COHERENCE IMAGING TECHNOLOGIES FOR THE MEASUREMENT OF TISSUE AND CELL BIOMECHANICS

BY

XING LIANG

DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Electrical and Computer Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Doctoral Committee:

Professor Stephen A. Boppart, Chair
Professor J. Gary Eden
Professor Michael F. Insana
Assistant Professor Gabriel Popescu
ABSTRACT

Biomechanical properties are important for living tissues and cells. They are indicators of functional changes and pathological variations in the micro-structure, such as during tumor development. The topic of this thesis falls at the intersection of biomechanics and optical imaging, and focuses on optical elastography, an optical sensing and imaging technique used to measure biomechanical properties at the tissue and cell levels. Optical coherence elastography, multiphoton elastography, and magnetomotive microscopy are developed, demonstrated, optimized, and applied at the tissue level to ex vivo breast cancer and in vivo skin, and at the cellular level to mouse macrophages in culture. Driven by scientific needs to engineer new quantitative methods that utilize the high micro-scale resolution achievable with optics, results of biomechanical properties were obtained from the biological samples. The results suggest potential diagnostic and therapeutic clinical applications. Results from these studies also help our understanding of the relationship between biomechanical variations and tissue/cell functional changes in biological systems. Therefore, the engineering of imaging techniques is employed to investigate biomechanics, as well as the feasibility for using these techniques to solve more scientific and clinical questions.
ACKNOWLEDGMENTS

This is a great opportunity to express my respect to everybody who contributed to this dissertation. I owe my deepest gratitude to my adviser, Professor Stephen A. Boppart, for his support, guidance, and mentoring through every step of this work. He also lighted up the way of conducting research for me, especially from his expertise in both engineering and medicine. I am grateful to my entire Ph.D. committee, including Professors J. Gary Eden, Michael F. Insana, and Gabriel Popescu, for their assistance throughout the work.

I am pleased to thank Marko Orescanin from Prof. Insana's Ultrasonic Imaging Lab as a great collaborator, who went through many studies with me and shared his wealth of knowledge. I thank Dr. Huafeng Ding from Prof. Popescu's Quantitative Light Imaging Lab. He has been a great collaborator and friend. I thank Dr. Brendan Kennedy from the Optical+Biomedical Engineering Lab at the University of Western Australia for a fruitful month of collaborative work.

I would like to record my gratitude to all my colleagues and friends from the Biophotonics Imaging Lab. I thank Drs. Daniel L. Marks and Amy L. Oldenburg for their initial guidance when I was a new member. I thank Robin Chelliyil, Dr. Zhi Jiang and Ben Graf for their help in the lab. I thank Dr. Marina Marjanovic, Dr. Haohua Tu, Dr. Steven G. Adie, Dr. Renu John, Vasilica Crecea, and Freddy Nguyen for working with me and sharing their expertise. Many thanks go to Eric J. Chaney, who is always ready to help me with animals and cells. I also thank the Beckman Institute for Advanced Science and Technology for fellowship and travel support.

I thank all my family, friends, and colleagues for their support and help throughout my Ph.D. study. My parents deserve special mention for their unshakable support and love. Last and most, I thank my wife, Jie Gao, for her love, dedication, persistent confidence, and understanding.
# TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION ........................................................................................................... 1

CHAPTER 2 BACKGROUND ........................................................................................................... 4

2.1 Biomechanics ..................................................................................................................... 4
2.2 Measurements of Tissue Biomechanics ........................................................................... 5
2.3 Measurements of Cell Biomechanics .............................................................................. 7
2.4 Elastography ..................................................................................................................... 10
2.5 Optical Coherence Tomography ...................................................................................... 12
2.6 Multiphoton Microscopy ................................................................................................. 23
2.7 Diffraction Phase Microscopy ......................................................................................... 24

CHAPTER 3 OPTICAL COHERENCE ELASTOGRAPHY FOR MAPPING
BIOMECHANICS ....................................................................................................................... 26

3.1 Dynamic OCE System ..................................................................................................... 27
3.2 OCE Mapping Technique ............................................................................................... 28
3.3 OCE Mapping Applied to Human Breast ....................................................................... 36
3.4 Discussion and Conclusion of OCE Mapping Technique ............................................... 42

CHAPTER 4 OPTICAL COHERENCE ELASTOGRAPHY FOR IMAGING
BIOMECHANICS ....................................................................................................................... 46

4.1 OCE Imaging System ...................................................................................................... 46
4.2 OCE Imaging Technique ................................................................................................. 48
4.3 OCE Imaging Applied to Breast Tumor ......................................................................... 53
4.4 Discussion and Conclusion of OCE Imaging Technique ................................................ 57

CHAPTER 5 ACOUSTOMOTIVE OCE FOR BIOMECHANICAL PROPERTY
MEASUREMENT ......................................................................................................................... 59

5.1 Acoustomotive OCE System ............................................................................................ 59
5.2 Acoustomotive OCE Technique ..................................................................................... 60
5.3 Acoustomotive OCE Applied on Gelatin Phantoms ....................................................... 62
5.4 Discussion and Conclusion of Acoustomotive OCE ...................................................... 64

CHAPTER 6 SURFACE WAVE PROPAGATION OCE FOR BIOMECHANICAL
PROPERTY MEASUREMENT ................................................................................................... 66

6.1 Surface Wave Propagation OCE System ......................................................................... 66
6.2 Surface Wave Propagation OCE Technique ................................................................... 68
6.3 Surface Wave Propagation OCE Applied to *In Vivo* Human Skin ................................ 70
6.4 Discussion and Conclusion of Surface Wave Propagation OCE Technique .................. 80
<table>
<thead>
<tr>
<th>CHAPTER 7 MULTIPHOTON ELASTOGRAPHY</th>
<th>................................................................. 82</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 Multiphoton Elastography System</td>
<td>............................................................................................................. 83</td>
</tr>
<tr>
<td>7.2 Multiphoton Elastography Technique</td>
<td>.............................................................. 86</td>
</tr>
<tr>
<td>7.3 Multiphoton Elastography Applied to <em>In Vivo</em> Human Skin</td>
<td>.............................................................. 87</td>
</tr>
<tr>
<td>7.4 Discussion and Conclusion of Multiphoton Elastography</td>
<td>.............................................................. 95</td>
</tr>
<tr>
<td>CHAPTER 8 OPEN PROBLEMS</td>
<td>.................................................................................. 97</td>
</tr>
<tr>
<td>CHAPTER 9 CONCLUSIONS</td>
<td>.......................................................................................... 109</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS</td>
<td>.............................................................................................. 111</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>................................................................................................................. 112</td>
</tr>
</tbody>
</table>
CHAPTER 1 INTRODUCTION

Mechanical forces play a significant role in biological tissue development, organization, and response to stimuli. At the cellular level, mechanical forces are important in guiding cell functions as they are actively sensed by living cells. Cell responses to mechanical stimuli frequently result in adaptive changes that modulate form and function. At the tissue level, biomechanical properties of living tissues and engineered tissues depend on their molecular building blocks which can shape and modify the cellular and extracellular structures under stress [1]. For example, forces of adhesion between layers of cells are known to regulate the extracellular growth of tissues [2] and cyclic mechanical strain regulates the development of engineered smooth muscle tissues [3]. Furthermore, pathological changes in the tissue micro-structure such as tumor invasion will lead to different biomechanical properties in tissues. At the organ and body level, biomechanical properties are related to numerous diseases and pathological changes, and also affect body functions. Figure 1.1 illustrates biomechanics from the cellular level (cytoskeleton) to the body level (body mechanics when shoveling).

![Figure 1.1 Biomechanics from cellular level to body level.](image)
Based on different measurement scales in biomechanics, many methods have been used to measure biomechanical properties as indicators of tissue health and disease. For example, physicians for centuries have routinely used palpation as a qualitative method to detect pathological changes such as tumor formation in tissues. To investigate and quantitatively measure biomechanical properties, investigators have utilized biomedical imaging technologies. Elastography is the technology which utilizes biomedical imaging technologies to investigate tissue biomechanical properties. Elastography technologies experienced intense developments during the last several decades, following the emerging imaging technologies, especially tomographic ones such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound imaging due to their unique abilities of generating slices of three-dimensional images. Based on different resolution and penetration scales, these biomedical imaging technologies and their corresponding elastography techniques were used for different purposes of biomechanical properties measurement, as shown in figure 1.2.

![Resolution and Penetration Scale](image)

**Figure 1.2 Biomedical imaging technologies and their scales.**

For measurement of biomechanical properties at tissue and cellular levels, optical coherence tomography (OCT) and microscopic based elastography techniques are most promising because
of their micron to nanometer resolutions, and up to millimeter-scale penetration depths, among these biomedical imaging modalities. This thesis focuses on elastography techniques based on OCT and microscopic technologies, to investigate biomechanics of breast tumor and skin. Breast and skin tissues often involve biomechanical variations during specific pathological changes. Breast tissue usually forms stiff lumps during the development of breast cancer, while the most common skin cancer, basal cell carcinoma, creates softer tissue than normal. To minimize the risks of such diseases, or to control the mortality of such diseases, early detection is the key, which involves high-resolution biomechanical property measurement and imaging.

The goal of this dissertation is to establish elastography techniques based on optical imaging technologies including OCT and microscopy, and to investigate their applications on measuring biomechanical properties at the tissue level and cellular level. Following an introduction in this chapter, Chapter 2 is background review in biomechanics, optical imaging techniques, and their applications in measuring biomechanics. Chapter 3 discusses the dynamic optical coherence elastography (OCE) mapping technique and applications on ex vivo human breast tumor. Chapter 4 discusses the study of the dynamic OCE imaging technique with applications to breast tumor tissues. Chapter 5 discusses acoustomotive OCE. Chapter 6 shows surface wave propagation OCE and application to in vivo human skin. Chapter 7 demonstrates a multiphoton elastography technique and application to in vivo human skin. Chapter 8 describes some open questions. Finally, conclusions are discussed in Chapter 9.
2.1 Biomechanics

Biomechanics is mechanics applied to biology, or the study of motion or equilibrium of biological materials and forces that cause such motion or equilibrium [4]. It is believed that the earliest book containing the concepts of biomechanics were the Greek classic *On the Parts of Animals* by Aristotle (384-322 B.C.) and a Chinese book, *Nei Jing (Internal Classic)* by anonymous authors (472-221 B.C.) [1]. They included primitive observations and understandings of biomechanics from animal and human bodies. Modern biomechanics developed as well as mechanics, which received its impetus from engineering, enabling measurements and analysis of biomechanics on atoms, molecules, cells, tissues, organs and individual organisms. In the studies of biomechanics, applied mechanics including thermodynamics and continuum mechanics, and mechanical engineering disciplines such as fluid mechanics and solid mechanics, play prominent roles. By applying the laws and concepts of these fields, biomechanical mechanisms and structures can be simulated and studied. For instance, in sports biomechanics, researchers apply laws of mechanics and physics to human performance in order to gain a greater understanding of athletic performance through modeling, simulation and measurement. These studies are also often multidisciplinary, which include elements of mechanical engineering, electrical engineering, physics, computer science and clinical studies.
2.2 Measurements of Tissue Biomechanics

In this dissertation, two tissue types are the main objects for the optical imaging techniques, namely breast and skin. Breast cancer comprises ~10% of all cancer incidence among women worldwide. It is the second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death. Early noticeable symptoms of breast cancer often include a palpable lump, pain, or discharge from the nipple [5]. Although X-ray mammography, which was introduced in the 1980s based on the contrast pattern of micro calcifications, has become a commonly used screening tool for breast cancer [6, 7], ultrasound elastography and MRI elastography are promising alternatives based on breast tissue biomechanical contrasts [8-11]. Optical imaging has also been implemented for mammography, which was achieved mainly by diffuse optical tomography techniques including both time-domain and frequency-domain methods [12-15]. Optical imaging of breast cancer can also be used for non-screening purposes such as to study breast tumor refractive indices and optical biopsies based on optical needle delivery methods [16, 17].

There are mainly four stages for breast tumor development. Stage 1 includes early-stage cellular changes, malignant growths, or carcinomas, prior to the formation of a solid mass. Stage 2 includes the process of primary tumor formation and growth. Stage 3 includes tumor metastases or cellular spreading to nearby organs, lymph nodes, or tissue via the bloodstream or the lymphatic circulation. Stage 4 includes metastatic tumor formation at distant locations. Treatments at the early stages have the highest chance for success, but are limited to the lack of
early-stage detection technologies. The tissue microenvironment of breast cancer is of great importance. Microenvironments can supply growth factors to fuel cell proliferation, and promote tumor cell migration through tissue. As tumors grow the microenvironment becomes increasingly turgid because of stromal reorganization. The connection between cell growth and the viscoelastic properties of the substrates on which they are attached has been firmly established, both in vitro and in vivo [18-20]. Studies have also revealed that tumor growth is driven by the stiffness of the stromal tissue, primarily by morphogenesis of nearby epithelial cells [21]. Therefore, it is critical to detect breast cancer at early stages for both diagnostic and therapeutic purposes, as well as to understand how biomechanical factors are related with tumor development. Elastography techniques using optical imaging modalities may provide a solution to quantitatively detect in vivo breast tumor development with micron-scale resolution.

Skin, as the largest organ of human body, is the other study tissue for measurements of biomechanics. Much of the research on human skin has mainly focused on reconstruction, transplantation, manipulation, and its use as a tool to assess pharmacologic functions of a variety of toxins, prescription drugs, and cosmetic treatments [22]. Skin also maintains many complex physiological functions essential to our survival: fluid homeostasis, immune surveillance, sensory detection, wound healing, and thermoregulation. The active and passive functions of skin are regulated by cells and structures located within the layered architecture. Biomechanical properties of skin are of great importance as they contribute to or are responsible for skin health and disease, structural integrity, cosmesis, and aging. Early studies on human skin biomechanics began in the 19th century [23], mainly focusing on skin mechanical anisotropy. From this realized
significance, further studies and investigations were conducted on skin biomechanical properties in the fields of skin aging [24, 25], plastic surgery [26, 27], sun exposure and skin cancer [28, 29], and cosmetics [30, 31]. With aging or pathological changes in human skin, mechanical properties will vary for different layers of skin and at regionally distinct sites, making quantitative measurements more important for diagnosis and for monitoring of interventions.

A number of techniques have been used for measuring skin mechanical properties. For example, ultrasound was used to determine skin thicknesses and mechanical properties in vivo [32]. A tangential traction method was used to determine the biomechanics of finger pad tissue in vivo [33]. Young’s modulus, initial stress, and index of non-elasticity of skin were characterized using a mechanical model under suction [34]. A twistometer was used to determine skin-related mechanical properties of human skin in vivo [25]. Strain-stress relationships were studied to determine the role of elastin in the mechanical properties of skin [35]. Wave propagation methods were also used to determine skin viscoelastic properties [36, 37]. A single-axis extension method was used to test the viscoelastic behavior of skin in vivo using a mechanical model [38].

2.3 Measurements of Cell Biomechanics

Cells constitute the basic unit of life and have a number of functions such as synthesis, storage and transport of molecules, the expression of genetic information, the recognition, transmission and transduction of signals, and so on. Biomechanical properties are extremely significant for specific cell types and their functions. For example, elastic deformations of human red blood
cells (with diameter of ~ 8 μm) are critical for them to flow through narrow capillaries, which usually have inner diameters smaller than 3 μm. Many normal and diseased conditions of cells depend on or are regulated by their mechanical environments and biomechanical properties, which inversely can provide important information about their biological and structural functions.

Biomechanical properties and behaviors can be interpreted by the cell tensegrity model, in which the cytoskeleton that mechanically stabilizes the cell is a tensed tensegrity framework composed of molecular struts, ropes and cables on the nanometer scale. In the model, forces are held by cytoskeletal microfilaments and intermediate filaments, and balanced by interconnected structural elements that resist compression from internal microtubule struts and extracellular matrix adhesions [39]. Cellular solids models are also used to estimate biomechanical properties of endothelial cells [40].

There are several ways for the cells to realize or react to mechanical stimuli or mechanical property changes. The first one is that cellular biomechanics, or mechanical stimulus of a cell, can be considered as another information transferring approach, compared with chemical and electrical stimuli. For example, the outer hair cell of the cochlea belongs to this cell type and can convert mechanical movements or forces into chemical or electrical stimuli [41]. The second way for cells to react to mechanical stimuli is that some cells transmit mechanical signals such as forces or deformations for some other functional use. Examples are from cardiology, where problems with the contractility of heart muscle cells result in heart failure, and problems with the stiffness of the blood vessel walls, which are also controlled by smooth muscle cells, can result
in hypertension and other cardiovascular diseases [1]. Another mechanism for cellular mechanical reaction is from some specific cell types, such as endothelial cells, which are subjected to forces as part of their physiological environment. An absence or malfunction of such forces might cause malfunctions in these cells, inducing an abnormal state of cells [42]. For example, biomechanical properties of cancer cells change during the development and metastasis of cancer. Furthermore, it is believed that tumor cells may experience a tensegrity switch prior to invasive growth, enabling them to migrate from a primary tumor to the surrounding tissues and bloodstream. A recent study showed obvious reduction of stiffness of exfoliated cells from patients suffering from three different types of cancers compared with healthy cells [43].

For breast cancer, which is one of our main target objectives, invasive carcinomas are characterized by tumor cells spreading to surrounding tissues by crossing the basement membrane, and migrating to remote locations by circulating from lymphatic and cardiovascular systems. However, the mechanism of biomechanical properties of cells and tissues affecting the metastatic translation at a cellular level is still not very clear. Therefore, the ability to measure and understand biomechanical properties of breast cancer cells and their microenvironments is of great importance and may be helpful for diagnostics, and subsequently therapeutic interventions.

There have been some techniques investigated to measure single cell biomechanical properties. Atomic force microscopy (AFM) and magnetic twisting cytometry (MTC) are common methods in which a portion of the cell is deformed. In AFM, a micro-tip at the free end of a flexible cantilever generates a local deformation on the cell surface and applied force is estimated [44]. In MTC, magnetic beads with functionalized surfaces are attached to a cell and a
magnetic field imposes a twisting moment on the beads, thereby deforming a portion of the cell [45]. Micropipette aspiration can be used to deform a cell by applying suction on the cell membrane [46]. Optical tweezers can also be used to apply forces on beads and deform a cell [47]. Recent efforts are interested in understanding more complex cell dynamics in cell populations, and a microfabricated system was used to record traction forces generated by a cell population [48, 49]. Studies have also revealed that the extracellular matrix has a significant role and effect on cell populations [50]. However, most experimental studies have focused on cellular biomechanics in vitro, which are most likely to be very different from the cellular biomechanics in living tissues.

2.4 Elastography

Among all the techniques measuring tissue biomechanical properties, cross-sectional imaging-based elastography techniques have become popular because of their added abilities for high-speed, high-resolution measurements, morphological changes in disease, and assessment of biomechanical properties. Elastography is, by definition, an imaging technology which measures tissue elastic properties [9].

Ultrasound elastography was the first technology to perform elastography and is widely studied in clinical diagnostic applications to image the biomechanical properties of soft tissues [8]. For example, the first in vivo application of ultrasound elastography was performed on breast and skeletal muscle [51]. In this study, an elastography system based on a linear array transducer suitable for in vivo scanning was used and an elastogram of a breast containing an 8 mm palpable
cancer nodule was shown clearly. Elastic modulus results were also quantified by ultrasound elastography [52]. Simple one-dimensional (1-D) ultrasound elasticity measurements were performed on muscle and liver and compared with independent and established mechanical measurements to investigate both the accuracy and consistency of ultrasound elasticity measurements. Transient elastography technique in ultrasound was studied later in vivo based on shear wave propagation speed. This study was conducted in vivo on patients who had palpable breast lesions [10]. An acoustic radiation force impulse was used as an internal excitation for elastography imaging to measure mechanical properties of the carotid and popliteal arteries in vivo and ex vivo [53].

MRI elastography has also been successfully applied in the clinical imaging field, especially in diagnosing breast cancer [11]. Acoustic shear waves were used as a mechanical excitation, an MRI-based method was used for imaging the propagation of these waves, and an algorithm for processing the wave images was used to generate quantitative images depicting tissue stiffness. This study was performed on multiple healthy and breast cancer specimens and the mean shear stiffness of breast carcinoma was measured to be 418% higher than the mean value of surrounding breast tissues.

Resolution and penetration of elastography techniques depend on their imaging modalities. For instance, the typical resolution of ultrasound elastography is between 125 and 200 µm [54], while resolution in MRI elastography is usually of millimeter scale [55]. The resolution and penetration of some imaging modalities are shown in figure 2.1. All the elastography techniques can be classified as static methods and dynamic methods, based on their temporal characteristics.
of excitation [56]. In static methods, mechanical excitations are considered to be slow and tissue displacements are usually measured as indications of tissue biomechanical properties [51]. Dynamic methods rely on solving wave equations, which in their differential form are local in character [10]. On the other hand, elastography techniques can also be classified as external and internal methods, based on their spatial characteristics of excitation. External excitation elastography methods often apply a stress to deform tissue from outside when imaging [51], while internal excitation elastography methods apply mechanical excitation directly into the region of interest in tissue. The radiation force of ultrasound is usually used as an internal excitation in elastography techniques [53].

2.5 Optical Coherence Tomography

OCT was used to detect biological tissues for the first time in 1991 [57]. Before that, similar
methods had been used in the related technologies of low-coherence interferometry (LCI) [58], optical coherence-domain reflectometry (OCDR) [59], and optical time-domain reflectometry (OTDR) [60]. LCI was used as an interferometric thickness gauge to measure variations in moving transparent films while OCDR and OTDR were used as 1-D optical evaluation tools to determine positions and magnitudes of reflection sites in fiber optic cables. OCT combined these interferometric techniques with broad-band near-infrared laser sources and a transverse scanning mechanism through a focusing lens to achieve two-dimensional (2-D) cross-sectional or three-dimensional (3-D) imaging. High resolution, non-invasive and real-time processing are the main features of OCT.

The principle of OCT is to depth-resolve optical scattering variations within a sample using optical interferometric techniques, in which the axial resolution is determined by the coherence length of the laser source while the transverse resolution is determined by the spot size of the incident beam. OCT is a noninvasive biomedical imaging modality and has been successfully applied in ophthalmology to image morphological changes in the retina [57]. This technology has also been investigated in other medical fields such as oncology, dermatology, cardiology, gastroenterology, neurosurgery, and developmental biology [61-65].

2.5.1 Development and theory of OCT

The early OCT studies mainly used time-domain OCT (TD-OCT) systems. A typical simplified interferometric setup is given in figure 2.2. A low-coherence near-infrared light source is split into two arms, namely reference and sample arms, using a beam splitter. The beams that return from the reference and sample arms are combined to produce an interference signal which
is detected using a photoelectric detector. Let $U_R$ be the optical field backscattered from the reference arm and $U_S$ be the field from the sample arm. Then the intensity detected by the detector will be

$$I_d = \left| \langle U_d \rangle \right|^2 = \left| \langle U_R(t + \tau) + U_S(t) \rangle \right|^2 = I_R + I_S + 2 \Re \left\{ \langle U_R(t + \tau)U^*_S(t) \rangle \right\},$$

where $\tau$ is the time delay between the two arms. The last term can be expressed as

$$\Re \left\{ \langle U_R(t + \tau)U^*_S(t) \rangle \right\} = \Re \{G(\tau)\} = |G(\tau)| \cos(2\omega_0\tau).$$

$G(\tau)$ is the temporal coherence function and can be written as

$$G(\tau) = \langle U_R(t + \tau)U^*_S(t) \rangle = \exp \left( \frac{\Delta \omega \tau}{4\sqrt{\ln 2}} \right) \exp(-i\omega_0\tau),$$

where $\omega_0$ is the central wavelength and $\Delta \omega$ is the wavelength deviation. When a perfect reflector is placed at the sample, $\Re \{G(\tau)\}$ can be recognized as the point spread function (PSF) or impulse response of the system, which is illustrated in figure 2.3a, and the detected signal is the envelope of the convolution of the PSF and the reflector structure function (a 1-D Dirac delta function), as shown in figure 2.3b-d.

In time-domain OCT, as the reference arm mirror is moved, the path length $h$ between the two arms is changed and hence the delay $\tau$ is changed. When there are multiple scatterers in the sample instead of a perfect reflector, the axial scan (A-scan) signal detected will resemble that in figure 2.4. This signal can be seen as a convolution of the point spread function and the structural function in the sample. By scanning the sample beam transversely, a 2-D image can be formed.
Figure 2.2 Schematic of typical time-domain OCT system. BS: Beam splitter; $U_r$: Optical field from the reference arm; $U_s$: Optical field from the sample arm.

Figure 2.3 Simulation of OCT signal from a perfect reflector. (a) Point spread function of an OCT system. (b) A 1-D Dirac delta function denoting a perfect reflector. (c) Detected OCT signal as dotted red line, which is the envelope of the convolution of a and b. (d) 1-D OCT image of (c).
Fourier-domain OCT (FD-OCT) is an extension of time-domain OCT [66]. A typical type of FD-OCT is spectral-domain OCT (SD-OCT), as shown in figure 2.5. Instead of the moving mirror, SD-OCT has a fixed mirror in the reference arm and a spectrometer as a detector. The structural information of the sample is obtained in the spatially encoded frequency components of the detected OCT signal and then reconstructed by the fast Fourier transform.

Since there are no physically moving parts, an SD-OCT system has a much higher stability and acquisition speed than a TD-OCT system. Another type of FD-OCT is swept-source OCT
(SS-OCT). In this technique, the spectral components are not encoded by spatial separation, but are encoded in time. The light source spectrum is either filtered or generated in single successive frequency steps [67, 68]. Due to the unique sweeping method, SS-OCT can achieve much faster acquisition speed than its counterparts, which can be as high as 370,000 A-scans per second [69].

2.5.2 Practical aspects of OCT

Axial resolution is a very important specification for OCT imaging since high axial resolution is critical to differentiate structural features of cells and tissues [70]. The axial resolution can be defined as the coherence length of the source, which can be written as

$$l_c = \frac{2 \ln 2}{n \pi} \frac{\lambda_0^2}{\Delta \lambda},$$

where $n$ is the refractive index of the sample, and $\lambda_0$ and $\Delta \lambda$ are the central wavelength and full width at half maximum (FWHM) bandwidth of Gaussian source spectrum, respectively. Thus, for example, if an OCT system has a Ti-Sapphire laser source with central wavelength of 800 nm and FWHM bandwidth of 100 nm, theoretically, this system has an axial resolution of about 2.8 μm in air.

The transverse resolution of OCT is determined by the objective lens in the sample arm and can be defined as the spot size of the sample beam, which can be written as

$$2w_0 = \frac{4f \lambda_0}{\pi D},$$

where $2w_0$ is the spot size diameter of the beam, $f$ is the focal length of the sample arm objective lens, and $D$ is the beam diameter incident onto the lens. However, there is a tradeoff between the transverse resolution and depth of field $b$, since $b$ can be written as
\[ b = 2z_0 = \frac{2\pi w_0^2}{\lambda_0}. \]  

From equation 2.6, one can observe that if the transverse resolution is improved, the actual depth of field of the beam is decreased, which blurs the area outside the focusing depth in the image. This tradeoff is intrinsic in OCT and microscopy in general, but may be solved numerically [71].

Signal to noise ratio (SNR) is another important specification for OCT images. SNR of an OCT system can be defined as the ratio of the signal power generated by a perfectly reflecting mirror to the noise of the system. The dominating noise sources are shot noise, excess intensity noise, and receiver noise. Receiver noise can either be calculated from manufacturer specifications or modeled as thermal noise. In the case of broad bandwidth light, the photocurrent noise has two terms: shot noise due to photocurrent variance and excess noise due to self-beating of broad-band light waves [72].

2.5.3 Optical coherence elastography

Optical elastography includes elastography techniques based on optical imaging modalities. With the inherent high-resolution of optical imaging technologies, optical elastography techniques are unique for measuring biomechanics at the micron-scale tissue level, the cellular level, and even the molecular level. The focus in this dissertation is on the development of optical elastography techniques and their biological and clinical applications, such as breast tumor and human skin biomechanical property measurements. Optical elastography techniques in this thesis are novel in terms of their mechanical excitation delivery methods, mechanical model for imaging, signal processing algorithms, and many results showing quantitative
high-resolution biomechanical properties for the first time.

One important optical elastography technique is optical coherence elastography, which is a novel elastography technology used to determine tissue biomechanical properties utilizing the \textit{in vivo} imaging modality OCT. The first OCE technique was reported in 1998 \cite{73}. The hardware and theory of operation of OCE were described and internal displacements indicating biomechanical properties of gelatin phantoms, pork meat and \textit{in vivo} skin were shown by using 2-D cross-correlation speckle tracking algorithm. Since then, slow external mechanical excitation and static elastography based on speckle tracking methods (or cross-correlation algorithms in OCE) have been established for this technology. The displacement sensitivity for this method was proportional to the axial resolution of the OCT system, which was usually several microns in tissues. The cross-correlation algorithms for OCE were then improved based on the scale of tissue displacements. Two approaches to speckle tracking for OCE were studied where one was appropriate for small speckle motions and the other for large, rapid speckle motions \cite{74}. Dynamic OCE was achieved using TD-OCT systems based on a Bessel frequency spectrum of the interferometric OCT signal, and performed on \textit{in vivo} human skin using a piezo ring \cite{75, 76}.

A phase-sensitive optical coherence elastography method was introduced later in 2007 \cite{77} when FD-OCT systems were widely used, from which phases of complex OCT data were more stable than TD-OCT systems. Deformations, strain rates, and strains of soft tissue were measured by this method in real time with a sensitivity at the nanometer scale. This method exploits the phase information available in the complex OCT images and measures the phase changes between successive B scans to resolve the instantaneous tissue deformations.
OCE techniques have been widely applied in different fields for biomechanical property measurements. For example, OCT has been investigated for intravascular imaging as a counterpart to intravascular ultrasound imaging, but can offer a higher spatial resolution. Intravascular OCE was studied to exploit prior information about arterial wall biomechanics to produce robust estimates of tissue velocity and strain [78]. An OCE system was studied for tissue elasticity reconstruction, and distribution results of four representative tissue block models in intravascular imaging [79]. Then a robust algorithm based on correlations between neighboring lines in the OCT image was used for an intravascular OCE study, and it offered detection of strains larger than 0.6% in response to an oscillatory force applied to the vessel wall [80]. OCE was also studied as a method for assessing the biomechanical properties of atherosclerotic plaque in which tissue phantoms and aorta were examined in vitro to quantify speckle modulation and measure the displacement and strain maps [81]. OCE was applied to measure the biomechanical properties of engineered and developing tissues as well. Spatially distributed mechanical displacements and strains were mapped in a representative model of a developing engineered tissue as cells began to proliferate and attach within a 3-D collagen matrix. Displacements were quantified by a cross-correlation algorithm on pre- and post-compression images, from which OCE was able to differentiate changes in strain over time, which corresponded with cell proliferation and matrix deposition as confirmed with histological observations. OCE was also performed in a complex developing tissue of the Xenopus laevis (African frog) tadpole [82].

Conventional OCE studies inherited a difficulty from static elastography as they were based on speckle tracking methods (or cross-correlation algorithms in OCE), which have an intrinsic
problem. Speckles are granular-shaped patterns in OCT images which result from the convolution of the beam point spread function and the scatterers in the tissue, as discussed in 2.5.1. The speckle pattern is determined by the distances between the scatterers inside the coherence volume [83]. So in conventional OCE, a speckle may not have the same signal amplitude or shape before and after deformation of the sample. Simulation of such a situation is shown in figure 2.6. In the figure, there are four 1-D Dirac delta functions in red in the z direction denoting four scatterers, in which each pair is irresolvable in the OCT signal, as denoted by the dotted red envelope. The corresponding 1-D OCT image is shown in figure 2.6c. After a compression is applied in the z direction, a possible case may happen as shown in figure 2.6b, where there are still four 1-D Dirac delta functions in red in the z direction, but according to destructive phase changes of OCT signals of these scatterers, the pair on the right side is no longer present in the OCT signals. The corresponding 1-D OCT image is shown in figure 2.6d. Under this circumstance, confusing results may happen for speckle tracking, as shown in figure 2.6f, compared with the ideal speckle tracking result in figure 2.6e. Thus, compared with the OCT resolution and sensitivity, it was difficult for conventional OCE to provide highly accurate quantitative biomechanical property results based on a cross-correlation algorithm.

Similar to other elastography technologies, OCE can also be grouped in static or dynamic methods, and external or internal methods, as shown in figure 2.7. Speckle tracking methods or cross-correlation algorithms are intrinsic difficulties for static OCE techniques, but can easily be avoided by dynamic OCE techniques, which are based on solving differential wave equations.
Figure 2.6 Possible cases of conventional OCE signal from multiple scatterers before and after compression.
2.6 Multiphoton Microscopy

As an optical imaging technology, OCT and thus OCE are proper for imaging and measuring biomechanical properties at the tissue level. However, to investigate biomechanical properties at the cellular level, optical microscopy technologies have inherent advantages because of their sub-micron resolution. In this dissertation, two microscopy technologies are used for measuring biomechanics, and are introduced in this section, including multiphoton microscopy and diffraction phase microscopy. Multiphoton microscopy (MPM) is a high-resolution imaging technique that relies on the non-linear excitation of endogenous or exogenous fluorescent molecules. In MPM, the energy of multiple photons may be absorbed simultaneously by fluorescent molecules and the energy is irradiated as a single photon. Practical two-photon microscopy and three-photon microscopy were demonstrated in the 1990s [84, 85]. However, two-photon excitation, which is used in the studies of this dissertation, is the most common technique for MPM, because the probability of the near-simultaneous absorption of two photons is the highest. In the studies, MPM is used for \textit{in vivo} human skin imaging, and assessing skin
biomechanical properties. MPM has been widely applied to investigate human skin in vivo including skin cancer diagnosis [86], skin aging [87], drug monitoring [88], among many other applications. However, few studies of MPM skin imaging have focused on human skin biomechanics. Thus, to the best of our knowledge, MPM is applied here to study in vivo human skin biomechanical properties for the first time.

2.7 Diffraction Phase Microscopy

Diffraction phase microscopy (DPM) with Fourier transform light scattering (FTLS) is another microscopy technique that is used in this dissertation to investigate cellular biomechanics. DPM is a quantitative phase microscopy technique which provides the phase shift associated with transparent structures from a single interferogram measurement. DPM can supply detailed structural information of cells with submicron resolution. FTLS is a novel approach to study static and dynamic light scattering, which combines the high spatial resolution (~0.5 μm) associated with DPM and intrinsic averaging of light scattering techniques [89]. The underlying principle of FTLS is to retrieve the phase and amplitude associated with a DPM image and numerically propagate this field to the scattering plane, as shown in figure 2.8. In contrast to MPM, DPM/FTLS has the ability to retrieve dynamic light scattering information from different angles. Given these features, DPM/FTLS is used to investigate single cell biomechanics.
Figure 2.8 Schematic setup of the DPM/FTLS microscope. BS, beam splitter; CCD, charge-coupled line-scan camera; S, sample; O, objective lens; M, mirror; TL, tube lens; I, iris; G, grating; L1, L2, Lenses; SF, spatial filter. Courtesy of Huafeng Ding.
CHAPTER 3 OPTICAL COHERENCE ELASTOGRAPHY FOR MAPPING

BIOMECHANICS

OCT has been widely used for biological and clinical applications. One promising application of OCT for breast tumor imaging is the intraoperative evaluation of breast tumor margins [90]. Briefly, in this study, OCT was used to assess surgical breast tumor margins by providing real-time microscopic images with micron-scale resolution and millimeter-scale penetration. This study demonstrated the feasibility of using OCT to evaluate the breast tumor margin status during the surgery, which is the key predictor of local recurrence [91-97]. Positive margins are the main causes for breast tumor local recurrence, which occur in at least 30% to 35% of cases [98]. However, highly scattering features such as connective tissues or surgical artifacts including cauterized tissue and superficial blood may cause false positives, since these features may have similar optical scattering features and OCT structural patterns. One method for differentiating these features is from their intrinsic biomechanical property variations. For this case, OCE as a complementary method also based on the OCT imaging technology may be of great importance to increase the sensitivity. Thus, the OCE technique is investigated to map tissue biomechanics for tumor margin detection. As introduced in section 2.2, variations in biomechanical tissue properties can indicate the level of breast cancer development, and biomechanical properties are also important indicators for understanding cell and microenvironment interactions, and their roles in facilitating or inhibiting tumor development and metastases.
3.1 Dynamic OCE System

3.1.1 OCT system

An SD-OCT system was used in the studies, as shown in figure 3.1. In this system, the low-coherence light source consists of a neodymium-doped yttrium orthovanadate (Nd:YVO₄) pumped titanium:sapphire laser, which has a center wavelength of 800 nm and a bandwidth of 100 nm, providing an axial resolution of ~3 µm in tissue or tissue phantoms. A 12.5 mm diameter, 40 mm focal length lens was used in the sample arm to provide a transverse resolution of 13 µm. The common average power incident on the samples was 10 mW. A line charge-coupled device (CCD) camera was used to detect the spectral interference signal with an acquisition rate of 25 kHz.

Figure 3.1 Schematic diagram of spectral-domain OCT system used in the dynamic OCE study.
3.1.2 External dynamic OCE system

The external dynamic OCE system is derived from the SD-OCT imaging system described in 3.1.1. In the OCE system, as shown in figure 3.2, a glass window stage was fixed in the sample arm of the OCE system to restrict the upper boundary of the tissue phantoms and tissues, yet enable optical imaging through the window. The sample stage was mounted on a mechanical wave driver (SF-9324, PASCO scientific, Roseville, CA), which provided a frequency range of 0.1 Hz to 5 kHz and a maximum amplitude of 7 mm at 1 Hz, decreasing with increasing frequency. The external driving waveform was programmed and synchronized with the image acquisition. Samples were mounted between the upper glass window and the sample stage, which are both of larger transverse size than the tissue phantom or tissue samples, with only minimal contact and force prior to data acquisition. To perform M-mode (motion mode, repetitive axial depth-scans into the tissue, acquired at a fixed transverse position over time) dynamic OCE, optical backscattering signals were acquired through the upper glass window while the driving mechanical perturbations were exerted simultaneously through the sample stage, compressing the sample vertically. Step and 20 Hz sinusoidal waveforms were used in the OCE experiments.

3.2 OCE Mapping Technique

Tissue phantoms with similar optical scattering and biomechanical properties to real tissues were used to calibrate the elastic moduli measurements obtained with OCE. Silicone-based tissue phantoms were chosen due to their permanence and the ability to vary their elastic moduli [100].
Phantoms were fabricated from pure polydimethylsiloxane (PDMS) fluid (50 cSt viscosity, ClearCo, Inc.), a room-temperature vulcanizing silicone, and its associated curing agent (General Electric RTV-615 A and B, respectively, Circuit Specialists, Inc.). To obtain different elastic moduli, a series of phantoms were fabricated with different concentration ratios of these three ingredients, which are listed in table 3.1.

Titanium dioxide (TiO$_2$) powder (Sigma-Aldrich, #224227, mean size 1 µm, <5 µm) were embedded with a concentration of 1 mg/g in the tissue phantoms to function as optical scatterers for OCE imaging. The phantom solutions were mixed in an ultrasonicator for 30 minutes at room temperature and then poured into 35 mm plastic Petri dishes, with a thickness of approximately 10 mm. All phantoms were cured at 80 °C for 8 hours and subsequently at room temperature for 24 hours. Two groups of phantoms were fabricated identically for measurement by indentation and by OCE seperately. The silicone tissue phantoms were sectioned into cubes of dimension approximately 10 mm × 16 mm × 10 mm (x-y-z directions).
Table 3.1 Measured elastic moduli for tissue phantoms.

<table>
<thead>
<tr>
<th>Phantom Concentration Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1:10:10</th>
<th>1:10:20</th>
<th>1:10:30</th>
<th>1:10:50</th>
<th>1:10:80</th>
<th>1:10:100</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_i$ (kPa)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>248.50±</td>
<td>112.20±</td>
<td>70.16±</td>
<td>44.38±</td>
<td>20.89±</td>
<td>14.58±</td>
</tr>
<tr>
<td>$E_s$ (kPa)</td>
<td>0.42</td>
<td>0.19</td>
<td>0.12</td>
<td>0.12</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>$E_m$ (kPa)</td>
<td>359.18±</td>
<td>167.33±</td>
<td>92.29±</td>
<td>45.08±</td>
<td>18.35±</td>
<td>12.74±</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.48</td>
<td>0.17</td>
<td>0.17</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>$E_m$ (kPa)</td>
<td>282.90±</td>
<td>106.14±</td>
<td>73.6±</td>
<td>23.32±</td>
<td>10.47±</td>
<td>8.55±</td>
</tr>
<tr>
<td></td>
<td>12.51</td>
<td>5.80</td>
<td>8.57</td>
<td>3.11</td>
<td>1.12</td>
<td>3.90</td>
</tr>
<tr>
<td>$\gamma_0$ (Pa·s)</td>
<td>0.23±</td>
<td>0.32±</td>
<td>0.20±</td>
<td>0.28±</td>
<td>0.22±</td>
<td>0.22±</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.19</td>
<td>0.05</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration ratio of the phantom is the mass ratio of PDMS curing agent RTV-615 B : PDMS RTV-615 A: pure PDMS fluid.  
<sup>b</sup>$E_i$ stands for the elastic modulus measured by the indentation method while $E_s$ and $E_m$ stand for the elastic moduli measured by step-driven OCE and sinusoidally driven OCE, respectively. Reprinted from reference [99].

3.2.1 Modeling and elastic moduli measurements of tissue phantoms by OCE

A dynamic OCE system shown in figure 3.2 was used to accurately measure the elastic moduli of tissue samples. To account for the characteristics of the mechanical wave driver and its mechanical coupling with the tissue, a Voigt body including a non-negligible mass was used to model the mechanical wave driver with a spring constant $k_0$ and a coefficient of viscosity $\gamma_0$ as shown in figure 3.3a. To quantify $k_0$ and $\gamma_0$, M-mode OCE was performed directly on the sample stage without any sample for 0.4 s. This process can be described by

$$m\ddot{z}_{ss}(t) + \gamma_0 \dot{z}_{ss}(t) + k_0 z_{ss}(t) = F(t),$$

(3.1)

in which $m$ is the mass of sample stage, $z_{ss}(t)$ is the displacement of the sample stage from the equilibrium value, and $F(t)$ is the driving step or sinusoidal force. Using M-mode OCE imaging, the displacement $z_{ss}(t)$ of the sample stage was extracted using a third-order spline interpolation
and peak value detection, and then the curve was fitted to the solution to equation (3.1)

\[ z_{ss}(t) = Re^{\lambda t} \cos(\mu t - \delta) \]  

(3.2)

using least-squares algorithm, where \( R \) is the amplitude of the vibration, \( \lambda = -\gamma_0 / 2m \) is the damping coefficient, \( \mu = \sqrt{4mk_0 - \gamma_0^2} / 2m \) is the damped oscillation frequency of the mechanical wave driver, and \( \delta \) is a phase factor. The spring constant \( k_0 \) and the coefficient of viscosity \( \gamma_0 \) of the wave driver can then be determined. Now that the wave driver has been characterized, one can consider its coupling with the tissue samples. The sample was modeled as a Voigt body coupled to the mechanical wave driver as shown in figure 3.3b. Tissue phantoms composed of silicone with varying stiffness were investigated using M-mode OCE. Figure 3.4c is a representative M-mode OCE image of a silicone tissue phantom with a step driving force applied.

From the acquired backscattered data, the damped motion of a single scattering particle was selected and displayed in the magnified image in figure 3.4d. In this figure, the dashed line denotes the driving waveform and the solid line denotes the fitted curve based on the track of the
particle motion, which is shown between the dashed and solid curves. The model therefore can be described by

![Figure 3.4 OCE images of silicone tissue phantoms.](image)

(a) B-mode OCT image of a representative silicone tissue phantom. Dashed arrow denotes the position of the laser beam for M-mode OCT imaging. (b) M-mode OCT image of the silicone tissue phantom at the laser beam position in a. (c) M-mode OCE image with a step-driven waveform. (d) Magnified image of the dotted range in (c). The dashed line represents the driving waveform and the solid line represents the fitted curve, while the image data between the curves shows the scattering particle movement. (e) M-mode OCE image with a sinusoidally driven waveform. (f) Magnified image of the dotted range in (e). The dashed line represents the driving waveform (amplitude rescaled) and the solid line represents the fitted curve, while the image data between the curves is due to the scattering particle movement. Reprinted from reference [99].
where \( k \) is the spring constant of the sample, \( \gamma \) is the coefficient of viscosity of the samples, \( m' \) denotes the total mass of the sample stage and sample, and \( z_{sp}(t) \) now denotes the displacement of the selected scattering particle from the equilibrium value. The depths of detection are usually less than 1 mm in this method and compared with the heights of the samples, the differences in displacement over the regions of interest can be neglected in terms of fitting the Voigt model.

In the same way, one can calculate \( k_0 + k \) from the fitted parameters of the solution to equation (3.3), and solve the elastic modulus of the sample using the Hookean strain-stress relationship

\[
E_s = kL / S = \left[ (\mu^2 + \lambda^2)m' - k_0 \right] L / S, \tag{3.4}
\]

where \( L \) and \( S \) are the thickness and the contact area of the samples, respectively. \( E_s \) represents the elastic modulus measured by the step-driven OCE. From equation (3.4), one can see that \( E_s \) is calculated from the oscillation frequency and damping coefficient of the scattering particle in the sample while amplitude information of the oscillation is not needed.

It is also possible to perform OCE using a sinusoidal driving waveform. Figure 3.4e is an OCE image of the silicone tissue phantom under sinusoidal excitation. In figure 3.4f, a magnified region of one particle is shown, along with a plot of the driving wave form and fitted curve. The solution to equation (3.3) then becomes

\[
z_{sp}(t) = Re^{\omega t} \cos(\mu t - \delta) + D \sin(\omega t - \alpha), \tag{3.5}
\]

where \( D \) is the amplitude for the driving wave and \( \alpha \) is another phase factor with \( \eta \) and \( \delta \) having the same meanings as before. By fitting the experimental data to equation (3.5), the elastic modulus measured by sinusoidally driven OCE (\( E_m \)) can be described by
where $E_m$ represents the elastic modulus measured by the sinusoidally driven OCE.

### 3.2.2 Phase-resolved method used for dynamic OCE

A phase-resolved method was used to measure the displacements in sinusoidally driven dynamic OCE experiments. Phase-resolved OCE was employed because it was somewhat difficult to extract and track the motion of a single scatterer in optically dense tissue, such as the neoplastic human breast tissue. The theoretical phase-resolved OCE method is similar to Doppler OCT [101]. The complex signal of a scatterer can be expressed as $\eta(z) = |\eta(z)|e^{i\phi(z)}$, with amplitude $|\eta(z)|$ and phase $\phi(z)$. When the sample is moving, the Doppler shift is shown as

$$
\Delta f_D = \frac{\Delta \phi(z)}{2\pi T} = \frac{\left[ \eta_{jT}(z) \cdot \eta_{j+1T}^*(z) \right]}{2\pi T},
$$

(3.7)

assuming the displacement is parallel to the incident beam direction. The moving speed of the scatterer then can be described as

$$
v = \frac{\lambda_0 \Delta f_D}{2\pi} = \frac{\lambda_0 \Delta \phi(z)}{4\pi \pi T}.
$$

(3.8)

Thus, the displacement of the scatterers within the laser beam is directly proportional to the interferometric phase angle change between two successive camera exposures, as shown by

$$
D = vT = \frac{\lambda_0 \Delta \phi(z)}{4\pi \pi} \propto \Delta \phi(z).
$$

(3.9)

This phase-resolved OCE method has been shown to have higher sensitivity than amplitude-based OCE methods [77]. Silicone tissue phantoms were measured by sinusoidally driven phase-resolved OCE and the results were verified to be the same as previous
amplitude-based OCE results. Then heterogeneous tissue with both tumor and normal adipose regions was imaged by sinusoidally driven phase-resolved OCE to show the ability to distinguish and spatially map different biomechanical regions in human tissue on the micro-scale. In these experiments, sinusoidal M-mode dynamic OCE phase data were recorded for every transverse position of the tissue, which can then be represented as a B-mode (brightness mode, two-dimensional mode with cross-sectional transverse scanning) OCE image. Each set of M-mode phase data represents the motion track of the scatters within the beam column. By scanning the laser beam transversely across the sample in steps of 15 μm, OCE data was obtained for every pixel within a two-dimensional tissue cross-section, with acquisition time of about 5 s for each frame. Subsequently, calculations were performed for each pixel to generate a 2-D spatial map of the elastic moduli. Approximately, the computation time is 7 s for each pixel and 10 hours for a 2-D spatial map. Compared with the amplitude-based OCE method, phase-resolved OCE is more sensitive to small displacements of scatterers in the tissue, providing sub-nanometer displacement sensitivity [74].

3.2.3 Verification measurements by the indentation method

Bounded indentation experiments were used to independently determine the elastic moduli of one group of the tissue phantoms. A TA.XT Plus Texture Analyzer (Texture Technologies Corp., Algonquin, IL) with a 6.35 mm radius steel sphere was used to perform the measurements. With an indenter velocity of 0.01 mm/s and displacement of 2 mm, the measurements were performed for two cycles for each tissue phantom under room temperature. A corrected Hertz contact mechanical model was used to calculate the elastic moduli [102].

35
3.2.4 Results from silicone phantoms

Measured elastic moduli using these M-mode dynamic OCE methods and the commercial indentation method for different silicone concentration ratios are shown in table 3.1 and plotted in figure 3.5. Viscosity values of these phantoms are also shown in table 3.1. In figure 3.5, it is observed that as the mass concentration ratio of the cross-linked to fluid silicone increases, the elastic modulus measured by OCE increases linearly with that measured by indentation method. Since the indentation method is a standardized way of measuring the elastic modulus of soft tissues, the elastic moduli data by OCE methods are calibrated with the indentation method data according to figure 3.5.

3.3 OCE Mapping Applied to Human Breast

3.3.1 Sample preparation

Human tissue samples were acquired from breast cancer patients under protocols approved by the Institutional Review Boards of the University of Illinois at Urbana-Champaign and Carle Foundation Hospital, Urbana, Illinois. The tumors were diagnosed by a board-certified pathologist as invasive ductal carcinomas via standard pathological evaluation techniques performed at Carle Foundation Hospital. All human tissues were resected into cubic shapes smaller than the sample stage and upper glass window, placed in a buffered saline solution, and stored in a cooler until imaging was performed within 2 hours.
3.3.2 Results from human tissues by dynamic OCE

Normal and neoplastic *ex vivo* human breast tissues were investigated using external dynamic OCE and were modeled using the same process as for the silicone tissue phantoms. The calculations of elastic moduli were also determined in the same manner. Both tumor and normal adipose tissue were investigated using the sinusoidally driven OCE method and their elastic moduli were subsequently determined, which are shown in table 3.2. The viscosity for *ex vivo* human breast tumor and adipose tissue are $0.23 \pm 0.02 \text{ Pa} \cdot \text{s}$ and $0.65 \pm 0.03 \text{ Pa} \cdot \text{s}$, respectively.

From these results, it can be seen that tumor tissue has an elastic modulus about 25 times
larger than that of adipose tissue, but the tumor tissue has a relatively smaller viscosity than adipose tissue. Furthermore, tissue samples containing a more heterogeneous combination of tumor and adipose tissue were similarly tested to demonstrate micro-scale optical mapping of elastic moduli using OCE. Images from tissue containing both tumor and normal adipose regions are shown in figure 3.6. Figure 3.6a shows a B-mode OCT image with adipose tissue on the left side and tumor tissue on the right side, corresponding with the histological image in figure 3.6b.

<table>
<thead>
<tr>
<th>Tissue type(^a)/ Measurement Position(^b)</th>
<th>tumor</th>
<th>adipose</th>
<th>t&amp;a(^c)/-1.5 mm</th>
<th>t&amp;a(^c)/-1.1 mm</th>
<th>t&amp;a(^c)/0 mm</th>
<th>t&amp;a(^c)/0.7 mm</th>
<th>t&amp;a(^c)/1.2 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>E(_s)(kPa)</td>
<td>10.68±0.86</td>
<td>0.42±0.17</td>
<td>4.14±0.42</td>
<td>2.69±0.24</td>
<td>4.59±0.26</td>
<td>12.92±1.20</td>
<td>14.15±3.45</td>
</tr>
</tbody>
</table>

\(^a\)Sinusoidally driven OCE experiments investigating three types of tissue: tumor tissue only, adipose tissue only, and the combination of tumor and adipose tissues. \(^b\)Measurement positions correspond to the transverse positions in figure 3.6a. \(^c\)T&a indicates the combination of tumor and adipose tissues. Reprinted from reference [99].

At different transverse positions, the sinusoidally driven OCE experiments were performed on the sample to test the spatial elastic modulus variation. Elastic moduli measured at five different transverse positions are shown in table 3.2. In figure 3.6b to e, two of the OCE images and associated magnified regions are shown, at positions indicated in figure 3.6 by the dotted arrows. When testing tissue containing multiple types, a large variation of elastic moduli between the tumor and adipose tissue regions was discerned, because each region exhibits different biomechanical properties. However, due to the fact that the current mechanical model does not account for the coupling between regions with different moduli, these values are only relative.
Figure 3.6 OCE of ex vivo human breast tissue. (a) B-mode OCT image of breast tissue. The left side of this image represents the adipose tissue while the right side of the image represents the tumor tissue. Dotted arrows denote the two positions which have corresponding OCE images. (b) Histology image corresponding to (a). (c) M-mode sinusoidally driven OCE image of the breast tissue at the laser beam position on the left side in (a). (d) Magnified image of the dotted range in (c). The dotted line represents the driving wave form and the solid line represents the fitted curve, while the one between them is the real particle motion track. (e) M-mode sinusoidally driven OCE image of the breast tissue at the laser beam position on the right side in (a). (f) Magnified image of the dotted range in (e). The dotted line represents the driving wave from and the solid line represents the fitted curve, while the one between them is the real particle motion track. Reprinted from reference [99].
These OCE results agree well with other ex vivo indentation measurements on breast tissue, in which adipose and tumor tissues are reported to have elastic moduli of 1.9 kPa and 12 kPa, respectively [103]. The results also agree with in vivo MRI elastography results for tumor tissues, which were reported to have elastic moduli in the range of 2 to 37 kPa, varying with different measurements. For normal adipose tissue, the measured moduli are lower than most of the values reported from in vivo MRI elastography studies [104, 105], possibly because the measurements were performed ex vivo.

3.3.3 Results from human tissues by phase resolved dynamic OCE

Using the phase-resolved dynamic OCE method, an elasticity map of tissue containing both tumor and normal adipose was provided and is shown in figure 3.7. The B-mode OCT image of the breast tissue is shown in figure 3.7a, along with the corresponding histology image shown in figure 3.7b. The measured elasticity map is shown in figure 3.7c along with the elasticity error map shown in figure 3.7d. In figure 3.7c and d, white areas denote pixels with OCE signals smaller than an amplitude threshold, and median filtering was performed on both images.

One can observe in figure 3.7 that the results from the phase-resolved OCE method correspond with the amplitude-based OCE results of table 3.2 and the apparent regions of tumor and adipose within the structural OCT image. The elasticity map allows for the differentiation of the tumor from the adipose on the micro-scale. Furthermore, elasticity differences on the tumor side of the OCE map (yellow and green colors) are observed, which cannot be differentiated on the OCT structural image alone. In comparison with histology, these elasticity differences are likely due to the presence of fibroblasts and tumor cells comprising the tumor tissue.
This ability to differentiate small biomechanical variations on the micro-scale may offer the potential for early-stage tumor detection, where it is often difficult to differentiate tissue types by the optical scattering properties alone, and certainly when these changes are too small to be detected by more traditional ultrasound or MRI elastography techniques, or by palpation.

Figure 3.7 Phase-resolved OCE map of human breast tissue elasticity. (a) B-mode OCT image of breast tissue. The left side of this image represents the adipose tissue while the right side of the image represents the tumor tissue. (b) Histology image corresponding to (a). (c) Map of elasticity by sinusoidally driven phase-resolved OCE. (d) Error map of elasticity by sinusoidally driven phase-resolved OCE. Unit for color bar is kPa. Reprinted from reference [99].
Currently, however, the mechanical model does not account for the couplings between tissue regions with different elastic moduli, and thus, the values on the elasticity map represent relative values of the elastic moduli of the tissue. Further refinement of these models could account for these couplings and improve the quantification of these measurements.

3.4 Discussion and Conclusion of OCE Mapping Technique

From the results on silicone tissue phantoms, the measurements from the step-driven OCE, sinusoidally driven OCE, and standard indentation method are slightly different, likely due to differences in the applied strain rate in these methods. It has been previously shown that the elastic modulus of a biological sample is dependent on the strain rate of its deformation, due to resistance originating from organic matrices in the sample [106]. Thus, for the tissue phantoms and tumor tissues, the larger the strain rate, the larger the measured elastic modulus. With strain rates much larger than those used in the indentation method, the elastic moduli measured by OCE can be treated as dynamic elastic moduli. These can be associated with the static elastic moduli according to the linear relationship shown in figure 3.5. In the range of 15 to 250 kPa, there exists the relationship of $E_s > E_m > E_i$ (where $E_i$ represents the elastic modulus measured by indentation method). In this study, the indentation method strain rate is fixed at 10 $\mu$m/s while the OCE strain rate is varying for different samples. For sinusoidally driven OCE, the strain rates range approximately from 3 mm/s to 17 mm/s for different samples. For step-driven OCE, the strain rate is as high as approximately 30 mm/s, but decreasing over the time of the measurement due to the exponential decay. This explains why the sinusoidally driven OCE is more closely
matched with the indentation method. Due to the relatively large strain rate and its rapidly changing nature in step-driven OCE, compared to the indentation method used to validate OCE, this method is not recommended and was not used for the subsequent tissue measurements.

System sensitivity is a critically important parameter to characterize the potential of the OCE system measurement. In the dynamic OCE system, the sensitivity can be described by the output error, which is the product of the output sensitivity function and parameter error [107]. The error can be estimated from fitting boundaries for the measured parameters, and then calculated the output error for the elastic moduli. According to equations for $E_s$ and $E_m$, the absolute elastic modulus output error in step-driven OCE is given by

$$
\sigma_{Es} = \sqrt{\left(\frac{\partial E}{\partial \lambda}\right)^2 \sigma_{\lambda}^2 + \left(\frac{\partial E}{\partial \mu}\right)^2 \sigma_{\mu}^2} = \left(2\frac{L_0}{S}m\right)\sqrt{\sigma_{\lambda}^2 + \mu^2 \sigma_{\mu}^2},
$$

while in sinusoidally driven OCE, the expression is

$$
\sigma_{Em} = \sqrt{\left(\frac{\partial E}{\partial \lambda}\right)^2 \sigma_{\lambda}^2 + \left(\frac{\partial E}{\partial \alpha}\right)^2 \sigma_{\alpha}^2}
= \left(2m'\omega \frac{L_0}{S}\right)\sqrt{\left(\frac{1}{\tan \alpha}\right)^2 \sigma_{\lambda}^2 + \left(\frac{\lambda}{\cos^2 \alpha}\right) \sigma_{\alpha}^2},
$$

assuming that the measurement errors of $m, S, L_0$, and $\omega$ are negligible. The terms $\sigma_{Es}, \sigma_{Em}, \sigma_{\lambda}, \sigma_{\mu},$ and $\sigma_{\alpha}$ are the errors for $E_s, E_m, \lambda, \mu,$ and $\alpha$, respectively. The estimates of the absolute elastic moduli output error were shown in tables 3.1 and 3.2. It can be seen that the relative output error can be as small as 0.08% for the step-driven method. However, the output error for sinusoidally driven OCE is larger than for the step-driven method and the error from the human tissue is worse than from the tissue phantoms. This is because in sinusoidally driven OCE, the coefficient
of viscosity is relatively too small to be accurately fitted, compared with the dominant driving waves. Thus, fitting of the step-driven OCE data usually had a higher R-squared value than for the sinusoidally driven OCE data, and the sensitivity was subsequently better in the former method. The silicone tissue phantoms used in these studies have TiO$_2$ microparticles in them as optical scatterers, so extracting particle tracking data by the spline algorithm is more straightforward than in human tissue, in which a single scatterer motion track can sometimes be difficult to extract, such as in the example shown for the neoplastic human breast tissue. The majority of tissues probed by optical imaging modalities such as OCT are optically heterogeneous, thereby facilitating the ability to locate and track single scatterers for this method.

In this chapter, the external dynamic OCE technique and its *ex vivo* application to breast tumor has been investigated. This technique is used to measure tissue biomechanical properties based on solving differential equations. Since no cross correlation algorithms or speckle tracking methods were used in this technique, the intrinsic deficiencies of these methods are no longer problematic in dynamic OCE. The features of OCE include quantitative measurements, micro-scale resolution, and high sensitivity.

For external dynamic OCE, low-frequency dynamic mechanical waves were used to drive tissue and measured elastic moduli were based on solutions to wave equations. Results from this OCE system have been calibrated with biomechanical tissue models, and validated against a standard indentation method for determining elastic moduli. Furthermore, by using phase-resolved displacement measurements, a biomechanical property distribution map was
computed, which showed the ability to differentiate tumor and normal regions of *ex vivo* human breast. This novel OCE system exhibited high resolution in both axial and transverse directions in accordance with OCT technology, and sensitivity in the elastic modulus as high as 0.08%. Utilizing *in vivo* OCT imaging, the novel OCE technology has potential to perform more accurately and with higher resolution in various other biomedical imaging applications, provided that the tissue is optically and mechanically accessible. This OCE technology may also contribute to understanding the micro-scale biomechanical tissue properties during the growth and development of engineered and natural biological tissues, and the biomechanical responses of these tissues following the application of mechanical or pharmacological stimuli. Additional studies are needed to further improve this system. More accurate tissue models such as finite element models can be developed, instead of the mass-weighted Voigt model, for different tissue imaging applications. Using this current methodology, every pixel in the OCE image or biomechanical map requires wave equation fitting and, as a result, the processing time is currently too long for real-time applications. Fortunately, these current computational limitations will be overcome as computing power continues to improve.
CHAPTER 4 OPTICAL COHERENCE ELASTOGRAPHY FOR IMAGING

BIOMECHANICS

In the previous chapter, dynamic OCE was studied to measure biomechanical properties. However, the applicability of these dynamic OCE techniques for real-time or \textit{in vivo} diagnostics is limited by their data acquisition and processing speeds. For OCE techniques, time-domain dynamic OCE inherently operates at slow image acquisition rates, while the previous spectral-domain dynamic OCE mapping technique needs a long acquisition time by taking M-mode OCT images per transverse location and a long curve-fitting processing time [75, 76, 99]. In this chapter, a novel dynamic OCE technique is developed to image biomechanical properties of breast tumor; the technique combines dynamic mechanical excitation with fast image acquisition and processing. B-mode images were acquired during sinusoidal mechanical compression excitation, and local strain rates were calculated to represent local biomechanical properties. Different excitation frequencies were used to highlight sample regions with distinct mechanical properties. This technique features fast image acquisition and processing speeds, and therefore has the potential for non-destructive volumetric imaging and real-time clinical applications. This chapter is adapted from reference [108].

4.1 OCE Imaging System

The OCE system, as shown in figure 4.1, was constructed based on the SD-OCT system described in the previous chapter. The average power incident on the samples was 10 mW. For
the OCE sample arm, a fixed 2° round wedge prism (PS810-B, Thorlabs, Newton, NJ) was used to restrict the upper boundary of the sample, and a piezoelectric (PZT) stack (AE0505D16F, Thorlabs, Newton, NJ) was used to bound the bottom of the sample. The PZT stack was driven by a single channel PZT driver (MDT694, Thorlabs, Newton, NJ) and used to sinusoidally displace the sample under frequencies of 20 Hz, 45 Hz, 100 Hz, and 313 Hz in the axial direction. Minimal contact and force were applied to the samples prior to data acquisition and a 30 V_{pp} sinusoidal voltage signal was applied to the PZT stack during data acquisition, providing 4.5 µm maximum displacement. The axial depth scans in the OCE images were collected by a CCD line-scan camera at acquisition line rates of 1 kHz and 10 kHz. The camera acquisition was synchronized with a transverse scanning galvanometer and the PZT stack excitation.

Figure 4.1 Schematic diagram of the dynamic spectral-domain OCE system. In the sample arm of the OCE system, the glass window is rigidly fixed to the table. The PZT (piezoelectric stack) is synchronized with data acquisition, and compresses the sample against the glass window. Reprinted from reference [108].
4.2 OCE Imaging Technique

4.2.1 Data acquisition and processing

OCE images were computed from B-mode OCT spectral datasets that were acquired during dynamic mechanical excitation and acquired with no mechanical excitation. In the transverse direction of the images, a number of cycles of sinusoidal oscillations from the samples were recorded according to the excitation frequency. These oscillations inside the samples were caused by the external excitation, and their amplitudes were determined by both the excitation amplitude and the local mechanical properties of the samples. To extract the mechanical properties of the samples, a custom processing algorithm (figure 4.2) was used to analyze the OCE images.

First, a reference background OCT image was subtracted from the datasets, and fast Fourier transformations were taken for each column of the datasets to calculate the complex OCT signals from the spectral data. The phase was then calculated for each corresponding pixel of the signal, and the phase difference between adjacent A-lines was computed, which was proportional to the scatterer velocities in the axial direction. The phase unwrapping along the transverse dimension was then performed and fast Fourier transformations were taken in the transverse direction to obtain the lateral phase evolution as the motion spectrum. A bandpass filter at the driving frequency and with an appropriate bandwidth was used to select the sample motion under each driving frequency. Thus, after inverse fast Fourier transformation, the sample motion velocities under the driving frequencies were extracted in the OCE phase signal for each pixel. Finally, strain rate calculations and down sampling were applied to generate the OCE images. To
improve specificity, the strain rate images were divided by the ones without any mechanical excitation, and shown in decibels. From the OCE data processing, amplitudes of vibration strain rates of the samples under driving frequencies in the sample were extracted for the OCE images. The strain rates can be expressed as

$$\epsilon'(x, z) = \frac{\Delta \phi(x, z) \lambda}{4\pi n \tau (z - z_0)},$$

(4.1)

where $x$ and $z$ denote the lateral and the axial direction, respectively, $\Delta \phi$ is the phase difference between each adjacent A-lines, $\lambda$ is the central wavelength of the source, $\tau$ is the time interval between two adjacent A-lines, and $z_0$ is the surface of the sample.

Figure 4.2 Signal processing flow chart for OCE on imaging biomechanics. Reprinted from reference [108].
4.2.2 Resonance frequency calibration

Strain rate is an important parameter for characterizing the mechanical properties of samples. It is related to measurements of moduli [109] and has been widely used for ultrasound myocardial imaging [110]. The excitation frequencies in this OCE technique were chosen to be around the resonant frequencies for the investigated samples, which are calibrated using M-mode OCE similar to the method described in section 3.2.1 and in reference [111]. Briefly, using the mechanical model given by figure 3.3 and equation 3.3, one can solve the resonance frequency as

\[
\mu_\parallel = \frac{\sqrt{(\mu_\parallel^2 + \lambda_\parallel^2)m'_\parallel - (\mu^2 + \lambda^2)m}}{m'_\parallel - m} - \left[ \frac{m'_\parallel - m\lambda_\parallel}{m'_\parallel - m} \right]^2, \tag{4.2}
\]

in addition to elastic modulus measured by the step-driven OCE, where \( \mu \) is the natural frequency of the mechanical wave driver, \( \mu_\parallel \) is the natural frequency of the sample, \( \mu'_\parallel \) is the natural frequency of the combination of these two, \( m \) is the mass of vibrator, and \( m'_\parallel \) denotes the total mass of the sample stage and sample. The resonance frequency for the mechanical wave driver can be derived as 22.76 Hz. The Young’s moduli and resonance frequency can also be derived for the rat tumor tissue and adipose tissue, as shown in table 4.1.

<table>
<thead>
<tr>
<th>Wave driver</th>
<th>Rat tumor</th>
<th>Rat adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus (kPa)</td>
<td>16.47 ± 1.13</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>Resonance frequency (Hz)</td>
<td>22.76</td>
<td>288.93</td>
</tr>
</tbody>
</table>

Table 4.1 Measured resonance frequencies for rat tumor and adipose tissues.
However, these chosen frequencies may be different from the calibrated resonant frequency for a particular tissue type because of the complexity of the sample geometry and boundary conditions. For example, the resonant frequency of rat adipose tissue was found to be ~39 Hz calculated from the M-mode OCE method, but in this study, we found a 45 Hz excitation frequency to be the most effective frequency to generate the greatest contrast for the adipose tissue. And for rat tumor tissue, we found 313 Hz to be the most effective frequency instead of ~289 Hz.

4.2.3 Data acquisition constraints and system resolution

The excitation frequencies are also limited by the axial scan rate, since the axial scan rate must be sufficiently fast to sample the excitations. This can be described as $f_A > 2f_E$, where $f_A$ is axial scan rate and $f_E$ is the excitation frequency. In this study, we have utilized four excitation frequencies (20 Hz, 45 Hz, 100 Hz, and 313 Hz). For the first three excitation frequencies, $f_A = 1$ kHz was used. However, for the 313 Hz excitation frequency, an $f_A$ of 1 kHz was too close to $2f_E$ by the Nyquist criterion, and thus an $f_A$ of 10 kHz was used. The CCD exposure time was set to be 100 µs for both axial scan rates, so that the imaging sensitivities were the same for all experiments, regardless of the excitation frequency.

The axial resolution of this OCE technique is the same as for OCT, which is the optical source coherence length. However, the transverse resolution of this OCE technique may be different, and is the larger value between the transverse OCT resolution and the resolution given by
\[ R_{OCE} = m \cdot d = \frac{f_A}{f_E} \cdot \frac{\Delta x}{N}, \]  

(4.3)

where \( \Delta x \) is the transverse scanning range, \( N \) is the number of axial scans in the raw OCT spectral data, \( d \) is the effective scanning distance for each A-line, and \( m = \frac{f_A}{f_E} \) is the down sampling factor. The calculated OCE lateral resolution is actually the effective scanning distance for each mechanical vibration cycle. One example is for the rat tumor tissue experiment. When \( f_A \) is 1 kHz, \( f_E \) is 45 Hz, \( \Delta x \) is 2 mm, \( N \) is 4000, the down sampling factor is 22, the effective scanning distance for each A-line is 0.5 µm, and the interval for each A-scan is 1 ms, as shown in figure 4.3a. Therefore \( R_{OCE} \) is 11 µm as calculated from equation (4.3). However, limited by the beam spot size, the transverse OCE resolution cannot be smaller than the transverse OCT resolution. Therefore, the effective OCE transverse resolution is 13 µm for this example. If \( f_A \) is doubled to 2 kHz, \( f_E \) is 45 Hz, \( \Delta x \) is 2 mm, and \( N \) is 4000, the interval for each A-scan is 0.5 ms, and thus the down sampling factor is 44. The effective scanning distance for each A-line remains unchanged to be 0.5 µm. Therefore, \( R_{OCE} \) is 22 µm as calculated from equation (4.3), which is larger than and thus not limited by the OCT transverse resolution. For this example, the effective transverse OCE resolution is 22 µm, as shown in figure 4.3b. If \( f_A \) is 1 kHz, \( f_E \) is 45 Hz, \( \Delta x \) is 2 mm, but \( N \) is 2000, the down sampling factor is 22, the effective scanning distance for each A-line is 1 µm, and the interval for each A-scan is 1 ms. The effective transverse OCE resolution is also 22 µm, as calculated by equation (4.3) and shown in figure 4.3c.
4.3 OCE Imaging Applied to Breast Tumor

4.3.1 Sample preparation

Samples imaged by OCE in this section include three-layer silicone tissue phantoms and rat tumor tissues. Silicone-based tissue phantoms were fabricated in a manner similar to that stated in section 3.2. Two concentration ratios of the three ingredients were used to obtain two different elastic moduli in the layers of the samples, which were 1:10:20 and 1:10:80 corresponding to elastic moduli of approximately 100 kPa and 10 kPa. Polystyrene microspheres (mean diameter
0.53 µm, Bangs Laboratories, Inc.) were embedded in the tissue phantoms with a concentration of 1 mg/g to function as optical scatterers for OCE imaging. Phantoms with polystyrene microspheres have relatively deeper penetration depth for OCT imaging due to smaller refractive index variations [100]. The phantom solutions were mixed thoroughly in an ultrasonicator for 30 minutes under room temperature and then poured into 9 cm plastic Petri dishes. Different layers of samples with different elastic moduli and thicknesses were fabricated separately after the curing process of the previous layer, which includes curing at 80 °C for 8 hours and subsequently at room temperature for 24 hours. The silicone tissue phantoms were sectioned into cubes of dimensions approximately 8 mm × 8 mm × 3 mm (x-y-z directions) for OCE imaging.

Female inbred Wistar–Furth rats (6-8 weeks old) (Harlan, Indianapolis, IN) were also used in this study. Experiments were performed under a protocol approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. The rats were individually housed, fed standard rat chow pellets, provided with water and food ad libitum, and kept on a 12-hr light–dark cycle. The induction of mammary tumors using the carcinogen N-methyl-N-nitrosourea (MNU) (50 mg/kg) was done as previously described [112]. Briefly, the MNU was injected intraperitoneally in the left side of the peritonea. A second injection of MNU into the right side of the peritonea was administered seven days later. Following MNU injections, animals were palpated weekly to determine mammary tumor development. Tumor with surrounding adipose tissues was excised and resected into cubic shapes with approximate dimensions of 4 mm × 3 mm × 3 mm for OCE imaging.
4.3.2 Results and discussion of OCE imaging on silicone phantoms

This dynamic spectral-domain OCE technique was first applied to the three-layer tissue phantoms. In the tissue phantoms, the top layer had an elastic modulus of ~100 kPa and a thickness of ~200 µm. The middle layer had an elastic modulus of ~10 kPa and a thickness of ~400 µm, and the bottom layer had an elastic modulus of ~100 kPa and a thickness of ~2400 µm, which is shown in the structural OCT image in figure 4.4a. The OCE images under driving frequencies of 20 Hz and 100 Hz are shown in figure 4.4b and c (green channel), with the structural OCT image as the background (red channel). Under 20 Hz excitation, the OCE signal is shown predominantly in the middle layer. When the excitation frequency is 100 Hz, the OCE signal is shown predominantly in the top and middle layers.

Strain rates as calculated in equation (4.1) in the OCE images are used to differentiate mechanical properties under different driving frequencies. If the mechanical properties of samples are in resonance with a driving frequency, the vibration strain rates will increase. This feature of OCE imaging makes it possible to mechanically characterize specific regions within heterogeneous samples. For OCE using 20 Hz excitation, the soft middle layer is near resonance, and thus the strain rate is high. The stiff top layer is far away from its resonance, and is not effectively compressed. The OCE image therefore has very little strain rate values in this top layer. The bottom layer is also off resonance, but is acting as a compressive plate. Thus in the OCE image, appreciable strain rate values are observed. However, the OCT signal for the bottom layer is noisier compared to the other two layers, and the OCT phase noise causes attenuation of
the OCE signal in this layer, for both excitation frequencies [66]. For OCE under 100 Hz excitation, the top and bottom layers are near resonance, which produces bulk motion, and yields large values of strain rates in the OCE image.

![Figure 4.4 OCT and OCE images of a three-layer tissue phantom. (a) Structural OCT image. Young's moduli are labeled for each corresponding layer. (b) OCE image under 20 Hz mechanical excitation. (c) OCE image under 100 Hz mechanical excitation. Scale bar applies to all the images. Reprinted from reference [108].](image)

4.3.3 Results and discussion of OCE imaging of breast tumor

The OCE results from rat tumor tissue are shown in figure 4.5. Figure 4.5a and c are the OCE images of the tissue under 45 Hz and 313 Hz excitations, respectively. The corresponding OCT structural and histological images are shown in figure 4.5b and d, respectively. From figure 4.5, one can observe that under 45 Hz excitation, the OCE image highlights predominantly the adipose tissue region (left side of image), while under 313 Hz, the OCE image highlights predominantly the tumor tissue region (right side of image). The 45 Hz and 313 Hz frequencies are near the calibrated mechanical resonances for rat adipose and tumor tissue, respectively. Therefore in the heterogeneous rat tumor tissue, different tissue types were selectively highlighted under different driving frequencies according to their different resonances. Furthermore, in figure 4.5a, the OCE image includes two regions from the right side (orange arrows) which corresponds to the highly scattering region from the OCT signal in figure 4.5b.
From the histological image in figure 4.5d, it is shown that this region corresponds to local areas of adipose cells and connective tissues (orange arrows) within the tumor, which can hardly be differentiated from the OCT scattering image. On the other hand, in figure 4.5c, the OCE image includes some regions from the left side (blue arrow) which corresponds to the less scattering region from the OCT signal in figure 4.5b. According to the histological image in figure 4.5d, those regions correspond to invading tumor tissues in the adipose side (blue arrow).

![Figure 4.5 OCE results on ex vivo rat tumor tissue. (a) OCE image under 45 Hz mechanical excitation. (b) OCT structural image of the tissue. (c) OCE image under 313 Hz mechanical excitation. (d) Corresponding histological image. Scale bar applies to all the images. Arrows are discussed in the text. Reprinted from reference [108].](image)

4.4 Discussion and Conclusion of OCE Imaging Technique

The image acquisition and processing speeds of this OCE technique have been dramatically improved compared with the OCE mapping method in chapter 3. The acquisition speed is 4 s per frame for a 1 kHz axial scan rate and 0.8 s per frame for a 10 kHz axial scan rate. The processing
speed is approximately 1 s per frame using Matlab on a PC with a dual core 2.0 GHz AMD Athlon™ CPU and 2 Gbytes of memory. With these higher acquisition and processing rates, this dynamic OCE technique has the potential for volumetric biomechanical imaging, and with state-of-the-art OCT hand held probes, there is also the potential for non-destructive in vivo and clinical applications using a ring actuator design [76].

In summary, a dynamic OCE technique has been demonstrated based on a phase-sensitive spectral-domain OCT system and audio-frequency external mechanical excitations. It provides micron-scale resolution, fast imaging and processing speeds, and non-destructive biomechanical imaging capability. Based on local mechanical properties, this technique has shown the ability to differentiate sample regions in silicone tissue phantoms and rat tumor tissues under different excitation frequencies, using strain rate as contrast.
CHAPTER 5 ACOUSTOMOTIVE OCE FOR BIOMECHANICAL PROPERTY MEASUREMENT

In the previous chapters, studies of dynamic OCE for mapping \textit{ex vivo} breast tumor tissue biomechanical properties have been reported. However, according to the characteristics of external excitation methods, all the external OCE studies may suffer from an inability to maintain a sterile \textit{in vivo} environment. This ability is important when measuring biomechanical properties under circumstances inside the human body or on the surface in trauma. In contrast to this, an internal elastography technique has been studied using radiation forces and ultrasound imaging on breast tumor [53]. Therefore, an internal and dynamic OCE method, namely acoustomotive OCE (AM-OCE), is introduced and studied in this chapter to show the feasibility of OCE techniques in an internal excitation method. This technique may also have the advantage for exciting and studying local biomechanical properties such as in the cancer microenvironment, instead of bulk excitation. This chapter is adapted from reference [113].

5.1 Acoustomotive OCE System

The SD-OCT system as described in section 3.1 was used and the average power incident on the sample was 10 mW. Acoustic radiation force was applied by a circular, 19-mm-diameter, f/1 lead zirconate titanate (PZT) element transmitting sine-wave bursts at the resonant frequency of 1 MHz. The PZT element was synchronized with the OCT system as step functions of radiation force were excited into the samples. A stainless steel sphere of 1.5 mm in diameter was
positioned on the beam axis at the 24.5-mm radius of curvature of the ultrasound source. A schematic of the experimental setup is shown in figure 5.1. An M-mode OCT image of the sphere was recorded at the position shown in figure 5.2a, and its amplitude and phase images were shown in figure 5.2b and c, respectively. The phase data were then used for the elastography analysis.

![Figure 5.1 Schematic diagram of AM-OCE. Reprinted from reference [113].](image)

5.2 Acoustomotive OCE Technique

5.2.1 Acoustomotive OCE data analysis

The recorded OCE data was first analyzed in the time domain, as described in section 3.2.1. The system comprised of a sphere embedded in gelatin was modeled as a Kelvin-Voigt body model with a mass, which can be expressed as $m \ddot{z}(t) + \gamma_0 \dot{z}(t) + k_0 z(t) = 0$, where $k_0$ and $\gamma_0$ represent a spring constant and a damping constant of the second order model, respectively, $z$ is the uniaxial displacement function of the sphere, and $m$ is the total mass on which the force acts,
which is the sum of sphere mass and added mass of surrounding gel \( m' = 2\pi a^3 \rho_g / 3 \), where \( a \) is the sphere radius and \( \rho_g \) is the gel density. The M-mode OCT image as shown in figure 5.2c was fitted according to the solution of the above equation and \( k_0 \) and \( \gamma_0 \) were estimated through the least square fitting procedure. The shear modulus can be calculated as \( \mu = k_0 / 6\pi a \), while the shear damping parameter can be calculated as \( \eta = \gamma_0 / 6\pi a \).

![Figure 5.2 AM-OCE data analysis. (a) B-mode OCT image of a sphere embedded in tissue phantom. (b) Amplitude of M-mode OCT image of the sphere recorded at the position shown by the arrow in (a). (c) Phase of the M-mode OCT image and its fitting curve in time domain. (d) Power spectrum of (c). Reprinted from reference [113].](image)

Following this, the OCE data was analyzed in the frequency domain. A fast Fourier transform was taken on the time-domain OCE data, generating its power spectrum as shown in figure 5.2d. In the spectrum, the peak frequency corresponds to the resonant frequency of the system with damping \( \omega_d \) which is related to \( k_0 \) as \( k_0 = m \left[ w_d^2 + (\gamma_0 / 2m)^2 \right] \), and the -3 dB bandwidth
Thus, by measuring the peak frequency and the -3 dB bandwidth, the material property shear modulus and shear damping parameter can also be determined.

5.2.2 Verification measurements by rheometer

The material properties of the samples were verified independently through oscillatory rheometer experiments. Parallel plate shear experiments were conducted on an AR-G2 rheometer (TA Instruments, New Castle, USA). Circular specimens, 25 mm in diameter and 2-4 mm in height, were molded from the same gelatin used to make the large samples containing spheres. At the same time of the AM-OCE experiments, the specimens were removed from the molds and attached to parallel plate fixtures using cyanoacrylate (Rawn America, Spooner, WI, USA). Five percent strain was applied over a frequency range from 0.1 Hz to 10 Hz with 10 sample points per decade of frequency.

5.3 Acoustomotive OCE Applied on Gelatin Phantoms

5.3.1 Sample preparation

Gelatin-based phantoms (250 bloom strength, Type B gelatin, Rouselot, Buenos Aires, Argentina) were constructed for AM-OCE measurements due to the need for larger sizes of samples. Gelatin powder and distilled water were heated in a water bath at a temperature between 62-68 °C for one hour and periodically stirred. When the sample was cooled to 50 °C, 0.1% weight-by-weight (w/w) of formaldehyde was added and thoroughly mixed. Liquid gelatin was poured into the sample mold (diameter 7.5 cm, height 5.5 cm). The stainless steel sphere was introduced just prior to gelling. This sphere is introduced to mimic a stiff tissue lesion such
as a small tumor. Samples were made for 3% and 4% w/w gel concentrations. Consequently, the congealed gelatin gels were homogenous except for isolated spheres. The spheres were positioned at least 1.5 cm from the sample case boundaries to minimize the influence of boundary effects on the measurement. OCE experiments were conducted after one day of gelation.

5.3.2 Results from Acoustomotive OCE

The AM-OCE results are shown in figure 5.3. In figure 5.3a, the shear modulus results of 3% and 4% gelatin samples measured by time-domain AM-OCE, frequency-domain AM-OCE and rheometer are plotted. The error bars for the time-domain AM-OCE results represent the calculation errors while the error bars for rheometer results represent the standard deviations. It can be seen that the AM-OCE results for both samples correspond well with the well-established rheometer results. In figure 5.3b, the shear damping parameter results for the 3% and 4% gelatin samples measured by time-domain AM-OCE and frequency-domain AM-OCE are shown. From the results, one can see that both the shear modulus and shear damping parameters of the gelatin samples double when their concentration increases from 3% to 4%.

Processing time was also recorded for comparison between time-domain and frequency-domain AM-OCE. Based on the post-processing procedure in Matlab (The Mathworks Inc.) and a sample number of 10, the AM-OCE processing time is about five times faster in the frequency domain than in the time domain. However, the frequency-domain AM-OCE calculations result in a larger (5-10%) calculation difference compared to the time-domain AM-OCE method.
5.4 Discussion and Conclusion of Acoustomotive OCE

For internal dynamic OCE, a new AM-OCE technique which uses dynamic and internal acoustic radiation forces for mechanical excitation has been experimentally demonstrated. Quantitative measurements of AM-OCE on gelatin-based phantoms were obtained and the results were verified with a commercial oscillatory rheometer. To investigate efficient processing procedures, analysis of AM-OCE data was performed in both the time domain and the frequency domain. The frequency-domain AM-OCE processing is about five times faster than the time-domain AM-OCE processing. Using the \textit{in vivo} imaging modality OCT for detection, this elastography technique has great potential for imaging biomechanical properties \textit{in vivo}, especially in quantitatively measuring biomechanical properties of microenvironments around tumor development with micron-scale resolution. Acoustic radiation forces have been
successfully applied for displacing small lesions that are stiffer than normal tissue (which was mimicked by the steel sphere) to enhance contrast in elastography for abdominal and breast cancers [114, 115]. For OCE studies on samples without any inclusions, this acoutomotive method is applicable too by using tightly focused acoustic waves [116]. Furthermore, the AM-OCE excitation force can be exerted from acoustic radiation originating from outside the body, which makes AM-OCE compatible with a wide range of OCT beam delivery techniques including endoscopes, catheters, and needle-based systems for biomechanical property measurements.
CHAPTER 6 SURFACE WAVE PROPAGATION OCE FOR BIOMECHANICAL PROPERTY MEASUREMENT

In previous chapters, quantitative measurements of biomechanical properties by external and internal dynamic OCE techniques and their applications on tissue phantoms and ex vivo breast tumor tissues have been demonstrated. Skin is the other main tissue of interest for biomechanical property measurements in this thesis research. Skin functions include protection from pathogens and injury, sensing external stimuli, heat regulation, control of evaporation, and water resistance. These functions are critical for human health and are due largely to the layered structure of human skin. In this chapter, the focus will be on a surface wave propagation OCE method and its in vivo application for skin structures and biomechanical property measurements. In addition, skin hydration process is discussed from the results of skin biomechanical property variations. The mechanical directionality of skin, represented by what is known as Langer’s lines, is also discussed as part of the surface wave propagation OCE results. This chapter is adapted from reference [117].

6.1 Surface Wave Propagation OCE System

OCE is used to quantitatively measure biomechanical properties of in vivo human skin, with mechanical surface waves as excitations. A surface wave is a mechanical wave that propagates along the interface between different media. Surface waves are widely used for measuring material mechanical properties [118, 119], or at a larger scale, in seismology where they are used
to study earthquakes [120, 121]. Mechanical surface waves include Love waves, in which movement is perpendicular to the direction of travel, and Rayleigh waves, in which movement is parallel to the direction of travel. In this section, a mechanical wave driver is used as the source of Love wave propagation on the surface of human skin, and OCE is used to detect the skin surface motion in order to quantitatively measure skin biomechanical properties.

The SD-OCT system as described in previous chapters was used for detection of surface wave propagation in skin, and the mechanical wave driver was used for external mechanical excitation. The mechanical wave driver was synchronized with the OCT system and sinusoidal waves were generated on the skin surface. A schematic of the experimental setup is shown in figure 6.1.

![Figure 6.1 Schematic of surface wave propagation OCE on skin. The mechanical wave driver is synchronized with the SD-OCT system and contacting the skin surface with minimum force. The OCT sample arm moves transversely on the skin surface. Reprinted from reference [117].](image)

The initial distance between the OCT sample arm beam and the mechanical wave driver was
chosen arbitrarily to be 16 mm. An M-mode OCT image was recorded at the first position and then the sample arm beam was moved away from the mechanical wave driver at a step distance of 2 mm before the next image was taken. The step distance was chosen to be less than one-half the wavelength of the surface wave for all frequencies to ensure the accuracy of the wave velocity calculation. Several step imaging positions were made for averaging data for each measurement. Phase data from OCT images were used for the skin displacement detection.

6.2 Surface Wave Propagation OCE Technique

6.2.1 Analysis algorithm for skin mechanical properties

The skin was modeled as an infinite elastic homogeneous layer as shown in figure 6.2. Waves generated by the harmonic excitation were polarized in the x-z plane and propagated in the x direction. The wave propagation was governed by the differential equations

\[
\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial z^2} = \frac{1}{c_L^2} \frac{\partial^2 \phi}{\partial t^2},
\]

(6.1)

\[
\frac{\partial^2 H_z}{\partial x^2} + \frac{\partial^2 H_z}{\partial z^2} = \frac{1}{c_T^2} \frac{\partial^2 H_z}{\partial t^2},
\]

(6.2)

where \( \phi \) and \( H_z \) are potentials in the z and x directions, respectively, and \( c_L \) and \( c_T \) are wave velocities for the longitudinal and shear directions, respectively. The solution for the displacement in the z direction can be expressed as

\[
u_z = \text{Re}\left(\frac{\partial \phi}{\partial z} + \frac{\partial H_z}{\partial x}\right) = \left(Ae^{-sz} - 2s\phi e^{-sz}\right)\cos(k_Rx - \omega t),
\]

(6.3)

where \( A, s, q \) are parameters for calculation, and \( k_R \) is the wave number of the surface wave propagating on the surface of the skin. By recording the displacements in the z direction for two
positions \( x_1 \) and \( x_2 \) in the x direction from OCT, the sinusoidal phase delay can be determined by

\[
\Delta \varphi = k_R (x_1 - x_2).
\] (6.4)

Thus, the surface wave velocity can also be calculated as \( v = \omega D / \Delta \varphi \), where \( \omega \) is the driving angular frequency and \( D \) is the step distance between \( x_1 \) and \( x_2 \). In the experiments, peaks of propagating surface waves were recorded for each location to calculate the surface wave velocity.

Figure 6.2 Schematic of surface wave propagation on skin. The mechanical wave driver moves along the x-axis by steps of \( D = 2 \) mm, and at each step, an M-mode OCT image is recorded and its phase shifts are used to calculate surface wave propagation velocity. Reprinted from reference [117].

Surface wave velocity is an important parameter for material mechanical properties and by which Young’s modulus can be determined, using the relationship of

\[
E = \rho v^2 \left( 2.618 + 1.332 \nu \right),
\] (6.5)
where $\nu$ is Poisson’s ratio for skin and $\rho$ is the mass density for skin [122]. By this method, one can quantitatively measure the Young’s moduli of \textit{in vivo} human skin by OCE.

6.2.2 Verification measurements from Cutometer

Measured Young’s moduli of \textit{in vivo} skin were verified using a commercial instrument (Cutometer® MPA 580, Courage Khazaka Electronic, Koln Germany). The Cutometer measurements were conducted after the OCE measurements using a 2 mm Cutometer probe fixed on the same skin area by a double-sided adhesive ring. The Cutometer experiments were measured with a pressure of 450 mbar. On-time, off-time and repetition numbers were 5 s, 3 s and 3, respectively. The parameter $U_r/U_f$ was used to represent the skin elastic moduli [123], and compared with the Young’s moduli results measured by OCE.

6.3 Surface Wave Propagation OCE Applied to \textit{In Vivo} Human Skin

6.3.1 Sample preparation

Silicone-based tissue phantoms were used again to calibrate the measurements of mechanical properties by the OCE system because of their similar optical scattering and biomechanical properties to human tissues. Phantoms were prepared from pure PDMS fluid (50 cSt viscosity, ClearCo, Inc.), a room-temperature vulcanizing silicone, and its associated curing agent (General Electric RTV-615 A and B, respectively, Circuit Specialists, Inc.). Different concentration ratios of these three ingredients were used to obtain layer structures with different stiffness and thickness in the samples. TiO$_2$ particles (Sigma-Aldrich, #224227, mean size 1 µm, maximum size 5 µm) were embedded with a concentration of 1 mg/g in the tissue phantoms to function as
optical scatterers for OCE imaging. The phantom solutions were mixed thoroughly in an ultrasonicator for 30 minutes under room temperature and then poured into 9 cm glass Petri dishes. Different layers of samples with different stiffness and thickness were fabricated separately after the curing process of the previous layer, which includes curing at 80 °C for eight hours and subsequently at room temperature for twenty four hours.

All in vivo experiments were done on the skin of a healthy male volunteer under room temperature and humidity. Informed consent was obtained from the subject. The skin sites were chosen as relatively flat regions on the volar forearm, dorsal forearm, and palm.

6.3.2 Measured skin thickness by OCT

Cross-sectional B-mode OCT images for skin sites on the volar forearm, dorsal forearm, and palm are shown in figure 6.3. From the B-mode images, structural features of human skin can be clearly discerned. For example, the skin over the palm has a thicker stratum corneum, shown in figure 6.3c, which can be compared with the histological image in figure 6.3d. Optical thickness of skin can be determined by B-mode OCT images. Physical thickness of the skin can then be estimated using the optical thickness divided by the refractive indices. By using refractive indices of $n=1.53$ and $n=1.39$ for the stratum corneum and epidermis, respectively [124], the physical thickness of skin layers at different sites can be determined. For the volar forearm, $T_{sc}=10 \mu m$ and $T_e=68.2 \mu m$ where $T_{sc}$ denotes the thickness of stratum corneum and $T_e$ stands for thickness of epidermis. The dermal layer usually is out of the range of OCT imaging penetration, which makes it difficult to measure the thickness of the dermal layer. For the dorsal forearm, $T_{sc}=10 \mu m$ and $T_e=56.8 \mu m$ while for the palm, $T_{sc}=129.9 \mu m$ and $T_e=73.8 \mu m$. At sites other than
the palm or sole, where the stratum corneum is thick, the stratum corneum thickness can be difficult to measure with OCT because the thickness is comparable to the coherence length (axial resolution, 2-5 µm) of the OCT system.

![B-mode OCT images of human skin from different sites.](image)

**Figure 6.3** B-mode OCT images of human skin from different sites. Images were acquired from the (a) volar forearm, (b) dorsal forearm, and (c) palm. (d) Histology of palm (adapted from reference [116]). Abbreviations: e, epidermis; ds, dermis; sc, stratum corneum. Reprinted from reference [117].

### 6.3.3 Skin Young’s moduli measurements by OCE

For OCE measurements, M-mode OCT images were taken at one transverse position of the skin. In figure 6.4a, the arrow denotes the fixed location of the OCT beam and figure 6.4b and c denote the amplitude and phase data of the M-mode OCT image at this position, respectively. By averaging over the range of interest (dotted line range in figure 6.4c), the phase of the optical data can be plotted as shown in figure 6.4d. The envelope ripples in figure 6.4d are due to very subtle motion artifacts, which will not affect the measurement results because the calculations
are based on sinusoidal phase differences between different measurement positions.

Using the sinusoidal phase changes from different positions of the skin measured by OCE, along with wave equation algorithms, the surface wave velocities can be calculated and, subsequently, the Young’s moduli of human skin. The results of measured Young’s moduli by OCE in this study are based on an average of six measurements and the error bars denote standard deviations. Figure 6.5a shows the results measured by OCE from different sites on human skin. The Young’s moduli from the volar forearm, dorsal forearm and palm are 101.18, 68.68 and 24.91 kPa, respectively. As shown in figure 6.5b, the OCE-measured Young’s moduli from different sites correspond well with the elasticity measured by Cutometer MPA580, which
is a well-characterized commercial skin stiffness measurement device. All measurements were conducted approximately orthogonal to Langer’s lines and with a driving frequency of 50 Hz. Langer’s lines describe the patterns of biomechanical anisotropy in human skin. Directions within skin and along (parallel to) Langer’s lines have the least flexibility (highest Young’s modulus) [23]. Calculations assumed a skin mass density of 1.02 g/cm$^2$ and Poisson’s ratio of 0.5.

6.3.4 Frequency dependence of OCE measurements

Figure 6.6 shows the OCE measurements of skin mechanical properties with different driving frequencies and under different hydration conditions. Skin measurements were acquired from the volar forearm of the volunteer. Skin hydration conditions include hydrated, dehydrated, and normal. A hydrated skin condition was produced by soaking normal skin in a water bath for
20 minutes, followed by a topical application of glycerin for 10 minutes. A dehydrated skin condition was produced by passing heated air from a commercial hair dryer over normal skin for 30 minutes. It can be observed from figure 6.6 that normal skin has a Young’s modulus of 101.20 kPa under a driving frequency of 50 Hz. This value decreases when the driving frequency increases. The Young’s modulus increases again with a driving frequency more than 300 Hz. The hydrated skin exhibited a smaller Young’s modulus under a driving frequency of 50 Hz, and when the driving frequency increased, the measured Young’s moduli increased as well, with larger values than the normal skin.

![Figure 6.6: Young's moduli measured by OCE under different driving frequencies and skin hydration conditions. Blue line denotes results from dehydrated skin, brown line denotes results on hydrated skin, and red line denotes results on normal untreated skin. Reprinted from reference [117].](image)

The dehydrated skin exhibited the largest Young’s modulus under a driving frequency of 50
Hz, and the value decreased dramatically with increasing frequency, with Young’s moduli comparable with the normal skin.

The frequency-dependent results from dynamic OCE measurements on human skin and skin phantoms are significant. From the literature, it was found that Young’s moduli measured with different frequencies correspond to the skin stiffness from different depths [125]. At a low surface wave driving frequency, dynamic skin mechanical properties were believed to be primarily due to the outer layer (stratum corneum), while at higher frequencies, the properties were believed to be dominated by the deeper layer (dermis). Based on this theory, the results can be understood as follows. For normal skin (as in figure 6.6), the measured Young’s modulus is 101.20 kPa under a 50 Hz driving frequency, which represents the mechanical properties of the stratum corneum. The measured Young’s modulus decreases until a driving frequency of 200 Hz and increases again, implying that the epidermis layer has a lower stiffness than the dermis layer.

For the hydrated skin, the measured Young’s modulus is only 23.01 kPa under a 50 Hz driving frequency, which indicates that the stratum corneum has been softened by the hydrating process. However, the hydrating process tends to increase the stiffness of the skin in the epidermis and dermis layers since the measured Young’s moduli are increasing when the driving frequency increases. From the literature, the hydration process does affect skin mechanical properties, but whether the process makes the deeper skin layers stiffer or less stiff is all subject dependent [126]. For the dehydrated skin, the measured Young’s modulus is 300.41 kPa under a 50 Hz driving frequency, but for higher frequencies, the measured Young’s moduli remain similar to those of normal skin. These findings support the physiology that the outer stratum corneum
serves to protect the deeper skin layers against dehydrating conditions.

6.3.5 OCE measurements on skin tissue phantoms

Similar OCE experiments were also performed on skin tissue phantoms. Three different tissue phantoms were fabricated with different number, thickness, and stiffness of layers, as shown in figure 6.7. Driving frequencies for these experiments were limited to less than 500 Hz to clearly differentiate the surface wave propagation in time. It can be observed from figure 6.7 that phantom 1 has a relatively high measured Young’s modulus under low driving frequency, and the value decreases until the driving frequency reaches 500 Hz. For phantom 2, the measured Young’s modulus decreases as the driving frequency increases. For phantom 3, the measured Young’s modulus is low under 50 Hz driving frequency, but the value increases at a driving frequency of 100 Hz and increases further at driving frequency of 400 Hz.

The relationship between driving frequency and skin measurement depth was verified by experiments on skin tissue phantoms (as in figure 6.7). Phantom 1 mimicked human skin with four layers. The first layer has the largest Young’s modulus of about 100 kPa, while the second layer has a lower value of 25 kPa. The third layer mimics the dermal layer in human skin and has a rather high Young’s modulus of 75 kPa, while the fourth layer is very soft (Young’s modulus of 8 kPa), playing the role of the hypodermal adipose layer of skin. The experimental results show a Young’s modulus of about 50 kPa below 100 Hz, denoting the first layer, and a rather low Young’s modulus between 200 to 400 Hz, denoting the second layer. The measurements from the third layer under a driving frequency of 500 Hz indicate an increase in the measured Young’s modulus. The results indicate a 500 Hz driving wave reaches a depth
around 250 μm in phantom 1. Results from phantoms 2 and 3 also follow the trends pertaining to layer thickness and stiffness within the phantoms.

6.3.6 Skin directionality measured by OCE

Figure 6.8 shows the measured OCE results acquired from different directions along the surface of human skin. The results were measured on the volar forearm, parallel and orthogonal to Langer’s lines, and with driving frequencies of 50 Hz and 600 Hz. The volar forearm was chosen for these measurements because the Langer’s lines are easily defined at this site to be nearly orthogonal to the long axis of the arm, as shown in figure 6.9 [127]. From figure 6.8, one can see that the measured Young’s modulus parallel to Langer’s lines can be differentiated from the Young’s modulus from the orthogonal direction under a driving frequency of 50 Hz. However,
when the driving frequency is increased to 600 Hz, the measured Young’s modulus parallel to
Langer’s lines is significantly larger and different than from the direction orthogonal to Langer’s
lines, with a ratio of 2.21. These findings correspond well with the anisotropy trend previously
reported [124]. From these results, it is obvious that OCE measurements show a larger difference
in the anisotropy of skin mechanical properties under high driving frequency rather than at lower
frequencies under 100 Hz. These results suggest the ability to resolve depth-dependent
biomechanical properties in human skin based on frequency-dependent mechanical waves.

Skin anisotropy measurements by dynamic OCE also support the findings in section 6.3.4.
Under a driving frequency of 50 Hz, the Young’s moduli between directions parallel and

![Figure 6.8 Young's moduli measured by OCE from different skin directions under 50 Hz and 600 Hz driving frequencies. The symbol // denotes direction parallel to Langer’s lines and ⊥ denotes direction orthogonal to Langer’s lines. * denotes p<0.05 and ** denotes p<0.0001. Reprinted from reference [117].]
orthogonal to Langer’s lines are comparable, but under a driving frequency of 600 Hz (corresponding to depths within the dermis), the measured Young’s modulus of skin parallel to Langer’s lines is significantly larger than the orthogonal value. This is likely due to the fact that anisotropic microstructure like collagen is located in the deeper dermal layer of skin, and not in the more superficial layers. However, this frequency-depth relationship is only relative, because factors such as thickness, stiffness, binding, and complex boundary conditions all contribute in ways that are not currently understood.

6.4 Discussion and Conclusion of Surface Wave Propagation OCE Technique

For external dynamic OCE application on \textit{in vivo} skin, the dynamic OCE technique was used to measure skin thickness and stiffness quantitatively. Skin layer thickness and Young’s moduli have been measured quantitatively on human skin \textit{in vivo} with a lab-based instrument. Direction-dependent and site dependent mechanical properties were measured and resolved \textit{in vivo} in human skin. Surface waves with different frequencies have been utilized to mechanically drive skin and Young’s moduli have been determined based on solutions to wave equations. A depth and driving frequency dependence theory on surface wave propagation can be
used to explain the results, which were also verified by results on polymer tissue phantoms. One assumption used in this study is that the skin can be modeled as pure elastic strips. From the experimental results, this assumption is valid because no significant decay in amplitude of surface wave propagation over distance was noticed within the range of amplitudes and frequencies used in this study. For specific experimental conditions, such as with a high-frequency driving wave, a viscoelastic mechanical model could be used for additional quantitative measurements [35, 128]. The quantitative Young’s moduli from skin mechanical property measurements vary over a large range of values reported in the literature, because of inconsistencies of measurement methods, skin conditions, mechanical models, and subjects [124]. The quantitative results of measured Young’s moduli by dynamic OCE on human skin in vivo agree well with many of these studies that report lower Young’s modulus values [34, 128, 129]. Compared with other previously used imaging technologies on human skin measurements such as ultrasound imaging, OCE can differentiate thickness with a resolution of several microns, which is critical for resolving different skin layers and their properties. With state-of-the-art OCT hand-held probes [130], this dynamic OCE technique has the potential in applications of clinical dermatology, plastic surgery, and cosmetic skin assessment. This technique may also find application where skin thickness and stiffness measurements are critical for interventions and devices, such as in transcutaneous micro-needle applications [131]. Further studies are needed to refine the 3-D mechanical modeling algorithms and generate high-resolution 2-D or 3-D maps of the mechanical properties of human skin.
CHAPTER 7 MULTIPHOTON ELASTOGRAPHY

Measurement of \textit{in vivo} skin biomechanics at the tissue level was studied and discussed in the previous chapter. Other modern technologies have also been investigated for human skin, especially for morphological, medical, and cosmetic purposes. For example, digital image correlation has been used for human skin displacement detection [132] and improvement in basal cell carcinoma detection based on variations in the biomechanical properties of skin [133]. A study investigating age-related skin changes in mechanical properties has also been performed based on speckle correlation [134]. However, most of these techniques concentrate on imaging the skin surface, which neglects depth-dependant information and the ability to resolve cellular features and the dynamics of cell populations. Skin mechanics depend significantly on the dynamic cellular morphology and microenvironment of deeper skin layers, as well as the outer-most stratum corneum, which is mainly composed of dead cells, but provides a protective barrier function. Cross-sectional imaging technologies such as ultrasound [32] and OCT have been utilized to study depth-dependant skin biomechanical properties, but resolution limitations have restricted these imaging modalities to extracting more tissue-level biomechanics from skin.

The use of reflectance confocal microscopy has had demonstrated success at imaging cellular features in \textit{in vivo} human skin [135, 136]; however, few studies have integrated mechanical excitations with confocal microscopy to investigate the biomechanical properties of cell populations in living skin.

In this chapter, the depth-dependant \textit{in vivo} biomechanics of different cell populations in
human skin is investigated using MPM. The skin biomechanical properties were estimated for cell populations in different skin layers using quasi-static external mechanical excitations. To the best of our knowledge, this is the first experimental demonstration of the depth-dependant cell population biomechanics in *in vivo* human skin.

7.1 Multiphoton Elastography System

For this study, a custom built integrated microscope system was used. This multi-channel microscope enables structural cell and tissue imaging utilizing optical coherence microscopy (OCM) and functional imaging utilizing MPM [137, 138]. The laser source of the microscope is a tunable Ti-sapphire laser (Mai Tai, Spectra Physics), which has a central wavelength of 770 nm, bandwidth of 12 nm, a pulse width of 150 fs, and a repetition rate of 80 MHz. The output from this laser is divided by a 90/10 beamsplitter into two beams. The higher power beam is coupled by a 0.4 NA aspheric lens into an 2 m-long photonic crystal fiber with a numerical aperture (NA) of 0.1 and a mode field diameter of 6 µm (LMA-8, Crystal Fibre A/S). The spectrally broadened OCM beam is collimated, and the linear polarization is rotated 90° by an achromatic half-waveplate before being recombined with the narrowband beam at the original beamsplitter. The linear polarization of the OCM beam after continuum generation is sufficiently maintained, enabling orthogonal polarization between the narrowband MPM beam and the broadband OCM beam. This allows the MPM beam to be blocked by a polarizer when acquiring the spectral interference pattern for OCM detection. The output of the dual-spectrum source is subsequently used for OCM and MPM imaging. Real-time A-scans from OCM images were used to locate the
skin surface, and MPM microscopy was used for skin imaging. The schematic of the integrated microscope system is shown in figure 7.1. The beam passes through a pair of scanning galvanometers and is expanded by a telescope to fill the back aperture of a 20X, 0.95 NA water immersion objective (Olympus). The focused light, with a mean excitation power of ~20 mW, generates a two-photon fluorescence signal from the sample, which is collected by the objective and directed to the detector using a dichroic mirror (Cold Mirror, CVI laser). The system parameters are comparable to those for a class 1M device according to the European laser safety regulations [139]. The detector consists of a photomultiplier tube (PMT) and filter to block the pump light. *En face* images are formed by detecting the raster scanned beam while focus depth is changed by the motorized stage on which the skin site is positioned. Acquisition time is ~ 4 s for one *en face* image with a field of view (FOV) of 260 µm × 260 µm.

MPM was first used to detect cellular features and cell populations in *in vivo* human skin. An image mosaic 600 µm × 600 µm in size was acquired by combining 16 MPM images of 160 µm × 160 µm FOV at each imaging depth. Once cellular features were identified, MPM was used to assess the biomechanical properties of the skin. At each region of interest on the skin, 3-D stacks of MPM images with a FOV of 260 µm × 260 µm were taken before and after a uniaxial deformation was applied. As shown in figure 7.1, the left arm of the volunteer was positioned on the stage and against the imaging window. During the experiments, the skin was held fixed at one end by double-sided tape to cover slip 1, and was deformed at the other end, which was fixed to cover slip 3 by double-sided tape.
Figure 7.1 Schematic of the multiphoton microscope used to image *in vivo* human skin. (a) The forearm was mounted on a motorized vertical stage, and the skin region to be imaged was fixed to cover slip 1 and 3 using double-sided tape. MPM images were acquired through the imaging window 2. A thin film of glycerin applied to the skin served as a lubricant between the skin surface and this window. (b) For biomechanical property measurements, a quasi-static lateral deformation was applied to cover slip 3 and the adherent skin. The surface area of skin that was deformed was 40 mm x 20 mm, and the image FOV (small black square) under the imaging window 2 was at most 600 µm x 600 µm for the MPM mosaics. Abbreviations: DM, dichroic mirror; F, filter; L, lens; O, objective; PMT, photomultiplier.
Images were taken through cover slip 2 and a layer of glycerin applied to the surface of the skin, which also acted as a lubricant between the imaging window and the skin. The three cover slips were at the same height on the skin surface. Over a 40 mm long and 20 mm wide region of interest on the skin, cover slip 2 was positioned in the center as the imaging window and the lateral translation of cover slip 3 provided a strain of 2.5%. After the deformation of the skin was performed, real-time A-scan OCM data was collected and used to locate the skin surface, so that the 3-D MPM image stacks before and after deformation were co-registered and contained the same depth-dependent cell population layers over in the region of interest.

7.2 Multiphoton Elastography Technique

Over each depth from the acquired 3-D MPM image stacks, the response of the skin was determined by elastic deformation to the uniaxial stretch. To assess the deformation between images at one depth, an image registration algorithm was used, which was implemented using ImageJ software and its plug-in bUnwarpJ. The details of this algorithm are introduced elsewhere [140]. Briefly, the skin images before deformation were considered as target images, while the images after deformation were source images. The deformation field was modeled as a B-spline function, determined by minimizing a pixelwise mean-square distance measure between the target and the source, and constrained by a vector-spline regularization. Once the deformation field was determined, it was then decomposed to yield average displacements along and across the deformation direction at one depth. The average displacements over depth were used as quantitative results of skin responses and were used to analyze the skin biomechanical properties.
The MPM image stacks before and after deformation were also reconstructed to demonstrate volumetric features of \textit{in vivo} human skin using Amira software.

7.3 Multiphoton Elastography Applied to \textit{In Vivo} Human Skin

7.3.1 Human subject measurement

All \textit{in vivo} MPM imaging experiments were performed on the left inner forearm skin of a healthy male volunteer under room temperature and humidity. Informed consent was obtained from the subject. The experiments consisted of measurements under two different states, namely under normal and hydrated skin conditions. The hydrated state was produced by immersing the skin in a water bath for 20 minutes, followed by a topical application of glycerin (G33-500, Fisher Scientific).

7.3.2 Cellular features from multiphoton microscopy of \textit{in vivo} human skin

Mosaics of MPM images from \textit{in vivo} human skin acquired from different depths are shown in figure 7.2. In figure 7.2a, an MPM image at a superficial depth of 3 µm is demonstrated. The contrast in this image is primarily due to autofluorescent signals from corneocytes, which have the main fluorophore keratin in the stratum corneum layer [141]. It is also obvious that microscopic skin furrows and a hair shaft are discernable in this image. Figure 7.2b is the MPM mosaic from a depth of 13 µm, showing cellular features of the stratum granulosum layer. Cellular features in this layer are characterized by an autofluorescent cytoplasm and nonfluorescent round nuclei [142], and features of skin furrows and the hair shaft are still evident. Figure 7.2c is the MPM mosaic acquired at a depth of 23 µm. In this image, cells in the stratum
spinosum layer can be clearly differentiated, in which NADH is the major fluorophore to yield contrast among the cellular features of this layer [87]. While these major features dominate the different skin layers as illustrated in figure 7.2, there are many other features present from other skin layers because of the frequent non-planar geometry of skin layers. Features such as hair shafts were avoided in the following experiments, because the large autofluorescent signal from hair frequently saturated the detector and electronics, making image registration problematic.

![Figure 7.2](image)

**Figure 7.2** Multiphoton image mosaics of *in vivo* human skin for detecting cellular features at different layers. Mosaics comprised a 600 x 600 µm area of skin. Imaging depths are (a) 3 µm, (b) 13 µm, and (c) 23 µm. Different cell types and populations are observed at different depths, and a single hair shaft is observed in the lower left corner of the images. Scale bar denotes 100 µm and applies to all images.

7.3.3 Skin biomechanical property analysis

Three-dimensional MPM image stacks of *in vivo* human skin were acquired and *en face* MPM images before and after deformation (uniaxial stretch) at each depth were processed to yield deformation fields. This process is demonstrated in figure 7.3. Six representative target MPM images acquired before deformation are shown in the left figure column, for imaging depths of 3 µm, 8 µm, 16 µm, 25 µm, 32 µm, and 37 µm.
Figure 7.3. Image processing for extracting skin biomechanical properties. For increasing depths (3-37 µm) beneath the skin surface, target images (left column) and source images (right column) are shown before and after deformation, respectively. Target images are overlaid with displacement vectors (middle column) to show the deformation fields. The length and orientation of each arrow represents the magnitude and direction of deformation at each arrow location, and the arrow length is on the same dimensional scale as the cellular features in the images. Image size is 260 µm × 260 µm for all images.
Their corresponding source images after the lateral deformation are shown in the right figure column. The elastic image registration algorithm as described above was applied to the image pairs at different depths to yield the deformation fields. Displacement vectors from the deformation fields are overlaid with the target images, as shown in the middle figure column. The displacement vectors are primarily oriented parallel to the deformation (stretch) direction (which was from left-to-right in the images), as shown for depths between 3-25 µm, but with decreasing amplitudes with increasing depth. However, the vectors turn orthogonal to the deformation direction at deeper locations, such as at 32 µm and 37 µm. Each displacement vector can be decomposed and projected on to the axes parallel and orthogonal to the deformation direction to quantify the deformation fields across the fields-of-view for these images.

The amplitudes of displacement in the parallel and orthogonal directions versus depth are plotted in figure 7.4. Figure 7.4a shows the results from normal human skin from the skin surface to a depth of ~40 µm. The amplitudes of the displacement vectors parallel to the stretch direction do not vary significantly for the first 15 µm in depth, but decrease quickly over greater depths, while the displacement amplitudes in the orthogonal direction increase at larger depths such as 37 µm. We applied a least-square linear fit to these two different regions, and the resulting slopes are clearly different. The slopes are indicative of the varying biomechanical properties between these different skin layers. The larger slopes indicate softer regions of tissue and smaller values of Young’s moduli.
Figure 7.4 Depth-dependent biomechanical property measurements for \textit{in vivo} human skin from MPM images. (a) Results from normal baseline-state human skin. (b) Results from hydrated human skin. Square data points and cross data points represent average displacement parallel and orthogonal to the stretching direction, respectively, at different depths. Dashed lines are linear fits for data from different regions in depth, and MPM images are shown for selected data points at the corresponding depths.
The results indicate that the more superficial region (0-15 µm) of human skin has a larger Young’s modulus than the deeper region (15-40 µm). From the literature, the stratum corneum layer is much stiffer in human skin, than the other parts of the epidermis [124], which corresponds to the slope results in figure 7.4a. In chapter 6 by dynamic OCE measurements on in vivo skin, the measured Young’s modulus of normal stratum corneum was found to be ~100 kPa, while the rest of the epidermis had a Young’s modulus of ~25 kPa. The same analysis was applied to hydrated skin, with results shown in figure 7.4b. It is obvious that under hydrated condition, the slope of the fitted line representing parallel displacement amplitude versus depth in the stratum corneum layer has a larger value than under the normal skin condition, which indicates that this layer becomes softer as a result of the hydration process. The slope representative of the rest of the epidermis has a smaller value than normal skin, which indicates that this region becomes stiffer from the hydration process.

From the literature, the hydration process affects not only the superficial stratum corneum, but also the skin mechanical properties deeper in the epidermis, and it may increase the Young’s modulus of this skin layer [124]. These results suggest that the stratum corneum of human skin does serve as the main barrier to protect the deeper layers of skin from the changing outer environment, such as hydrating conditions, but that the mechanical properties of deeper skin layers may also be affected following hydration for extended periods of time.

Hydration is the most important factor determining biomechanical properties in human skin layers. Keratinocytes are transported from the stratum basale outwards through the epidermis. With the same process, the keratinocytes mature, become flattened and lose water. The skin
hydration decreases from ~70% at deeper epidermal regions to ~30% at the lower stratum corneum and to ~10% at the surface of the skin [143]. Less hydration in the stratum corneum makes this outer layer stiffer than the deeper layers. Another factor for the mechanical variation comes from intercellular material on the skin surface. In the stratum corneum, the proteins of the corneocyte cell membranes are tightly connected by a lipidic intercellular glue, which makes this skin layer more rigid [124]. However, the stratum corneum is also able to absorb an additional amount of water, because of the semipermeability of the corneocyte membrane. Thus, when immersed in water, the stratum corneum will become more hydrated than normal, and subsequently become less stiff (exhibits a lower Young’s modulus).

For isotropic and homogeneous samples, a uniaxial stretch applied across areas significantly larger than the image field-of-view should not create any significant deformation orthogonal to the stretch direction. However, it is not unexpected to visualize deformations in the orthogonal direction from this study on in vivo human skin because of the highly heterogeneous and irregular papillary layer structures present at the dermal-epidermal junction, and because of the directionality of collagen in the dermis, which defines the Langer’s lines of human skin [127]. The complex mechanical connections and boundary conditions in human skin will affect the directionality of deformations, especially in the deeper layers around these dermal papillae, and their effects on adjacent skin layers.

*In vivo* human skin studies are generally affected by motion artifacts, including respiration motion, cardiac pulse motion, and other involuntary motion. In this study, however, the imaged skin regions were placed in contact with a rigidly fixed microscope sample arm, which prevented
large amplitude motion artifacts. The motion artifacts present were estimated quantitatively from experiments similar to those described above. Briefly, two 3-D *in vivo* MPM stacks were acquired from the same region as above, but without mechanically stretching the skin. Then, deformation fields were taken, and displacement amplitudes from the parallel and orthogonal directions were calculated, which were 1.4 µm and 2.5 µm, respectively. Therefore, these motion artifacts contribute only 0.5% and 0.9%, respectively, to the results parallel to and orthogonal to the deformation directions shown in figure 7.4.

7.3.4 3-D image reconstruction

The MPM image stacks were reconstructed to illustrate volumetric structures of *in vivo* human skin. As shown in figure 7.5, features such as the microscopic skin furrows, papilla, and cells in the stratum spinosum are indicated by arrows and can be clearly discerned in the reconstructed images. Although these full 3-D data sets are not compatible with the current 2-D image registration algorithm, these features may be used for 3-D image registration in future studies, and thus be used to extract the 3-D biomechanical properties of populations of cells in different cell layers of human skin at this microscopic scale.
Three-dimensional multiphoton microscopy was used to image human skin \textit{in vivo}, and skin biomechanical properties were estimated by cell population deformations at different skin layers.

7.4 Discussion and Conclusion of Multiphoton Elastography

Three-dimensional multiphoton microscopy was used to image human skin \textit{in vivo}, and skin biomechanical properties were estimated by cell population deformations at different skin layers.
Cellular features were first identified from larger MPM mosaics, and MPM image stacks were subsequently acquired before and after stretch along the skin surface. An elastic registration algorithm was applied to the *en face* images to calculate deformation fields and displacement amplitudes both in parallel and orthogonal to the stretching direction. The results from normal skin showed that the stratum corneum layer is significantly stiffer than the rest of the epidermis. However, when the skin is hydrated, the stratum corneum layer becomes softer, while the remainder of the epidermis becomes stiffer. The results are primarily due to a lower degree of hydration in the stratum corneum and a higher degree of hydration in the rest of the epidermis, as well as the fact that hydration of the stratum corneum can be increased by immersing the skin in water. These results correspond well with the literature, and reflect the physiological function of the stratum corneum. Three-dimensional MPM images were reconstructed to demonstrate volumetric features of *in vivo* human skin, and future studies will utilize full 3-D data to extract biomechanical properties between skin layers. This technique is promising for quantitative measurements of the *in vivo* biomechanical properties of human skin, including the investigation of the barrier function of the stratum corneum under different conditions. This same diagnostic image-based methodology can also be used in other organs and tissues to assess the *in vivo* biomechanical properties of populations of cells under conditions of health and disease.
CHAPTER 8 OPEN PROBLEMS

A number of open problems have emerged from these studies. First, the OCE techniques in this dissertation come with some assumptions in mechanical models, which may not be ideal for heterogeneous biological samples. Finite element method may be one of the solutions to this problem. However, to simulate 4-D biological samples at a millimeter scale with micron or nanometer resolution may be challenging.

Another open question is to investigate cellular biomechanics. From the studies in previous chapters, measurements of biomechanical properties using dynamic OCE at the tissue level and using MPM elastography at the cell population level are investigated. However, understanding the biomechanical properties for single cells is also of great importance, especially for understanding the basic science for certain diseases such as breast cancer development. The viscoelastic properties of exfoliated tumor cells are reported to be considerably softer than those of cells in normal tissue [20]. Thus, it is important to understand how the cancer cells are triggered mechanically to switch to metastatic states and invade into surrounding tissues or migrate to distant locations by circulating in the lymphatic system or the bloodstream. It is also important to consider the role of tumor cell stiffness in the dynamic sense. For instance, cellular biomechanics is found to be related with the loss of cell polarity and cancer development, for example, as in metastatic cancer processes in *Drosophila* [144, 145]. A magnetomotive
microscopy technique is briefly introduced in this chapter for studying cellular biomechanics with high resolution, and is presented as a future direction with some preliminary results.

Magnetic excitations are one of the internal mechanical excitation methods used as an OCT imaging contrast mechanism. Magneto motion has been used for functional contrast enhancements in OCT by applying magnetic fields to tissues with magnetic contrast agents, such as magnetic nanoparticles (MNPs) [146], and has also been used to investigate the mechanical properties of tissue phantoms [147]. MNPs usually are composed of biocompatible iron oxide (magnetite Fe₃O₄ or maghemite γ-Fe₂O₃) which exhibit magnetic susceptibilities greater than $10^5$ times larger than human tissues. These particles have been used as MRI contrast agents for molecular imaging purposes [148-152]. For studies in this chapter, hydrophilic MNPs (Ocean NanoTech #SHP-20) with maghemite cores (exact ratio unknown) and polymer coatings with COOH-terminated outer surface were used.

The schematic experimental setup for magnetomotive microscopy is shown in figure 8.1. A permanent magnet with a diameter of 5 mm and a length of 30 mm (D4E, K&J Magnetics, Jamison, PA) is used to mechanically excite nanoparticles in the cells on the bottom of 35 mm Petri dishes. The axial magnetic field of the permanent magnet is 200 Gauss at a distance of 10 mm away from the magnet tip, which is measured by a Hall probe (3M 12-2-2-0.2T, Sentron AG, Switzerland). Nanoparticles in Petri dishes can be modulated by the magnetic field from the permanent magnet. In the experiments, the permanent magnet is spatially displaced by a mechanical wave driver (described in chapter 3), which is controlled by a function generator. Sinusoidal wave forms are used to spatially modulate the permanent magnet, with a maximum
spatial displacement of 7 mm and a frequency range of 0.5-2 Hz. The magnetic field generated by the permanent magnet can be approximately modeled as the field from a current loop, as shown in figure 8.2. In this model, \( i \) is the current of the loop, and \( a \) is the radius of the loop. The on-axis field \( B_z \) can be calculated as

\[
B_z = \frac{1}{\pi \sqrt{Q}} B_0 \left[ E(k) \frac{1-\alpha^2 - \beta^2}{Q - 4\alpha} + K(k) \right],
\]

where the parameters are defined as \( \alpha = x / a \), \( \beta = z / a \), \( \gamma = z / x \), \( Q = (1+\alpha)^2 + \beta^2 \), and \( k = \sqrt{4\alpha / Q} \). Variables \( x \) and \( z \) are the transverse and axial components of the displacement from the center of the current loop to the field measurement point, respectively.

![Diagram](image)

Figure 8.1 Magnetomotive microscope setup schematic. A permanent magnet is used to mechanically excite the cells in a Petri dish during microscopic imaging. The permanent magnet moves perpendicular (in and out) to the paper.

\( E(k) \) and \( K(k) \) denote the complete elliptic integral function, of the first kind and the second kind, respectively, and can be written as

\[
K(k) = \int_0^\frac{\pi}{2} \frac{d\theta}{\sqrt{1-k^2 \sin^2 \theta}} = \int_0^1 \frac{dt}{(1-t^2)(1-k^2 t^2)},
\]

(8.2)
\[ E(k) = \int_0^{\pi} d\theta \sqrt{1-k^2 \sin^2 \theta} = \int_0^1 \frac{\sqrt{1-k^2 t^2}}{\sqrt{1-t^2}} dt \quad (8.3) \]

\[ B_0 \] is the magnetic field at the center of the coil as \( B_0 = i \mu_0 / 2a \) and can be calculated from \( B_x \), which is measured as 200 Gauss when \( z \) is 25 mm. With the known \( B_0 \) of the current loop, the off-axis field \( B_x \) can be calculated as

\[ B_x = \frac{\gamma}{\pi \sqrt{Q}} B_0 \left[ E(k) \frac{1+\alpha^2 + \beta^2}{Q-4\alpha} - K(k) \right] \quad (8.4) \]

When \( x \) is sinusoidally modulated by the mechanical wave driver while \( z \) remains unchanged, the off-axis magnetic field can be calculated by equation (8.4). Given the magnitude of \( x \) is 5 mm, driving frequency is 1 Hz, \( z \) is 25 mm, one period of \( B_x \) at a fixed point is simulated as shown in figure 8.3. Figure 8.3b shows the sinusoidal displacement \( x \). In figure 8.3a, the blue curve denotes the simulated result from equation (8.4), and the red curve is a sinusoidal waveform with

---

![Diagram](image.png)

**Figure 8.2** Schematic of magnetic field generated by a current loop.
the same magnitude and frequency as the blue curve. Figure 8.3 suggests that at one point in the Petri dish, the off-axis magnetic field is similar but not the same as a sinusoidal wave form, when the permanent magnet is spatially modulated sinusoidally in the $x$ direction.

![Figure 8.3 Simulated off-axis magnetic field by sinusoidally varying, spatially modulated permanent magnet. (a) Magnetic field simulated from equation (8.4) (blue curve) and simulated sinusoidal curve with the same magnitude and frequency (red curve). (b) Displacement of the permanent magnet.](image)
By applying this magnetic field, the MNPs can be mechanically modulated. The force on one MNP is expressed as

\[ F_p = V_p M_p \frac{\partial B_x}{\partial x}, \]  

(8.5)

where \( V_p \) is the volume of the MNP, \( M_p \) is the magnetization of the MNP, and \( B \) is the applied magnetic field. When the MNPs are distributed in cells, the force exerted on a cell will then become

\[ F_{\text{eff}} = (N_p V_p M_p + VM_m) \frac{\partial B_x}{\partial x}, \]  

(8.6)

where \( N_p \) is the number of MNPs within the cell volume \( V \) and \( M_m \) is the magnetization of the cell. For simplicity, an infinitesimally small pure elastic cubic volume with sides of length \( d \) can be used to model the cell reacting to the forces.

If the bottom surface of the cell is stationary and the MNPs are well distributed in the cell, the cell elongates along the \( x \) direction and the top surface is displaced \( \Delta x \) from its original position, which can be derived using Hooke’s law:

\[ \Delta x = \frac{F_{\text{eff}}}{d \cdot E}, \]  

(8.7)

where \( E \) is the Young’s modulus of the cell. Using experimental values of \( M_p = 105 \text{ emu/g Fe} \), \( E = 0.5 \text{ kPa} \) and \( AB = 15 \text{ T/m} \), the displacement along the \( x \) direction is on the scale of hundreds of nanometers, which is close to the optical resolution of microscopes, but sufficient for optical sensitivities of quantitative phase images from DPM and phase-resolved magnetomotive OCT (MM-OCT). Therefore, by using this magnetomotive microscopy method, cells in Petri dishes
can be deformed by the MNPs, which are modulated by a magnetic field with a known waveform. The cellular deformations can be detected by microscopes to investigate cellular biomechanics.

Compared with the other methods, magnetomotive microscopy has some advantages to mechanically excite cells and measure cell biomechanics. First, this magnetomotive microscopy method can be applied not only to \textit{in vitro} cells lines, but also to cells in living tissues. For instance, this method can be used along with microscopic techniques on live animal models with surgically implanted optical windows or exposed tissues. Second, MNPs can be functionalized to target different cellular receptors. For example, antibody-conjugated MNPs have been demonstrated for targeting molecules from mammary tumors in an animal model [153]. Third, in addition to being a measurement tool, this magnetomotive microscopy method also has the potential to function as a therapeutic treatment from induced hyperthermia by magnetic fields [154]. Finally, MNPs can be fluorescently labeled or combined with luminescent quantum dots to provide fluorescent information from microscopic imaging techniques as a validation of magnetomotive signal [155].

DPM with FTLS, as described in section 2.7, is used as an example microscopy in this study as an \textit{in vitro} tool to investigate cellular biomechanics based on an average of dynamic scattering from the cells in the field of view. The DPM system is built on a commercial inverted microscope (Axio Observer Z1, Carl-Zeiss), and provides incubation conditions of 37 \textdegree C temperature and 5\% CO\textsubscript{2} during imaging. Microscopic images were taken by a CCD camera (Axio Cam MRm, Carl-Zeiss), and 10X magnification was used for the DPM system, providing
a field of view of 120 µm × 120 µm. During the imaging, 1 Hz and 2 Hz sinusoidal oscillations were used to drive the permanent magnet by a function generator, with a 10 V_{pp} amplitude. Images were also taken without magnetic field modulations. The MNP were suspended in a saline solution with a concentration of 1 mg/ml and added to the Petri dishes, followed by incubation for 6 hours. Then, the cell cultures were washed 3 times using PBS (phosphate buffered saline). Fresh media was then added before magnetomotive microscopy imaging. Cells with an incubation time of 24 hours were also investigated with the magnetomotive microscope system. Microscopic images were taken during the magnetic modulation at a frame rate of ~5 Hz. Image sets of time-lapse amplitude and phase images were taken at each region of interest. Then the scattered far field was obtained by Fourier transform in space, which is determined by the angular scattering of particles and the structure field. Temporal fluctuations of the optical field scattered at a particular angle were then determined. The scattered intensity was chosen at 40˚ as a representative angle for light scattering. No obvious differences were noticed from the experimental results using different scattering angles. Therefore, besides quantitative microscopic phase images of cells, a peak value from the driving frequency is expected to appear in the FTLS dynamic scattering power spectrum, as an indication of the cellular biomechanical properties from different cell lines, given the consistently applied magnetic field for different samples.

The mouse macrophage cell line TIB-67 (American Type Culture Collection, Manassas, Virginia) is used in this study. This cell line was taken from a female adult mouse. Cells were seeded in 35 mm glass bottom Petri dishes and allowed to grow in an incubator at 37 °C, 5% CO₂
in Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute medium (RPMI) 1640 (complete growth medium with 10% fetal bovine serum) (ATCC). Two dishes of macrophages were tested by the magetomotive microscopic method. The first group of macrophages were cultured with MNPs, and incubated for 48 hours. Before imaging, these macrophages were washed three times by PBS, and provided with fresh media. The second group was a control, and was not cultured with MNPs but with PBS only. Media changes were performed with the control group before imaging just as with the MNP-exposed group. Upon imaging, the majority of macrophages in the Petri dishes were observed to be morphologically healthy, and well attached to the glass bottoms of the dishes. To avoid artifacts in FTLS analysis, the permanent magnet was positioned so as not to block the light from the microscope. No mechanical movement was noticed from the Petri dishes or media alone by bright field microscopic observation.

The DPM images of macrophages are shown in figure 8.4. Figure 8.4a is the intensity image of a macrophage, while figure 8.4b is the quantitative phase image of a macrophage.

![Figure 8.4 DPM images of macrophage. (a) Intensity image from DPM before background (dark dots) subtraction. (b) Quantitative phase image from DPM of a macrophage. Unit for color bar is radians.](image)
Based on the time-lapse DPM quantitative phase image sets for the two dishes of macrophages, the power spectrum results of FTLS at 40° on the macrophage are shown in figure 8.5. The first row of figure 8.5 represents FTLS power spectra from the first dish of macrophages, which were fed with MNPs before imaging. Figure 8.5a shows the power spectrum without magnetic modulation, from which we can see that no obvious light scattering signal was detected, except for the DC components. Figure 8.5b shows the power spectrum with 1 Hz magnetic modulation, which indicates that light scattering from the cell structures or MNPs was modulated by the magnetic field, and the degree of the macrophage deformation can be represented by the magnitude of the power spectrum at 1 Hz. Figure 8.5c shows the power spectra with 2 Hz magnetic modulation, which gives power spectrum magnitude similar to that of the 1 Hz case. The second row of figure 8.5 shows the FTLS power spectrum of the second dish of macrophages, with no magnetic modulation, 1 Hz magnetic modulation, and 2 Hz magnetic modulation. Since there were no MNPs present in this Petri dish, the macrophages were not mechanically modulated by the magnetic fields, and thus no peaks in the power spectrum were detected.

It is suggested from the magnetomotive microscope results on macrophages that this method can be used to detect cellular biomechanical properties, based on using the MNPs as nanotransducers, DPM as an imaging modality, and FTLS as an analysis tool. The magnitude of the peaks in the FTLS power spectrum can be used as an indication of how much the cells are deformed by the nanotransducers, and thus can be a representative parameter for the
measurement of cellular biomechanical properties.

Figure 8.5 FTLS power spectra of macrophages. (a)-(c) Power spectra with nanoparticles. (d)-(f) Power spectra without nanoparticles. (a) and (d) are power spectra with no magnetic field modulation. (b) and (e) are power spectra with 1 Hz magnetic field modulation. (c) and (f) are power spectra with 2 Hz magnetic field modulation. Arbitrary unit for y axis.

For magnetomotive microscopy and FTLS as measurement tools for cell biomechanical properties, the mechanisms by which MNPs become attached or internalized by cells is significant. For macrophages, this mechanism is relatively straightforward. The MNPs were engulfed by the macrophages (as shown in the transmission electron microscopy image in figure 8.6) as the role of these cells is to phagocytose cellular debris and pathogens. However, to answer more scientific questions, this magnetomotive microscopy technique must be applicable to more cell types. Studying variations in cancer cell biomechanics during development and metastasis is one example. Potential further studies for measuring biomechanical properties of cancer cell lines using magnetomotive microscopy may include immunological studies on
functional nanoparticles targeted to cell membrane receptors to determine how a sufficient number of nanotransducers can be attached to the membranes of different cell lines.

Figure 8.6 Transmission electron microscopy image of a macrophage. Black regions denoted by the red arrows indicate nanoparticles engulfed by the macrophage.
CHAPTER 9 CONCLUSIONS

This dissertation demonstrates a series of work on optical imaging technologies and applications for measuring biomechanical properties of tissues and cells. Mechanical properties such as Young’s modulus are studied for ex vivo breast tumor tissue, in vivo human skin and in vitro cell line, for which optical imaging technologies are practically applied to measure biomechanical properties. OCT, multiphoton microscopy, and diffraction phase microscopy are used as optical imaging modalities, based on techniques which include dynamic OCE mapping and imaging, acoustomotive OCE, surface wave propagation OCE, multiphoton elastography, and magnetomotive microscopy. The biomechanics investigated in this dissertation range from the scale of cell population to tissues. The biomechanics of cells and tissues, especially in vivo dynamic biomechanics which reflect the properties close to reality, are of great importance for their functionalities, pathological changes, and reactions to external stimuli. The results of the studies are also potential indicators of how cell and tissue function is affected by variations in mechanical properties. Although imaging techniques have been investigated in biomechanics, the optical elastography techniques discussed in this thesis are unique for their capabilities in dynamic and in vivo measurements, and also application to practical topics such as breast tumor and skin clinical applications. Therefore, the engineering to employ optical imaging to investigate biomechanics has been demonstrated, as well as the feasibility for using these techniques to solve more scientific and clinical questions.

The techniques shown here are feasible for biological applications. The dynamic OCE
mapping and imaging techniques are promising for investigating tumor biomechanical properties during development with micron-scale resolution. Combining the tissue level study with the cellular biomechanics investigation, a more thorough and complete understanding of how the mechanical properties of cancer cells and their microenvironments interact during development may be achieved. The idea is also applicable for skin studies, and in particular, for investigating the development of basal cell carcinoma using OCE methods at the tissue level and MPM for cellular studies.

The techniques shown here are feasible for clinical applications. With successful intraoperative OCT for breast tumor margin detection, the dynamic OCE imaging technique can be used as a simultaneous but complementary method to increase the sensitivity of the tumor margin assessment. Dynamic OCE imaging may also be combined with balloon endoscopy techniques to measure biomechanics inside the human body. Further clinical studies are needed to demonstrate early tumor detection and to improve our understanding about the role of biomechanics in tumor development. OCE imaging of human skin is very promising for dermatology purposes, including the detection of basal cell carcinoma, differentiation of Langer's lines, and investigation of skin water resistance functions.
LIST OF PUBLICATIONS


REFERENCES


