\beta} \partial x_{\beta}} \right] = \rho \frac{\partial^2 u_j}{\partial t^2} \tag{4-14}
$$
The wave speeds were computed for both cases too.

For $M=1$, without losing generality, the waves was assumed to propagate along $x$, axis. For the longitudinal wave

$$C_1 = \sqrt{\sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \tau_{\alpha 1} \tau_{\beta 1} \rho}$$

(4-15)

Similarly the shear speed is

$$C_2 = \sqrt{\sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \tau_{\alpha 1} \tau_{\alpha 2} \tau_{\beta 1} \tau_{\beta 2} \rho}$$

(4-16)

For $M=2$, the explicit expressions for wave speeds in the discrete-represented media are very complicated. Only the dispersion equations are listed below.

For longitudinal waves

$$- \rho \omega^2 = -(A_{11} - \frac{1}{2} A_{14}) k^2 + \frac{\eta^2}{4} (A_{11} - \frac{\sqrt{2}}{2} A_{44} + \frac{1}{4} A_{44}) k^4$$

(4-17)

where $\omega$ is the radial frequency of the wave, and $\eta$ is the internodal distance between any two nodes assuming the distance is constant throughout the medium.

Similarly for shear wave

$$- \rho \omega^2 = - \frac{A_{14}}{4} k^2 + \frac{\eta^2 A_{44}}{32} k^4$$

(4-18)
4.3 Micro-architecture Model for the Biological Tissue Layer

Consider the three-dimensional arrangement of nodes given in Figure 4.2. Each node is mechanically related to six other nodes in each octant. Figure 4.2 shows the configuration of the nodes in the first octant. The doublet vectors (the unit vectors along the direction connecting each doublet) are as follows:

\[
\begin{align*}
\tau_1 &= (1,0,0) & \tau_4 &= (0,1/\sqrt{2},1/\sqrt{2}) \\
\tau_2 &= (0,1,0) & \tau_5 &= (1/\sqrt{2},0,1/\sqrt{2}) \\
\tau_3 &= (0,0,1) & \tau_6 &= (1/\sqrt{2},1/\sqrt{2},0)
\end{align*}
\]

The physical nanoscale properties are given by

\[
\begin{bmatrix}
A_{11} & A_{12} & A_{13} & A_{14} & A_{15} & A_{16} \\
A_{22} & A_{23} & A_{24} & A_{25} & A_{26} & A_{26} \\
A_{33} & A_{34} & A_{35} & A_{36} & A_{36} & A_{36} \\
A_{44} & A_{45} & A_{46} & A_{46} & A_{46} & A_{46} \\
(\text{Sym.}) & & & & & \\
A_{66} & & & & & \\
\end{bmatrix}
= 
\begin{bmatrix}
A_{11} & A_{12} & A_{13} & 0 & A_{15} & A_{16} \\
A_{12} & A_{12} & A_{13} & 0 & A_{15} & A_{16} \\
A_{13} & A_{13} & A_{13} & 0 & A_{15} & A_{16} \\
0 & A_{12} & A_{13} & A_{13} & 0 & A_{16} \\
(\text{Sym.}) & & & & & \\
0 & & & & A_{14} & \\
\end{bmatrix}
\]

with the condition that

\[
A_{15} = -\frac{1}{2} A_{44}
\]

The main reason for choosing this particular arrangement is that the micro-architecture and physical properties specified thereby yields an isotropic constitutive
relation at the macro level, if the elastic constants in continuum mechanics relate to the micro elastic constants according to the following:

\[ \lambda = A_{11} - A_{44} \]
\[ \mu = \frac{1}{4} A_{44} \]  

(4-22)

The wave speeds at the first scale \((M=1)\), can be calculated as below:

\[ C_1 = \sqrt{\frac{1}{\rho} \sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \tau_{\alpha 1} \tau_{\beta 1}} = \sqrt{\frac{A_{11} - \frac{1}{2} A_{44}}{\rho}} = \sqrt{\frac{\lambda + 2\mu}{\rho}} \]  

(4-23)

\[ C_2 = \sqrt{\frac{1}{\rho} \sum_{\alpha=1}^{n} \sum_{\beta=1}^{n} A_{\alpha \beta} \tau_{\alpha 2} \tau_{\beta 2}} = \sqrt{\frac{A_{44}}{4\rho}} = \sqrt{\frac{\mu}{\rho}} \]  

(4-24)

which are in exact agreement with those derived in continuum mechanics.

The number of independent micro level elastic constants is reduced to two while choosing this arrangement. These constants are \(A_{11}\) and \(A_{44}\) respectively.

For more analysis on the micro-architecture, please refer to [Ferrari, 2000].
Figure 4.2: Micro-architecture of the doublets associated with the node at the origin
4.4 Numerical solutions to the continuity conditions

Time-harmonic plane waves were the solutions to the wave propagation equations defined in (4-2) and (4-14), under continuum mechanics and doublet mechanics respectively. Therefore, the expressions for the displacement of all nine ways in the system can be written in the following format:

Incident S wave:

\[
\begin{align*}
\mathbf{u}^{(0)} &= \begin{bmatrix} u_{1}^{(0)} \\ u_{2}^{(0)} \end{bmatrix} = \begin{bmatrix} A_0 \cos \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t)) \\ A_0 \sin \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t)) \end{bmatrix} \\
\end{align*}
\] (4-25)

Incident P wave:

\[
\begin{align*}
\mathbf{u}^{(0)} &= \begin{bmatrix} u_{1}^{(0)} \\ u_{2}^{(0)} \end{bmatrix} = \begin{bmatrix} A_0 \sin \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t)) \\ -A_0 \cos \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t)) \end{bmatrix} \\
\end{align*}
\] (4-26)

Reflected S wave:

\[
\begin{align*}
\mathbf{u}^{(1)} &= \begin{bmatrix} u_{1}^{(1)} \\ u_{2}^{(1)} \end{bmatrix} = \begin{bmatrix} -A_1 \cos \theta_1 \exp(ik_1(x_1 \sin \theta_1 + x_2 \cos \theta_1 - c_1 t)) \\ A_1 \sin \theta_1 \exp(ik_1(x_1 \sin \theta_1 + x_2 \cos \theta_1 - c_1 t)) \end{bmatrix} \\
\end{align*}
\] (4-27)

Reflected P wave:

\[
\begin{align*}
\mathbf{u}^{(2)} &= \begin{bmatrix} u_{1}^{(2)} \\ u_{2}^{(2)} \end{bmatrix} = \begin{bmatrix} A_2 \sin \theta_2 \exp(ik_2(x_1 \sin \theta_2 + x_2 \cos \theta_2 - c_2 t)) \\ A_2 \cos \theta_2 \exp(ik_2(x_1 \sin \theta_2 + x_2 \cos \theta_2 - c_2 t)) \end{bmatrix} \\
\end{align*}
\] (4-28)

Transmitted S wave in layer:

\[
\begin{align*}
\mathbf{u}^{(3)} &= \begin{bmatrix} u_{1}^{(3)} \\ u_{2}^{(3)} \end{bmatrix} = \begin{bmatrix} A_3 \cos \theta_3 \exp(ik_3(x_1 \sin \theta_3 - x_2 \cos \theta_3 - c_3 t)) \\ A_3 \sin \theta_3 \exp(ik_3(x_1 \sin \theta_3 - x_2 \cos \theta_3 - c_3 t)) \end{bmatrix} \\
\end{align*}
\] (4-29)
Transmitted P wave in layer:

\[
\begin{bmatrix}
u^{(4)}_1 \\ u^{(4)}_2
\end{bmatrix} = \begin{bmatrix}
A_4 \sin \theta_4 \exp(ik_4(x_1 \sin \theta_4 - x_2 \cos \theta_4 - c_4 t)) \\
-A_4 \cos \theta_4 \exp(ik_4(x_1 \sin \theta_4 - x_2 \cos \theta_4 - c_4 t))
\end{bmatrix}
\] (4-30)

Reflected S wave in layer:

\[
\begin{bmatrix}
u^{(5)}_1 \\ u^{(5)}_2
\end{bmatrix} = \begin{bmatrix}
-A_5 \cos \theta_3 \exp(ik_5(x_1 \sin \theta_3 + x_2 \cos \theta_3 - c_3 t)) \\
A_5 \sin \theta_3 \exp(ik_5(x_1 \sin \theta_3 + x_2 \cos \theta_3 - c_3 t))
\end{bmatrix}
\] (4-31)

Reflected P wave in layer:

\[
\begin{bmatrix}
u^{(6)}_1 \\ u^{(6)}_2
\end{bmatrix} = \begin{bmatrix}
A_6 \sin \theta_4 \exp(ik_6(x_1 \sin \theta_4 + x_2 \cos \theta_4 - c_4 t)) \\
A_6 \cos \theta_4 \exp(ik_6(x_1 \sin \theta_4 + x_2 \cos \theta_4 - c_4 t))
\end{bmatrix}
\] (4-32)

Transmitted S wave:

\[
\begin{bmatrix}
u^{(7)}_1 \\ u^{(7)}_2
\end{bmatrix} = \begin{bmatrix}
A_7 \cos \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t)) \\
A_7 \sin \theta_0 \exp(ik_0(x_1 \sin \theta_0 - x_2 \cos \theta_0 - c_0 t))
\end{bmatrix}
\] (4-33)

Transmitted P wave:

\[
\begin{bmatrix}
u^{(8)}_1 \\ u^{(8)}_2
\end{bmatrix} = \begin{bmatrix}
A_8 \sin \theta_2 \exp(ik_2(x_1 \sin \theta_2 - x_2 \cos \theta_2 - c_2 t)) \\
-A_8 \cos \theta_2 \exp(ik_2(x_1 \sin \theta_2 - x_2 \cos \theta_2 - c_2 t))
\end{bmatrix}
\] (4-34)

where \(u^{(j)}_i\) is the displacement associated with the \(j\)th wave that propagates along \(x_i\) axis.

The continuity of stresses and displacements at the upper interface of material discontinuity is shown in Figure 4.3.
4. Continuity of shear stress

\[ \sigma_{21}^{(0)} + \sigma_{21}^{(1)} + \sigma_{21}^{(2)} = \sigma_{21}^{(3)} + \sigma_{21}^{(4)} + \sigma_{21}^{(5)} + \sigma_{21}^{(6)} \]  

(4-38)

where \( \sigma_{m}^{(n)} \) is the stress associated with the \( n \)th wave (the waves are numbered from 0 to 9): normal stress if \( m=22 \), shear stress if \( m=21 \); \( u_{m}^{(n)} \) is the displacement associated with the \( n \)th wave: normal displacement if \( m=2 \), shear displacement if \( m=1 \).

At the interface of \( x_z=-h \), where \( h \) is the thickness of the thin layer, the following continuity conditions must be satisfied:

5. Continuity of normal displacement:

\[ u_1^{(7)} + u_1^{(8)} = u_1^{(3)} + u_1^{(4)} + u_1^{(5)} + u_1^{(6)} \]  

(4-39)

6. Continuity of normal stress:

\[ \sigma_{22}^{(7)} + \sigma_{22}^{(8)} = \sigma_{22}^{(3)} + \sigma_{22}^{(4)} + \sigma_{22}^{(5)} + \sigma_{22}^{(6)} \]  

(4-40)

7. Continuity of shear displacement:

\[ u_2^{(7)} + u_2^{(8)} = u_2^{(3)} + u_2^{(4)} + u_2^{(5)} + u_2^{(6)} \]  

(4-41)

8. Continuity of shear stress

\[ \sigma_{21}^{(7)} + \sigma_{21}^{(8)} = \sigma_{21}^{(3)} + \sigma_{21}^{(4)} + \sigma_{21}^{(5)} + \sigma_{21}^{(6)} \]  

(4-42)

These eight linear equations obtained from the continuity conditions at both interfaces were employed to solve for the eight unknown reflection/transmission coefficients, which were defined as the ratios of the magnitudes of the reflection/transmission over the incident wave:

\[ R_s(f) = \frac{M_R(f)}{M_i(f)} \]  

(4-43)
where $R_s(f)$ is the reflection/transmission coefficient at frequency $f$, $M_R(f)$ is the magnitude of the reflection/transmission at frequency $f$, and $M_i(f)$ is the magnitude of the incidence at frequency $f$.

A MatLab program was created to give numerical solutions for the reflection coefficients at any frequency, given that the physical properties of the glass were known and those of the tissue were arbitrarily specified.

The reflection spectrum was generated by computing the reflection coefficients for multiple frequencies within a certain range, which was therefore a function of the specific set of physical properties of the tissue.

4.5 Inverse Algorithm: Least Square Minimization

As discussed before, the reflection spectra are fully defined by the properties of the thin layer. Therefore these properties can be determined through inversion from the experimental data in combination with the theoretical model. With continuum mechanics model the reconstructed properties were density $\rho$ and Lame’s constants: $\lambda$ and $\mu$. Conversely, with the doublet mechanics model the reconstructed properties were density $\rho_2$, micro modulus $A_{11}$ and $A_{44}$, and the intermodal distance $\eta$. The nodal orientation vectors $\tau_{ab}$ were pre-specified according to the micro-architecture of choice. It is noted that in the reconstruction with doublet mechanics model, the macro level observable/measurable (i.e., the reflection coefficients from the thin layer) was directly
defined by the micro level properties, which made it possible for the inversion on these properties directly from the macro level measurements.

A least square method was employed to minimize the sum of the squared deviations between the experimental data points and the calculated reflection coefficients at the same frequency. The minimization problem was essentially a search for the optimal combination of the parameters that satisfied

\[
\min_{\mathbf{x}} \frac{1}{2} \sum_{i=1}^{m} (|R_i^x| - |R_i^r|)^2
\]  

(4-44)

where \(x\) are the reconstructed parameters. \(n\) is the number of the parameters to be found, \(m\) is the number of data points at different frequencies, and \(R^x\) and \(R^r\) are the experimental reflection coefficients and simulated reflection coefficients, respectively.

A two-tailed, unequal variance, student T-test was performed on the reconstructed values to test whether there was difference between normal and malignant tissue.
CHAPTER 5
RESULTS

Specimens:

Experimental measurements on the reflection spectra from the sandwich structure were conducted on tissue samples from goat mammary glands and human breasts. The purpose of studying goat mammary glands was to develop the sample preparation protocol rather than measuring the physical properties of goat tissue. Therefore, the results from the goat mammary gland tissue are not presented in this dissertation.

There were ten tissue specimens obtained from five breast cancer patients who went through breast mastectomy or surgical removal of tumor. The specimens are listed in Table 5.1.
Table 5.1: The breast tissue specimens that were used in this study (*IDC: Invasive Ductal Carcinoma).

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Specimen ID no</th>
<th>Specimen size</th>
<th>Cancer type</th>
<th>Age</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0110C271f</td>
<td>1 cm²</td>
<td>IDC*</td>
<td>80</td>
<td>White</td>
</tr>
<tr>
<td></td>
<td>0110C272f</td>
<td>1 cm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0201C175j</td>
<td>1 cm²</td>
<td>IDC*</td>
<td>58</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>0201C173v</td>
<td>0.4 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0202C027a</td>
<td>1 cm²</td>
<td>IDC*</td>
<td>43</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>0202C026a</td>
<td>1 cm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0206C076d</td>
<td>1 cm²</td>
<td>IDC*</td>
<td>49</td>
<td>Black</td>
</tr>
<tr>
<td></td>
<td>0206C075g</td>
<td>1 cm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0206C088a</td>
<td>0.54 g</td>
<td>IDC*</td>
<td>50</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>0206C087c</td>
<td>0.3 g</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.1 Experimental Data on the Reflection Spectra

Specimen 1:

Time: 12-19-01

Tissue samples: 0110C271f and 0110C272f

![Reflection Spectra Graph]

**Figure 5.1** The reflection spectra for specimen 1 (T1, T2, T3: reflection spectra from three malignant tissue sections; N1, N2, N3: reflection spectra from three normal controls.)
Specimen 2:

Time: 05-09-02

Samples: 0201C175j and 0291C173v

Figure 5.2 The reflection spectra for specimen 2 (T1, T2, T3: reflection spectra from three malignant tissue sections; N1, N2, N3: reflection spectra from three normal controls.)
Specimen 3:

Time: 05-14-02

Samples: 0110C271f and 0110C272f

Figure 5.3 The reflection spectra for specimen 3 (T1, T2, T3: reflection spectra from three malignant tissue sections; N1, N2, N3: reflection spectra from three normal controls. *note: There was a procedure error in the preparation for the tumor tissue sample whose spectrum was plotted in dashed line: T1. The two short sides of the glass were not sealed by glue; therefore, the thickness of the sample was not accurate.)
Specimen 4:

Time: 07-16-02

Samples: 0206C076d and 0206C075g

Figure 5.4 The reflection spectra for specimen 4 (T1, T2, T3, T4: reflection spectra from three malignant tissue sections; N1, N2, N3, N4: reflection spectra from three normal controls).
Specimen 5:

Time: 08-30-02

Samples: 0206C088a and 0206C087c

Figure 5.5 The reflection spectra for specimen 5 (T1, T2, T3: reflection spectra from three malignant tissue sections; N1, N2, N3: reflection spectra from three normal controls).
5.2 Reconstruction on the Reflection Spectra (in comparison with experimental data)

The reconstruction of the reflection spectra through the inverse algorithm was shown below. In the figures, curves of circles are experimental measurement of the reflection spectra; curves of solid lines are theoretical predication/reconstruction based on either model. RESNORM is the magnitude of the residual. For illustration, the comparison of theoretical prediction and experimental data for specimen two and five are presented in the following figures.
Figure 5.6 Comparison of the spectra obtained from theoretical prediction and real experimental data for a normal section from specimen 1. (RESNORM = 0.1539)

Figure 5.7 Comparison of the spectra obtained from theoretical prediction and real experimental data for a malignant section from specimen 1. (RESNORM = 0.0924)
Figure 5.8 Comparison of the spectra obtained from theoretical prediction and real experimental data for a normal section from specimen 5. (RESNORM = 0.1429)

Figure 5.9 Comparison of the spectra obtained from theoretical prediction and real experimental data for a malignant section from specimen 5. (RESNORM = 0.2589)
5.2 Reconstruction on tissue properties and statistical analysis

Property reconstruction based on both models was performed on the same sets of the reflection spectra from the experimental measurements. For each pair of samples (i.e., the malignant tissue and its normal control), a two-tailed, unequal variance student T-test was conducted to test whether any of the reconstructed properties was significantly different (P<0.05). It is noted that Young's modulus $E$ were computed from the Lamé's constants ($\lambda$ and $\mu$). Therefore, it is not an independent parameter. The results are shown below.
Specimen 1:

Time: 12-19-01

Samples: 0110C271f and 0110C272f

### Table 5.2 Continuum mechanics reconstruction for specimen 1 (please note that E is not an independent variable, but computed from λ and μ).

<table>
<thead>
<tr>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ρ (g/cm³)</td>
<td>1.0692</td>
</tr>
<tr>
<td>λ (Gpa)</td>
<td>2.5335</td>
</tr>
<tr>
<td>μ (Gpa)</td>
<td>0.0380</td>
</tr>
<tr>
<td>E (Gpa)</td>
<td>0.1134</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0192</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

### Table 5.3 Doublet mechanics reconstruction for specimen 1.

<table>
<thead>
<tr>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ρ (g/cm³)</td>
<td>0.8357</td>
</tr>
<tr>
<td>A₁₁ (Gpa)</td>
<td>2.1987</td>
</tr>
<tr>
<td>A₄₄ (Gpa)</td>
<td>0.0307</td>
</tr>
<tr>
<td>attn 1.</td>
<td>0.1260</td>
</tr>
<tr>
<td>attn 2.</td>
<td>0.0990</td>
</tr>
<tr>
<td>η (mm)</td>
<td>0.0053</td>
</tr>
</tbody>
</table>

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Specimen 2:
Time: 05-09-02
Samples: 0201C175j and 0291C173v

<table>
<thead>
<tr>
<th></th>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>1.0861</td>
<td>0.9985</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>2.4246</td>
<td>2.3245</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0352</td>
<td>0.0544</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.1051</td>
<td>0.1620</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0201</td>
<td>0.0139</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0063</td>
<td>0.0097</td>
</tr>
</tbody>
</table>

Table 5.4 Continuum mechanics reconstruction for specimen 2.

<table>
<thead>
<tr>
<th></th>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.8777</td>
<td>0.8695</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>2.0500</td>
<td>2.1782</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.0309</td>
<td>0.0593</td>
</tr>
<tr>
<td>attn. 1.</td>
<td>0.1145</td>
<td>0.1474</td>
</tr>
<tr>
<td>attn. 2.</td>
<td>0.0836</td>
<td>0.1589</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0052</td>
<td>0.0062</td>
</tr>
</tbody>
</table>

Table 5.5 Doublet mechanics reconstruction for specimen 2.

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Specimen 3:

Time: 05-14-02

Samples: 0110C271f and 0110C272f

<table>
<thead>
<tr>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ρ (g/cm³)</td>
<td>0.9299</td>
</tr>
<tr>
<td>λ (Gpa)</td>
<td>1.9101</td>
</tr>
<tr>
<td>μ (Gpa)</td>
<td>0.0572</td>
</tr>
<tr>
<td>E (Gpa)</td>
<td>0.1699</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0051</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0156</td>
</tr>
</tbody>
</table>

Table 5.6 Continuum mechanics reconstruction for specimen 3.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>ρ (g/cm³)</td>
<td>0.8777</td>
</tr>
<tr>
<td>A_11 (Gpa)</td>
<td>2.0500</td>
</tr>
<tr>
<td>A_44 (Gpa)</td>
<td>0.0309</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.1145</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0836</td>
</tr>
<tr>
<td>η (mm)</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

Table 5.7 Doublet mechanics reconstruction for specimen 3.
Specimen 4:

Time: 07-16-02

Samples: 0206C076d and 0206C075g

<table>
<thead>
<tr>
<th></th>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>1.0235</td>
<td>1.0962</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>2.3109</td>
<td>2.5066</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0357</td>
<td>0.0354</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.1066</td>
<td>0.1057</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0355</td>
<td>0.0503</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0077</td>
<td>0.0068</td>
</tr>
</tbody>
</table>

Table 5.8 Continuum Mechanics reconstruction for specimen 4.

<table>
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<tr>
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<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.8603</td>
<td>0.8408</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>2.0398</td>
<td>2.0311</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.0383</td>
<td>0.0538</td>
</tr>
<tr>
<td>attn 1.</td>
<td>0.1260</td>
<td>0.1378</td>
</tr>
<tr>
<td>attn. 2.</td>
<td>0.0914</td>
<td>0.0798</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0056</td>
<td>0.0070</td>
</tr>
</tbody>
</table>

Table 5.9 Continuum Mechanics reconstruction for specimen 4.
Specimen 5:
Time: 08-30-02
Samples: 0206C088a and 0206C087c

<table>
<thead>
<tr>
<th>Tumor (Invasive Ductal Carcinoma)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>ρ (g/cm³)</td>
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</tr>
<tr>
<td>λ (Gpa)</td>
<td>2.2111</td>
</tr>
<tr>
<td>μ (Gpa)</td>
<td>0.0236</td>
</tr>
<tr>
<td>E (Gpa)</td>
<td>0.0706</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0378</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

Table 5.10 Continuum Mechanics reconstruction for specimen 5.

<table>
<thead>
<tr>
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<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
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<tr>
<td>ρ (g/cm³)</td>
<td>0.8335</td>
</tr>
<tr>
<td>A_{11} (Gpa)</td>
<td>2.0520</td>
</tr>
<tr>
<td>A_{44} (Gpa)</td>
<td>0.0557</td>
</tr>
<tr>
<td>attn 1.</td>
<td>0.1546</td>
</tr>
<tr>
<td>attn. 2.</td>
<td>0.0655</td>
</tr>
<tr>
<td>η (mm)</td>
<td>0.0053</td>
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</table>

Table 5.11 Continuum Mechanics reconstruction for specimen 5.
Statistical Analysis:

Specimen 1:

Time: 12-19-01

Samples: 0110C271f and 0110C272f

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>( \rho ) (g/cm(^3))</td>
<td>0.9796</td>
<td>0.0788</td>
<td>0.8728</td>
</tr>
<tr>
<td>( \lambda ) (Gpa)</td>
<td>2.2989</td>
<td>0.2201</td>
<td>1.6848</td>
</tr>
<tr>
<td>( \mu ) (Gpa)</td>
<td>0.0438</td>
<td>0.0072</td>
<td>0.0381</td>
</tr>
<tr>
<td>( E ) (Gpa)</td>
<td>0.1304</td>
<td>0.0214</td>
<td>0.1135</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0130</td>
<td>0.0112</td>
<td>0.0000</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0108</td>
<td>0.0036</td>
<td>0.0200</td>
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</table>

Table 5.12 Statistic analysis on continuum mechanics reconstruction for specimen 1.

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>( \rho ) (g/cm(^3))</td>
<td>0.8315</td>
<td>0.0233</td>
<td>0.8147</td>
</tr>
<tr>
<td>( A_{11} ) (Gpa)</td>
<td>2.1637</td>
<td>0.0571</td>
<td>1.7836</td>
</tr>
<tr>
<td>( A_{44} ) (Gpa)</td>
<td>0.0523</td>
<td>0.0192</td>
<td>0.2202</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.1457</td>
<td>0.0194</td>
<td>0.0675</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.1465</td>
<td>0.0420</td>
<td>0.0706</td>
</tr>
<tr>
<td>( \eta ) (mm)</td>
<td>0.0065</td>
<td>0.0011</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

Table 5.13 Statistic analysis on doublet mechanics reconstruction for specimen 1.
Specimen 2:

Time: 05-09-02

Samples: 0201C175j and 0291C173v

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>1.0087</td>
<td>0.0729</td>
<td>0.9232</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>2.3180</td>
<td>0.1099</td>
<td>1.9200</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0419</td>
<td>0.0109</td>
<td>0.0252</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.1284</td>
<td>0.0322</td>
<td>0.0753</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0135</td>
<td>0.0069</td>
<td>0.0017</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0077</td>
<td>0.0018</td>
<td>0.0046</td>
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</table>

Table 5.14 Statistic analysis on continuum mechanics reconstruction for specimen 2.

<table>
<thead>
<tr>
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<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.8711</td>
<td>0.0060</td>
<td>0.9070</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>2.1131</td>
<td>0.0641</td>
<td>1.9545</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.0319</td>
<td>0.0269</td>
<td>0.0703</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.1150</td>
<td>0.0321</td>
<td>0.1395</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0904</td>
<td>0.0654</td>
<td>0.1813</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0047</td>
<td>0.0017</td>
<td>0.0064</td>
</tr>
</tbody>
</table>

Table 5.15 Statistic analysis on doublet mechanics reconstruction for specimen 2.
Specimen 3:

Time: 05-14-02

Samples: 0110C271f and 0110C272f

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>1.0264</td>
<td>0.0339</td>
<td>1.0109</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>2.1597</td>
<td>0.2190</td>
<td>2.1959</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0337</td>
<td>0.0031</td>
<td>0.0279</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.1006</td>
<td>0.0093</td>
<td>0.0833</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0385</td>
<td>0.0120</td>
<td>0.0830</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0068</td>
<td>0.0011</td>
<td>0.0055</td>
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</table>

Table 5.16 Statistic analysis on continuum mechanics reconstruction for specimen 3.

<table>
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<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.8561</td>
<td>0.0154</td>
<td>0.8674</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>2.1424</td>
<td>0.0493</td>
<td>1.9723</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
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<td>0.0305</td>
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<tr>
<td>attn. 1</td>
<td>0.1598</td>
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<td>0.1562</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.2557</td>
<td>0.0797</td>
<td>0.0921</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0065</td>
<td>0.0015</td>
<td>0.0052</td>
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</table>

Table 5.17 Statistic analysis on doublet mechanics reconstruction for specimen 3.
Specimen 4:

Time: 07-16-02

Samples: 0206C076d and 0206C075g

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std Dev</td>
<td>Mean</td>
</tr>
<tr>
<td>( \rho \text{ (g/cm}^3 )</td>
<td>1.0451</td>
<td>0.0364</td>
</tr>
<tr>
<td>( \lambda \text{ (Gpa)} )</td>
<td>2.3647</td>
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</tr>
<tr>
<td>( \mu \text{ (Gpa)} )</td>
<td>0.0365</td>
<td>0.0013</td>
</tr>
<tr>
<td>( E \text{ (Gpa)} )</td>
<td>0.1090</td>
<td>0.0039</td>
</tr>
<tr>
<td>attn. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>attn. 2</td>
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<td></td>
</tr>
</tbody>
</table>

**Table 5.18** Statistic analysis on continuum mechanics reconstruction for specimen 4.

<table>
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<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std Dev</td>
<td>Mean</td>
</tr>
<tr>
<td>( \rho \text{ (g/cm}^3 )</td>
<td>0.8560</td>
<td>0.0171</td>
</tr>
<tr>
<td>( A_{11} \text{ (Gpa)} )</td>
<td>2.0449</td>
<td>0.0190</td>
</tr>
<tr>
<td>( A_{44} \text{ (Gpa)} )</td>
<td>0.0478</td>
<td>0.0078</td>
</tr>
<tr>
<td>attn. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>attn. 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta \text{ (mm)} )</td>
<td>0.0064</td>
<td>0.0006</td>
</tr>
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</table>

**Table 5.19** Statistic analysis on doublet mechanics reconstruction for specimen 4.
Specimen 5:

Time: 08-30-02

Samples: 0206C088a and 0206C087c

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>$p$ (g/cm$^3$)</td>
<td>1.0086</td>
<td>0.0561</td>
<td>1.0644</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>2.1314</td>
<td>0.0887</td>
<td>2.4113</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0346</td>
<td>0.0130</td>
<td>0.0442</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.1032</td>
<td>0.0385</td>
<td>0.1317</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.0380</td>
<td>0.0199</td>
<td>0.1037</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0062</td>
<td>0.0032</td>
<td>0.0083</td>
</tr>
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</table>

Table 5.20 Statistic analysis on continuum mechanics reconstruction for specimen 5.

<table>
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<th></th>
<th>Tumor</th>
<th>Normal</th>
<th>T Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>$p$ (g/cm$^3$)</td>
<td>0.8421</td>
<td>0.0074</td>
<td>0.8736</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>2.0250</td>
<td>0.0235</td>
<td>1.9720</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.0562</td>
<td>0.0006</td>
<td>0.0092</td>
</tr>
<tr>
<td>attn. 1</td>
<td>0.1358</td>
<td>0.0163</td>
<td>0.1723</td>
</tr>
<tr>
<td>attn. 2</td>
<td>0.0759</td>
<td>0.0090</td>
<td>0.0836</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0059</td>
<td>0.0006</td>
<td>0.0076</td>
</tr>
</tbody>
</table>

Table 5.21 Statistic analysis on doublet mechanics reconstruction for specimen 5.

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CHAPTER 6

DISCUSSION

6.1 Wave Dispersion Caused by Micro-architecture

One of the salient features of doublet mechanics modeling is that it intrinsically accounts for the dispersion phenomenon at high frequencies. Dispersion is the phenomenon that the propagation speed of the waves is dependent on the frequency. The two major types of dispersion are geometrical and material dispersion. Waves propagating in free space are usually assumed to be non-dispersive in continuum mechanics, meaning that wave speed is a constant regardless of the frequency it travels at. Most waves in solid material are dispersive, nevertheless, especially for high frequency waves in granular media. In doublet mechanics, the material dispersion is intrinsically built into the governing equations of the wave propagation, without resorting to explicit parameters to account for the relationship between wave speed and frequency.

Under doublet mechanics, the governing equation of plane elastic wave propagation for $M=1$ case essentially converges to that under continuum mechanics (Equation 4-13). The $M=2$ equation (Equation 4-14) has an extra term compared to that of $M=1$ case.
which is the derivative of displacement to its forth order. It is this term that introduced the \textit{intrinsic} dispersion to wave propagation in the discrete media. That is, the dispersive nature of the wave propagation was not accounted for through externally imposed terms or curves, but was naturally built in through the multi-scale governing equations.

The wave speeds versus frequency were calculated under the framework of doublet mechanics to study the trends and the degree of the dispersion that was introduced by the discrete presentation of material. The internodal distance was varied in a range to test its effect on dispersion.

According to the experimental measurements from this study, the micro elastic constants and the density were chosen as follows, which are close to the average of the reconstructed values.

\[ A_{11} = 2.0 \text{ Gpa}; \]
\[ A_{44} = 0.1 \text{ Gpa}; \]
\[ \rho = 1.0 \text{ g/cm}^3. \]
6.1.1 The dispersion of longitudinal waves

The dispersion relation for longitudinal waves is as follows:

\[
\frac{\omega}{c_L} = \frac{2\eta \sqrt{(4A_{11} - 2\sqrt{2}A_{11} + A_{11}) (2A_{11} - A_{11} + \sqrt{4A_{11}^2 - 4A_{11}A_{44} + A_{44}^2 - 4\rho_\omega^2 \eta^2 A_{11} + (2\sqrt{2} - 1) \rho_\omega^2 \eta^2 A_{44}})}}{4\eta^2 A_{11} - (2\sqrt{2} - 1) \eta^2 A_{44}}
\]

(6-1)

Figure 6.1 Dispersion of longitudinal waves in doublet mechanics.
Figure 6.1 shows a plot of the magnitude of the longitudinal wave speeds versus frequency at the interested range used for experimental studies in this project. It displayed that for internodal distance $\eta$ less than 1 $\mu$m, the curve is essentially a flat line, i.e., the elastic longitudinal wave is essentially non-dispersive; for internodal distance $\eta$ greater than 1 $\mu$m and less than 15 $\mu$m, the curves are not straight lines, i.e., the waves are dispersive with high frequency retardation: the greater the internodal distance, the stronger the dispersion; for internodal distance $\eta$ greater than 15 $\mu$m, it is shown that magnitude of the wave speeds decline as the frequency becomes higher, however, incline from certain frequency point on. Further investigation on the dispersion relation shows that the latter increment was caused by "attenuation" type of mechanism—the magnitude of the imaginary part of the wave speed becomes larger and larger. In summary,

1. For $\eta < 1$ $\mu$m, non-dispersive;
2. For $1$ $\mu$m $< \eta < 15$ $\mu$m, dispersive, the greater the particles size, the stronger the dispersion;
3. For $\eta > 15$ $\mu$m, strongly dispersive, possibly attenuation at higher frequencies.

6.1.2 Dispersion of shear waves

The dispersion relation for shear waves is as follows:

$$\frac{\omega}{C_5} = \frac{2\sqrt{A_{44}(A_{14} - \sqrt{A_{14}^2 - 2\rho\omega^2\eta^2A_{11})}}}{\eta A_{14}}$$

(6-2)
Figure 6.2 Dispersion of shear waves in doublet mechanics.

Figure 6.2 shows similar trends of the dispersion for shear waves as those for longitudinal waves. It is also observed that the degree of dispersion and attenuation at high frequencies are even stronger for shear waves.

6.2 The Effect of Microstructural Properties on the Overall Reflection Spectra

Computer simulations were conducted to study the effect of every individual microstructural parameter on the overall reflection spectra [Liu et al, 2002]. The objective
was to understand the role of each parameter in determining the characteristics of the spectral curves such as the magnitude of the minima or the distance between the minima. To study the effect of one parameter, the values of all other parameters were fixed, and the value of the parameter of interest was varied in a certain range. The spectra were plotted in one figure to display the change introduced by the variation of the parameter.

6.2.1 Effect of micromoduli

The micromodulus $A_{11}$ is varied ±10% to study its effect on the reflection spectrum.

![Figure 6.3 The effect of the $A_{11}$.](image)
Figure 6.3 shows that increase in micromodulus $A_{11}$ results in shifting of the overall spectrum to the right (higher frequency), and vice versa. The changes in micromodulus $A_{11}$ affected the location of the minima in the curves. Nevertheless, the magnitude of the minima and the distance between the minima remained unchanged.

Similarly the effect of modulus $A_{44}$ was studied by varying its magnitude to ±10% of the original. The result is shown in Figure 6.4.

![Figure 6.4 The effect of $A_{44}$ with 10% variation.](image)

The above figure shows that the magnitude of $A_{44}$ affected the overall reflection spectra to a much lesser degree compared to that of $A_{11}$. In other words, $A_{44}$ is a less sensitive parameter in terms of determining the reflection spectrum. To study its general
effect, a greater amount of change (20%) was introduced and the result is shown in Figure 6.5.

![Figure 6.5](image)

**Figure 6.5** The effect of $A_{44}$ with 20% variation.

Figure 6.5 shows that increase in micromodulus $A_{44}$ resulted in the shift of the minima to the left (lower frequency) and reduced depth (increased magnitude), and vice versa. Therefore changes in micromodulus $A_{44}$ affected the location and the magnitude of the minima.

### 6.2.2 Effect of internodal distance

The internodal distance of the thin layer was varied ±20% to study its effect on the reflection spectrum (Figure 6.6).
Figure 6.6 The effect of particle size at lower frequencies.

Figure 6.6 shows that the internodal distance had minimal effect on the overall reflection spectrum at the relatively low frequency range (lower than 9 MHz). It has an effect of shifting the location of the second minimum without changing the position of the first one. Therefore, it has an effect on the distance between the two minima. For a much higher frequency range, the simulation result is shown in Figure 6.7.
Figure 6.7 The effect of particle size at higher frequencies.

It appears that at higher frequencies the internodal distance has an appreciable effect on the reflection spectrum. With increased internodal distance, the reflection spectrum shifts to its left. This effect is similar to that of micromodulus $A_{1/1}$.

6.3 Statistical Analysis on Reconstructed Properties

In Chapter 5: Results, the pair wise comparison of the reconstructed parameters for both the malignant tissue and its normal controls from each individual patient were presented. The resulted $P$ values on each tissue properties were calculated.
Attention was focused on the comparison of density, (micro) elastic constants, and internodal distances. The magnitudes of the attenuation coefficients (the imaginary portion of elastic constants) are subject to unknown factors such as the comparative size of the tissue sample and the condition of the bonding. Therefore, the comparison between these parameters was not considered.

Biological soft tissue is generally regarded as approximately incompressible material. The measurement and reconstruction showed that this statement was true for the breast tissue samples used in this study. The reconstructed values for Young's modulus and shear modulus obeyed the following relationship:

\[ E = 3\mu \]  

Therefore, these two parameters were not completely independent. Due to the above reason, the comparison of only one of the parameters was considered.

The P-values below 0.05 were bolded and starred in the following tables. From the reconstructed properties of the first specimen, it is found that only one parameter (\(\lambda\)) was significantly different according to the continuum model; conversely, there were three parameters that were significantly different (Tables 6.1-6.2) in doublet mechanics analysis. Similar trend, i.e., there were equal or more properties that could be differentiated by doublet mechanics model, was found in the reconstruction of the properties of other specimens 2, 3, and 5. The specimen 4 is the only case that two parameters were significantly different in continuum mechanics, while only one in doublet mechanics.
Specimen 1:
Time: 12-19-01
Samples: 0110C271f and 0110C272f

Table 6.1 P values from continuum mechanics reconstruction on specimen 1.

<table>
<thead>
<tr>
<th>T Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.1298</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>≈0.0342</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.6046</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.5975</td>
</tr>
</tbody>
</table>

Table 6.2 P values from doublet mechanics reconstruction on specimen 1.

<table>
<thead>
<tr>
<th>T test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.6813</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>≈0.0015</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>≈0.0035</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>≈0.0091</td>
</tr>
</tbody>
</table>
Specimen 2:

Time: 05-09-02

Samples: 0201C175j and 0291C173v

Table 6.3 P values from continuum mechanics reconstruction on specimen 2.

<table>
<thead>
<tr>
<th>Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.1695</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>0.0178</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.0994</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.0986</td>
</tr>
</tbody>
</table>

Table 6.4 P values from doublet mechanics reconstruction on specimen 2.

<table>
<thead>
<tr>
<th>Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.1024</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>0.0494</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.1176</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.2306</td>
</tr>
</tbody>
</table>
Specimen 3:

Time: 05-14-02

Samples: 0110C271f and 0110C272f

**Table 6.5** P values from continuum mechanics reconstruction on specimen 3.

<table>
<thead>
<tr>
<th>Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.5602</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>0.8068</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.5964</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.5951</td>
</tr>
</tbody>
</table>

**Table 6.6** P values from doublet mechanics reconstruction on specimen 3.

<table>
<thead>
<tr>
<th>Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.3423</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>$\leq$0.0167</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>$\leq$0.0028</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.2926</td>
</tr>
</tbody>
</table>
Specimen 4:

Time: 07-16-02

Samples: 0206C076d and 0206C075g

Table 6.7 P values from continuum mechanics reconstruction on specimen 4.

<table>
<thead>
<tr>
<th>T Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.6962</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>$\neq0.0367$</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>$\neq0.0079$</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>$\neq0.0080$</td>
</tr>
</tbody>
</table>

Table 6.8 P values from doublet mechanics reconstruction on specimen 4.

<table>
<thead>
<tr>
<th>T test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.1269</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>$\neq0.0009$</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>0.1195</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>0.0983</td>
</tr>
</tbody>
</table>
Specimen 5:

Time: 08-30-02

Samples: 0206C088a and 0206C087c

Table 6.9 P values from continuum mechanics reconstruction on specimen 5.

<table>
<thead>
<tr>
<th>T Test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.2345</td>
</tr>
<tr>
<td>$\lambda$ (Gpa)</td>
<td>0.0545</td>
</tr>
<tr>
<td>$\mu$ (Gpa)</td>
<td>0.4001</td>
</tr>
<tr>
<td>$E$ (Gpa)</td>
<td>0.3988</td>
</tr>
</tbody>
</table>

Table 6.10 P values from doublet mechanics reconstruction on specimen 5.

<table>
<thead>
<tr>
<th>T test</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (g/cm$^3$)</td>
<td>0.0964</td>
</tr>
<tr>
<td>$A_{11}$ (Gpa)</td>
<td>0.0598</td>
</tr>
<tr>
<td>$A_{44}$ (Gpa)</td>
<td>$&lt;0.0361$</td>
</tr>
<tr>
<td>$\eta$ (mm)</td>
<td>$&lt;0.0205$</td>
</tr>
</tbody>
</table>
6.4 Comparison of Continuum and Doublet Mechanics Models

The models of the first and second scale doublet mechanics were developed for comparison with continuum mechanics. It was shown that the first scale doublet mechanics model was exactly the same as the continuum model in terms of predicting the reflection spectra, given that the parameter values were specified for both simulations with the restraints of (4-22). It is noted that doublet mechanics takes a different set of parameters that are considered as independent. For the first scale of the doublet mechanics model, even though the micro-structural parameter “η” does not appear in any of the modeling equations, the defining parameters were still those of the micro-architecture and micro elasticity. Therefore, the specified architecture posed a constraint on the macro counterparts of the elastic constants.

The second scale modeling in doublet mechanics obviously yielded different results compared to first scale and continuum mechanics modeling. Simulations were carried out to observe the magnitude of the interface continuum stresses that were calculated from the nodal stresses. It was found that the second scale modeling introduced higher order terms of the internodal distances to the formula. Therefore, at very small internodal distance, the second scale modeling converges to the first order, therefore continuum mechanics except that the former allows access to node level elasticity and physical properties. There was an intermediate range of the internodal distance, where the stresses were substantially affected by it; also an upper limit of the internodal distance before the overall dynamics was changed. It is interesting to note that
this dependence on the magnitude of the internodal distance is similar to that found in dispersion.

It is also noted that in the continuum mechanics modeling, viscoelasticity was not considered. In the framework of viscoelasticity, dispersive properties could be studied. That might yield a continuum model that also possesses comparable number of independent variables as in the doublet mechanics (doublet mechanics has an additional parameter \( \eta \)). Further studies are needed to verify whether viscoelasticity could be sufficient. However, doublet mechanics would still be advantageous because it does not require an explicit parameter or equation to model the dispersion cause by discreteness of the material—it is intrinsically accounted for.

6.5 Limitations

Although the experimental setup was carefully chosen based on previous studies of characterization on adhesive materials using the same device, further studies are still needed to find the optimal combination of the settings for the special demands of microstructural accounting measurement of the properties of biological tissue.

1. The frequency range of the transducers

The transducers used in this study had central frequency at 10 MHz. The \(-6\)dB bandwidth of the transducers was 7-13 MHz. From the experimental measurement it was
found that the local minima for tissue were usually located at about 6 and 11 MHz (Please see the reflection spectra in chapter 5). It is obviously that the first minimum fell off the −6dB bandwidth of the transducers. That had introduced significant noise to the lower minimum portion of the reflection spectra. Transducers with 7.5 MHz as their central frequency should be obtained and tested.

2. The material of the embedding structure

Glass was used to form the embedding layers for the thin tissue sections. Its material properties (density: 2.51 g/cm³; Lame’s constants: 26.4 and 29.02 Gpa) are very different from those of the biological tissue (density: ~1.0g/cm³; Lame’s constants: ~2.5 and ~0.05 Gpa). The large difference in these properties caused an almost total reflection of ultrasonic waves at the glass and tissue interface. This reflection is desirable at the second interface, but not the first one. The reason is: it is more desirable that a sufficient amount of waves could be transmitted into the tissue layer to interact with it. That generally entails a better chance to obtain information on it from the reflections.

Materials with properties closer to biological tissue should be tested as the top piece of the embedding layers. Polymer is recommended. The incident oblique beams took 17 degrees in this study. This angle could be varied within the range of the two mode conversion angles to achieve the optimal experimental sensitivity. In combination with the material that would be used as the top substrate, experimental studies are necessary to find the new optimal incident angle.
3. The beam size of the transducers

The ultrasound beams had the size at about the diameter of the transducers used, which was 0.5 inch (12.5 mm). This limited the useful size of the tissue sections. Because the latter had to be equal or greater in size compared to the ultrasound beams in order to attain an accurate measurement. With the practice of screening mammography, the breast cancers that are surgically removed are reducing in size. The minimum diameter of 12.5 mm has been a severe restricting factor in terms of procuring sufficient tissue samples for the study. This directly dictated the number of specimens that could be tested and reported in this study. Besides, the majority of the samples that were used to obtain data had diameter around 10 mm, which is less than 12.5 mm. This insufficiency in size may also contribute to unknown noise factors that were not taken into account in this study.

Smaller beam size (diameter) and direct contact mode transducers should be tested in order to address this problem.

4. The inverse algorithm

Solving the inverse problem of wave propagation is notoriously complex and ill conditioned. The inversion algorithm adopted in this study was built upon a routine least square method (LSM) available through MatLab 6.1. The stability of the convergence of the LSM was tested for the reconstruction of the properties for known materials including water and adhesive. The results showed that the method was fairly stable for the initial guesses that were within 20% deviation from the true values. Since the true values of the properties of the tissue were difficult if not impossible to be measured directly, the
stability test for tissue was not relevant in this sense. However, further studies on the inverse problem and its numerical method of least square minimization are recommended in order to ensure the accuracy and stability of the reconstruction.

5. The comparatively low measurement sensitivity to certain parameters

According to the theoretical simulation, the reflection spectrum was not greatly sensitive to small changes (±10%) of elastic constant $A_{44}$ and internodal distance $\eta$. The experimental measurement and reconstruction confirmed that the values of these two parameters were sensitive to noise (large standard deviations among the tissue sections from the same block). This situation suggests modification on the overall design of the non-destructive method. The integration of measurements from multiple angles (e.g., applying normal incident beams in addition to the oblique incidence) or enlarged amount of the pre-straining on the thin tissue layer may also provide possibilities to enhance the measurement sensitivity.
CHAPTER 7

CONCLUSIONS

In this study, theoretical models for solving the forward problem of wave propagation in a multi-layered medium were constructed from both conventional continuum mechanics and doublet mechanics. The experimental protocol for preparation and quantitative measurement on thin biological tissue specimens was developed. Very thin sections of breast tissue were obtained and tested by an ultrasonic non-destructive evaluation system. An inverse algorithm was designed for the reconstruction of the relevant physical properties of the investigated tissue sections to obtain objective and quantitative interpretation on the measurement results.

The main element of innovation in this project is the application of a microstructure-accounting mechanical field theory (doublet/nano mechanics) in combination with the use of a characterization-mode ultrasound testing, for the deconvolution of otherwise inaccessible information from the tissue samples. The differential analysis on tissue properties indicated that the microstructure quantities including the micro elastic constants and internodal distance could be considered as a new set of physical parameters that potentially aid cancer detection and diagnosis [Liu et al, 2002].
7.1 Significance of Micro-Architecture

The theoretical simulations from the microstructure-accounting model in doublet mechanics has demonstrated that:

1) There is a direct relationship between the existence of the micro-architecture and the degree of dispersion. The general pattern of the dispersion is the high frequency retardation—the wave speeds decrease with the increase of the frequency of the waves. In particular, the greater the magnitude of the internodal distance, the severer the high-frequency retardation. This phenomenon is consistent with the results discussed in the fifth chapter of [Ferrari et al, 1997];

2) The mechanical responses of tissue as in the format of reflection spectra, which were observed through an ultrasonic non-destructive evaluation system, are fully determined by the micro level properties of tissue. The theoretical simulation showed that changes in any single parameter affected the overall response to a certain degree. The micro elastic constant $A_{11}$ appeared to be the most influential parameter, while the effects of the other elastic constant $A_{44}$ and internodal distance $\eta$ were less prominent.
7.2 The Feasibility of Ultrasound-Based Non-Destructive Evaluation

The ultrasonic measurement showed that the spectral responses from normal and abnormal tissue appeared to be appreciably and consistently different, by which the feasibility and reliability of the adopted NDE method for biological tissue testing is demonstrated. The spectral differences include the following aspects:

1) The locations of the two minima, as determined by the corresponding frequencies of the lowest reflection coefficients shown on the spectrum plots. Minimal reflection coefficient indicates the existence of resonance within the tissue layer at certain particular frequency, which is determined by the layer geometry (thickness) and the physical properties of the tissue. According to the theoretical simulation results the micro elastic constant $A_{11}$ has the greatest influence on the location of the minima. Therefore, a difference in the locations indicated the difference in $A_{11}$. The results showed that the minima in the spectra generated by the malignant specimens were located at higher frequencies as compared to those by the normal specimens, indicating higher $A_{11}$ values for malignant tissue;

2) The distance between the two minima, as defined by the difference in the two frequencies corresponding to the two adjacent minima. Many factors including both elastic constants and the intermodal distance could have contributed to the difference. It is noted that according to
the simulation in doublet mechanics, the internodal distance has a unique effect on the distance between the minima. Variation in its magnitude causes the distance to change while the location of the first minimum is unaffected. All other parameters change the distance by simultaneously affecting the locations of both minima. The measurement results showed that the distance for malignant tissue was slightly greater than that for the normal, indicating that malignant tissue had slightly smaller internodal distance if the latter were the sole affecting factor.

3) The depth of the minima, as defined by the magnitude of the minima. The theoretical simulation indicated that the micro elastic constant $A_{44}$ had the greatest effect on the magnitude, with smaller $A_{44}$ corresponding to smaller/deeper minima. The measurement results showed that the reflection spectra of the malignant tissue generally (with one exception) had greater depth (smaller minima) than those of the normal tissue, indicating a smaller $A_{44}$ for malignant tissue.
7.3 The Advantages of Micro-Structure Accounting Analysis

The differential analysis of continuum and doublet mechanics showed that the discrete-based theoretical framework might be advantageous in terms of its ability to separate normal tissue from the abnormal.

1) The range of the least square errors (RESNORM values from the inverse algorithm) was similar for both continuum and doublet mechanics prediction. This indicated that both models were in good agreement with the actual experimental measurement [Ferrari et al, 2001].

2) The reconstructed values of the parameters such as density and Young's modulus were generally comparable to the values found in literature [Krouskop et al, 1998]. This study utilized high frequency elastic plane waves as the probe for measuring the mechanical properties of tissue. It is commonly recognized that the bulk modulus obtained through high frequency longitudinal wave interrogation is not pronouncedly different between normal and abnormal tissue. The results from continuum mechanics reconstruction were in agreement with the above statement. The values of \( \lambda + 2\mu \) were close (around 5-10% variance) between normal and abnormal tissue.

1) The statistic analysis on the reconstructed values of tissue parameters showed that generally the P values for parameters in doublet mechanics reconstruction were lower than those in continuum mechanics. For four
out of five specimens, there were more parameters from doublet mechanics reconstruction that were significantly different (P<0.05) compared to continuum mechanics reconstruction. These results suggested that doublet mechanics might be more powerful and beneficial for the separation of abnormal tissue from its normal counterparts. One possible mechanism could be doublet mechanics modeling offers a new set of parameters that are microstructure relevant and (therefore) potentially more discriminative.
CHAPTER 8
FUTURE WORK

The first step of the future work is to optimize this approach from different perspectives including sample preparation protocol, ultrasonic measurement system, as well as the theoretical model. The focus of this chapter is to outline the future directions that are envisioned from this study.

8.1 Tissue Analysis at the Molecular Level

It has become of great importance to assess the status of molecular markers especially in the field of tumor pathology. In the case of breast cancer, molecular alterations are being incorporated into the development of new treatment strategies [Ross, 1998; Ravdin, 1999; Tsongalis, 2000; Mass, 2000; Piccart, 2001]. There are two major groups of breast cancer biomarkers: 1. Prognostic markers, which can be defined as factors correlated with patient outcome. This group includes: PCNA, thymidine labeling index, S-phase, her-2/neu, p53, VEGF, uPA, PAI-1, Cb. CD. CL, cadherin E. etc; 2. Predictive markers, which predicts response or resistance to a specific therapy. This
group includes: estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu [Duffy, 2001].

Of special interest is the biomarker Her-2/neu gene, which is overexpressed in 15-20% of primary invasive breast cancers [Hung et al, 1999]. Generally, overexpression of Her-2/neu is associated with worse patient outcome, especially in patients with node-positive breast cancers [Mitchell, et al, 1999]. Thus, Her-2/neu status has been tested as a prognostic marker. It is also very possibly a predictive marker because overexpression of Her-2/neu can cause resistance to hormone therapy and response to therapeutic antibody, Herceptin [Mitchell et al, 1999, Pegram et al, 1998]. In fact, assay of Her-2/neu is currently mandatory in deciding whether or not to treat breast cancer patients with Herceptin.

IHC and fluorescence in situ hybridization (FISH) are the most common Her-2/neu tests used [Hanna, 2001]. IHC is a cheaper and simpler method compared to FISH. The latter is recognized as a complicated procedure that requires special training to perform and interpret, with the associated consequence of high cost and limited access [Unger, et al, 1999; Dabb, 2002]. Nevertheless, studies have shown that IHC is appropriate only for initial Her-2/neu assessment; for patients with tumor scored as 2+ it would be beneficial to perform FISH test for more accurate assessment and thus to avoid improper prognostication and treatment [Kakar et al., 2000]. IHC categorizes stain intensity as 0, 1+, 2+ and 3+, corresponding to increasing expression ratio. In fact, it is the interobserver agreement that is not satisfactory for current IHC performance. More specifically, it was found that the interobserver agreement was good for 0 and 3+ but
poor for 1+ and 2+ categories [Thomson, 2001]. Therefore, subjective interpretation impacts the sensitivity and specificity, and thus the accuracy, of IHC.

The main and most important objective of further optimization of these methodologies is to achieve good interobserver and interlaboratory concordance. In current practice of IHC, as the need for reliable quantitative analysis becomes increasingly pressing, an objective quantitative method is indeed essential for proper use of the biochemical markers as prognostic and therapeutic-predictive factors [Taylor et al., 2002]. An expansion of this study is proposed herein to address these needs.

It is hypothesized that the biochemical information of molecular expressions on the biopsy tissue could be translated and further detected and analyzed as quantifiable physical properties. Nanoparticles that are capable of both targeting antigens and enhancing detection could be developed to test this hypothesis.

Nanoparticles in certain size range and with appropriate material properties have long been used to target molecular entities as immuno probes. The rapid, automated, and quantitative detection of nanoparticle distributions is however an elusive goal, simply because of their size. Towards this goal, it may be advantageous to employ the contrast in physical properties with respect to the biological tissue. For example, the density of gold is about 20 times greater than that of normal biological tissue; the elastic modulus, about 50 times. Instead of directly visualizing the location of the particles by optical means, a method based upon this study could be developed to quantitatively and automatically "read" the tissue slides and "detect" the particles through the change in physical properties caused by the specific localization of the particles upon antigen recognition. In
other words, the nanoparticles comprising appropriate choices of materials and size will be detected through their own special physical properties, by employing the similar quantitative method described in this dissertation. Thus a novel system that will supplement and potentially improve current methods of analysis of molecular expressions in breast biopsies could be developed.

8.2 *In Vivo* Applications—Cancer Imaging

Early detection of cancer through screening technologies holds substantial promise for reducing mortality. The National Cancer Institute (NCI) recently reaffirmed the value of screening X-ray mammography after due consideration over the debate [NCI website, 2002]. Nevertheless, there is still a pressing need for new and better modalities that could overcome the hurdles regarding the current gold standard for breast cancer early detection.

X-ray imaging enjoys a good resolution, however, mammography based on this technology suffers from low specificity and sensitivity that negatively affects the physicians' confidence to use it as the sole screening tool [Keith et al. 2002]. Studies have shown that benign biopsies are as high as 75-90% [Kerlikowske et al. 1995; Mushlin et al. 1998]. This is directly associated with the huge unnecessary pain and cost for the patients. In addition, X-ray imaging procedure involves ionizing radiation, which itself is a carcinogenic risk. It is therefore not recommended for young women. This
situation has created an important incentive for the development of novel technologies to improve detection, diagnosis and even treatment for breast cancer.

Non-ionizing modalities such as magnetic resonance imaging and ultrasound are being studied intensively with promising results. Other imaging technologies, such as optical tomography, scintimammography, position emission tomography, thermography, electrical potential measurements, electrical impedance imaging, are also under active development.

The long-term objective of this project is to translate the \textit{in vitro} method to \textit{in vivo} application, where the data acquisition and its quantitative analysis could be performed at the whole organ level as an imaging tool for more powerful non-invasive detection and diagnosis for breast malignancy.

A possible scenario is described as follows. The transducer system could be designed as a ring structure to be placed on top of the breast during examination (Figure 8.1). The transducers could be either emitters or receivers, or dual-functional. The field of the ultrasonic wave propagation could be monitored and recorded by the transducers at the receiver mode. Multiple reflections and transmissions could be recorded as needed. The properties of the tissue layer where the transducer ring is located could be inversed by modeling the media as discrete matter through doublet mechanics. The transducer ring could be moved upward or downward to examine different coronal planes of the breast. The spatial map of the parameters reconstructed from experimental data in conjugation with a Doublet model could be visualized as images for physicians to interpret. The quantitative information of the parameters at any interested area would be available simultaneously, and which may further enhance the accuracy of non-invasive diagnosis.
8.2 Biochemical-mechanical Contrast Agents

The long-term vision for nanoparticle conjugates is also not restricted to \textit{in vitro} applications. Studies have shown that particles of certain size range (i.e., $< 10$ micron) are safe for IV injection [Latres, et al, 1992; Conhaim et al, 1998]. It is envisioned that tumor marker targeting nanoparticles could be developed into ultrasound contrast agents for \textit{in vivo} molecular imaging. Animal safety studies could be conducted to characterize the ranges of materials, shapes, sizes, and concentrations that are appropriate for IV injection. Coupled with the potential outcome of the research proposed in 8.1, these studies will provide the platform for the investigation of nanoparticles as \textit{in vivo}, molecularly-targeted, minimally-invasive mechanical contrast agents.
To further highlight the region that contains cancer or pre-cancer cells for early detection, nanoparticles can be designed with the following properties: 1. The particles are coated with antibody that will specifically target biomarkers on either cancer cells or angiogenic blood vessels. In this way, they will concentrate preferentially in the vicinity of cancer; 2. The particles have distinct mechanical properties compared to biological tissue in general. Specifically, the material of the particles could be bioglass or silicon, or other materials that have a Young's modulus several orders higher than normal or even malignant tissue. In that case, the contrast in elastic properties could be used for detection; 3. The shape of the particles could be arbitrary due to the flexibility of microfabrication. Certain geometry of the particles could introduce enhanced reflection and scattering of ultrasonic waves through the interfaces, and serve as another possible mechanism for enhanced detection.

8.4 Single Platform: Remote-Activation Of Therapeutic Agents By Ultrasound

Systemic administration of pharmaceutical agents — such as toxic chemicals commonly used in chemotherapy for cancer patients — is associated with the well-known disadvantage of severe adverse side effects on normal tissue. Targeted drug delivery schemes are thus in great need to remedy this situation. The hypothesis underlying these approaches is that the therapeutics can be managed in such a way so that its potency will only be released at the site of concern.
The nanoparticles described in 8.1 and 8.3 are endowed with the capability of targeting through antigen recognition. These particles could be further derivatized to be drug delivery vehicles by incorporating therapeutic agents within them. Possible approaches for derivatization of such particles are: 1) to fabricate hollow particles and then render drugs within the reservoir; 2) to fabricate mesh particles and then impregnate them with drugs.

Ultrasound has been exploited to produce therapeutic effects by using its energy to create mechanical vibrations as in lithotripsy [Zhu et al., 2002], enhanced drug delivery [Ng et al., 2002], hyperthermia [Kohrmann et al., 2002], enhanced thrombolysis [Behrens et al., 2001] or other physiochemical changes [Reher et al., 2002], and so on. In light of the drug-loaded, targeting nanoparticles, ultrasound could again be used to facilitate the release of therapeutics from the particles in vivo.

Specific designs in both the nanoparticles and the ultrasound are introduced to achieve this goal.

First, the nanoparticles can be designed to be sensitive to ultrasound energy for breakage. This could be fulfilled through the following mechanisms:

1) A mechanical weakness is built in the configuration of the nanoparticles, so that the latter would be vulnerable to vibration energy, but fairly stable otherwise.

2) A biodegradable material is used for the mesh structure of the nanoparticles, while the degradation could be substantially enhanced by remote administration of ultrasound.
3) A two material cap-and-reservoir structure is devised (shown in Figure 8.2). The dimension of the structure could be up to a few microns. The material difference of the two pieces could cause difference in their response to mechanical vibration. Therefore, the bonds between the two pieces would be broken fairly easily by ultrasound waves, and thus release the drugs within.

![Figure 8.2 A two material cap-and-reservoir structure for drug delivery](image)

The dispensation approaches of ultrasound energy could also take several forms. The frequency could be from low-to-medium; the intensity could be from low to high and focused; the device could be catheter-based to external—according to the combinational design of the nanoparticles and the therapeutic ultrasound.

In principle, the imaging technique can also be interfaced with the tumor killing approaches described above to more precisely administer tissue destruction at the tumor site. This integrated system offers better controllability in both space (localized) and time.
(arbitrary delay). Thus, a single platform system with non-invasive quantitative detection/diagnosis and remotely controlled drug delivery is envisioned.
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APPENDIX

DOUBLET/NANO MECHANICS

For the ease of visualization, a granular interpretation of the theory is introduced. The solid is modeled as a regular array of equal-sized elastic spheres of diameter $d$, the centers of which form one of the fourteen Bravais lattices. It is noted that the theory itself does not require such an interpretive aid. Any space filling assemblies of Fedorov polyhedra would be equally sufficient.

Each lattice node is assumed with a rotation and a translation vector. The displacements of the particles are assumed to vary little at the lengths on the order of their separations. Two smooth vector fields of the translation function $u(X,t)$ and the rotation function $\Phi(X,t)$ are introduced, where $X$ is the position vector of an arbitrary point in the body and $t$ is time. The vector fields of the displacements are assumed to coincide with the real translation and rotation of the granular particles at the node $a$, where $X=x$.

The incremental vectors $\Delta u_a$ and $\Delta \Phi_a$ are defined. For simplicity, only that pertains to translation is presented below:

$$\Delta u_a = u(x + \zeta_a, t) - u(x, t) \quad (A-1)$$

It represents an increment of the translation vector $u$ in a transition from an arbitrary node $a$ to the adjacent node $b_a$ (Figure A-1).
The increment vector may be expanded in a convergent Taylor series in a neighborhood of an arbitrary node $a$ whose position vector is $x$. Truncating this series at the $M$-th term, it is obtained that

$$\Delta u_a = \sum_{\chi=1}^{M} \frac{(\eta_a)^\chi}{\chi!} (\tau_a \cdot \nabla)^\chi u(x,t)$$  \hspace{1cm} (A-2)$$

where $X=x$. The order $M$ at which the series is truncated defines the degree of the approximation in doublet mechanics.

Figure A-1: Translations of the doublet nodes $a$ and $b_a$. $\eta_a$ is the distance between the two doublet nodes, $\tau_a$ is the unit vector along the original direction from node $a$ to $b_a$, and $\zeta_a$ is the new direction vector after the deformation.
Based on the above assumptions, the axial microstrain is obtained

$$
\varepsilon_a = \tau_{ai} \sum_{x=1}^{M} (\eta_a)^{x-1} \tau_{ak} \cdots \frac{\partial^2 u_i}{\partial x_k \cdots \partial x_k} (A-3)
$$

It follows that the first approximation \((M=1)\) for the axial microstrain takes the form

$$
\varepsilon_a = \tau_{ai} \tau_{aj} \varepsilon_{ij} \quad (A-4)
$$

where \(\varepsilon_{ij} = \frac{1}{2} \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right)\), which resembles the continuum mechanics results.

The second approximation \((M=2)\) takes the form

$$
\varepsilon_a = \tau_{ai} \tau_{aj} \frac{\partial u_i}{\partial x_j} + \frac{1}{2} \eta_a \tau_{ai} \tau_{aj} \frac{\partial^2 u_i}{\partial x_j \partial x_k} \quad (A-5)
$$

in expansion, it becomes:

$$
\varepsilon_a = \tau_{ai}^2 \frac{\partial u_i}{\partial x_i} + \tau_{ai} \tau_{aj} \left( \frac{\partial u_i}{\partial x_i} + \frac{\partial u_j}{\partial x_i} \right) + \tau_{aj}^2 \frac{\partial u_i}{\partial x_j} \\
+ \frac{\eta_a}{2} \left( \tau_{ai}^3 \frac{\partial^2 u_i}{\partial x_i^3} + 2 \tau_{ai} \tau_{aj} \frac{\partial^2 u_i}{\partial x_i \partial x_j} + \tau_{aj}^2 \frac{\partial^2 u_i}{\partial x_j^2} \right) \\
+ \tau_{ai} \tau_{aj} \left( \frac{\partial^2 u_i}{\partial x_i \partial x_j} + 2 \frac{\partial^2 u_i}{\partial x_i \partial x_j} \right) \quad (A-6)
$$

Internal generalized axial microstress \(p_a\) that is associated with \(\varepsilon_a\) is postulated. The microstress-microstrain constitutive relationship presented below is a simplified version derived from doublet thermomechanics [Ferrari et al. 1997]:

$$
p_a = \sum_{\beta=1}^{n} A_{ab} \varepsilon_{\beta} \quad (A-7)
$$
where $A_{ap}$'s are the micro-level elastic moduli. This relationship is obtained under the assumption that the particle interactions are longitudinal (central), so that the shear and torsional microstresses vanish everywhere.

The transition from microstresses to macrostresses is achieved by applying equilibrium equations and the resulting relationship is

$$
\sigma_{ij}^{(M)} = \sum_{\alpha=1}^{n} \tau_{\alpha k} \sum_{x=1}^{M} (-1)^{x+1} \frac{(\eta_{\alpha})^{x-1}}{x!} \frac{\partial^{x-1}(p_{\alpha})}{\partial x_k \partial x_k} 
$$

The first approximation ($M=1$) for the continuum stress takes the form

$$
\sigma_{ij} = \sum_{\alpha=1}^{n} \tau_{ij} \tau_{\alpha k} p_{\alpha} 
$$

It is further derived that the macromoduli for $M=1$ case can be calculated as

$$
C_{ijkl} = \sum_{\alpha,\beta=1}^{n} A_{ij} A_{\alpha k} A_{\beta l} [\tau_{\alpha k} \tau_{\beta l}] 
$$

which shows that for $M=1$ the macromoduli are summations of the micromoduli weighted by the coefficients of micro-architecture.

The second approximation ($M=2$) for the continuum stress takes the form

$$
\sigma_{ij} = \sum_{\alpha=1}^{n} \tau_{ij} \left( \tau_{\alpha k} p_{\alpha} - \frac{1}{2} \eta_{\alpha} \tau_{\alpha k} \tau_{\alpha k} \frac{\partial p_{\alpha}}{\partial x_k} \right) 
$$

The more general variants of the theory are introduced in [Ferrari et al. 1997].