Ultrasonic Characterization of Corneal and Scleral Biomechanics

DISSERTATION

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By

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Abstract

Cornea and sclera are the major load-bearing tissue of the eye and the biomechanics of the cornea and sclera have been shown to be critical in the understanding, diagnosis and management of glaucoma. It is therefore important to non-invasively measure the mechanical properties of the cornea and sclera and examine their effects in glaucoma. The current study was designed to investigate the effect of corneal stiffness on intraocular pressure (IOP) and central corneal thickness (CCT) measurements and develop ultrasound-based techniques for non-invasive characterization of corneal and scleral biomechanics under physiological loadings and configurations.

We first examined the effect of the natural variation in speed of sound in cornea on the measurement of corneal thickness, which is an important parameter affecting the IOP measurement and a risk factor of glaucoma. The effect of the variation in corneal stiffness on IOP measurement was examined experimentally, and a non-invasive ultrasound method for measuring acoustic impedance was used to estimate the corneal stiffness and potentially provide corrections for IOP measurement. An ultrasound strain imaging method based on speckle tracking was developed to characterize the mechanical response of the sclera under IOP elevations, and the performance of the ultrasound method was evaluated both experimentally and using simulations. The mechanical
responses of the porcine and human sclera under IOP elevations were then examined by this ultrasound strain imaging method. The variance of speed of sound in cornea was shown to potentially produce significant error in corneal thickness measurement using the current clinical setting of speed of sound in ultrasound pachymetry. Corneal acoustic impedance was significantly correlated with the speed of sound in cornea and could potentially be used to improve corneal thickness measurement accuracy. The effect of corneal stiffness on IOP measurement was found to be significant, and the corneal acoustic impedance was significantly correlated with the IOP measurement error and the corneal stiffness measured through uniaxial tests. This correlation may provide necessary corrections for clinical IOP measurement. The ultrasound speckle tracking method developed for noninvasive measurement of through-thickness distributive strains of the sclera demonstrated excellent accuracy and high signal-to-noise ratio in both experimental and simulation results. The porcine and human sclera showed anisotropic, nonlinear, heterogeneous mechanical responses under IOP elevations.

The current study demonstrated significant effects of corneal biomechanics on CCT and IOP measurement and the complexity of sclera biomechanics in response to IOP loadings. This research also established the feasibility of the proposed ultrasound methods as useful experimental and clinical tools to characterize corneal and scleral biomechanics non-invasively. Future studies should implement these techniques for measurement of corneal and scleral biomechanical properties in glaucoma patients.
Dedication

This document is dedicated to my family.
Acknowledgments

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Table of Contents

Abstract ........................................................................................................................................... ii

Dedication ......................................................................................................................................... iv

Acknowledgments ............................................................................................................................ v

Vita ..................................................................................................................................................... vi

Publications ........................................................................................................................................ vi

Fields of Study ................................................................................................................................. vii

Table of Contents ............................................................................................................................. viii

List of Tables .................................................................................................................................... xii

List of Figures ................................................................................................................................... xiii

Chapter 1 Introduction ..................................................................................................................... 1

1.1 Ocular anatomy and physiology .............................................................................................. 1

1.2 IOP and CCT measurement in glaucoma ................................................................................ 7

1.3 Corneal and scleral biomechanics .......................................................................................... 12

1.4 Ultrasound elastography ......................................................................................................... 19

1.5 Specific Aims ............................................................................................................................ 22
Chapter 2 Variation of speed of sound in cornea

2.1 Introduction

2.2 Materials and Methods

2.2.1 Sample preparation

2.2.2 Acoustic impedance measurement

2.2.3 Speed of sound measurement

2.2.4 Data Analysis

2.3 Results

2.4 Discussion

2.5 Summary

Chapter 3 Effect of Corneal Stiffness in IOP measurements

3.1 Introduction

3.2 Methods

3.2.1 Sample preparation

3.2.2 Acoustic impedance measurements

3.2.3 Goldmann applanation tonometry (GAT) and Tonopen measurements

3.2.4 Uniaxial tensile test of corneal strips

3.2.5 Statistical analysis
3.3 Results ............................................................................................................................................... 56
3.4 Discussion ......................................................................................................................................... 66
3.5 Summary ............................................................................................................................................ 71

Chapter 4 Ultrasonic strain mapping on porcine sclera ................................................................. 72
4.1 Introduction ......................................................................................................................................... 72
4.2 Methods ............................................................................................................................................... 74
  4.2.1 Experimental setup and testing protocol ...................................................................................... 74
  4.2.2 Ultrasound speckle tracking algorithm ....................................................................................... 77
  4.2.3 Experimental validation of displacement measurement .......................................................... 78
  4.2.4 Theoretical analysis of the signal-to-noise ratio in ultrasound strain mapping > ......................... 79
  4.2.5 Analysis of strain estimation accuracy using simulated RF data .............................................. 82
4.3 Results ............................................................................................................................................... 84
  4.3.1 Through-thickness strain distribution in porcine posterior sclera .............................................. 84
  4.3.2 Experimental validation of displacement measurement .......................................................... 88
  4.3.3 Theoretical analysis of the SNR in strain estimation ................................................................. 89
  4.3.4 Analysis of strain estimation accuracy using simulated data ..................................................... 91
4.4 Discussion .......................................................................................................................................... 95
4.5 Summary .......................................................................................................................................... 101
List of Tables

Table 3.1 GAT and Tonopen readings at various true IOP levels in canine eyes........... 58

Table 3.2 Secant moduli of 8 human cornea samples measured using uniaxial tensile test.................................................................................................................................................... 68

Table 4.1 Average scleral strains at different pressure levels. (*: For lateral strains, the circumferential was significantly smaller than the meridian at 45 mmHg, P<0.05; †: The axial strains were significantly greater than the lateral strains at all pressure levels, P<0.01)..................................................................................................................................................... 88

Table 4.2 The peak SNRe and the -3dB range of the SNRe for different kernel sizes.... 90

Table 4.3 Simulated vs. calculated strains under uniform compression or extension ..... 92

Table 4.4 Calculated strains for small heterogeneities (2% simulated strain) of various sizes surrounded by a background with 1% simulated strain. .................................................... 94

Table 5.1 Demographic information and postmortem time of the human cadaver eyes 104
List of Figures

**Figure 1.1** Gross anatomy of the eye (from www.nei.nih.gov) with blue lines showing light focusing on retina ................................................................. 2

**Figure 1.2** A cross-section view of the cornea (www.vetmed.vt.edu).................... 4

**Figure 2.1** System and setup for measurement of corneal acoustic impedance.......... 31

**Figure 2.2** Curvature coefficient (ratio of the reflection amplitude from a curved surface against that from a flat surface) as a function of radius of curvature. ...................... 34

**Figure 2.3** Substitution method for measuring corneal speed of sound. (a) Ultrasound measurements with cornea intervening; (b) ultrasound measurements without cornea intervening. (t1 is the time-of-flight for the ultrasound reflection from the anterior cornea; t2 is the time-of-flight for the ultrasound reflection from the posterior cornea; t3 is the time-of-flight for the ultrasound reflection from the flat reflector with the cornea intervening; and t4 is the time-of-flight for the ultrasound reflection from the reflector without the cornea intervening). The dimensions (except the distance from the transducer to the cornea) were drawn in approximate proportion to the actual size ....................... 35

**Figure 2.4** Correlation between acoustic impedance and speed of sound in fresh canine corneas (solid line: regression line; dashed line: 95% confidence interval). .................. 38

**Figure 2.5** Correlation between speed of sound and density in fresh canine corneas..... 39
Figure 2.6 Scatter plot of speed of sound and corrected ultrasound pachymetry readings showing a general positive correlation between these two parameters. .......................... 40

Figure 2.7 Correlation of speed of sound in the two eyes of the same dogs............. 41

Figure 2.8 Scatter plot of acoustic impedance and speed of sound in groups of different ages (Puppy: less than one year old; Young: one to five years old; Old: above five years old.) .......................................................................................................................... 42

Figure 3.1 The schematics of the experimental set-up for GAT measurements.......... 54

Figure 3.2 The average stress-strain relationship for control and corneal stiffening groups. ............................................................................................................................................................................. 59

Figure 3.3 Scatter plots of GAT readings versus post-treatment corneal acoustic impedance at different levels of IOPMan (■: corneal stiffening; ▲: control)............. 60

Figure 3.4 Scatter plots of Tonopen readings versus post-treatment corneal acoustic impedance at different levels of IOPMan (■: corneal stiffening; ▲: control).......... 61

Figure 3.5 Scatter plots of GAT readings versus post-treatment corneal secant modulus at 1% strain at different levels of IOPMan (■: corneal stiffening; ▲: control)........... 62

Figure 3.6 The scatter plots of Tonopen readings versus post-treatment corneal secant modulus at 1% strain at different levels of IOPMan (■: corneal stiffening; ▲: control). ............................................................................................................................................................................. 63

Figure 3.7 Linear correlation between post-treatment corneal acoustic impedance and secant modulus at 1% strain.................................................................................................................................................. 64

Figure 3.8 Scatter plots of GAT readings versus post-treatment CCT at different levels of IOPMan (■: corneal stiffening; ▲: control). ............................................................................................................................................... 65
Figure 3.9 Scatter plots of Tonopen readings versus post-treatment CCT at different levels of IOPMan (■: corneal stiffening; ▲: control) ................................................................. 66

Figure 4.1 Schematics of experimental setup. ........................................................................ 75

Figure 4.2 Schematics for sclera shell mounting chamber ....................................................... 76

Figure 4.3 (a) The experimental protocol of preconditioning and IOP loading; (b) the scanning orientations for ultrasound data acquisition. The circumferential cross-section was about 2 mm from the ONH. ......................................................................................... 77

Figure 4.4 Displacement vector fields in a posterior porcine sclera obtained from ultrasound speckle tracking. (a) A cross-sectional ultrasound image of the sclera at 5 mmHg; (b) displacement field at 15 mmHg; (c) displacement field at 30 mmHg; (d) displacement field at 45 mmHg. .................................................................................................................. 85

Figure 4.5 Strain images of porcine sclera: (a) axial strain at 15 mmHg (dashed rectangle indicates the region of interest at posterior pole); (b) lateral strain at 15 mmHg; (c) axial strain at 30 mmHg; (d) lateral strain at 30 mmHg; (e) axial strain at 45 mmHg; (f) lateral strain at 45 mmHg ........................................................................................................................................ 86

Figure 4.6 Average scleral strains at different pressure levels. ............................................... 87

Figure 4.7 Comparison of the displacements calculated from speckle tracking and the actuator output: (a) calculated axial displacement vs. actuator output; (b) calculated lateral displacement vs. actuator output ........................................................................................................ 89

Figure 4.8 SNRe vs. strain for different kernel sizes .............................................................. 90

Figure 4.9 A simulated ultrasound image of the sclera using the Field II Ultrasound Simulation Program. ...................................................................................................................... 91
Figure 4.10 The approximated SNRe curves of the strain maps based on simulated data. .......................................................................................................................................................................................... 93

Figure 4.11 Displacement vector field and strain maps calculated from simulated RF signals for top 1% and bottom 2% strains. ......................................................................................................................... 93

Figure 4.12 Strain images of a simulated sclera with an inhomogeneous region. Row (a) and (b) are axial and lateral strains, respectively, for an inhomogeneous layer with decreasing thickness: 1) 500 µm, (2) 250 µm, (3) 150 µm, and (4) 50 µm; Row (c) and (d) are axial strains and lateral strains, respectively, for an inhomogeneous zone with decreasing width: (1) 2 mm, (2) 1 mm, (3) 400 µm, and (4) 200 µm. ........................................... 95

Figure 5.1 Schematics for sclera shell mounting and ultrasonic measurements............ 105

Figure 5.2 Diagram of the four quadrants examined on posterior sclera (N: nasal quadrant; S: superior quadrant; T: temporal quadrant; I: inferior quadrant) ............... 106

Figure 5.3 Illustration of the radial and the tangential directions (red dashed arrows) in relation to the axial and lateral directions (black dashed arrows) and an example of the angle $\theta$ between the two coordinates ........................................................................................................... 108

Figure 5.4 Example of an ultrasound image of human sclera with boundaries fitted to circles and the inside equally divided into three layers (the outer layer, the middle layer, and the inner layer). .......................................................................................................................................................... 109

Figure 5.5 Radial strain of all human sclera samples including both the meridian and the circumferential scans at different pressure levels ................................................................. 111

Figure 5.6 Tangential strain of all human sclera samples including both the meridian and the circumferential scans at different pressure levels ............................................................ 112
Figure 5.7 Tangential strain vs. pressure at the meridian and the circumferential scanning directions (*: P < 0.05) .............................................................................................................. 113

Figure 5.8 Radial strain vs. pressure at the meridian and the circumferential directions .............................................................................................................................................................................. 114

Figure 5.9 Average tangential strain of the outer layer, the middle layer and the inner layer.............................................................................................................................................................................. 115

Figure 5.10 Average radial strain of the outer layer, the middle layer, and the inner layer .............................................................................................................................................................................. 116

Figure 5.11 The average tangential strain vs. pressure in the four quadrants measured 117

Figure 5.12 The average radial strain vs. pressure in the four quadrants measured ..... 118

Figure 5.13 Average tangential strain in all four quadrants vs. pressure at different scanning directions.............................................................................................................................................................................. 119

Figure 5.14 Average radial strain in all four quadrants vs. pressure at different scanning directions.............................................................................................................................................................................. 119
Chapter 1 Introduction

This chapter introduces the clinical and research findings in corneal and scleral biomechanics as necessary background information that has motivated the dissertation research. A brief review of the relevant ultrasound techniques employed in this research is also provided.

1.1 Ocular anatomy and physiology

The eye is an important organ that gives the vision. The light from outside first travels through the cornea, the anterior transparent part of the ocular shell, and gets initially focused by the cornea. It is then further focused when passing the lens and finally converges on the retina, which contains photoreceptors that can convert the light signals into electrochemical signals and pass them to the retina ganglion cells and their axons, the nerve fibers. All the nerve fibers converge to a bundle at the optic nerve head (ONH) of the posterior eye to exit the ocular shell and convey the signals to the brain (Figure 1.1). To obtain optimal vision, the power of the lens can be adjusted through a process called accommodation and the pupil size can be controlled to limit the amount of light going through. The delicate structure of the eye is important to maintain its stable function of vision. The eye is filled mostly with vitreous humor posterior to the lens and aqueous humor at the anterior part. The fluid pressure inside the eye is called intraocular
pressure (IOP). IOP is important for maintaining the shape and function of the eye, since the human eye is a boneless structure constituted of soft tissues. The cornea-sclera envelope provides the necessary mechanical support to withstand the IOP and protect the inner structure of the eye.

Figure 1.1 Gross anatomy of the eye (from www.nei.nih.gov) with blue lines showing light focusing on retina

As the transparent part of the ocular shell, the cornea is essentially an avascular connective tissue containing many collagen fibrils. The shape of the cornea is an important factor in determining the quality of vision since the cornea is the major light-refracting structure of the eye. The transparency of the cornea is likely maintained by the highly organized collagen fibers in the stroma. The collagen fibrils have a small, uniform diameter and are positioned with a high degree of order, which produces destructive
interference of the scattered light and constructive interference of the transmitted light throughout all visible wavelengths [1]. The cornea is generally considered to be composed of five layers. They are the epithelium, the Bowman’s membrane, the stroma, the Descemet's membrane, and the endothelium, from outer to innermost (Figure 1.2). The epithelium is about 50 µm thick and mainly composed of about five to seven stacks of epithelial cells [2]. The Bowman’s layer is a thin layer anterior to the stroma with randomly arranged collagen fibrils. It is absent in some animals, such as rabbit, cat and dog [3, 4]. The presence of Bowman’s membrane provides anchoring for the collagen fibrils in anterior stroma and is believed to help stabilize corneal shape [4]. The stroma of the cornea takes up about 90% of the corneal thickness and is mainly composed of collagen fibrils that are mostly collagen type I with a small amount of type V and others [4]. The structure and organization of the collagen fibrils is important in determining both the mechanical and optical properties of cornea [5, 6], which motivated extensive studies on the corneal collagen fibril structure [7-9]. The collagen fibrils in the corneal stroma are mainly organized in the form of lamellae with a thickness of about 2µm and a width up to 0.2mm [10, 11]. These lamellae are mostly aligned in parallel to the surface except that some interweave to form a bow structure at the anterior stroma which may provide additional mechanical strength [9]. In addition to the difference in organization through thickness, the collagen fibrils have different preferred distribution directions in the center cornea (cross-shaped orthogonal arrangement) than in the peripheral cornea (circumferential arrangement) [4]. Descemet’s membrane is a thin limiting layer, and the
endothelium is a single layer of cells that are critical in maintaining the hydration level and thus the transparency of the stroma through their active transport [4].

Figure 1.2 A cross-section view of the cornea (www.vetmed.vt.edu)

The sclera, the white coat of the eye, is connected to the cornea through the limbus and comprises about 85% of the outer tunic of the eye. It provides a tough support to other ocular components such as the retina and the optic nerve head. The thickness of the human sclera is not uniform, with the posterior pole being the thickest (1-1.35mm), and the thickness decreasing gradually to 0.4-0.6mm at the equator and increasing again to 0.8mm around limbus [12]. The overall organization of collagen fibers in the sclera is more random than those in the cornea, which makes the sclera an opaque structure. From outer to innermost, the sclera may be divided into four layers: episclera, stroma, lamina
fusca, and endothelium. The episclera is a thin, loose connective tissue with vascular structure. The strength of the sclera stroma is dominated by bundles of collagen fibrils. The fibrils are less uniform in diameters (25 to 230 nm) than those in cornea, and the sclera fibril bundles branch and interlace extensively and exhibit wide-ranging dimensions, up to 50 µm wide and 6 µm thick [11]. Increased interweaving and density of fibers replaces the lamellar arrangement in the deep sclera. There are variations in the collagen fibrils through the tissue depth. In the outer sclera, fibrils are thicker but the bundles are narrower and thinner than those in deeper regions. In the inner sclera, the collagen fibrils are smaller in diameter, wavy and intermingled, and the bundles show a wide range of widths (1-50µm) and thickness (0.5-6µm) [4]. In the lamina fusca layer of the sclera, the collagen bundles are again smaller and branch extensively to insert into the underlying choroidal stroma [12]. There are scattered elastic fibers within or between the collagen bundles throughout the stroma [12]. The sclera is also traversed by blood vessels and nerves.

There is an opening on the posterior sclera, the sclera canal, through which retinal ganglion cell axons exit the eye. In the sclera canal region the ganglion cell axons converge to form a bundle and there are also supporting connective tissues (peripapillary sclera and lamina cribrosa) and cells, all of which are usually referred to as the optic nerve head (ONH). The lamina cribrosa is a fenestrated connective tissue which allows axons to pass through and connects tightly to the peripapillary sclera to maintain the relative continuity of the ocular shell. Mechanically the ONH has been considered as a relatively weaker spot on the ocular shell [13, 14].
With the cornea, sclera and ONH together to form the shell of the eye, its structure is made stable by the positive intraocular pressure inside it. The intraocular pressure is normally maintained by the balancing between the production and the outflow of the aqueous humor inside the eye. The aqueous humor is secreted by the ciliary body (ciliary epithelium) and contains necessary nutrients for the cornea and lens [15]. Its composition may be altered by reabsorption when entering the posterior chamber and the aqueous humor flows through pupil into anterior chamber. There are two main outflow pathways for the aqueous humor to be drained from the eye, the conventional trabecular meshwork pathway (about 90% aqueous humor) and the uveo-scleral pathway (about 10% aqueous humor) [16]. In the trabecular meshwork pathway, the aqueous humor can exit through trabecular meshwork into the Schlemm’s canal and collector channels before it gets into the intrascleral and episcleral venous plexuses. The other pathway is through the ciliary body, along interstitial spaces of ciliary muscle and choroid or the suprachoroidal space through the sclera or vascular channels of the sclera into the connective tissue of the orbit, from where it is drained via the veins. The rate of outflow through the trabecular meshwork is pressure dependent with higher outflow rate associated with higher intraocular pressure. Once the outflow pathways are pathophysiologically impaired and the outflow resistance is increased, the fluid starts to accumulate in the anterior chamber and the IOP rises until the aqueous secretion is balanced by the outflow again at an elevated IOP level.
1.2 IOP and CCT measurement in glaucoma

Elevated IOP is associated with higher risk of glaucoma, which is characterized by the irreversible damage to the retinal ganglion cell axons within the ONH. Glaucoma is the second leading cause of blindness worldwide after cataract, and the population affected has been estimated to reach about 79.6 million by 2020 [17]. Although glaucoma is usually thought to occur at elevated levels of IOP, it actually can also develop at normal IOP, which has been called normal tension glaucoma or low tension glaucoma. In general, glaucoma can be divided into two main categories, open-angle glaucoma and angle-closure glaucoma, based on the state of the angle between the iris and the cornea through which the aqueous humor travels to the trabecular meshwork. Closed-angle glaucoma is the result of a narrow or closed angle in the anterior chamber that impedes the outflow and thus leads to IOP elevation and damages to the retinal ganglion cell axons. Open-angle glaucoma is characterized by open angles in the anterior chamber and can be further divided into two major categories: high-tension glaucoma (IOP > 21 mmHg) and normal-tension glaucoma (IOP is within normal range of 10 - 21 mmHg).

For glaucoma patients, the only effective treatment to date is to reduce the IOP through either pharmacological or surgical treatments. However, it has to be acknowledged that sometimes even lower than normal IOP may still fail to stop the progression of the damages to the ganglion cell axons, which suggests significant risk factors other than IOP.

Because IOP is a critical parameter in the diagnosis and treatment of glaucoma [18], it is important to measure the IOP accurately in a convenient way for clinical routines. The use of Goldmann applanation tonometry (GAT) for assessing the IOP has
been the clinical “gold standard” worldwide, since it was first introduced by Dr. Goldmann in the 1950s [19]. In GAT measurements, the cornea is flattened over a defined area and the required force is used to estimate IOP based on the Imbert-Fick law (at equilibrium, Pressure = Force/Area) [20]. The Imbert-Fick law assumes perfectly elastic and thin membranes [19]. Nevertheless, the cornea is not an ideal thin membrane and exerts some resistance to the applanation. In addition, the surface tear film may also exert some forces on the tonometer tip. The measured pressure could thus deviate from the prediction of the Imbert-Fick law [21, 22]. Goldmann proposed an applanation area of 3.06 mm in diameter and it has been generally believed that such applanation area would allow the cancellation between the effect of the tear film traction and the corneal resistance, which would lead to a relatively accurate measurement of IOP for human corneas with average dimensions and properties [19, 21]. However, not all corneas satisfy the calibration conditions of GAT, due to the natural variances among individuals in corneal thickness, radius of curvature and biomechanical properties. Central corneal thickness (CCT) and corneal curvature have long been suspected to affect the accuracy of GAT measurements. Numerous studies have reported a significant effect of CCT on the accuracy of GAT with the measurement error ranging from 0.11 to 0.71 mmHg for each 10 μm deviation from the population mean [22-26]. The effect of corneal curvature has also been reported with the GAT errors ranging from 0.57 to 1.14 mmHg per 1 mm change in radius of curvature [27-29]. There has been an increasing interest in the effect of corneal stiffness on GAT measurements since theoretical modeling suggested that
corneal stiffness could potentially play a larger role than corneal thickness and curvature [27, 29-31].

Other than the GAT, there are also many other techniques developed to measure the IOP, such as palpation, manometry, indentation tonometry, noncontact air puff applanation tonometry, contour-matching tonometry, and MacKay-Marg tonometry. Palpation is the oldest way of estimating IOP by just putting fingers over the eyeball and eyelid, which could still be useful when other tonometry is unavailable or subject to gross error [32]. Manometry is an invasive technique that can precisely measure the pressure inside the eye, and it has served as a reference pressure for the calibration of other tonometers such as GAT, dynamic contour tonometry, and indentation tonometry [19, 33-35]. However, it is usually limited to cadaver eyes or eyes undergoing enucleation or surgeries [36]. The indentation tonometry was developed before the GAT and the basic principle is that a known force will indent a fluid filled sphere with low internal pressure to a greater degree than those with high internal pressure. Due to its relatively large volume displacement during the measurement, the indentation tonometry is greatly affected by the rigidity of the ocular shell and there is also a significant increase in the pressure during indentation [37]. The noncontact air puff tonometry works on the same principle as Goldmann, except that the force is applied by air puff and the condition of applanation is determined by an optical sensor [38]. The air puff is relatively painless and requires no cornea anesthesia. The Reichert Ocular Response Analyzer (ORA), a recent air puff tonometer, not only measures the IOP but also estimates parameters related to corneal properties such as corneal hysteresis (CH) and corneal resistance factor (CRF)
In order to minimize the effect of cornea geometry on tonometry, Pascal Dynamic Contour Tonometry (DCT) uses a probe with concave surface matching the normal cornea and the pressure is measured with an external pizo-electric pressure sensor [40]. The DCT has been reported to be less affected by corneal thickness than the GAT, although still statistically significant [41], and the effect of curvature has been reported to be more significant than using GAT [42]. The standard GAT requires a slit-lamp for taking measurements and thus is not suitable for handheld measurement. Portable tonometers based on the Goldmann principle have been designed to facilitate clinical screening [43, 44]. Tonopen is also a handheld instrument that measures IOP based on the MacKay-Marg principle [45], in which both the indentation and applanation were combined during the measurement and the IOP was estimated at the applanation stage. Tonopen has been reported to be suitable for accurate measurement on corneas with surface abnormality [46]. It has also been shown to produce comparable measurements with GAT on normal corneas [47-50], with the effect of CCT reported to be less significant than in GAT [51].

Aside from the IOP, the corneal thickness has attracted an increasing attention due to its importance in glaucoma diagnosis and management. The importance of the CCT mainly comes from two aspects in glaucoma: its influence on IOP measurement using tonometry, and its direct effect as a glaucoma risk factor. The influence of CCT in the IOP measurements using tonometric methods has been documented extensively [22-26]. The average CCT among normal population has been reported to be about $536 \pm 31$ µm in one study [52] and it is now generally accepted that a thicker cornea tends to
induce overestimation of IOP using applanation tonometry. The quantitative effect of CCT on IOP measurement error was however inconsistently reported in the literature, varying from 0.11 to 0.71 mmHg per 10 μm deviation [22-26]. It is possibly due to the fact that other factors could be confounded in the IOP measurement error, such as corneal hydration [53], disease condition [52], and corneal stiffness [27, 30, 31]. Moreover, CCT has been identified as an independent risk factor in glaucoma development [18, 54, 55]. Although the direct connection between the CCT and glaucomatous damage has not been well understood, it has been suspected that difference in CCT may be an indication of ocular structural variation among eyes, and if such variation presents in lamina cribrosa it may contribute to the fact that some eyes are more susceptible than others to glaucomatous damage [56]. It has been found that the CCT negatively correlates with the size of optic disc [57, 58]. However, it should also be noted that this hypothesis has remained controversial as the CCT was not found to be correlated with the deformation of the optic disc surface [59] and the correlation between the thickness of lamina cribrosa and the CCT was not statistically significant either [60]. Nevertheless, a clinical model based on statistical studies suggested a 20 μm thinner in CCT could increase the risk of glaucoma by 3 points [61], which is clinically significant. These studies warrant the clinical need for accurate measurement of CCT in glaucoma management. In addition, the CCT is also a critical parameter in refractive surgeries such as LASIK [62]. Keratoconus treatment using UVA-riboflavin collagen cross-linking has been suggested to be only applied on corneas thicker than 400 μm [63] and it is thus critical to accurately determine the corneal thickness before applying the treatment.
In clinics, the measurement of CCT is usually based on either acoustic method, such as in ultrasound pachymetry, or optical method, such as in optical pachymetry and optical coherence tomography (OCT). Ultrasound pachymetry has been considered the clinical “gold standard”. During the measurement of ultrasound pachymetry, ultrasound waves propagate perpendicular to the central cornea surface and are reflected at the anterior surface and the posterior surface. The CCT is estimated based on the time of flight of ultrasound between the anterior corneal surface and the posterior corneal surface, with assumed speed of sound typically at 1640 m/s. The optical pachymetry utilizes light scattered from anterior and posterior corneal surfaces, with similar assumption of constant refractive index [64]. Recent developments in optical coherence tomography are available for imaging the profile of the cornea and measuring the thickness from cross-sectional images [65].

1.3 Corneal and scleral biomechanics

The corneal mechanical properties may play an important role in affecting the accuracy of applanation tonometry. For example, Liu and Roberts’s analytical model assuming isotropic linear elasticity showed that a variation in corneal Young’s modulus from 0.1 to 0.9 MPa could introduce a difference greater than 10 mmHg in GAT measurements [27]. Elsheikh et al.’s numerical models assuming nonlinear material properties generally produced a similar level of effects of corneal stiffness on IOP measurements [30, 31]. Orssengo and Pye proposed a mathematical model to determine the true intraocular pressure and cornea elasticity from cornea dimensions and
applanation pressure from GAT [29]. These modeling results have provided valuable insight into the role of corneal biomechanics in GAT measurement accuracy. The motivation to study corneal biomechanics is not only limited to understanding the error sources in IOP measurement. Corneal biomechanics is also critical in understanding how the cornea would respond to the IOP loading under pathophysiological conditions such as in keratoconus [66], or during and after corrective refractive surgeries [67, 68]. The cornea mechanical properties have also been shown to play an important role in damping the IOP insults at a given volume displacement [69, 70].

*Ex vivo* studies on corneal biomechanics have shown that the cornea exhibits non-linear stress-strain behavior with typical stiffening at higher strain level [71-73]. A wide range of Young’s modulus can be found in the literature with reports varying from about 0.25 MPa to 57 MPa [71, 73-83], which could be partially due to the variation in the testing conditions and protocols employed in different studies. The cornea also shows significant viscoelastic properties [84], and exhibits anisotropic behavior that is consistent with the preferred orientation of stromal fibrils in the nasal-temporal and superior-inferior directions at the center cornea [85]. There are many factors and conditions that could affect and alter the corneal stiffness. It has been widely shown that the cornea is stiffened with aging [86, 87], which may be due to the increase in collagen fibril diameter with age [88] and, more importantly, the accumulation of nonenzymatic cross-linking between collagen fibrils [89]. The keratoconus corneas have been reported to be substantially weaker than normal corneas [76], although inconsistent findings were also reported [90]. A recent therapeutic procedure for keratoconus corneas employing
riboflavin and UVA irradiation to induce cross-linking has been reported to significantly stiffen the corneas [91, 92]. There are also some other conditions, such as swelling [53], ectasia [90], and wound healing [67], which could alter the corneal mechanical properties.

Previous studies of corneal stiffness employed uniaxial tensile strip tests extensively [66, 81, 91, 93-96]. For tensile tests, the cornea is usually cut into rectangular strips and clamped between two mechanical grips, from which the tensile loading is applied. Both the load (stress) and deformation (strain) are recorded and the mechanical properties of the sample can thus be calculated by knowing the dimensions of the sample. The tensile test is intrinsically a destructive method which requires cutting corneal strips out, and the uniaxial loading applied may not represent the in vivo loading conditions either. Inflation tests have been utilized to study the cornea mechanical properties at its natural configurations [71, 73]. During the inflation tests, the loading is applied through the fluid pressure inside, which is more physiological than that in the tensile tests. But it typically requires precise measurement of the tiny surface displacement and a mathematical model with simplified assumptions to derive the material properties. Ocular rigidity, defined as the change in intraocular pressure divided by the change in ocular volume, was first proposed by Friedenwald [97] and used to characterize the overall compliance of the ocular shell which however involves combined effects of both the cornea and the sclera. The usage of the ORA to measure corneal parameters (i.e., CH and CRF) related to corneal biomechanics has become quite popular in recent years [39]. The ORA measures the corneal response to applanation and indentation by a rapid air pulse, which is a convenient way of obtaining measurements in vivo. The CH represents the
absolute difference between the applanation pressure $P_1$ ($1^{\text{st}}$ applanation when air pressure increases) and $P_2$ ($2^{\text{nd}}$ applanation when air pressure drops). The CRF is derived from empirical analysis of the relationship between $P_1$, $P_2$, and CCT. However, the measured CH and CRF are indirectly related to corneal stiffness, and it has been reported that the CH tends to be lower on thinner corneas and higher IOPs [98, 99] and the CRF may also be affected by corneal thickness [99]. Recently, a supersonic shear imaging method has been developed to measure the shear wave propagation speed in cornea and thus infer the material properties [100]. There are some safety concerns associated with this method and its validity may still need further confirmation. It should also be acknowledged that aside from the experimental characterization of cornea biomechanics, there have been fiber-matrix biomechanical models [101, 102] developed to better understand the mechanical behavior of corneas. There have been approaches combining experimental and numerical models [103] proposed to reconstruct and characterize the corneal biomechanical properties.

Characterization of scleral biomechanical properties is important for understanding glaucomatous damage to the eye. The optic nerve head (ONH) is the principal site of damage in glaucomatous vision loss. It remains controversial whether the glaucomatous damage is the result of a direct mechanical failure or insufficient vascular perfusion [104, 105]. But it is generally believed that the mechanical environment of the ONH is critical in both mechanisms since it could not only induce direct mechanical loading and deformation on neuron cells [106], but also mediate the IOP-related blood flow and cellular responses [107]. Since the sclera is the major load-bearing tissue in the
eye and the ONH is confined by the peripapillary sclera, scleral mechanical properties could play an important role in affecting the mechanical environment of the ONH. The effect of sclera mechanical properties on ONH deformation has been studied through several computational models [13, 14, 108-110]. The motivation to study scleral biomechanics is not just limited to understanding the glaucomatous damage; the scleral biomechanics could also provide insights into another prevalent eye disease, myopia. Changes in sclera mechanical properties have been reported to be implicated in the myopia progression [111]. For example, sclera thinning and softening has been observed during the early development of myopia in tree shrew eyes [112]. In mammalian eyes developing myopia, the sclera has been reported to creep much faster under constant loading [113, 114]. The sclera mechanical factors involved in both glaucoma and myopia development might provide further insight into the reported association between both diseases [115].

The mechanical behavior of the sclera has been characterized as nonlinear [71], viscoelastic [116], heterogeneous [117, 118] and likely anisotropic [119, 120]. The stiffening of sclera with increased strain could be due to the fact that collagen fibers uncrimp and strengthen the sclera during the stretch [121], which is protective for the ocular shell to avoid severe deformation at high IOP loading. Downs et al.’s study demonstrated the quick stress relaxation of rabbit and monkey scleras when they were held under constant strain [116]. Fazio et al reported significant regional variation in posterior sclera strains under inflation with temporal and inferior quadrants being more compliant [118]. Recent microstructure studies of scleral collagen fibrils indicated a
preferred circumferential fiber alignment around the ONH [122, 123], which suggests a stiffer response along the circumferential direction than the meridian direction on peripapillary sclera. However, a bi-axial tensile test study did not show significant anisotropy on human sclera samples [124]. Theoretical simulations showed the accuracy of bi-axial tensile tests could be affected by the conditions of sample mounting during the test [125]. Recent studies using surface optical tracking of displacement and strain in combination with inverse reconstruction have suggested anisotropic fiber orientation and stiffness on peripapillary sclera on both monkey [119] and human eyes [120]. Similar to the cornea, there are also many conditions and factors that could alter the scleral mechanical properties, such as aging, collagen cross-linking, or diseases. Scleral stiffening with aging has been widely reported in monkey [121], mouse [126], and human eyes [120, 127]. Collagen cross-linking through UVA/riboflavin, glyceraldehyde, or glutaraldehyde treatment can significantly increase the scleral stiffness [128]. Monkey eyes with early glaucoma have been found to exhibit an increased relaxation time and higher equilibrium modulus [116]. It has also been reported that scleral tangent modulus could decrease with small chronic IOP elevation and increase with large chronic IOP elevations in monkey eyes [119]. In glaucoma eyes, there was a stiffer meridian response in the peripapillary sclera than the normal eyes, and some glaucoma eyes had slower circumferential creep rates [120]. It is still not well understood whether the difference between the glaucomatous eye and the normal eye serves as a baseline risk factor to axon damage, or it is secondary to the glaucomatous damage [120]. A recent study showed stiffer sclera could alter the susceptibility to experimental glaucoma and reduce retinal
ganglion cell loss in a mouse model [129], which may suggest a baseline risk factor with soft sclera. More studies are needed to elucidate the role of sclera mechanical properties in glaucoma. The sclera stiffness can be altered through treatment inducing collagen cross-linking [128], which could potentially be useful in the treatment of glaucoma if the sclera stiffness is identified as risk factor.

Similar to the studies on corneal biomechanics, the sclera mechanical properties have also been mostly characterized on dissected tissue specimens using uniaxial tensile test [87, 106, 127, 130-132]. Biaxial mechanical tests have been recently used on sclera samples to interrogate the potential anisotropy [124]. Compression tests on scleral strips have been occasionally used and the scleral compressive modulus was found to be much lower than the tensile modulus [133, 134]. In order to study regional variation or anisotropy of sclera at physiological loading conditions, inflation tests on intact scleral shells have been employed to investigate their mechanical response to fluid pressure elevation [71, 126, 135-137]. Surface optical tracking techniques, such as optical flow [135], digital image correlation [126, 136] and electronic speckle pattern interferometer (ESPI) [118, 138], were typically employed during the inflation tests to map the sclera surface displacements and strains. Inverse finite element modeling has been proposed to reconstruct the mechanical properties from the measured surface strains [138, 139]. The uniaxial tensile tests and biaxial tensile tests are both invasive measurements and are difficult if not impossible to implement for in vivo measurement of sclera stiffness. The inflation tests and surface optical tracking have also been limited to laboratory studies so
far and there is still lack of *in vivo* measurement techniques for assessing the scleral stiffness.

1.4 Ultrasound elastography

Ultrasound imaging is one of the standard clinical imaging modalities and has been widely used since late 50s. The intrinsic non-invasive and non-radioactive characteristic of the low energy ultrasound employed makes it favorable for many *in vivo* imaging applications, i.e., in cardiac imaging and fetal imaging [140]. Ultrasound is essentially a type of mechanical wave and its propagation is related to the mechanical properties of the medium (i.e., soft tissues). For example, the speed of sound is determined by the bulk modulus (the compressibility in volume) and the density of the medium. The reflectivity of ultrasound on the interface between two different types of media is affected by their difference in acoustic impedance, the product of the density and the speed of sound. The scattering of ultrasound in soft tissue is dependent on the microstructure of the tissue. Because these ultrasound related properties may be altered during some diseases progression and the backscattered ultrasound signals could thus reflect such change, there have been many studies that characterized tissue properties by analyzing the ultrasound signals obtained from the tissue, such as the intensity of the ultrasound echoes [141, 142] or spectral parameters [143]. The speed of sound in soft tissue is generally assumed to be about 1540 m/s in ultrasound imaging systems, but in reality it varies among different tissues. For example, the speed of sound in cornea is generally considered to be around 1640 m/s in clinical ultrasound pachymetry. The speed
of sound in different soft tissues has been extensively characterized [144-146], since it is not only critical in obtaining the accurate measurement of tissue dimensions from ultrasound images, but also provide information about the condition of the tissue, such as hydration [147], defect [148], or diseases [149].

Since 1990s, there has been an increasing interest on imaging soft tissue elasticity using ultrasound, and the techniques of ultrasound elastography has been proposed [150, 151]. In ultrasound elastography, ultrasound speckle tracking is typically employed to image the distributive displacements and strains of soft tissue in response to internal or external loading [151, 152]. The ultrasound speckles are the natural characteristics in the ultrasound images/signals caused by the local interference pattern of ultrasound scattering in soft tissue. Although the ultrasound speckles are related to the ultrastructure of tissue, they are difficult to be directly interpreted to infer the tissue structure. The ultrasound speckles are stable local intrinsic patterns that can sustained from scan to scan. The basic idea of ultrasound speckle tracking is to trace these patterns in ultrasound signals from one frame to the next so as to obtain the displacement field of the tissue under deformation. The strain can thus be calculated from the spatial gradient of the displacement field in the tissue [153]. The techniques employed in ultrasound speckle tracking include cross-correlation algorithms [151, 152], sum of absolute differences [154], or optic flow methods [155]. Based on the fact that the diseased tissue usually has altered stiffness [156], ultrasound elastography has been successfully applied to image pathologies in soft tissues including breast [156], heart [157], blood vessels [158], liver [159], kidney [160] and prostate [156, 160, 161]. The ways of inducing tissue
deformation generally fall into two categories: quasi-static compression [151, 152] and dynamic vibration [162, 163]. During static compression, the tissue elasticity is generally reflected by the static strain map obtained from ultrasound speckle tracking. In dynamic vibration, the tissue motion over time can be measured through ultrasound speckle tracking and the measurement of viscoelasticity is made feasible [164], or the stiffness (i.e., shear modulus) can also be directly inferred by mapping the propagation speed of shear wave [165].

The performance, i.e., the signal-to-noise ratio (SNR) of ultrasound elastography, can be analyzed theoretically and through simulations [166-170]. Varghese et al. proposed a theoretical framework for studying the SNR at different strain levels and used the term, strain filter, to indicate the ‘band-pass’ characteristics of the SNR vs. strain relationship [167, 170]. It has been shown that the SNR in the axial strain image is dependent on the strain levels. At very low strains, the SNR is relatively low since the signal (strain) is low as compared to the noise. While at very high strains, the SNR is also low due to the de-correlation noise induced by large deformation, which deteriorate the performance of ultrasound speckle tracking and introduces significant noise in the strain image. Therefore, there is an optimal range of strain for the ultrasound speckle tracking algorithm to be optimally applied. The relationship between the SNR and the strain can be affected by system characteristics (e.g., transducer center frequency and bandwidth), the signal-to-noise ratio in the raw radiofrequency (RF) signals, or the signal processing parameters (e.g., kernel size and overlap) during ultrasound speckle tracking [167, 170]. It is thus possible to choose appropriate transducers for specific applications or tune the
signal processing parameters for the optimal performance. There is a trade-off between the SNR in strain images and the resolution when changing the kernel size since larger kernel size could increase the SNR in strain images but deteriorate the resolution [166]. It is also noted that the SNR of the measured lateral strain is generally much worse than the SNR of the measured axial strain since there is lack of phase information in the lateral direction which is perpendicular to the ultrasound propagation [171]. Synthetic lateral phase has been proposed to improve the SNR in the lateral strain [172]. A least-square strain estimator has been shown to reduce the noise in strain maps when calculating the strain out of the displacement field [153].

With high frequency ultrasound, it is possible to obtain high-resolution ultrasound signals/images on the relatively thin ocular shell (i.e., the cornea and sclera). It has been demonstrated that the ultrasound elastography using high frequency ultrasound (i.e., 50MHz) could provide microscopic through-thickness compressive strain images on cornea [173]. The speckles within the sclera ultrasound are much more significant than those in the cornea. It is thus possible to use ultrasound elastography methods to image the scleral deformation under IOP elevation at high resolution.

1.5 Specific Aims

We hypothesize that the corneal and sclera mechanical properties play an important role in the pathogenesis and management of ocular diseases such as glaucoma. The corneal mechanical properties are important in both diagnosis and treatment of glaucoma. The sclera mechanical properties are crucial for understanding glaucomatous
damage and identifying potential glaucoma risk factors. As a non-invasive method of probing soft tissues, ultrasound is a promising technique that can help to broaden our knowledge about corneal and sclera biomechanical properties. Therefore, the specific aims of the current studies are as follows:

**Aim 1:** To test the hypothesis that the accuracy of the corneal thickness measurement by using ultrasound pachymeter is influenced by the variation in speed of sound in cornea. The objective was to examine the magnitude and variance of speed of sound in canine cornea models, and explore the potential correlation between the acoustic impedance and the speed of sound in cornea that may provide correction for corneal thickness measurement.

**Aim 2:** To test the hypothesis that IOP measurement by applanation tonometry is affected by corneal stiffness. The purpose of this study was to experimentally examine the effect of corneal modulus on applanation tonometry using a canine eye model, and to explore the potential correlation between the acoustic impedance and the applanation tonometry error for correction in IOP measurement.

**Aim 3:** To develop a non-invasive ultrasonic method to assess sclera mechanical response under IOP loadings. The purpose of this study was to develop the method, evaluate its performance through experiment and simulations, and to obtain initial measurement of through thickness strains on animal eye models (i.e., porcine eyes).

**Aim 4:** To examine the human sclera biomechanical response to IOP elevation using the ultrasound method developed. The purpose of this study was to measure the
through-thickness deformation of human sclera and to examine the potential anisotropy between the meridian and the circumferential directions around the ONH.

1.6 Outline

The contents of the following chapters are as follows:

Chapter 2 described the study that investigated the variance of speed of sound in cornea and the correlation between acoustic impedance and speed of sound. This work has been published in *Ultrasound in Medicine and Biology* [145]. This study used fresh canine eye model to measure the variance of the speed of sound in cornea. The acoustic impedance was measured using A-mode ultrasound and the correlation between the speed of sound and the acoustic impedance was examined for potential correction in speed of sound measurement.

Chapter 3 focused on examining the effect of corneal stiffness on intraocular pressure measurement. This work has been published in *Investigative Ophthalmology and Vision Science* [174, 175]. The study examined the GAT and Tonopen measurements on enucleated canine eyes, and compared them with the direct manometric measurements. The corneal stiffness was measured by uniaxial tensile tests and the acoustic impedance was measured through ultrasound method. The stiffness of cornea was also altered by collagen cross-linking and the effect of changing in stiffness and changing in IOP measurement was examined.

Chapter 4 describes the development of an ultrasound technique on the sclera to obtain sclera strain mapping non-invasively. This work has been published in *Journal of*
Biomechanical Engineering [176]. This study presented the theoretical analysis, experimental and simulation results to validate the method developed. Measurements on porcine scleras were also conducted and the sclera strains along the meridian scanning direction and the circumferential direction were compared.

Chapter 5 showed initial measurement of sclera strain imaging on human cadaver eyes using the ultrasound strain imaging method developed in the previous chapter. This study includes through thickness strain imaging on human sclera, analysis of potential through thickness heterogeneity in deformation and examination of the anisotropy of scleral response to IOP elevation along the meridian and the circumferential directions.
Chapter 2 Variation of speed of sound in cornea

The content discussed in this chapter has been published in *Ultrasound in Medicine and Biology* [145].

2.1 Introduction

Accurate measurement of central corneal thickness (CCT) has become clinically important because of its increasing role in glaucoma diagnosis and management. Many studies have documented a significant influence of CCT in the intraocular pressure (IOP) measurements using tonometric methods, where the measurement error could range from 0.2 – 1.4 mmHg for each 20 µm deviation of CCT from the population mean [22-26]. Thickness is an important parameter in corneal structural stiffness which contributes to the corneal resistance to applanation. More recently, CCT has been found an independent risk factor for glaucoma development [18, 54]. According to a currently-adopted clinical model for predicting glaucoma risk, a 20 µm thinner CCT could increase the risk by 3 points [61], equivalent to an increase of IOP from 23 mmHg to 26 mmHg. This is clinically significant because even a 1 mmHg increase in IOP was found to increase the hazard ratio for glaucoma progression by 11% [177]. These results emphasize the need for accurate determination of CCT.
CCT is measured by either acoustic or optical methods. Ultrasound pachymetry is considered the clinical gold standard and measures CCT based on the assumed speed of sound. Similar assumptions about refractive index are made for optical methods. The use of a uniform speed of sound or refractive index is believed to generally apply in the population. Nevertheless, the extent of the variance and potential CCT measurement errors are not well-understood. If the inter-subject variance was large enough to introduce a CCT measurement error in the range of 10’s of microns, it could have clinically significant implications to the risk profiling for glaucoma patients and suspects.

The variance of speed of sound in healthy human soft tissue was generally within ±5% [178]. If a ±5% variance in speed of sound is present in the human corneas, it will translate into an error as large as ±25 microns in ultrasound pachymetry readings for a 500 µm thick cornea. Silverman et al. reported a range of 1600 m/s to 1616 m/s for the speed of sound in eight fresh, non-swollen bovine corneas. These eyes were collected from animals in a commercial abattoir whose age was likely similar because it was the typical practice of commercial abattoirs to euthanize animals at a certain age. In addition, the sample size (n=8) was fairly small. To our best knowledge, few studies have examined the variance of speed of sound in fresh normal corneas in animals of a large age span.

Since the 1960s, measurements of speed of sound have been carried out in postmortem dissected human corneas [179-183]. Most of the studies reported a mean speed of sound in the range of 1553 to 1575 m/s, with one study reporting a mean value of 1639 m/s [181]. Clinical instruments typically assume a speed of sound at 1640 m/s,
which was motivated by achieving good agreement with optical methods [184]. The lower speed of sound values measured in human corneal buttons may result from substantial corneal swelling due to the contact of the corneas with saline prior to or during acoustic measurements. Postmortem human corneas are known to rapidly absorb water due to the dysfunctional endothelia [185]. Silverman et al. examined the effect of hydration on speed of sound in bovine corneas and reported a decrease from 1605 m/s in non-swollen corneas to 1563 m/s in fully-swollen corneas when corneal thickness increased from 970 µm to 1579 µm in average [147]. In addition, it is often the case that a period of time (typically ranging from 6 to 48 hours) has passed between the time of death and the time of eye recovery from human donors. Although the exact nature has not been determined, corneal tissue may have undergone some degradation during this period of time which may explain the difference between the speed of sound used in vivo and measured ex vivo. Therefore, it is important to understand the population variance of speed of sound in non-swollen, fresh corneas immediately recovered after death. This accounted for one motivation for using canine eyes in the present study. Because of their availability immediately after euthanasia, the canine eyes can be measured at their natural, non-swollen, minimally degraded state.

Speed of sound is related to acoustic impedance in that acoustic impedance is the product of speed of sound and density. Theoretically, if corneal density varied little across subjects, speed of sound will be linearly correlated with acoustic impedance with the density being the slope. If corneal density differs significantly across subjects, the correlation will be altered or no longer exist. Su et al.’s reported a small variance of
density directly measured in bovine corneas [186]. The animals were reported to be of similar age (about two years old). Currently, there is lack of experimental data detailing the relationship between corneal speed of sound, acoustic impedance, and density in normal eyes of a large age span.

The objective of the present study is to examine the magnitude and variance of speed of sound in fresh, normal canine corneas. A secondary goal is to explore the relationship between corneal speed of sound and acoustic impedance. Canine corneas were used in this study not only because of their availability within a short postmortem time from animals of a large age span but also because of their similarity to human corneas. According to our initial tests and reported data [73], the thickness and biomechanical properties (i.e., modulus) of canine corneas are close to those of human’s, making it a good animal model prior to extensive studies in less available human donor eyes.

2.2 Materials and Methods

2.2.1 Sample preparation

Thirty four fresh canine globes were collected immediately after euthanasia from healthy dogs that were humanely euthanized for population control purposes at a local animal shelter. The use of the biological specimens from the animal shelter was approved by the university's Institutional Animal Care and Use Committee (IACUC). Age was recorded based on birthdates or estimated by experienced veterinarians based on anatomical information such as that of teeth for those without birth records. The animals
were categorized into three age groups: puppy (below one year old, six dogs), young (one to five years old, four dogs), and old (above five years old, four dogs). Three dogs in this study were undetermined in age and not included in the analyses related to age effects.

Corneal epithelia were carefully removed using a surgical blade before all measurements to reduce the effect of potentially degrading epithelial integrity over time commonly seen in postmortem eyes. This manipulation was feasible according to our preliminary tests that showed minimal alterations in the acoustic reflections at the ultrasound frequencies used in this study comparing the signals from corneas initially with intact epithelium and then after epithelial removal. CCT was first measured in the globes using an ultrasound pachymeter (DGH-550 PACHETTE 2, DGH Technology, Inc., Exton, PA). Three measurements were recorded and the average was used for further analysis. All measurements were completed within two hours postmortem at room temperature (21±1°C).

2.2.2 Acoustic impedance measurement

Corneal acoustic impedance was first measured in the intact globe, while maintaining an IOP around 15 mmHg using a saline column connected to the vitreous chamber through a needle (Figure 2.1). The globe was immersed briefly in a saline bath during the acoustic measurements. An unfocused ultrasound transducer (XMS-310, Panametrics-NDT, Waltham, MA) with an element size of 2 mm was used. The nominal frequency of the transducer was 10 MHz and the -6dB bandwidth was 8.2 MHz (5.6 to 13.8 MHz). Based on the nominal frequency, the calculated near field distance in saline
at room temperature was 6.7 mm and the half angle spread between -6 dB points was 0.0386 (2.2°). The calibration sample and the corneas were placed at a distance of 7.5 mm from the transducer surface during measurements. Short-duration ultrasonic pulses were emitted along the optical axis of the eye and the reflected ultrasound signals were collected by the transducer. The position of the transducer was adjusted by using precision linear stages (1 μm step size; Newport, Irvine, CA) to ensure the accurate alignment of the transducer with respect to the cornea by maximizing the amplitude of the reflected signals. Ultrasound reflections were sampled by a digitizer (500MHz/8-bit; DP105; Acqiris, Monroe, NY) and stored in a computer for further analysis. The duration of the immersion in saline for each globe was less than 2 minutes to prevent corneal swelling.

Figure 2.1 System and setup for measurement of corneal acoustic impedance.
The acoustic impedance of the cornea was determined from the amplitude of the ultrasound reflections, based on the following equation:

\[ R = \frac{A_s}{A_0} = \frac{Z_c - Z_w}{Z_c + Z_w}, \]  

(2.1)

where \( R \) is the reflection coefficient, \( A_s \) is the amplitude of reflected ultrasound signal, \( A_0 \) is the amplitude of incident ultrasound signal, \( Z_w \) is the acoustic impedance of the coupling media (i.e., saline or aqueous humor), and \( Z_c \) is the acoustic impedance of the cornea. A flat calibration sample (Soflens-59, Bausch & Lomb, Rochester, NY) with experimentally determined speed of sound and density was used to calibrate the amplitude of the incident ultrasound beam \( A_0 \). The speed of sound of the calibration sample was measured at room temperature using the substitution method. The density was measured in the bulk material using volume displacement based on Archimedes’ principle. The pulser-receiver settings were identical during the calibration and the corneal measurements. The anterior reflection of the cornea, well separated from the posterior reflection in time, was used as \( A_s \) for consistency with the calibration. The acoustic impedance calculated from the anterior reflection was used to represent that of the entire cornea because no internal reflections were detected at the ultrasound frequencies used in this study indicating minimal changes in acoustic impedance within the corneal stroma.

The measured amplitudes were all adjusted for corneal curvature effects. A flat steel plate and six solid steel spheres of various radii (McMaster-Carr, Aurora, OH) were used to characterize the curvature effect on the reflection amplitudes. The experimental setup for the transducer and the pulser/receiver was the same as used in measuring
corneas with one exception that the receiver attenuation was increased to avoid signal saturation. Both the plate and the spheres were made of the same material (E521000 steel alloy) and all surfaces were polished. The radii of curvature of the spheres were 4, 6, 7, 8, 9, and 10.32 mm (or 13/16") with a diameter tolerance of ±.0001". Each sample was placed at the distance of 7.5 mm to the transducer surface. The orientation of the sample and the transducer were adjusted for optimal alignment as in the measurement of corneas. Three measurements were taken from each sample and the average was used for further analysis. The curvature coefficient for each radius was determined by dividing the reflection amplitude from the sphere with that from the flat plate. Our results showed a strong linear effect of radius of curvature on the reflection amplitude (Figure 2.2). Based on the linear regression found from the experimental data, a coefficient of 0.416 was used for correcting the curvature effect of an average canine eye with a radius of curvature of 8.5 mm [187].
2.2.3 Speed of sound measurement

After completion of the acoustic impedance measurements, corneal buttons with a 3-mm-wide ring of sclera were dissected immediately. The corneal lamellae were kept intact to minimize potential changes in corneal properties. The speed of sound was measured by using the substitution method which has been described in detail elsewhere [147, 188]. Briefly, a plastic plate was used as a flat reflector. Two sets of ultrasonic reflections were acquired with and without the cornea intervening (shown in Figure 2.3). The transducer and the plastic reflector were maintained at the same position for both measurements.

![Figure 2.2 Curvature coefficient (ratio of the reflection amplitude from a curved surface against that from a flat surface) as a function of radius of curvature.](image)
Figure 2.3 Substitution method for measuring corneal speed of sound. (a) Ultrasound measurements with cornea intervening; (b) ultrasound measurements without cornea intervening. (t1 is the time-of-flight for the ultrasound reflection from the anterior cornea; t2 is the time-of-flight for the ultrasound reflection from the posterior cornea; t3 is the time-of-flight for the ultrasound reflection from the flat reflector with the cornea intervening; and t4 is the time-of-flight for the ultrasound reflection from the reflector without the cornea intervening). The dimensions (except the distance from the transducer to the cornea) were drawn in approximate proportion to the actual size.

The envelop of the ultrasound radiofrequency signals was defined using the analytic signal magnitude [147, 189] and peaks corresponding to tissue interfaces and reflector interfaces were detected. The distances between the peaks were used for calculating the time-of-flight (t1, t2, t3, and t4, as described in Figure 2.3). The speed of sound in cornea (Vc) was calculated from the measured time-of-flight and the speed of sound in saline (Vs saline) according to:
where $t_1$ is the time-of-flight for the ultrasound reflection from the anterior cornea; $t_2$ is the time-of-flight for the ultrasound reflection from the posterior cornea; $t_3$ is the time-of-flight for the ultrasound reflection from the flat reflector with the cornea button intervening; and $t_4$ is the time-of-flight for the ultrasound reflection from the reflector without the cornea intervening. The speed of sound in saline ($V_{\text{saline}}$) was determined by dividing a measured distance between the transducer and the reflector by the time-of-flight for ultrasound to travel in saline. The distance was determined by the displacement of the transducer controlled by a precision linear stage (1 μm resolution; Newport, Irvine, CA). Three different distances (5, 10, and 15 mm) were used and each measurement was repeated three times. A value of 1501.4±1.8 m/s for saline speed of sound at room temperature was obtained in this study, which was slightly higher than the published value of 1495.4 m/s at 21°C [190]. The substitution method was also repeated in 21 globes to determine the repeatability of speed of sound measurement. Repeatability expressed as the coefficient of variation (COV), which is the ratio of the standard deviation to the mean expressed as a percentage, was 0.25 ± 0.20% (range: 0-0.7%). This level of repeatability was consistent with previous reports [180].

2.2.4 Data Analysis

Density was calculated by dividing the measured acoustic impedance with the measured speed of sound. CCT, speed of sound, density, and acoustic impedance were
described as mean ± SD with 95% confidence intervals. Pearson’s correlation between corneal speed of sound and acoustic impedance, between density and speed of sound, and between speed of sound and CCT were calculated (SAS software, ver. 9.1; SAS Institute Inc., Cary, NC). The differences in corneal speed of sound between the three age groups were evaluated using two-sample t-tests.

2.3 Results

The mean speed of sound in the thirty-four canine corneas (seventeen pairs) was 1577 ± 10 m/s (range: 1553 to 1594 m/s). The mean acoustic impedance was 1.71 ± 0.03 MPa•s/m (range: 1.63 to 1.77 MPa•s/m). The ultrasound pachymetry readings of the CCT (assuming speed of sound = 1640 m/s) was 568 ± 68 μm (range: 451 μm to 720 μm). If we “correct” the CCT based on the measured speed of sound for each individual cornea, the CCT was 547 ± 68 μm (range: 431 μm to 695 μm). The corrected CCT was smaller in all eyes compared to the ultrasound pachymetry readings because the measured speed of sound was also smaller than the assumed value in all eyes.

A strong linear correlation was observed between the acoustic impedance and the speed of sound in the canine corneas (R = 0.84, P < 0.001). Figure 2.4 shows the scatter-plot of speed of sound versus acoustic impedance. The linear regression line is: Y = 1083X, where Y the acoustic impedance (in Pa•s/m) and X is the speed of sound (in m/s). The dashed lines show the 95% confidence interval of the fitted line.
Figure 2.4 Correlation between acoustic impedance and speed of sound in fresh canine corneas (solid line: regression line; dashed line: 95% confidence interval).

The density values calculated from the measured speed of sound and acoustic impedance showed a mean value of 1.083 g/cm³ with a range from 1.042 to 1.112 g/cm³. There was also a positive correlation between speed of sound and density (R = 0.69, P < 0.001; Figure 2.5).
Figure 2.5 Correlation between speed of sound and density in fresh canine corneas.

The Pearson correlation between speed of sound and the ultrasound pachymetry measured CCT from the intact globe was 0.49 (P=0.003). Since the ultrasound pachymetry assumed a uniform speed of sound of 1640 m/s, the CCT readings were recalculated using the speed of sound measured for each cornea. The Pearson correlation between speed of sound and the corrected CCT was 0.53 (P=0.001). Figure 2.6 shows the scatter plot of corneal speed of sound versus the corrected pachymetry readings.
Figure 2.6 Scatter plot of speed of sound and corrected ultrasound pachymetry readings showing a general positive correlation between these two parameters.

The corneas were also randomly assigned into two groups so that each group contained one eye from each animal. The comparison of speed of sound between the two corneas of the same dog is shown in Figure 2.7. A strong correlation was observed (R = 0.89, P<0.001).
The difference in speed of sound between the puppy group and the other two groups was statistically significant (P<0.001, two-sample t-tests). There was no significant difference in speed of sound between the young and the old groups (P>0.05, two-sample t-test). Figure 2.8 shows the comparison of speed of sound and acoustic impedance in all three groups. The puppy group occupied the lower left quadrant (lower speed of sound and lower acoustic impedance), while the young and old groups overlapped in the upper, right quadrant. The highest speed of sound and the highest acoustic impedance were found in the oldest animal in the present study (10 years old). The average speed of sound in these three age groups was $1567 \pm 9$ m/s, $1582 \pm 4$ m/s,
and 1585 ± 4 m/s, respectively; and the corresponding acoustic impedance was 1.68 ± 0.03 MPa•s/m, 1.72 ± 0.01 MPa•s/m, and 1.74 ± 0.02 MPa•s/m, respectively.

Figure 2.8 Scatter plot of acoustic impedance and speed of sound in groups of different ages (Puppy: less than one year old; Young: one to five years old; Old: above five years old.)

2.4 Discussion

The CCT (568 ± 68 μm) measured with a clinical pachymeter was comparable to those reported from in vivo studies in canine eyes [191, 192] indicating minimal swelling of the corneas. The speed of sound in the canine corneas, 1577 ± 10 m/s, was similar to that in porcine corneas [94, 180, 182, 188], bovine corneas [147, 193], and
human corneas measured postmortem [179, 180, 182, 183]. This value was lower than what is assumed for human corneas for in vivo measurements, even if the temperature effect is corrected for (the measurements were performed at room temperature while in vivo measurements are done at body temperature) [182]. We performed a preliminary examination on the temperature dependence of corneal speed of sound in four canine corneas. When temperature varied from 20°C to 34°C, the speed of sound increased in average by 14 m/s indicating a 1 m s⁻¹ °C⁻¹ effect similar to that reported for human crystalline lens [194]. This temperature effect would predict a mean speed of sound around 1590 m/s at body temperature (34°C) for canine corneas, which was still significantly lower than that assumed for human corneas in clinical ultrasound pachymetry. If the speed of sound value used in clinical ultrasound pachymetry is correct for human corneas in vivo, the lower values measured in the fresh canine eyes may indicate species difference in this parameter given that the postmortem changes were likely small.

The standard deviation of speed of sound in the current study was less than 1% of the mean value, indicating generally small variance of this parameter in normal canine corneas. This result favors the assumption of a single value in measuring normal eyes. If we consider the minimal and maximal values, the CCT measurement error would be less than 10 μm for a 500 μm cornea assuming the readings were calibrated against mean speed of sound. However, if the calibration of the ultrasound pachymeters deviated significantly from the true speed of sound, there could be systemic error in CCT measurements. Additionally, future studies are needed to examine corneal speed of sound
in eyes with pathologies to evaluate whether there are significant changes in the diseased conditions. Pathological conditions may change tissue acoustic properties because of altered microstructure and mechanical properties [144, 195, 196]. It was shown in the past that the discrepancy between ultrasound pachymetry and optical measurements of corneal thickness became more pronounced in keratoconic [197, 198], edematous [199], and post-LASIK corneas [200, 201], indicating a potential alteration in speed of sound, refractive index, or both.

There appeared to be a trend of slower speed of sound in thinner corneas, but with a weak correlation (R = 0.53, P < 0.01, Figure 2.6). Age appears to be a confounding factor for this correlation. For example, we observed generally thin corneas with low speed of sound in canines less than one year old, whose corneas may have not fully grown and still contain immature collagen fibers. There were a few older canine eyes that had thin corneas but without a corresponding low speed of sound. Therefore, the association between corneal thickness and speed of sound may be explained by the age influence on both parameters. We also observed a strong correlation between the two eyes of the same dog, indicating the microstructure and composition of the non-pathological corneas could be subject to the same factors in the same individual.

A potentially significant effect of age on speed of sound was noted in the studied animals. The puppy canines had significantly lower speed of sound as compared to the older canines. The age effect however may not be linear across all ages and the speed of sound may plateau after a certain age, because there was no significant difference between the young and the old groups. A possible explanation for this observation is the
age-associated changes in corneal microstructure including collagen fiber maturation [202] and increase of corneal cross-linking [89]. Both of these microstructural changes could affect acoustic parameters such as speed of sound and acoustic impedance. The increase of total collagen content and cross-linking leads to an increase in corneal stiffness [86]. We previously reported a correlation between corneal stiffness (i.e., Young’s modulus measured by uniaxial tensile tests) and acoustic impedance in fresh canine corneas [96]. In another study, we observed a significant increase in speed of sound due to riboflavin-UVA cross-linking in porcine corneas [203]. These results are consistent with the findings in the current study, indicating speed of sound may increase with corneal stiffness.

We observed a strong correlation between acoustic impedance and speed of sound in normal canine corneas. The standard engineering approaches for determining speed of sound such as the substitution method are not applicable in vivo. The finding of a strong linear correlation between acoustic impedance and corneal speed of sound may suggest an approach to correct the speed of sound setting in clinical ultrasound pachymeters based on the evaluation of the acoustic impedance, which can be measured by the same device with additional signal processing software. Abnormal acoustic impedance (either too small or too large) may serve as an indicator for abnormal speed of sound and alert the physician to potential inaccuracy in corneal thickness readings. It is noted that the present study assumed a homogeneous cornea with the speed of sound and acoustic impedance unchanged throughout the thickness. Some pathological conditions may induce differential changes in the anterior and posterior cornea [204] so these parameters
may exhibit more complicated patterns of variation through thickness, which warrants future studies.

Corneal density was calculated to explore whether this parameter was constant in the measured samples which may have underlined the strong correlation between speed of sound and acoustic impedance. The calculated canine corneal density (1.083±0.02 g/cm$^3$) appeared to be higher than bovine corneal density (1.06±0.004 g/cm$^3$) reported in Su et al’s study [186], but within the range of the published data for porcine, bovine, and human corneas [94, 186, 205]. The agreement between our study and previous reports suggested the reliability of the acoustic impedance measurement whose accuracy was important for the density estimation. Our study revealed a slightly larger variance in density. This was likely due to the larger age span in the studied animals. In Su et al’s study, the animals were all about two years old and also their study used bovine eyes which may differ from canine eyes. The present study included animals from weeks to about 10 years old spanning almost the entire age range of canines. As discussed before, age influences corneal microstructure, and therefore likely has an effect on density as well. Furthermore, the interesting correlation between speed of sound and density in the cornea may be related to the collagen-dominant structure of the cornea. Conceivably, a higher density and a higher speed of sound may both be an outcome of a greater concentration of collagen. Our results indicated that the density was likely not constant across all corneas. A limitation of the present study was that the density was calculated rather than directly measured. The density for an individual cornea is difficult to accurately determine because of its small volume and the necessity for storage in
solutions [186]. The cornea also has a strong tendency to absorb water when the stroma is dissected and exposed to aqueous solutions [206], which could alter density during measurements involving immersion. These issues likely result in experimental errors reaching the level of the sample-to-sample difference [188] and thus affect the ability to detect its correlations with other parameters.

2.5 Summary

In summary, we found that speed of sound varied to a noticeable extent in healthy canine corneas and was significantly lower in canines less than one year old. The strong correlation between speed of sound and acoustic impedance may be exploited to non-invasively detect abnormal speed of sound in healthy corneas for more accurate determination of corneal thickness. Future studies are needed to understand how ocular pathologies may further alter corneal speed of sound.
Chapter 3 Effect of Corneal Stiffness in IOP measurements

The content discussed in this chapter has been published in *Investigative Ophthalmology and Vision Science* [174, 175].

3.1 Introduction

Intraocular pressure (IOP) is of fundamental importance in the management of glaucoma. Lowering IOP has long been the mainstay of glaucoma treatment, and accurate measurement of IOP is thus important.

It is known that the measurement of the current clinical standard, Goldmann applanation tonometry (GAT), could deviate substantially from the true IOP depending on corneal properties. The potential inaccuracy is intrinsic to the design of the Goldmann tonometer, which assumes human corneas to be perfectly extensible (i.e., no resistance to deformation), infinitely thin, and completely dry [207]. Variations of corneal thickness and stiffness could introduce clinically significant errors in IOP readings. Experimental and clinical data has clarified a positive correlation between central corneal thickness (CCT) and measured IOP. The measurement error was however inconsistently reported, with values ranging from 0.11 to 0.71 mmHg per 10 μm deviation from the population mean of central corneal thickness [22-26]. The difference in the correction algorithms may be related to different experimental conditions in the studies; however, it is generally
acknowledged that corrections based solely on central corneal thickness are not sufficient because there are other influential confounding factors in IOP measurements, most notably corneal stiffness. Theoretical analysis suggested that the variance in corneal stiffness could potentially introduce larger errors in tonometric measurements [27]; moreover, different corneal stiffness would result in different “slopes” necessary for correcting the effect of central corneal thickness [27].

Tonopen is a handheld instrument that measures IOP based on the MacKay-Marg principle. In one study, tonopen was found to be accurate for corneas with surface abnormality [46]. Other studies showed that tonopen was capable of producing comparable measurements with GAT on normal corneas [47-50]. The effect of CCT was reported to be less significant for Tonopen measurements compared to GAT [51]. The effect of corneal stiffness on Tonopen measurements has not been fully determined.

Tensile tests have been widely adopted in characterizing the mechanical properties of soft tissue including the cornea [84, 95, 208, 209]. Previous study has reported a correlation between GAT/Tonopen readings and corneal secant modulus measured by uniaxial tensile tests on canine corneas [210]. The purpose of the present study was to test the hypothesis that an altered corneal stiffness directly leads to a change in GAT and Tonopen readings. In addition, we explored the potential using of a non-invasive ultrasound method to obtain an estimate of corneal stiffness which may be used for correcting clinical measurements of tonometry.

Canine eyes were used in this study because their corneal thickness was close to that of human eyes. Other frequently used animal models including porcine or bovine
eyes have corneal thickness much greater than that of human’s. In addition, canine eyes can be obtained immediately after death and tested within hours to minimize swelling and other postmortem changes, which is important for preserving corneal properties close to the in vivo conditions. Canine eyes resemble human eyes in corneal thickness and radius of curvature although both parameters are slightly larger in the canine eye. The canine cornea has multi-layers including epithelium, stroma, Descemet’s membrane and endothelium, but the Bowman’s membrane found in the human cornea is absent from the canine cornea [3].

Corneal stiffening can be induced by corneal collagen cross-linking. Irradiation with ultraviolet light in the presence of photosensitizer has been reported to increase corneal stiffness [92, 211]. Corneal stiffening can also be achieved through chemical cross-linking using glutaraldehyde or other fixative agents [211-213]. In the present study, we chose to use glutaraldehyde treatment because it does not require the removal of corneal epithelium which was useful for obtaining reliable GAT measurements. Our preliminary tests showed that glutaraldehyde penetration was sufficient without the removal of corneal epithelium when it was co-administered with benzalkonium chloride (BAK), which is known to facilitate transepithelial transport [214]. Glutaraldehyde treatment also provides an adjustable experimental procedure to achieve the desired extent of stiffening [213].

Corneal stiffness was characterized first by an ultrasound method in the intact eye and then tensile tests in the dissected tissue strips. It has been shown that corneal acoustic impedance was correlated with corneal tangent or secant modulus [96]. The ultrasound
method was thus used in the present study as a non-invasive measurement of the corneal stiffness. Direct measurement of corneal secant modulus was also carried out on dissected corneal strips using uniaxial tensile tests.

3.2 Methods

3.2.1 Sample preparation

Twenty fresh canine globes were obtained within one hour postmortem from ten healthy dogs that were humanely euthanized for population control purposes at a local animal shelter. Globes from the same animal were randomly assigned into two groups: a control group and a corneal stiffening group. The eyes in the corneal stiffening group were treated with corneal immersion in a phosphate buffered saline (PBS) solution with 1% glutaraldehyde and 0.025% BAK for 1 hour. The glutaraldehyde concentration and the immersion time were chosen to induce a desired level of corneal stiffening based on initial tests. The sclera was not in contact with the solution during corneal treatment. Wet gauze was wrapped around the sclera to maintain its hydration during corneal treatment. For the control group, the corneas were immersed in PBS for 1 hour. CCT was measured using an ultrasound pachymeter (DGH-550 PACHETTE 2, DGH Technology, Inc., Exton, PA) prior to and after the treatment. CCT was also checked after ultrasonic measurements and tonometric measurements. Three readings were recorded and the average was used for further analysis. Corneal stiffness was measured by using the ultrasound method before and after treatment in each eye. GAT and Tonopen measurements were collected after treatment at different controlled IOP levels. Uniaxial
tensile tests were conducted on corneal strips prepared from all globes after the completions of the measurements mentioned above. All measurements were completed within 8 hours postmortem. Details of the ultrasound measurements, tonometric measurements, and tensile tests are elaborated below.

3.2.2 Acoustic impedance measurements

Corneal acoustic impedance was measured in the intact globe before and after treatment for both control group and corneal stiffening groups, following the methods described in previous chapter and published paper [96]. Briefly, the globe was immersed in a saline bath and an unfocused ultrasound transducer (XMS-310, Panametrics-NDT, Waltham, MA) was used to measure the ultrasonic reflection from the cornea. Short-duration ultrasonic pulses were emitted along the optical axis of the eye and the reflected ultrasound signals were collected by the transducer. The position of the transducer was adjusted by using precision linear stages (1 μm step size; Newport, Irvine, CA) to ensure accurate alignment of the transducer with respect to the cornea by maximizing the amplitude of the reflected signals. The reflections were sampled by a digitizer (500MHz/8-bit; DP105; Acqiris, Monroe, NY) and stored for further analysis. The acoustic impedance of the cornea was determined from the amplitude of the ultrasound reflections from the cornea and the ultrasound reflection measured from a calibration sample (Soflens-59, Bausch & Lomb, Rochester, NY) with known acoustic properties, based on the following equation:

\[ R = \frac{A_c}{A_0} = \frac{Z_c - Z_w}{Z_c + Z_w}, \] (3.1)
where \( R \) is the reflection coefficient, \( A_s \) is the amplitude of the reflected ultrasound signal, \( A_0 \) is the amplitude of the incident ultrasound signal, \( Z_w \) is the acoustic impedance of the coupling media (i.e., saline or aqueous humor), and \( Z_c \) is the acoustic impedance of the cornea. The amplitude of the ultrasonic reflection was adjusted for the curvature effect as described in previous chapter and a previous study [215].

3.2.3 \textit{Goldmann applanation tonometry (GAT) and Tonopen measurements}

GAT measurements were performed in the treated globes using an experimental setup as in Figure 3.1. Similar measurement setup on \textit{ex vivo} globes has been described previously [33, 210, 216]. Briefly, the globe was placed in a holder padded with moistened gauze. The holder was affixed to a plastic plate that was vertically mounted on the headset of a standard slit lamp (Topcon, Oakland, NJ). A 22G needle was inserted into the anterior chamber of the globe from the limbus. Through the needle and a tubing system, the anterior chamber was connected to a saline column which controlled the intraocular pressure and also a pressure sensor (Omega Px154, Omega Engineering Inc., Stamford, CT) which monitored the IOP in real time. The pressure was first set to 10 mmHg by adjusting the height of the saline column and confirmed by the pressure sensor readings. A drop of fluorescein solution (Fluorox, Altaire Pharmaceuticals, Inc., Aquebogue, NY) was gently spread onto the cornea surface using a cotton tip. Fluorescein was used to visualize the Goldmann mires for slit lamp examination, as in the clinical measurements. IOP was measured by using a Goldmann tonometer (AT900, Haag Streit, Switzerland) and the slit lamp according to the standard protocol. The
Goldmann tonometer tip was then temporarily moved away from the cornea for Tonopen measurements (Tono-pen XL, Reichert, Inc.) under the same pressure setting. Both GAT and Tonopen were calibrated prior to experiments. The intraocular pressure was adjusted to the levels of 15 mmHg, 20 mmHg, 30 mmHg, and 40 mmHg. The corresponding pressure sensor readings and the GAT and Tonopen measurements were recorded. Each pressure measurement was repeated three times and the average was used for further analysis.

Figure 3.1 The schematics of the experimental set-up for GAT measurements.
3.2.4 Uniaxial tensile test of corneal strips

After completing the pressure measurements, the globes in both control group and cross-linking group were dissected and cornea strips (3.5mm by 18mm) were prepared along the nasal-temporal direction. Uniaxial tensile tests were performed on the corneal strips using a Rheometrics System Analyzer III (RSA-III, New Castle, DE) with a displacement resolution of 0.05 μm and a force resolution of 20 μN. The tissue strip was coupled between a motor and a transducer that measures the resultant force generated by sample deformation. The initial sample length between the two gripping jaws was approximately 10 mm. Sample width and thickness were measured by using a high resolution ultrasound imaging system (Vevo660, VisualSonics Inc., Toronto) and input into the RSA control panel. A 55-MHz ultrasound scanning probe was used with an axial resolution of 30 μm and a lateral resolution of 62.5 μm. A pre-load of 20 mN was applied to each sample to precondition and flatten the tissue. The sample was then subject to a constant strain rate of 0.1% per second until strain reached about 6%. The stress-strain data were stored on the hard disc of the computer for further processing. The strain rate was selected from the typical values used in the literature [94, 211, 217]. Secant modulus at 1% strain was calculated and used as the stiffness measure based on tensile tests in further analysis.

3.2.5 Statistical analysis

The CCT and acoustic impedance were summarized before and after the treatment for the control group and the corneal stiffening group. The after-treatment tensile
modulus was summarized for both groups. The changes in CCT and corneal stiffness were tested using paired t-test for each group and the difference between the two groups was tested using a mixed model to account for the dependency among observations in the same eye. GAT and Tonopen measurements were summarized as Mean ± SD at each IOP level (10, 15, 20, 30, or 40 mmHg) for each group. The differences in tonometric measurements were tested using a mixed model to account for the dependency of the measurements in the same eye. In both the control group and the corneal stiffening group, the correlations between GAT/Tonopen measurement and the corneal stiffness measurement (i.e., secant modulus or acoustic impedance) were evaluated at each IOP level by using Pearson correlation coefficients. The influence of CCT on the tonometric measurements was also explored using Pearson correlation at different IOP levels. SAS (Version 9.12, SAS Institute Inc., North Carolina) was used for all data analysis.

3.3 Results

The mean CCT in the control group was 663.2 ± 53.7 µm before all measurements and 693.1 ± 47.9 µm after 1 hour immersion in PBS. The mean CCT in the corneal stiffening group was 659.9 ± 63.3 µm and 674.2 ± 49.9 µm, respectively. There was a small but statistically significant increase in CCT after treatment in both groups (p’s < 0.05, paired t-test). No statistically significant difference was found in CCT between the control and the corneal stiffening group at baseline or after treatment (p = 0.90 and 0.40, two-sample t-test).
The acoustic impedance (AI) in the control group was $1.71 \pm 0.02$ MPa•s/m at baseline and $1.71 \pm 0.01$ MPa•s/m after 1 hour immersion in PBS. The AI in the corneal stiffening group before and after treatment were $1.70 \pm 0.02$ MPa•s/m and $1.74 \pm 0.02$ MPa•s/m, respectively. No statistically significant difference was found in AI between the control and the corneal stiffening groups at baseline ($p = 0.76$, two-sample t-test). No significant change in acoustic impedance was found in the control group after PBS treatment ($p = 0.10$, paired t-test). Acoustic impedance increased significantly in the corneal stiffening group after treatment ($p < 0.001$, paired t-test), which was significantly higher than that in the post-treatment control group ($p < 0.01$, two-sample t-test).

The mean GAT and Tonopen readings corresponding to various manometric IOPs ($IOP_{Man}$) were summarized in Table 3.1. The corneal stiffening group had statistically higher IOP measurements than the control group for both GAT and Tonopen measurements at IOP levels of 10, 15, 20, 30, and 40 mmHg ($p < 0.001$). In the control group, both GAT and Tonopen underestimated the intraocular pressure while in the corneal stiffening group, both tonometric methods measured closer to the manometric pressure.
Table 3.1 GAT and Tonopen readings at various true IOP levels in canine eyes.

<table>
<thead>
<tr>
<th>IOP&lt;sub&gt;Man&lt;/sub&gt; (mmHg)</th>
<th>GAT (mmHg)</th>
<th>Tonopen (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=10)</td>
<td>Corneal Stiffening (n=10)</td>
</tr>
<tr>
<td>10</td>
<td>2.2 ± 1.0</td>
<td>10.1 ± 3.6</td>
</tr>
<tr>
<td>15</td>
<td>6.8 ± 1.4</td>
<td>15.6 ± 4.1</td>
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<td>21.1 ± 5.6</td>
</tr>
<tr>
<td>30</td>
<td>20.3 ± 1.5</td>
<td>30.7 ± 5.3</td>
</tr>
<tr>
<td>40</td>
<td>29.0 ± 1.8</td>
<td>40.7 ± 6.0</td>
</tr>
</tbody>
</table>

The stress-strain curves obtained from uniaxial tensile tests were plotted in Figure 3.2. Both groups showed typical nonlinearity. Secant modulus was calculated as the ratio of stress and strain at a given strain level. The mean secant modulus at 1% strain was 1.72 ± 0.41 MPa in the control group and 3.03 ± 0.61 MPa in the corneal stiffening group. The difference in secant modulus was statistically significant (p < 0.001).
The correlation between the GAT/Tonopen readings and the stiffness measurements was explored in the combined data set of the control and the corneal-stiffened eyes. A significant positive correlation was found between the GAT readings and the acoustic impedance at each pressure level (Figure 3.3, R = 0.77, 0.77, 0.72, 0.73, and 0.77 for IOP_{Man} = 10, 15, 20, 30, and 40 mmHg, p’s < 0.001). The slopes of the linear regression between GAT and acoustic impedance were 160.9, 180.3, 200.6, 209.8, and 247.2 mmHg/(MPa•s/m) at 10, 15, 20, 30, and 40 mmHg, respectively. Significant correlations were also found between the Tonopen readings and the acoustic impedance at all pressure levels (Figure 3.4, R = 0.70, 0.69, 0.69, 0.79, and 0.60 for IOP_{Man} = 10, 15, 20, 30, and 40 mmHg; p’s < 0.01). The slopes of the linear regression between Tonopen readings and acoustic impedance were smaller than the corresponding slopes between

**Figure 3.2** The average stress-strain relationship for control and corneal stiffening groups.
GAT and acoustic impedance, with values of 116.1, 108.3, 103.7, 135.1, and 115.1 mmHg/(MPa•s/m) at 10, 15, 20, 30, and 40 mmHg.

Figure 3.3 Scatter plots of GAT readings versus post-treatment corneal acoustic impedance at different levels of IOPMan (■: corneal stiffening; ▲: control).
Figure 3.4 Scatter plots of Tonopen readings versus post-treatment corneal acoustic impedance at different levels of IOPMan (■: corneal stiffening; ▲: control).

The GAT readings were found to be significantly correlated with the secant modulus (Figure 3.5, R = 0.87, 0.89, 0.83, 0.85, and 0.86 for IOP\textsubscript{man} = 10, 15, 20, 30, and 40 mmHg, p’s < 0.001). The slopes of the linear regression between GAT and secant modulus were 4.9, 5.7, 6.3, 6.7, and 7.6 mmHg/MPa at 10, 15, 20, 30, and 40 mmHg. There was also a significant correlation between the Tonopen readings and the secant modulus...
modulus (Figure 3.6, R = 0.73, 0.78, 0.81, 0.82, and 0.75 for \( IOP_{\text{man}} = 10, 15, 20, 30, \) and 40 mmHg; p’s < 0.001). The slopes of the linear regression between Tonopen readings and secant modulus were smaller than the corresponding slopes between GAT and secant modulus, with values of 3.3, 3.3, 3.3, 3.8, and 3.9 mmHg/MPa at 10, 15, 20, 30, and 40 mmHg.

![Figure 3.5](image)

**Figure 3.5** Scatter plots of GAT readings versus post-treatment corneal secant modulus at 1% strain at different levels of \( IOP_{\text{man}} \) (■: corneal stiffening; ▲: control).
Figure 3.6 The scatter plots of Tonopen readings versus post-treatment corneal secant modulus at 1% strain at different levels of IOPMan ($\square$: corneal stiffening; $\triangle$: control).
The acoustic impedance was significantly correlated with the secant modulus in the measured canine corneas (Figure 3.7, R=0.80, p<0.001).

Figure 3.7 Linear correlation between post-treatment corneal acoustic impedance and secant modulus at 1% strain.

No correlation was found between the GAT readings and CCT (Figure 3.8, R=-0.02, 0.04, -0.01, 0.01, and 0.02, p=0.94, 0.86, 0.98, 0.96, and 0.93 for IOP\textsubscript{Man}=10, 15, 20, 30, and 40 mmHg, respectively). There was also no correlation between the Tonopen readings and CCT (Figure 3.9, R=0.11, 0.03, 0.01, 0.15, and 0.12, p=0.64, 0.89, 0.98, 0.52, and 0.6, respectively).
Figure 3.8 Scatter plots of GAT readings versus post-treatment CCT at different levels of IOPMan (■: corneal stiffening; ▲: control).
3.4  Discussion

The primary finding of the present study was that corneal stiffening significantly increased GAT and Tonopen readings in the canine eyes. In the normal canine eyes, GAT and Tonopen both underestimated IOP. For example, the average GAT reading was 6.8
mmHg at a true pressure of 15 mmHg and the underestimation was more pronounced at higher pressures. This level of underestimation by GAT was reported in a previous study on porcine eyes [216]. The potential factors underlying this large underestimation were discussed in our previous publication [210]. Primarily, GAT is calibrated to the dimensions and properties of the human eye. After corneal stiffening through chemical cross-linking, GAT reading was increased to 15.6 mmHg, considerably closer to the true pressure of 15mmHg. A similar increase in Tonopen readings was also found.

Uniaxial tensile tests showed that the glutaraldehyde-treated corneas had a significantly higher secant modulus than those in the control group (3.03 ± 0.61 MPa vs. 1.72 ± 0.41 MPa). In this study, we found the secant modulus in the control dog corneas (n = 10) was from 1.15 to 2.53 MPa. This range (i.e., 2.53 - 1.15 = 1.38 MPa) was comparable to the difference between the control and glutaraldehyde stiffened dog corneas (i.e., 3.03 - 1.72 = 1.31 MPa). Cartwright et al [218] reported that the stiffness of the human cornea doubled between the ages of 20 and 100 years. In the present study, glutaraldehyde treatment increased corneal stiffness by a factor of about 1.8, which was comparable to the age-related stiffening in human corneas. Our laboratory performed mechanical testing on several human corneas (n = 8, unpublished data) following the same protocol described in this paper and found that the 1% secant modulus ranged from 1.70 to 3.15 MPa (Table 3.2). Therefore, the glutaraldehyde treatment used in the present study appeared to induce a corneal stiffness change that is within the physiological variance of corneal stiffness. Future studies are needed to fully characterize the range of corneal stiffness in both normal and diseased human eyes.
Table 3.2 Secant moduli of 8 human cornea samples measured using uniaxial tensile test

<table>
<thead>
<tr>
<th>ID</th>
<th>1% Secant Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18337OS</td>
<td>1.70</td>
</tr>
<tr>
<td>18337OD</td>
<td>3.15</td>
</tr>
<tr>
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</tbody>
</table>

The ultrasound method also detected significantly higher acoustic impedance in the stiffened corneas. The strong correlation between corneal acoustic impedance and secant modulus (Figure 3.7), previously reported by our laboratory [96], was confirmed in the present study. Importantly, we found a strong correlation between acoustic impedance and GAT/Tonopen readings, suggesting that the measurement of acoustic impedance may serve as a non-invasive approach to estimate corneal stiffness and guide the clinical interpretation of IOP measurements using these tonometric methods. For instance, a higher than normal acoustic impedance measurement may suggest the possibility of overestimation of IOP using GAT. However, corneal stiffness alone is not sufficient in calibrating GAT or tonopen readings, because other parameters such as corneal thickness, curvature, as well as the interactions between corneal properties and the true pressure could all affect tonometric readings. It is noted that the linear regression slopes between acoustic impedance and tonometric errors were dependent on the true pressure. This pressure-dependence, reflecting the nonlinear nature of corneal
biomechanics, makes correction algorithms based on a single factor likely unattainable. More sophisticated algorithms incorporating several factors may be needed to predict true pressures from tonometric measurements. A possible approach could be to optimize the true pressure estimates from patient-specific computational models of clinical GAT measurement that incorporate nonlinear corneal responses and fit the predicted GAT readings, as well as corneal thickness, curvature and stiffness to the measured data.

Our results showed that an increase of corneal modulus from 1.72 to 3.03 MPa was associated with an increase of GAT readings from 6.8 to 15.6 mmHg (an 8.8 mmHg increase) when the true IOP was 15 mmHg. A previous analytical model suggested an increase of GAT readings by 17 mmHg when corneal modulus increased from 0.1 to 0.9 MPa [27]. The current experimental data agreed qualitatively with the theoretical prediction confirming that a clinically significant difference in GAT readings could result from plausible individual difference in corneal stiffness. The model however assumed linear isotropic mechanical properties of the cornea while in reality the cornea is nonlinearly viscoelastic and has strong anisotropy due to the preferential collagen fiber alignment in the direction parallel to the surface. These limitations of the analytical model may explain the quantitative difference in the predicted errors when compared with the experimental results found in this study. Future efforts are needed to develop theoretical models that can more accurately represent corneal biomechanical characteristics that are relevant in GAT measurements.

In the present study, no statistically significant correlation was observed between CCT and GAT/Tonopen readings at any pressure level (Figure 3.8 and Figure 3.9).
Although this result was possibly due to the small sample size, the undetectable correlation with CCT contrasted with the strong correlation with corneal stiffness and indicated that the effect of corneal stiffness may be greater than CCT within clinically relevant ranges of each parameter. Future studies are needed to further elucidate the effects of CCT and corneal stiffness on tonometric measurements of the human eye and the interactions of these parameters in a larger sample size.

Our results showed that Tonopen also underestimated IOP in control canine eyes but the underestimation was smaller compared to GAT. For example, at a true IOP of 15 mmHg, the average underestimation was 2.3 mmHg. This result was consistent with previous reports that Tonopen was relatively reliable for canines at normal pressure levels [219]. Our results also showed that Tonopen readings were significantly higher in the eyes with stiffened corneas, indicating the effect of corneal stiffness on Tonopen measurements. The smaller slopes of the linear regressions between Tonopen readings and secant modulus suggest that corneal stiffness may have less influence on Tonopen than GAT.

The limitations of the present study are as follows. First, the experimental setup in the present study adopted an “open stock” system to maintain the IOP level while in the living eye the applanation procedure may induce a significant IOP rise. The “open stock” system was helpful in studying the relationship between equilibrium pressures and their tonometric readings [34]. The results of this study therefore should be interpreted with the understanding of potentially more complicated processes in actual clinical situations [26]. Second, corneal curvature was not measured. Although it is unlikely that corneal
curvature was significantly different between the two groups before treatment, it could be
differentially altered during treatment. Previous analytical and numerical models
suggested that corneal curvature had a much smaller influence on tonometric readings
than corneal thickness and stiffness [27, 30]. Therefore, the main outcome of the present
study will likely remain the same if the information of the curvature is made available.
Third, canine eyes were used in the present study. Although sharing similar corneal
thickness, canine corneas differ from human corneas in microstructure and biomechanical
properties. Future studies are needed to investigate the effect of altered corneal stiffness
on tonometric errors in human eyes.

3.5 Summary

In summary, the current study found a significant increase in both GAT and
Tonopen measurements of IOP in canine eyes with stiffened corneas. These results
provide experimental evidence to support the hypothesis that corneal stiffness
significantly influences tonometric readings. The GAT/Tonopen errors appeared to be
correlated with the corneal tensile modulus. Corneal acoustic impedance may be used as
a surrogate for corneal tensile modulus and provide a noninvasive method for estimating
tonometric errors associated with abnormal corneal stiffness.
Chapter 4 Ultrasonic strain mapping on porcine sclera

The content discussed in this chapter has been published in *Journal of Biomechanical Engineering* [176].

4.1 Introduction

Characterization of scleral biomechanical properties is important for understanding prevalent ocular diseases such as glaucoma and myopia. The mechanical environment of the ONH is believed to be critical for retinal ganglion cell pathophysiology in glaucomatous damage [106]. Recent computational models have shown that scleral mechanical properties may play an important role in affecting the mechanical environment of the ONH [109, 110]. It has also been proposed that changes in sclera mechanical properties are implicated in myopia progression [111]. For example, scleral thinning and weakening have been reported in the early development of myopia in tree shrew eyes [112]. Increased scleral creep has also been reported in mammalian eyes developing myopia, particularly at the posterior pole of the eye [113, 114]. The understanding of scleral mechanical properties and their alterations may provide insight into the potential association between glaucoma and myopia [115].

The sclera is the major load-bearing tissue in the eye and consists primarily of collagen fibers, more than 90% of which are Type I [12]. Sclera mechanical properties
have been characterized on dissected tissue specimens using uniaxial [87, 106, 127, 130-132] or biaxial mechanical tests [124]. These studies have demonstrated that the mechanical behavior of the sclera is typically nonlinear, viscoelastic and anisotropic. Compression tests on scleral strips offered additional information of scleral biomechanics and revealed a much lower compressive modulus than tensile modulus [133, 134]. Inflation tests have been used to investigate the mechanical behavior of the intact sclera under intraocular pressure loadings [71, 126, 135-137]. Surface optical tracking in combination with inverse finite element modeling has been used to measure scleral surface strains and reconstruct the mechanical properties [138, 139]. Digital image correlation was recently used to measure the response of sclera surface under inflation [126, 136]. The inflation tests and surface strain measurements have provided a delineation of sclera surface response under intraocular pressure (IOP) elevation. However, to the best of our knowledge, the distributive and average strains throughout the thickness of the sclera during intraocular pressure elevation have not been reported. These data may provide further insights into the mechanical responses of the sclera in vivo, particularly those of the posterior sclera that are likely involved in the disease processes of glaucoma and myopia.

Ultrasonic strain mapping (i.e., ultrasound elastography) has been developed to measure the distributive displacements and strains of soft tissue in response to internal or external loading [151, 152]. This method has been primarily used to image pathologies in soft tissues including breast [156], heart [157], blood vessels [158], liver [159], kidney [160] and prostate [156, 160, 161], based on the fact that diseased tissue usually has
altered stiffness [156]. Ultrasound strain mapping is achieved using speckle tracking
techniques applied to ultrasound signals acquired at both undeformed and deformed
states. Ultrasound speckle tracking estimates tissue displacements using either cross-
correlation algorithms [151, 152], sum of absolute differences [154], or optic flow
methods [155]. The strain is calculated as the spatial gradient of the displacement field
[153]. Tissue deformation is typically induced by quasi-static compression [151, 152] or
dynamic vibration [162, 163].

The signal-to-noise ratio (SNR) of ultrasound elastography has been extensively
studied in the past [166-170]. It has been shown that the strain image SNR is dependent
on the strain levels, system characteristics (e.g., transducer center frequency and
bandwidth), as well as signal processing parameters (e.g., kernel size) [167, 170].

Previous work has demonstrated the feasibility of using high frequency ultrasound
[173] and optical coherence tomography [220] for corneal strain mapping. In this study,
we report a speckle tracking method based on high frequency ultrasound radiofrequency
data analysis to achieve cross-sectional strain mapping in the sclera during elevations of
IOP. Porcine sclera was used and the accuracy and signal-to-noise ratio (SNR) of the
strain estimation were evaluated both theoretically and experimentally.

4.2 Methods

4.2.1 Experimental setup and testing protocol

Five porcine globes were obtained from a slaughterhouse within 24 hours
postmortem. The samples were stored in phosphate buffered saline (PBS) solution at 4°C
prior to experiments. The posterior scleral shells were dissected at about 2 mm posterior to the limbus and the vitreous, retina, and choroid were carefully removed. The sclera shells were mounted onto a custom-built pressurization chamber and the IOP was controlled by a saline column and confirmed by using a pressure sensor (Figure 4.1). The sclera was clamped on a custom-built chamber with two O-rings that sandwiched the sclera in between (Figure 4.2). Plastic screws were used to tighten the O-rings. The chamber was clamped to a metal ring mount to prevent undesired rotation or other motion, and the chamber with the ring mount was placed in a saline bath. The posterior pole, i.e., the intersection of the optical axis and the sclera, was ensured to situate at the apex of the sclera mount. The ultrasound scans were taken from the posterior pole region in the temporal quadrant adjacent to the optic nerve head.

Figure 4.1 Schematics of experimental setup.
Figure 4.2 Schematics for sclera shell mounting chamber

A high-frequency ultrasound system (Vevo660, VisualSonics Inc., Toronto) with a 55-MHz transducer was employed to perform cross-sectional scanning at the posterior pole and the raw radiofrequency (RF) ultrasound signals were sampled by a digitizer (500 MHz, DP105; Acqiris, Monroe, NY). Before measurements, each scleral shell was subject to preconditioning consisting of 5 cycles of pressurization from 5 to 45 mmHg in 60 seconds, and the pressure was resumed to 5 mmHg for 360 seconds. IOP was then gradually increased from 5 to 45 mmHg at steps of 5 mmHg, with 360 seconds for equilibration prior to the acquisition of the RF signals at each step (Figure 4.3(a)). Ultrasound scans at two different directions were performed: one tangential to the ONH (the circumferential direction) and the other perpendicular to the first (the meridian direction), as shown in Figure 4.3(b).
Figure 4.3 (a) The experimental protocol of preconditioning and IOP loading; (b) the scanning orientations for ultrasound data acquisition. The circumferential cross-section was about 2 mm from the ONH.

4.2.2 Ultrasound speckle tracking algorithm

The ultrasound RF signals were first filtered using a band-pass filter with a bandwidth of 100 MHz centered at 55 MHz to remove noise. A correlation-based speckle tracking algorithm [221], which has been widely used in the ultrasound elastography field, was applied to the RF signals obtained at two consecutive pressure levels. Briefly, a kernel in the original signal A centered at $(i_0, j_0)$ was compared with a series of kernels in the deformed signal B near the neighborhood of the original kernel. The correlation coefficient between the original kernel in signal A and the kernel in signal B centered at $(i_0+l, j_0+m)$ is calculated as follows:
\[ \rho_{l,m}(i_0, j_0) = \frac{\sum_{i = i_0 - (M/2)}^{i_0 + (M/2)} \sum_{j = j_0 - (N/2)}^{j_0 + (N/2)} (a_{i,j} - \bar{a})(b^*_i + l,j + m - \bar{b}^*)}{\sqrt{\sum_{i = i_0 - (M/2)}^{i_0 + (M/2)} \sum_{j = j_0 - (N/2)}^{j_0 + (N/2)} |a_{i,j} - \bar{a}|^2 \sum_{i = i_0 - (M/2)}^{i_0 + (M/2)} \sum_{j = j_0 - (N/2)}^{j_0 + (N/2)} |b_{i,j} + l,j + m - \bar{b}^*|^2}} \]  

where \( a \) and \( b \) are the values in signal A and B, and the \( \bar{a} \) and \( \bar{b}^* \) are the average values of the corresponding kernels. The size of the kernel was \((M+1) \times (N+1)\) data points, corresponding to a region of \((M+1) \times (N+1)\) pixels in the ultrasound image.

The correlation coefficients were calculated for a search region around \((i_0, j_0)\) in signal B by varying \(l\) and \(m\), and interpolated using a spline function to achieve sub-pixel tracking. The location with the largest correlation coefficient magnitude was used to determine the displacement vector. The displacement fields were accumulated from 5 mmHg to 45 mmHg. A least-square strain estimator was used to calculate the strains in both the axial (along the ultrasound beam) and lateral (perpendicular to ultrasound beam) directions [153]. Although these two directions were selected in the present study, strains along any arbitrary direction could be obtained from the displacement field. Average strains along the axial and lateral directions were obtained within a region of interest at the posterior pole (about 2 mm wide and 1 mm thick). The strains at the two scanning orientations (i.e., circumferential and meridian) were compared. The strain fields were smoothed for plotting the strain images following the typical procedures used in the ultrasound elastography field.[151, 221]

### 4.2.3 Experimental validation of displacement measurement
The accuracy of the ultrasound speckle tracking method in calculating tissue displacements was evaluated experimentally by comparing the calculated displacements with known displacements induced by a motorized actuator (TRA25CC, Newport Corporation, Irvine, CA) [220]. The actuator has a resolution of 0.2 µm and an accuracy of ±5 µm for the travel distance of 20 mm. A porcine sclera shell was mounted onto the pressurization chamber as in Figure 4.1 and the chamber was clamped to a metal ring mount and immersed in a saline bath placed on a positioning stage. The transducer was displaced first laterally and then axially by 100 µm at steps of 10 µm using the actuator to introduce controlled rigid-body displacement between the transducer and the sclera. The alignment of the transducer movement with respect to the ultrasound beam was ensured by careful visual inspection. In an additional experiment, two ultrasound scans were acquired consecutively on the same sample without any displacement. Ultrasound RF data was acquired at each step and the ultrasound speckle tracking algorithm was used to calculate the displacement field. The average displacement within the region of interest was compared with the mechanical displacement induced by the actuator. The standard deviation of the displacement within the region of interest was calculated to evaluate the uncertainty in displacement measurement.

4.2.4 Theoretical analysis of the signal-to-noise ratio in ultrasound strain mapping

Previous studies have shown that the primary sources of noise in strain estimation based on ultrasound speckle tracking are: the electronic and quantization noise, which is present in ultrasonic scanning and data acquisition; and the decorrelation noise, which
results from the distortion of the speckles during tissue deformation [167]. The performance of ultrasound speckle tracking in strain estimation is typically evaluated by the signal-to-noise ratio of the strain image ($SNR_c$) [166, 167, 170, 222], which is defined as the ratio between the true strain and the overall noise/variance.

Conceptually, the actual strain level would affect the $SNR_c$ because very small true strains are likely less distinguishable from electronic noise while very large strains tend to distort the ultrasound speckles and deteriorate the correlation for speckle tracking. Therefore, for a given ultrasound system with a given data processing scheme, there typically exists an optimal intermediate range of strains that can be accurately measured by speckle tracking.

The concept of “strain filter” has been used to determine the theoretical bound of $SNR_c$ at different levels of strain, which corresponds to the tightest bound of noise in strain estimation [167]. This bound consists of several segments as described in Eq. (2), integrating the Cramér-Rao lower bound ($\sigma^2_{CRLB}$) and the Barankin bound ($\sigma^2_{BB}$) into the Ziv-Zakai lower bound ($\sigma^2_{ZZLB}$). From Eq. (2), it can be seen that $\sigma^2_{ZZLB}$ is determined by $\sigma^2_{CRLB}$ when the post-integration signal to noise ratio (i.e., $B \cdot T \cdot SNR_c$) is large, and by $\sigma^2_{BB}$ when the post-integration signal to noise ratio is moderate [167]:
where $SNR_c$ is the combination of the sonographic signal-to-noise ratio ($SNR_s$) associated with electronic noise and the correlation signal-to-noise ratio ($SNR_\rho$) associated with deformation-related decorrelation noise as defined in Eq. (3), $B$ is the absolute bandwidth of the ultrasound system, $T$ is the temporal length of the kernel, $s$ is the mean value of the estimated strain, $\Delta T$ is the temporal separation between adjacent kernels, and $Threshold_1$ and $Threshold_2$ are exponential transitions between the segments [222].

$$SNR_c = \frac{SNR_\rho SNR_s}{1 + SNR_\rho + SNR_s} \quad (4.3)$$

$\sigma^2_{CRLB}$ and $\sigma^2_{BB}$ can be calculated using Eq. (4) and Eq. (5).

$$\sigma^2_{CRLB} = \frac{3}{2\pi^2 T (B^3 + 12 B f_0^2)} \left( \frac{1}{\rho^2} \left( 1 + \frac{1}{SNR_s} \right)^2 - 1 \right) \quad (4.4)$$

$$\sigma^2_{BB} = \frac{12 f_0^2}{B^2} \sigma^2_{CRLB} \quad (4.5)$$

where $f_0$ is the center frequency of the ultrasound transducer and $\rho$ is the correlation coefficient determined by tissue stretch ratio, ultrasound frequency, pulse width and correlation kernel size [169]. The parameters $\gamma, \delta, \mu$ and $\eta$ are defined as follows [222]:
\[ \gamma \approx 0.46 \] (4.6)

\[ \delta = \zeta / 2 \] (4.7)

\[ \mu = \frac{2.76}{\pi^2} \left( \frac{f_0}{B} \right)^2 \] (4.8)

\[ \eta = \frac{6}{\pi^2} \left( \frac{f_0}{B} \right)^2 \left[ \varphi^{-1} \left( \frac{B^2}{24f_0^2} \right) \right]^2 \] (4.9)

where \( \varphi^{-1}(\gamma) \) is the inverse of \( \varphi(\gamma) = \frac{1}{\sqrt{2\pi}} \int_\gamma^\infty e^{-\mu^2/2} d\mu \), and \( \zeta \) is the larger value of solutions for \( (\zeta / 2) \varphi(\sqrt{\zeta / 2}) = (12\pi / BsT)^2 \).

In the present study, the center frequency \( f_0 \) of the transducer was 55 MHz. The relative bandwidth of the transducer was 100% and thus \( B \) is 55 MHz. Several different temporal kernel lengths (i.e., \( T \)) which corresponded to 50, 100, 150, or 200 \( \mu \)m kernels, were investigated in order to identify the optimal \( T \). A 50% kernel overlap (\( \Delta T = T/2 \)) was adopted to achieve the best trade-off between \( SNR_e \) and spatial resolution of the strain map [166].

4.2.5 Analysis of strain estimation accuracy using simulated RF data

The accuracy of the ultrasound speckle tracking algorithm in estimating scleral strains was evaluated using simulated ultrasound RF data where arbitrary patterns of strain were introduced in the “deformed” signal. The original RF data of a scleral cross-section were generated using the Field II Ultrasound Simulation Program [223, 224] with a customized MATLAB sub-routine. The simulation program convolves the point spread function (PSF) of the ultrasound transducer used in this study with a 3D cloud of
randomly distributed scatterers that simulate the sclera. Forty scatterers per resolution cell was used in order to simulate Rayleigh scattering [225] and form fully developed speckles [170] as seen in the ultrasound images of the sclera. Random noise that corresponds to an $SNR_s$ of 38-dB was added to the RF data to simulate electronic noise in the ultrasound system (this level of $SNR_s$ was typically observed in our experiments). The scatterers were then displaced to simulate uniform axial and lateral compression or extension at strain levels ranging from 0.1% to 15%. The “deformed” scatterer cloud was then convolved with the transducer point spread function to generate the “deformed” ultrasound RF signals using the simulation program. The “undeformed” and the “deformed” RF data were processed by the speckle tracking algorithm to calculate the displacement fields and corresponding strain images. The calculated average strains were compared with the predefined “true” strains induced in the scatterers.

In another simulation, the scatterers were divided into two equal-sized layers with the top layer subject to 1% uniform axial compression and lateral extension, while the bottom layer subject to 2% axial compression and lateral extension. The strain images were computed to examine how well the speckle tracking algorithm could differentiate the different strains in these two layers.

In a third simulation, the scatterers were divided into three regions (layers or zones, as defined below). In the first scenario, the scatterers were divided into three layers with the top and bottom layers subject to 1% uniform axial and lateral compression or extension, while the middle layer subject to 2% axial and lateral compression or extension. The total thickness of the three layers was 1 mm and the top and bottom layers
had the same thickness. The middle layer was assigned with an increasingly smaller thickness (500 µm, 250 µm, 100 µm, and 50 µm) to evaluate the capability of the ultrasound speckle tracking algorithm in detecting small heterogeneities along the axial direction. In the second scenario, the scatterers were divided into three zones with the left and right zones subject to 1% uniform axial and lateral compression or extension, while the middle zone subject to 2% axial and lateral compression or lateral extension. The total width of the three zones was 4 mm and the left and right zones had the same width. The middle zone was assigned with an increasingly smaller width (2 mm, 1 mm, 400 µm, and 200 µm) to evaluate the capability of the ultrasound speckle tracking algorithm in detecting small heterogeneities along the lateral direction. The average calculated strains within the inhomogeneous regions were obtained as an indication of the accuracy and sensitivity of the ultrasound speckle tracking algorithm for detecting small inhomogeneous areas in a simulated sclera.

4.3 Results

4.3.1 Through-thickness strain distribution in porcine posterior sclera

A cross-sectional ultrasound image of the posterior pole of a porcine sclera is presented in Figure 4.4(a), showing the typical ultrasonic appearance of the sclera with bright and evenly distributed speckles. The boundaries of the sclera (the interior and exterior surfaces) were readily discernible.

The displacement fields relative to the average displacement of the entire the sclera at pressure elevations from 5 to 15, 30, and 45 mmHg are shown in Figure 4.4(b),
(c), and (d), respectively. The displacement fields showed a clear trend of compression along the axial direction and extension along the lateral direction. The corresponding strain images are presented in Figure 4.5. In the present study, the spatial resolution of the strain images (defined as the distance between two adjacent kernels used in the ultrasound speckle tracking algorithm) was 75 µm (axial) by 100 µm (lateral).

Figure 4.4 Displacement vector fields in a posterior porcine sclera obtained from ultrasound speckle tracking. (a) A cross-sectional ultrasound image of the sclera at 5 mmHg; (b) displacement field at 15 mmHg; (c) displacement field at 30 mmHg; (d) displacement field at 45 mmHg.
The average axial and lateral strains within the region of interest were calculated at all pressure levels for the measured porcine sclera (Table 4.1). Figure 4.6 shows the average strains along the circumferential and the meridian directions. The average axial
strains were greater in magnitude than the average lateral strains in both directions at all pressure levels (P’s < 0.01). In general, the circumferential axial strains were greater than the meridian axial strains, while the circumferential lateral strains were smaller than the meridian lateral strains. At 45 mmHg, the circumferential lateral strains were significantly smaller than the meridian lateral strains (1.2 ± 0.6% vs. 2.2 ± 0.7%, P < 0.05). No significant difference was found at other pressure levels.

**Figure 4.6** Average scleral strains at different pressure levels.
Table 4.1 Average scleral strains at different pressure levels. (*: For lateral strains, the circumferential was significantly smaller than the meridian at 45 mmHg, P<0.05; †: The axial strains were significantly greater than the lateral strains at all pressure levels, P<0.01)

<table>
<thead>
<tr>
<th>Strain</th>
<th>15mmHg</th>
<th>25mmHg</th>
<th>35mmHg</th>
<th>45mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meridian</td>
<td>-2.4 ± 0.8%</td>
<td>-3.4 ± 1.2%</td>
<td>-4.2 ± 1.2%</td>
<td>-5.1 ± 1.5%</td>
</tr>
<tr>
<td>Circum</td>
<td>-3.1 ± 1.6%</td>
<td>-4.1 ± 1.8%</td>
<td>-5.0 ± 1.9%</td>
<td>-5.9 ± 2.2%</td>
</tr>
<tr>
<td>Lateral†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meridian</td>
<td>1.4 ± 0.6%</td>
<td>1.8 ± 0.7%</td>
<td>2.1 ± 0.6%</td>
<td>2.2 ± 0.7% *</td>
</tr>
<tr>
<td>Circum</td>
<td>1.1 ± 0.5%</td>
<td>1.1 ± 0.5%</td>
<td>1.2 ± 0.6%</td>
<td>1.2 ± 0.6% *</td>
</tr>
</tbody>
</table>

4.3.2 Experimental validation of displacement measurement

The comparison between the actuator output and the calculated displacement is presented in Figure 4.7. At an actuator output of 10 μm, the average displacements calculated from speckle tracking was 10.9 ± 0.4 μm (axial) and 9.4 ± 2.2 μm (lateral), respectively. At an actuator output of 100 μm, the calculated displacements were 102.8 ± 0.4 μm (axial) and 105.5 ± 1.7 μm (lateral), respectively. The standard deviation of the calculated displacement was consistent across different actuator output, i.e., below 1 μm for the axial direction and about 2 μm for the lateral direction.
Figure 4.7 Comparison of the displacements calculated from speckle tracking and the actuator output: (a) calculated axial displacement vs. actuator output; (b) calculated lateral displacement vs. actuator output.

In the experiment of acquiring two consecutive images on the same sclera without moving the sample or introducing deformation, the average displacement calculated from speckle tracking was $-0.42\pm0.25 \, \mu m$ (axial) and $0.65\pm0.92 \, \mu m$ (lateral). Further calculations of the strains showed an average axial strain of $(-0.008\pm0.020)\%$ and an average lateral strain of $(-0.001\pm0.080)\%$. These results suggested that the ultrasound speckle tracking algorithm was robust to random environmental noise.

4.3.3 Theoretical analysis of the SNR in strain estimation

The $SNR_e$ bounds as a function of strain (i.e., the strain filters) corresponding to different kernel sizes (50-200 $\mu m$) are presented in Figure 4.8.
Figure 4.8 SNRe vs. strain for different kernel sizes.

The peak $\text{SNR}_e$ and the -3dB range of the strain filter (i.e., the range of strains corresponding to an $\text{SNR}_e$ that is greater than 70% of the peak value) is presented in Table 4.2. With an increased kernel size, the peak $\text{SNR}_e$ increases while the -3dB range shifts to the lower strain levels. This means that a larger kernel size gives higher $\text{SNR}_e$ in measuring small strains, at the cost of a decreased spatial resolution of the strain images and compromised performance at large strains.

Table 4.2 The peak SNRe and the -3dB range of the SNRe for different kernel sizes

<table>
<thead>
<tr>
<th>Kernel size</th>
<th>Peak $\text{SNR}_e$</th>
<th>Low cutoff strain</th>
<th>High cutoff strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μm</td>
<td>8.73</td>
<td>0.49%</td>
<td>28%</td>
</tr>
<tr>
<td>100 μm</td>
<td>12.74</td>
<td>0.26%</td>
<td>15%</td>
</tr>
<tr>
<td>150 μm</td>
<td>15.71</td>
<td>0.17%</td>
<td>10%</td>
</tr>
<tr>
<td>200 μm</td>
<td>18.19</td>
<td>0.13%</td>
<td>7.5%</td>
</tr>
</tbody>
</table>
4.3.4 *Analysis of strain estimation accuracy using simulated data*

Figure 4.9 shows a simulated image of the sclera with typical ultrasonic scattering patterns. The comparison between the calculated strains and the simulated strains are presented in Table 4.3. For axial strains, the % error \((|\text{simulated strain} - \text{calculated strain}| / \text{simulated strain})\) was smaller than 3% for all strain levels between 0.1 to 5%. For lateral strains between 1 to 5%, the % error was smaller than 5%. For lateral strains between 0.1 to 0.75%, the % error was 16-40%. For strains larger than 5%, the % error was larger than 15% for both axial and lateral directions.

![Simulated Ultrasound Image](image)

**Figure 4.9** A simulated ultrasound image of the sclera using the Field II Ultrasound Simulation Program.
Table 4.3 Simulated vs. calculated strains under uniform compression or extension

<table>
<thead>
<tr>
<th>Simulated Strain</th>
<th>Compression (Calculated)</th>
<th>Extension (Calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axial</td>
<td>Lateral</td>
</tr>
<tr>
<td>0.1%</td>
<td>0.10 ± 0.05%</td>
<td>0.07 ± 0.09%</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.49 ± 0.07%</td>
<td>0.37 ± 0.16%</td>
</tr>
<tr>
<td>1%</td>
<td>0.99 ± 0.09%</td>
<td>1.0 ± 0.40%</td>
</tr>
<tr>
<td>2%</td>
<td>2.0 ± 0.14%</td>
<td>2.1 ± 0.7%</td>
</tr>
<tr>
<td>5%</td>
<td>4.9 ± 0.44%</td>
<td>5.0 ± 1.1%</td>
</tr>
</tbody>
</table>

Integrating the results at different levels of strains, an $SNR_e$ curve was approximated by dividing the mean strain (signal) by the standard deviation of the strain (noise). The $SNR_e$ curves (Figure 4.10) resembled the theoretical analysis (Figure 4.8) showing the pattern of a low $SNR_e$ at very small or very large strains and a plateau region with a high $SNR_e$ for the intermediate range of strains. The $SNR_e$ curves were comparable between the two types of loading (e.g., compression vs. extension). For both loading types, the axial direction had a much higher $SNR_e$ than the lateral direction.
Figure 4.10 The approximated SNRe curves of the strain maps based on simulated data.

Figure 4.11 shows the calculated displacement field and strain images from the simulation with 1% axial compression and lateral extension of the top layer and 2% axial compression and lateral extension of the bottom layer. The ultrasound speckle tracking algorithm could clearly differentiate the strains in these two layers.

Figure 4.11 Displacement vector field and strain maps calculated from simulated RF signals for top 1% and bottom 2% strains.
Table 4.4 summarizes the simulation results of the average calculated strains of a small inhomogeneity that has larger strains (2%) than the surrounding background (1% strain) in a simulated sclera. The strain maps are shown in Figure 4.12. For axial strains, the % error of the strain estimation for the inhomogeneous area was lower than 20% for a layer thicker than 150 µm or a zone larger than 400 µm; and the % error of the strain estimation for the background was lower than 5%. For lateral strains, the % error of the strain estimation for the inhomogeneous area was lower than 25% for a layer thicker than 150 µm or a zone larger than 1 mm; however, the % error of the strain estimation for the background was typically higher (i.e., 10-15%). Figure 4.12 shows visually clear contrast of the inhomogeneous area in the axial strain maps.

**Table 4.4** Calculated strains for small heterogeneities (2% simulated strain) of various sizes surrounded by a background with 1% simulated strain.

<table>
<thead>
<tr>
<th>Type of heterogeneities</th>
<th>Size</th>
<th>Compression</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>axial</td>
<td>lateral</td>
</tr>
<tr>
<td>Axial Layers</td>
<td>500 µm</td>
<td>-1.90%</td>
<td>-1.93%</td>
</tr>
<tr>
<td></td>
<td>250 µm</td>
<td>-1.68%</td>
<td>-1.90%</td>
</tr>
<tr>
<td></td>
<td>150 µm</td>
<td>-1.64%</td>
<td>-1.74%</td>
</tr>
<tr>
<td></td>
<td>50 µm</td>
<td>-1.25%</td>
<td>-1.39%</td>
</tr>
<tr>
<td>Lateral Zones</td>
<td>2 mm</td>
<td>-1.94%</td>
<td>-1.98%</td>
</tr>
<tr>
<td></td>
<td>1 mm</td>
<td>-1.82%</td>
<td>-1.55%</td>
</tr>
<tr>
<td></td>
<td>400 µm</td>
<td>-1.62%</td>
<td>-1.07%</td>
</tr>
<tr>
<td></td>
<td>200 µm</td>
<td>-1.37%</td>
<td>-0.75%</td>
</tr>
</tbody>
</table>
Figure 4.12 Strain images of a simulated sclera with an inhomogeneous region. Row (a) and (b) are axial and lateral strains, respectively, for an inhomogeneous layer with decreasing thickness: 1) 500 µm, (2) 250 µm, (3) 150 µm, and (4) 50 µm; Row (c) and (d) are axial strains and lateral strains, respectively, for an inhomogeneous zone with decreasing width: (1) 2 mm, (2) 1 mm, (3) 400 µm, and (4) 200 µm.

4.4 Discussion

In this study, we developed a new experimental method based on ultrasound speckle tracking to obtain cross-sectional strain maps of the posterior sclera during elevations of intraocular pressure. Theoretical analysis, simulation, and experimental validations were performed to examine the accuracy and the signal-to-noise ratio of this method for strain estimation.

The strain maps from the porcine sclera (Figure 4.5) during IOP elevation from 5 to 45 mmHg showed negative axial strains and positive lateral strains. On average, the
axial and lateral strains increased nonlinearly with the pressure increase (Figure 4.6), which was consistent with the results in a previous study that reported nonlinear surface strains in porcine sclera [138].

Our results showed that the axial strains were on average more than twice as large as the lateral strains. For the area within the region of interest shown in Figure 4.5, the axial and lateral directions were approximately corresponding to the radial and tangential directions. The results suggested that there was significant compression of the sclera during pressure elevation. Previous studies have reported a much smaller compressive modulus than tensile modulus in human and porcine sclera [133, 134]. The large magnitude of the compressive strains suggested that compression, although not the primary form of mechanical loading in sclera, may warrant further study in understanding scleral mechanobiology. It is also of interest to note that the axial/compressive strains were typically heterogeneous through the thickness of the measured porcine sclera, where larger strains were found in the outer and inner layers and smaller strains were found in the middle layer of the sclera. Previous studies have reported different collagen bundle size and arrangement in the inner, middle, and outer layers of the sclera [11, 12]. Scleral collagen bundles vary in size from the inner to outer layers with more narrower and thinner bundles in the outer layer [11]. In the innermost layer adjacent to the uvea, the collagen bundles are again smaller and blend into the underlying choroidal stroma [12]. These microstructural differences might be responsible for the observed heterogeneous strains. Conversely, the larger compression at the inner and outer layers might be associated with the poroelastic properties of the sclera (i.e., fluid being displaced out
from the surfaces of the sclera during IOP elevation). Poroelasticity has been frequently observed in other soft tissues such as articular cartilage [226], ligament, and tendon [227]. The heterogeneity of the through-thickness compression was consistently found in most of the porcine sclera we have tested and also in human sclera (data not shown), suggesting a need to further elucidate the underlying mechanisms of this phenomenon and its implications to scleral biomechanics and pathophysiology.

We obtained cross-sectional strain maps along both the circumferential and the meridian directions to investigate if ultrasound speckle tracking could detect anisotropic properties as reported in previous studies [138]. Our results showed that the average lateral strains along the meridian direction were larger than those from the circumferential direction, which was consistent with previous reports and agreed with the typical microstructural observations that collagen fibers are preferentially aligned along the circumferential direction close to the ONH [228]. Previous modeling work has suggested a significant impact of the collagen fiber orientation in the peripapillary sclera region on IOP-induced deformation of ONH [139]. Circumferential arrangement of collagen fibers around the ONH could significantly reduce canal expansion which is protective of the structural stability of the ONH [139].

In order to validate the accuracy of the ultrasound speckle tracking method in estimating scleral strains, we first examined how well the method could detect absolute displacement of a porcine sclera induced by an actuator. Our results showed that the displacements calculated from ultrasound speckle tracking agreed well with the actuator output (Figure 4.7). For example, when the actuator displacement was 10 μm, the average
calculated displacement of the ultrasound speckles were 10.9 ± 0.4 μm (axial) or 9.4 ± 2.2 μm (lateral). Assuming the actuator output was the true displacement, this result showed that the high-resolution ultrasound method was capable of tracking displacements at the accuracy of sub-micron to micron scale with an uncertainty (i.e., standard deviation) of the same scale. Our results also showed a larger variance in the calculated lateral displacement than axial displacement. This was consistent with previous reports that axial speckle tracking typically had better performance than lateral speckle tracking due to the additional phase information and higher sampling rate in the RF data along the axial direction [171]. At larger displacements, there was a small discrepancy between the actuator output and the ultrasound measurements. This is likely due to the potential small misalignment between the transducer movement and the ultrasound beam, as well as the inaccuracy of the actuator itself.

The theoretical analysis of the strain filter, i.e., the $SNR_e$ as a function of strain, showed the typical band-pass characteristics found in other ultrasound elastography systems [166, 167, 170, 222]. The -3dB range of the strain filter was from 0.17% to 10%, which adequately covers the strain levels observed in sclera. Girard et al reported an average maximum Lagrangian strain of 1.3% at 10 mmHg and 3.1% at 45 mmHg in porcine sclera shells [135]. Woo et al reported a strain level of 0.5% on human sclera at 45 mmHg [71]. These strain values fall within the -3dB range reported above. In the present study, a larger than 10% axial strains was sometimes observed in the porcine sclera when IOP was raised to 45 mmHg. This strain was determined accumulatively based on the speckle tracking results at multiple intermediate pressures and the strains
corresponding to the intermediate steps were well within the -3dB range. The current strain filter predicted a maximum $SNR_e$ of 15.7, which corresponds to 0.06% noise at a 1% actual strain. It is noted that this was predicted from the theoretical analysis without any further signal processing applied (e.g., filtering or smoothing). In practice, the $SNR_e$ can be further improved by applying least-square fitting on strain estimation [153] or low-pass filtering in both displacement fields and strain images [221], at the cost of decreased spatial resolution of the strain maps.

Based on the simulation experiments, the calculated strains matched well with the simulated strains in both the axial and lateral directions under either compression or extension for uniform samples (Table 4.3). The $SNR_e$ estimated from the simulation results was consistent with the theoretical analysis demonstrating a band-pass characteristic with the optimal strain levels ranging from 0.5% to 6%. The $SNR_e$ was generally lower in the lateral direction than in the axial direction, which was consistent with the aforementioned better performance of speckle tracking along the ultrasound propagation direction (i.e., axial direction) [171]. It is noted that the scanning direction can be adjusted within the limits of tissue geometry so that the axial direction could be aligned towards the direction of interest to optimize the performance along that direction. The simulation results in two layers with different strains demonstrated the sensitivity of using ultrasound speckle tracking to detect heterogeneous tissue responses. The simulation results of an inhomogeneous area with increasingly smaller size showed that the axial strains were sensitive to an inhomogeneity of a few hundreds of microns, suggesting the suitability of this approach for acquiring axial deformation data of
heterogeneous tissue at a fine resolution. The lateral strains, due to the lower $SNR_e$ discussed above, suffered from more noise which made it more difficult to clearly differentiate the inhomogeneous areas unless the size is approaching the mm level. Future studies are needed to analyze how the ultrasound speckle tracking algorithm performs for strain contrasts at other levels and for stiffer heterogeneities that have lower strains than the background.

The limitations of the present study are as follows. First, only 2D cross-sectional data was obtained and analyzed. Potential out-of-plane motion such as sample rotation could result in erroneous strain measurements. In the present study, the correlation coefficients for speckle tracking were high (generally over 0.8), suggesting minimal out-of-plane motion. Future 3D full-field studies are needed to fully characterize 3D strains and validate the results. This can be achieved by using 3D ultrasound scanning and 3D speckle tracking algorithms. Second, the present study only examined axial and lateral strains. Other types of strains including the principal strains and shear strains may provide additional insights into the mechanical behavior of the sclera and better description of potential tissue heterogeneity, and thus should be investigated in the future. In addition, the present study only examined one region of the posterior sclera (i.e., the temporal quadrant near the ONH), which may not be reflective of the general state of strains near the ONH but in different quadrants. Future studies are needed to examine potential regional heterogeneity in different quadrants of the posterior sclera. Third, the present study only examined the elastic responses of the sclera. New testing protocols should be developed to evaluate the viscoelastic properties which will provide important
data for understanding the time-dependent scleral properties and their involvement in glaucoma disease processes. Fourth, the porcine sclera may have experienced some swelling prior to the experiments due to the storage in PBS, which could affect the measured strains (particularly, the axial strains). The swelling however was likely small because of the low concentration of proteoglycan and substantial interweaving of the collagen fibers in the sclera.[206]

4.5 Summary

In summary, this study established the feasibility of a new experimental method for non-invasive measurement of distributive internal strains of the sclera at high accuracy and high signal-to-noise ratio.
Chapter 5 Ultrasonic strain mapping on human sclera

5.1 Introduction

As introduced in the previous chapters that the scleral mechanical properties, especially those of the peripapillary sclera, play an important role in affecting the mechanical environment of the ONH [229, 230]. It has been reported that human sclera is typically nonlinear [71], viscoelastic [120], heterogeneous [117, 118]. The anisotropy of the human posterior sclera has been suggested by microstructure analysis of collagen fibers showing preferred circumferential alignment around ONH [122, 123]. However, Eilaghi et al.’s bi-axial tensile tests did not show significant anisotropy on the tested human sclera samples [124], which might be affected by how the samples were dissected and mounted during the biaxial test [125]. Results from surface optical tracking and inverse reconstruction have showed anisotropic fiber orientation and stiffness in the peripapillary sclera of monkey [119] and human eyes [120]. Regional variation in the scleral mechanical properties at human posterior sclera has been reported with the temporal and inferior quadrants to be more compliant than other quadrants [118]. Similar to what has been found in animal eyes [121, 126], the human sclera stiffens with age as well [120, 127]. Disease conditions could also alter the sclera mechanical properties. Coudrillier et al.’s study showed stiffer meridian response in the peripapillary sclera in
glaucoma eyes than in normal eyes, and some glaucoma eyes had slower circumferential creep rates [120].

Our previous work has demonstrated the feasibility of using ultrasound speckle tracking method to measure the through thickness strains of porcine sclera [174]. In this study, posterior human scleras were examined using the ultrasound speckle tracking method to characterize the strain distribution throughout the thickness. The strains at two scanning orientations (circumferential and meridian) were compared, and the stains at different depth in the thickness were also compared. The regional variance of strains in four quadrants (the nasal, the temporal, the superior, and the inferior) of the posterior sclera was also examined on some of the tissue samples.

5.2 Methods
5.2.1 Specimen preparation

Eleven human globes from 7 subjects (aged 57 to 73 years old with the average at 66.3 years old based on information on 5 subjects available) were obtained from Central Ohio Lions eye bank. The samples were stored in moist chamber at 4°C prior to experiments. All samples were tested within 48 hours after they were obtained from the eye bank. The post mortem time of the samples at experiment varied from 2 days to 13 days (Table 5.1).
Table 5.1 Demographic information and postmortem time of the human cadaver eyes

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Eye Tested</th>
<th>Age</th>
<th>Gender</th>
<th>Post Mortem Time when Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>OD, OS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>OD, OS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>OS</td>
<td>57</td>
<td>F</td>
<td>13 days</td>
</tr>
<tr>
<td>D</td>
<td>OD, OS</td>
<td>73</td>
<td>F</td>
<td>10 days</td>
</tr>
<tr>
<td>E</td>
<td>OS</td>
<td>61</td>
<td>F</td>
<td>5 days</td>
</tr>
<tr>
<td>F</td>
<td>OD, OS</td>
<td>68</td>
<td>M</td>
<td>2 days</td>
</tr>
<tr>
<td>G</td>
<td>OS</td>
<td>66</td>
<td>M</td>
<td>9 days</td>
</tr>
</tbody>
</table>

5.2.2 Experimental setup and measurement protocol

Similar to the procedure described in the previous chapter, the posterior scleral shells were dissected at about 2 mm posterior to the limbus and the vitreous, retina, and choroid were carefully removed. The sclera shells were mounted onto a custom-built pressurization chamber (Figure 4.2). Different from the experimental setup in the previous chapter, the pressure inside the shell was controlled by a closed-loop PID pressurization system that is composed of a computer, a syringe pump (Standard Infuse/withdraw PHD Ultra Syringe Pump, Harvard Apparatus, Holliston, MA) and a pressure sensor (Advanced Research Venous Pressure Transducer P75, Harvard Apparatus, Holliston, MA) (Figure 5.1). The pressure sensor reads the pressure inside the chamber and output it to the computer. The computer compares the pressure measured with the pre-set pressure target and changes the pumping speed of the syringe pump accordingly at real time to have the measured pressure follow the pre-set pressure target. The computer interface driving the pump and the sensor was implemented in Labview (National Instruments Corporation, Austin, TX).
Similar to the previous study on porcine sclera, a high-frequency ultrasound system (Vevo660, VisualSonics Inc., Toronto) with a 55-MHz transducer was employed to perform cross-sectional scanning at the posterior pole, and the raw radiofrequency (RF) ultrasound signals were sampled by a digitizer (500 MHz, DP105; Acqiris, Monroe, NY). Before measurements, each scleral shell was subject to preconditioning consisting of 5 cycles of pressurization from 5 to 45 mmHg in 60 seconds, and the pressure was resumed to 5 mmHg for 360 seconds. IOP was then gradually increased from 5 to 15 mmHg at steps of 2.5 mmHg and then 15 to 45 mmHg at steps of 5 mmHg, with 360 seconds allowed for equilibration prior to the acquisition of the RF signals at each step. Ultrasound scans at two different directions were performed: one tangential to the ONH
(the circumferential direction) and the other perpendicular to the first (the meridian direction), as shown in the previous chapter Figure 4.3(b). For three out of the eleven samples, after the two scans at the posterior pole (temporal to the ONH) the other three quadrants, the inferior, the nasal and the superior, were also measured at a randomized order (Figure 5.2). Ultrasound scans at the meridian direction and the circumferential direction were both applied in a random order in all four quadrants examined.

**Figure 5.2** Diagram of the four quadrants examined on posterior sclera (N: nasal quadrant; S: superior quadrant; T: temporal quadrant; I: inferior quadrant)

### 5.2.3 Displacement and Strain calculation

The ultrasound speckle tracking algorithm used for obtaining the displacement has been detailed in the previous chapter and our previous publication [231]. Briefly, a cross-correlation based speckle tracking algorithm was applied to the RF signals acquired

106
at two consecutive pressure levels. The displacement was determined by locating the maximum value in the cross-correlation coefficient map around the neighborhood of a speckle window. A least-square strain estimator was used to calculate the strains in both the axial (along the ultrasound beam) and lateral (perpendicular to ultrasound beam) directions. In addition, the radial and tangential stains were calculated from the axial and lateral strain based on the transform of the global axial-lateral coordinate to the local radial-tangential coordinate (Figure 5.3) using the following equations.

\[
\varepsilon_x' = \frac{\varepsilon_x + \varepsilon_y}{2} + \frac{\varepsilon_x - \varepsilon_y}{2} \cos 2\theta + \varepsilon_{xy} \sin 2\theta 
\]  
(5.1)

\[
\varepsilon_y' = \frac{\varepsilon_x + \varepsilon_y}{2} - \frac{\varepsilon_x - \varepsilon_y}{2} \cos 2\theta - \varepsilon_{xy} \sin 2\theta 
\]  
(5.2)

\[
\varepsilon_{xy} = \frac{1}{2} \left( \frac{\partial u_j}{\partial x_i} + \frac{\partial u_i}{\partial x_j} \right) 
\]  
(5.3)

Where \( \varepsilon_x' \) and \( \varepsilon_y' \) were the radial and tangential strains, respectively. The \( \varepsilon_x \) and \( \varepsilon_y \) were the axial and lateral strains, respectively. The \( \varepsilon_{xy} \) was the axial-lateral shear strain that can be calculated from the axial displacement \( u_i \) and the lateral displacement \( u_j \) using the equation 5.3. The \( \theta \) was the angle between the local tangential direction and the lateral direction. The angle \( \theta \) was derived as following: first, both the inner boundary and the outer boundary of sclera were identified on the undeformed B-mode images by manually selecting 9 points on each boundary; the 9 points on each boundary were then divided into three groups of three points and each group corresponded to a unique circle that passed through all three points; the origins of all the 6 circles obtained were then averaged to define the best fitted global origin; the global axial-lateral coordinates can
then be transformed into the polar coordinates with the global origin from the fitted circles and the local $\theta$ can thus be determined from the angle in the polar coordinate for each point in the original axial-lateral coordinates.

![Diagram](image)

**Figure 5.3** Illustration of the radial and the tangential directions (red dashed arrows) in relation to the axial and lateral directions (black dashed arrows) and an example of the angle $\theta$ between the two coordinates

To investigate the through thickness strain variation, the sclera were artificially divided into three layers with equal thickness for each layer: the outer layer, the middle layer, and the inner layer. For each sclera, the average radial strains or tangential strains within each layer were averaged and compared.
Figure 5.4 Example of an ultrasound image of human sclera with boundaries fitted to circles and the inside equally divided into three layers (the outer layer, the middle layer, and the inner layer).

5.2.4 Statistical analysis

The through thickness radial strains were compared with the tangential strain using paired t-test. At the posterior pole, the strains between the circumferential scanning direction and the meridian scanning direction were compared using paired t-test. The strains within the three layers were compared using paired t-tests. The average radial or tangential strains within the four quadrants were also compared using paired t-tests. It is noted that the statistical analysis methods (i.e., paired t-tests) were chosen due to the explorative nature of the current study. More sophisticated statistical analysis using linear mixed models with repeated measures are needed for more rigorous statistical analysis.

5.3 Results
The average through thickness radial strains at different pressure levels for all sclera samples measured were summarized and plotted in Figure 5.5. The average through thickness tangential strains at different pressure levels were summarized and plotted in Figure 5.6. The average radial strain among all samples was -0.30% ± 0.33%, -0.68% ± 0.54%, -1.16% ± 0.83%, -1.59% ± 1.03%, -2.03% ± 1.27%, -2.54% ± 1.54%, -3.00% ± 1.66%, -3.49% ± 1.88% at pressure levels of 5, 10, 15, 20, 25, 30, 35, 40, and 45 mmHg, respectively. The average tangential strain among all samples was 0.11% ± 0.06%, 0.20% ± 0.09%, 0.28% ± 0.10%, 0.34% ± 0.11%, 0.38% ± 0.12%, 0.42% ± 0.11%, 0.45% ± 0.13%, and 0.49% ± 0.13% at pressure levels of 5, 10, 15, 20, 25, 30, 35, 40, and 45 mmHg, respectively. There was a large inter-subject variance in both the radial strains and the tangential strains among all samples tested. At the same pressure level, the radial compression was much larger in magnitude than the tangential stretching (i.e., 3.49% radial compression vs. 0.49% tangential extension at 45 mmHg, P < 0.001, paired t-test).
Figure 5.5 Radial strain of all human sclera samples including both the meridian and the circumferential scans at different pressure levels
Figure 5.6 Tangential strain of all human sclera samples including both the meridian and the circumferential scans at different pressure levels.

The average tangential strains in the meridian scanning direction and the average tangential strains in the circumferential direction were plotted on Figure 5.7. The tangential strains in the circumferential direction were significantly smaller than the tangential strains in the meridian direction at pressure levels of 10, 15, 40, and 45 mmHg (P = 0.018, 0.014, 0.029 and 0.029, respectively, paired t-test). Although linear mixed models are needed to verify the results, our data showed a trend that the circumferential tangential strains were consistently lower than the meridian tangential strains at all pressure levels. The average radial strains in the meridian scanning direction and the average radial strains in the circumferential direction were plotted on Figure 5.8. There was no significant difference between the radial strains along the meridian direction and
along the circumferential direction at any pressure level. The radial strains appear to be almost linear with respect to pressure changes while the tangential strains showed nonlinear increases with pressure.

Figure 5.7 Tangential strain vs. pressure at the meridian and the circumferential scanning directions (*: P < 0.05)
Figure 5.8 Radial strain vs. pressure at the meridian and the circumferential directions

The average tangential stains and radial strains within the outer, the middle, and the inner layer of all sclera samples were shown in Figure 5.9 and Figure 5.10, respectively. The tangential strain in the outer layer was significantly lower than in the inner layer at pressure levels of 30, 35, 40, and 45 mmHg ($P = 0.0398$, $0.0273$, $0.0238$ and $0.01$, respectively, paired t-tests) and was significantly lower than the middle layer at pressure levels of 20, 25, 30, 35, 40, and 45 mmHg ($P = 0.0076$, $0.0025$, $0.0011$, $0.0003$, $0.0002$ and $0.00008$, respectively, paired t-tests). No statistically significant difference was found in the tangential strain between the middle layer and the inner layer at any pressure level tested. The radial strain in the middle layer was significantly higher in magnitude than in the inner layer at pressure levels of 20, 25, 30, 35, 40, and 45 mmHg ($P = 0.0091$, $0.0038$, $0.0022$, $0.0015$, $0.0028$ and $0.0028$, respectively, paired t-tests) and was significantly higher than in the outer layer at pressure levels of 40 and 45 mmHg ($P$
= 0.046 and 0.021, respectively, paired t-tests). No statistically significant difference was found in the radial strains between the inner layer and the outer layer at any pressure level measured.

**Figure 5.9** Average tangential strain of the outer layer, the middle layer and the inner layer
Figure 5.10 Average radial strain of the outer layer, the middle layer, and the inner layer.

The average tangential strains within the nasal, temporal, superior and inferior quadrants of the three samples tested were plotted in Figure 5.11. The average radial strains in the four quadrants were plotted in Figure 5.12. The temporal tangential strains seemed to be the largest among the four quadrants, while the nasal tangential strains appeared to be lower than the others. At 45 mmHg, the temporal tangential strains were significantly greater than the nasal or the inferior tangential strain (P = 0.0062 and 0.0298, respectively, paired t-test). The temporal radial strains were also greater than the other quadrants’. At 45 mmHg, the temporal radial strains were significantly greater than those in the superior or the inferior quadrants (P = 0.0195 and 0.0107, respectively, paired t-test).
Figure 5.11 The average tangential strain vs. pressure in the four quadrants measured.
The average radial strain vs. pressure in the four quadrants measured.

Figure 5.13 and Figure 5.14 show the comparison between the tangential strains and the radial strains in the meridian scanning directions and the circumferential directions on the three samples measured if the nasal, temporal, superior, and inferior quadrants were lumped together in the analysis. The meridian tangential strains appeared to be greater than the circumferential tangential strains although the difference did not achieve statistical significance with the current sample size ($P = 0.08$). The radial strains seemed to be similar between the two directions ($P = 0.74$). This trend was consistent with the previous findings in the temporal quadrant only analysis (i.e., posterior pole) with a larger sample size ($n = 11$) except that the latter showed statistically significant difference in the tangential strains between the two scanning directions.
Figure 5.13 Average tangential strain in all four quadrants vs. pressure at different scanning directions.

Figure 5.14 Average radial strain in all four quadrants vs. pressure at different scanning directions.
5.4 Discussion

Consistent with the results on porcine sclera in the previous chapter, the ultrasound speckle tracking showed negative radial strains (compression) and positive tangential strains (extension) when the IOP was elevated. In general, the tangential strains increased non-linearly with the pressure increase (Figure 5.6), which was consistent with previous studies that reported non-linear surface strains on human scleras [71, 118, 120]. The non-linearity in the strain-pressure relationship may be explained by the fact that the collagen fibers in sclera would uncrimp and strengthen the sclera during the stretch when IOP is elevated [121]. The radial strains seem to be almost linear when compared to the tangential strain, which may be explained by the lack of the collagen fibers along the radial direction in sclera. This is also different from the slightly non-linear increase in the radial strain with pressure increase in the porcine sclera in the previous chapter, but the radial strains in the human sclera were generally much smaller than those in the porcine sclera, so the strain-pressure relationship may become more non-linear as the strain or pressure further increases in the human sclera.

The radial strains were much higher in magnitude than the tangential strains (about more than 6 times in average at 45 mmHg), which may suggest that there was compression of the sclera during the pressure elevation. Previous studies have reported a compressive modulus orders of magnitude smaller than the tensile modulus in human and porcine sclera [133, 134] so it is reasonable that the sclera is much easier to be compressed in radial direction than being stretched tangentially. Although the tangential stress ($\sigma$) is about 6 times higher in magnitude than the radial compressive stress ($P$) at
the inner boundary, based on a rough estimation from the Laplace equation \( \sigma = PR/(2T) \) assuming a thickness (T) of 1 mm and radius of curvature (R) of 12 mm, our results suggested that the radial compression could be substantially greater than the tangential extension. It should be noted that the current study only examined the tangential strains and the radial strains in the two cross-sections scanned thus it may not necessarily reflect the actual volume change in the 3D circumstance. It should also be acknowledged that this study employed some sclera samples with more than a week’s post mortem time and there might be some level of swelling in the samples prior to the measurements. It is generally believed that the collagen fibers which are the major load-bearing component to sustain the tangential strains are relatively more stable than the proteoglycans post mortem [232]. If we consider that the tangential strains were mostly affected by the collagen fibers and the radial strains are affected by the proteoglycans, the measured tangential strains should be less affected by the post mortem time than the radial strains. Nevertheless, the large magnitude of the compressive strains, if confirmed in future in vivo studies, could have important implications in the mechanobiology.

The sclera showed stiffer response in the circumferential direction than in the meridian direction at the posterior pole of the sclera. This finding may be explained by the relatively preferred fiber orientation along the circumferential direction on the posterior sclera close to the ONH [122, 123]. This is also consistent with a previous report using surface strain measurements [120]. Circumferential arrangement of collagen fibers around the ONH could significantly reduce canal expansion but could not prevent the posterior bulging under IOP elevation [139]. It has been shown that glaucomatous
eyes have stiffer response in the meridian direction than the normal eyes [120], which may indicate the active remodeling of the collagen fibers and alternations of the fiber alignment during the progression of glaucoma. The current study showed significant difference along the radial and circumferential directions at the posterior pole in the 11 samples, but the difference in the 3 samples combining all four quadrants around the ONH was not statistically significant and may require further studies on a larger sample size.

The current study showed smaller tangential strains in the outer layer than in the middle layer or the inner layer, and the radial strains in the middle layer was higher in magnitude than those in the inner or the outer layer. The difference in the strains at different depth might be related to the microstructure difference of sclera through its thickness. As discussed in the previous chapter, different collagen bundle size and arrangement in the inner, the middle, and the outer layers of the sclera have been reported in previous studies [11, 12]. The heterogeneity of the radial strains and the tangential strains through-thickness was consistently found in most of the human scleras tested, which warrants further studies to elucidate the underlying mechanisms and its implications to scleral biomechanics and pathophysiology. It should also be noted that the stress distribution throughout the thickness could vary although the sclera is often considered thin membrane. With the assumption of homogeneous and isotropic properties and the simplified geometry of a uniform spherical shell, the radial stress would be highest at the inner boundary and decrease to zero at the outer boundary, and the tangential stress would be highest at the inner boundary and decrease to minimum at the
outer boundary. The radial stress $\sigma_r$ and the tangential stress $\sigma_c$ can be estimated using the following equations [134].

$$\sigma_r = \text{IOP} \left[ 1 - \left( \frac{b}{r} \right)^3 \right] / \left[ \left( \frac{b}{a} \right)^3 - 1 \right] \quad (5.4)$$

$$\sigma_c = \text{IOP} \left[ 2 + \left( \frac{b}{r} \right)^3 \right] / \left[ 2 - 2 \left( \frac{b}{a} \right)^3 \right] \quad (5.5)$$

The $a$ and $b$ are the inner and outer radii of the shell, respectively, and $r$ is the radial position measured from the center of the sphere. Based on this estimation, the tangential stress at the inner boundary is roughly 1.1 times larger than that at the outer boundary, assuming inner radii and outer radii of 11 mm and 12 mm, respectively. Future studies using finite element analysis based on experimentally measured geometry and properties are needed to obtain more accurate prediction of the stress distribution through the thickness of the sclera, without relying on the highly-simplifying assumptions. Although the through-thickness difference in the stresses may partially account for the through-thickness difference in the strains found in current study, our results indicated that the surface strain measurements could underestimate the actual strains inside the sclera. Future studies are needed to elucidate the implications of the variations in strains through the thickness of the sclera and their potential consequences to the pathophysiology of the retina which is close to the inner surface of the sclera.

The difference in both the radial and the tangential strains between the four quadrants were consistent with the previous study showing that the temporal quadrant is more compliant than the others [118]. The temporal area has been found to have increased prevalence of glaucomatous damage [118] and greater thickness than other quadrants [120]. The current finding in quadrant difference would need further studies on
a larger sample size and glaucoma samples to explore its potential role in glaucoma progression. It should also be acknowledged that the temporal quadrant was always the first quadrant to scan in the current study so as to make the posterior pole strain on these three samples comparable to the other 8 samples which did not go through all four quadrants scanning. It has been reported that human scleras exhibited hysteresis in the strain response during inflation and deflation [120], a clear indication of viscoelasticity. It is thus important to have the sclera fully recovered before the next loading cycle can be repeated. There was 6 minutes allowed between the scans in the current studies but the time required for full recovery of human sclera has not been fully understood or reported in the literature. Future studies should identify the time required for full recovery of the sclera when the IOP is brought back to the baseline, and should employ fully randomized design in the scanning scheme of the four quadrants.

5.5 Summary

The current study found a significant anisotropic response and heterogeneous strain distribution throughout the thickness of human sclera during IOP elevation. There was also initial evidence of regional variation in the scleral response to IOP elevation. The results broadened our knowledge of human sclera mechanical response to IOP and could provide further insight into the understanding of glaucoma related sclera biomechanics.
Chapter 6 Summary and future work

6.1 Summary

The objective of this study was to estimate the corneal and sclera biomechanical properties using non-invasive ultrasonic method for better management and understanding of glaucoma. Four specific aims were accomplished in this study. A summary of the work for each aim was presented in this section.

**Aim 1:** To test the hypothesis that the accuracy of the corneal thickness measurement by using ultrasound pachymeter is influenced by the variation in speed of sound in cornea. The objective was to examine the magnitude and variance of speed of sound in canine cornea models, and explore the potential correlation between the acoustic impedance and the speed of sound in cornea that may provide correction for corneal thickness measurement.

This aim has been successfully achieved, and the variation of speed of sound in cornea has been examined. The speed of sound in cornea showed significant correlation with the acoustic impedance, which can be measured non-invasively and thus provides a potential approach to make corrections on CCT measurements. The age effect on speed of sound in corneas may provide more insight into the development of corneal microstructure.
**Aim 2:** To test the hypothesis that IOP measurement by applanation tonometry is affected by corneal stiffness. The purpose of this study was to experimentally examine the effect of corneal modulus on applanation tonometry using a canine eye model, and to explore the potential correlation between the acoustic impedance and the applanation tonometry error for correction in IOP measurement.

This study successfully demonstrated a significant effect of corneal stiffness on applanation tonometry through experimental investigations. The correlation between acoustic impedance and IOP measurement error provides a potential way of making corrections on IOP measurements in clinics.

**Aim 3:** To develop a non-invasive ultrasonic method to assess sclera mechanical response under IOP loadings. The purpose of this study was to develop the method, evaluate its performance through experiment and simulations, and to obtain initial measurement of through thickness strains on animal eye models (i.e., porcine eyes).

The ultrasonic strain imaging method was developed for sclera and it has been shown to be able to measure the scleral strains at relatively high SNR. The initial results on porcine eyes demonstrated significant larger axial compression than lateral extension during IOP elevation, and the response of sclera was nonlinear to pressure increase and anisotropic in the tangential strains between the meridian direction and the circumferential direction.

**Aim 4:** To examine the human sclera biomechanical response to IOP elevation using the ultrasound method developed. The purpose of this study was to measure the
through-thickness deformation of human sclera and to examine the potential anisotropy between the meridian and the circumferential directions around the ONH.

This goal has been satisfactorily achieved and the study showed much greater radial compression on human sclera under IOP elevation than tangential extension. Also the human scleras showed heterogeneous deformation through thickness and anisotropic response in the tangential strains along different scanning directions. The regional variation in scleral strains was also examined. These findings provided important information to improve future mechanical modeling of human sclera and may offer further insight into mechanobiology studies for better understanding glaucomatous damage on neuron axons.

6.2 Future work

Based on the results of the current study, some further steps could be taken to advance our knowledge about human cornea and sclera biomechanics using non-invasive ultrasonic methods.

In chapter 2, the acoustic impedance showed significant correlation with the speed of sound in canine cornea. The future work can be to measure the speed of sound in human corneas and examine its correlation with acoustic impedance. The potential age effect in the speed of sound should also be carefully examined on human samples with large sample size.

The acoustic impedance could potentially be used as surrogate for the corneal stiffness and was significantly correlated with the applanation tonometry error as
demonstrated in chapter 3. Since the error in applanation tonometry could be affected by the combined effect of corneal thickness, corneal curvature and corneal stiffness, future studies can be designed to collect more data on human corneas so as to determine the correction equations for IOP measurement using all these factors.

The scleral biomechanical response to IOP loadings can be accurately measured by the ultrasonic speckle tracking method as discussed in chapter 4 and 5. Larger sample size of good quality human cadaver eyes will be needed to conduct more measurements and compare the regional difference along the posterior sclera, which could potentially identify weak spot on the human sclera. Future studies on glaucomatous human eyes will also be necessary to directly examine the potential glaucomatous risk factors on sclera or glaucomatous damage on sclera. The current ultrasound method is non-invasive and could potentially be used for \textit{in vivo} measurements, with some modifications in the experimental setup. The current ultrasound system has limited penetration depth but is enough to take measurement on the exposed anterior sclera. With lower frequency ultrasound (i.e., 20MHz) the posterior sclera can be imaged with compromised resolution. The current strategy of inducing IOP elevation was invasive and can only be applied during surgery for \textit{in vivo} measurement. Future improvement may employ some other non-invasive methods of manipulating IOP, such as changing the sitting position, drinking test, or other devices that can flatten, indent or suck the ocular shell. Future development on 3D strain imaging would also help to reduce the potential out of plane motion artifact presented in the 2D strain imaging during \textit{in vivo} measurement.
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