Development of optical coherence elastography toward intraoperative guidance of breast cancer surgery

Kelsey M. Kennedy

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Abstract

Biological tissue has intrinsic mechanical properties that are linked to its structure and function and altered by the onset of disease. Techniques that create images based on tissue mechanical contrast, referred to as elastography, can aid in the detection, diagnosis, and treatment of disease. In this research, an emerging optical elastography technique, optical coherence elastography (OCE), is further developed to visualize tissue mechanics on a microscopic scale. We present compression OCE techniques with unprecedented microstrain sensitivity and with the added capability to measure absolute values of stiffness. In addition, using a novel needle-based probe, we perform OCE at previously unreached depths in tissue. These advances are made with a view toward facilitating the clinical application of OCE, and particularly toward improved guidance of breast tumour resection during breast-conserving surgery.

To advance the clinical viability of OCE, the image contrast must be improved and characterized. In the first aspect of this work, the first quantitative analysis of mechanical contrast in OCE is performed. This relied on the development of tissue-mimicking imaging targets, or phantoms, to enable analysis using samples with controlled mechanical and structural properties. Finite element models of OCE were developed and outputs compared to experimental measurements. The results demonstrate a theoretical sensitivity to differences in tissue stiffness as small as 0.2% using our system. We also investigate fundamental mechanical limitations on image quality, such as stress non-uniformity.

The second part of this work develops methods to measure stress and provide absolute measurements of tissue stiffness in OCE, extending its clinical potential by enabling longitudinal studies and inter-sample comparison. Firstly, we introduce a novel, compliant stress sensor for high-resolution tactile imaging of tissue, in which the stress distribution at the tissue surface is used as contrast, mimicking the manual palpation that clinicians commonly use to detect disease. We call this technique “optical palpation” and demonstrate its utility for generating tissue contrast in breast cancer tissues. We then combine optical palpation imaging with depth-resolved strain imaging to enable the first quantitative maps of tissue stiffness using compression OCE, and obtain preliminary measurements in human breast and lymph node tissues.
Finally, conventional OCE techniques are limited to imaging the first 1 to 2 mm of tissue in depth, a major limitation in the technique's potential as a surgical guidance tool. We demonstrate, for the first time, OCE measurements deep within tissue by implementing OCE via an optical needle probe. We perform this needle OCE technique in phantoms and in human breast cancer samples, demonstrating its ability to detect tissue interfaces based on mechanical contrast.

With further refinement, these techniques may aid in the intraoperative detection of the boundary of tumour to help ensure full removal of malignant tissues, which is critical to the success of breast-conserving surgery.
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Statement of contribution

This thesis contains results of the research that I, Kelsey Kennedy, performed within the School of Electrical, Electronic, and Computer Engineering at The University of Western Australia between February 2011 and July 2014. Except where indicated below and throughout the text, all work and writing are my own.

The chapters of this thesis are primarily derived from eight published works: four published, first-author journal articles (two full-length and two letters); one published, full-length, co-authored journal article; two published, co-authored review articles; and one published, co-authored book chapter\(^1\). I am the sole author of the remainder of the document.

Sections 5.2, 6.1, 7.2, 9.2 and 9.3 are reproductions of publications, modified only in formatting to be consistent with the remainder of this document. Chapters 1 and 2 are an amalgamation of a published review article and a book chapter, modified to include the most current information from the literature and to provide more specific context for the research aims of this thesis. A small portion of Chapter 4 is derived directly from a co-authored review article. I am the sole author of Chapter 8. Listed below are the contributions of each author to each publication.

1. Brendan F. Kennedy, **Kelsey M. Kennedy (30%)**, and David D. Sampson,
   **(parts of Introduction, Section 1.2, majority of Section 2.2, Section 2.3.2.)**

Brendan F. Kennedy (BFK) is the principal author of this invited review paper. Contributions of Kelsey M. Kennedy (KMK) were: to generate all original figures within the review; to assist BFK with literature review and determining the organization and scope of the article; to write Section 5 of the article; to edit manuscript drafts.

David D. Sampson (DDS) guided the overall organization and scope of the article, and supervised drafting of the manuscript.

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\(^1\) This thesis was prepared according to The University of Western Australia’s guidelines on “Thesis as a series of papers” ([http://www.postgraduate.uwa.edu.au/students/thesis/series](http://www.postgraduate.uwa.edu.au/students/thesis/series), Accessed 7 July 2014).
2. Brendan F. Kennedy, Kelsey M. Kennedy (20%), Amy L. Oldenburg, Steven G. Adie, Stephen A. Boppart, and David D. Sampson, “Optical Coherence Elastography,” in Optical Coherence Tomography: Technology and Applications, W. Drexler, J.G. Fujimoto, Editors. (Springer), in press. (Section 1.1, parts of Section 2.2, majority of Sections 2.3 and 2.4)

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The portions of this article included in Chapter 4 correspond mainly to those portions that KMK led in the original article; therefore, the majority of the text and all figures are her own work.
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4. Brendan F. Kennedy, Maceij Wojtkowski, Maceij Szkulmowski, Kelsey M. Kennedy, Karol Karnowski, and David D. Sampson,
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   2014.

Book chapters
10. Brendan F. Kennedy, Kelsey M. Kennedy, Amy L. Oldenburg, Steven G. Adie,
    Stephen A. Boppart, and David D. Sampson,
    “Optical Coherence Elastography,” in *Optical Coherence Tomography Technology and

First-Author Conference Presentations
1. Kelsey M. Kennedy, Shaghayegh Es’haghian, Lixin Chin, Robert A. McLaughlin,
   David D. Sampson, and Brendan F. Kennedy
   “Quantitative compression optical coherence elastography enabled by a high-resolution
   stress sensor”
   SPIE Photonics West BiOS – Optical Elastography and Tissue Biomechanics I
   San Francisco, CA
   February 2014

2. Kelsey M. Kennedy, Shaghayegh Es’haghian, Lixin Chin, Robert A. McLaughlin,
   David D. Sampson, and Brendan F. Kennedy
   Australia New Zealand Conference on Optics and Photonics
   “High-resolution stress sensor for quantitative optical coherence elastography”
   Australia New Zealand Conference on Optics and Photonics
   Perth, Australia
   December 2013

3. Kelsey M. Kennedy, Brendan F. Kennedy, Robert A. McLaughlin, and David D.
   Sampson
   “Probing elastic contrast in human tissues using optical coherence elastography”
   Biophotonics ’13 Summer School - Poster Presentation
   Ven, Sweden
   June 2013

4. Kelsey M. Kennedy, Robert A. McLaughlin, Brendan F. Kennedy, Alan Tien, Bruce
   Latham, Christobel M. Saunders, and David D. Sampson
   “Measuring elastic contrast in human tissues using OCT needle probes”
   SPIE Photonics West BiOS – Optical Coherence Tomography and Coherence Domain
   Optical Methods in Biomedicine XVII - Oral Presentation
   San Francisco, CA
   February 2013

5. Kelsey M. Kennedy, Chris Ford, Brendan F. Kennedy, Mark B. Bush, and David D.
   Sampson
“Evaluating image contrast in optical coherence elastography using finite element analysis”
SPIE Photonics West BiOS – Dynamics and Fluctuations in Biomedical Photonics – Invited Oral Presentation
San Francisco, CA
February 2013

6. Kelsey M. Kennedy, Robert A. McLaughlin, Brendan F. Kennedy, Alan Tien, Bruce Latham, Christobel M. Saunders, and David D. Sampson
Combined Biological Sciences Meeting - Poster Presentation
“Probing the mechanical properties of healthy and diseased tissues using a microscope-in-a-needle”
Perth, Australia
August 2012

7. Kelsey M. Kennedy, Robert A. McLaughlin, Brendan F. Kennedy, Alan Tien, Bruce Latham, Christobel M. Saunders, and David D. Sampson
“Needle-based optical coherence elastography: a novel technique for probing mechanical properties of deep tissues”
Australian Conference on Microscopy and Microanalysis - Oral Presentation
Perth, Australia
February 2012

8. Kelsey M. Kennedy, Brendan F. Kennedy, Robert A. McLaughlin, and David D. Sampson
“A new method of optical biopsy: demonstration of mechanical contrast in deep tissue using an optical coherence elastography needle probe”
Asia-Pacific Optical Sensors Conference - Oral Presentation
Sydney, Australia
January 2012

9. Kelsey M. Kennedy, Brendan F. Kennedy, Robert A. McLaughlin, and David D. Sampson
“Detection of mechanical interfaces in soft tissue with an optical coherence elastography needle probe”
Asia-Pacific Symposium on Optical Coherence Tomography - Oral Presentation
Taipei, Taiwan
November 2011

10. Kelsey M. Kennedy, Brendan F. Kennedy, Robert A. McLaughlin, and David D. Sampson
“A novel technique to identify stiff lesions in soft tissue using miniaturized optical imaging probes”
Combined Biological Sciences Meeting - Oral Presentation
Perth, Australia
August 2011
# List of acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>ARF</td>
<td>acoustic radiation force</td>
</tr>
<tr>
<td>BCS</td>
<td>breast-conserving surgery</td>
</tr>
<tr>
<td>CCD</td>
<td>charge-coupled device</td>
</tr>
<tr>
<td>CTE</td>
<td>contrast transfer efficiency</td>
</tr>
<tr>
<td>DCIS</td>
<td>ductal carcinoma <em>in situ</em></td>
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<tr>
<td>DR</td>
<td>dynamic range</td>
</tr>
<tr>
<td>FD-OCT</td>
<td>Fourier-domain optical coherence tomography</td>
</tr>
<tr>
<td>FEM</td>
<td>finite element model</td>
</tr>
<tr>
<td>FF-OCT</td>
<td>full-field optical coherence tomography</td>
</tr>
<tr>
<td>FFT</td>
<td>fast Fourier transform</td>
</tr>
<tr>
<td>FWHM</td>
<td>full-width at half-maximum</td>
</tr>
<tr>
<td>GM</td>
<td>galvanometer mirror</td>
</tr>
<tr>
<td>GPU</td>
<td>graphics processing unit</td>
</tr>
<tr>
<td>GRIN</td>
<td>gradient index</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>haematoxylin and eosin</td>
</tr>
<tr>
<td>IDC</td>
<td>invasive ductal carcinoma</td>
</tr>
<tr>
<td>ILC</td>
<td>invasive lobular carcinoma</td>
</tr>
<tr>
<td>LCIS</td>
<td>lobular carcinoma <em>in situ</em></td>
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<tr>
<td>MEMS</td>
<td>micro-electro-mechanical systems</td>
</tr>
<tr>
<td>MM</td>
<td>magnetomotive</td>
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<tr>
<td>MNP</td>
<td>magnetic nanoparticle</td>
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<tr>
<td>MRE</td>
<td>magnetic resonance elastography</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NA</td>
<td>numerical aperture</td>
</tr>
<tr>
<td>NIRF</td>
<td>near-infrared fluorescence</td>
</tr>
<tr>
<td>OCE</td>
<td>optical coherence elastography</td>
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<tr>
<td>OCM</td>
<td>optical coherence microscopy</td>
</tr>
<tr>
<td>OCT</td>
<td>optical coherence tomography</td>
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<tr>
<td>PAI</td>
<td>photoacoustic imaging</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
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<tr>
<td>PARS</td>
<td>paired-angle rotation scanning</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>PVA-C</td>
<td>polyvinyl alcohol cryogels</td>
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<tr>
<td>ROLL</td>
<td>radioguided occult lesion localization</td>
</tr>
<tr>
<td>SAW</td>
<td>surface acoustic wave</td>
</tr>
<tr>
<td>SD-OCT</td>
<td>spectral-domain optical coherence tomography</td>
</tr>
<tr>
<td>SMF</td>
<td>single-mode fibre</td>
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<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
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<tr>
<td>SS-OCT</td>
<td>swept-source optical coherence tomography</td>
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<tr>
<td>STdOCE</td>
<td>joint spectral-and-time-domain optical coherence elastography</td>
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<tr>
<td>SW</td>
<td>shear wave</td>
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<tr>
<td>TD-OCT</td>
<td>time-domain optical coherence tomography</td>
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<tr>
<td>TFM</td>
<td>traction force microscopy</td>
</tr>
<tr>
<td>WLS</td>
<td>weighted-least squares</td>
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Introduction

The onset of disease in the human body is typically coupled with a change in the mechanical properties of tissue: a stiff lesion in the breast may be indicative of breast cancer; escalated contractility of the airway muscles caused by pulmonary disease makes breathing difficult; and a hardened plaque in the arteries, under the right amount of pressure, could rupture and lead to a heart attack. For centuries, physicians have assessed the mechanical properties of tissues using manual palpation, feeling for changes in tissue stiffness that help them to form a diagnosis. The link between disease and mechanical changes in tissue, however, begins at the cellular level, at a scale too small to be probed by human touch, and mechanical changes can occur deep within the body, in areas inaccessible by palpation.

Motivated by the prevalence of palpation as a clinical tool, the advent of medical imaging has been accompanied by the development of techniques to create images of the mechanical properties of tissue. This suite of techniques, known as elastography, allows clinicians to visualize mechanical contrast within tissue in a more quantitative manner, in three dimensions, and in some cases with better spatial resolution than manual palpation. The word elastography was first introduced by Ophir et al. in 1991 [1], in using ultrasound to capture the deformation of tissue under an externally applied, compressive load. From the measured deformation, mechanical properties may be estimated and mapped onto an image known as an elastogram. Ultrasound elastography and magnetic resonance elastography (MRE) have been developed extensively over the past 23 years, with clinical success in applications such as diagnosis of liver disease [2] and breast lesions [3], and systems are commercially available from a range of vendors including General Electric and Siemens [4].

The spatial resolution and penetration depth of elastography depend on the underlying modality. Ultrasound elastography and MRE [5] map mechanical properties with spatial resolutions of 100s μm [6] and 1-3 mm [7], respectively, and with penetration depths from a few centimetres to whole-body imaging, as illustrated in Fig. 1. Whilst these scales are very suitable for screening and diagnostic imaging, orders of magnitude higher resolution is required for imaging at the scales of disease origin and progression, i.e., at the cellular and extracellular level. The most prominent
technique for mechanical imaging of cells and their environments is atomic force microscopy (AFM), which uses micro-indenters that scan across a tissue surface and can map tissue stiffness with nanometre-scale spatial resolution [8]. Other techniques such as optical tweezers [9] and traction force microscopy [10] have also been developed to measure the stiffness of individual cells, as well as forces that cells impart on each other and their environment. However, conversely to the clinical modalities, these technologies are constrained to laboratory imaging, as they are impractical to employ in vivo and are often too slow to meet the demands of clinical scenarios.

Elastography at the scale of tissue microstructure, with spatial resolutions of 1-10s μm, can potentially bridge the gap between established laboratory and clinical mechanical imaging techniques and contribute to both fundamental investigations of the mechanics of disease and clinical applications. Development of elastography at this intermediate scale has only begun relatively recently, primarily using optical imaging to provide superior resolution than that of typical clinical modalities (Fig. 1). Measuring the bulk mechanical properties of tissues with optics actually dates back to at least the 1950s [11], where surface optical techniques, such as photoelastic stress analysis and electronic speckle pattern interferometry, were developed for the non-destructive evaluation of materials and hard tissues, such as bone [12]. The first optical techniques to create images based on mechanical properties, i.e., optical elastography, were introduced in 1998, with independent reports by Jacques et al. [13] and Schmitt [14]. Schmitt first proposed optical coherence elastography (OCE) in employing optical coherence tomography (OCT) [15] to detect depth-resolved sample deformation induced by quasi-static compression. Since the first demonstration of OCE, advances in the stability and sensitivity of OCT systems have dramatically increased the accuracy with which displacement can be measured [16]. This improvement, together with the commercial and clinical success of ultrasound elastography and magnetic resonance elastography, has led to a resurgence of interest in OCE [17, 18] and related optical elastography techniques [19-24].
This research aims to further develop OCE toward clinical translation. From a clinical imaging perspective, OCE offers distinct features in comparison to ultrasound elastography and MRE, including:

1) *Imaging spatial resolution*: OCE has the potential to provide imaging spatial resolution of 1–10 μm, much higher than that of (clinical) ultrasound elastography and magnetic resonance elastography. Such resolution provides mechanical contrast on length scales that have been little studied to date, with the potential to resolve the microscopic heterogeneity in mechanical properties known to be present in diseases such as cancer.

2) *Acquisition speed*: OCE provides for two-dimensional (2-D) image acquisition rates at 10s to 100s of kHz [25], and potentially even higher. Such high acquisition rates promise three-dimensional (3-D) *in vivo* elastography [26]. These rates are much higher than for conventional ultrasound elastography (limited by the speed of sound) or MRE (limited by sensitivity), although multi-kilohertz 2-D scan rates have been achieved in ultrasound elastography with supersonic shear imaging [27].
3) **Mechanical sensitivity:** Sub-nanometre displacement sensitivity [28] should enable the measurement of smaller changes in mechanical properties than with other forms of elastography. This sensitivity has the potential to better differentiate subtle changes in mechanical properties that occur, *e.g.*, between benign and malignant tumours.

In breast cancer in particular, elastography of tissue microstructure may enable surgeons to detect small extensions of tumour that are otherwise missed by manual palpation or other clinical imaging modalities. Complete removal of the cancerous tissue is imperative to the success of breast-conserving surgery (*i.e.*, removal of the tumour and a margin of healthy tissue, sparing the remainder of the breast) for treatment of breast cancer. Tumour found within 5 mm of the edges of the resected tissue, as determined by post-operative pathology, is correlated with an increased rate of local recurrence of cancer and often necessitates a second surgery [29]. Currently, 23% of patients that undergo breast-conserving surgery must return for a second operation due to insufficient tissue removal the first time [30]. OCE could provide a means to intraoperatively assess the boundaries of excised tissue, checking the margins for remaining tumour, and guiding the surgeon’s excision to ensure all tumour is completely removed. The research in this thesis extends the capabilities of OCE for clinical imaging in general, with a special emphasis toward providing guidance of breast cancer surgery. Chapter 3 provides background on guidance techniques in breast cancer surgery and further motivates the development of OCE toward this goal.

OCE has not yet been systematically applied to any clinical application; rather, OCE research to date has largely focused on implementing new techniques for loading tissue and measuring displacement. The development of OCE is tracing that of ultrasound elastography and MRE 15 years ago, when these technologies were on the brink of demonstrating clinical utility after about a decade of technical development (the first commercialization of elastography for clinical use was by Hitachi in 2003 [31]). This is reflected by the number of papers in the fields of ultrasound elastography and optical elastography, respectively, as generated by keyword searches in Web of Science, shown in Fig. 2. After initial demonstration of its efficacy for breast and liver disease diagnosis, the number of clinical studies using ultrasound
elastography has contributed to its exponential growth in terms of number of publications.

![Optical elastography papers](image1)

![Ultrasound elastography papers](image2)

*Fig. 2. Number of ultrasound elastography papers published in past 20 years with inset of optical elastography papers published in past 15 years. Based on this, the status of development of optical elastography is at that of ultrasound elastography 15 years ago. Data obtained from key word searches in Web of Science, accessed 13 May 2013.*

Before OCE can realize its potential in clinical applications, several key advances must take place, including in the areas listed below:

1) *Elastogram fidelity*: To construct elastograms in OCE, simplified models of tissue mechanics are employed such that tissue behaviour may be more readily described through mathematical relations. However, tissue is very mechanically complex, and when these simple assumptions break down, the resulting images contain artefacts that limit their reliability and clinical utility. Although several techniques for performing OCE have been proposed, no studies have yet evaluated the fidelity of the images to the underlying tissue properties, an essential step toward establishing OCE as a clinically useful imaging tool.

2) *Displacement measurement*: Measurement of mechanical properties over a large range and with high sensitivity is ultimately limited by the measurable displacement using OCT. Improvements in the displacement sensitivity of OCT have led to a resurgence of interest in OCE techniques in recent years, but there remains significant scope for improvement, including implementation of phase-unwrapping algorithms to extend the measurable dynamic range.

3) *Quantitative measurements*: OCE techniques based on quasi-static compression measure relative mechanical contrast, based on strain, whilst wave-based
techniques can directly estimate absolute values of tissue mechanical properties, e.g., stiffness, albeit with lower spatial resolution. The former may be sufficient in clinical applications where contrast between tissue types within an image is desirable; however, quantitative measurements are needed to enable inter-sample comparison and longitudinal studies. Enabling high-resolution, quantitative maps of tissue stiffness using compression OCE requires either computationally intensive inverse methods to predict local stiffness or direct measurement of the stress distribution applied to the tissue, which has not been demonstrated to date.

4) **Imaging penetration**: OCE penetrates only millimetres into tissue, which is much less than for ultrasound elastography and magnetic resonance elastography. This small penetration depth is insufficient for imaging most pathological lesions *in vivo*, including breast tumours. As a result, OCE studies so far have been limited to phantoms, *ex vivo* samples, *in vivo* human skin [26, 32-34] and *in vivo* mouse cornea [35]. Recently, however, OCT needle probes have been introduced [36], exploiting fibre optics to deliver the light via the tip of a needle, and enabling OCT imaging in deep, solid tissues. Implementation of OCE into a needle probe could greatly extend its potential clinical applications.

**Research aims**

The overall objective of this research is to facilitate clinical implementation of OCE for guidance of breast cancer surgery. Three aspects of original work will be undertaken toward achieving this goal:

1) Evaluation and improvement of the image quality in OCE, including assessment of the fidelity of image contrast to the underlying tissue properties and extension of the measurable range of mechanical properties;

2) Enabling high-resolution, quantitative images of tissue stiffness by development and implementation of a method to measure stress applied to a sample;

3) Design and development of the first needle-based OCE device to enable *in vivo* measurements of mechanical contrast deep in solid tissues.

**Structure of thesis**

This thesis is divided into three major parts: **Part I** provides the background to this research; **Part II** presents an analysis of image quality in OCE from a mechanical point
of view and describes advances to enable the first high-quality elastograms of breast tissue microstructure; and **Part III** presents two novel concepts that may further facilitate clinical translation, including a method to perform quantitative compression OCE and a needle-based OCE method to overcome the imaging depth limitation. A brief outline of each of the chapters within these parts is given below.

**Part I – Optical coherence elastography: principles**

**Chapter 1 – Mechanical properties of tissue**
The first chapter defines the mechanical properties of tissue and presents the analytical expressions that link stress, strain, and mechanical properties. This analysis and its associated assumptions about the mechanical behaviour of tissue form the basis of image formation in OCE. We go on to consider the determinants of the mechanical properties across length scales and how these are altered by the onset of disease.

**Chapter 2 – Optical coherence elastography approaches**
Chapter 2 presents a comprehensive review of the OCE techniques reported to date. Techniques are classed based on their loading mechanism and method for estimating tissue displacement. Various probes that have been proposed to facilitate *in vivo* imaging are also reviewed. Throughout, we draw comparisons between OCE and ultrasound elastography and MRE, as well as motivate the further development of the particular type of OCE pursued in this research, phase-sensitive compression OCE. Finally, we provide details of the OCE setups used throughout the subsequent chapters of this thesis.

**Chapter 3 – Optical coherence elastography for guidance of breast cancer surgery**
In this chapter, we consider the mechanical properties of breast tissues, both normal and malignant, and argue that imaging these properties at the scale probed by OCE could aid localization of cancerous tissue during breast cancer surgery. We describe current clinical challenges in breast cancer surgery and the guidance techniques currently at the disposal of clinicians. We also review several technologies that have recently been proposed for guidance of breast-conserving surgery, including OCT, and highlight how OCE may fill the need for additional and complementary tissue contrast.
Part II – Analysis and improvement of image quality in optical coherence elastography

Chapter 4 – Tissue-mimicking phantoms
Artificial imaging targets, or phantoms, with known mechanical properties and structure are a key enabler for the analysis of OCE image quality, as well as the validation of new techniques presented in this thesis. In Chapter 4, we provide an overview of phantom materials that have been used previously in OCT and OCE [37], and highlight the advantages of silicone as an ideal phantom material for use in OCE. We explain how the optical properties, mechanical properties, and structure of silicone phantoms may be controlled to simulate a range of soft tissues and, in particular, demonstrate the ability to tune the stiffness of silicones over a wider range than previously shown in OCE.

Chapter 5 – Mechanical limitations on contrast
Clinical translation of OCE requires characterization of the fidelity of elastograms, that is, the accuracy with which they depict the underlying mechanical properties of a sample. In Chapter 5, we perform the first quantitative analysis of the contrast in phase-sensitive compression OCE, focusing on limitations imposed by sample mechanics [38]. Finite element analysis is used to simulate OCE experiments, validating the contrast measured experimentally, and elucidating sources of artefacts. This approach provides a framework for analysis of mechanical contrast in other OCE techniques. We conclude that, despite providing only relative mechanical contrast, phase-sensitive compression OCE is promising for its exquisite sensitivity to variations in tissue stiffness.

Chapter 6 – Optical coherence micro-elastography
Chapter 6 presents improvements to the image acquisition scheme and signal processing in compression OCE to enable optical coherence micro-elastography [39], a 3-D technique with microstrain sensitivity and the highest lateral resolution of OCE techniques to date. We demonstrate results on freshly excised human breast tissues from breast cancer patients and reveal a first look at the mechanical heterogeneity of the breast on this microstructural scale. Prospects for using the strain contrast in these images to detect regions of malignancy are discussed.
Part III: Novel techniques toward clinical translation of optical coherence elastography

Chapter 7 – Development of a stress sensor for optical coherence elastography
Mapping the absolute values of tissue stiffness using compression OCE requires measurement of the stress distribution on the sample. Stress mapping, also known as tactile imaging, has been developed using electronic sensors for robotics applications, but has not previously been proposed for mapping mechanical contrast in OCE. In this chapter, we develop a novel stress sensor for high-resolution, OCT-based tactile imaging, known as optical palpation [40]. We explore the potential of this novel technique to provide mechanical contrast on its own, ahead of using it for quantitative measurements in Chapter 8. The sensor consists of a compliant silicone rubber layer with known stress–strain behaviour. OCT is used to measure the local strain in the sensor under a compressive load, from which the local stress at the sample surface is calculated and mapped onto an image. We present results demonstrating the detection of a feature embedded 4.7 mm below the sample surface, well beyond the depth range of OCT, as well as delineation of a tumour boundary in freshly excised human breast tissue.

Chapter 8 – Quantitative compression optical coherence elastography
In the penultimate chapter, we combine the 3-D strain measurements provided by optical coherence micro-elastography (Chapter 6) with the high-resolution stress maps provided by optical palpation (Chapter 7) to enable the first quantitative compression OCE technique. We present preliminary results in tissue-mimicking phantoms and freshly excised human lymph node tissue, demonstrating the potential for improved contrast over the strain elastograms in compression OCE. With further refinement, this technique could greatly extend the clinical potential of compression OCE, enabling inter-sample comparison and longitudinal studies.

Chapter 9 – Needle optical coherence elastography
Finally, we overcome the imaging depth limitation of standard OCE and greatly extend its potential for in vivo imaging by implementing OCE via a needle probe. The needle probe design is described in the context of previous OCT needle probe designs. We explain how we use insertion of the needle itself as a loading mechanism and present
proof-of-principle results in phantoms, demonstrating the ability to localize tissue interfaces based on mechanical contrast [41]. The chapter culminates with the application of needle OCE in freshly excised breast cancer tissues [42], representing the first OCE measurements acquired deep within human tissue, and demonstrating promising contrast between normal and cancerous tissues based on their mechanical response to needle insertion.

**Conclusion and perspectives**
A final section summarizes the contributions of this research and discusses future directions to continue the translation of OCE toward clinical implementation.
I.

Optical coherence elastography: principles
Chapter 1

Mechanical properties of tissue

The goal of elastography is to map local mechanical properties, typically elasticity, or stiffness, from a set of measured displacements. Given the complex and highly variable composition of tissue, this link between measured displacement and local elasticity is not readily apparent. Tissue exhibits varying degrees of viscoelasticity (time-dependent response to a load), poroelasticity (presence of fluid-filled pores or channels), and mechanical anisotropy, as well as a nonlinear relationship between elasticity and the applied load [43, 44]. As a starting point in establishing the link between elasticity and displacement, a number of simplifying assumptions are usually made about tissue behaviour and structure. Most commonly, tissue is approximated as a linear, elastic solid with isotropic mechanical properties [45-48]. The assumption of linearity has been shown to be valid in some tissues for the level of strain (typically <10%) applied in elastography [49].

In Section 1.1, using the above assumptions, we define constitutive equations that describe tissue deformation in continuum mechanics. We present equations that relate applied load, resulting deformation, and elasticity for quasi-static and dynamic loading, following the derivations provided in [43], [45], and [50]. In Section 1.2, we describe key determinants of tissue elasticity, namely, the constituents and structural organization of tissue, and consider how disease alters these determinants across length scales. Such an understanding is vital if OCE is to be successfully applied.

1.1. Governing equations of tissue deformation

First, let us consider the physical principles that govern tissue deformation. We use the conventional quantities defined in continuum mechanics to link tissue displacement, deformation, load and elasticity [50]. We describe the load applied to tissue and the resulting deformation using stress and strain tensors. A constitutive equation is then used to define the elasticity by relating these tensors.

1.1.1 Stress tensor

The application of a mechanical load generally results in stress acting throughout a
tissue. To analyse the stress, we consider a volume, subject to an arbitrary number of external forces, \( \mathbf{F}_m \), as illustrated in Fig. 1.1(a). These external forces give rise to internal forces distributed throughout the volume. To define the corresponding stress, we divide the volume into two portions (I and II), using the plane \( S \) which passes through an arbitrary point, \( P \), with unit normal vector \( \mathbf{n} \). Considering I, we assume this portion is in equilibrium under the action of the external forces \( \mathbf{F}_1 \) and \( \mathbf{F}_2 \), and the internal forces distributed over the plane \( S \) representing the actions of II on I. To obtain the stress acting in the area \( \Delta A \) in the plane \( S \) containing \( P \), we assume that the forces acting in this area can be reduced to a resultant force \( \Delta \mathbf{F} \), where the limiting direction of \( \Delta \mathbf{F} \) is perpendicular to \( S \). The stress vector, \( \sigma_n \), acting at this point is defined as:

\[
\sigma_n = \lim_{\Delta A \to 0} \frac{\Delta \mathbf{F}}{\Delta A} \tag{1.1}
\]

The S.I. unit of stress is the Pascal, equivalent to \( \frac{N}{m^2} \). Equation (1.1) defines the particular case where the direction of the resultant force, \( \Delta \mathbf{F} \), is also the direction of the stress vector. More generally, the direction of the stress vector is inclined to \( \Delta A \), and is described by two components: a normal stress perpendicular to \( \Delta A \) and a shear stress acting in the plane of \( \Delta A \). Consider the infinitesimal cubic element located at the point \( P \), shown in Fig. 1.1(a), with faces parallel to the coordinate axes. Each component of stress acting on the cube is highlighted in Fig. 1.1(a). Two subscripts are used for each component. The first indicates the direction of the normal to the plane and the second indicates the direction of the stress component. The total stress acting on the cube is described by a second-order tensor:

\[
\mathbf{\sigma} = \begin{bmatrix}
\sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz}
\end{bmatrix} \tag{1.2}
\]
1.1.2 Strain tensor

The strain tensor describes each component of the deformation resulting from an applied load. Consider the x-axis of the infinitesimal cube presented in Fig. 1.1(a). In Fig. 1.1(b), this axis is shown (i) before and (ii) after compressive deformation. The component $u_x$ describes the displacement at the point $A$. The initial length along the x-axis, $|AB|$, is given by $dx$. After deformation, this length [$|ab|$ in Fig. 1.1(b)] is given by $dx + \frac{\partial u_x}{\partial x} dx$. The normal strain is defined as the unit contraction:

$$
\varepsilon_{xx} = \frac{|ab| - |AB|}{|AB|} = \frac{\partial u_x}{\partial x}.
$$

The same analysis holds for the normal strain in the y- and z-axes, $\varepsilon_{yy}$ and $\varepsilon_{zz}$, defined as $\frac{\partial u_y}{\partial y}$ and $\frac{\partial u_z}{\partial z}$, respectively. In OCE, the strain defined in Eq. (1.3), is often referred to as the local strain [51]. As the strain is a ratio of lengths, it is dimensionless. By convention, tensile strains are positive and compressive strains are negative. Following this convention, the quantity $\frac{\partial u_x}{\partial x} dx$ in Fig. 1.1(b) is negative, and therefore,
the compressive strain $\varepsilon_{xx}$ is also negative. Analogously to stress, the strain has both normal and shear components. The $xy$-plane of the cube in Fig. 1.1(a) is illustrated in Fig. 1.1(c). After deformation, the area $dxdy$ takes the form of a parallelogram in the general case. The shear strain is defined as the change in angle between two axes that were originally orthogonal. From Fig. 1.1(c), the shear strain, $\varepsilon_{xy}$, is given by $\alpha + \beta$. For small displacement gradients, we have $\alpha = \frac{\partial u_y}{\partial x}$ and $\beta = \frac{\partial u_x}{\partial y}$, where $u_y$ is the displacement along the $y$-axis at the point $A$, allowing the engineering shear strain, $\varepsilon_{xy}$, to be defined as $\frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y}$. By interchanging $x$ and $y$, and $u_x$ and $u_y$, it can be demonstrated that $\varepsilon_{xy} = \varepsilon_{yx}$. Shear strain components in the $xz$ and $yz$ planes can be defined in a similar manner. The infinitesimal strain tensor describing each component of strain can then be expressed as:

$$
\varepsilon = \begin{bmatrix}
\varepsilon_{xx} & \varepsilon_{xy} & \varepsilon_{xz} \\
\varepsilon_{yx} & \varepsilon_{yy} & \varepsilon_{yz} \\
\varepsilon_{zx} & \varepsilon_{yz} & \varepsilon_{zz}
\end{bmatrix} = \begin{bmatrix}
\frac{\partial u_x}{\partial x} & 0.5\left(\frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y}\right) & 0.5\left(\frac{\partial u_z}{\partial x} + \frac{\partial u_x}{\partial z}\right) \\
0.5\left(\frac{\partial u_y}{\partial y} + \frac{\partial u_x}{\partial x}\right) & \frac{\partial u_y}{\partial y} & 0.5\left(\frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z}\right) \\
0.5\left(\frac{\partial u_z}{\partial z} + \frac{\partial u_x}{\partial z}\right) & 0.5\left(\frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z}\right) & \frac{\partial u_z}{\partial z}
\end{bmatrix}, \quad (1.4)
$$

where the shear components are scaled by 0.5, as $\varepsilon_{xy} = \varepsilon_{yx}$, $\varepsilon_{xz} = \varepsilon_{zx}$, and $\varepsilon_{yz} = \varepsilon_{zy}$. It should also be noted that in dynamic elastography techniques, the strain rate is often measured [26]. Strain rate is defined as the rate of change of strain with time and is easily obtained from the expressions defined in Eq. (1.4).

1.1.3 Constitutive equation for a linearly elastic solid

Having defined the load applied to tissue using the stress tensor and the resulting deformation using the strain tensor, a constitutive equation relating the two is used to define tissue elasticity. In elastography, the most commonly used constitutive equation is that of a linearly elastic (or Hookean) solid in which the relationship between stress and strain is linear and strain is independent of the rate at which the load is applied. The constitutive equation for a linearly elastic solid is:
\[ \sigma_{ij} = C_{ijkl} \varepsilon_{kl}, \]  

where \( i, j, k \) and \( l \) define each tensor component. As \( \sigma \) and \( \varepsilon \) are second-order tensors, \( C \) is a fourth-order tensor, consisting of 81 elastic constants, referred to as the elasticity tensor. To simplify the analysis, it is frequently assumed that the elasticity is isotropic, \( i.e., \) elasticity can be described without reference to direction. This imposes maximum symmetry on the tensor, reducing 81 elastic constants to two and resulting in the isotropic linear elastic constitutive equation, defined as:

\[ \sigma_{ij} = \lambda \varepsilon_{kk} \delta_{ij} + 2\mu \varepsilon_{ij}, \]  

where \( \lambda \) and \( \mu \) are the elastic constants, also known as the Lamé constants, and \( \delta_{ij} \) is the Kronecker delta (equal to 1 if \( i = j \) and 0 otherwise). As \( \varepsilon \) is dimensionless, the unit for \( \lambda \) and \( \mu \) is that of stress, \( i.e., \) the Pascal (Pa). It should be noted that constitutive equations that more accurately model the nonlinear, viscoelastic response of soft tissue to loading have also been proposed [44]. However, their complexity has restricted their use in OCE.

### 1.1.4 Elastic properties

A number of descriptors may be derived from the Lamé constants for a material's elastic properties. In this section, we define these descriptors, namely, Young's modulus, shear modulus, bulk modulus and Poisson's ratio, and describe their relevance to OCE.

**Young's modulus, \( E \)** characterizes the elasticity of a material subjected to uniaxial stress, \( i.e., \) only one normal stress component in Eq. (1.2) is non-zero. Uniaxial stress is generally a good approximation for the loads applied in OCE. For a load acting along \( z \), \( E \) is defined as \( \frac{\sigma_{zz}}{\varepsilon_{zz}} \), \( i.e., \) the ratio of normal (axial) stress to normal (axial) strain. In terms of the Lamé constants, it is expressed as \( \frac{\mu(3\lambda + 2\mu)}{(\lambda + \mu)} \) and has the same unit as stress, \( i.e., \) the Pascal.

**Shear modulus, \( G \)** is the ratio of shear stress to shear strain, \( e.g., \) \( \frac{\sigma_{xz}}{\varepsilon_{xz}} \). It is equal to the Lamé constant, \( \mu \), and its unit of measurement is the Pascal.

**Bulk modulus, \( K \)** is defined as the ratio of hydrostatic pressure, \( \sigma \), to fractional volume change, \( \frac{\Delta V}{V} \). Hydrostatic pressure describes the situation when all shear
stress components are zero and the normal stress components are equal, \( i.e., \sigma = \sigma_{xx} = \sigma_{yy} = \sigma_{zz} \). \( K \) is a measure of the compressibility of a material and defined as \( \lambda + \frac{2}{3} \mu \). Its unit of measurement is the Pascal.

Poisson’s ratio, \( \nu \), is defined as the ratio of the normal strain along the axis of stress to the normal strain in each orthogonal axis. In compression, it is the ratio of elongation per unit breadth to contraction per unit length, \( i.e., -\frac{\varepsilon_{xx}}{\varepsilon_{zz}} \), where the negative sign results from the division of a tensile normal strain, \( \varepsilon_{xx} \), by a compressive normal strain, \( \varepsilon_{zz} \). It is defined in terms of the Lamé constants as \( \frac{\lambda}{2(\lambda + \mu)} \). As it is a ratio of two strains, it is dimensionless.

The Lamé constants, Young’s modulus, shear modulus, bulk modulus and Poisson’s ratio are related. Only two of these parameters are independent for an isotropic, linearly elastic material and, thus, measurement of any two determines the other parameters. Although soft tissues in general exhibit poroelastic or bi-phasic mechanical behaviour, for practicality in elastography, they are typically approximated as nearly incompressible solids (\( i.e., \) their volume is conserved upon loading), resulting in a Poisson’s ratio of very close to but not exceeding 0.5. The bulk modulus of soft tissues, which is another measure of compressibility, has been reported to vary by less than 15% from that of water [45]. Thus, the goal of most OCE techniques is to estimate Young’s modulus or shear modulus, which have a much larger dynamic range than bulk modulus, as well as correspond most closely to what is sensed by manual palpation of the tissue. It is readily shown that \( E = 3G \), under the assumption that \( \nu = 0.5 \) [50]. Therefore, Young’s modulus is the most common property used to characterize tissue elasticity, and the most commonly probed property in OCE. The Young’s modulus of soft tissues extends from several kPa, in very soft tissues such as adipose [52], to hundreds of kPa to MPa, as in hard tumours [49].

1.1.5 Equations of motion

In many OCE techniques, as well as in many ultrasound and magnetic resonance elastography techniques, a dynamic (sinusoidal) load is applied to the sample. In this case, inertia must be taken into account. The constitutive equation of motion for a
linearly elastic solid is given by Navier’s equation as [50]:

$$\rho_0 \frac{\partial^2 \mathbf{u}}{\partial t^2} = (\lambda + \mu)(\nabla(\nabla \cdot \mathbf{u})) + \mu \nabla^2 \mathbf{u},$$

(1.7)

where \(\rho\) is the density of the tissue and \(\mathbf{u}\) is the displacement vector. Transverse and longitudinal waves can propagate independently in the material: S (shear) waves and P (pressure) waves, respectively. For shear wave propagation, there is no volume change in the material. The dilatation term \((\nabla \cdot \mathbf{u})\) is therefore zero and Eq. (1.7) becomes:

$$\nabla^2 \mathbf{u} = \frac{1}{c_s^2} \mathbf{u},$$

(1.8)

where \(c_s\), the shear wave speed, is defined as \(\sqrt{\frac{\mu}{\rho}}\). Pressure waves are irrotational, i.e., \(\nabla \times \mathbf{u} = 0\), allowing \(\mathbf{u}\) to be written in terms of a potential, \(\psi\), such that \(\mathbf{u} = \nabla \psi\). The wave equation then becomes

$$\nabla^2 (\nabla \psi) = \frac{1}{c_p^2} \left(\nabla \psi\right),$$

(1.9)

where the P-wave speed, \(c_p\), is defined as \(\sqrt{\frac{\lambda + 2\mu}{\rho}}\), and \(\rho\) for soft tissue is typically assumed to be \(\sim 1000 \text{ kg/m}^3\) [48].

For soft tissues, the pressure wave speed, typically several thousand m/s, is orders of magnitude faster than the shear wave speed, typically several m/s [48]. The focus in dynamic OCE to date has mainly been to measure elasticity from the shear wave properties [33, 53-55]. There are several reasons for this: firstly, as the P-wave speed depends on variations of the bulk modulus, it has a much lower dynamic range in tissue than S-waves; secondly, the high speed of P-waves makes their detection challenging.

When a dynamic load is applied at the surface of the sample, a surface wave results. When generated by a load applied orthogonal to the surface, these surface acoustic waves are thought to behave as Rayleigh waves, which contain surface longitudinal and vertical shear components that propagate typically at a few m/s and evanescently penetrate an elastic medium. Such waves are prominent in techniques developed in the fields of non-destructive evaluation [56] and seismology [57]. In this case, the
surface acoustic phase velocity, \( c_p \), in an elastic, homogeneous half-space is related to the Young’s modulus by [58]

\[
c_p = \frac{0.87 + 1.12\nu}{1 + \nu} \sqrt{\frac{E}{2\rho(1 + \nu)}}
\]  \hspace{1cm} (1.10)

In all forms of elastography, the goal is to map the local elasticity throughout a sample. Techniques for estimating the local elasticity in OCE depend on the specific loading scheme, and examples of quasi-static loading, and shear wave and SAW generation, amongst others, are discussed in detail in Chapter 2.

1.2. Determinants of tissue mechanical properties

1.2.1 Tissue constituents

The mechanical properties of tissue are determined by its constituent materials and their structural organization. Values of the Young’s modulus of a selection of tissue constituents, structures, and whole organs are displayed in Fig. 1.2. This chart shows variations in Young's modulus over more than eight orders of magnitude (from 10s Pa to GPa), illustrating the inherent high contrast potentially available in elastograms.

Fig. 1.2. Reported values and ranges of Young’s modulus for selected tissues and tissue constituents. Values obtained from [2, 34, 49, 59-62].

Tissue is a composite material, and its constituents can be classified as cellular
(e.g., blood, adipose, and epithelial cells); polymer (e.g., collagen); elastomer (e.g., elastin); and ceramic (e.g., dentin) [63]. The Young’s modulus of tissue is partially dependent on the density of these constituents; materials of higher density are typically stiffer and, therefore, have a larger Young’s modulus [62].

The constituents are arranged in a hierarchy of load-bearing structures, each with unique mechanical properties, and distributed over many orders of magnitude, from the molecular level on the nanometre scale, to the tissue level on the centimetre scale. This structural organization is inextricably linked to tissue function; e.g., tendon is made up of bundles of parallel fibres, enabling it to perform its primary function of transferring load between muscle and bone. In a study demonstrating this structure–property relationship, atomic force microscopy measurements were performed on porcine articular cartilage [64]. Large and small indenter tips were used to probe the cartilage elasticity at microscopic and nanoscopic scales, respectively. The nanoscopic measurements revealed a 100-fold lower Young’s modulus (20 kPa) in comparison to the microscopic measurements, highlighting the importance of structural integrity and scale in determining mechanical properties.

1.2.2 The impact of disease

Disease modifies both the constituent elements and the structure of tissue at each level in the structural hierarchy, resulting in corresponding scale-dependent variations in mechanical properties. In nanobiomechanics, nano- and microscale methods, such as scanning-probe microscopy, laser-based manipulation and micropipette aspiration, enable the measurement of mechanical properties in, and between, individual cells [65]. Studies using such tools are revealing the complex interplay that exists between the genesis of disease and cellular mechanics [66]. For example, metastatic malignant cells from a patient with ductal carcinoma were shown to have a Young’s modulus of 0.5 kPa, some four times lower than that of healthy cells [67]. It has been shown that this lower stiffness leads to increased deformability that facilitates the tumour cell’s migration and metastasis [68]. At the other extreme, manual palpation has been used for centuries as a macroscopic diagnostic tool and, more recently, ultrasound elastography and magnetic resonance elastography have provided improved diagnostic capabilities in a range of diseases [45-47, 69]. At this macroscale, tumour has been reported to have a Young’s modulus of hundreds of kPa
This much higher value can be attributed to the stromal response associated with tumour progression, which results in the production of large quantities of collagen, causing a stiffening of the stroma surrounding tumour cells [59]. Such multiscale studies reveal scale-dependent variations in Young’s modulus of several orders of magnitude in tumour and its constituents.

OCE probes tissue on a scale between the cellular and whole organ scales. Investigation of the mechanical properties of tissue on this mesoscopic scale (10s–100s μm) has the potential to impact on the understanding, diagnosis and treatment of diseases and conditions. In cancer, OCE may have the resolution and sensitivity to detect small extensions of tumour, which could enable detailed assessment of tumour margins, as is investigated in this thesis. Chapter 3 provides more details of the mechanical properties of breast tissue. In cardiovascular disease, atherosclerotic plaque mechanics have been linked to plaque rupture. Ultrasound elastography has been proposed to measure plaque biomechanics [70], but OCE could provide higher resolution measurements on a scale more relevant to the disease morphology [71, 72]. OCE also has potential in measuring mesoscopic mechanical properties in diseases of the eye (e.g., glaucoma [73], keratoconus [74], and presbyopia [75]), in dermatology [26, 33, 51, 76] and in obstructive lung disease [77].

In the next chapter, we review techniques proposed in OCE research to date and provide examples of OCE’s potential to visualize tissue mechanics on the mesoscopic scale.
Chapter 2

Optical coherence elastography approaches

This chapter will review OCE techniques developed and implemented to date and place into this context the OCE techniques described in this thesis. Following a basic introduction to the principles of OCT, OCE approaches are described in terms of the various loading techniques used, the reported methods for measuring displacement, and the probe designs that have been implemented for performing measurements. This review draws from our recently published book chapter and review article on OCE [17, 78]. Throughout, we highlight the similarities and distinctions between OCE and more established elastography techniques, mainly ultrasound elastography and MRE. Finally, we provide the details of the particular OCT system and OCE setup used in this thesis.

2.1. Principles of optical coherence tomography

OCT is a high resolution imaging modality capable of producing real-time, in vivo images of tissue microstructure. With a typical resolution of 1-15 μm [79], OCT can provide images on a scale approaching that of histology, enabling its valuable comparison with the clinical gold standard. OCT operates in a manner analogous to ultrasound, directing near-infrared light waves instead of sound waves into tissue to construct images of sub-surface structure. A schematic of a typical OCT system is shown in Figure 2.1. Near-infrared light from a broadband light source is split into two paths. One path goes to the sample that is being imaged, while the other goes to a reference mirror. Light reflected from the reference arm and a fraction of the light backscattered by the sample recombines in an interferometer and the interference signal is detected. A coherent interference signal will only be detected when the light travels the same distance in the reference and sample arms; i.e., when the optical pathlengths are matched to within the coherence length of the light source. Thus, a reflectivity profile in depth of the sample can be built by scanning the reference mirror. This is how the original and simplest mode of OCT, called time-domain OCT (TD-OCT), operates; the reference mirror is mechanically stepped to change the optical pathlength and produce a 1-D axial scan known as an A-scan. Two or three-
dimensional images, known as B-scans and C-scans, are constructed using beam positioning mirrors to move the beam across the sample. The intensity, the amplitude of the backscattered electric field multiplied by the amplitude of the constant reference electric field, is typically plotted in an OCT image.

Fig. 2.1. Schematic of a TD-OCT system, in which an axial depth scan (A-scan) is built up in time by mechanically stepping the reference reflection to modify the optical pathlength difference between the sample and reference arms. Transverse scanning of the beam is used to build up a 3-D structural image.

TD-OCT suffers from long acquisition time and low sensitivity, making 3-D in vivo imaging impractical for many applications. Frequency-domain OCT (FD-OCT) was subsequently developed and overcomes these issues by eliminating the need for mechanical scanning in the reference arm. Instead, the reflectivity profile (A-scan) is encoded as a function of wave number in the detected spectrum, and the depth-resolved information is reconstructed by performing a fast Fourier transform (FFT) [80]. This way, the entire depth scan is obtained using a single computation rather than mechanically stepping the reference arm in time. FD-OCT also offers improved phase stability and sensitivity over TD-OCT [16]. Two methods have primarily been used for performing FD-OCT. The first, known as spectral-domain OCT (SD-OCT), employs a spectrometer to analyse the spectral content of the signal. The second, known as swept-source OCT (SS-OCT) steps the frequency of the light source in time, such that the spectral content is also encoded in time.

OCT uses near-infrared light, most commonly in the wavelength range 800-1300 nm, which is also known as the “diagnostic window,” due to relatively low absorption by water and tissue constituents such as melanin and haemoglobin within this range. The imaging depth of OCT typically ranges between 0.5 to 2.5 mm in scattering tissues, depending on the tissue type and the wavelength used.
OCT axial resolution, $\Delta z_{\text{OCT}}$, can be defined by the coherence length of the source, and is given by the full-width at half-maximum (FWHM) of the OCT axial response. Assuming a Gaussian source with a FWHM bandwidth of $\Delta \lambda$, and mean wavelength $\bar{\lambda}$, the axial resolution is [79],

$$\Delta z_{\text{OCT}} = \frac{2 \ln 2 \bar{\lambda}}{\pi \Delta \lambda}. \quad (2.1)$$

The lateral resolution in OCT is decoupled from the axial resolution and is determined by the focusing optics of the sample arm. It is defined as the $\frac{1}{e^2}$ diameter of the beam at its waist, $W_0$. For a beam with a $\frac{1}{e^2}$ waist diameter of $W_0'$, incident upon a lens with focal length $f$, the lateral resolution, $\Delta x_{\text{OCT}}$, is given by [81],

$$\Delta x = 2 \sqrt{\ln 2 W_0} = \frac{2 \sqrt{\ln 2 f \bar{\lambda}}}{\pi W_0'}. \quad (2.2)$$

There is an inherent trade-off between lateral resolution and the Rayleigh range, or useful depth-of-focus, in OCT and in general in microscopy. In OCT, where depth sectioning deep into tissue is desirable, a focused beam with relatively a low numerical aperture, $NA = \frac{\lambda}{\pi f W_0}$ [81], is used to achieve reasonable resolution over a large depth-of-focus.

One of the main features of OCT exploited in this work is its exquisite sensitivity to sample motion. Methods for measuring displacement using OCT are reviewed in Section 2.3.

### 2.2. Loading techniques

OCE techniques have employed a wide variety of loading methods. These methods may be static/quasi-static or dynamic (continuous wave or pulsed) and applied to the tissue either internally or externally [82]. In each technique, the resulting displacement is measured and a mechanical model of deformation is used to estimate a mechanical property of the tissue. In this section, we consider the OCE techniques that have been prominent to date, paying particular attention to: compression OCE using quasi-static, external loading; surface acoustic wave (SAW) OCE, which uses dynamic loading; and magnetomotive (MM) OCE, which uses internal loading. Key elements of these techniques are shown in Fig. 2.2. We also consider other recently
proposed techniques employing shear wave (SW) imaging, acoustic radiation force (ARF) loading and swept-frequency loading, respectively. Selected performance parameters of OCE techniques are summarized in Table 2.1, based either on published performance or on our estimates. (ARF techniques are not included, as they may be considered as variants of compression [83], SW [84] or frequency-swept loading [85] techniques.) These techniques are compared and contrasted in the following sections, including with respect to ultrasound elastography and magnetic resonance elastography.

Fig. 2.2. Illustrations of loading schemes and elasticity estimation for three OCE techniques: (a) Compression: (top to bottom) concentric loading and detection in a bi-layer sample; resulting displacement versus depth; the corresponding local strain, $\varepsilon_i$; (b) SAW: (top to bottom) periodic loading and off-axis detection; amplitude decay with depth for high and low SAW frequencies, $f_1$ and $f_2$, respectively; phase velocity, $c_p$, is frequency-dependent in a layered sample (higher frequency SAW depends more on elasticity of top layer, and lower frequency SAW on elasticity of both layers); (c) MM: (top to bottom) magnetic nanoparticles (MNPs) embedded in a homogeneous sample move upward in response to a step application of the magnetic field, imparting a localized load to the surrounding sample; applied magnetic field; corresponding sample response versus time, where $f_n$ is the natural frequency of oscillation and $T_n$ is the period.

26
<table>
<thead>
<tr>
<th>Frequency Range</th>
<th>Loading</th>
<th>Quantitative</th>
<th>Lateral Resolution</th>
<th>Axial Resolution</th>
<th>Dynamic Range Reported (max/min measured)</th>
</tr>
</thead>
<tbody>
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<td>0.2-800 Hz (51)</td>
<td>Yes</td>
<td>No</td>
<td>Same as OCT</td>
<td>Not known</td>
<td>40-120 mm (38)</td>
</tr>
<tr>
<td>1-300 Hz (21)</td>
<td>Yes</td>
<td>Same as OCT</td>
<td>Same as OCT</td>
<td>Not known</td>
<td>660~1000 mm (86)</td>
</tr>
<tr>
<td>1-5 kHz (25, 54)</td>
<td>Yes</td>
<td>Not known</td>
<td>Same as OCT</td>
<td>Not known</td>
<td>1-6 mm/2.4, -7 mm/450~14</td>
</tr>
<tr>
<td>10-400 Hz (87)</td>
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<td>Not known</td>
<td>Not known</td>
<td>Not known</td>
<td>1-6 mm/2.4, -7 mm/450~14</td>
</tr>
<tr>
<td>0.1-6 kHz (32, 85)</td>
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<td>Same as OCT</td>
<td>Same as OCT</td>
<td>Not known</td>
<td>1-6 mm/2.4, -7 mm/450~14</td>
</tr>
</tbody>
</table>

**Table 2.1:** Some Demonstrated and Estimated Imaging Parameters for OCT Techniques
2.2.1 Compression techniques

In compression OCE, an external compressive load is applied to the whole region being imaged. Typically, a step change in this load is applied between acquisitions (of either OCT A-scans or B-scans). The local axial strain (i.e., the strain measured over a small depth range), \( \varepsilon \), is estimated, as illustrated in Fig. 2.2(a), by measuring the change in displacement, \( \Delta u_z \), over an axial depth range, \( \Delta z \) [89]:

\[
\varepsilon = \frac{\Delta u_z}{\Delta z}.
\]

(2.3)

The elastogram is a map of this local strain, which is a relative measure of mechanical properties. Such relative measures have proven effective in other forms of compression elastography [1, 3, 45, 46]. Calculating the Young’s modulus from the local strain cannot be done without knowledge of the corresponding local stress (see Section 1.1.4). In principle, local stress could be measured at the sample surface, but how to directly measure stress within the sample is not known. Indirect approaches, which attempt to match the measured local strain to a prediction based on an assumed stress distribution (so-called inverse methods), are possible [90]. An experimental method for measuring stress at the sample surface is proposed and demonstrated in Chapter 7 of this thesis.

The lateral resolution in compression OCE typically matches that of the underlying OCT system. However, the axial resolution is set by choice of \( \Delta z \), and is typically 5–10 times lower than the underlying OCT axial resolution, dictated by the trade-off between strain axial resolution and sensitivity [88]. Strain axial resolution is improved in proportion to improvements in the OCT system axial resolution. Improvements in strain axial resolution may also be possible by further advances in signal processing.

The major factors that determine the strain measurement accuracy are how displacement is determined from the OCT data and how local strain is estimated from the displacement [88]. The techniques used to measure displacement are discussed in Section 2.3. A number of methods have been used to estimate strain from the measured displacement. Finite-difference methods were the first to be implemented [14]. Analogously to ultrasound elastography [89], improved strain estimation can be obtained from least-squares fitting [51, 88]. In [88], our group showed that Gaussian-smoothed, weighted-least-squares fitting provides an improvement in both sensitivity
and signal-to-noise ratio (SNR) of 7 dB over ordinary least squares, and 12 dB over the finite-difference method. For all methods investigated in [88], a limit on strain axial resolution of 40 μm was obtained.

Compression OCE has also used dynamic loading [26, 32, 51, 91-93]. In this case, the vibration amplitude resulting from a sinusoidal external load (demonstrated so far in the range 20–800 Hz) is measured and the dynamic strain is calculated from the change in vibration amplitude versus axial depth, analogously to the ultrasound elastography method described in [94] for quasi-static loading. In some instances [26, 92], the strain rate, defined as the rate of change of strain with time, has been calculated, rather than the strain. The relationship between strain and strain rate is analogous to that between displacement and velocity.

A number of other OCT-based techniques to measure a material’s response to compressive loads have been reported. Several studies have used OCT to measure a sample’s response to micro-indentation [73, 95-97]. These techniques have employed point measurements, but may well be extended to provide 2-D or 3-D elastograms in the future. In another technique, polarization-sensitive OCT was used to measure the spatially resolved stress in very stiff (Young’s modulus in the GPa range), etched polymers [98]. The photoelastic effect was utilized to quantify permanent stress. This technique would seem suitable for hard tissues such as bone, but it is unclear if it is relevant to soft tissue.

An advantage of compression OCE over other OCE techniques is that entire tissue volumes can be scanned. To demonstrate this, Fig. 2.3 shows 3-D visualizations of an OCT image and the corresponding strain elastogram. The sample is a soft silicone phantom containing two stiff, rectangular prism-shaped inclusions [99]. Scattering contrast is provided by titanium dioxide mixed into the silicone (see Chapter 4 for phantom fabrication details). The strain axial resolution is 75 μm, and the lateral resolution is 11 μm. The inclusions are more readily visible in the elastogram than in the OCT image, suggesting the potential of compression OCE to provide improved contrast. The example is somewhat artificial, however, as the mechanical and scattering contrast in this case can be independently set.
Compression OCE has demonstrated the highest elastogram spatial resolution of OCE techniques proposed thus far [88]. Recently, compression OCE has been performed using high-resolution, full-field OCT, with the promise of further improvement in the spatial resolution [100].

The innate absence of a measurement of local stress in compression OCE, which precludes the direct measurement of absolute elasticity, may limit its suitability for certain applications, e.g., those involving longitudinal studies. The impact of nonuniform stress in compression OCE is investigated in detail in Chapter 5.

2.2.2 Surface acoustic wave techniques

For techniques using surface acoustic waves, as illustrated in Fig. 2.2(b), a transient (pulsed) or periodic load generates surface waves that are detected using OCT after propagating typically at a few metres per second over a lateral distance of typically ~0.5–20 mm [101, 102], which sets the lateral resolution. Axial discrimination is available from the dependence of the evanescent penetration on frequency. The dependence of the phase velocity on Young’s modulus, in turn, produces dispersion, which depends in a complex way on structure, including in the axial dimension.
The relationship between the phase velocity of the SAW and the Young’s modulus is given in Eq. (1.10). The phase velocity, in the range 1–20 m/s [103, 104], has been measured using several methods, each utilizing phase-sensitive OCT. Manapuram et al. [35] applied a transient load to the tissue and determined the phase velocity from the time taken for a pulse to travel 0.5–1 mm across the corneal surface. In [86], in which a transient load was also applied, the phase velocity was determined from the OCT phase delay, as measured by cross-correlation [105].

Various loading methods have been used to generate SAWs. Contact methods include a metal rod or piezoelectric transducer in direct contact with the sample [21, 33, 35, 86, 106], as shown in Fig. 2.2(b). Non-contact methods include thermoelastic expansion induced by light absorption [101, 103] and a pulsed stream of focused air [104]. The relative merits of the different reported loading mechanisms are not yet clear. One important consideration is that the minimum depth probed is inversely proportional to the surface area over which the load is applied [86], making access to shallow depths challenging for some methods.

The SAW decays exponentially in depth, with an effective penetration depth, \( z_{\text{SAW}} \), approximately equal to the wavelength, \( \lambda_{\text{SAW}} \), i.e., [86]:

\[
z_{\text{SAW}} = \lambda_{\text{SAW}} = c_p f_{\text{SAW}},
\]

where \( f_{\text{SAW}} \) is the SAW frequency. The penetration depth can exceed 10 mm [21]. A consequence of this wavelength dependence is that any variation of elasticity with depth leads to dispersion [107], with high frequencies more dependent upon the superficial layers and low frequencies on the deeper layers. As the phase velocity is influenced by both layer thickness and the ratio of Young’s modulus in different regions [107], inverse methods, such as that proposed by Mohan et al. [21], are required to isolate the depth-resolved Young’s modulus. Inverse methods not only have the potential to provide quantitative depth resolution, but also to greatly exceed the penetration depth of OCT and, therefore, of compression OCE. For example, in [86], the depth-resolved Young’s modulus was estimated in the cutaneous fat below the human skin surface, as shown in Fig. 2.4. A hard nodule is identified, and the method generates contrast to depths of 4 mm, well beyond that provided by OCT.
Fig. 2.4. (a) OCT image of human forearm skin in vivo with a hard nodule in the middle and (b) the corresponding SAW OCE elastogram (colour scale in Pa). Adapted from [86]. ©2012 The Optical Society

The main attractions of SAW OCE are: the potential to measure tissue mechanical properties at depths beyond the OCT imaging limit; its suitability for non-contact (air-pressure or photothermal) loading on delicate tissues such as the cornea [101, 102]; and its potential to directly quantify the Young’s modulus in homogeneous tissues, albeit with relatively low spatial resolution. Surface acoustic waves have been studied in ultrasound elastography [58], albeit to a far less extent than shear waves propagating in a bulk sample [45, 47]. Optical elastography techniques employing surface acoustic waves have also been proposed based on laser speckle imaging [108, 109] and on digital holography [21, 106]. These imaging modalities appear well suited to this form of elastography as, unlike in compression elastography, optical depth sectioning is not required. In fact, the frequency-dependent penetration depth endows such surface imaging methods with depth sectioning when combined with the appropriate inverse method.

SAW OCE may ultimately be most useful for those applications requiring an absolute measurement of Young’s modulus when tissue heterogeneity is not an issue. One of the most promising applications may be to the cornea [101], however, many of the potential confounders and key assumptions mentioned above have yet to be investigated.
A drawback of SAW OCE is the order of magnitude lower lateral resolution (~500 µm) in comparison to compression techniques that can achieve the OCT lateral resolution (~10 µm). This limitation is imposed by the relatively long wavelength (>10 mm), which sets the minimum propagation distance necessary to detect the time delay and dispersion of the surface acoustic waves. Detection of surface waves using full-field OCT (FF-OCT) has recently been proposed for higher-resolution modulus maps [110], but this technique apparently remains limited by the need for a very high-frame-rate camera to sample shorter-wavelength surface waves. A further limitation is that the minimum depth probed is determined by the maximum detectable frequency. For the setup presented in [86], the Young’s modulus could not be estimated at depths <1 mm.

### 2.2.3. Shear wave techniques

The use of surface waves, considered in the last section, has been more prominent to date than the use of bulk shear waves, but recently several groups have begun to examine them utilizing phase-sensitive OCT [25, 54, 55, 111, 112]. The SW speed, $c_s$, defined in (5), is calculated using the expression:

$$c_s = \frac{\omega \Delta r}{\Delta \phi},$$  \hspace{1cm} (2.5)

where $\omega$ is angular frequency and $\Delta \phi$ is the phase shift of the SW over a distance $\Delta r$. The Young’s modulus can then be calculated using Eq. (1.7), Eq. (1.8), and $E = 3G$, assuming incompressible tissues.

A key issue is the differentiation of bulk shear waves from surface acoustic waves, since at the depths probed by OCT both forms may present. Razani et al. avoided surface acoustic waves by employing a transmission geometry to measure the average Young’s modulus of homogeneous gelatin phantoms [55] and subsequently of carotid artery [113]. The authors used a focused ultrasound beam in burst mode to generate the shear wave. Such use of ARF as a loading mechanism is discussed in Section 2.2.5.

Recently, Song et al. have used piezoelectric point loading, similar to that previously used for SAW generation, to generate shear waves [25]. By operating the transducer in the kilohertz regime (5 cycle bursts of 5 kHz sine waves), surface acoustic waves are expected to attenuate rapidly with depth, such that the detected sub-surface lateral motion is expected to relate directly to bulk SW propagation. This
study has begun the important process of examining the various factors influencing the observed OCT path-length differences when surface and SW components are simultaneously generated. This technique has also been extended to use non-contact, ultrasonic loading as a shear wave source [114]. Air-puff excitation has also been as a non-contact method for inducing shear waves in *ex vivo* rabbit cornea [112] and mouse cardiac muscle [115].

Another challenge in using SW to estimate local mechanical properties is the potential for reflection of shear waves by mechanical heterogeneities within the tissue, which can interfere with the incident shear wave and cause artefacts in the reconstruction of local shear modulus. Nguyen *et al.* have proposed a pulse-compression, time-gated approach to image SW propagation using phase-sensitive OCT, where digital compression of a chirped pulse excitation improves the SNR of the displacement measurement, and time gating allows separation of the incident and reflected waves to reduce artefacts in reconstruction [116]. Fig. 2.5 shows a quantitative elastogram of a stiff inclusion ( \( E = 15 \text{ kPa} \) ) embedded in soft agar matrix ( \( E = 4 \text{ kPa} \) ), generated using this pulse-compression approach, in which loading was by a piezoelectric transducer in contact with the sample on the left-hand side. The reconstruction has avoided reflection artefacts, but some artefacts are seen after the inclusion, possibly due to diffraction of the shear wave by the inclusion.

![Image of OCT image and shear modulus map](image)

**Fig. 2.5.** (a) OCT image and (b) shear modulus map of an agar phantom containing a stiff inclusion. The modulus map was generated using a pulse-compression, time-gated approach to measure shear wave propagation. Adapted from [116]. ©2014 SPIE.
Whilst SW methods in OCE are in the early stages of development, closely related methods are commonly used in ultrasound elastography and MRE [69, 117]. It remains to be seen whether shear waves can be effectively utilized in the shallow-penetration regime accessed by OCE, and in highly heterogeneous tissues such as breast. Similarly to SAW OCE, the need to sample shear wave propagation over its wavelength ($\sim 1$ mm) [114] limits the lateral resolution of SW techniques.

2.2.4 Magnetomotive techniques

MM-OCE employs magnetic nanoparticles (MNPs) distributed in the tissue and actuated using an external magnetic field to produce localized, nanometre-scale tissue displacements [87, 118-122]. MM-OCE has no direct analogue in either ultrasound elastography or MRE. It originated from MM-OCT [123], a technique providing molecular image contrast by detecting the motion of targeted functionalized nanoparticles in a time-varying magnetic field [124]. MM-OCT and MM-OCE use similar experimental setups involving an on-off switched solenoid placed above the tissue, as shown in Fig. 2.2(c), as well as similar acquisition methods. The key distinction is that MM-OCT uses the motion of MNPs to provide additional OCT contrast, whereas MM-OCE uses the time-dependence of the motion to determine the Young’s modulus. Elastograms have yet to be demonstrated in MM-OCE using MNPs: only point measurements of a tissue’s mechanical properties have been presented (although elastograms were generated using a macroscopic magnetic actuator embedded in phantoms in [119]).

The local magnetic gradient force, $F$, per unit volume, $V$, in tissue containing MNPs resulting from the gradient magnetic field, $B$, is given by:

$$\frac{F}{V} = [(M_{MNP} + M_T) \cdot \nabla]B,$$  \hspace{1cm} (2.6)

where $M_{MNP}$ and $M_T$ are the volume magnetizations due to the MNPs and tissue, respectively. These magnetizations are opposite in direction: positive (paramagnetic) for MNPs and negative (diamagnetic) for tissue. As a result, the net magnetic force can also be either positive or negative. To avoid ambiguity, it is necessary to ensure that $M_{MNP} > M_T$ [120]. As the magnetic susceptibility of MNPs is $>105$ times larger than that of tissue, this sets a threshold MNP fractional volume of $10^{-5}$ [120], which is an important consideration in the feasibility of applications. To the extent to which the
MNPs are mechanically coupled to the tissue, the magnetic force is assumed to cause tissue deformation on the nanometre scale. A key issue is that the magnitude of tissue deformation is dependent not only on the mechanical properties of the tissue, but also on the MNP concentration.

To determine the Young's modulus, the tissue can be modelled as an under-damped harmonic oscillator according to a Voigt model [93] and its dynamic response, induced by oscillating MNPs, detected with phase-sensitive OCT [87]. As the MNPs contribute negligible inertia compared with the tissue, the measured frequency response can be related directly to the Young's modulus.

A schematic illustration of MM-OCE is shown in Fig. 2.2(c). In the first demonstration [87], the under-damped oscillation of magnetite MNPs (concentration 2 mg/g, average diameter 25 nm) was measured and used to characterize the Young's modulus of silicone phantoms. Results obtained from specific locations within three phantoms are shown in Fig. 2.6. The natural frequency of the phantoms showed a linear dependence on the square root of the Young's modulus [87].

![Fig. 2.6. Normalized measured displacements from samples of different Young's moduli following a step (off-to-on) transition of the applied magnetic field at t = 0. The "0/1" labels on the vertical axis, respectively, indicate the minimum and maximum of the normalized amplitudes of the displacement traces. Taken from [87]. © 2009 The Optical Society.](image)

An advantage of MM-OCE over other techniques is the ability to perform measurements in small samples, as the localized tissue loading avoids the geometrical constraints of external loading. Additionally, the low force applied in MM-OCE makes it suited to the measurement of very soft tissues that are unsuited to measurement by other OCE techniques. These advantages were recently exploited to perform cellular scale MM-OCE, using combined optical coherence microscopy and multiphoton microscopy to target microbeads engulfed by macrophages and measure their
displacements using phase-sensitive detection [118]. Fig. 2.7 shows a combined structural image along with plots of micro bead displacements within the cell.

![Image](image.png)

Fig. 2.7. (a) Co-registered optical coherence microscopy (grayscale) and two-photon excited fluorescence microscopy (red) of a mouse macrophage that has phagocytosed two fluorescent microspheres. The location of the optical beam for recording MM-OCE displacements is indicated by the green arrow. (b) Plot of sinusoidal axial displacement of the microspheres as calculated from phasesensitive data. (c) Frequency spectrum of the magnetomotive signal, showing a peak corresponding to the 5 Hz modulation of the external magnetic field. Taken from [118]. ©2014 IEEE.

A disadvantage of MM-OCE, however, is that only very small displacements can be induced, and measuring them with high dynamic range may be challenging. In addition, the requirements to disperse MNPs at relatively high concentrations throughout the tissue and to achieve a high magnetic field gradient in the tissue present challenges, particularly to in vivo applications. As alluded to above, it remains to be seen if MM-OCE can provide image contrast between different tissues and disease states. To date, only point measurements of mechanical properties have been presented using MNPs, but MM-OCE imaging should be feasible.

2.2.5 Acoustic radiation force techniques

OCE based on loading using ARF has also been demonstrated [53, 55, 83, 85, 125, 126]. A force is generated in the direction of propagation of the ultrasound beam, depending linearly on its absorption and reflectance, and localized by its focus [117, 127]. To observe the effect of the resulting local displacement, the ultrasound carrier
is modulated, typically in burst mode. Depending on the frequency of the burst, the micrometre-scale axial displacement that is generated may be accompanied by orthogonal, radially propagating shear waves. The ultrasound focus may be up to many centimetres in the tissue and achieve a focal spot in the range of hundreds of micrometers to millimetres [55, 127].

The localized displacement in the sample has been imaged using phase-sensitive OCT. In one scheme, termed ARF-OCE, which represents a variant of compression OCE, axial strain in excised human coronary arteries was measured using on-off loading with a 4 MHz transducer at 500 Hz [83]. In another scheme, described in the previous section on shear-wave OCE, the SW velocity was used to determine Young's modulus in phantoms loaded by 400-μs bursts of 20 MHz ultrasound [55]. A variant of ARF loading has also been demonstrated in which the relaxation of layered phantoms was measured using phase-sensitive OCT after ultrasonic excitation by varying frequencies from 3.7 MHz (to probe deeper layers) to 25 MHz (to probe for superficial layers) to access the depth-resolved dynamic response of the sample [128].

Many ARF-based techniques have been developed for ultrasound elastography and magnetic resonance elastography, including acoustic radiation force impulse imaging [127], shear wave elasticity imaging [129] and supersonic shear imaging [27]. In these forms of elastography, using acoustic loading instead of external quasi-static loading increases the displacement amplitudes achievable at locations many centimetres deep within the tissue, a major issue for compression ultrasound elastography [127]. This advantage is of limited relevance to OCE, as it is generally straightforward to achieve adequate displacement amplitudes at the shallow depths probed by OCT (1–2 mm). Another advantage of acoustic loading exploited in ultrasound elastography is that the ultrasound beam creates an effective point load relative to the resolution of the ultrasound imaging system. A point load effectively decouples the measurement of mechanical properties at each spatial location in point-by-point imaging. In OCE, such spatial decoupling does not occur, as the ARF-induced load extends over a region far larger than the volumetric point spread function of the OCT system. For example, in [55], the depth of field of the ultrasound signal was ~3 mm and the full-width at half-maximum beam width was ~0.25 mm, an order of magnitude larger than the OCT resolution. A further important point in practice is the requirement for contact with
the sample in order to provide acoustic impedance matching, usually via a liquid or thin polymer interface between the sample and the ultrasound transducer [114].

2.2.6 Swept-frequency loading techniques

Applying a frequency sweep of the external load in dynamic OCE has been used to demonstrate additional contrast that probes the temporal response of samples [92, 130]. Low frequencies are more sensitive to viscoelasticity, whereas, high frequencies probe the purely elastic response. Sweeping the frequency also probes mechanical resonances. Such techniques have only been recently introduced in OCE. Sweeping the frequency from 20 Hz to 1000 Hz in a phase-sensitive, compression OCE system showed variations in the vibration amplitude and mechanical phase images that were attributed to the resonant frequencies of the rat mammary tissue sample [92, 130]. This technique, termed spectroscopic OCE in [130], has provided preliminary evidence of contrast identifying tumour in an animal model. In a recent study, a form of spectroscopic OCE has been combined with ARF loading and termed resonant ARF-OCE [85]. In this report, extending earlier work [83], the authors probed tissue phantoms and post-mortem human coronary artery at frequencies up to ~6 kHz. In a variant of MM-OCE, termed magnetomotive resonant acoustic spectroscopy, the magnetic oscillation frequency was swept from 0–500 Hz [122]. For known sample geometry, the Young’s modulus and viscous damping coefficient of samples may be determined. This variant has been used to measure the elasticity of blood clots, which could be used in the assessment of vascular defects in cardiovascular diseases [120, 121].

An important consideration for the future development of swept-frequency loading techniques is the extent to which the frequency responses of different regions within the tissue are coupled. Computational models will be useful in assessing this. Also, this technique acquires an elastogram at each frequency probed, implying much larger data sets. This may limit its applicability, particularly in in vivo scenarios.
2.3. Displacement estimation techniques

The accuracy of the displacement measurement is pivotal in many OCE techniques, as, in concert with the validity of the assumptions on the nature of tissue deformation, it determines the accuracy of measurements of the mechanical property of interest (typically Young’s modulus). In this section, we describe the two predominant techniques that have been employed: speckle tracking and phase-sensitive detection. Additionally, we describe a technique that our group recently developed based on the Doppler spectrum arising under dynamic loading that shows improved resilience to noise. Some performance parameters of these displacement estimation techniques are summarized in Table 2.2.

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<th>Table 2.2. OCE DISPLACEMENT ESTIMATION METHODS</th>
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2.3.1 Speckle tracking

OCT speckle is the fine-scale, mottled intensity pattern generated by the coherent summation of backscattered optical wavefields arising from sub-resolution sample scatterers [131]. Speckle’s utility for measuring displacement in OCE arises because each speckle realization is dependent upon the precise location of scatterers within the sample. As the scatterers translate, within a certain range, so does the associated speckle pattern. An example of this is shown in Fig. 2.8, in which a small portion of an OCT speckle pattern acquired from a homogeneously scattering phantom under compressive load (applied from above) is shown in false colour. The pattern is shown for four increments in load, increasing from Fig. 2.8(a) to Fig. 2.8(d). Translation of a single bright speckle is highlighted by a black outline on each figure part. The speckle
pattern's shift between successive acquisitions is equal, then, to the sample displacement. The shift is typically evaluated by calculating the cross-correlation of a small portion of successive B-scans acquired during loading [14]. Speckle tracking was used in initial OCE investigations [14, 71, 72, 132-134] and remains in wide use in ultrasound elastography [47].

Fig. 2.8. Speckle pattern of a silicone phantom (logarithmic intensity scale) under increasing compressive load (applied from above) from (a) to (d). Image dimensions 50 µm x 50 µm. Black outline highlights the evolution of an individual speckle.

The major limitation of speckle tracking is its low dynamic range. The minimum measurable displacement is determined by the spatial resolution and the spatial sampling in the underlying OCT system. For cross-correlation techniques that use a central difference approach and incorporate no a priori information about the sample displacement, this has been shown to correspond to 0.5 pixels [135]. Smaller displacements, with a minimum limited by the Cramer Rao lower bound, are theoretically achievable, assuming small displacements (<0.5 pixel), unbiased, Gaussian noise, and high OCT SNR; simulations have demonstrated the ability of a maximum likelihood estimator to accurately estimate displacements as small as 0.1 pixel under these assumptions [136]. The maximum measurable displacement is set by speckle decorrelation. Perfect translation of the speckle pattern without its alteration assumes that the relative positions of the sub-resolution scatterers remain fixed whilst they translate. The correlation needed to assess translation is gradually lost as the translation increases; such gradual decorrelation of the speckle pattern with increasing translation is evident in the example in Fig. 2.8. A practical upper limit on this has been found empirically to be equal to ~0.5 times the resolution of the OCT system [137]. Assuming an OCT image pixel size of 2 µm and isotropic spatial resolution of 10 µm results in a very low displacement dynamic range of ~5. Additionally, as the displacement is calculated over a certain window within the B-
scan, the spatial resolution of the displacement measurement is lower than the OCT image resolution, typically by a factor of 5–10 [14, 72].

An advantage of speckle tracking is that motion can be tracked in more than one spatial dimension, making feasible the simultaneous measurement of axial and shear deformation. Speckle tracking in OCE in three dimensions has yet to be demonstrated, but it has been proposed and implemented in ultrasound elastography [138]. A drawback in ultrasound elastography is the long acquisition time, limited by the speed of sound, for 3-D imaging. The order-of-magnitude higher acquisition speed of OCE alleviates this problem and may enable provision of additional OCE contrast in anisotropic tissues, such as skin and muscle, as has already been demonstrated in ultrasound [139] and MRE [140]. This ability to track displacement in multiple dimensions may well sustain interest in speckle tracking, despite the significantly lower dynamic range.

Recently, a novel technique has been proposed to estimate strain directly from the speckle decorrelation [141, 142]. Such decorrelation caused by strain has previously been demonstrated as a means of speckle reduction in OCT [137, 143]. This technique avoids the need to directly estimate tissue displacement. It seems likely that co-registration methods will be required to isolate the speckle decorrelation caused by strain from other sources, such as gross motion, as was required in [137, 143]. A method closely related to speckle tracking, in compression OCE using full-field OCT, has recently been proposed [100]. In this method, digital volume correlation [144] was used to measure tissue displacement. Whilst not solely dependent upon speckle, the efficacy of this technique is largely determined by speckle correlation between consecutive volumes. A digital volume correlation approach to OCE has also recently been used to measure strain in three dimensions and estimate both Young's modulus and Poisson's ratio in phantoms undergoing tensile loading [145].

2.3.2 Phase-sensitive detection
Speckle tracking utilizes changes in the intensity of an OCT image with loading. In Fourier-domain (FD) OCT, after performing a Fourier transform on the detected spectral fringes, the depth-resolved complex signal is obtained. This provides the opportunity to analyse the phase of the detected signal. OCT phase is generally random in soft tissue; however, it is temporally invariant if the sample is stationary. If
a sample is subjected to mechanical loading, its axial displacement between two A-scans, acquired from the same lateral position, results in a phase shift, $\Delta \phi$. The displacement, $u_z$, along the axis of the incident beam is defined as [146]

$$u_z = \frac{\Delta \phi \bar{\lambda}}{4\pi n} \quad (2.7)$$

where $\bar{\lambda}$ is the mean wavelength of the source and $n$ is the average refractive index along the beam path. The phase difference is calculated either between two successive A-scans in a B-scan (requiring high axial sampling density) [146] or between two A-scans acquired in the same lateral position in successive B-scans [147]: the latter is illustrated in Fig. 2.9 with experimental data acquired from a silicone phantom containing a stiff inclusion (data generated from same phantom as used in Fig. 5.3; see Chapter 5 for experimental details). Phase-sensitive detection was initially developed for Doppler flow velocity measurement in OCT [148]. Indeed, the phase-sensitive technique is based on the Doppler shift; thus, unlike speckle tracking, only the axial component of the displacement can be detected. Phase-sensitive detection is used in the majority of recent reports on OCE.

Fig. 2.9. Illustration of phase-sensitive detection in compression OCE with experimental data from a silicone phantom containing a stiff inclusion. (a) The phase of OCT B-scans acquired at the same lateral position before (1) and after (2) sample loading; (b) phase difference between the B-scans shown in (a). As the load was applied from above, the phase difference is a maximum at the top of the image.

If we assume that the maximum measurable displacement is set by the maximum unambiguous phase difference, i.e., $2\pi$, then this corresponds to half the source centre wavelength (in the sample medium). The minimum measurable displacement is determined by the phase sensitivity of the OCT system, which, in the shot-noise limited regime, is related to the OCT SNR (intensity ratio) and is approximated as [149].
Park et al., in their work on flow imaging [150], demonstrated good agreement between Eq. (2.8) and experimental results for OCT SNR >30 dB. It is important to emphasize, however, that this approximation is only valid for large SNR.

Another source of phase noise is introduced under the successive A-scan scenario if the temporally displaced beams are not precisely overlapped in space. In practice, to minimize phase noise due to scanning, dense sampling is performed along the axis used to calculate the phase difference. A further source of phase noise is introduced by mechanical instabilities in the system, such as jitter in the scanning mirrors. Under the conditions of negligible phase noise due to beam overlap and mechanical instabilities, and maximized OCT SNR, a displacement sensitivity of ~20 pm has been reported [28]. Techniques employed to minimize mechanical noise include the use of a common-path interferometer and a reference reflector within the sample arm. In practice, the noise due to offset of the beam overlap is often appreciable, due to the requirement to laterally scan across the sample, such that typical displacement sensitivities lie in the range 0.1-1 nm.

A key advantage of phase-sensitive detection over speckle tracking is its larger dynamic range. If we consider the phase difference between two A-scans acquired using an OCT system with mean wavelength of 1300 nm, and minimum OCT SNR of 30 dB, the displacement dynamic range is >60, almost 20-fold larger than that achievable using the cross-correlation method for speckle tracking described in Section 2.3.1. Additionally, the spatial resolution of displacement matches that of the underlying OCT system; the displacement is not estimated in a predefined window, as it is in speckle tracking. A major limitation is imposed by phase wrapping, which invalidates the assumption of a linear relationship between the phase difference and displacement described in (2.7). Phase jumps of $2\pi$ occur not only when the desired phase difference is close to $2\pi$, but also as the result of noise when the OCT SNR is low. In the case of dynamic loading, phase wrapping can be reduced by faster acquisition since, for constant loading frequency, the displacement occurring between acquisitions is reduced. At low OCT SNR, our group has shown that the application of intensity thresholding and weighting, to give preference to the phase difference
estimated from pixels with high SNR, is also effective [88]. Chapter 6 presents further improvements to the dynamic range of displacement measurements using phase-sensitive detection.

2.3.3 Doppler spectrum detection

A major limitation of phase-sensitive detection is the large drop-off in accuracy with decreasing SNR (Eq. (2.8)). Recently, with the goal of reducing this drop-off, our group proposed a technique for extracting the displacement from the measured intensity under dynamic mechanical loading. Joint spectral and time domain optical coherence elastography (STdOCE) improves vibration amplitude measurement in dynamic OCE [91] by adapting the technique previously proposed for Doppler flow imaging [151]. STdOCE provides more accurate vibration amplitude measurements than the phase-sensitive technique in the case of low OCT SNR (<20 dB), thereby extending the depth range of accurate dynamic OCE measurements.

Dynamic loading results in an amplitude spectrum of frequency tones described by Bessel functions of the first kind. In STdOCE, a Fourier transform is performed in the time domain, i.e., across multiple A-scans. If the A-scan rate is much higher than the loading rate, the result is a Doppler spectrum containing frequency tones, which can be used to extract the vibration amplitude at each depth in the sample.

Figure 2.10 demonstrates the superiority of STdOCE over a representative technique for phase-sensitive detection [28]. Data was acquired using the spectral domain OCT system and OCE loading setup detailed at the end of this chapter (Section 2.5). For both cases, a 50 Hz sinusoidal wave was applied to the actuator. See [91] for details of phantom fabrication and signal processing. In Fig. 2.10(a), a structural OCT image of a soft phantom containing a stiff inclusion is shown. The inclusion is located in the centre of the image and is indicated by the labelled arrow. Below the inclusion, a shadow artefact, corresponding to a region of low OCT SNR, is also labelled. A plot of the OCT SNR at the lateral position indicated by the vertical red arrow in Fig. 2.10(a) is shown in Fig. 2.10(d). Vibration amplitude images generated using STdOCE and phase-sensitive OCE are shown in Figs. 2.10(b) and 2.10(f), respectively. In both images, the stiff inclusions are denoted by the lower rate of change in vibration amplitude with depth, i.e., lower strain. Vibration amplitude plots generated using both techniques, at the lateral position indicated by the red arrow in Fig. 2.10(a), are
shown in Fig. 2.10(e). Both plots match well until a depth of ~600 μm. At depths >600 μm, the decrease in vibration amplitude is higher for the phase-sensitive technique (red). This is caused by an underestimation of the vibration amplitude in the low OCT SNR region below the inclusion. In comparison, the decrease in vibration amplitude with depth measured with STD OCE below the inclusion is the same as that above the inclusion, as expected for these mechanically uniform regions. The strain elastograms corresponding to Figs. 2.10(b) and 2.10(f) are shown in Figs. 2.10(c) and 2.10(g), respectively. The artificially high strain in the phase-sensitive elastogram (Fig. 11(g)) is clearly visible below the inclusion. As the elastogram is used as a surrogate for elasticity, this leads to errors in the interpretation of elastograms.

Fig. 2.10. Soft phantom containing a stiff inclusion: (a) OCT structural image; (b) Vibration amplitude image; and (c) Elastogram for STD OCE; (d) OCT A-scan; and (e) Vibration amplitude plots for STD OCE (blue) and phase-sensitive OCE (red) at the lateral position indicated by the red arrow in (a), where the dashed lines in (d) and (e) indicate the boundaries between the soft bulk and hard inclusion; (f) Vibration amplitude image; and (g) Elastogram for phase-sensitive OCE. Reproduced from [91].

2.4. Potential probes for in vivo OCE
Translation of OCE techniques to clinical use requires further development of practical probes that enable simultaneous loading and imaging of tissue. This section reviews initial efforts to implement OCE in vivo, using probes for imaging superficial tissues such as skin, and catheter-based probes for imaging within the body. The potential for implementation in OCT needle probes is also introduced.
2.4.1 Imaging superficial tissues

Most initial OCE studies performed imaging and loading of small samples from opposite sides [32, 93, 146]. Whilst this configuration may be sufficient for imaging small ex vivo tissue samples or phantoms, imaging and loading from the same side is essential for in vivo imaging. In the first OCE paper [14], loading and imaging were performed from the same side using an annular piezoelectric actuator, but an A-scan rate of 11 Hz and decorrelation of speckle due to sample movement limited the in vivo capabilities of this set-up. A same-side OCE set-up was later proposed using a similar design [51], as shown in Fig. 2.11. An annular piezoelectric transducer is fixed to an imaging window and coupled directly to the sample, enabling simultaneous, concentric actuation and imaging of the tissue. This ring actuator design can be used to apply microscale displacements to tissue over a wide range of frequencies, and has been used in compression and spectroscopic OCE studies [26, 130], including the first in vivo 3-D OCE measurements, which used human skin as the target tissue [26], as seen in Figs. 2.11(b)-(d).

![Fig. 2.11. (a) Schematic of a ring actuator proposed for imaging and loading from the same side in compression OCE. (b) OCT; (c) OCE; and (d) overlaid images of in vivo skin on the middle finger acquired using this ring actuator design. In (a), the stratum corneum (SC), living epidermis (LE), imaging plate and a sweat gland are labelled. Image dimensions are 1.4 mm × 1.4 mm. Adapted from [26].](image)

To perform SW or SAW OCE requires either an impulse load or vibrational load applied to the tissue. SAW OCE on skin in vivo has been achieved using a mechanical shaker coupled directly to the tissue [34]. The mechanical shaker employed a piezoelectric element to introduce an impulse load to the tissue, and the resulting surface waves were measured by the adjacent OCT probe. For applications where direct contact of the probe with tissue is not desirable, such as imaging of the cornea, a non-contact implementation of SAW OCE has also been proposed using a remote laser pulse to generate surface waves [101].
Other loading techniques for superficial tissues have included an air puff system for measuring the elastic response of the cornea [73, 104] as well as an annular suction device which applies a suction force concentric with the OCT imaging to measure elasticity of skin in tension [76].

2.4.2 Imaging within the body: Catheter-based OCE

Implementation of OCE using miniaturized, fibre-based probes for endoscopic and intravascular imaging presents new challenges for developing loading schemes that can be deployed in such confined spaces. One natural approach to overcoming this issue is to use physiological deformation of the tissue as the "loading" mechanism. This is especially suitable for tissues that undergo regular changes in luminal pressure, such as airways and blood vessels. This concept has been used for measurement of the mechanical properties of the airway wall using anatomical OCT (aOCT), a long-range OCT technique for profiling of hollow organs [152]. Here, changes in airway dimensions versus pressure were measured in patients with and without obstructive lung diseases to investigate the correspondence of airway wall compliance with pathology.

Luminal pressure changes have also been proposed as a loading technique for performing intravascular OCE [71, 153]. Although physiological deformation of tissue is convenient to measure, quantitative measurement of mechanical properties requires controlled loading of the tissue. An OCT imaging catheter that achieves this has been proposed for applying acoustic radiation force at localized points deep in the body [154]. A catheter design incorporating a piezoelectric ultrasound transducer into the distal probe head has the advantage that direct contact with the tissue is not required, as the acoustic radiation force may be focused to induce a tissue "push" at a desired distance from the probe. Such a probe design, shown in Fig. 2.12, has been realized for combined OCT and ultrasound structural imaging of arteries [154], but not yet been used to perform elastography. However, the same group has demonstrated OCE using a concentric ARF loading and OCT detection, further facilitating translation of OCE into catheter-based probes [155]. Another proposed technique for catheter-based OCE incorporates a small loading device in the distal end of the probe, which may consist of a controlled liquid jet or mechanical indenter for
compressing adjacent tissues [156]. Despite the several proposed embodiments of catheter-based OCE, results acquired using such a probe have yet to be reported.

![Diagram of combined ultrasound and OCT probe](image)

**Fig. 2.12.** (a) Schematic of combined ultrasound and OCT probe that may be suitable for performing catheter-based ARF-OCE measurements. PTFE: polytetrafluoroethylene; GRIN: graded-index. Adapted from [154]. ©2012 SPIE.

The recent development of OCT needle probes has allowed three-dimensional OCT imaging deep within solid tissues, extending potential OCT applications to include, e.g., biopsy and surgical guidance and monitoring of interstitial procedures [157, 158]. However, at the outset of this thesis, implementation of OCE in a needle probe had not been accomplished. In Chapter 9 we will present an overview of OCT needle probe designs and describe the development of the first needle-based OCE technique.

### 2.5 OCE system used in this thesis

All OCE measurements in this work were performed using compression for loading and phase-sensitive detection for displacement estimation. As described in Section 2.2.1, compression OCE has some key advantages over other loading techniques: it has the highest resolution reported to date, as it maintains the native OCT lateral resolution; and its straightforward implementation facilitates 3-D scanning of tissue volumes. For displacement estimation, phase-sensitive detection provides a much larger dynamic range than speckle tracking and requires far less data than the Doppler spectrum technique, making it an attractive method for detecting a wide range of mechanical contrast within tissue in a time- and data-efficient manner. These features make phase-sensitive compression OCE a good candidate for translation into clinical scenarios. In this section, we provide details of the OCT system and OCE setup used throughout this thesis.
2.5.1 OCT system
A schematic diagram of the custom-built, fibre-based FD-OCT system used for all measurements in this thesis is presented in Fig. 2.13. The system employs a superluminescent diode source (S-840-B-I-20, Superlum, Ireland) with mean wavelength of 835 nm and 50 nm bandwidth, illuminating the sample with 10 mW of optical power. The spectrometer (Hyperion 830, Thorlabs HL AG, Germany) incorporates a high-speed, complementary metal-oxide semiconductor (CMOS) line scan camera (spL4096-140 km, Basler Vision Technologies, Germany), with the optical spectrum dispersed by a 1500 lines/mm transmission grating over 1800 pixels in a wave number-linear arrangement. The measured axial and lateral resolutions (full-width at half-maximum irradiance) are 7.8 μm (in air) and 11 μm, respectively. The measured OCT sensitivity is 102 dB for an exposure time of 36 μs.

For the majority of OCE measurements in this thesis, the system was operated in a common-path configuration [159], with the reflected beam from the back surface of a 2-mm thick imaging window used as a reference (CPR in Fig. 2.13), which was fixed to the top surface of the ring actuator for OCE loading (RA in Fig. 2.13). (For needle OCE, the fibre-glue interface at the distal end of the probe provided a reference reflection; refer to Chapter 9 for details). The alternative dual-arm configuration used a separate reference mirror (DAR in Fig. 2.13).

Lateral scanning in the sample arm was performed in a raster fashion by a xy galvo-scanning mirror pair (GM in Fig. 2.13) contained within an enclosed scan head. The x and y axes refer to the fast and slow lateral scanning directions, respectively, and the z axis refers to the direction of light propagation. Note that this same OCT system was used for needle OCE measurements, but with the sample arm replaced by the needle probe. The sample arm for needle-based OCE, including the probe design, is detailed in Chapter 9.
2.5.2 OCE hardware and scanning protocol

For compression OCE, samples were mechanically loaded using a piezoelectric ring actuator (Piezomechanik, Germany) to enable same-side imaging and loading, as described in Section 2.4.1. A schematic and photograph of this setup are shown in Fig. 2.14. A 2-mm thick, 20-mm diameter, glass imaging window was rigidly fixed to the ring actuator, which has a 15-mm aperture. For imaging of phantoms or small ex vivo samples, a rigid plate (RP in Fig. 2.13) was fixed opposite to the imaging window and used to preload the sample and hold it in place during imaging. The configuration shown in Fig. 2.14 can be used for in vivo imaging, where underlying bones can provide a rigid back surface for compression [51].

Fig. 2.13. OCT system schematic showing both common-path reference (CPR) and dual-arm reference (DAR) reflectors. SLD, superluminescent diode; RP, rigid plate; RA, ring actuator; L, lens; GM, galvanometer mirror pair.

Fig. 2.14. Ring actuator for compression OCE (shown in a configuration for in vivo imaging). (a) Probe schematic, including lens, ring actuator, and imaging window. (b) Photograph of set up.
A 5-Hz square wave, synchronized with lateral scanning as shown in Fig. 2.15, was then applied to the actuator, producing axial displacements with amplitudes up to 2.2 µm. The loading frequency was chosen to remain in the quasi-static loading regime, i.e., low enough to avoid wave propagation in the sample. The resulting sample displacement was calculated from the phase difference between A-scans at the same lateral position acquired in successive B-scans (i.e., between the uncompressed and compressed states). Note that by using this procedure, the phase sensitivity is not affected by the lateral oversampling in the x-direction, as is the case when the phase difference is measured between A-scans acquired within one B-scan [150]. However, when performing 3-D OCE measurements, scanning in the y-direction degrades phase sensitivity, and oversampling in this axis is required to minimize this reduction in phase sensitivity.

![Graph](image)

Fig. 2.15. Saw-tooth pattern of lateral scanning beam synchronized with motion of ring actuator (RA). Adapted from [88].

To facilitate clinical scanning, the system was housed on a portable trolley with dimensions (height × width × depth) of 1.20 m × 0.7 m × 0.9 m, as shown in Fig. 2.16.
Fig. 2.16. FD-OCT system on portable trolley for clinical scanning.
Chapter 3

Optical coherence elastography for guidance of breast cancer surgery

Breast cancer is the most widely diagnosed form of invasive cancer in women in the United States, Europe, and Australia [160-162]. It is also the leading cause of cancer-related deaths in women in Europe [161], and second only to lung cancer in the United States and Australia [160, 162]. One of the most prominent challenges in the diagnosis and treatment of the disease is localization of the tumour. Identification of suspicious lesions forms the basis of screening programs, and pinpointing the lesion is important for guiding biopsy and obtaining an accurate diagnosis. At the stage of surgical removal of the tumour, its precise localization is again imperative to ensure that all the cancerous tissue is removed. Some of the major techniques used to detect and localize breast cancer are listed in the diagram in Fig. 3.1.

![Tools for detecting breast cancer](image)

Fig. 3.1. Select tools for detecting breast cancer at the stages of screening, diagnosis, and surgery.

The work in this thesis focuses on detecting the precise location and extent of the tumour at the time of surgery, with the aim of developing a tool to guide a surgeon’s resection of the lesion. Surgical treatment of breast cancer may require a mastectomy, i.e., complete removal of the breast, depending on the size and spread of the tumour. If the cancer is localized within the breast, women who meet the appropriate criteria
may elect to undergo breast-conserving surgery (BCS). Instead of complete removal of the breast, BCS involves a lumpectomy, or surgical resection of the tumour along with a margin of the surrounding healthy tissue, usually followed by radiation therapy. Women may choose BCS to avoid the cosmetic and psychological difficulties, as well as the risk of wound infection associated with mastectomy [163]. A major disadvantage of BCS, however, is the risk of local recurrence of cancer if not all of the malignant tissue is removed. The status of the tumour margin, the distance between the tumour and the boundary of the excised tissue, is a key predictor for local recurrence. A negative tumour margin, illustrated in Fig. 3.2(a), is the desired result, in which postoperative histological analysis shows no cancerous tissue at the boundary of the excised lesion, suggesting that the cancerous tissue is totally enclosed within the excised tissue. Fig. 3.2(b) illustrates the case of a positive margin in which cancerous tissue is found at the boundary of the excised tissue, indicating that residual disease remains in the patient, and a second surgery may be required. It has been shown that tumour margins of <5 mm are indicative of residual disease and an increased risk of local recurrence [29]. However, it should be noted that the definition of a clinically acceptable margin varies between hospitals, and may be anywhere in the range 0-5 mm. Currently, approximately 23% of women who undergo BCS must return for additional surgery due to insufficient margins [30]. Improved guidance of tumour excision and intraoperative assessment of tumour margins have the potential to reduce the need for re-excision.

![Fig. 3.2. Illustrations of (a) negative and (b) positive tumour margins following breast surgery. Available www.breastcancer.org/symptoms/diagnosis/margins. Used with permission from breastcancer.org.](image)

In the following sections, we motivate the development of OCE as a potential tool for intraoperative guidance of BCS. We describe the mechanical properties of breast
tissue across different scales, and consider the mechanical heterogeneity of the breast on the scale probed by OCE. We then review current and recently proposed techniques for guidance of BCS, including OCT.

3.1. Mechanical properties of the breast
3.1.1 Breast tissue constituents and structure

The breast consists primarily of adipose, or fatty tissue, and fibroglandular tissues including connective tissues (primarily a combination of collagen and elastin), lobular units (consisting of groups of lobules that produce milk in lactating women) and ducts (vessels for transporting milk). Blood vessels, nerves, and lymphatic vessels are also found throughout the breast. Fig. 3.3 highlights some of the main elements of breast anatomy.

![Diagram of breast anatomy](image)

**Fig. 3.3.** Elements of breast anatomy. Illustration by Patrick J. Lynch, medical illustrator.

Breast cancer initiates in the epithelial cells of the breast, *i.e.*, the cells that line the inner walls of the lobules and ducts. Malignant cells that are confined within the ducts or lobules are referred to as ductal carcinoma *in situ* (DCIS) or lobular carcinoma *in situ* (LCIS), respectively. If malignant cells have broken through the basement membrane of the epithelium into the surrounding stroma, this is known as invasive ductal carcinoma (IDC), which also the most common type of breast cancer [164], or invasive lobular carcinoma (ILC). Invasive cancers have the potential to metastasize, or spread to other organs within the body. Lymph nodes are typically the first site outside the breast to which the cancer may spread; thus, during breast cancer surgery,
the sentinel (or first-draining) lymph node is commonly assessed for metastatic cancer intraoperatively, a procedure known as sentinel lymph node biopsy [165].

3.1.2 Imaging the mechanical properties of breast cancer

Like many diseases, breast cancer modifies the mechanical properties of tissue, from the cellular to the macroscopic scales. Considering again the tools for detection of breast cancer listed in Fig. 3.1, we note that manual palpation is utilized at all stages of diagnosis and patient treatment, from self-examination, the broadest form of breast cancer screening, to palpation by the surgeon to help guide resection of the tumour. The prevalent use of palpation in the clinical management of breast cancer helped to motivate much of the early work in ultrasound elastography and MR elastography (MRE) of breast cancer. Both techniques have shown promise in providing additional contrast to complement the underlying imaging technique and to aid in the diagnosis of breast lesions [3, 69, 140, 166]. Examples of ultrasound elastography and MRE of the breast compared to the underlying modalities are shown in Figs. 3.4(a) and 3.4(b), respectively.

![Ultrasound and Elastogram](image1)

![MRI and Elastogram](image2)

**Fig. 3.4.** (a) Ultrasound image (left) and elasticity map (right) of breast tissue containing a 5-mm lesion diagnosed as invasive ductal carcinoma. The lesion is difficult to detect in the ultrasound image, but is clearly delineated in the elasticity map. Adapted from [166] with permission from Elsevier. (b) MR magnitude image (left) and shear modulus map (right) of a breast with a cancerous lesion, showing added contrast in the modulus map. Adapted from [140] with permission from Elsevier.

Distinguishing healthy and malignant breast tissue based on mechanical properties is not a binary problem. Breast cancer can develop through varied pathways and
manifest with a range of mechanical properties. Benign lesions within the breast can also exhibit stiffness values in the range of some malignant tumours. Fig. 3.5 depicts data acquired from indentation testing of 169 breast tissue samples and demonstrates the wide range of stiffness values found within normal and malignant tissue types [167].

![Graph showing stiffness variation in breast tissue types](image)

Fig. 3.5. Stiffness variation in breast tissue types. Data from indentation testing (5-mm diameter cylindrical indenter) of 169 breast tissue samples: 71 normal fat, 26 normal fibroglandular tissue, 16 fibroadenoma, 12 low-grade invasive ductal carcinoma (IDC), 4 invasive lobular carcinoma (ILC), 4 ductal carcinoma \textit{in situ} (DCIS), 4 fibrocystic disease, 21 intermediate grade IDC, 9 high-grade IDC, 1 invasive mucinous carcinoma (IMC), 1 fat necrosis. Data from [169].

Importantly, the mechanical properties of the breast depend not only on the tissue type, but also the scale on which the properties are probed. The data in Fig. 3.5 were obtained using a 5-mm diameter, cylindrical indenter on samples that appeared macroscopically homogeneous. On the other extreme of scale, indentation of breast cancer tissues using atomic force microscopy (AFM) has revealed the mechanical heterogeneity of the cellular makeup within normal breast tissue, benign, and malignant lesions [168]. Fig. 3.6 shows histograms of stiffness values measured using AFM in each of these tissue types, obtained by biopsy of breast cancer patients. This data shows a distribution of stiffness values within each tissue type, including a multimodal distribution for malignant tumour, which can be attributed to the mixture of cell types, normal and malignant, making up the tissue.
Fig. 3.6. Stiffness variation in breast tissue types on the cellular scale. Data from AFM of breast biopsy samples. Top left: stiffness distribution for normal breast tissue is unimodal. Top right: post-AFM H&E-stained section reveals the terminal ductal lobular unit of a normal breast fenced by interstitial connective tissue. Middle left: biopsy-wide histogram for a benign lesion reveals a unimodal but broader stiffness distribution with an increase in stiffness compared with the healthy biopsy. Middle right: H&E-stained section reveals extensive fibrotic stroma interspersed with fibroblasts typical for fibroadenoma. Bottom left: heterogeneous stiffness distribution with a characteristic soft peak for malignant tumour tissue is consistent with histopathology (bottom right), revealing an invasive breast carcinoma with infiltrating nests of cancer cells that have evoked a dense fibrous tissue response. Scale bar for all images, 50 μm. Reprinted from [168] by permission from Macmillan Publishers Ltd.

These studies across spatial scales result in disparate values of stiffness for malignant breast tissue. At the macroscale, tumour tissues are typically stiffer than healthy tissues, which can likely be attributed to desmoplasia, the rapid production of collagen as stroma to structurally support the growing tumour. This dense stroma causes many tumours to feel stiff to the touch [169]. However, the AFM study reveals that individual tumour cells tend to be softer than normal epithelial cells in the breast,
as shown by the characteristic peak for the malignant cells in Fig. 3.6 [168]. Reports have suggested that this lower stiffness facilitates their migration and metastasis [68].

OCE probes mechanical properties at a scale between those of cellular-scale, laboratory techniques and macroscopic clinical modalities. Applied to breast imaging, OCE is expected to be sensitive to mechanical contrast due to the microstructural components of the breast. That is, OCE cannot probe the stiffness of individual cells and intracellular spaces, nor is it expected to obtain a single stiffness value for a macroscopically homogeneous sample, as those in Fig. 3.5. Rather, OCE is expected to visualize the mechanics of groups of cells and structures such as lobules, ducts, and blood vessels. Fig. 3.7 illustrates this niche of OCE within mechanical imaging modalities of the breast. In the next section, we will consider the utility of imaging mechanical properties on this scale for guidance of breast-conserving surgery.

![Image](image.png)

*Fig. 3.7. OCE has potential to visualize groups of cells and microstructures within breast tissue, between the cellular and macrostructural scales probed by other mechanical imaging techniques.*

### 3.2. Guidance techniques for breast-conserving surgery

Clinicians take several measures to ensure that breast-conserving surgery results in a clear margin. Currently, surgeons manually palpate the tissue to find the boundaries of the tumour and to guide excision, as many breast tumours manifest as stiff lesions. However, some cancers present as nonpalpable lesions, i.e., they are too small or soft to detect through touch or may have a permeative growth pattern that results in a poorly delineated mass with small extensions of malignancy [170]. In these nonpalpable cases, hookwire guidance is commonly used, in which a wire is placed in the tumour mass under radiological guidance prior to surgery. The surgeon then excises an area of tissue around the wire according to the tumour size estimated by
preoperative imaging. However, tissue can deform between preoperative imaging and surgery, causing displacement of the wire. An alternative method for localization of nonpalpable lesions is radioguided occult lesion localization (ROLL), in which a small radioactive seed is injected into the tumour under radiological guidance, and the surgeon uses a gamma probe to detect the seed during surgery [171]. This technique has been shown to provide faster and more accurate removal of nonpalpable lesions than hookwire guidance [172]. However, these techniques do not provide the surgeon with the precise location of the tumour boundaries to guide excision. Preoperative imaging can also be imprecise for localizing tumour boundaries; extensions of malignancy beyond the bulk tumour mass are not always detectable using conventional medical imaging modalities such as ultrasound, x-ray, and magnetic resonance imaging [163]. Intraoperative, in vivo ultrasound guidance of excision has been shown to reduce re-excision rates for invasive cancers [173], but ultrasound typically cannot visualize DCIS or small multifocal cancers [174]. Furthermore, if the size of the lesion is overestimated, then an unnecessarily large amount of tissue may be removed, degrading the preservation of cosmesis.

Once the lesion is excised, intraoperative assessment of tumour margins is sometimes performed using frozen-section analysis, but this process is time-consuming, taking ~25 min on average [175] and, importantly, has had only limited success in reducing overall re-excision rates [176]. Intraoperative imaging of the excised lesion has also been performed using ultrasound and X-ray and has resulted in some improvement to the rate of clear margins [163]. However, ultrasound is not suitable for non-infiltrating cancers such as DCIS [177], and X-ray lacks specificity in detecting benign versus malignant lesions [163]. Thus, there remains a need for an intraoperative guidance technique that can ensure that adequate tumour margins are obtained and, ultimately, reduce the number of re-excisions performed. Such a technique could be in vivo guidance of excision, ex vivo assessment of the excised tumour margins, or a combination.

Recently, several optical imaging techniques have demonstrated potential for imaging of breast cancer. Optical techniques have the advantages of being safe (non-ionizing) and relatively low cost and can provide structural and functional information in images of breast tissues. Optical techniques that have been proposed for
Intraoperative assessment of breast tumour margins include Raman spectroscopy [178], diffuse reflectance spectroscopy [179, 180], and spatial frequency domain imaging [181]. In addition, the development of near-infrared molecular probes sensitive to growth factors involved in breast cancer [182] has led to developments in near-infrared fluorescence (NIRF) optical imaging [183] to illuminate the cancer cells and ensure their removal during surgery. However, as these methods rely on analysis of diffuse light transport, they are limited in their ability to resolve important morphological features with depth. Photoacoustic imaging (PAI) [184] also has potential for intraoperative tumour assessment, as it can be used for functional and molecular imaging of tissue and can image tumour microvasculature [185]. The majority of PAI studies in breast cancer have focused on providing improved screening and diagnosis [186, 187], but the potential for intraoperative margin assessment has recently been demonstrated using a miniaturized intraoperative system [188]. Initial results in a mouse model of breast cancer showed the ability to localize the tumour boundaries in three dimensions, with a penetration depth of up to 2.3 mm; however, the authors suggested that exogenous contrast agents such as iron oxide nanoparticles should be used to improve contrast.

In the past ten years, several studies have shown potential for OCT in imaging breast cancer [189]. OCT and its extensions, including OCE, may be suitable for intraoperative tumour assessment compared to other optical techniques due to the combination of depth-sectioning capability with microscale resolution and the ability to be implemented in compact, fibre-based probes.

A number of studies have investigated the ability of OCT, optical coherence microscopy (OCM), and full-field OCT (FF-OCT) to visualize structural features in normal and malignant breast tissues [190-192]. These studies found that OCT can identify structures such as ducts, lobules, and vessels. They also showed that adipose is clearly distinguishable from other tissues due to its characteristic texture and low backscattering within the cytoplasm of the adipose cells. In addition, regions of invasive tumour were often identified by much higher and heterogeneous backscattering than normal fibroglandular tissues of the breast [189].

The OCT setups for breast imaging in [190-192] could be used to image the margins of the excised tissue immediately following excision, providing assessment of the first
1-2 mm in depth in the tissue. However, this small penetration depth limits the utility of bulk optics setups for *in vivo* imaging of the lesion prior to excision. To perform OCT deep within solid tissues, OCT needle probes have recently been developed [36]. Our group has shown the potential for needle OCT imaging of tumour margins in freshly excised breast tissues, with promising initial results [158, 193]. An example of an OCT image of a tumour margin in a human mastectomy sample, obtained using a 640-μm-diameter OCT needle probe, is shown in Fig. 3.8(b), along with corresponding histology, Fig. 3.8(a). The tumour boundary is clearly delineated in the OCT image. Other features, including a blood vessel, connective tissue (stroma), and the characteristic honeycomb structure of adipose tissue, are also visualized in the OCT image.

Fig. 3.8. (a) H&E histology of a tumour margin; (b) longitudinal reconstructed OCT image of the tumour margin, with the horizontal axis aligned with the direction of needle retraction during scanning. Taken from [158] with permission. ©2012 IEEE.

Whilst OCT has shown potential for differentiating tumour from healthy tissue in breast cancer, contrast between tissue types is not always apparent based on OCT intensity. For example, carcinoma *in situ* and normal breast lobules both appear as highly optically scattering regions in OCT, possibly because both tissues have high cellular density [192]. To address the need for additional contrast in OCT imaging of cancer, several methods for tissue classification have been proposed, such as measurement of refractive index [194], optical attenuation [195], spatial frequency content [196], and fractal analysis based on textural differences [197].

The potential to differentiate tissues based on the wide range of mechanical properties within the breast on this scale remains largely untapped. Preliminary results using OCE have shown potential for differentiating gross regions of tumour versus non-tumour in excised breast tissues based on, respectively, elasticity using a viscoelastic model [93], mechanical resonance [130], and strain [198]. An elastogram

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and corresponding histology of excised human breast tumour from [93] is shown in Fig. 3.9. This represents, in the author’s opinion, the most convincing OCE result in breast tissue prior to commencement of this research.

Fig. 3.9. (a) H&E histology and (b) elasticity map of excised human breast tissue containing a region of fat on the left and tumour on the right. Adapted from [93]. ©2008 The Optical Society.

A goal of this thesis is to elucidate mechanical limitations of the image quality in OCE and to improve upon the existing OCE image quality, with an emphasis on visualizing breast tissue mechanics. In parallel, we aim to develop and apply practical methods to facilitate clinical implementation, including a stress sensor to allow quantitative measurements of mechanical properties (Chapters 7, 8) and an OCE needle probe to enable in vivo measurements (Chapter 9). As demonstrated throughout the following chapters, this synergy between improving fundamentals and developing practical tools ultimately results in the ability to visualize compelling mechanical contrast in breast cancer tissues using OCE. It also helps to establish the proof-of-principle for methods that may have an impact on guidance of breast cancer surgery and feasibly be translated to other clinical and preclinical applications of OCE.
II.
Analysis and improvement of image quality in optical coherence elastography
Chapter 4

Tissue-mimicking phantoms

Development and validation of OCE methods require that we perform measurements on artificial imaging targets, known as phantoms, with controllable optical, mechanical, and structural properties. It is necessary to alter these properties to mimic a range of tissue properties. In this research, phantoms with known mechanical properties and structure are essential for the analysis of contrast in compression OCE presented in Chapter 5, and the validation of the new techniques described in Chapters 7, 8, and 9. In this chapter, we review phantom materials and fabrication techniques used for OCT and OCE, and, in particular, describe the fabrication of silicone phantoms.

4.1. Review of potential phantom materials for OCE

Fabrication of tissue-mimicking phantoms for the development of OCE requires a material with the following features:

1) Durability: Benchmarking OCE techniques and system performance requires imaging targets that maintain their properties over time, allowing comparison of measurements, and avoiding the need to refabricate and recharacterize batches of phantoms. Specifically, we desire a material that may be used reliably for at least one month.

2) Mechanical properties in the range of soft tissues: We wish to control the mechanical properties, namely the Young’s modulus, to mimic that of a range of tissues, from the sub-kPa range to the MPa range.

3) The ability to decouple the optical and mechanical properties: we desire to demonstrate the potential to visualize mechanical contrast using OCE for a given level of optical contrast in OCT.

Many early OCT phantoms were based on hydrogels, two of the most common being agar [199] and gelatin [200]. These semisolid matrices allow for the inclusion of both organic and nonorganic additives as optical scatterers. The tissue-like mechanical properties of these hydrogels have been utilized extensively in ultrasound elastography [201] and were used in early OCE experiments [14]. More recently,
homogeneous agar phantoms have been used to test the accuracy of quantitative surface wave and shear wave OCE techniques [25, 112, 203]. However, there are several major issues with hydrogel phantoms. They have a short durability on the order of a week, which limits their capacity for long-term system development, and are not rigid at room temperature [204], which makes forming them into complex shapes impractical. Resin phantoms, by contrast, are very durable; they can be used for years whilst maintaining their optical properties [205] and have the potential to be fabricated into complex shapes. However, resin phantoms are typically much stiffer than soft tissue, which limits their utility in OCE.

Poly(vinyl alcohol) cryogels (PVA-C) have been used extensively to fabricate phantoms for other medical imaging modalities, particularly ultrasound and MRI [206]. The mechanical properties of PVA-C may be controlled over the range found in tissue. In biomedical optics, PVA-C phantoms have been used for photoacoustic imaging [207], diffuse optical imaging [208], and optical elastography [209]. However, their use in OCT has been less extensive. A disadvantage of PVA-C phantoms for elastography is that their mechanical properties and optical properties are linked, making them unsuitable for our development of OCE; however, some OCE studies have employed PVA-C for initial validation of techniques [110, 210].

Silicone is a convenient base material for flexible and straightforward fabrication of phantoms. It is readily compatible with a wide range of suitable scatterers for adjustment of the optical properties. The mechanical properties can be adjusted over a wide range by controlling the amount of cross-linking within the silicone formulation, and can be done independently of incorporating optical scatterers. Silicone is also well suited for fabrication of phantoms with complex structures due to its low viscosity prior to curing and high toughness, i.e., resistance to fracture.

A disadvantage is that silicone is not compatible with organic materials, such as tissue constituents. In simulating the optical properties of tissue, a convenient option is to employ material systems to which tissue constituents may be readily added [204]. Fibrin phantoms [211] meet this need, providing a transparent organic matrix to which both organic and inorganic scatterers and absorbers may be added. Fibrin is a naturally occurring protein in humans that provides structural support for blood clots. Fibrin is readily synthesized and has a shelf life of up to one month. However,
since we do not desire to implement organic constituents into phantoms in this research, silicone phantoms are more suitable, as they provide a larger range of achievable mechanical properties and are more suitable for the development of structured phantoms. Fig. 4.1 shows a comparison of the achievable stiffness range in silicone, fibrin, and PVA materials compared to tissue, highlighting the advantage of silicone for mimicking a range of tissues.

![Comparison of range of Young's modulus of phantom materials and tissue.](image)

Having identified silicone as the most suitable phantom material for this study, in the following sections, we describe how the optical properties, mechanical properties, and structure are controlled in the fabrication of these phantoms.

### 4.2 Fabrication of silicone phantoms for OCE

Silicone phantoms, previously reviewed in the context of biomedical optics by Pogue and Patterson [204], were first introduced in the context of OCT by Oldenburg et al. [123], who made use of their mechanical properties to demonstrate magnetomotive contrast in OCT. Commercial silicone kits, that include compound and catalyst, can be purchased from numerous manufacturers. When mixed, the two components cure at room temperature. Curing can also be accelerated by heating [212]. Specific fabrication details for the individual silicone phantoms used in this research are provided in the methods sections of Chapters 5-9.

#### 4.2.1 Controlling optical properties

Silicone provides a soft matrix to which one can integrate a variety of scatterers and absorbers. These additives are introduced prior to adding the catalyst. For OCT phantoms, the following scatterers have been reported: titanium dioxide (TiO₂) [51, 213], silica microspheres [214], alumina [215], and more recently, gold nanoshells [216]. Reported absorbing agents include carbon black [215] and dye [213].
A major challenge in fabricating a silicone phantom is obtaining a homogeneous distribution of scatterers and absorbers without aggregates, sedimentation, or air bubbles. Different techniques can be used separately or in combination to ensure a homogeneous distribution of scatterers and absorbers in the silicone matrix: e.g., sonication, thinning of silicone before curing with hexane followed by evaporation, and degassing under vacuum.

All phantoms used in this research incorporate TiO\(_2\) (powder, particle size <5 \(\mu\)m, Sigma-Aldrich, St. Louis, Missouri, USA) in varying concentrations (weight per volume of silicone) to provide optical contrast between features. We ensure homogeneous dispersion of the particles by first oven drying the powder to reduce particle aggregation, and by \(~1\) hour of sonication once incorporated into the uncured silicone. Longer sonication times are needed for more viscous silicone compounds and for lower concentrations of TiO\(_2\). Our goal in altering the scattering properties of the silicones is to provide contrast between features within OCT images, providing a baseline structural image against which to compare OCE images. However, we do not measure the quantitative optical properties (e.g., attenuation coefficient) of the phantoms, as the main focus of our phantom studies is on mechanical contrast, rather than optical contrast.

4.2.2. Controlling mechanical properties

Careful control of the mechanical properties, and in particular, the Young’s modulus, of phantom materials in OCE is imperative for validating new techniques and analysing mechanical contrast. Thus, these properties must be independently measured to provide a benchmark for comparison to OCE measurements.

The mechanical response of materials can be measured by performing a standard uniaxial load test using a materials testing machine, such as Instron (Norwood, MA, USA), as shown in Fig. 4.2(a). These devices apply either a tensile or compressive load to a sample positioned between two rigid plates. In the tensile case, a sample is clamped between two fixtures, and one fixture is moved away from the other at a constant rate (strain rate), stretching the sample. In the compressive case, a moving plate compresses the sample against an underlying stationary plate. In both tensile and compressive cases, during loading, a sensor measures the overall force applied to the sample, whilst a separate sensor measures the displacement introduced to the
sample. The stress-strain curve may then be generated from the recorded force and displacement data, as shown in Fig. 4.2(b) for a silicone phantom under compression. All mechanical characterization of phantoms in this thesis were performed on an Instron materials testing machine (model 5848) using a load cell (model 2530-427) calibrated to an accuracy of 0.01 N over a range 1-100 N. Silicones, and many tissues [49], exhibit nonlinear stress-strain behaviour. Therefore, the Young’s modulus will be different for different parts of the curve. For some nonlinear materials, including some soft tissues, the stress-strain behavior is most linear at low strains (<0.1 strain) [49], and the Young’s modulus may be estimated by performing a linear fit to the stress strain data at these low strains. For comparison purposes across silicone phantoms, we have reported the Young’s modulus values for all silicone materials in this chapter from a linear fit from 0-0.1 strain (as shown in Fig. 4.2(b)). The tangent modulus, i.e., the slope of the tangent to the stress-strain curve at a specified stress or strain [217], may also be reported for nonlinear materials. In the compression OCE experiments throughout this thesis, preloads were applied to bring the compression plate into contact with the sample; thus, the resulting strain contrast in the image depended on the preload applied. This is discussed further in Chapter 5, and a method for normalizing for preload and accessing the tangent modulus is reported through Chapters 7 and 8 through the use of a stress sensor.

If a compressive or tensile force is held constant, a creep curve such as that in Fig. 4.2(c) (simulated data using a Kelvin-Voigt model of creep deformation) may also be generated to characterize viscoelastic (time-dependent) behaviour. Other methods for testing the viscoelastic response of materials include a stress relaxation test (measuring time-dependent stress for a constant strain), or dynamic mechanical analysis that uses opposite rotation of two plates contacting the specimen (measuring the time lag of the material response to a periodic load). In the compression OCE experiments throughout this thesis, we ensured that the elastic, not the viscoelastic, response of samples were measured by waiting up to one minute after application of a preload to allow the viscous motion to cease. Live visualization of the phase difference between B-scans, using custom-built software, allowed confirmation that the sample was static before beginning OCE data acquisition. During acquisition, the sample was actuated with a frequency on the order of 2-25 Hz, whereas the viscoelastic strain
response to a step load typically occurs over a time scale of several seconds for these phantom materials, such that only the elastic response was captured.

Fig. 4.2. Characterizing the stress-strain response of materials. (a) Photograph of Instron compression test setup. (b) Representative stress-strain curve of a silicone sample, highlighting the region from which Young’s modulus was estimated. (c) Representative creep curve of a silicone sample.

The mechanical properties of silicone phantoms are controlled by varying the cross-link density; i.e., by varying the ratio of catalyst to cross-linker. This was explored by Jiang et al. [218] and later implemented in phantoms for magnetomotive OCT [123] and OCE [51]. Importantly for OCE, the optical and mechanical properties of silicone phantoms can be controlled independently [218].

To obtain phantoms mimicking a range of tissue stiffness values for use in OCE, we have acquired several commercially available silicones and measured their Young’s modulus for varying combinations of cross-linker and catalyst. For all data included in Tables 4.1-4.3, measurements were performed using the Instron system described above, on cylindrical samples with 15-mm diameter and 4-5-mm height, without any optical scatterers incorporated. The sample diameter was chosen to match that used in compression OCE experiments. The sample surfaces were lubricated using silicone fluid for both Instron and OCE experiments. As the Instron machine had a compensated temperature range of 0-50 degrees Celsius, the room temperature was assumed to not affect measurements. Samples were loaded at a rate of 0.005 strain/s.

Table 4.1 illustrates the effect of varying the ratio of cross-linker to catalyst for a particular commercially available silicone, Wacker Elastosil® 601 (all values are reported for a linear fit to the stress-strain curve from 0-0.1 strain.) This material has similar mechanical properties to other silicones that have been used for fabrication of OCT phantoms [123, 215]. Using this silicone, a Young’s modulus range of ~100 kPa to ~5 MPa is achievable, which covers a wide range of tissue stiffness. However, very soft
tissues such as adipose, brain and liver, have Young’s modulus below 10 kPa [219]. To further reduce the stiffness of silicone phantoms to be in the range of very soft tissues, silicone fluid such as polydimethylsiloxane (PDMS) oil may be added prior to curing [93, 123, 212]. Silicone fluid does not participate in the curing process, but remains as a fluid within the cross-linked polymer network of the cured silicone, resulting in a softer material. Table 4.2 lists the measured Young’s modulus of Wacker Elastosil® 601 silicone with varying concentrations of silicone fluid. By adding PDMS oil, a Young’s modulus as low as 10 kPa was achieved.

<table>
<thead>
<tr>
<th>Mixing ratio</th>
<th>Elastic modulus (kPa)</th>
</tr>
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<tbody>
<tr>
<td>1:5</td>
<td>4910</td>
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<tr>
<td>1:15</td>
<td>3060</td>
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<tr>
<td>1:20</td>
<td>1483</td>
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<tr>
<td>1:30</td>
<td>1008</td>
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<tr>
<td>1:40</td>
<td>286</td>
</tr>
<tr>
<td>1:50</td>
<td>127</td>
</tr>
</tbody>
</table>

Table 4.1. Young’s modulus of various mixing ratios (cross-linker : catalyst) for Wacker Elastosil® 601 silicone phantoms.

<table>
<thead>
<tr>
<th>Mixing ratio</th>
<th>Elastic modulus (kPa)</th>
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</thead>
<tbody>
<tr>
<td>1:10:10</td>
<td>316</td>
</tr>
<tr>
<td>1:10:20</td>
<td>122</td>
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<tr>
<td>1:10:30</td>
<td>81</td>
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<td>38</td>
</tr>
<tr>
<td>1:10:50</td>
<td>23</td>
</tr>
<tr>
<td>1:10:60</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 4.2. Young’s modulus of various mixing ratios (cross-linker : catalyst : PDMS oil) for Wacker Elastosil® 601 silicone and Wacker AK50 PDMS silicone fluid.

Whilst addition of silicone fluid provides a convenient solution for adjusting the mechanical properties of phantoms, the polymer network for any given silicone elastomer can hold only a limited percentage weight of silicone fluid before reaching saturation. Manufacturers of silicones have suggested that exceeding this limit can result in a gradual exudation of the oil [212], leading to large variations of the phantom’s mechanical properties over time. This is a major limitation of this phantom fabrication technique.

Silicone with very low hardness, such as silicone gels, liquid silicone rubbers, and some elastomers provide a more durable and reliable alternative to silicone fluid.
phantoms for simulating very soft tissues. Such silicones have not previously been implemented in OCT phantoms, but are commonly used in biomechanics studies of deformation of very soft tissues [220, 221]. In order to have the ability to mimic very soft tissues, we fabricated a variety of silicone gels and liquid silicone rubbers and measured their Young’s modulus. The results in Table 4.3 show that the Young’s modulus of these materials is in the range of very soft tissues such as adipose, and much lower than the silicone materials presented in Table 4.1. The mechanical properties of these silicones could also be finely adjusted by adding small quantities of silicone fluid, if needed, without the concern of exudation of the fluid. However, these very soft silicones tend to be sticky compared to their stiffer counterparts, and generous lubrication is needed to ensure that the phantoms do not stick to the compression plates during OCE measurements.

| TABLE 4.3. YOUNG’S MODULUS OF SELECT SOFT SILICONES |
|-------------------------------|-----------------|-----------------|
| Product                      | Mixing ratio | Elastic modulus (kPa) |
| Wacker LR3003/03             | 1:1           | 18.1            |
| Wacker P7676                 | 1:1           | 6.4             |
| Wacker SilGel 612            | 1:1           | 1.6             |
| Nusil LS3441                 | 1:1           | 1.3             |

4.2.3. Creating 3-D structures

Phantoms that incorporate 2-D and 3-D structures with known geometry allow validation and verification of system performance and techniques. For example, such phantoms can enable the spatial resolution of an OCT system or OCE technique to be determined. As the development and applications of OCT continue to advance, the need for phantoms with complex shapes is growing for both inter-laboratory and inter-system comparison. Phantoms that mimic the more complex microstructure of tissues are also valuable to assess new techniques for particular applications.

In other imaging modalities such as ultrasound, 2-D and 3-D structured phantoms have received much attention [222, 223] and, indeed, are now available commercially [224]. In comparison, the OCT community is in the relatively early stages of developing phantoms with complex shapes. Simple bi-layer phantoms representing morphological change in 1-D have been proposed with different materials, including agar, gelatin, PVA, fibrin and silicone [51, 200, 210, 211]. Phantoms containing randomly dispersed inclusions, used to validate various speckle-reduction techniques,
have also been proposed [225].

Silicone lends itself well to the fabrication of phantoms with complex shapes, due to its low viscosity prior to curing and high toughness. Phantoms have been developed for point-spread function measurements at controlled positions, using femtosecond laser subsurface micro-inscription [226]. Structures can easily be formed in silicone phantoms through sequential moulding, i.e., by curing each desired feature sequentially. For a layered phantom, this involves stacking layers by adding and curing one layer at a time [51, 123]. Strong inherent adhesion between layers ensures fabrication of a solid block bearing the desired features.

A further advance, made by our group, has been the fabrication of 3-D-structured silicone phantoms using a more advanced and highly flexible soft-lithographic moulding technique known as replica moulding [227]. In this technique, a phantom containing the microscopic, 3-D letters “O B E L” was fabricated and its use was demonstrated in assessing feature contrast enhancement and the associated effects on image resolution. This method provides for well-defined, reproducible and independently controllable structures with a wide range of structures possible, but it requires access to complex and costly soft lithography apparatus.

The structured phantoms in this thesis were primarily made by manually cutting features using a scalpel under microscope guidance. Where indicated, inclusions were cut using a femtosecond laser. The width and thickness of individual inclusions and layers were monitored using a commercial OCT system (model OCS1300SS, Thorlabs, USA). A sequential moulding process was used to create the 3-D inclusion phantoms that feature throughout this thesis; specific details of their fabrication are provided in the methods sections of Chapters 5-9.

4.3. Discussion and conclusion

Phantoms play a key role in the development of new OCE techniques, and their importance is likely to become more prominent as OCE techniques mature and standard imaging targets for benchmarking systems are required. There is also a prospect to use phantoms to compare the existing OCE techniques reviewed in Chapter 2. Performing each technique on the same phantom containing, for example, inclusions with a range of mechanical properties, would allow the advantages and
disadvantages of each technique to be elucidated in terms of spatial resolution, sensitivity, and accuracy. An example of such a phantom is shown in Fig. 4.3. Using the silicone materials described above, we were able to create four inclusions of similar size, shape, and scattering properties, but varying stiffness (decreasing stiffness from left to right in the images; the Young’s modulus ratio for inclusion to background is labelled above each inclusion). The inclusions were embedded in a soft, homogeneous silicone matrix. Fig. 4.3(a) is an OCT B-scan of the phantom, and Fig. 4.3(b) is the corresponding strain elastogram generated using phase-sensitive compression OCE described in Chapter 5. This provides just one example of a phantom that can provide rich information on the capabilities of an OCE technique, including spatial resolution and sensitivity to variations in mechanical properties.

![OCT B-scan and strain elastogram](image)

Fig. 4.3. (a) OCT B-scan and (b) strain elastogram produced using phase-sensitive compression OCE for a silicone phantom containing inclusions embedded in a soft matrix. The Young’s modulus ratio (inclusion:matrix) is labelled above each inclusion in the elastogram.

In conclusion, we have reviewed phantom materials used in OCT, motivated the use of silicone phantoms for development of OCE, and described methods for controlling the optical properties, mechanical properties, and structure of silicone phantoms. The mechanical characterization of silicone materials provided in Section 4.2.2 resulted in a range of silicones to mimic the stiffness of tissues from very soft (<10 kPa) to stiff tissues (MPa). To our knowledge, this is the first time such a library of phantom materials has been obtained and characterized for use in OCE. The flexibility to alter the mechanical properties of silicones is a key enabler for the studies presented in the following chapters.
Chapter 5

Mechanical limitations on contrast

5.1. Elastogram fidelity

As reviewed in Chapter 2, research activity in OCE has experienced rapid growth in the past 10 years, since the maturation of FD-OCT [16]. Several variants of loading mechanisms and potential applications have been proposed, and intriguing preliminary images have demonstrated mechanical contrast in tissues such as the cornea [112, 228] and human skin [26, 229]. However, despite the recent upsurge in the number of proposed techniques and applications, no studies have investigated the fundamental limitations of a given optical technique, in terms of its sensitivity to mechanical contrast and its accuracy in depicting the underlying mechanical properties of the sample. Every OCE implementation relies on some assumptions about the mechanical response of tissue to an applied load, as outlined in Chapter 1. Use of such assumptions simplifies the characterization of tissue, and may in some cases prove sufficient for producing clinically useful images. However, tissue behaviour in general is more complex, exhibiting varying degrees of nonlinearity, viscoelasticity, mechanical anisotropy, and geometric complexity. When simple assumptions break down in the presence of such complexities, the resulting elastograms contain mechanical artefacts, limiting their fidelity to the underlying tissue properties and, ultimately, their potential clinical utility.

For OCE to advance toward clinical implementation, it is necessary to test the validity of assumptions employed in the various techniques, understand where they break down, and quantify the impact of artefacts on image contrast and reliability. Such quantitative assessment requires comparison of the measured sample deformation in OCE to that predicted by mechanical models. Discrepancies between the two will highlight sources of mechanical artefacts and allow their effects on image contrast to be assessed. In addition to serving as an investigative tool, modelling of tissue deformation may also be used as part of an iterative elasticity reconstruction process to produce quantitative elastograms [90].
Accurate modelling of tissue deformation involves solving for complex geometries and heterogeneous elasticity fields, and, therefore, numerical methods are preferred over analytical methods for accurate representation of tissue deformation. The predominant numerical method for modelling tissue deformation is finite element modelling. Here, the tissue geometry is divided into a mesh of individual elements, and the governing equations of motion are solved for each element. Finite element models (FEMs) have been used in OCE to validate new methods for estimating velocity and strain [132], to validate new loading techniques for generating elastographic contrast [119, 230], and to estimate stress and strain fields in artery walls under luminal pressure [71], but has not yet been used to evaluate the limitations of mechanical artefacts on image contrast. Such investigations have been performed extensively in ultrasound elastography [231-234]. However, the impact of mechanical artefacts on image contrast in OCE will differ from those in ultrasound elastography due to different limits on measurable displacement and an increased sensitivity to boundary conditions, as images are generally limited to the first few millimetres of tissue.

In this chapter, we use FEMs to investigate sources of mechanical artefacts and evaluate their effects on image contrast in compression elastography. This represents the first study devoted to analysis of mechanical contrast in OCE. We focus on fundamental mechanical limitations on contrast (i.e., for a given set of imaging system parameters), and in particular we investigate the impact of stress nonuniformity on mechanical contrast in compression OCE. FEMs of the physical OCE experiments serve to validate measurements, as well as elucidate sources of artefacts in the elastograms. The model provides not only idealized strain images, but also simulates the stress distribution within each sample. Once initial validation of the model with experiments has been obtained, sample geometry, boundary conditions, and relative stiffness of features can be varied in the model to analyse how the stress distribution changes. This aids in identifying a range of mechanical parameters for which strain images are reliable; that is, for which the assumption of uniform stress distribution is most valid.
5.2 Analysis of mechanical contrast in optical coherence elastography

Kelsey M. Kennedy¹, Chris Ford², Brendan F. Kennedy¹, Mark B. Bush³, and David D. Sampson¹,⁴

Abstract: We present optical palpation, a tactile imaging technique for mapping micrometre- to millimetre-scale mechanical variations in soft tissue. Optical coherence elastography (OCE) maps the mechanical properties of tissue microstructure and has potential applications in both fundamental investigations of biomechanics and clinical medicine. We report the first analysis of contrast in OCE, including evaluation of the accuracy with which OCE images (elastograms) represent mechanical properties and the sensitivity of OCE to mechanical contrast within a sample. Using phase-sensitive compression OCE, we generate elastograms of tissue-mimicking phantoms with known mechanical properties and identify limitations on contrast imposed by sample mechanics and the imaging system, including signal-processing parameters. We also generate simulated elastograms using finite element models to perform mechanical analysis in the absence of imaging system noise. In both experiments and simulations, we illustrate artefacts that degrade elastogram accuracy, depending on sample geometry, elasticity contrast between features, and surface conditions. We experimentally demonstrate sensitivity to features with elasticity contrast as small as 1.1:1 and calculate, based on our imaging system parameters, a theoretical maximum sensitivity to elasticity contrast of 1.002:1. The results highlight the microstrain sensitivity of compression OCE, at a spatial resolution of tens of micrometers, suggesting its potential for the detection of minute changes in elasticity within heterogeneous tissue. © 2013 Society of Photo-Optical Instrumentation Engineers (SPIE); Received July 2, 2013; accepted October 14, 2013; published online November 12, 2013.

¹ Optical + Biomedical Engineering Laboratory, The University of Western Australia, Crawley, WA 6009
² Department of Mechanical Engineering, Curtin University, Perth, WA 6102
³ School of Mechanical and Chemical Engineering, The University of Western Australia, Crawley, WA 6009
⁴ Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, Crawley, WA 6009
5.2.1 Introduction

When tissue becomes diseased, it undergoes changes in its constituent materials and microstructure, which translates to changes in its mechanical properties [1]. Optical coherence elastography (OCE) [2] is an emerging imaging technique that probes mechanical contrast on a microscale. To perform OCE, a mechanical load is applied to tissue, and optical coherence tomography (OCT) is used to measure the resulting displacements, from which variations in elasticity, or stiffness, are estimated and mapped into an image known as an elastogram. OCE probes mechanical contrast on a length scale (tens of micrometres) considerably smaller than that probed by other elastography techniques, such as ultrasound (hundreds of micrometres) [3] and MRI (∼1 mm) [4]. It also provides a depth-resolved capability, unlike cellular-scale and nanoscale elasticity mapping techniques such as atomic force microscopy [5] and optical tweezers [6]. As this unique length scale is relevant to the progression of many diseases, OCE has the potential to become a valuable tool for imaging of tissue microarchitecture, with possible applications ranging from fundamental studies of biomechanics to numerous aspects of clinical medicine. So far, OCE has been proposed for a number of clinical applications, such as assessing the vulnerability of atherosclerotic plaques [7, 8] guiding surgical resection of soft tissue tumours [9], and monitoring changes in corneal elasticity with age and progression of disease [10–12].

A number of techniques have been proposed to date for performing OCE and may be grouped according to the type of loading used. The first reported technique is compression OCE, in which a compressive load is applied to a sample, and the resulting strain is estimated and mapped into a strain elastogram [2, 13–18]. Strain provides a relative measure of elasticity under the assumption of a uniform stress field within the sample. More recently, shear wave and surface wave techniques have been proposed, which measure the phase velocity of a propagating wave generated using either vibration or impulse loading [19–22]. Unlike compression OCE, such methods directly estimate sample elasticity. However, compression OCE maintains the native lateral resolution of the OCT system, whereas shear and surface wave techniques have considerably lower lateral resolution (0.5 to 1 mm), as they assume tissue homogeneity for the length over which the shear wave speed is calculated. Compression elastography is also straightforward to implement and has been
extensively used for clinical imaging in ultrasound elastography [23]. In addition to compression and shear/surface wave OCE, techniques have been proposed that use internal, localized loading generated by a magnetic implant [24] or by magnetic nanoparticles embedded in the sample [25].

A variety of methods for measuring displacement using OCT have also been proposed, including speckle tracking [2], phase-sensitive OCE [26], and use of the Doppler spectrum [27].

Despite the breadth of the proposed techniques and potential clinical applications, the limitations on mechanical contrast that can be detected in OCE elastograms remain poorly understood. The contrast in elastograms is determined by a combination of the true elasticity distribution within the tissue, the employed imaging system parameters, and the assumptions made about tissue behaviour in the elastogram reconstruction process. To better understand the ability of OCE to detect mechanical contrast in tissue and to enable more useful interpretation of OCE elastograms, some fundamental questions must be addressed:

1. How accurately do OCE elastograms represent the elasticity distribution within a sample?
2. What is the sensitivity of OCE elastograms to variations in elasticity within a sample?
3. What factors limit elastogram accuracy and sensitivity?

In this article, we address these questions in the context of phase-sensitive compression OCE. We present strain elastograms of tissue-mimicking phantoms with varying geometries and a range of mechanical contrast between features and identify limitations on elastogram contrast imposed by built-in aspects of tissue mechanics. We focus our analysis on the detection of features within mechanically heterogeneous samples, as this aspect is the most relevant for assessing the ability of OCE to differentiate normal versus diseased or compromised regions within biological tissue. Thus, relative, rather than absolute, measurements of elasticity are the focus of our analysis.

We employ finite element models (FEMs) in this study to generate simulated elastograms and analyse the impact of varying mechanical parameters on contrast, independent of imaging system noise and signal processing techniques. We also use
FEMs to test the validity of assumptions made about sample behaviour in the elastogram formation process and to analyse how the breakdown of these assumptions impacts on elastogram accuracy.

We then consider how elastogram contrast is limited by imaging system and signal processing parameters and quantify the sensitivity and range of mechanical contrast achievable using our compression OCE system. In so doing, we keep the imaging and signal processing parameters constant throughout this study, as our objective is to investigate the impact of varying mechanical parameters, and how the fundamental mechanics combine with the imaging system capabilities to determine contrast.

5.2.2 Compression OCE

In this section, we describe the deformation of a sample undergoing compression, highlight the assumptions made about tissue mechanical behaviour in the elastogram formation process, and describe how elastogram accuracy may be measured. We also define expressions for measures of elastogram quality in phase-sensitive compression OCE, including resolution, sensitivity, and dynamic range (DR).

**Tissue deformation in compression elastography**

A compressive load applied to a sample is described in terms of stress, $\sigma = F/A$, where $F$ is the applied force and $A$ is the cross-sectional area over which the force is applied. The resulting bulk deformation of the sample along the axis of compression is quantified by strain, $\varepsilon = \Delta l / l_0$, where $\Delta l$ is the change in length and $l_0$ is the original length of the sample. The axial compression of a sample is generally accompanied by some lateral expansion, to conserve volume. This shape change is characterized by Poisson’s ratio, $\nu$, which is equal to 0.5 for a completely incompressible material and commonly assumed to be in the range $\sim$0.49 to 0.5 for most soft tissues [28].

If we make the assumption that tissue deforms in a linearly elastic manner, which has been reported to be an accurate approximation for strains $< 0.1$ in tissues such as breast and prostate [29], stress and strain are related through a three-dimensional (3-D) set of elastic constants. In compression elastography, the applied load can be approximated as uniaxial, i.e., occurring along one axis of the sample. Under the further assumption that the sample is isotropic, i.e., its response to stress is direction independent, the uniaxial stress and strain are related through one elastic constant,
defined as the Young’s modulus, \( E = \sigma / \varepsilon \). The Young’s modulus is commonly used to characterize the elasticity of tissues and, for soft tissues, has been reported to range from hundreds of Pascals (Pa) for healthy liver tissue [30], to tens and hundreds of kPa for healthy and malignant breast tissues [29], to a few MPa for arterial wall tissues [31].

The objective of compression OCE is to form a map of Young’s modulus within the sample – a so-called elastogram. The equation for Young’s modulus given above describes a “bulk” response of a sample, but a map of Young’s modulus requires knowledge of the “local” stresses and strains throughout the sample. Local strain is obtained by calculating the spatial derivative of the measured displacement, i.e., the change in displacement per unit length of the sample. Local stress, on the other hand, cannot be directly measured at depth within the sample. As a result, the elastogram in compression OCE is typically a map of strain, which gives a relative measure of Young’s modulus, under the assumption that stress is uniformly distributed throughout the sample. However, in practice, stress concentrations arise at feature boundaries within heterogeneous samples [32] and at the sample surface where friction is present. Thus, strain elastograms are subject to mechanical artefacts that limit their accuracy in representing the true elasticity distribution in a sample.

**Quantification of elastogram accuracy**

A measure of elastogram accuracy (fidelity to the true elasticity distribution) is the contrast transfer efficiency (CTE), which was defined by Ponnekanti et al. [32] in ultrasound elastography as the ratio of strain contrast observed between features in an elastogram, \( C_s \), to true elasticity contrast of the features, \( C_e \): \( CTE = C_s / C_e \). We employ this definition of CTE to quantify elastogram accuracy in this article and address the first question in the introduction: “How accurately do OCE elastograms represent the elasticity distribution in a sample?”

In compression elastography, the CTE of strain elastograms depends on the validity of the assumption of uniform distribution of stress. This assumption holds for the trivial case of a mechanically homogeneous sample undergoing uniaxial compression, which results in a uniform strain field but provides no elasticity information without a measurement of the applied stress. We consider here the more relevant case of a
mechanically heterogeneous sample, in which the aim is to differentiate features based on mechanical contrast within an image. In particular, we consider two heterogeneous sample geometries: bilayer (soft on stiff) and stiff inclusions embedded in a soft matrix. Analytical expressions for the CTE of strain elastograms have been derived for each of these geometries, assuming an infinite medium of isotropic, linear elastic material undergoing uniaxial compression, in which each material in the structure has equal Poisson’s ratio. For a layered structure, by considering an equivalent system of springs, it can be shown that the stress is uniformly distributed among the layers, such that the strain contrast between layers is inversely proportional to the Young's modulus contrast between layers [33]. In other words, a strain elastogram of a layered geometry is expected to have $\text{CTE} = 1$.

Kallel et al. [34] used an analytical solution to the elasticity equations to derive expressions for the CTE of strain elastograms of an inclusion geometry. They found that for the case of an inclusion perfectly embedded (bonded to its surroundings) in a homogeneous matrix, the contrast between the strain in the inclusion, $\varepsilon_{\text{inclusion}}$, and strain in the matrix, $\varepsilon_{\text{matrix}}$, (at large distances from the inclusion), $C_o = \varepsilon_{\text{inclusion}}/\varepsilon_{\text{matrix}}$, is related to the true modulus contrast between the inclusion and matrix, $C_t = E_{\text{inclusion}}/E_{\text{matrix}}$, by

$$\frac{1}{C_o} = \left[ \frac{1 - 2\nu}{C_t + (1 - 2\nu)} + \frac{2}{1 + C_t(3 - 4\nu)} \right]. \quad (5.1)$$

Note that $C_o$ depends neither on the size of the inclusion nor on its depth below the surface, but only on the modulus contrast between the inclusion and matrix and on the Poisson’s ratio, $\nu$, which, as discussed above, falls in the range $\sim 0.49$ to 0.5 for most soft tissues. In this case, Eq. (5.1) may be approximated as $C_o = 1/2 + C_t/2$. This indicates that for increasing elasticity contrast between a stiff inclusion and a soft matrix, the observed strain contrast is also expected to increase but that the CTE will plateau at just over 0.5 for large modulus contrast [32, 34]. This is illustrated and explained in detail in Section 5.2.4. Equation 5.1 also applies to the case of a soft inclusion embedded in a stiff matrix; however, we limit the analyses in the present study to consider stiff inclusions.

The analysis of elastogram accuracy described above, first introduced in ultrasound elastography, is independent of the spatial resolution scale of the employed
elastography technique and so may be applied to compression OCE. A key difference in OCE, however, is an increased sensitivity to surface effects (friction and coupling with the compressor) as images are generally limited by the penetration depth of OCT to the first 1 to 2 mm in dense tissues. Furthermore, optical interferometric detection in OCE will translate to a higher sensitivity to deformation than in ultrasound elastography, and the higher spatial resolution and smaller field of view in OCE will mean that a different scale of tissue structures is probed. We further examine some of these issues in the discussion in Section 5.2.5.

Finite element modelling is used in this study for addressing mechanical aspects of the third question proposed above: “What factors limit the accuracy and sensitivity?” Once shown to produce results that accurately model the physical system, FEMs enable ready variation of mechanical parameters, including sample geometry, mechanical properties, and surface friction. The model predicts the effects of these parameters on the resulting strain distribution in the absence of imaging system noise. Importantly, FEMs also provide estimates of the resulting stress distribution, allowing identification of sources of mechanical artefacts in strain elastograms.

**Strain elastogram performance parameters in compression OCE**

In addition to artefacts arising from built-in aspects of mechanical behaviour, elastogram contrast depends on imaging system noise and parameters used in signal processing, particularly in the strain estimation process. Our group recently defined performance parameters for strain elastograms in compression OCE, in terms of the mean and variance of the measured displacement values [15]. Here, we summarize those definitions for reference in this article.

In phase-sensitive OCE, local displacement, $u_z$, is calculated as the change in phase, $\Delta \phi$, between A-scans or B-scans [26], scaled by the mean wavelength of the source in the sample, $\lambda/n$, where $n$ is the refractive index of the sample, i.e., $u_z = \frac{\Delta \phi \lambda}{4\pi n}$. The local strain, $\varepsilon_i$, is then estimated as the gradient of displacement over a range in depth: $\varepsilon_i = \frac{\Delta u_i}{\Delta z}$. In this equation, $\Delta z$ is the strain spatial resolution, defined as $\Delta z = m d_z$, where $m$ is the number of OCT image pixels over which strain is estimated, and $d_z$ is the axial pixel size.
The strain sensitivity, \( S_e \), is defined as the standard deviation of strain \( (\sigma_e) \) and, in phase-sensitive OCE, depends fundamentally on the phase stability of the OCT system. The strain dynamic range, \( DR_e \), is defined as the ratio of the maximum strain to the strain sensitivity. In this article, we assume that the maximum detectable strain is that due to a phase change of \( \pi \) radians within one strain resolution (the axial distance over which the strain is estimated). Thus, the maximum measurable displacement, \( u_{z,\text{max}} \), is equal to \( \lambda/4n \). Under this assumption, the strain DR is defined as

\[
DR_e = \frac{u_{z,\text{max}}}{\sigma_e} = \frac{\lambda}{4n\sigma_e\Delta z}
\]  

(5.2)

The strain sensitivity and DR, as defined above, in combination with the contrast transfer functions detailed in the previous section, will determine the ultimate sensitivity of OCE strain elastograms to variations in elasticity within a sample. We present an analysis of this sensitivity in Sec. 5.2.4.

5.2.3 Methods

**Phantom fabrication and characterization**

Tissue-mimicking phantoms with controllable structure and mechanical properties were fabricated using a range of silicone elastomers, namely, combinations of Elastosil® RT601, Elastosil® P7676, and AK50 Silicone Fluid (Wacker, Germany) [35]. Three types of phantoms were constructed for this study: homogeneous, bilayer, and inclusion. All phantoms were cylindrical with 15-mm diameter and were made \(~1\) mm thick such that OCT could readily image the entire depth of the phantom to facilitate comparison to the FEM. Representative OCT B-scans of each phantom type are shown in Fig. 5.1. All OCT images have been scaled to physical dimensions using a refractive index of 1.4 for the silicone materials [35]. The bilayer phantoms comprise two layers (soft and stiff) of approximately equal thickness. The inclusion phantoms consist of a soft silicone matrix containing a stiff silicone inclusion in the form of a rectangular prism embedded 300 to 350 \( \mu m \) below the surface. The inclusions were cut by hand from a bulk of cured silicone to sizes in the range of 300 to 500 \( \mu m \). This range was set by the achievable tolerances. (Smaller inclusions than those used here could be fabricated using soft lithography techniques previously used to fabricate 3-D
structured phantoms [36].) To ensure that layers and inclusions were distinguishable in OCT images, titanium dioxide particles were added to the uncured silicones in concentrations of 0.8 mg/mL for the homogeneous phantoms, matrix materials, and top layers, and 2.5 mg/mL for the inclusions and bottom layers.

![OCT images](image)

Fig. 5.1. Optical coherence tomography (OCT) B-scans of (a) homogeneous, (b) bilayer, and (c) inclusion phantoms. Scale bar applies to both dimensions. Dashed lines in (b) and (c) indicate feature boundaries.

The Young’s modulus of the cured silicone was controlled by varying the volumetric ratio of crosslinker, catalyst, and non-crosslinking silicone fluid [35]. To measure the Young’s modulus of each silicone, stress-strain curves were obtained using standard compression tests (Instron, Norwood, Massachusetts). The Young’s modulus was then estimated by linear regression against the stress-strain curves, as described in further detail below.

**Compression OCE system and measurements**

Compression OCE measurements were performed using a fibre-based, Fourier-domain OCT system. The light source is a superluminescent diode with a central wavelength of 835 nm and bandwidth of 50 nm. The measured axial resolution is 8.5 μm. The lens in the sample arm has a working distance of 25 mm and provided a measured lateral resolution of 11 μm. The measured sensitivity is 102 dB at an exposure time of 36 μs. B-scans were acquired with 1000 × 2048 (x × z) pixels over a 6 × 3 mm field of view and at a line rate of 10 kHz.

The sample arm comprises an imaging window fixed to a ring-actuator set-up described previously [14, 15], enabling loading and imaging from the same side. A
preload was applied to each phantom using an upper brass plate, of larger surface area than the phantom, to ensure uniform contact between the phantom, the upper plate, and the imaging window (lower plate). The amount of preload required was dependent on the magnitude of variations in the surface topography in each phantom, which arose due to imperfections in the manual fabrication process of the phantoms, and typically ranged between 50 and 150 μm, as measured using OCT. The preload served to ensure optimal transfer of the load from the ring actuator during measurements.

The ring actuator introduced displacements of 80 to 120 nm to the sample surface using a square wave function at 5 Hz, synchronized to the OCT B-scan acquisition (at 10 Hz), as described in [15]. This loading frequency was chosen to remain in the quasi-static loading regime, i.e., low enough to avoid wave propagation in the sample. The local displacement in the sample was measured by taking the phase difference between consecutive B-scans, i.e., between the compressed and uncompressed states. Displacement data were derived from the average of 50 sets of these B-scans. Temporal averaging served to reduce the variance of the measured displacement at each point and was performed in the complex plane in order to reduce systematic underestimation of displacement due to averaging of phase differences obtained at positions with low OCT signal-to-noise ratio (SNR) [37]. The phase sensitivity of the system in a scanning configuration (calculated as the standard deviation of the measured phase difference between sequential B-scans of a stationary sample, over 50 pairs of B-scans) was 25 mrad at an OCT SNR of 50 dB, corresponding to a displacement sensitivity of 1.2 nm. Discrepancy between the measured sensitivity and the predicted shot noise–limited sensitivity (3.2 mrad at 50 dB) [38] is attributed to galvanometer lateral positioning error between B-scans.

The local strain in the sample was estimated from the measured displacements using a weighted-least squares (WLS) algorithm and represented in a strain elastogram. In WLS strain estimation, the measured displacement values are weighted based on the underlying OCT SNR at each point, since the phase variance and, therefore, the displacement measurement accuracy, depends on the OCT signal intensity. (We demonstrated in [15] that WLS strain estimation improves the strain sensitivity and DR over previously reported strain estimation techniques.) We applied
Gaussian smoothing to the strain elastograms, using an 18 × 7-μm window (width × height) to further improve strain sensitivity. The axial strain spatial resolution (axial distance over which the slope of axial displacement versus depth is calculated) was 90 pixels, corresponding to a physical length of 92 μm, using a group refractive index of 1.4. The lateral strain spatial resolution was equal to the lateral resolution of the OCT system (11 μm).

**Finite element model**

Model description

FEMs of the compression OCE experiments were developed using the simulation software Abaqus (Dassault Systèmes, Providence, USA, version 6.10.1). To construct each model, a geometry was defined and material properties were assigned to deformable regions within the model. The geometry was then divided into discrete (finite) elements, in which an approximate solution to the governing equilibrium equations was determined, subject to the application of a known displacement along the boundary corresponding to the lower plate (imaging window) in the experiments. The solution provided the displacements, strains, and stresses on each of the finite elements [39]. The spacing of the elements (mesh size) ranged from 5 to 20 μm, with the finer mesh in areas where large variations in stress and strain were expected, e.g., around inclusions. The model assumed linear elastic behaviour of all materials.

An axisymmetric model was employed, in which a two-dimensional cross-sectional geometry was defined and then rotated about a central axis to obtain a 3-D visualization of the sample. This model yields an effective 3-D solution without the added computational complexity of solving the governing equations in three dimensions. Although inclusions were shaped as rectangular prisms in the experiments, rather than cylinders as in the axisymmetric model, measurements were made in a plane close to the centre of the inclusions such that the measured deformation approximated that through the centre plane of a cylinder.

**Determination of model inputs**

Inputs to the models of each experiment included:
• phantom geometry (total thickness, thickness of layers, inclusion dimensions, depth of inclusion below sample surface), as measured using OCT before application of preload;
• modulus contrast between features, determined from estimates of Young's modulus from the stress-strain curve of each silicone (further details below);
• amount of displacement introduced to the sample by the preload, as measured using OCT;
• amount of nanometre-scale displacement introduced to the sample surface by the actuator; and
• the friction conditions present in the experiment (further details below).

Compression testing of the bulk silicones typically revealed a nonlinear stress-strain relationship, as illustrated by the representative stress-strain curve in Fig. 5.2. However, as a relatively small range of strain (≤0.12) was used in OCE experiments, the silicones were modelled as linear elastic. The Young’s modulus of each material was estimated based on the bulk strain due to the preload. For example, in an inclusion sample, the change in thickness of the matrix was measured from OCT images taken before and after preload, from which a bulk strain on the matrix was estimated. The Young's modulus was estimated by the linear regression from 0 to this bulk strain on the stress-strain curve of the matrix material (See Fig. 5.2). Similarly, the change in an inclusion thickness due to preload was determined and its modulus approximated using the same fitting procedure on the stress-strain curve of the inclusion material. This same procedure was used to estimate Young’s modulus of the silicones in the bilayer phantom. Table 5.1 lists the estimated Young’s modulus of each silicone as well as the modulus contrast between features (inclusion:matrix or bottom:top layer) for all phantoms used in this study.

Unless otherwise stated, the coefficients of friction used were 0.1 at the phantom-glass imaging window interface and 0.5 at the phantom-brass plate interface. These values were empirically determined to give the best quantitative matches between the modelled and measured displacements. The difference in the degree of friction between the upper and lower surfaces is expected, as the imaging window is smoother than the brass upper plate, and because silicone fluid (Wacker AK50) was applied to lubricate the phantom-imaging window interface. Exceptions to these conditions are
specified, where relevant, in Section 5.2.3. Finally, a Poisson’s ratio of 0.49 was assumed for all silicones [40].

![Stress-strain curve](image)

**Fig. 5.2.** Representative stress-strain curve obtained from compression testing of silicones used in phantoms. Inset shows an example of a linear fit (black line) used to estimate Young’s modulus.

**Table 5.1. Bulk Mechanical Characterization of Silicone Phantom Materials.**

<table>
<thead>
<tr>
<th>Phantom number</th>
<th>Feature</th>
<th>Young’s modulus (kPa)</th>
<th>Modulus contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Homogeneous</td>
<td>20</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Bottom layer</td>
<td>520</td>
<td>37:1</td>
</tr>
<tr>
<td></td>
<td>Top layer</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Inclusion</td>
<td>837</td>
<td>45:1</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Inclusion</td>
<td>153</td>
<td>10:1</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Inclusion</td>
<td>100</td>
<td>5:1</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Inclusion</td>
<td>34</td>
<td>2:1</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Inclusion</td>
<td>20</td>
<td>1.1:1</td>
</tr>
<tr>
<td></td>
<td>Matrix</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

### 5.2.3 Results

**Comparison of measured and simulated deformation in compression OCE**

Figures 5.3(a)–(c) present the structural OCT images, along with the measured and simulated displacement maps resulting from compression OCE experiments and FEMs, respectively, for (top to bottom) homogeneous, bilayer, and inclusion phantoms (Phantoms 1 to 3 in Table 5.1). Figure 5.3(d) is a comparison of the measured and simulated displacement versus depth taken from the positions indicated by the
dashed lines in Figs. 5.3(b) and 5.3(c). Silicone fluid was used to lubricate the upper and lower surfaces to minimize friction, and these surfaces were modelled as frictionless in the FEM, in order to simplify the mechanical system in the first instance.

Qualitatively, the measured and simulated displacement maps show good correspondence, and the displacement traces in Fig. 5.3(d) verify a good quantitative match. These traces also allow a clear interpretation of the displacement trends in each phantom. In each case, compression was introduced from the top of the image, resulting in maximum displacement at the top surface and zero displacement at the opposite surface, where the phantom was compressed against a rigid, unmoving plate. For the homogeneous case, a linear decrease in displacement with depth is observed, corresponding to a uniform local strain throughout the phantom.

In the bilayer phantom, two slopes are observed, with greater slope, corresponding to higher strain, in the softer top layer. The location of the change in slope corresponds to the physical interface between the layers. The slight discrepancy between the experiment and simulation in the displacement at the layer interface is likely to be due to an imperfect interface between the two silicone layers that arose during curing.

Finally, in the inclusion phantom, large displacement gradients, corresponding to higher strain, are observed in the matrix material, surrounding a region of bulk displacement, or low strain, in the stiff inclusion. The slight discrepancy in the displacement of the inclusion seen here is likely due to a slight discrepancy in the depth at which the inclusion was situated in the model versus in the actual phantom.

**Accuracy of strain elastograms in representing elasticity**

The agreement between measured and simulated displacement demonstrated in Fig. 5.3 confirms the validity of the assumption of linear elastic behaviour of these materials over the range of employed strains. In this section, we compare measured and simulated strain elastograms for varying geometries, friction conditions, and modulus contrasts. We also use FEMs to simulate the corresponding stress distributions and, thus, aid our analysis of mechanical artefacts in elastograms and their impact on accuracy.
Fig. 5.3. Comparison of measured and simulated displacement maps in (top to bottom): homogeneous; bladder; and induration phantoms. (a) OCT image; (b) experimental measurement; (c) simulated displacement map; and (d) displacement versus depth for the lateral positions indicated by the blue and black dashed lines in (b) and (c), respectively.
Nonuniform stress due to friction
As described in Section 5.2, axial compression of a soft tissue sample is accompanied by lateral expansion (the Poisson effect). In the ideal case of zero friction between the sample and compressor the sample is free to slip along this interface and expand laterally under axial compression. This is the case for the phantoms shown in Fig. 5.3, as the surfaces were well lubricated to minimize friction. When friction is present, this lateral expansion is restricted. This is illustrated in the plot of displacement versus depth, Fig. 5.4(a), for a homogeneous phantom in which no lubrication was used at the surfaces. The silicone used here, like many soft tissues, is inherently adhesive and is expected to undergo little or no slipping at the boundary in the absence of lubricant. In the experimental elastogram, Fig. 5.4(b), this restricted motion manifests as bands of low strain at the top and bottom surfaces, which are also seen in the simulated elastogram, Fig. 5.4(c), for which a no-slip condition was defined at the surfaces in the model.

The simulated stress, Fig. 5.4(d), reveals a nonuniform distribution, with increasing stress toward the centre of the sample. This trend is attributed to the increasing restriction on lateral motion of the sample with distance from the free vertical edges.

Nonuniform stress due to inhomogeneity
Figure 5.5 presents the experimental and simulated elastograms, and simulated stress distribution of the same bilayer and inclusion phantoms as in Figure 5.3. The experimental strain elastograms were generated using the displacement maps in Figs. 5.3(c) and 5.3(d).

The elastograms of the bilayer phantom, Figs. 5.5(a) and 5.5(b), show layers of high and low strain, corresponding to the soft and stiff layers, respectively. Importantly, the stress distribution, Fig. 5.5(c), is uniform in this sample. There are no stress concentrations or strain artefacts apparent at the interface of the two materials. This confirms that the observed strain contrast should match the true modulus contrast between the layers as predicted by the mechanical analysis presented in Section 5.2.
Fig. 5.4. Effect of friction on strain and stress distribution in a homogeneous sample. (a) Measured (blue) and simulated (black) displacement versus depth along the central vertical axis of the sample, (b) measured strain elastogram, (c) simulated strain elastogram, and (d) simulated stress map for a homogeneous sample with friction present at both surfaces.

In the elastograms of the inclusion phantom, Figs. 5.5(d) and 5.5(e), the stiff inclusion is clearly distinguishable as a region of very low strain, and higher strain is observed in the soft matrix. However, the strain in the matrix is not uniform throughout the elastogram even though this is a mechanically uniform material. This is due to variations in stress due to the presence of the stiff inclusion as seen in the simulated stress map in Fig. 5.5(f). Higher stresses above and below the inclusion manifest as regions of higher strain in the elastogram. In addition, at the vertical edges of the inclusion, a region of high stress is observed, adjacent to a region of lower stress in the matrix material. This can be attributed to a stress-shielding effect, in which the two materials at this location experience similar loads transferred from the material above, but the deformation of the matrix is restricted by that of the stiff inclusion. As
the stress at this interface is not effectively distributed to the matrix, the inclusion experiences higher stress. This manifests in the elastograms as regions of low strain in the matrix adjacent to the sides of the inclusion. These stress and strain patterns are consistent with those shown for inclusion phantoms in ultrasound elastography studies [33].

Fig. 5.5. Measured strain elastogram, simulated strain elastogram, and simulated stress map, respectively, for (a)-(c) a bilayer sample with modulus contrast 37:1 and (d)-(f) an inclusion sample with modulus contrast 45:1.

As made apparent in Figs. 5.5(d) and 5.5(e), strain artefacts degrade the fidelity of the elastogram to the true distribution of modulus. The severity of these artefacts, however, depends on the modulus contrast between the inclusion and matrix. This is demonstrated in Fig. 5.6, where the modelled stress and strain are shown for Phantoms 3 to 7 listed in Table 5.1, from highest to lowest modulus contrast (top to bottom). Note that the colour scale for the stress maps was adjusted in each case, depending on the preload strain applied to the sample, to facilitate comparison of stress distribution due solely to changes in modulus contrast. The inclusion sizes in each set of images in Fig. 5.6 correspond to those used in the actual experiments (in the range 300-500 μm in height and width); however, recall that the resulting strain contrast is independent of the inclusion size [Eq. (5.1)]. This sequence of images illustrates that although the stress concentrations and strain artefacts decrease with decreasing modulus contrast, the contrast between inclusion and background in the
elastogram also diminishes. In the following sections, we consider the limits on this contrast imposed by both mechanical and imaging parameters.

Fig. 5.6. Simulated strain elastograms and stress maps for varying degrees of contrast between inclusion and matrix. Modulus contrasts (inclusion:matrix): (a) 45:1, (b) 10:1, (c) 5:1, (d) 2:1, (e) 1.1:1.

**Contrast transfer efficiency**

Figure 5.7 compares, using Eq. (5.1), the true modulus contrast, $C_\tau$, and predicted strain contrast, $C_\nu$, to the strain contrast observed between the inclusion and matrix in the OCE experiments. The curve for the predicted strain contrast was generated for a Poisson’s ratio of 0.49. The average strains in the elastograms were calculated for regions both within the inclusion and in the matrix at least 500 µm away from the
inclusion. Fifty strain pixels were used to calculate the average and standard deviation of strain in each feature, all at the same depth in the sample to minimize the effects of decreasing OCT SNR with depth on the standard deviation of strain. Error bars reflect the standard deviation of contrast between the inclusion and matrix. The contrast values are plotted as amplitude ratios on a logarithmic scale, \( C_o \) (dB) = \( 20 \log \left( \frac{\epsilon_{\text{matrix}}}{\epsilon_{\text{inclusion}}} \right) \), following the convention used in ultrasound elastography studies of contrast [32, 34, 41, 42].

![Graph showing contrast vs. modulus contrast](image)

Fig. 5.7. Simulated strain elastograms and stress maps for varying degrees of contrast between inclusion and matrix. Modulus contrasts (inclusion:matrix): (a) 45:1, (b) 10:1, (c) 5:1, (d) 2:1, (e) 1.1:1.

Note that for modulus contrasts >10:1 (20 dB), the observed contrast maintains an approximately constant offset of \( \sim 6 \) dB from the true modulus contrast. In other words, the CTE reaches a maximum of just over 0.5 for modulus contrasts of 10:1 and greater. This can be explained by considering the interaction of the inclusion and matrix under compression. A stiff inclusion resists deformation, and because it is mechanically coupled to the surrounding matrix, this causes a perturbation to the stress and strain in the matrix. This manifests as stress and strain concentrations, as illustrated in Figs. 5.5 and 5.6. Increasing inclusion modulus increases these perturbations on the matrix, also illustrated in Fig. 5.6. Above a certain contrast, the inclusion can be regarded as a rigid body, \( i.e., \) it undergoes very little strain; thus, the perturbations it causes to the matrix remain fixed regardless of increases in modulus contrast [32]. Low contrast inclusions, which more easily deform with the matrix under compression and cause little perturbation on the matrix strain, will result in
strain elastograms that more accurately represent the true mechanical contrast. This reiterates that a fundamental limitation on strain elastogram accuracy, previously described in [32] and [34], also applies to OCE.

**Sensitivity of strain elastograms to variations in elasticity**

The CTE limits the accuracy of mechanical contrast in strain elastograms, but to determine the smallest detectable mechanical contrast, *i.e.*, the elastogram sensitivity, we must consider the limitations of the employed imaging system and strain estimation process. Using the definitions for elastogram performance parameters presented in Sec. 5.2, we can estimate the strain sensitivity (*S*<sub>e</sub>) and DR for the measurements performed in this article. These values are summarized in Table 5.2. Note that these values are specific to the phase stability (25 mrad) and wavelength (835 nm) of the employed OCT system, the axial strain spatial resolution (92 μm), and the strain estimation technique (WLS with Gaussian smoothing).

**Table 5.2. Estimated elastogram performance parameters (DB calculated using 20 log(e)).**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ε&lt;sub&gt;max&lt;/sub&gt;</strong></td>
<td>1600 με (-56 dB)</td>
</tr>
<tr>
<td><strong>ε&lt;sub&gt;min&lt;/sub&gt; (S&lt;sub&gt;e&lt;/sub&gt;)</strong></td>
<td>2.4 με (-112 dB)</td>
</tr>
<tr>
<td><strong>DR</strong></td>
<td>667 (56 dB)</td>
</tr>
</tbody>
</table>

The reported strain DR of 56 dB is based on a theoretical maximum displacement corresponding to a phase change of π radians within one strain resolution (in this case, 1600 με over 92 μm). However, the actual maximum displacement used in the experiments corresponded to a phase change of π radians over the entire depth of the phantom (~1 mm) to avoid the need for any phase unwrapping. This decreases the actual strain DR for these measurements to ~36 dB and points to the potential improvement to be gained in the strain DR through implementation of robust phase unwrapping algorithms.

*S*<sub>e</sub> was calculated as the standard deviation of 500 strain pixel values over a 50 × 600 μm region in an elastogram of a homogeneous phantom, and at a depth with an average OCT SNR of 20 dB. *S*<sub>e</sub> degrades with decreasing OCT SNR. For example, in the same phantom, *S*<sub>e</sub> of 3.7 and 5.6 με was calculated at depths of average OCT SNR of 12
and 8 dB, respectively. $S_e$ also depends on the strain axial resolution and is expected to improve with a tradeoff of lower resolution [15].

$S_e$ limits the minimum detectable contrast in strain elastograms, i.e., to distinguish two features in a strain elastogram, the difference in strain between two features must be greater than $S_e$. Figure 5.8 illustrates this problem of feature detectability. In Figs. 5.8(a)–(c), experimental elastograms of inclusion phantoms with decreasing modulus contrast are shown, along with plots, Fig. 5.8(d), of the simulated (solid lines) and experimental (dotted lines) strain values through the locations indicated by the dashed lines in the elastograms. This plot illustrates the degradation of strain contrast due to noise in the experimental versus simulated strain. Still, the inclusions are readily detectable in each elastogram, including the inclusion with small modulus contrast of 1.1:1, Fig. 5.8(c). The measured strains in the inclusion and matrix for this phantom were 90 and 120 με, respectively, a difference sufficiently greater than the strain sensitivity to make the inclusion readily detectable.

The strain sensitivity, in combination with the CTE of a particular geometry, ultimately determines the sensitivity to variations in elasticity. For layered samples, as well as for inclusion samples with low modulus contrast between the inclusion and matrix, such as that in Fig. 5.8(c), the strain contrast closely approximates modulus contrast (CTE close to 1). It follows that the strain sensitivity is approximately equal to the modulus sensitivity in these cases. Considering our best-case scenario, with $S_e$ of 2.4 με and maximum strain of 1600 με, a modulus contrast of $\varepsilon_{\text{max}}/(\varepsilon_{\text{max}} - S_e)$, corresponding to ~1.002:1, could theoretically be detected in a strain elastogram. In the phantoms shown in Fig. 5.8, where the matrix material has Young’s modulus ~18 kPa, this would translate to the ability to detect a change in elasticity as small as 36 Pa.
Fig. 5.8. Impact of strain sensitivity on detectable contrast. Experimental strain elastograms for modulus contrasts (a) 45:1, (b) 5:1, and (c) 1:1:1. (d) Plots of experimental (dotted lines) and simulated (solid lines) strain in the samples at depths specified by the dashed lines in the elastograms.

5.2.4 Discussion

The measurements and simulations presented here aid in the understanding of mechanical contrast by illustrating the limitations imposed by sample mechanics and the image formation process. In the field of ultrasound elastography, several studies have investigated the combined impact of mechanics and imaging on the resulting contrast in strain elastograms [32–34]. However, there are some key differences in the limits on contrast at the scale of OCE.

First, a consideration specific to OCE highlighted in this work is the importance of mechanical conditions at the sample surface, as elastograms in OCE are limited to the first 1 to 2 mm of tissue. In compression OCE, in particular, friction decreases the
amount of axial strain, hence, increasing the apparent stiffness of the material. It also introduces surface artefacts in strain elastograms, such as those in Figs. 5.4(b) and 5.4(c), which could potentially be misinterpreted as regions of higher Young’s modulus in what is actually a mechanically homogeneous sample. For a thick sample, this band of low strain could potentially dominate the entire OCE field of view. Even in noncontact OCE techniques where such friction is absent, such as those that use laser pulse [22] or air puff excitation [9] an uneven sample surface, such as that expected to manifest in tissue, can induce complex motion and is expected to cause simple models of mechanical behaviour to break down. The importance of such surface effects represents an added challenge in advancing OCE techniques toward practical clinical use.

Second, the displacement sensitivity of OCT, especially using phase-sensitive detection, results in a very high sensitivity to changes in tissue elasticity. Although the DR of strain for compression OCE found in this article is similar to that reported for compression elastography using ultrasound [42] the microstrain sensitivity of OCE should enable detection of more subtle increments in tissue elasticity, as illustrated in Fig. 5.8. Together with the high spatial resolution of OCE, this suggests the potential to differentiate tissues within an elastogram that may not be distinguishable with the sensitivity of ultrasound and magnetic resonance (MR) elastography. This has important implications for providing mechanical contrast between, for example, healthy, premalignant, and malignant tissues of the breast, which have been reported to exhibit elasticity values in the narrow range of 0.2 to 2 kPa, as measured by atomic force microscopy [43].

The capacity to detect the mechanical heterogeneity in tissue will also vary depending on the spatial scale being probed. The scale of tissue probed by ultrasound and MR elastography is closer to that probed by manual palpation but with the important advantage of being able to probe deep within the body. On the other hand, OCE should be able to resolve mechanical heterogeneity on a finer length scale but within a smaller field of view. An indication of the impact of spatial resolution on detectable mechanical contrast is seen in the elastograms of the inclusion phantom in Figs. 5.5(d) and 5.5(e). The lower axial strain spatial resolution (92 μm) employed in the experiment, compared to the element spacing used in the FEM (10 μm), tends to
blur the true edges of the inclusion in the experimental elastogram. This also accounts for the absence of very localized variations in strain in the experiment versus the simulation, *e.g.*, small points of high strain visible at the corners of the inclusion in the simulation are not resolved in the experiment. A study of the impact of spatial resolution on mechanical contrast is beyond the scope of this current work, but such an investigation would provide additional insight into the unique tissue contrast OCE can offer.

A limitation of the present study was the simplification of the response of nonlinear materials to a linear elastic model of behaviour. However, we took account of the nonlinearity of the materials by estimating their effective Young’s moduli based on bulk strain due to preload. Input of this effective Young’s modulus into a linear elastic FEM gave similar results to input of the stress-strain curve into a nonlinear FEM. Thus, we chose to use only linear elastic models to simplify the mechanical analysis in this study. Furthermore, all linear fits to the stress-strain curves to estimate modulus had an error ≤95 Pa, which is on the order of our modulus sensitivity for these materials, so this error had negligible impact on the final comparison of elastogram contrast to “true” contrast. However, there was some uncertainty in the estimation of bulk strain of each phantom, as we assumed that the bulk strain measured in the imaging plane (B-scans) was representative of the bulk strain of the matrix and inclusions out of the plane. The asymmetry of the inclusions (~50 μm tolerance was achievable in cutting each face of the inclusion) could have resulted in a discrepancy between the actual effective modulus of each silicone and the calculated “true” modulus. This explanation most likely accounts for discrepancies between the observed strain contrast in elastograms and the strain contrast predicted by the CTE in Fig. 5.7.

Unlike the silicones used here, it has been found that many tissues more closely follow linear behaviour at such low strains [29]. In applications of OCE where high strains (>0.1) may be difficult to avoid, such as in arterial tissues, models for elastogram reconstruction that incorporate tissue nonlinearity have been proposed [44].

The qualitative nature of strain elastograms was demonstrated in several cases in this article through the presence of geometry and contrast-dependent strain artefacts. The CTE was used to quantify the impact of these artefacts on elastogram accuracy.
For the particular geometry of an inclusion in a matrix, it is feasible that, for a given value of Poisson’s ratio, a correction factor could be applied, based on Eq. (5.1), to quantify the true modulus contrast from a strain elastogram. However, while it provides a suitable first approximation of an inhomogeneous tissue structure for the purposes of this study, this simplistic inclusion geometry and the contrast transfer function derived for it are not expected to accurately represent the much more complex microstructure found in tissue. Thus, in tissue, we can expect a degradation of the CTE with higher modulus contrast between adjacent tissues, i.e., with a higher degree of stress nonuniformity.

Quantitative estimation of modulus distribution using compression OCE will require implementation of more advanced methods, such as approaching elastogram reconstruction as an inverse problem. Avenues toward quantitative OCE using inverse methods have been proposed, including a model-based approach, in which an analytical or numerical model (such as FEM) is used to iteratively reconstruct a quantitative modulus image [7, 44, 45]. More recent studies have moved toward quantification of modulus by implementing transient loading techniques in which modulus may be extracted directly from the velocity of shear waves or surface waves in the sample [10, 19–21]. However, these techniques come at a loss of resolution, as they assume tissue homogeneity for the length over which the shear wave speed is calculated. This assumption may not be suitable for imaging organs with heterogeneous, complex structures, such as the breast.

Despite currently providing only relative measurements, compression OCE is expected to remain an attractive technique for clinical translation, as it is relatively straightforward and practical to implement and provides higher spatial resolution than other OCE techniques. Furthermore, the relative mechanical contrast in strain elastograms may often be sufficient for detecting variations due to changes in tissue pathology, especially for clinical applications focused on detecting the boundaries of diseased tissue, where both diseased and healthy tissue lie within the imaging field of view.

The analysis presented here provides a framework for evaluation of the sensitivity and accuracy of contrast in other OCE techniques. Finite element modelling is expected to be an essential tool for testing the validity of assumptions made about
sample behaviour in various OCE techniques and for analysing how variables such as geometry, surface conditions, loading rate, and detection schemes impact on contrast.

5.2.5 Conclusion
We have performed an evaluation of mechanical contrast in strain elastograms produced using phase-sensitive compression OCE, highlighting the limitations imposed by mechanical deformation and by imaging system noise and signal processing parameters. We have illustrated these limitations by presenting experimental and simulated elastograms of tissue-mimicking phantoms with a range of mechanical properties. Based on our analysis, we summarize the following answers to the questions proposed in the introduction as they apply to compression OCE:

1. How accurately do OCE elastograms represent the elasticity distribution within a sample?
   • Layered structures and structures with low elasticity contrast tend toward uniform stress and high accuracy (CTE ~ 1).
   • Structures with high elasticity contrast and/or surface friction have nonuniform stress and lower accuracy (CTE < 1).
   • For an inclusion geometry, CTE plateaus at 0.5 for elasticity contrast >10:1.

2. What is the sensitivity of OCE elastograms to variations in elasticity within a sample?
   • For our imaging and strain estimation parameters, we were able to measure an elasticity contrast of 1.1:1 and predicted a maximum sensitivity to elasticity contrast of 1.002:1.

3. What factors limit elastogram accuracy and sensitivity?
   • Mechanical factors: geometry, friction, elasticity contrast between features.
   • Imaging factors: displacement sensitivity, resolution, strain estimation parameters.

Despite mechanical artefacts limiting compression OCE to providing relative measurements of elasticity, the microstrain sensitivity demonstrated here, together with its high spatial resolution, has not previously been attainable by other methods. Access to this new regime for tissue mechanical measurements suggests that phase-
sensitive compression OCE is a very promising technique for providing novel and clinically meaningful tissue contrast.

5.2.6 References for Section 5.2


5.3. Discussion

This study has provided an improved understanding of the role of sample mechanics in image formation in compression OCE. The main source of mechanical artefacts in compression OCE is nonuniform stress within the sample; this imposes fundamental limits on the dynamic range of mechanical contrast that may be visualized in strain elastograms. In severe cases, large spatial variations in stress could potentially mask mechanically disparate features, leading to misinterpretation of elastograms. In Chapters 7 and 8, respectively, we present a method to measure stress in OCE, and a method to combine this stress measurement with compression OCE measurements to quantify Young’s modulus. We reiterate, however, that the qualitative contrast provided by strain elastograms has clinical potential on its own, especially in cases where the goal is to differentiate features based on mechanical contrast within the field of view of a single image, as may be the case in delineating the extent of malignant tissue in excised breast lesions.
Another limiting factor in the mechanical contrast in strain elastograms, not considered in depth in this study, is the dynamic range of measurable displacements. This is dictated at a minimum by the phase sensitivity of the system, and at a maximum by phase unwrapping, and ultimately by speckle decorrelation. The phase sensitivity reported here of 50 mrad, corresponding to a displacement sensitivity of 1.2 nm, is expected to be difficult to achieve in practice, given the variable OCT SNR within tissue samples. In addition, compression of tissue samples causes shifting of sub-resolution scatterers, which is also expected to set a practical limit on displacement sensitivity in OCE, as phase-sensitive measurements of displacement are based on a frozen speckle model [135].

To improve the displacement sensitivity, and, thus, the strain sensitivity in the OCE measurements in this study, images were formed by averaging 100 B-scans (50 uncompressed/compressed pairs). Whilst this enabled excellent strain sensitivity (2.4 με), such oversampling for temporal averaging is impractical for rapid 3-D scanning of tissue volumes, as may be necessary for in an intraoperative scenario. Furthermore, in this chapter and in the majority of OCE studies to date, phase unwrapping has not been implemented, as robust phase unwrapping algorithms for OCE have not been proposed.

In the next chapter, we demonstrate several key improvements to phase-sensitive compression OCE, including a new acquisition method incorporating weighted averaging of the complex OCT signal, and a custom phase-unwrapping algorithm to enable 3-D elastograms with microstrain sensitivity whilst preserving the OCT lateral resolution in the en face plane. We also consider the importance of using common-path interferometry for phase stability, especially in a portable system as that used in clinical settings (a common-path setup was used in this chapter, but its impact on phase sensitivity was not considered). This improved technique, called optical coherence micro-elastography, brings us a step closer to realizing the version of OCE that may ultimately be used for intraoperative guidance of breast cancer surgery.
Chapter 6

Optical coherence micro-elastography

This chapter describes key improvements in the image acquisition scheme and signal processing of compression OCE to enable optical coherence micro-elastography. The microstrain sensitivity, high lateral resolution, and 3-D capabilities of this technique result in elastograms that reveal the microscale mechanical heterogeneity of breast tissues with exquisite detail and provide significant additional contrast over OCT imaging alone. Compared to previous OCE images of breast tissues, such as that in Fig. 3.9, the images presented here represent a considerable improvement in image quality and demonstrate good correspondence to features in co-registered histology.

6.1. Optical coherence micro-elastography: mechanical-contrast imaging of tissue microstructure

Brendan F. Kennedy¹, Robert A. McLaughlin¹, Kelsey M. Kennedy¹, Lixin Chin¹, Andrea Curatolo¹, Alan Tien², Bruce Latham³, Christobel M. Saunders², and David D. Sampson¹,⁴

Abstract: We present optical coherence micro-elastography, an improved form of compression optical coherence elastography. We demonstrate the capacity of this technique to produce en face images, closely corresponding with histology, that reveal microscale mechanical contrast in human breast and lymph node tissues. We use phase-sensitive, three-dimensional optical coherence tomography (OCT) to probe the nanometre-to-micrometer-scale axial displacements in tissues induced by compressive loading. Optical coherence micro-elastography incorporates common-path interferometry, weighted averaging of the complex OCT signal and weighted least-squares regression. Using three-dimensional phase unwrapping, we have increased the maximum detectable strain eleven-fold over no unwrapping and the minimum detectable strain is 2.6 με. We demonstrate the potential of

¹ Optical+Biomedical Engineering Laboratory, School of Electrical, Electronic & Computer Engineering, The University of Western Australia, Crawley, WA 6009
² School of Surgery, The University of Western Australia, Crawley, WA 6009
³ PathWest, Perth, WA 6000
⁴ Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, Crawley, WA 6009
6.1.1 Introduction

Key to the advancement of the optical microscopy of tissue has been the exploration of sources of contrast aimed at improving the visualization of structure and providing information on function. On length scales from the molecular (sub-nanometre) to many millimetres, elastic scattering is a source of contrast that provides information on the structure, size and motion of tissue constituents [1], and spectroscopy provides information on molecular composition [2]. Over the same length scales, the mechanical properties of tissue are a rich alternative to optical sources of contrast [3]. Such properties govern the mechanical interactions between cells and their environment, which, in concert with chemical interactions, determine how they grow, differentiate and migrate. The impairment of a cell’s capacity to respond to mechanical forces contributes to the pathogenesis of diseases such as cancer [4], and leads to differences in the mechanical properties of normal and malignant tissue. Tumour cells are known to be commonly softer than their normal counterparts and, at the same time, tumours commonly cause the generation of additional collagen-dense stroma making them feel stiffer on the macroscale [5]. The result of this innate heterogeneity is that, on the microscopic scale, malignant tissues often have a broader stiffness distribution than normal tissues [5].

The measurement of the mechanical properties of cells and tissues on the nano- and microscale, using techniques such as atomic force microscopy [5] and optical tweezers [6], has contributed greatly to our understanding of the role of mechanical interactions in disease. On the macroscale, physicians have used palpation as a means of diagnosis for centuries. The advent of medical imaging, such as ultrasound and magnetic resonance imaging, has provided the means for elastography, the use of imaging to map mechanical properties [7–9]. In elastography, the tissue is mechanically loaded and imaged to measure its deformation. For the class of methods based on compressive loading, the displacement vector between image acquisitions is used to estimate components of the local strain tensor (change in length per unit
length), which are displayed in images (elastograms) that represent relative tissue stiffness [9]. Elastography has a more-than-twenty-year history [10] during which many loading methods and means of extracting mechanical properties have been explored, and clinical applications have emerged, e.g., in breast cancer [11] and liver fibrosis [12].

Amongst optical elastography methods [13–16], the use of optical coherence tomography (OCT) to measure displacement, termed optical coherence elastography (OCE), has been the most prominent [14, 16–26]. Despite much recent progress, optical elastography has not yet demonstrated the required spatial resolution, or mechanical sensitivity and dynamic range, to adequately reveal the microscale structure of tissue. In this paper, we aim to address this with optical coherence micro-elastography, which improves on existing compression OCE techniques [22, 26] by incorporating common-path interferometry [27], averaging of the complex OCT signal and three-dimensional phase unwrapping. Additionally, optical coherence micro-elastography is performed using a portable system, facilitating clinical imaging. In en face micro-elastograms compared against en face OCT images and histology, we demonstrate high mechanical contrast maintained over a large dynamic range that is complementary to the optical contrast and reveals additional tissue contrast in human breast cancer and lymph node samples.

6.1.2 Methods

**Optical coherence micro-elastography system**

Our optical coherence micro-elastography system employs a Fourier-domain OCT system described previously [28]. To perform clinical scanning, the system was housed on a portable trolley with dimensions (height × width × depth) of 1.20 m × 0.7 m × 0.9 m. It uses a superluminescent diode light source with central wavelength of 835 nm and 50-nm bandwidth, and illuminates the sample with 10 mW of optical power. The system was configured in common-path [27], with the reflected beam from the back surface of a 2-mm thick imaging window (IW in Fig. 6.1(a)) used as a reference. The axial and lateral resolutions (full-width at half-maximum irradiance) were measured to be 7.8 μm (in air) and 11 μm, respectively, and the sensitivity was measured to be 102 dB for an exposure time of 36 μs.
We used phase-sensitive, three-dimensional OCT to measure the axial displacement of a sample in response to compressive loading. The sample is compressed using a ring actuator, which is rigidly coupled to the imaging window (Fig. 6.1(a)) [29]. To ensure full contact with the imaging window, the sample was statically preloaded by displacing the rigid upper plate after initial contact by a further 0.1-1 mm, corresponding to strain values in the range 0.02-0.2, within the normal range used in compression testing of soft tissue [9]. A 1-minute delay before imaging reduced the effects of viscoelastic creep deformation in the sample [30] to a negligible level. Subsequently, a 5-Hz square-wave loading [Fig. 6.1(b)], applied collinearly with the imaging beam [22], was synchronized with the lateral scanning and produced an axial displacement amplitude of up to 2.2 μm in the ring actuator. At each lateral (xy)
position of a three-dimensional complex OCT data set, the phase difference between two A-scans in consecutive B-mode frames reveals the sample’s depth-resolved axial displacement [Fig. 6.1(c)] [19]. The displacement sensitivity is 0.34 nm, calculated as the standard deviation of 500 displacement measurements acquired from the same position on a stationary adhesive tape phantom with corresponding OCT SNR of 50 dB [Fig. 6.2(a)] [22, 31]. As shown in Fig. 6.2(a), this is >40-fold higher displacement sensitivity than achieved using the dual-arm configuration in otherwise the same setup. For each B-mode micro-elastogram, 1000 A-scans were recorded in an acquisition time of 0.1 s. For scanning in the y-direction, oversampling was used to provide phase correlation between consecutive B-mode frames and enabled weighted averaging of the complex OCT signal. (The y-sampling density is four B-mode frames per micrometre for Figs. 6.3 and 6.5, and 0.9 B-mode frames per micrometre for Figs. 6.6-6.8, corresponding to 3-D micro-elastogram acquisition times of 2000 s and 500 s, respectively). Three-dimensional micro-elastograms were generated, wherein each voxel represents the local strain [Fig. 6.1(c)], i.e., the change in displacement over a specified axial range at each depth position [22]. The chosen axial range, Δz, lies between 100 μm and 215 μm in the micro-elastograms presented in this paper. The lateral resolution of the micro-elastogram (11 μm here) matches that of the corresponding OCT image.

Strain sensitivity, in common with OCT sensitivity, is a measure of the system sensitivity and not a measure applicable to any given image. Whereas in OCT this sensitivity may be determined by reflection from a mirror, in compression OCE, this measure depends on the sample used to determine it. The system strain sensitivity [Fig. 6.2(b)] is determined using a method similar to one reported previously [22, 28]. 200 B-mode elastograms acquired at the same y-position in an optically and mechanically homogeneous phantom were averaged. Strain sensitivity is defined as the standard deviation of 100 strain measurements calculated over a lateral range of 100 μm acquired from the central region of the phantom. This provides an estimation of the maximum achievable system strain sensitivity, the strain sensitivity floor, measured to be 2.6 με for our system. This strain sensitivity is >30-fold higher than that achieved using the same method with a dual-arm configuration [Fig. 6.2(b)]. For this measurement, the sample was subjected to a local strain of ~0.05 με,
corresponding to the mean strain observed in Fig. 6.2(b). This approach ensures that all factors influencing the system strain sensitivity, such as actuator response, are included in the sensitivity measurement.

Fig. 6.2. (a) Displacement and (b) strain sensitivity measurements, respectively, for common-path, \( \sigma_{d, cp} \) and \( \sigma_{r, cp} \) (red), and dual-arm, \( \sigma_{d, da} \) and \( \sigma_{r, da} \) (blue), configurations of our portable imaging system determined using (a) an adhesive tape phantom and (b) a scattering silicone sample.

**Phantom fabrication**

Heterogeneous tissue-mimicking phantoms with well-determined optical and mechanical properties and structure were fabricated using two-component room-temperature vulcanizing silicone [32, 33]. The optical properties were controlled by adding titanium dioxide particles (refractive index ~2.5, average diameter 1 \( \mu \)m) to the silicone in concentrations in the range 0.8-2.5 mg/mL. To ensure uniform particle distribution, the mixture was placed in an ultrasonic bath for 25 minutes. Phantoms were then oven-cured at 90°C for ~30 minutes. For each phantom, the Young’s modulus of the soft bulk silicone material, measured using a materials testing system [33], was 20 kPa and that of the inclusion was 850 kPa. The phantom used to generate the images in Fig. 6.3 has dimensions of 15 \( \times \) 15 \( \times \) 1.5 mm. The cubic inclusion in this phantom has approximate dimensions 0.2 \( \times \) 0.2 \( \times \) 0.2 mm and was cut by hand using a scalpel. The dimensions of the ‘star’ phantom used to generate the images in Fig. 6.5 are 15 \( \times \) 15 \( \times \) 3 mm. The stiff, star-shaped inclusion was cut from a cube of cured silicone with approximate dimensions 10 \( \times \) 10 \( \times \) 2 mm using a femtosecond laser to an equivalent circular diameter of 5 mm and a thickness of 2 mm. To embed the inclusions, we used a procedure described in detail previously [33]. Briefly, a layer of soft silicone was cured in a mould. The thickness of this layer determined the depth of the inclusion from the lower boundary of the phantom and is 1.1 mm and 0.75 mm for the phantoms presented in Figs. 6.3 and 6.5, respectively. In both cases, the inclusion
was placed on the layer and soft silicone from the same batch as the layer was poured over the inclusion to the desired thickness. Upon curing, this resulted in a soft silicone phantom with a stiff inclusion embedded within.

**Tissue preparation, histology, and co-registration**

Informed consent was obtained from the patients and the study approved by the Human Research Ethics Committee of Royal Perth Hospital, Perth, Western Australia. In total, 45 samples were excised from 15 patients undergoing mastectomy, or mastectomy with axillary clearance, and were imaged within 2-3 hours of excision. After excision, the fresh tissue was dissected into samples of approximate dimensions 1.5 cm × 1.5 cm × 0.5 cm. The samples were kept hydrated in phosphate-buffered saline prior to imaging. After imaging, each sample was fixed in 10% neutral buffered formalin and then embedded in paraffin and sectioned following the standard histopathology protocols used at Royal Perth Hospital. The haematoxylin and eosin-stained sections were digitally micrographed and co-registered with the corresponding en face OCT images and en face micro-elastograms using a custom three-dimensional visualization tool [34] that enabled the extraction of arbitrary imaging planes. The planes selected for the micro-elastograms were those determined manually by inspection to correspond most closely to the histological section. The OCT images corresponding most closely were chosen from within the axial range of the corresponding micro-elastogram. A voxel-to-voxel comparison of the en face micro-elastograms and OCT images must take into account the poorer elastogram axial resolution.

**Signal processing in optical coherence micro-elastography**

In Fig. 6.3, the signal processing chain is illustrated using experimental data acquired from a phantom containing the stiff inclusion, described above. In existing phase-sensitive compression OCE techniques [19, 22], the displacement, \( d \), is determined from the phase difference, \( \Delta \phi \), between loaded and unloaded B-mode pairs, according to: \( d = \Delta \phi \lambda_0 / 4\pi n \) [19], where \( \lambda_0 \) is the central wavelength of the light source and \( n \) is the tissue refractive index. In optical coherence micro-elastography, the displacement sensitivity is improved by averaging \( q \) complex quotients, \( Q_k = W_k \exp(i\Delta \phi_k) \), for
\[ k = 1, 3 \ldots 2q - 1 \], where \( k \) is the B-scan index [Figs. 6.3(a)-6.3(c)], extracted from a three-dimensional OCT dataset [35]. \( Q_k \) incorporates weighting by the OCT signal-to-noise ratio (SNR; intensity ratio), \( W_k \), and the phase difference, \( \Delta \phi_k \), [Fig. 6.3(b)] of the \( k \)-th loaded and unloaded B-mode pair. The weighting factor assumes similar signal levels between the unloaded and loaded states. \( q \) pairs acquired within a lateral \( y \)-range of

Fig. 6.3. Signal processing steps used in optical coherence micro-elastography illustrated with experimental data from a structured phantom. (a) \( q \) loaded \( (C_L) \) and unloaded \( (C_U) \) complex OCT B-mode frame pairs are acquired within a \( y \)-range of \(~6 \text{ mm}\). (b) The phase difference, \( \Delta \phi_k \), and weighting \( W_k \), are calculated for each pair of B-mode scans, for \( k = 1, 3 \ldots 2q - 1 \). (c) The weighted average phase difference, \( \Delta \phi_{av} \), is calculated by averaging \( q \) complex quotients, \( Q_k \). (d) The unwrapped phase difference is calculated using a three-dimensional phase unwrapping algorithm, described in the section below. Negative phase difference indicates decreasing displacement with depth [Fig. 6.1(c)]. (e) A B-mode micro-elastogram is calculated from the rate of change of the unwrapped phase difference with depth, using weighted least-squares linear regression over axial range, \( \Delta z \). Increasing negative strain indicates increasing amounts of compression of the sample in response to the load. In the micro-elastogram, the scale is in millistrain, me. Scales bars, 200 mm.
~6 μm [Fig. 6.3(a)] are averaged to improve the displacement sensitivity whilst, unlike previous OCE methods [16, 21], retaining the OCT lateral resolution. Additionally, we use a custom three-dimensional phase unwrapping algorithm [Fig. 6.3(d)], described in detail in the next section. We use the algorithm to correct up to five wrapping discontinuities, thereby, extending the maximum measurable displacement from 0.15 μm to 2.2 μm, representing an eleven-fold improvement on the maximum measurable strain. To calculate local strain from the unwrapped phase difference, we utilize weighted least-squares regression [Fig. 6.3(e)], assigning a weight to each phase difference based on the OCT SNR, which improves upon ordinary least-squares and gradient methods [22]. Repeating the steps shown in Fig. 6.3 at ~6-μm intervals in the y-direction provides a three-dimensional micro-elastogram, as presented in Section 6.1.3.

Phase unwrapping algorithm

As phase is modulo 2π, phase-sensitive OCE techniques have been limited to maximum measurable displacements between two acquisitions of ~0.3-0.46 μm [16]. To increase the maximum detectable strain, we implemented a custom three-dimensional phase unwrapping algorithm, illustrated using the flowchart in Fig. 6.4. This algorithm takes advantage of the features of optical coherence micro-elastography as follows. Firstly, the common-path interferometer configuration results in a known phase difference at the initial axial coordinate of a scan, providing a well-defined starting condition. As the sample is physically coupled to the common-path reference reflector at the point corresponding to the first en face plane (z = 0 μm, k = 1), the phase difference at this initial point is known to be 0 radians. Secondly, as the displacement determined with phase-sensitive detection is exclusively in the axial direction [18] and because, for a sample under a compressive load, the magnitude of the phase difference increases with depth, the direction along which phase unwrapping should be performed is always known. Thirdly, the measured phase difference at each voxel is affected by additive Gaussian white noise with variance approximately equal to the inverse of the OCT signal-to-noise ratio (SNR) [31]. This relationship allows the SNR to be used to weight the corresponding phase difference, minimizing the effects of noise on the unwrapped phase (the weight
corresponds to $W_{ijk}$ in Fig. 6.4). Fourthly, the displacement induced by loading is assumed to be uniform over local regions in the acquired data. The phase difference is, thus, unwrapped based on the weighted-average phase difference in local neighbourhoods.

In the flowchart shown in Fig. 6.4, we index the $xyz$ coordinates of the data volume by $ijk$. The algorithm commences at the $en face$ plane $k = N_z + 1$, where $N_z$ is the number of axial voxels used to calculate the axial-weighted mean phase, $\mu_{z,ijk}$, which is used to determine if a wrapping discontinuity has occurred. We use $N_z = 10$, corresponding to $\sim 14$ $\mu$m in depth. The phase difference in the first $N_z$ $en face$ planes is assumed to be free of phase wrapping. For each subsequent $en face$ plane, each voxel is first unwrapped axially (corresponding to $\Delta\phi_{z,ijk}$ in Fig. 6.4) by subtracting an integer multiple of $2\pi$ to minimize the difference between the phase difference of the voxel, $\Delta\phi_{av,ijk}$, and $\mu_{z,ijk}$ of the $N_z$ preceding voxels. After axial unwrapping, every voxel in the $en face$ plane is laterally unwrapped (corresponding to $\Delta\phi_{uw,ijk}$ in Fig. 6.4) by subtracting a multiple of $2\pi$ to minimize the difference between the phase difference of each axially unwrapped voxel and the lateral-weighted average phase difference, $\mu_{xy,ijk}$, of the voxels within a $(2R + 1) \times (2R + 1)$ neighbourhood (where we have chosen $R = 5$). We have demonstrated that the algorithm can correct up to five wrapping discontinuities.
Fig. 6.4. Flowchart for the three-dimensional phase unwrapping algorithm.

The upper limit on the number of wrapping discontinuities that can be corrected is, in principle, very large, approaching a third of the total number (2048 in our case) of axial voxels [36]. However, for displacements corresponding to more than five wrapping discontinuities, we observed speckle decorrelation artefacts between B-mode acquisitions that reduce the phase sensitivity, setting a practical upper limit on the measurements reported here. The algorithm was tested and validated on both phantoms and tissue. The algorithm was found to perform sub-optimally in areas where the assumption of uniaxial compression did not apply, i.e., close to tissue boundaries and where the tissue was not fully in contact with the compression plate.

6.1.3 Results

Figures 6.5(a) and 6.5(b) compare a three-dimensional OCT image and an optical coherence micro-elastogram ($\Delta z = 100 \text{ \mu m}$) of an optical and mechanical tissue-mimicking phantom containing a stiff, star-shaped inclusion, described in Section 6.1.2
and illustrated in Fig. 6.1(a). Perspective views of the OCT and optical coherence micro-elastography volumes are presented at a depth of ~500 μm. In Fig. 6.5(b), the star-shaped inclusion is clearly distinguished from the bulk material based on the measured local strain. As observed in this example, optical coherence micro-elastography provides similar spatial resolution in the en face plane to the corresponding OCT image. A cutaway view in Fig. 6.5(b) reveals the local strain contrast obtained in the xz-plane. The axial strain spatial resolution is lower than the axial OCT resolution as, for each pixel, the local strain is estimated over a depth range of 100 μm. Despite this, high contrast is also observed in this plane between the star and the surrounding bulk material.

Fig. 6.5. (a) Three-dimensional OCT perspective view of the phantom obtained from a depth of ~500 μm. (b) Corresponding perspective view of the three-dimensional micro-elastogram displaying the local strain, and a cutaway view revealing a B-mode micro-elastogram through the central region of the inclusion. OCT data are displayed on a log intensity scale, and local strain is displayed in millistrain, mε. Scale bars (arrows), 0.5 mm.

To demonstrate the potential of optical coherence micro-elastography on tissue, in Figs. 6.6, 6.7, and 6.8 we present three representative micro-elastograms co-registered with histology. Figure 6.6 shows the OCT image [Fig. 6.6(a)], micro-elastogram [Δz = 215 μm, Fig. 6.6(b)], and histology [Fig. 6.6(c)] of fresh breast tissue excised from a patient with invasive ductal carcinoma. The micro-elastogram and OCT image are oriented en face and mosaicked from two overlapping data sets, each with xyz dimensions 6 × 6 × 2.25 mm. Observable features of breast microarchitecture in the micro-elastogram include adipose, smooth muscle, ducts, blood vessels and tumour. Ducts and blood vessels appear
Fig. 6.6. Optical coherence micro-elastography of malignant human breast tissue. (a) *En face* OCT image at a depth of ~100 μm. (b) Corresponding *en face* micro-elastogram. (c) Histology, co-registered with OCT and micro-elastogram. A, adipose; D, duct; M, smooth muscle; T, region densely permeated with tumour; and V, blood vessel. In the micro-elastogram, the scale is in millistrain, mε. The insets show a 2.5× magnification of the blue-dotted boxes.

as regions of high negative strain, indicating that they are more compressible than surrounding tissue. Inverted (positive) strain is observed at feature boundaries, including in each of the labelled ducts (D), and acts to accentuate these features. The magnified inset of Fig. 6.6 highlights a feature (blood vessel) present in the micro-elastogram but difficult to discern in the OCT image. A region of tumour (T) in the bottom left of the image is distinctive in the micro-elastogram but difficult to discern.
in the OCT image. Figure 6.6 demonstrates that the improvements in spatial resolution, mechanical sensitivity, and dynamic range enable optical coherence micro-elastography to reveal contrast in malignant tissue that is complementary and, in some aspects, superior to OCT.

Figure 6.7 shows images of tissue excised from a patient with low/intermediate-grade ductal carcinoma in situ. In the micro-elastogram [Fig. 6.7(b)], there is clear delineation of several ducts infiltrated with malignant cells, labelled T, which appear as focal variations in strain. Additionally, several uninvolved lobules, labelled L, are visible in the micro-elastogram. Each of these features is more clearly visible in the micro-elastogram than in the corresponding OCT image [Fig. 6.7(a)], and is also visible in the histology [Fig. 6.7(c)]. In comparison, the strain throughout the central region (stroma) presents as more uniform, suggesting that the tissue is more mechanically uniform in these regions.

![Fig. 6.7. Optical coherence micro-elastography of human breast tissue diagnosed as ductal carcinoma in situ at a depth of ~100 μm. (a) En face OCT image; (b) En face micro-elastogram; and (c) Histology, co-registered with OCT and micro-elastogram. The micro-elastogram presents additional contrast compared to the OCT image. L, lobule; and T, tumour in a duct. In the micro-elastogram, the scale is in millistrain, mε.](image)

Figure 6.8 shows the OCT image [Fig. 6.8(a)], micro-elastogram [Δz = 215 μm, Fig. 6.8(b)], and histology [Fig. 6.8(c)] of an uninvolved lymph node excised from a patient undergoing axillary clearance. Observable features of lymph node microstructure in the micro-elastogram include capsule, follicles, medulla, and paracortex. Follicles appear in the micro-elastogram as regions of heterogeneous strain. These features are much less apparent in the OCT image. In comparison, the medulla presents as a region of homogeneous strain, indicating that it is a more mechanically uniform structure. The paracortex appears as a smooth texture which transitions between these two regions.
Fig. 6.8. Optical coherence micro-elastography of human lymph node tissue. (a) En face OCT image at a depth of ~140 μm. (b) Corresponding en face micro-elastogram. (c) Histology, co-registered with OCT and micro-elastogram. C, capsule; F, follicle; M, medulla; and P, paracortex. In the micro-elastogram, the scale is in millistrain, mE.

6.1.4 Discussion

The results presented in this paper demonstrate the potential of optical coherence micro-elastography in imaging excised breast and lymph node tissue. OCT has previously been proposed as a tool in breast cancer surgery for both intraoperative assessment of tumour margins [37] and lymph nodes [34]. The complementary, and in some cases superior, contrast provided by micro-elastography suggests that it has potential to improve the contrast provided by OCT in these clinical scenarios. Optical coherence micro-elastography could potentially be used to establish margins on excised breast cancer tissues, as has been proposed for OCT [37]. To establish its efficacy in such an application requires further studies aimed at ascertaining its sensitivity and specificity.

In addition to breast cancer, optical coherence micro-elastography has the potential to improve detection of a number of diseases that alter the microscale mechanical properties of tissue. Indeed, preliminary measurements in optical elastography have already begun to explore this possibility in areas such as cardiology [38], dermatology [20, 29], and ophthalmology [39]. The clinical application of optical coherence micro-elastography may also be facilitated by translating the developments presented here to needle [40, 41] and endoscopic [42] versions of elastography, potentially providing a pathway to microscale mechanical contrast in vivo. The relatively long acquisition times employed here require improvement to make in vivo applications practical. This could be achieved by performing in vivo measurements only in 1-D or 2-D, thereby, achieving shorter acquisition times. Alternatively, higher speed scanning mechanisms could be implemented to facilitate in vivo 3-D application.
Micro-elastograms as presented here employ compression elastography and, thus, rely on relative mechanical contrast provided by strain rather than absolute measurement of a tissue’s elastic modulus, without recourse to inverse methods. By comparison, related optical elastography techniques, e.g., those based on surface acoustic wave [23] and shear wave [43] generation, provide a more direct path to absolute measurements of Young’s modulus. Such quantification of tissue mechanical properties is useful in assessing changes in disease over time and enables comparison of the mechanical properties of different samples. However, using these techniques, it has so far not been possible to resolve the microscale structures visible in the results presented here using optical coherence micro-elastography. It may also be possible to quantify tissue elasticity at the spatial resolution provided by optical coherence micro-elastography. For compression elastography, this requires knowledge of both the stress and the strain distributed throughout the tissue. An initial step towards this goal may be possible by coupling optical coherence micro-elastography with optical palpation [44], a technique our group has recently proposed, in which a compliant sensor is used to provide a high-resolution map of the stress distributed across a tissue’s surface (Chapter 7).

6.1.5 Conclusion
In conclusion, we have demonstrated that optical coherence micro-elastography can reveal the mechanical heterogeneity of tissue at the OCT lateral resolution (11 μm demonstrated here), with microstrain sensitivity and large dynamic range. Through close correspondence with OCT images and histology, we have demonstrated that the mechanical contrast in human malignant breast and non-involved lymph node tissues extends and complements that available from OCT. Beyond the opportunity to expand our understanding of tissue mechanics and the role it plays in biology, optical coherence micro-elastography may enhance the capability to detect a range of diseases, such as cancer, atherosclerosis, and glaucoma, by probing tissue mechanical properties on an intermediate scale between nanoscopic and macroscopic methods.

6.1.6 References for Section 6.1


6.2 Discussion: mechanical heterogeneity of the breast on the microscale

The micro-elastograms of breast and lymph node tissues shown in this chapter reveal the highly heterogeneous mechanical properties of the tissues. The results demonstrate a good correspondence with features in histology but also highlight a dramatic increase in complexity with the tissue structure, compared to the simplistic phantom structures analysed in Chapter 5. At the scale of ultrasound elastography and MRE, breast lesions have typically been modelled as inclusions with different stiffness from the background tissue. In Chapter 5, we used such “inclusion” geometries to help us understand sources of artefacts in OCE. But the elastograms of breast tissue in this chapter illustrate that such simplistic geometries do not accurately represent breast tumours on the microscale.

As discussed in Chapter 3, as cancer develops and spreads, it permeates and breaks up the normal structures of the breast tissue. A recent study of breast tissues on the cellular scale showed that the mixture of cell types in malignant tissues produces a heterogeneous distribution of stiffness values compared to normal tissues (see Fig. 3.6) [170]. In the micro-elastogram in Fig. 6.6, the area containing invasive cancer cells produced a heterogeneous strain pattern. This strain pattern is characterized by intermixed regions of negative (in the direction of the applied load) and positive (in the direction opposite to the applied load) local strain and is possibly due to a combination of the compromised structure of the invaded region and the varied mechanical properties of the tissue constituents, namely, malignant cells and fibrous tissue.

Another example that illustrates this association of malignancy with mechanical heterogeneity is shown in the result in Fig. 6.9. This, like the example in Fig. 6.6, is
from a patient with poorly differentiated, high-grade tumour. The histology for this sample, Fig. 6.9(a), shows a region of mature stroma, *i.e.*, the more dense and dark pink connective tissue in the central region of the sample, surrounded by a mix of immature stroma (lighter pink) and diffuse tumour cells (purple). The OCT image, Fig. 6.9(b), and the corresponding micro-elastogram, Fig. 6.9(c), have been co-registered to histology using custom software as described above. The contrast in the OCT may be due to the differing backscattering properties of the stroma and the small regions of cancer cells throughout the sample. In the micro-elastogram, regions of highly fluctuating strain surround a region of relatively uniform strain. The heterogeneous strain pattern may arise from the dispersed groups of cancer cells that permeate the immature stroma, consistent with the heterogeneity seen in the region of invasive tumour in Fig. 6.6. The region of more uniform strain may correspond to the mature stroma, which is characterized in the histology by a lack of malignant cells, and, thus, is expected to be more structurally and mechanically homogeneous.

Fig. 6.9. Optical coherence micro-elastography of human breast tissue diagnosed as high-grade invasive ductal carcinoma. (a) Histology, co-registered with OCT and micro-elastogram. (b) En face OCT image at a depth of ~215 μm. (c) Corresponding en face micro-elastogram. The area of uninvolved mature stroma in the central region of the histology image corresponds to a region of relatively uniform strain in the micro-elastogram, and the diffuse tumour and immature stroma surrounding the uninvolved region manifest as large fluctuations in strain.

Much of the mechanical contrast observed in the images in this chapter follows the model for compression OCE described in Section 2.2.1; strain is in the direction of the applied load and is inversely related to elasticity. Excised breast is, however, a structurally complex tissue that contains cavities, such as ducts and blood vessels, and has surface roughness characterized by localized peaks and troughs caused by the tissue’s structure at the excision surface. This structural heterogeneity causes more complex tissue deformation and can lead to local strain in the direction opposite to the
applied load, as observed in Figs. 6.6-6.9. Consider the case of a localized “trough” in the tissue surface, where no axial force is applied due to the gap between the compression plate and the tissue. The adjacent tissues, which have been axially loaded, can undergo lateral expansion and effectively squeeze the tissue below the trough laterally, such that it undergoes axial expansion and results in positive local strain. A similar effect may also occur if the assumption that all constituent materials in breast tissue are incompressible is invalid (Incompressibility causes lateral expansion in response to an axially applied load and is quantified by Poisson’s ratio).

Varying Poisson’s ratio between tissue types can allow the more incompressible tissue to expand laterally into the less incompressible tissue and can result in the incompressible tissue being forced upwards, causing a gradient of displacement with depth opposite to the direction of the applied load. A combination of these mechanisms cause the positive local strain observed in regions of invasive tumor visible in Figs. 6.6 and 6.9, characterized by adjacent regions of positive and negative local strain.

Breast cancer can manifest in many forms and spread through varied pathways in the breast, and each type of breast cancer will present diverse structural characteristics and mechanical properties. OCE will not be able to localize malignancy in all types of breast cancer, particularly those in which malignant and benign tissues present similar mechanical properties. For example, in the case of the low-to-intermediate-grade tumour in Fig. 6.7, features corresponding to lobular carcinoma in situ are not obviously distinguishable from the normal, uninvolved lobules within the image. As cancer and normal tissues are expected to have different elasticity, quantifying the elasticity of these features, rather than relying on the relative contrast based on strain, may improve the ability of OCE to detect malignancy. In Chapter 7, we introduce a method for measuring stress to provide a pathway toward quantitative compression OCE. However, the initial results in invasive tumours, Fig. 6.6 and Fig. 6.9, show the potential to differentiate malignant regions based on strain. More extensive investigations are needed to determine whether this strain signature for malignant tissues is repeatable, and several more measurements in each type of breast cancer are necessary to establish the efficacy of optical coherence micro-elastography for localizing tumour boundaries.
III.

Novel techniques toward the clinical translation of optical coherence elastography
Chapter 7

Development of a stress sensor for optical coherence elastography

7.1 Tactile imaging

Strain imaging of tissue microstructure using compression OCE can reveal the mechanical heterogeneity within human breast tumours. As shown in Chapter 6, the strain elastograms produced by optical coherence micro-elastography have potential to visualize areas of malignancy in breast tissue based on textural differences where cancer cells have infiltrated the tissue structure. However, the elastograms presented so far in this thesis can only, at best, indicate the relative Young’s modulus of tissues in a sample under the assumption of uniform stress. In the case of nonuniform stress, not even a relative measure of stiffness can be derived from a strain elastogram, as mechanical artefacts, such as those illustrated in Chapter 5, become prevalent. A method to measure the stress distribution would make it possible to perform quantitative imaging of Young’s modulus using compression OCE and expand its potential in a number of applications that require quantification for inter-sample comparison or longitudinal measurements.

Imaging the stress field at a tissue surface forms a field of research known as tactile imaging. The goal of these techniques is typically to mimic the sensation of touch by the human fingers; mechanoreceptors within the skin sense pressure and transmit this tactile information to the brain [235]. This is the physical process behind manual palpation used by clinicians to detect tissue lesions. In robotics applications, tactile imaging and sensing technologies have been developed toward building an “electronic skin,” which would allow robots to more comprehensively interact with their environments [236]. These technologies typically employ some flexible medium, such as soft polymers, to conform to objects and incorporate discrete tactile sensing nodes, or “taxels,” for instance based on piezo-resistors, to measure the stress distribution [237]. Tactile imaging has also been proposed for diagnostic imaging of breast lesions.
[235, 238, 239]. However, the spacing of the discrete sensing nodes in these techniques limited their spatial resolution to ~1 mm.

In this chapter, we develop the first OCT-based tactile imaging technique, termed optical palpation, to enable high-resolution mapping of the stress at a tissue surface. Building on previous methods for tactile imaging, we employ a sensor consisting of a compliant silicone rubber and use OCT to detect its deformation. We explore the potential of optical palpation to visualize mechanical contrast in tissue-mimicking phantoms and in human breast tissues. We demonstrate that optical palpation has some practical advantages over OCE as a mechanical imaging technique, as it can be performed independently of the sample’s optical properties, and it is sensitive to features beyond the imaging depth of OCT. These advantages, as well as a promising initial image of a human breast tumour margin, suggest that optical palpation has potential for clinical imaging on its own. But in the context of this thesis, it also provides an important step toward combining stress measurements with strain measurements to obtain absolute values of elasticity in compression OCE (Chapter 8).
7.2. Optical palpation: optical coherence tomography-based tactile imaging using a compliant sensor

Kelsey M. Kennedy¹, Shaghayegh Es’haghian¹, Lixin Chin¹, Robert A. McLaughlin¹, David D. Sampson¹,², and Brendan F. Kennedy¹

Abstract: We present optical palpation, a tactile imaging technique for mapping micrometre- to millimetre-scale mechanical variations in soft tissue. In optical palpation, a stress sensor consisting of translucent, compliant silicone with known stress–strain behaviour is placed on the tissue surface and a compressive load is applied. Optical coherence tomography (OCT) is used to measure the local strain in the sensor, from which the local stress at the sample surface is calculated and mapped onto an image. We present results in tissue-mimicking phantoms, demonstrating the detection of a feature embedded 4.7 mm below the sample surface, well beyond the depth range of OCT. We demonstrate the use of optical palpation to delineate the boundary of a region of tumour in freshly excised human breast tissue, validated against histopathology.

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7.2.1 Introduction

The mechanical properties of biological tissue are linked to its structure and function and are altered by disease. Clinicians commonly manually palpate tissue to feel for changes in stiffness that may indicate malignancy. To provide mechanical contrast in tissue in a more repeatable, objective manner, imaging techniques have been developed to generate maps of tissue stiffness. These mechanical imaging techniques may be broadly divided into two categories. The first and most prominent is elastography, which uses medical imaging to measure the deformation of tissue under a static or dynamic load, from which a map of strain or elasticity may be estimated [1,2]. Another technique is tactile imaging, which uses an external sensor to map

¹ Optical+Biomedical Engineering Laboratory, School of Electrical, Electronic & Computer Engineering, The University of Western Australia, Crawley, WA 6009
² Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, Crawley, WA 6009
stress at the tissue surface under compression, mimicking the physical process of manual palpation [3]. Elastography has primarily been based on ultrasound [1] and magnetic resonance imaging [2], with spatial resolutions on the orders of 100 μm and 1 mm, respectively. In tactile imaging, previous methods have used arrays of piezoresistive [3] or capacitive [4] sensors, and the spatial resolution has been limited by the spacing of the discrete sensing nodes, typically >1 mm [5]. However, on a smaller scale, the onset of diseases such as cancer causes mechanical changes in tissue microstructure long before changes manifest on the length scales of these mechanical imaging techniques. Therefore, mechanical imaging on the microscale has the potential to provide insights into the mechanics of a range of diseases and conditions, e.g., cancer, atherosclerosis, and ocular diseases, with applications in early disease detection, surgical guidance, and monitoring of therapeutics.

Over the past 15 years, a high-resolution optical elastography technique has emerged called optical coherence elastography (OCE) [6–10], based on optical coherence tomography (OCT), with spatial resolutions of 10–100 μm and nanoscale displacement sensitivity. An optically based tactile imaging technique has also recently been proposed that detects light scattering from a deformable polymer waveguide using a CCD camera with 9.8-μm pixel size [11]. Beyond clinical applications, a major focus in tactile imaging research has been on the development of artificial skin for robotics applications [5], and recent advances using nanowire technologies have enabled stress sensors with spatial resolutions as fine as 2.7 μm [12]. However, these have not been demonstrated for soft tissue imaging.

Here, we present optical palpation, the first OCT-based tactile imaging technique, with the goal of enabling microscale tactile imaging of soft tissues. In optical palpation, we utilize a stress sensor consisting of a layer of translucent, compliant silicone rubber. The sensor is placed on the tissue surface and a compressive load is applied. OCT is used to measure sensor deformation between pre- and post-compression images, from which local strain values are determined.

In this Letter, we describe the optical palpation technique and show results in tissue-mimicking phantoms, demonstrating the ability to visualize mechanical contrast and to detect features beyond the imaging depth of OCT. We also present
initial optical palpation images of human breast cancer tissue, showing differentiation between malignant tumour and adipose.

7.2.2 Method

Figure 7.1 illustrates the principle of optical palpation. A Fourier-domain OCT system, described previously [13], was used for imaging, employing a superluminescent diode with central wavelength 835 nm and a 3-dB bandwidth of 50 nm. The measured axial resolution is 8.5 μm, and the measured lateral resolution is 11 μm, which sets the best-case spatial resolution of optical palpation. The system was operated in a common-path configuration, using the far surface of the imaging window as the reference reflection. To ensure uniform loading, we used OCT to verify that this surface was parallel to the underlying rigid plate. Figures 7.1(a) and 7.1(b) show schematics of the imaging setup before and after compression, respectively, for a phantom comprising a stiff inclusion (Young’s modulus, $E = 1.5$ MPa) embedded 1 mm below the surface in soft silicone ($E = 20$ kPa). Corresponding OCT B-scans recorded pre- and post-compression are shown in Figs. 7.1(c) (inclusion not visible) and 7.1(d). To provide optical contrast in this phantom, the inclusion was made of transparent silicone, and titanium dioxide (TiO$_2$) scatterers were added to the matrix (0.8 mg/mL). The stress sensor, which is made of translucent silicone and has an unloaded thickness of 1 mm, presents a low level of intrinsic scattering in the OCT images. The sensor has a Young’s modulus of 20 kPa, which is in the range of soft tissue, allowing it to conform to tissue surfaces and deform with the tissue under a load.

The original sensor thickness, $l_0(x,y)$, and compressed sensor thickness, $l(x,y)$, are measured at each lateral position in the B-scans, using a Canny edge detector to detect the sensor-sample interface. Note that the upper boundary of the sensor is in contact with the imaging window (reference arm) and corresponds to the top of the B-scan. The local axial strain, $\varepsilon(x,y)$, is determined from the change in sensor thickness. The minimum detectable change in thickness is limited by the OCT axial resolution (here, 8.5 μm), and the maximum is limited by the OCT depth range (here, 2.9 mm in air). The local stress values corresponding to the local strain values are obtained from the stress-strain curve of the sensor material, Fig. 7.1(e). These curves are generated by independent compression testing of the sensor materials (Instron, Norwood,
Massachusetts; see Section 7.3 for discussion of sensor characterization and repeatability). The local stress is estimated at each lateral position in an OCT C-scan, resulting in a 2-D, en face projection of stress on the sample, analogous to the stress detected by the fingers during manual palpation. Figure 7.1(f) shows the en face stress map for the inclusion phantom.

Fig. 7.1. (a), (b) Schematics of the optical palpation setup for an inclusion phantom and (c), (d) corresponding OCT B-scans before and after compression, respectively. (e) The stress–strain curve of the sensor material, used to estimate the local stress from the measured local strain, $\varepsilon(x,y)$. (f) En face stress map (optical palpation image) of the inclusion phantom.

7.2.3 Results

To assess the accuracy of the stress measurement in optical palpation, we applied incremental loads to the stress sensor over a known area and calculated the average stress using the procedure detailed above. The results are plotted in Fig.7.2 for a range of applied stresses from 1 to 5.2 kPa. This range of stresses is relevant to that expected to be measured in soft tissue; for example, assuming an elastic response, 10% strain applied to soft breast tissue with a Young’s modulus of 20 kPa is expected to give a stress of 2 kPa. The average stress values were calculated from 100 × 100 pixels over a 2 × 2 mm field of view. The error bars indicate the standard deviation of
the measured stress over this region. The measured and expected stress values are in close agreement (<10% error for all data points).

![Graph showing measured vs. expected stress](image)

Fig. 7.2. Validation of stress measurements. The black line indicates ideal sensor (i.e., expected = measured stress), and the blue stars indicate actual measured stress.

To compare the contrast provided by optical palpation to that provided by OCT, we fabricated a soft silicone phantom (\( E = 20 \) kPa) containing a stiff, star-shaped inclusion (\( E = 4 \) MPa). The phantom had a diameter of 20 mm and a thickness of 3 mm, and the inclusion had an equivalent circular diameter of 5 mm and a thickness of 2 mm. The inclusion was made of transparent silicone and embedded equidistant (0.5 mm) from the top and bottom surfaces of the phantom, oriented such that the star shape was in the en face plane. On one side of the inclusion, a low concentration of TiO\(_2\) scatterers was added to the matrix (0.8 mg/mL), providing optical contrast with the star but allowing sufficient imaging depth such that the star was visible in the OCT image. On the opposite side, a high concentration of TiO\(_2\) (20 mg/mL) was added to the silicone matrix such that the OCT signal was rapidly attenuated and the star was occluded in the OCT image.

Optical palpation was then performed from each side of the phantom. Figures 7.3(a) and 7.3(b) show en face OCT images below the low and high scattering surfaces, respectively. These images were constructed from points 500 \( \mu \)m past the sensor-phantom interface. As expected, in the OCT image, the star is clearly visible on the low scattering side, and completely occluded on the high scattering side. The corresponding optical palpation images, Figs. 7.3(c) and 7.3(d), however, both provide excellent mechanical contrast of the star, illustrating that the contrast in optical palpation is independent of that in OCT. However, comparing Figs. 7.3(a) and 7.3(c), it
is apparent that OCT is able to resolve the star boundaries more sharply than optical palpation. This is due to the mechanical coupling of features in optical palpation and is discussed in more detail below.

![Comparison of OCT and optical palpation](image)

**Fig. 7.3.** Comparison of the contrast in OCT and optical palpation. (a), (b) *En face* OCT images 500 µm below the low and high scattering surfaces of the phantom, respectively, and (c), (d) the corresponding optical palpation images.

Optical palpation is also able to detect mechanical contrast from features situated beyond the depth range of OCT. To demonstrate this, we embedded a spherical stainless steel inclusion in a soft silicone phantom (E = 20 kPa), 4.7 mm from the surface [Surface B in Fig. 7.4(a)]. An *en face* OCT image showing the outline of the inclusion, Fig. 7.4(c), was acquired 0.8 mm past Surface A, in the plane indicated by the dashed line in Fig. 7.4(a). Optical palpation was then performed from Surface B, far from the inclusion. Approximately 30% strain was applied to the phantom such that, after compression, the top of the inclusion was 3.2 mm below the surface [Fig. 7.4(b)], a depth >50% beyond the imaging range of the OCT system (for a refractive index of 1.4 for silicone, the spectrometer-limited depth range of the system is 2.1 mm). However, the perturbation in local stress due to the inclusion is readily visualized in the optical palpation image, Fig. 7.4(d).
Fig. 7.4. Optical palpation detects a feature situated 4.7 mm below the sample surface. Setups for (a) OCT imaging and (b) optical palpation imaging (post-compression), (c) en face OCT image 0.8 mm past Surface A, (d) optical palpation image generated from Surface B, 3.2 mm above the inclusion after compression. The black dotted circle indicates the actual diameter of the inclusion.

Finally, to demonstrate optical palpation in tissue, we performed measurements on freshly excised human breast tissue from a 41-year-old female breast cancer patient undergoing a mastectomy. A tissue volume (approximate dimensions 2 × 1.5 × 0.5 cm) comprising regions of stiff tumour and normal adipose was cut from the mastectomy sample for imaging. The tissue was kept hydrated in saline prior to imaging, and measurements were completed within four hours of excision. Following measurements, histological sections were prepared in the en face imaging plane 400 μm below the tissue surface using standard histopathological techniques.

The optical palpation image of the breast tissue, Fig. 7.5(a), contains distinct regions of high and low local stress, indicating underlying stiff and soft regions of tissue. The corresponding histology, Fig. 7.5(b), confirms the presence of invasive tumour (invasive ductal carcinoma) on the left and adipose tissue on the right. These regions are separated by a thick band of desmoplastic stroma (dense connective tissue) at the edge of the tumour. Stress variations within the region of tumour may be attributed to its heterogeneous structure. Areas of necrotic tissue, as identified in the histology, correspond to lower local stress, suggesting that these regions are softer than the surrounding tissue. This result suggests that optical palpation can distinguish
regions of tumour from adipose based on mechanical contrast, as well as visualize mechanical heterogeneity of the tumour microenvironment.

![Optical palpation image](image)

Fig. 7.5. (a) Optical palpation image of human breast cancer tissue and (b) corresponding histology prepared in the imaging plane. DS, desmoplastic stroma; N, necrosis.

### 7.2.4 Discussion and conclusion

In this proof-of-principle demonstration of optical palpation, the detected mechanical contrast depends on the size, depth, and relative stiffness of features. For example, the measured stress due to the deep inclusion in Fig. 7.4 was \( \sim 9 \) kPa, compared to \( >200 \) kPa for the more superficial inclusion in Fig. 7.3, despite the former having higher relative stiffness. In previous tactile imaging studies, inverse methods using finite element modelling have been proposed to decouple these parameters and reconstruct the size, depth, and stiffness of a lesion [4]. Another approach used an empirical method to extract lesion parameters from a series of tactile images obtained at increasing applied stresses [3]. These methods could feasibly translate to optical palpation. In contrast with previous techniques, in optical palpation a 3-D structural
OCT image of the tissue is acquired in parallel with tactile information. This complementary information may be used as additional input to such inverse methods.

The spatial resolution of optical palpation is ultimately limited by the OCT lateral resolution (in this case 11 μm) but also depends on a number of mechanical factors, including the shape and depth of features. For example, the stress detected at the surface due to deeply situated features will be diffused over a wider area than for features close to the surface. The resolution is also linked to the compressibility of the sensor material. For a nearly incompressible material (Poisson’s ratio ~0.5), such as the silicone used here, axial compression of the sensor is accompanied by its lateral expansion to conserve volume, effectively blurring feature boundaries as detected by the stress measurement. To assess the extent to which these mechanical factors can cause blurring, we performed a step response test [14] in a phantom comprising a column of stiff silicone (\( E = 4 \) MPa) cured to an adjacent column of soft silicone (\( E = 20 \) kPa). At the interface of the columns, the distance required for the step response of the stress to rise from 10% to 90% was 180 μm. Despite this coupling of sample mechanics and spatial resolution, the results presented here show that optical palpation has potential to provide tissue contrast that is additional to that in OCT, and at comparable length scales. In the future, the limitation on resolution imposed by mechanical coupling may be overcome by implementing the inverse methods described above, enabling estimation of the true feature size. The resolution may also be improved by optimizing the compressibility of the compliant sensor material. Finite element modelling of optical palpation will aid in identifying the optimal sensor mechanical properties, which can be tuned by altering the ratio of silicone, crosslinker, and silicone fluid used in fabrication [15].

The result in Fig. 7.3 highlights an advantage of optical palpation over OCE techniques for visualizing mechanical contrast: that the stress measurements are independent of the optical properties of the sample. In OCE, accurate measurement of depth-resolved displacements using either speckle tracking or phase-sensitive methods relies on a high (>20 dB) OCT signal in the sample [16]. This can be a confounding factor, e.g., in the presence of blood, which highly scatters near-infrared light and can severely limit OCT imaging depth and quality. As optical palpation relies only on the detection of the sensor-sample interface, a projection of local stress can be
mapped independently of the OCT signal beyond this interface. On the other hand, given sufficient OCT signal in the sample, it may be possible to perform compression OCE [6, 13, 16, 17] in parallel with optical palpation, simultaneously acquiring local strain values in the sample and local stress values at the surface, which could then be combined to obtain a quantitative map of elasticity via Hooke’s law.

In conclusion, we have demonstrated optical palpation, an OCT-based tactile imaging technique for visualizing mechanical contrast in soft tissue. We presented results demonstrating the ability of optical palpation to detect features beyond the OCT imaging depth, as well as preliminary results delineating the boundary between regions of tumour and adipose in human breast tissue. Further studies are needed to assess the ability of optical palpation to delineate tumour from tissue types with more similar mechanical properties, such as benign connective tissue. For future in vivo studies, a handheld optical palpation probe is being developed. The compliant sensor could also potentially be miniaturized and implemented on the distal end of endoscopic or needle-based probes [18] to enable optical palpation in a variety of clinical scenarios.
7.2.5 References for Section 7.2

7.3 Discussion

A critical step in optical palpation is relating the measured sensor deformation to stress. The stress-strain behaviour of the sensor material was characterized using the Instron compression testing setup described in Section 4.2.2. This setup employed a load cell calibrated to an accuracy of 0.01 N over a range 1-100 N. Nine samples of the most commonly used sensor material, Elastosil P7676 in a catalyst:crosslinker:fluid ratio of 2:1:0.3, were characterized. Curves are shown for three samples from each of three separate batches, indicated by the blue, red, and green dashed lines. The mean curve, indicated by the black solid line, is used to estimate stress in elastography experiments. As a measure of repeatability, the slope of a linear fit to each stress-strain curve from 0-0.2 strain results in a mean of 1.5 kPa and a standard deviation of 0.1 kPa (<7%). The ability to produce sensors with repeatable stress-strain behaviour is even more critical in the quantitative elastography technique presented in Chapter 8, where inter-sample comparison based on quantification of elasticity could be biased if there are large errors in the stress estimation technique. The repeatability of the curves in Fig. 7.6, especially at strains <0.2, lends confidence that stress measurements using optical palpation may be combined with strain measurements in the sample to yield accurate estimates of sample elasticity.

![Stress-strain curve](Image)

*Fig. 7.6. Stress-strain curve repeatability for silicone sensor material. Curves are shown for three samples from each of three separate batches, indicated by the blue, red, and green dashed lines. The mean curve is indicated by the black solid line.*
To translate optical palpation toward clinical use, the choice of sensor material must be further considered, both to improve spatial resolution and to enable practical use in a clinical setting. Looking to the field of traction force microscopy (TFM), soft materials, such as polyacrylamide gel (PAG), are to sense forces imparted by cells [10]. The Poisson’s ratio of PAG can be made lower than that of silicone (~0.3 compared to ~0.5) [240], which should enable improved spatial resolution; however, the forces imparted in TFM are much smaller than that required for optical palpation. For clinical imaging, the sensor should have sufficient mechanical toughness to withstand a range of forces; i.e., it must not be so soft or compressible that it collapses or tears under a load. Using phase-sensitive detection, smaller stresses should be measurable, but for clinical imaging a preload will be needed to conform the layer to the irregular surface of tissues. This corresponds to up to 20% strain in our experience, or stresses in the kPa range for the sensor used here; PAG may lack the toughness to withstand these stresses. A further consideration in choosing a sensor material is biocompatibility. Many medical-grade, non-toxic, soft silicone elastomers are available and should be suitable for this application.

The ability to map stress with high spatial resolution is an essential step toward directly estimating local Young’s modulus within tissue using compression OCE. In the next chapter, we combine this optical palpation technique with the phase-sensitive, compression OCE technique presented in Chapters 5 and 6 to perform the first quantitative compression OCE method.
Chapter 8

Quantitative compression optical coherence elastography using a compliant stress sensor

8.1. Introduction

Compression OCE can map tissue strain with high lateral resolution, providing relative mechanical contrast of tissue microstructure. However, in many applications, the qualitative nature of strain imaging precludes its utility for inter-sample comparison or longitudinal imaging, e.g., to monitor a patient’s response to treatment over time. In the case of breast cancer surgery, this relative contrast based on strain can limit the ability to distinguish between tissue types, including regions of solid tumour cells and solid stroma; although their absolute elasticity values are expected to differ, both produce a homogeneous strain signal. To access absolute values of tissue elasticity using compression OCE, the local stress must be determined throughout the tissue. Methods that solve an inverse problem, e.g., using an iterative method incorporating a finite element model of the tissue, have been proposed for reconstructing elasticity in ultrasound elastography and in OCE [241, 242]. However, these techniques are generally computationally intensive, and finding an accurate and unique solution to the inverse problem has proven challenging [243].

In Chapter 7, we demonstrated an experimental method for measuring stress at a tissue surface using a compliant stress sensor and OCT detection [40]. This method, called optical palpation, uses the OCT structural image to measure the deformation of the sensor at each lateral position, under compression against a tissue surface. The known stress-strain behaviour of the sensor material is then used to estimate the corresponding stress. The resulting stress map is a projection of the stress on the tissue at each lateral position. We have shown that this technique provides mechanical contrast independently of the OCT signal in the sample and demonstrated its ability to visualize a tumour boundary in excised human breast cancer tissue.

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1 Barbone and Bamber [243] showed that knowledge of stress at the sample boundary may lead to a well-posed inverse problem. The stress sensor used here could provide input to such a method.
Here, we combine optical palpation with phase-sensitive, compression OCE to provide the first maps of elasticity using compression OCE. We demonstrate experimentally that this technique can estimate Young’s modulus with sub-kPa sensitivity. We present a preliminary result in excised human lymph node tissue, and show that, in addition to providing quantification, quantitative imaging can provide superior mechanical contrast to strain imaging.

As the stress sensor does not provide depth-resolved measurements of stress, the initial quantitative elastograms are 2-D, *en face* projections of elasticity at each lateral position. However, in cases where the stress can be assumed constant with depth, the surface stress measurement may serve as an offset to the underlying strain to obtain the depth-resolved elasticity. In Section 8.3.2, we demonstrate 3-D quantitative compression OCE in heterogeneous phantoms.

### 8.2 Method for quantitative compression OCE

Figure 8.1 illustrates the working principle of quantitative compression OCE using the compliant stress sensor. The sensor is placed between the compression plate and the sample, and a preload is applied to ensure uniform contact between the sensor and sample. The total strain on the system (sensor plus sample) due to preload is typically between 1-20%. Schematic illustrations of the unloaded and preloaded setup are shown in (i) and (ii) in Fig. 8.1(a). In this example, the sample is a homogeneous silicone phantom with stiffness ~10 times that of the sensor and with titanium dioxide (TiO₂) scatterers incorporated (2 mg/mL) to provide optical contrast with the sensor (0.33 mg/mL). The original sensor thickness, \( l_0(x,y) \), and preloaded sensor thickness, \( l(x,y) \), are measured at each lateral position using a Canny edge detector to detect the sensor-sample interface. The change in thickness of the sensor due to the preload is used to calculate the preloaded sensor strain at each lateral position (0.1 in the example in Fig. 8.1). The corresponding local stress values are then estimated from the stress-strain curve of the material, Fig. 8.1(d). These local stress values are used to form optical palpation images, as described in the previous chapter [40].

From the pre-loaded state, strain imaging is performed on the system, Fig. 8.1(a)-(iii). A piezoelectric ring actuator that is fixed to the imaging window applies stepwise, nano- to microscale displacement to the sensor surface, synchronized with the
B-scan acquisition, as in the compression OCE techniques in Chapters 5 and 6 [38, 39, 51, 88]. The local, depth-resolved displacements in the sensor and the sample are calculated from the phase difference between corresponding A-scans in sequential (uncompressed and compressed) B-scans. The local, depth-resolved strain, $\varepsilon_i(x,y,z)$, is estimated in both the sensor and the sample using weighted-least-squares regression to the displacement [88] and incorporating phase unwrapping as detailed in Chapter 6 [39]. A B-scan view of the local strain calculated in the sensor and underlying phantom is seen in Fig. 8.1(c). The phantom in this example is ~10 times stiffer than the sensor, and so exhibits ~10 times lower strain.

At each lateral position, the local strain in the sensor due to the actuation can be used to estimate the corresponding increase in stress, $\sigma_{\text{sensor}}(x,y)$, as seen in Fig. 8.1(d). The initial bulk stress is known, based on the measured strain in the sensor due to preload, as described above. The incremental increase in stress due to the smaller actuation can be defined by the measured local strain in the sensor, and the tangent modulus [slope of the tangent line to the stress-strain curve; indicated by the black line in the inset in Fig. 8.1(d)] of the sensor material:

$$\sigma_{\text{sensor}}(x,y) = E'_{\text{sensor}}(x,y)\varepsilon_{\text{sensor}}(x,y).$$  \hfill (8.1)

where $\varepsilon_{\text{sensor}}(x,y)$ is the average local strain of the sensor over the depth range $z = 0$ to $z = l(x,y)$. This stress is divided by local strain in the sample to calculate elasticity:

$$E(x,y) = \frac{\sigma_{\text{sensor}}(x,y)}{\varepsilon_{\text{sample}}(x,y)}.$$  \hfill (8.2)

This is repeated at each lateral position and mapped onto an en face image.

Two types of quantitative elastograms may be generated: a 2-D projection of elasticity, or a 3-D map of elasticity, in which stress is assumed to be constant with depth. In the former, $\varepsilon_{\text{sample}}(x,y)$ is defined as the mean local strain in the sample over the depth range $z = l(x,y)$ to $z = z_{\text{max}}$, where $z_{\text{max}}$ is chosen based on the sample attenuation. In the phantom experiments in this chapter, $z_{\text{max}}$ is 1.05 mm, and in the tissue results, $z_{\text{max}}$ is between 0.8 and 1.05 mm. Thus, the elasticity values in the 2-D images may be interpreted as the average elasticity of the sample over this depth. In Section 8.3, drawing on the study of stress distributions using finite element analysis in Chapter 5, we show examples of 3-D quantitative images in structured phantoms.
where stress may be approximated as constant with depth. However, all tissue results shown here are 2-D projections of elasticity, as the validity of uniform stress with depth in breast and lymph node tissues has not been established.

![Diagram](image)

Fig. 8.1. (a) Schematics of the experimental steps in quantitative compression OCE, including (i) the unloaded system, (ii) the preloaded system, and (iii) phase-sensitive strain imaging. (b) OCT B-scan and (c) strain elastogram of the stress sensor and a homogeneous phantom with stiffness ~10 times that of the sensor. The mean local strain over the depth ranges of the sensor and sample, respectively, are used to calculate $\varepsilon_{\text{sensor}}(x,y)$ and $\varepsilon_{\text{sample}}(x,y)$, according to the equations below (c). (d) The stress–strain curve of the sensor material is used to estimate the local stress due to preload. In the inset, the incremental increase in stress during strain imaging, $\sigma_{\text{sensor}}(x,y)$, is estimated based on the tangent modulus (tangent to the curve at the preload stress).

### 8.3 Results

In this section, we provide estimates of the sensitivity and accuracy of quantitative compression OCE for estimating elasticity of homogeneous phantoms. We also present preliminary results in structured phantoms and freshly excised human lymph node.

#### 8.3.1 Accuracy and sensitivity

To assess the accuracy with which elasticity is quantified using this technique, we performed measurements on homogeneous phantoms with expected elasticity in the range 5-180 kPa, as measured using standard compression tests on an Instron
materials testing system with 50-mm diameter platens. All phantoms were cylindrical, 15 mm in diameter and 1 mm thick. 2D quantitative micro-elastograms were generated using by averaging the strain in the sample over a depth of 0.6 mm past the sensor-sample interface. Figure 8.2(a) plots the expected elasticity values versus those measured using OCE. The solid line represents an ideal system (expected = measured), the diamonds represent the mean measured elasticity for a 6 × 6 mm (x × y) region across four independent measurements of each sample (indicating accuracy), and the error bars represent the mean error across the four measurements (indicating repeatability). In each case, the measured elasticity matched to within 8% of the expected elasticity, and inter-measurement variability was <12%. Repeatability degraded slightly with higher elasticity, which is attributed to the resistance of stiffer samples to conform evenly to the compression plates, resulting in inconsistent boundary conditions across measurements.

The elasticity sensitivity is the minimum detectable $E$ in an elastogram. It can be defined as the standard deviation of the measured $E$ within a homogeneous sample. We performed quantitative OCE over a 5 × 5 mm region in a homogeneous phantom with expected $E = 5.7$ kPa. The resulting en face elastogram is shown in Fig. 8.2(b). The standard deviation of $E$ in the image is 0.43 kPa, suggesting that this technique is able to measure differences in $E$ within an image with sub-kPa sensitivity.

![Fig. 8.2. (a) Expected vs. measured elasticity ($E$) in homogeneous phantoms. Black line is ideal (expected = measured), and error bars represent repeatability over four sets of measurements. (b) Quantitative elastogram of a homogeneous phantom with $E = 5.7$ kPa.](image)

8.3.2 Heterogeneous phantoms

In cases where nonuniform stress is present in a sample, strain imaging can give an inaccurate and incomplete visualization of mechanical contrast. To illustrate the
advantage of quantitative imaging over strain imaging for detecting mechanical contrast, we performed strain imaging (Chapter 6) and quantitative compression OCE on a phantom comprising a column of stiff silicone (\( E = 1.5 \text{ MPa} \)) cured to an adjacent column of soft silicone (\( E = 25 \text{ kPa} \)). When a compressive load is applied to this sample, the strain of the soft column is restricted to that of the stiff column; i.e., the stiff column acts as a “stress shield” preventing deformation of the soft column. Fig. 8.3(a) shows the setup for strain imaging. Fig. 8.3(b) is an OCT image of the loaded sample, and Fig. 8.3(c) is the measured strain along the depth indicated by the dashed line in 8.3(b). No contrast is detected in the strain measurement for these two mechanically disparate materials.

In quantitative imaging of the sample, Fig. 8.3(d), the stress sensor is much more compressed over the stiff column than over the soft column, indicating higher stress, as seen in the OCT image of the loaded sample, Fig. 8.3(e). The measured \( E \) along the depth indicated by the dashed line in (e), plotted in Fig. 8.3(f), shows greatly improved contrast compared to strain imaging. The artefact at the interface of the columns (~3 mm) is due to the incompressibility of the stress sensor; compression of the sensor above the stiff column results in expansion of the adjacent material to conserve volume, producing tensile stress at this interface. The measured \( E \) values otherwise closely correspond to the expected values.

![Fig. 8.3. (a) Setup for strain imaging; (b) OCT image of the loaded phantom; (c) measured strain along the axis indicated by dashed line in (b). (d) Setup for quantitative compression OCE; (e) OCT image of the loaded phantom; (f) measured \( E \) along the axis indicated by dashed line in (e). (c) and (f) are plotted over the same dynamic range to show improvement in contrast using quantitative compression OCE.](image-url)
To demonstrate that elasticity may be accurately mapped in 3D in cases in which stress is approximately uniform with depth, we performed 3D quantitative OCE on an inclusion phantom, Figs. 8.4(a)-(c), and a layered phantom, Figs. 8.4(d)-(f). The inclusion phantom comprises an inclusion in the shape of a rectangular prism (\(E = 150\) kPa) embedded at the surface of a soft matrix (\(E = 5.7\) kPa); its structure is seen in the OCT image of the loaded sensor and sample, Fig. 8.4(a). The strain elastogram of the system (sensor and sample) is shown in Fig. 8.4(b) and illustrates the higher strain (and, therefore, higher stress) in the sensor above the stiff inclusion. Fig. 8.4(c) is a B-scan view of the resulting quantitative elastogram. The layered phantom comprised a soft layer (\(E = 25\) kPa) cured on top of a stiff layer (\(E = 500\) kPa). Figs. 8.4(d)-(f) show an OCT B-scan of the loaded phantom, the corresponding strain elastogram, and quantitative elastogram, respectively. A Gaussian filter was applied to the strain images using a \(20 \times 10\) μm \((x \times z)\) window to improve the strain sensitivity [88].

The measured \(E\) values for the soft matrix in the inclusion phantom, Fig. 8.4(c), and the soft layer in the layered phantom, Fig. 8.4(f), closely match the expected values. The \(E\) values for the stiff features, however, are much noisier than for the soft features. This may be attributed to the low strain, and therefore, low strain SNR, measured within these features, resulting in low \(E\) SNR. The impact of the relative strain signal in the sensor and sample is discussed further in Section 8.4.

Fig. 8.4. (a) OCT B-scan, (b) corresponding strain elastogram, and (c) corresponding quantitative elastogram of an inclusion phantom. (d) OCT B-scan, (e) corresponding strain elastogram, and (f) corresponding quantitative elastogram of a layered phantom. Dashed lines in all images indicate the sensor-sample interface. Dotted lines in (a) and (d) indicate the boundaries of the inclusion and layers, respectively. The sensor material is blanked out in (c) and (f).
Figure 8.5 shows *en face* views of the 3-D OCT [Fig. 8.5(a)], strain elastogram [Fig. 8.5(b)], and quantitative elastogram [Fig. 8.5(c)] of the same inclusion sample as in Fig. 8.4, further demonstrating the 3-D capability of the technique. These images were taken from a depth 90 μm below the sensor-sample interface.

![Fig. 8.5](image)

**Fig. 8.5.** (a) *En face* OCT, (b) corresponding strain elastogram, and (c) corresponding quantitative elastogram of the inclusion phantom in Fig. 8.4 at a depth 90 μm below the sensor-sample interface.

### 8.3.3 Results in *ex vivo* human lymph node

To demonstrate the ability of quantitative compression OCE to map elasticity in tissue, we performed measurements on a freshly excised human lymph node. The lymph node was excised from a breast cancer patient undergoing a mastectomy and axillary clearance, kept hydrated in saline, and imaged within three hours of excision. Following measurements, histological sections were prepared in the *en face* imaging plane using standard histopathological techniques.

Fig. 8.6(a)-(d) show the *en face* OCT, optical palpation image, *en face* strain elastogram, and quantitative elastogram (2-D projection), respectively, for the lymph node sample. Fig. 8.6(e) is an overlay of the quantitative elastogram on the OCT image, demonstrating a mechanism by which the mechanical and structural information may be combined. The OCT and strain images were extracted from a depth 70 μm below the sensor-sample interface.

The corresponding histology, Fig. 8.6(f), reveals that this lymph node was involved, *i.e.*, contained metastatic cancer cells. The region infiltrated with cancer cells (bottom left) exhibits higher elasticity than the remainder of the lymph node. The quantitative elastogram provides improved contrast between the involved and uninvolved region of the lymph node compared to both the OCT and the strain elastogram. The elasticity values are higher than reported values for lymph node stiffness using ultrasound elastography [244], and may be due to the relatively large preload (corresponding to
~60% strain) that was placed on the sample in order to bring the sample surface into complete contact with the sensor. The nonlinear, poroelastic mechanical properties of lymph node tissue may account for these elevated elasticity values; the tissue is expected to experience an increase in interstitial fluid pressure under a load, increasing its apparent stiffness [245, 246]. In addition, the elasticity of the tissue constituents of lymph node may be different on the microscale than on the scale probed by ultrasound elastography. This result indicates that quantitative compression OCE may have potential for providing intraoperative assessment of sentinel lymph node involvement, which is significant in deciding whether to perform axillary clearance [247] to prevent further spread of the cancer.

Fig. 8.6. (a) En face OCT, (b) optical palpation image, (c) strain elastogram, (d) quantitative elastogram, (e) overlay of the quantitative elastogram on the OCT image, and (f) corresponding H&E histology of a freshly excised human lymph node containing metastatic cancer.
8.4. Discussion

We have employed a compliant stress sensor to enable the first demonstration of quantitative compression OCE. This method combines the optical palpation technique presented in Chapter 7 with the optical coherence micro-elastography technique presented in Chapter 6. Initial results in heterogeneous phantoms and excised human lymph node suggest that quantitative compression OCE can map elasticity with microscopic resolution and sub-kPa sensitivity.

The successful combination of these techniques required some modifications to the stress sensor design compared to that used in optical palpation. Firstly, whilst optical palpation can be performed independently of the OCT signal in the sample, quantitative compression OCE requires sufficient OCT SNR to accurately estimate the sample strain. Thus, the thickness and optical attenuation of the sensor should be low enough to allow adequate OCT SNR in the underlying sample. On the other hand, unlike in optical palpation, in which the structural OCT image is used to estimate sensor strain, in quantitative compression OCE, phase-sensitive displacement measurements are performed to estimate sensor strain. This requires OCT SNR $>>1$ in the sensor for optimal strain sensitivity. To perform optical palpation on its own, no scatterers need be incorporated into the sensor; however, for the quantitative measurements in this chapter, the sensor had 0.33 mg/mL TiO$_2$ incorporated in order to give mean OCT SNR $\sim$15 dB in the sensor. Thus, the relative scattering of the sensor and the sample must be optimized in order to maximize the strain sensitivity in each, and, consequently, maximize the elasticity sensitivity.

The relative mechanical properties of the sensor and sample also impact on the achievable elasticity sensitivity and dynamic range. If we consider a case in which the sensor is much stiffer than the sample, the sensor will exhibit relatively low strain SNR, leading to greater noise in the stress estimation and, consequently, in the quantitative elastogram. Conversely, a sample that is much stiffer than the sensor will exhibit low strain SNR and similarly result in a noisy estimate of elasticity. The latter was the case in the 3-D phantom results in Fig. 8.4 and 8.5; the level of strain in the stiff features approached the noise floor of our system [38, 39], resulting in large spatial variations in the estimates of elasticity within these features. The sensitivity measurement of 0.43 kPa demonstrated in Fig. 8.2(b), however, was acquired from a
sample that had matched stiffness to the sensor, allowing optimization of the strain SNR in both the sensor and the sample, as they both experience the same amount of strain. Practical application of the technique will require optimization of the sensor design for a given application. For measurements in breast tissue, for example, the sensor stiffness should be in a range similar to that expected in breast tissues, and the optical backscatter of the sensor should be chosen to allow adequate OCT SNR in both sensor and sample.

One of the current limitations of the technique is its suboptimal accuracy and repeatability in measuring the elasticity of homogeneous samples, as seen in Fig. 8.2(a). A limiting factor is the degree to which the measurement conditions in the material characterization using Instron match those in the OCE experiments. For instance, different surface areas were used to measure $E$. Using Instron, the stress and strain were effectively integrated over the whole phantom surface, whilst the imaging experiments considered only a 2 mm × 2 mm central region in the phantom. As stress is expected to increase toward the sides of the phantom in the presence of friction [38], the average $E$ calculated from an image of the entire phantom is, likewise, expected to be larger than one calculated from the centre of the phantom. Future characterization will ensure that imaging is performed over the entire phantom surface for more fair comparison to the gold standard. In addition, finite element modelling, such as that performed in Chapter 5, of both the Instron measurements and the OCE setup will help to identify discrepancies between these quantitative measurements.

The repeatability may be improved by more carefully controlling the boundary conditions, i.e., the friction between the sensor, sample, and compression plates. In both Instron measurements and OCE experiments, silicone fluid is used to lubricate all interfaces. This is done to maximize slip between interfaces, and ensure that the uniaxial compression model is closely followed, as it breaks down in the presence of friction [38]. However, between measurements, we observed varying amounts of friction at these interfaces, resulting in varying amounts of axial strain, and as a result, varying measures of elasticity for the same sample. Mechanically fixing the stress sensor to the upper compression plate could alleviate this variability at one of the interfaces; however, the model for estimating elasticity would need to be adjusted to
account for no slip at this boundary. Finite element modelling of the setup would also facilitate this process.

The 2-D, en face quantitative elastogram shown in Fig. 8.6 demonstrates the ability to map elasticity of tissue microstructure; however, the image reconstruction assumes that the tissue is mechanically homogeneous with depth, effectively degrading the resolution of the technique. Extension to 3-D imaging, as demonstrated in Figs. 8.4 and 8.5, allows elasticity values to be assigned to discrete features in depth but requires the assumption of constant stress with depth. Using finite element analysis, we showed in Chapter 5 that for layered structures, in the absence of friction, the stress is expected to be completely uniform [38]. For samples comprising an inclusion in a homogeneous background, the stress is also mostly uniform, except at the boundaries of the inclusion. For inclusions with stiffness closely matched to that of the background material, the stress approaches uniformity [38]. Thus, in tissue, this assumption of constant stress may be valid in layered structures, such as the skin [33] or the epithelial tissues of the airway [248]. In more geometrically complex tissues such as the lymph node shown here, stress is expected to vary with depth. However, the surface stress and depth-resolved strain measurements in this technique, as well as the structural information afforded by OCT, could serve as inputs to inverse methods that may be able to solve for the 3-D distribution of Young’s modulus [241, 243].

In conclusion, we have extended the capabilities of compression OCE, enabling quantitative mapping of Young’s modulus by combining strain measurements with stress measurements provided by optical palpation. This opens up possibilities for applications that require longitudinal imaging or inter-sample comparison, for example, intraoperative assessment of sentinel lymph node status during breast cancer surgery. Initial results in an involved lymph node have demonstrated the ability of the technique to map lymph node stiffness, which may serve as an indicator for the presence of metastasis.
Chapter 9

Needle optical coherence elastography

OCT, and by extension, OCE, are limited to an imaging depth of 1-2 mm in most biological tissues, including breast tissues. The elastograms presented so far in this thesis were acquired using standard raster scanning with external focusing optics, enabling superficial, 3-D imaging of excised tissue samples. During breast cancer surgery, this setup could be used to image the margins of a lesion immediately following excision, and could potentially provide intraoperative feedback to the surgeon as to whether cancerous tissue is present within the first 1-2 mm of the excision boundary. Such information could guide the decision of whether to remove additional tissue. However, this small penetration depth is insufficient for imaging the full extent of breast lesions in depth. The ability to probe the microscale mechanical properties deep within the tissue could potentially allow the surgeon to localize the tumour boundary in vivo, prior to excision, helping them to plan where to cut.

To access features within the body, OCT has been incorporated into endoscopic and intravascular probes, but these do not allow imaging of deep, solid tissues, as is needed for imaging of the breast. Recently, OCT needle probes have been introduced [157, 249-253], which incorporate micro-optics into a hypodermic needle, allowing imaging to be performed wherever a needle may be inserted. OCT needle probes have enabled 3-D structural imaging [158, 250, 251], as well as Doppler measurements [254], deep in solid tissue, yet implementation of OCE into a needle probe has not previously been accomplished.

In this chapter we describe the development of needle OCE to enable the first measurements of microscale mechanical contrast deep within solid tissues. We provide a brief review of OCT needle probe technology and establish an initial design for the OCE needle probe. In Section 9.2, we present the concept and proof-of-principle results for needle OCE (published as K. M. Kennedy et al., Optics Letters, 2012) [255], and finally, in Section 9.3, we demonstrate its capacity to measure mechanical contrast deep within human breast tissues (published as K. M. Kennedy et al., Journal of Biomedical Optics, 2013) [42].
9.1. OCE needle probe design

The design of an OCT needle probe may be considered as two major components: the scanning mechanism and the focusing optics. We consider these components below, briefly describing designs reported in the literature, and describing our design choices for the OCE needle probe design. A more comprehensive review of OCT needle probes is presented in [36]. In addition, when adapting the design of OCT needle probes to OCE, we must consider a third component: the loading mechanism. We propose to use forces imparted by the needle probe during insertion as a loading mechanism, and consider the needle-tissue interaction aspects of the design in Section 9.1.3.

9.1.1 Scanning mechanism

OCT needle probes may be categorized as forward-facing, in which the light beam exits the tip of the needle along the axis of the optical fibre; or side-facing, in which the beam is deflected perpendicularly to exit through an imaging window etched into the body of the needle. Examples of each are shown in Fig. 9.1. The choice of beam orientation usually depends on the application. For instance, Liang et al. proposed a probe for neurosurgical guidance that used a forward-facing orientation to detect and avoid blood vessels ahead of the needle trajectory [256]. Our group has proposed a side-facing probe for structural OCT imaging of breast tumour margins [158], in which the probe may be rotated and pulled back over several centimetres to build up a 3-D image of the tissue morphology.

In our implementation, we use a forward facing configuration, allowing the probe to simultaneously load the tissue and measure its deformation as it is inserted. Further details of this loading mechanism are given in Section 9.1.3.

(a) 

(b)

Fig. 9.1. OCT needle probes may be configured as (a) forward-facing or (b) side-facing.

To perform 2-D or 3-D imaging using a needle probe requires mechanical scanning of the beam. This may be done in one of two ways: by “beam scanning,” implementing moving parts within the probe to scan the beam, allowing the exterior of the probe to
remain stationary during scanning; or by “probe scanning,” fixing the internal components and mechanically scanning the entire needle probe.

In endoscopic probes, beam-scanning mechanisms have incorporated actuators to oscillate the fibre tip [257] or MEMS mirrors to deflect the beam [258]; however, miniaturization of these designs to the sub-millimetre diameter of most medical needles is challenging. One design that overcomes the need for these components, illustrated in Fig. 9.2(a) uses counter-rotating wedged gradient-index (GRIN) lens pairs to scan the beam laterally in a forward-facing configuration, a technique called paired-angle-rotation scanning (PARS) [259]. This design has been implemented in a needle with 0.82-mm diameter. Another novel beam-scanning approach, illustrated in Fig. 9.2(b) scans the beam using galvanometer mirrors at the proximal end of long GRIN relay rods that are enclosed in the needle [256, 260]. This avoids the need to miniaturize the scan mechanism to fit inside the needle, allowing implementation into needle probes as small as 0.74-mm diameter [256].

![Diagram of beam-scanning OCT needle probe designs](image)

**Fig. 9.2.** Examples of forward-facing, beam-scanning OCT needle probe designs. (a) Paired-angle rotation scanning (PARS) needle [259]. (b) Scanning by galvanometer mirrors housed at the proximal end of the probe (dashed rectangle) onto a GRIN relay rod in the needle [256]. Reproduced with permission from [36]. ©Springer Science + Business Media 2013.

The smallest, and so most minimally invasive, designs reported to date have used probe scanning perform 2-D and 3-D imaging. Most of these designs scan the probe by rotating it and pulling it back, building a 3-D cylindrical volume. Our group has employed this design to create the smallest-ever 3-D scanning OCT needle probe, with just 310-μm outer diameter [250], as illustrated in Fig. 9.3(a). Alternatively, probe scanning may be performed by rapidly translating the probe for 2-D lateral scanning, and by rotating the probe more slowly for 3-D imaging. These designs are
advantageous for capturing dynamic motion in the tissue within a 2-D image. They have been proposed for Doppler imaging [254] and, more recently by our group, for dynamic imaging of alveoli within the lungs [261]. An example of the linear scanning needle developed in our group is shown in Fig. 9.3(b). It uses a smaller inner needle that linearly scans within an outer, stationary needle, such that the rapid linear motion does not damage the adjacent tissue.

Fig. 9.3. Examples of side-facing, probe-scanning OCT needle probe designs. (a) The smallest 3-D scanning OCT needle probe, employing a rotation/pull-back scanning mechanism [250]. (b) OCT needle probe employing rapid linear scanning to perform dynamic imaging of the lungs [261]. Adapted with permission from [36]. ©Springer Science + Business Media 2013.

The needle OCE design used in this thesis translates the forward-facing probe along the beam axis, acquiring depth-resolved scans versus time/needle position. Extensions of the design to 2-D or 3-D needle OCE are feasible using the mechanisms described above. The compact, forward-facing designs illustrated in Fig. 9.2, are particularly attractive, as they are compatible with the needle-induced loading mechanism employed in this thesis.

9.1.2 Probe optics

The design of the fibre probe determines optical parameters of the needle probe, such as the lateral resolution and working distance, and can greatly impact on the imaging sensitivity of the probe. The optical design typically consists of a length of single-mode fibre (SMF) connecting the OCT scanner to the distal focusing optics. Several options exist for the distal focusing optics, including GRIN rod lenses [157], GRIN fibre lenses [250, 262, 263], or ball lenses [264]. These are typically fusion spliced onto the end of the connecting SMF. Of these options, GRIN fibre lenses allow the most compact
design. In addition, accurate modelling of GRIN fibre lenses is possible using the beam propagation method [265].

In this work, we use phase-sensitive measurements to estimate tissue displacement, extending the methods described for conventional OCE in the previous chapters. For this reason, a further consideration in the design of the OCE needle probe is the phase stability of the system. As shown in Chapter 6, a common-path OCT configuration greatly improves the phase-stability in fibre-based systems [39]. A challenge in using a common-path configuration in a needle probe is realizing a reliable reference reflection. We observed in previous OCT needle designs within our group that the interface between the probe end-face and the glue encasing the fibre probe produces a reflection suitable for common-path imaging [158]. The beam profile at this fibre-glue interface may be controlled by altering the length, or pitch, of GRIN fibre at the distal end of the probe [262]. To determine the GRIN fibre length needed to maximize the reference reflection, and thus, optimize the probes sensitivity, we simulated the beam profile for various lengths of GRIN fibre using the ray-matrix formalism for Gaussian beams [266].

The results of the simulation are shown in Fig. 9.4, assuming the refractive index of water \((n = 1.33)\) for the sample medium. We found that a probe with a GRIN fibre length of 270 \(\mu\text{m}\), spliced directly to SMF, results in flat wavefronts at the probe end-face, which should give optimal back-coupling into the SMF. This design has no focus, as the beam diverges past the probe end-face. However, for initial needle OCE measurements, the phase stability was given priority over the lateral resolution.

![Fig. 9.4. Simulated beam profile in water \((n=1.33)\) for a GRIN fibre probe with GRIN length of 270 \(\mu\text{m}\). Predicted beam diameter at the probe end-face is 21 \(\mu\text{m}\) and the working distance is 580 \(\mu\text{m}\).](image)
9.1.3 Needle-tissue interaction

When a needle is inserted into tissue, the needle imparts a number of forces on the tissue that affect the amount of resultant tissue deformation. Firstly, the needle imparts a cutting force that has a magnitude proportional to the fracture toughness of the tissue [267]. Toughness is a parameter that describes a material’s capacity to absorb energy and plastically deform under stress before fracturing. This differs from stiffness in that a tissue that is stiff, or does not greatly deform under an applied stress, may not be tough, that is it may easily fracture under a concentrated load such as needle insertion. Additionally, when the needle is not fully cutting the tissue but is compressing it prior to puncture, a compression force acts directly on the axis of insertion of the needle. The relative magnitude of cutting and compression forces depends on tissue toughness and stiffness, as well as on the needle tip geometry and sharpness. For instance, it has been found that a trocar or triangular tip causes greater deformation than a bevel or angled tip [267]. In the case of a flat needle end-face, it has been shown that needle insertion causes a ring-shaped crack in the tissue, and the tissue deformation ahead of the needle may be approximated as compression of a column of tissue [268]. This is illustrated in the diagram in Fig. 9.5.

![Diagram of needle insertion](image.png)

Fig. 9.5. Insertion of a needle with a flat tip into tissue induces a ring-shaped crack in the tissue surrounding the needle, and compresses a column of tissue ahead of the needle.

We chose to use a flat end-face in our needle probe design in order to maximize compression, rather than cutting, of the tissue ahead of the needle. Firstly, the deformation of the tissue due to compressive loading provides information on the stiffness of tissues, which is consistent with our previous implementations of
compression OCE. A sharp needle tip, on the other hand, would induce cutting that is related to the toughness of the tissue. Models linking displacement due to cutting and tissue toughness have not been developed in OCE, and tissue toughness has not been studied sufficiently to show that it could provide contrast between diseased and healthy tissues. Furthermore, measuring the tissue deformation due to cutting would be challenging using OCT, as the cutting is expected to cause gaps ahead of the needle tip. This, in turn, would result in lateral motion in addition to axial motion. As phase-sensitive OCT only measures axial motion, this would introduce additional errors in measurements of tissue displacement.

In summary, our probe design for needle OCE uses a forward-facing needle with a flat end-face to compress the tissue ahead of the needle tip. It incorporates an all-fibre optical design, comprising a length of GRIN fibre fusion spliced to the SMF, with the GRIN fibre length chosen to optimize the reference back-reflection at the fibre-glue interface in a common-path OCT configuration. A microscope image of the internal fibre probe and a photograph of the finished needle probe are shown in Fig. 9.6.

![Fig. 9.6. Photograph of the complete needle OCE probe. Inset: microscope image of the fibre probe.](image-url)
9.2. Needle optical coherence elastography for tissue boundary detection

Kelsey M. Kennedy¹, Robert A. McLaughlin¹, Brendan F. Kennedy¹, and David D. Sampson¹,²

Abstract: We incorporate, for the first time, optical coherence elastography (OCE) into a needle probe and demonstrate its ability to measure the microscopic deformation of soft tissues located well beyond the depth limit of reports to date. Needle OCE utilizes the force imparted by the needle tip as the loading mechanism and measures tissue deformation ahead of the needle during insertion. Measurements were performed in tissue-mimicking phantoms and ex vivo porcine trachea. Results demonstrate differentiation of tissues based on mechanical properties and highlight the potential of needle OCE for in vivo tissue boundary detection.

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9.2.1 Introduction

The mechanical properties of biological tissues are influenced by and can be indicative of pathological state [1]. When tissue becomes diseased, its microstructure undergoes changes that can translate to changes in its local mechanical properties. Clinicians commonly palpate tissue to detect variations in mechanical properties, such as a stiff tumour in soft breast tissue. However, palpation is limited by its subjectivity and low resolution. Elastography is a technique that provides maps of the mechanical properties of tissues by measuring local deformations in tissue undergoing loading. Ultrasound elastography has been developed extensively over the last 20 years and can measure mechanical properties of tissues with ~100 μm resolution [1]. More recently, elastography has been achieved on a microscale with optical coherence elastography (OCE) [2], which is an extension of the imaging modality optical

¹ Optical+Biomedical Engineering Laboratory, School of Electrical, Electronic & Computer Engineering, The University of Western Australia, Crawley, WA 6009
² Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, Crawley, WA 6009
coherence tomography (OCT). OCT is capable of 1–10 μm resolution, at least an order of magnitude higher than ultrasound, yet is limited to a penetration depth of 1-2 mm in turbid biological tissues. This has restricted the application of OCE to ex vivo and epithelial in vivo tissues [2–5].

In the past decade, the development of OCT needle probes has allowed structural [6], Doppler [7], and 3-D [8–10] imaging deep in solid tissues. To overcome the imaging depth limitation and extend the potential of OCE as a guidance and diagnostic tool, we have integrated, for the first time, OCE into a needle probe. Our technique utilizes insertion of the needle itself as the loading mechanism and employs a forward-facing probe design to measure tissue deformation ahead of the needle tip. In this letter, we present the first needle OCE data, demonstrating detection of mechanical variations in a tissue phantom and in freshly excised porcine trachea.

9.2.2 Methods

Figure 9.7 illustrates the OCE needle probe design. The probe consists of a 270-μm segment of gradient-index (GRIN) fibre spliced to a length of single-mode fibre (SMF) and coupled to a fibre-based spectral-domain OCT (SD-OCT) system in a common-path configuration (central wavelength 836 nm, source bandwidth 50 nm). The distal optics are encased within a 20-gauge needle (outer diameter 900 μm) and fixed using optical adhesive. The GRIN fibre length was chosen to maximize the reference backreflection power and, thus, the probe’s sensitivity, as determined from simulations using the paraxial ray-matrix formalism for Gaussian beams. The measured beam profile exhibited a 1/e² beam diameter of 19 μm at a distance of 20 μm from the probe end face.

![Fig. 9.7. Spectral-domain OCT system connected to the forward-facing OCE needle probe (SLD: superluminescent diode; PC: polarization controller; FFT: fast Fourier transform; MTS: motorized translation stage; SMF: single-mode fibre; GRIN: gradient-index fibre).](image-url)
Light exits the needle tip, such that during insertion, 1-D OCT depth scans (A-scans) are acquired ahead of the needle. Translation of the tissue relative to the probe end face induces a Doppler shift captured in sequential OCT spectra. The induced phase shift, \( \Delta \phi(z,t) \) is related to the local displacement, \( u_z(z,t) \), in the sample by \( u_z(z,t) = \Delta \phi(z,t) \frac{\lambda}{4\pi n} \), where \( z \) is the distance from the needle tip, \( t \) is time, \( \lambda \) is the mean wavelength of the source, and \( n \) is the refractive index of the sample [11]. The phase sensitivity of the employed SD-OCT system, measured for a static reflector with 50 dB SNR, is 7.7 mrad, or 340 pm displacement, which is on the order of the shot noise-limited phase sensitivity (3.2 mrad at 50 dB) [12]. Discrepancy from theory may be attributed to mechanical jitter of the sample arm components.

The phase-sensitive approach used here to quantify tissue deformation is sensitive only to sample translations along the beam axis. Therefore, to maximize sensitivity to tissue mechanical response, we wish to maximize the axial component of tissue displacement ahead of the needle tip. The magnitude and direction of tissue displacement due to needle insertion are influenced by needle tip geometry. A sharp tip penetrates tissue by forming a crack that opens to allow the needle shaft to pass through [13]. Formation of the opening crack results in tissue displacement that is mostly orthogonal to the direction of needle insertion. A flat tip, by contrast, penetrates the tissue by forming a ring-shaped crack with approximately the same dimensions as the needle tip [13], which encompasses a column of tissue in front of the needle. This column of tissue is compressed upon further needle advancement, resulting in displacement with a large component in the direction of insertion; i.e., along the beam axis. Thus, a flat tip was implemented to maximize detectable tissue displacement.

Soft tissue phantoms were fabricated from a combination of room-temperature vulcanizing silicone and polydimethylsiloxane (PDMS) fluid. Phantom stiffness was controlled by adjusting the volumetric ratio of silicone catalyst, curing agent, and PDMS fluid, and the desired scattering was obtained by adding titanium dioxide particles to the uncured silicone. The phantom used here comprised a stiff, highly scattering inclusion in a soft, low scattering matrix, as illustrated in the schematic in Fig. 9.8(a). The mechanical response of each phantom component was measured
independently using a standard compression test (Instron, USA). The Young’s modulus was calculated from the resulting stress-strain curves (<0.1 strain) to be 4 MPa and 23 kPa for the hard and soft components, respectively.

9.2.3 Results
The needle probe was inserted in the soft matrix at 50 μm/s using a piezoelectric translation stage with 2 nm resolution, and A-scans were acquired at 5 kHz whilst moving toward the highly scattering inclusion, as seen in the motion-mode OCT image in Fig. 9.8(b). To extract sample displacements, the phase difference was found between two A-scans separated by an increment of needle displacement. This increment varied depending upon the local elastic properties of the sample, and was chosen to maximize tissue displacement while remaining within π radians to avoid ambiguity due to phase wrapping. Figures 9.8(c)–(e) show OCT intensity profiles and corresponding measured sample displacements for three needle probe positions during insertion. To reduce the effects of phase errors induced by mechanical noise of the moving stage and increase the accuracy of the measured displacements, averaging was performed over 1000 A-scans for each set of plots. The effects of spatial averaging were considered negligible as this corresponds to a needle displacement of only 10 μm.

During insertion, compression of the soft phantom ahead of the needle results in maximum tissue displacement at the needle tip and a decrease in displacement with distance from the needle. The stiff inclusion, by contrast, appears to move as a rigid body, exhibiting bulk displacement with approximately zero spatial derivative. Defining the derivative of displacement as local strain [1] and fitting two lines to the data, it was found that strain in the soft phantom varied between 0.37 and 0.96 microstrain, whereas strain in the stiff inclusion was consistently <0.02 microstrain. Thus, abrupt changes in strain indicate the location of the mechanical interface, whilst the magnitude of strain is dictated by the boundary conditions and local mechanical properties of the sample. This is demonstrated in the progression of Figs. 9.8(c)–(e); over time, the abrupt change in strain occurs nearer to the needle tip, indicating that the needle is indeed approaching a mechanical interface. Also, as the needle advances towards the inclusion, the soft phantom becomes more compressed between the
needle and the stiff inclusion, which manifests as increasing strain (slope) across the three plots.

In each corresponding OCT intensity profile, the boundary of the highly scattering inclusion is clearly defined as a change in the rate of signal attenuation. Importantly, in all cases, the optically detected interfaces match the locations of change in strain.

![Diagram](image)

**Fig. 9.8.** (a) Schematic of probe insertion into phantom; (b) motion-mode image (dashed line indicates interface); OCT A-scans and measured displacements at distances (c) 570 μm; (d) 450 μm; and (e) 300 μm from the inclusion. The red stars denote the locations of interfaces, and the black lines indicate the linear fits used to approximate strain.

In a second experiment, needle OCE was performed in a freshly excised posterior section of porcine tracheal wall. This tissue has a layered structure with varying mechanical properties, comprising soft mucosal and submucosal layers, a tougher muscle layer, and a stiff cartilage layer [14]. After excision, the sample was kept hydrated in saline and imaged within 6 hours. The needle probe was inserted from the luminal side of the trachea at 50 μm/s and an A-scan rate of 5 kHz. After measurements, the sample was fixed in formaldehyde, and haematoxylin and eosin (H&E) sections were prepared in a plane parallel to the direction of needle insertion.

Three distinct slopes are observed in the measured displacement (averaged over
500 A-scans) in Fig. 9.9(b), with changes at 170 μm and 370 μm, indicating mechanical interfaces. The location of these interfaces, denoted by the red stars, were determined by visual inspection of the displacement data. These interfaces are less apparent in the corresponding OCT intensity profile in Fig. 9.9(a); however, changes in signal attenuation do occur at location closely matched to the mechanically detected interfaces. In addition, a representative section of the obtained histology, Fig. 9.9(d), reveals well-defined layers of mucosa, submucosa, and muscle. The layer thicknesses closely parallel those observed in the needle OCE data, matching to within 50 μm. Discrepancies are due to tissue shrinkage caused by fixation in formaldehyde, as well as compression of the tissue by the needle probe, as the data in Figs. 9.9(a) and 9.9(b) was obtained once the needle had moved 180 μm past the tissue surface. The displacement trend observed is consistent with the expected tissue behaviour; that is, compression of the soft mucosa and submucosa, followed by bulk motion of the tougher muscle layer.

![Fig. 9.9. Results in ex vivo porcine tracheal wall. (a) OCT and (b) measured displacement at a depth in tissue of 180 μm. Interface locations are denoted by the red stars. (c) Photograph of needle probe being inserted into sample. (d) H&E histology of the tracheal wall at approximate location of needle insertion. Scale bar =100 μm.](image)

In conclusion, we have developed a technique, needle OCE, and presented the first
published OCE measurements obtained within a solid sample. By measuring the mechanical response of the tissue to needle insertion, needle OCE can detect tissue interfaces with high resolution, as demonstrated here in tissue phantoms and \textit{ex vivo} porcine tissue. Whilst these particular samples exhibited both optical and mechanical contrast, the two are not simply related; this technique can detect mechanical variations independent of optical contrast. The probe may be adapted to accommodate a variety of tissue loading mechanisms and optical delivery schemes. For instance, focused ultrasound may be delivered via the needle to perform acoustic-ocmotive-OCE \cite{2011}; 2-D and 3-D measurements may be possible using other probe designs \cite{2007-2011}; and a handheld design may facilitate clinical translation of the technique. Based on the proof-of-principle results presented here, we anticipate that needle OCE has the potential to detect tissue boundaries \textit{in vivo}, enabling new possibilities in biopsy guidance or delineation of diseased tissue.

\section*{References for Section 9.2}

9.3. Needle optical coherence elastography for measurement of microscale mechanical contrast deep within human breast tissues

*Kelsey M. Kennedy¹, Robert A. McLaughlin¹, Brendan F. Kennedy¹, Alan Tien², Bruce Latham², Christobel M. Saunders², and David D. Sampson¹, ⁴*

*Abstract:* Optical coherence elastography (OCE) is an emerging imaging technique that probes microscale mechanical contrast in tissues with the potential to differentiate healthy and malignant tissues. However, conventional OCE techniques are limited to imaging the first 1 to 2 mm of tissue in depth. We demonstrate, for the first time, OCE measurements deep within human tissues using needle OCE, extending the potential of OCE as a surgical guidance tool. We use needle OCE to detect tissue interfaces based on mechanical contrast in both normal and malignant breast tissues in freshly excised human mastectomy samples, as validated against histopathology. Further, we demonstrate the feasibility of *in situ* measurements >4 cm from the tissue surface using ultrasound guidance of the OCE needle probe. With further refinement, our method may potentially aid in accurate detection of the boundary of the tumour to help ensure full removal of all malignant tissues, which is critical to the success of breast-conserving surgery.

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9.3.1 Introduction

Breast cancer is the most commonly diagnosed cancer in females worldwide and the second leading cause of cancer-related deaths in females in North America, Europe, and Australia [1]. A common surgical treatment is breast-conserving surgery, or lumpectomy, in which the tumour and a margin of healthy surrounding tissue are excised, while the remainder of the breast is preserved. The efficacy of breast-conserving surgery in the treatment of breast cancer relies on obtaining clear tumour
margins, *i.e.*, the boundary of the excised tissue must be free of malignancy. Tumour margins are currently assessed by postoperative histological examination. Positive and close tumour margins (tumour cells found at the boundary of the excised tissue and within 1 to 5 mm of the boundary, respectively) are correlated with an increased risk of local recurrence of the disease, and often necessitate additional surgery or aggressive postoperative radiation treatment [2]. Currently, approximately 23% of women who undergo breast-conserving surgery must return for additional surgery due to insufficient margins [3]. Improved guidance of tumour excision and intraoperative assessment of tumour margins have the potential to reduce the need for re-excision.

Currently, surgeons manually palpate, or feel, the tissue to find the boundaries of the tumour and to guide excision, as many breast tumours manifest as stiff lesions. However, some cancers present as nonpalpable lesions, *i.e.*, they are too small or soft to detect through touch or may have a permeative growth pattern that results in a poorly delineated mass with small extensions of malignancy [4]. In these nonpalpable cases, hookwire guidance is commonly used, in which a wire is placed in the tumour mass under radiological guidance prior to surgery. The surgeon then excises an area of tissue around the wire according to the tumour size estimated by preoperative imaging. However, tissue can deform between preoperative imaging and surgery, and extensions of malignancy beyond the bulk tumour mass are not always detectable using conventional medical imaging modalities such as ultrasound, x-ray, and magnetic resonance imaging, making this technique imprecise [5]. Furthermore, if the size of the lesion is overestimated, then an unnecessarily large amount of tissue may be removed, degrading the preservation of cosmesis. Once the lesion is excised, intraoperative assessment of tumour margins is sometimes performed using frozen-section analysis, but this process is time-consuming, taking ~25 min on average [6] and, importantly, has had only limited success in reducing overall re-excision rates [7]. Intraoperative ultrasound guidance of excision has been shown to reduce re-excision rates for invasive cancers [173], but ultrasound typically cannot visualize *in situ* cancers or small multifocal cancers [174]. Thus, new intraoperative techniques have the potential to improve upon guidance of breast-conserving surgery and, ultimately, reduce the number of re-excisions performed.
Optical coherence tomography (OCT) provides three-dimensional (3-D) images of tissue microstructure with a high resolution of ~10 μm and a penetration depth in scattering tissue of 1 to 2 mm and can be performed in vivo, making it a suitable candidate for intraoperative detection of tumour boundaries [8–10]. However, the contrast in the OCT images, produced largely by optical scattering, is sometimes insufficient for tissue differentiation. For example, collagenous connective tissues of the breast can appear similar to dense tumour [8–10]. Additional contrast mechanisms would assist OCT to more readily distinguish tumour from normal breast tissues.

The changes in tissue’s constituent materials and microstructure that occur with the onset of breast cancer lead to changes in the local mechanical properties of the tissue. The spread of cancer cells in the breast is often accompanied by desmoplasia, the rapid production of collagen as stroma to structurally support the rapidly growing tumour. It is the presence of this dense stroma that causes many tumours to feel stiff to the touch [11]. On a cellular level, individual tumour cells actually tend to be softer than normal epithelial cells in the breast [12]. Reports have suggested that this facilitates their migration and metastasis [13]. These mechanical characteristics in malignant tumours make them distinct from healthy tissues. Thus, probing the mechanical properties of tissue during breast-conserving surgery could potentially provide additional contrast between healthy and malignant tissues and aid identification of tumour boundaries.

Optical coherence elastography (OCE) is an extension to OCT that probes the mechanical properties of tissue on the microscale [14, 15]. OCE is performed by applying a load to tissue and measuring the resulting local displacements within the tissue using OCT. From the displacements, a distribution of mechanical properties may be estimated. OCE is an optical analogue of elastography techniques previously developed using ultrasound and magnetic resonance imaging. These techniques map mechanical properties with coarser spatial resolution than OCE, but over a wider field-of-view, and have been shown to provide additional contrast to complement the underlying imaging technique and to aid in the diagnosis of breast lesions [16–18]. OCE has been demonstrated in vivo, [19–21] proposed for soft tissue tumour detection [22], and shown potential for providing contrast between malignant and normal
tissues of the breast [23, 24]. However, OCE has so far been restricted to imaging the first 1 to 2 mm of tissue in depth, which limits its utility for the detection of tumour boundaries situated farther below the tissue surface. Preliminary results measuring the velocity of surface acoustic waves [25, 26] have shown potential to probe depth-dependent mechanical properties beyond the penetration depth of OCT; sensitivity to changes in stiffness up to 55 mm below the surface has been reported using holographic detection [25]. However, these techniques come at a loss of lateral resolution (0.5 to 1 mm), primarily due to the wavelength of the generated surface wave, typically >10 mm.

The recent development of OCT needle probes [27–32] has enabled imaging of tissue microstructure, including breast tumour margins [27, 29] centimetres below the tissue surface. We recently developed a technique to perform one-dimensional (1-D) OCE measurements via an OCT needle probe [33]. Our technique, needle OCE, detects mechanical contrast by measuring the depth-resolved mechanical response of tissue to needle insertion. Our initial results demonstrated the detection of boundaries in tissue-mimicking phantoms and in ex vivo porcine airway wall [33].

Here, with a view toward improving guidance of breast-conserving surgery, we report the use of needle OCE to detect tissue boundaries in human breast tissues. We demonstrate the ability of needle OCE to distinguish tissue types in freshly excised (not fixed) human breast tissue samples comprising adipose, connective tissues and tumours. These needle OCE measurements are the first obtained deep within tissue, as previous measurements were obtained by, in effect, indenting the tissue surface [33]. We also demonstrate the feasibility of needle OCE to detect tumour boundaries in situ, using ultrasound guidance of the needle probe [27] to detect a tumour boundary at a depth of several centimetres below the tissue surface in a human mastectomy sample. The results demonstrate the first mechanical contrast in tissue detected at such depths using OCE and point to the potential of needle OCE as an intraoperative guidance tool.

9.3.2 Methods

Figure 9.10 illustrates the OCE system and forward-facing needle probe design employed in this study. The distal end of the optical fibre probe, designed to operate in the 800-nm wavelength range, consists of a single-mode fibre fusion spliced to a 270-
μm length of gradient-index (GRIN) fibre. A common-path interferometric configuration was used with the GRIN end face serving as the reference backreflection. The length of the GRIN fibre was selected such that a collimated beam would result at the fibre exit, maximizing the reference backreflection power and, thus, the probe’s sensitivity. This fibre length was determined from simulations using the paraxial ray-matrix formalism for Gaussian beams [34]. The measured 1/e² beam diameter was 19 μm at a distance of 20 μm from the bare probe end face, and the measured depth of focus in air was 310 μm. The optics are fixed inside a hypodermic needle using an optical adhesive (Norland, Cranbury, New Jersey). Two needle gauges (G) were used in this study: 20G (0.9-mm outer diameter) and 22G (0.7-mm outer diameter).

Fig. 9.10. Schematic of spectral-domain optical coherence tomography (SD-OCT) system and forward-facing needle probe (SLD: superluminescent diode; PC: polarization controller; FFT: fast Fourier transform; MTS: motorized translation stage; SMF: single-mode fibre; GRIN: gradient-index fibre).

To perform the measurements, the needle probe was coupled to a custom-built, portable, spectral-domain OCT (SD-OCT) system. The light source is a superluminescent diode with a central wavelength of 835 nm and a bandwidth of 50 nm. The measured axial resolution is 8.5 μm in air. A motorized translation stage was used to advance the needle at 50 μm/s for all measurements, and A-scans were acquired at a rate of 5 kHz during insertion. To measure the tissue deformation ahead of the needle during insertion, we employed a phase-sensitive method, calculating the depth-resolved tissue displacements from the phase difference between sequential A-scans [35], as illustrated in Fig. 9.11. The phase sensitivity of the system was measured to be 7.7 mrad at an OCT signal-to-noise ratio (SNR) of 50 dB,
corresponding to a displacement sensitivity of 340 pm.

The magnitude and direction of tissue displacement ahead of the needle depend on the needle tip geometry as well as on the tissue mechanical properties. As the phase-sensitive method detects only the axial component of tissue motion (i.e., along the incident light beam), it is important to maximize this axial motion for optimal sensitivity to mechanical contrast. We therefore employed a flat needle tip, which maximizes compression of the tissue ahead of the needle [36] resulting in a large axial component of displacement. Note that a sharp needle tip was not used, as this would maximize the degree of tissue cutting directly ahead of the tip, potentially resulting in gaps between the needle probe and the tissue and complicating measurement and interpretation of axial tissue displacements.

![Diagram of tissue displacement measurement](image)

Fig. 9.11. Method for the measurement of tissue displacement in needle optical coherence elastography (OCE). (a) Measurements of the phase of the OCT A-scans are acquired at two incremental needle probe positions. (b) The phase difference between the two A-scans, $\Delta \phi = \phi_1 - \phi_2$, is linearly related to axial tissue displacement.

Upon needle insertion, compression of the tissue ahead of the needle results in maximum tissue displacement at the needle tip and a decrease in displacement with distance from the needle. We note that although compression can alter the group refractive index of tissue, potentially offsetting the measured displacement values, this effect will be negligible for the small strains induced by the nanometre-scale
movement of the needle between A-scans. Strain may be estimated in these measurements as the derivative of displacement versus depth [37]. It is inversely related to the Young’s modulus, or stiffness, under the assumption of uniform stress in the region of tissue probed. In [33], we demonstrated in phantoms containing inclusions of known Young’s modulus that a change in strain determined by a needle OCE measurement indicates a mechanical interface. It was also demonstrated that the relative strains depend not only on the relative Young’s moduli of the tissues, but also on the proximity of the needle to a mechanical interface. For example, as the needle approaches a stiff lesion, the soft tissue between the needle tip and the lesion will become more compressed, resulting in higher strain. Thus, such strain measurements can localize tissue interfaces, but can only give a qualitative estimate of the relative Young’s modulus of tissues without the knowledge of the local stresses in the sample [38, 39].

**Experiments in Human Breast Tissue**

Excised human mastectomy samples from patients undergoing breast cancer surgery were scanned immediately following excision (n = 5), and all scans were completed within four hours of excision. The Human Research Ethics Committee of Royal Perth Hospital approved the study, and informed consent was obtained from the patients. Two types of scanning were performed, as illustrated in Fig. 9.12. In the first experimental setup, to assess the ability of needle OCE to detect contrast between various breast tissues, small samples (~1 cm³ in volume) comprising distinct regions of tissue were cut from four of the breast mastectomy specimens. This allowed the needle probe to be inserted into the samples with a known trajectory, facilitating later histological validation. However, we observed that the insertion of the needle probe into such small samples does not elicit a response representative of the tissue *in situ* because of the significant impact of boundary conditions in small samples. Therefore, these small samples were embedded in agar solution to simulate the tissue being held within a larger sample, as seen in Fig. 9.12(a). This also served to reduce bulk motion of the sample, such that only tissue displacement due to insertion was measured. The agar solution was heated to liquid form and then placed on ice to rapidly cool to room temperature, forming a soft solid. During cooling, the tissue was embedded in the agar
solution and held using forceps in an orientation suitable for scanning while the surrounding agar solidified. Samples were kept hydrated in a saline solution prior to embedding in agar and scanning. Following measurements, the tissue samples were bisected along the axis of needle probe insertion, and haematoxylin and eosin (H&E)-stained sections were prepared in the plane of the bisection using standard pathology techniques.

Fig. 9.12. Schematics of experimental setups for needle OCE measurements in human breast tissues. (a) Setup for small excised breast tissue samples and (b) photograph of an experiment. Samples were embedded in agar solution to simulate needle insertion into a large mass of tissue. (c) Setup for in situ ultrasound-guided measurements in a full mastectomy sample. Guide needle was used to aid positioning of the needle OCE probe and to facilitate histological sectioning following measurements.

In the second experimental setup, to demonstrate the feasibility of needle OCE for in situ measurements, measurements were performed on one full mastectomy sample, in which the tumour was situated several centimetres below the tissue surface. Ultrasound guidance was used to direct the needle toward the tumour mass, following the protocol described in [27]. Placement of the needle probe in proximity to the tumour was achieved by first inserting a sharp, 18G hollow guide needle toward the tumour under ultrasound guidance, as illustrated in Fig. 9.12(c). Unlike the needle probe, which used a flat tip to compress tissue, the guide needle employed a sharp tip.
to facilitate initial puncture and penetration into tissue. The 22G needle probe was then inserted through the guide needle to acquire measurements while advancing toward the tumour. An additional purpose of the guide needle was to indicate the exact location scanned for subsequent histological validation. For this reason, it was left in situ following needle OCE measurements. A slice was taken along the length of the guide needle, and H&E-stained sections were prepared from this face following the procedure used in [27].

9.3.3 Results

**Needle OCE of excised human breast tissue**

Figure 9.13 shows the results of needle OCE measurements in two breast samples [scanned using the setup in Fig. 9.12(a)] comprising adipose and normal stroma [Figs. 9.13(a)–9.13(d)] and adipose and tumour [Figs. 9.13(e)–9.13(h)], respectively. The H&E histology sections taken along the needle insertion path in these two samples verify the presence of the two tissue types. In each case, the 20G needle probe was inserted into the adipose and advanced toward the stroma or tumour, respectively. Motion-mode (M-mode) images of the structural OCT A-scans acquired versus time during needle insertion are shown in Figs. 9.13(b) and 9.13(f), in which the adipose tissue is distinguishable as a region of modulated signal due to higher reflectance at the cytoplasmic membrane of individual fat cells. The regions of stroma and tumour present as more homogeneously backscattering regions in these M-mode images. The M-mode images show the tissue interface drawing closer as the needle advances in the tissue. These trends in the backscattering signatures of the tissues are also apparent in the OCT A-scans, Figs. 9.13(c) and 9.13(g), which are the average of 500 A-scans at the time points indicated by the dashed lines in the M-mode images. Note that the needle displacement during the acquisition of 500 A-scans is only 5 μm, which is less than the axial resolution of the OCT system. Thus, these averaged A-scans provide, in effect, instantaneous 1-D structural information at the indicated time points.

The corresponding displacement traces at these time points are shown in Figs. 9.13(d) and 9.13(h). The black lines indicate least-squares fits to the displacement data in the two predominant tissue regions seen in the histology (in these cases, adipose/stroma and adipose/tumour). The fluctuations in the measured
displacement in the adipose may be attributed to the corresponding fluctuations in OCT SNR as the phase variance, and, therefore, the variance of the measured displacements increase with lower OCT SNR. However, averaging of the 500 A-scans served to narrow this variance and to increase the displacement measurement accuracy. Thus, the fluctuations in displacement may also be a genuine effect of the differing mechanical responses of the outer membranes and lipid interiors of the individual cells. In either case, displacements measured at points with high SNR (corresponding to the adipose cell membranes) provide sufficient data to indicate the overall trend of displacement within the regions of adipose. Locations of sharp changes in the slope of the displacement, visually estimated from the displacement data, are denoted by red stars, which have also been placed at the same depth locations in the OCT M-mode images and A-scans (connected by the dotted red lines), to show the close correspondence of locations of the tissue interfaces detected using each type of data. In Fig. 9.13(d), a rapid decrease in displacement of the adipose with distance from the needle indicates high strain in this soft tissue. This strain was calculated as the slope of the fitted line to be 110 microstrain (με). By contrast, the dense stroma exhibits an approximately constant displacement with depth or ~0 strain, indicating that it is pushed away from the needle as a bulk. This is expected for tissue made up of such densely packed collagen [40]. The tissue composition is confirmed by the histology in Fig. 9.13(a).

In the case of needle insertion toward the tumour, Fig. 9.13(h), compression of the adipose is again apparent; the strain in this region, as calculated from the slope of the black line, is 150 με. The tumour also exhibits a small amount of strain (~30 με) rather than a bulk motion, as exhibited by the dense stroma in Fig. 9.13(d). This data suggest that portions of tissue consisting of intermingled, heterogeneous regions of tumour and collagen, as seen in the enlarged inset of histology for this sample in Fig. 9.13(e), may be more deformable than tissue that is homogenously and densely packed with mature collagen fibres. As the local response of tissue to the needle is dependent on both the tissue constituents and the structural integrity with which the constituents are arranged, we hypothesize that this deformability may be a consequence of the compromised structural integrity of tissue infiltrated with areas of invasive malignant growth and also the relative softness of individual cancer cells [12].
Of the five excised tissue samples scanned in this study, the results presented in Fig. 9.13 are those in which the greatest mechanical contrast was observed. In two of the samples, we did not identify an apparent optical nor mechanical interface in the M-mode data, likely because an interface was not actually reached by the needle probe tip. In the remaining sample, we identified an optical interface between adipose and non-adipose tissue, but the OCT signal diminished too quickly in the non-adipose tissue to acquire an accurate estimate of displacement.

Fig. 9.13. Needle OCE of excised breast tissue samples comprising (a–d) adipose and dense stroma and (e–h) adipose and tumour. (a, e) Representative haematoxylin and eosin (H&E) histology section along the path of needle insertion, with inset in (e) showing tissue constituent detail (scale bar on inset 100 μm); (b, f) M-mode structural OCT image during needle insertion; (c, g) Average OCT A-scans at time point indicated by dashed lines in M-mode images; and (d, h) corresponding displacement ahead of the needle tip at these time points. The red stars indicate locations of the tissue interfaces estimated from the displacement data, and the slopes of the least-squares fitted black lines indicate local strain.
Tumour Boundary Detection Using Ultrasound-Guided Needle OCE in a Mastectomy Sample

Figure 9.14 shows an ultrasound image (sonogram) captured immediately prior to needle OCE measurements taken from within a full mastectomy sample. The needle probe can be seen protruding from the guide needle toward the tumour mass, which presents as a hypoechoic region in the image. The image demonstrates that the measurements were acquired when the needle was >4 cm beyond the tissue surface.

![Sonogram](image)

Fig. 9.14. Sonogram captured during ultrasound-guided needle OCE in a human mastectomy sample.

The results of these ultrasound-guided measurements are shown in Fig. 9.15. An M-mode image of the OCT A-scans acquired during insertion, Fig. 9.15(a), shows the characteristic modulated OCT signal generated from adipose cells as well as a region of more homogeneously backscattering tissue corresponding to tumour. A histology section prepared along the path of needle insertion, Fig. 9.15(b), also shows these two tissue types.
Fig. 9.15. Ultrasound-guided needle OCE results in a human mastectomy sample. (a) M-mode OCT image during needle insertion toward tumour. (b) Histology acquired along path of needle insertion. Cyan and green brackets mark the estimated regions of tissue scanned at $t = 1$ s and $t = 18$ s, respectively. (c, d) Average OCT A-scans and (e, f) corresponding displacement for time points indicated by the dashed cyan and green lines in (a). The red stars indicate locations of the tissue interfaces estimated from the displacement data, and the slopes of the least-squares fitted black lines indicate local strain.

Figures 9.15(c) and 9.15(d) show the average of two sets of 500 OCT A-scans acquired at times 1 and 18 s, respectively, during needle insertion, as denoted by the cyan and green dashed lines in the M-mode image. The estimated locations in the tissue in which these scans were acquired are denoted by the cyan and green brackets on the histology image. Note that between these two time points, the needle advanced a total of 850 μm (17 s at 50 μm/s). However, as seen in the M-mode image, the tumour interface moves only ~100 μm closer to the needle tip, indicating that the needle was pushing the tumour away instead of continuously cutting through the tissue. The scan locations indicated in the histology image were estimated based on this observation.

In the displacement traces, Figs. 9.15(e) and 9.15(f), the adipose tissue exhibits higher strain than the tumour, which moves as a bulk with ~0 strain, indicating that this tumour interface is more rigid than that measured in Fig. 9.13(h). Also, as the needle advances, the adipose becomes more compressed between the needle and the tumour, which manifests as a slight increase in strain between the two time points [from 60 με in Fig. 9.15(e) to 80 με in Fig. 9.15(f)]. At each time point, the tissue interface can be easily detected based on a sharp change in strain, demonstrating the ability of the technique to detect the instantaneous location of the tumour boundary relative to the needle tip throughout the insertion. Also, although the region of adipose
is easily identified in the OCT data, it is challenging to identify the boundary of the
tumour based on the changes in the structural OCT signal. Importantly, the
displacement data offers better contrast between the two tissue regions and more
convincing identification of the tumour boundary.

9.3.4 Discussion

The results presented here demonstrate the ability of needle OCE to detect mechanical
contrast between tissue types deep in human breast cancer samples. Using phase-
sensitive OCT to measure the deformation of tissue due to needle insertion, tissue
boundaries can be detected at depths several centimetres below the surface of the
tissue.

The mechanical contrast detected by needle OCE is complementary to the
scattering contrast detected using OCT and may in some cases be superior, as the
scattering contrast alone can be inadequate for distinguishing regions of normal and
malignant tissues [8–10, 29]. This potential for improved contrast is demonstrated in
the results in Fig. 9.15, where the boundary between adipose and tumour tissue in the
mastectomy sample is notably more distinct in the displacement traces than in the
individual OCT A-scans. We anticipate that the combined interpretation of the
mechanical and scattering contrasts will enable more sensitive tumour boundary
detection than either type of contrast on its own. Parametric analysis of OCT data
could be incorporated to further enhance tissue contrast [41]. The addition of
molecular contrast, e.g., that provided by fluorescence imaging, could also strengthen
sensitivity to malignancy, and this may be possible with the recent development of a
dual-modality needle probe that performs OCT and fluorescence imaging [42].

Further studies are needed to assess the impact of needle insertion speed, tissue
geometry, and stiffness contrast between features on the sensitivity of needle OCE to
tissue interfaces. This can be achieved by performing measurements in a range of
phantoms with controllable structural and mechanical properties [43]. Comparison of
the measurements to finite element simulations of tissue deformation due to needle
insertion would also improve understanding of the contrast detectable using needle
OCE [38, 39]. Such modelling studies, as well as continued measurements in larger
numbers of breast tissue samples, will be essential for eventually establishing
quantified criteria for automated detection of tumour boundaries using this technique.
Furthermore, the measurements here provide qualitative information about the relative stiffness of the tissues, which in many cases may be sufficient for detecting the presence of malignant tissue. However, a measurement of stress imparted to the tissue may allow a quantitative assessment of tissue stiffness, which may enable more specific identification of malignant versus benign breast tissues than contrast based on deformation alone. Such a stress measurement could feasibly be achieved by incorporating a load cell on the proximal end of the needle.

In three of the four displacement measurements presented here, namely, in cases of needle insertion toward dense stroma in Fig. 9.13(d) and toward the tumour in the mastectomy samples in Figs. 9.15(e) and 9.15(f), we observed bulk motion (very low strain) of the non-adipose tissues. We hypothesize that this is partially due to the high stiffness of these tissues, as they were detectable by manual palpation, and may also be a result of the tissues’ resistance to puncture by the needle probe. Although we have assumed here that the measured displacement is due to compression of the tissue ahead of the flat needle tip, insertion of the needle is also known to induce crack propagation [36]. Therefore, the displacement measurements are likely a function of both the tissue's stiffness and resistance to puncture (fracture toughness), and the measurement of axial strain may not always be sufficient for differentiating tissue types. This is another example where comparison of measurements to mechanical models of needle insertion is expected to aid in the understanding of tissue contrast.

The 1-D depth-resolved measurements provided by the current method can be used to detect mechanical contrast and to localize tissue boundaries. However, for the technique to eventually be used as a guidance tool, greater spatial sampling of the tissue would provide clearer delineation of tissue boundaries. Imaging the spatial variance of mechanical properties may also improve the technique’s differentiation of malignant tissue from healthy stroma. Both tissue types exhibited bulk motion in the local measurements in this article but are known to present different degrees of spatial heterogeneity [12]. A number of scanning schemes have been proposed for optical needle probes that enable formation of 3-D structural images, including forward-facing designs [44, 45], in which the beam is scanned laterally from the front of the needle tip, as well as side-facing designs [31, 46, 47], in which the beam exits from the side of the needle and the needle is rotated to acquire a two-dimensional (2-
Implementation of elastography measurements into such an imaging probe design is expected to facilitate the translation of needle OCE toward clinically useful measurements. In particular, a forward-facing design, such as those proposed in [44] or [45], would preserve the ability to measure tissue deformation due to needle insertion ahead of the needle tip. The alternative 2-D implementation of OCE in a side-facing design will present additional challenges in developing strategies for synchronous tissue loading and detection of displacement. A potential solution may be to deliver focused ultrasound via the needle, applying an acoustic radiation force to the tissue [48, 49].

A handheld implementation of the probe may be necessary for clinical use, although the needle's actuating motion may still be automated for OCE acquisition. Such a handheld scenario may require faster acquisition speeds to reduce sensitivity to low-frequency motion artefacts, such as those potentially caused by involuntary motion of the surgeon or patient. External tracking of the needle probe position could be used to identify involuntary motions and to allow the acquired data to be corrected accordingly [50]. Furthermore, practical clinical use of needle OCE will require real-time interpretation of data, which may be facilitated by the conversion of the acquired signal to an audio output [51].

Finally, breast-conserving surgery represents only one type of cancer surgery in which precise guidance of tumour resection and assessment of margins are critical for predicting patient outcomes. In resection of cancerous tumours of the brain [52] and prostate [53], for example, guidance of surgery is imperative not only to ensure complete tumour removal, but also to prevent damage to healthy tissues to preserve vital functions and quality of life. We anticipate that in addition to breast-conserving surgery, needle OCE may have applications in surgical guidance for other soft tissue tumours, in which probing mechanical properties may also provide additional contrast between normal and malignant tissues.

9.3.5 Conclusion
Needle OCE has significant potential for the in situ assessment of tumour margins. This article presents the first needle OCE measurements of human breast tissues, showing an ability to detect the boundary between normal adipose and malignant tissue with results validated against co-located histology. We have demonstrated the potential of
this technique to detect a tumour interface deep within a mastectomy sample with measurements acquired >4 cm below the tissue surface. These preliminary results demonstrate the feasibility of this technique to measure contrast based on the mechanical properties of tissue, complementing the optical contrast in the corresponding OCT A-scan data, with the potential to improve guidance of breast-conserving surgery.

9.3.6 References for Section 9.3


9.4. Conclusion
Prior to the commencement of this research, OCE experiments reported in the literature were limited to superficial tissues such as skin. We have developed and demonstrated a needle OCE technique to probe the microscale mechanical properties of deep, solid tissues, greatly expanding the potential of OCE for in vivo imaging. Our needle OCE method exploits the tissue deformation that is inherent to needle insertion and measures this deformation during insertion to probe mechanical contrast. The results presented here in tissue-mimicking phantoms and freshly excised human breast tissues demonstrate that needle OCE can detect mechanical interfaces deep within tissue, and point to its potential for localizing tumour boundaries for intraoperative guidance of breast cancer surgery. We have also shown a pathway toward in vivo measurements, by performing needle OCE in a full mastectomy sample under ultrasound guidance. However, a main limitation of the needle OCE measurements acquired in breast tissue so far is that the detected interface was always between adipose and non-adipose tissue, such as normal stroma or tumour. These interfaces are likely the best-case scenario for measuring mechanical contrast, but are also most easily identifiable using palpation or visual inspection. To rigorously test the capability of needle OCE to detect tumor boundaries, the full spectrum of tissue interfaces expected in breast tissues should be studied, especially the less obvious interface between normal stroma and malignant tumor. With further development, including extension of the design presented in this chapter to incorporate 2-D and 3-D imaging, needle OCE has great potential as a tool for guidance of biopsy and surgical procedures.
Conclusion and perspectives

OCE techniques to date have shown promise for imaging the mechanical properties of tissues on a spatial scale between that of individual cells and whole organs. Visualizing the mechanical properties of breast tissues on this scale could provide a means to localize areas of malignancy, potentially improving guidance of breast-conserving surgery. In this research, we have taken several steps to progress OCE toward realizing its potential as a clinical imaging tool. We investigated fundamental mechanical and imaging system parameters that limit image quality in OCE. In parallel, we developed novel tools to facilitate practical clinical application, including a method for measuring stress using OCT and the first needle-based OCE technique. The results demonstrate compelling mechanical contrast of the microstructure in human breast tissues. Below, we summarize the key results of this research, discuss some limitations of the current studies, and provide our perspectives on areas for further development in OCE.

Significance of research outcomes

Despite a recent upsurge in the number of proposed OCE methods and potential applications [17, 18], very few studies have addressed the issue of elastogram fidelity, i.e., how accurately elastograms represent the underlying mechanical properties of a sample. In the first aspect of this research, we performed the first analysis of mechanical contrast in compression OCE. To aid our investigation, we developed tissue-mimicking phantoms with a wide range of mechanical properties, as described in Chapter 4. Then, in Chapter 5, we employed finite element models (FEMs) to simulate OCE experiments. This provided a means to validate experimental measurements, as well as to analyse the impact of nonuniform stress on mechanical contrast. We found that surface friction is a key source of artefacts specific to the shallow imaging regime of OCE, in contrast to ultrasound elastography and MRE. We also showed that nonuniform stress limits the fidelity of strain elastograms when imaging a stiff inclusion in a soft background. Although the results highlight the qualitative, rather than quantitative, nature of strain elastograms in compression OCE, they also point to the exquisite microstrain sensitivity of the technique and indicate a theoretical sensitivity to changes in stiffness as small as 0.2%.
In Chapter 6, we improved upon the compression OCE techniques used in Chapter 5 by altering the image acquisition scheme and signal processing chain. We implemented a weighted-averaging approach to enable 3-D imaging with excellent strain sensitivity, whilst maintaining the OCT lateral resolution in the en face plane. Furthermore, we increased the maximum measurable strain eleven-fold by introducing a novel phase-unwrapping algorithm. The resulting technique, optical coherence micro-elastography, was demonstrated in freshly excised human breast tissues. The micro-elastograms of breast tissues visualized tissue microstructures such as ducts and blood vessels, and indicated that regions containing malignant cells may produce a characteristically heterogeneous strain pattern compared to uninvolved tissues. Furthermore, the contrast between features in the micro-elastograms was often superior to that in OCT images. However, the images further highlighted the relative, rather than absolute, mechanical contrast in strain elastograms, and indicated the need for a stress measurement to better enable inter-sample comparison and assessment of changes in disease over time.

Motivated by this need for a measurement of stress in OCE, in Chapter 7 we developed the first OCT-based tactile imaging technique, termed optical palpation. This technique drew inspiration from the field of tactile imaging for robotics applications, in which electronic sensors have been used to mimic the tactile sensation experienced by human skin [235]. Our stress sensor consists of a compliant silicone layer with known stress-strain behaviour, which allows mapping of the stress distribution at the sample surface under a compressive load. In Chapter 7, we explored the ability of optical palpation to map stress in heterogeneous phantoms and in excised human breast tissue. We showed that optical palpation has potential clinical utility on its own, as unlike OCE, it can be performed independently of the sample’s optical properties, and it is sensitive to features beyond the penetration depth of OCT. We also demonstrated delineation of a breast tumour boundary using optical palpation. However, as optical palpation is not a depth-resolved technique, it cannot decouple the size, depth, and mechanical contrast of features without resorting to the use of inverse methods [238, 269].

Chapter 8 brought together the strain imaging and stress imaging afforded by optical coherence micro-elastography and optical palpation, respectively, to enable
the first quantitative compression OCE technique. This overcomes the limitation of relative contrast in strain elastograms, opens possibilities for inter-sample comparison and longitudinal studies, and is expected to allow more improve accurate identification of malignant versus benign breast tissues in elastograms. Initial results in a homogeneous phantom showed sub-kPa sensitivity to variations in Young’s modulus. Building on our study of stress uniformity in Chapter 5, we also demonstrated the potential for 3-D imaging of Young’s modulus in layered and inclusion structures. A preliminary result in excised human lymph node highlighted a region containing malignant cells based on elevated Young’s modulus and demonstrated improved contrast over the strain elastogram of the tissue.

Finally, one of the major limitations of OCE as a clinical imaging tool is its limited penetration depth of only 1-2 mm in tissue. In Chapter 9, we presented needle OCE, enabling the first measurements of microscale mechanical contrast deep within solid tissues, and extending the abilities of OCE for in vivo imaging of breast lesions. Needle OCE employs insertion of the needle itself as a loading mechanism. We demonstrated the ability to localize mechanical interfaces in tissue-mimicking phantoms and in excised human breast cancer tissues. The potential for in situ imaging was demonstrated using ultrasound guidance of the needle probe in a full mastectomy sample. These initial results demonstrated detection of the tumour boundary based on mechanical contrast.

**Study limitations and future work**

Together, the technical developments in this research have significantly enhanced the viability of OCE as a clinical imaging tool, particularly toward providing intraoperative guidance of breast cancer surgery. However, before the techniques presented here are adapted for clinical use, several technical limitations need to be addressed. In addition, the efficacy of OCE for improving outcomes of breast cancer surgery must be established. In this section, we list some key limitations, suggest pathways for improvement, and recommend future clinical studies.

**Mechanical limitations**

Compression OCE is an attractive technique for clinical use, as it has relatively high lateral resolution compared to surface wave [86] and shear wave [111] techniques,
and it is relatively straightforward to implement for 3-D imaging. But bulk compression inherently causes mechanical coupling of the tissue response. This presents challenges in accurately representing the local mechanical properties of the tissue, as the deformation in one region is coupled to that of the surrounding regions. As shown in Chapter 5, this can manifest as strain artefacts caused by nonuniform stress in the sample. However, additional artefacts were seen in the micro-elastograms of breast tissue presented in Chapter 6, where inverted (positive) strain was measured at the boundaries of features such as ducts and vessels. We hypothesize that this could be due to the incompressibility of the tissue; compression of one region causes expansion of an adjacent region to conserve volume. Whilst this inverted strain helps to accentuate features in the micro-elastograms, it also highlights the complexity of interpreting mechanical contrast based on strain. The addition of a stress measurement to perform quantitative OCE, proposed in Chapter 8, partially alleviated this problem; however, because the stress sensor itself is also incompressible, it suffers from similar mechanical coupling issues, and inverted strain can also be measured in the sensor. This resulted in portions of the quantitative elastograms giving erroneous Young’s modulus values, as the accurate calculation of Young’s modulus relies on compressive strain in both the sensor and the sample.

To overcome this issue of mechanical coupling and provide more localized measurements of mechanical properties in OCE, a micro-indentation method could be used to actuate the tissue, as opposed to bulk compression. A small number of previous studies have shown the potential to use OCT to measure micro-indentation for assessment of tissue mechanical properties [73, 95-97]. This would require adaptation of our mechanical model to estimate stiffness based on, for example, Hertz contact theory [270], rather than based on strain. A disadvantage of indentation methods is the need to mechanically scan the indenter to form an image, potentially making an indentation technique too slow for intraoperative tumour margin assessment. One possibility, however, is to perform wide-field compression OCE to provide an initial assessment of the sample, and then use an indentation technique to provide more localized information about the mechanical properties of suspicious regions.
Finite element modelling will continue to play an important role in validating OCE measurements using these various loading mechanisms and in understanding the strengths and limitations of each. Furthermore, the potential of FEMs to be used in iterative solutions to the inverse problem, as demonstrated in ultrasound elastography [271], has been little explored in OCE. This may provide an alternative path toward quantitative compression OCE.

Imaging system limitations
The current acquisition speed of the 3-D, phase-sensitive techniques presented in Chapters 6-8 is 0.1 s per B-scan, corresponding to 500 s for a volumetric scan of $6 \times 6 \times 2$ mm. This should be improved to facilitate rapid intraoperative assessment of tumour margins. In the current method, the phase difference is measured between consecutive compressed and uncompressed B-scans, and a practical upper limit on imaging speed is set by the need to remain in the quasi-static regime ($<10$ Hz to avoid inducing mechanical waves in the sample [272]). This could be overcome by calculating the phase difference between consecutive C-scans instead of B-scans. Our group has very recently demonstrated that such an approach can acquire volumetric strain elastograms ($5 \times 5 \times 2$ mm) in 5 s, with similar phase sensitivity to that using the approach in Chapter 6 [273]. However, this technique has not yet been tested on breast tissues, and it is unclear whether the high fluid content of breast tissue will cause phase instabilities between C-scan acquisitions. Using the fastest OCT systems available will give this technique the best chance for success; volumetric acquisition times of just 25 ms have been demonstrated using high-speed swept sources [274]. Again, however, a quasi-static loading frequency of 5 Hz sets a practical limit on speed of 0.2 s per volumetric scan.

We envision that OCE may be incorporated into the clinical workflow of breast cancer surgery in one or both of the following ways: by performing in vivo imaging prior to and during excision using needle OCE, guiding the surgeon’s decision as to where to cut; or by imaging the margins of the excised tissue using a bulk optics setup, providing feedback to the surgeon as to whether further tissue should be removed. Another option using the bulk optics setup may be to scan the surgical cavity in addition to imaging the excised lump following excision, effectively doubling the amount of tissue sampled. This approach has recently been implemented in a clinical
study of the efficacy of OCT for tumour margin assessment [275]. Any of these imaging schemes require modification to the current probe designs to facilitate practical clinical use. For example, adequate sampling of the excised lump using the bulk optics setup will require a wider imaging field of view. One solution may be to use multiple OCT systems, or a dual beam OCT system [276], with the two beams positioned at opposing surfaces of the sample. Either the beams or the sample could be translated in the \( x \) and \( y \) directions to scan the entire sample surface, and rotated to image all sample faces.

For needle imaging, a handheld implementation of the probe should be developed for \textit{in vivo} imaging, providing an ergonomic device for use by the surgeon. As we demonstrated in Chapter 9, ultrasound guidance could be used to guide the needle tip toward the suspected lesion boundary. However, the actuating mechanism of the needle should remain automated in order to more reliably deform the tissue and interpret its response.

Real-time display and interpretation of the large amounts of data generated by OCE is crucial for its translation into clinical scenarios. Live visualization of the data could be achieved by employing graphics processing units (GPUs), as has been done for OCT [277] and Doppler OCT [278]. To interpret the data, having a trained radiologist present in the operating theatre to read OCE images during surgery is likely unrealistic for many hospitals. Instead, automated tissue classification algorithms to highlight areas of malignancy would greatly facilitate data interpretation. The local Young's modulus estimated using the quantitative technique in Chapter 8 could provide one parameter for tissue classification, but we expect that Young's modulus values will overlap for several tissue types. However, the preliminary results in Chapter 6 indicate that malignant tissues produce characteristically heterogeneous mechanical contrast; thus, textural analysis such as that proposed for OCT imaging of breast cancer [197] could be combined with Young's modulus values to potentially classify tissue types.

\textbf{Future clinical work}

In parallel to improving the technical aspects of OCE as outlined above, its efficacy as a surgical guidance tool must be established by systematic application to a large number of breast tissue specimens. The key measures for clinical efficacy are sensitivity,
defined as the percentage of positive margins correctly diagnosed as positive; and specificity, defined as the percentage of negative margins that are correctly diagnosed as negative. A clinical study determining sensitivity and specificity should incorporate all types of breast tumours (e.g., in situ, invasive, and benign tumours) to ascertain whether OCE is better suited to detection of particular subtypes of breast cancer.

The mechanical contrast in OCE is complementary to the structural information in OCT, and combined interpretation of the images will provide better sensitivity to areas of malignancy than either type of imaging on its own. In addition, several other extensions to OCT have shown potential for improving contrast between breast tissues. Parametric imaging of the optical properties of lymph nodes has shown potential for differentiating tissue types, including tissues containing metastatic tumour cells [195, 279]. Molecular contrast provided by combined OCT and fluorescence imaging [280] could also provide a means to specifically target malignant cells within the OCT field of view. Combined OCT and fluorescence imaging has also recently been demonstrated via an OCT needle probe [281]. Other extensions to OCT that may improve contrast for breast imaging include polarization-sensitive OCT to quantify tissue birefringence [282] and Doppler OCT for imaging of tumour microvasculature [283]. Any of these techniques could feasibly be combined with OCE to provide rich information on the structural, mechanical, and functional properties of the tissue.

The OCE techniques developed in this thesis have potential in a broad range of clinical and preclinical applications. Besides breast-conserving surgery, OCE and needle OCE could provide guidance of a number of surgical procedures. In resection of tumours of the brain [284] and prostate [285], for example, precise guidance is needed not only to ensure complete tumour removal, but also to preserve tissue structures that are critical to function and quality of life. Another promising application of OCE may be in assessment of skin conditions such as burn scars, where manual palpation is often used to qualitatively indicate a scar’s healing progression, but quantitative measures could provide a more reliable assessment of response to treatment [286-288].

The unique spatial scale of OCE, between the cellular and whole organ scales, gives it potential to bridge the gap between mechanical imaging techniques that have been
confined to the laboratory, such as AFM, and the ultrasound elastography and MRE technologies applied in the clinic. For instance, the cellular and extracellular mechanics of muscle tissues have been studied to understand the origins and progression of muscular dystrophy [289]. In parallel, the macroscale mechanical properties of muscles have been measured in humans with the disease [290]. OCE has potential to monitor muscle mechanics at the scale of disease progression, and to do so in vivo, e.g., in a mouse model. Our group has previously shown the potential for OCT to assess muscular dystrophy in a mouse model of the disease [291], including in an OCT needle probe [292], and we are currently exploring the potential of OCE to provide additional contrast of the diseased tissues.

Finally, an underexplored area in OCE is application to the rapidly emerging field of tissue engineering. Here, the mechanical properties of the synthetic tissue constructs are critical for predicting viability in the body [133, 293]. OCE could provide a tool to assess the mechanical properties of the engineered tissue across all stages of development, from monitoring changes in mechanical properties during fabrication and cell proliferation to longitudinal examination of the implanted tissue in the body.

**Final remarks**

This research has made several key advances toward the clinical translation of OCE. The compression OCE techniques developed in this thesis can visualize tissue mechanical properties on the microscale with unprecedented sensitivity and with the added ability to quantify stiffness. Using needle OCE, these measurements can be performed at previously unreached depths in tissue. With further development, OCE may provide improved intraoperative assessment of tumour margins in breast-conserving surgery and, ultimately, reduce the number of patients that must undergo a second surgery.
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