A hybrid approach to determining cornea mechanical properties using a combination of inverse finite element analysis and experimental techniques

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A hybrid approach to determining cornea mechanical properties using a combination of inverse finite element analysis and experimental techniques

by

Maryam Haghighi Abyaneh

Thesis submitted for the award of Doctor of Philosophy

November 2013
Abstract

It is of great clinical importance to predict the behaviour of the cornea in various diseases and post-surgical recovery. Therefore, a numerical model that is able to simulate the corneal behaviour, considering corneal material properties obtained from individuals is highly desirable. In this work a combined numerical-experimental technique has been developed that can characterize the mechanical properties of a cornea properties from two aspects: time-dependency and spatial variation. Initially, an analysis of the material properties of porcine corneas was performed to investigate the time-dependant behaviour of the cornea. A simple stress relaxation test was used to determine the viscoelastic properties of a cornea and a rheological model was built based on the Generalized Maxwell (GM) approach. A validation experiment using nano-indentation showed that an isotropic GM model was insufficient for describing the corneal time-dependent behaviour when exposed to a complex stress state. A technique was proposed that takes into account the microstructural composition of the cornea and is based on a combination of nano-indentation experiment, isotropic and transversely isotropic numerical models, and an inverse finite element method. The good agreement using this method suggests that this is a promising technique for measuring the time-dependent properties of the cornea. The spatial variation of the properties was then investigated. This time, the long–term structural response of the cornea was targeted. A full field displacement response of a loaded cornea was evaluated from Optical Coherence Tomography (OCT) volume reconstructions of the cornea using Digital Volume Correlation (DVC). The inverse finite element method was employed with two models sequentially; first, a radially partitioned model and then a circumferentially partitioned model, in order to recover the elastic parameters in radial and circumferential directions. The good agreement using this method suggests that this is a promising and reliable technique for identifying the distribution of the corneal properties.

In this research, we have shown that it is possible to determine the local time-dependent properties of the cornea and the in-depth (2D) distribution of the properties using the hybrid technique. This technique has the potential to be implemented in vivo. However, further work should focus on the feasibility of this technique in practice.
Acknowledgements

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Finally and for most, I would like to thank my family, father, mother and Minche, for everything.
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<th>Description</th>
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<tr>
<td>AAC</td>
<td>Artificial Anterior Chamber</td>
</tr>
<tr>
<td>ACT</td>
<td>Artificial Corneal Teriphinate</td>
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<tr>
<td>ALE</td>
<td>Arbitrary Lagrangian-Eulerian</td>
</tr>
<tr>
<td>DCT</td>
<td>Dynamic Contour Tonometry</td>
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<tr>
<td>DIC</td>
<td>Digital Image Correlation</td>
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<tr>
<td>DVC</td>
<td>Digital Volume Correlation</td>
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<tr>
<td>FEM</td>
<td>Finite Element Model</td>
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<tr>
<td>GAT</td>
<td>Goldman Applanation Tonometry</td>
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<td>GM</td>
<td>Generalized Maxwell</td>
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<tr>
<td>IFEM</td>
<td>Inverse Finite Element Method</td>
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<tr>
<td>Infl.</td>
<td>Inflation</td>
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<td>IOP</td>
<td>Intra-Ocular Pressure</td>
</tr>
<tr>
<td>I-S</td>
<td>Inferior-Superior</td>
</tr>
<tr>
<td>LASIK</td>
<td>Laser-Assisted In-Situ Keratomileusis</td>
</tr>
<tr>
<td>L-M</td>
<td>Levenberg-Marquardt</td>
</tr>
<tr>
<td>MRE</td>
<td>Magnetic Resonance Elastography</td>
</tr>
<tr>
<td>NI</td>
<td>Nano-Indentation</td>
</tr>
<tr>
<td>N-T</td>
<td>Nasal-Temporal</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical Coherence Tomography</td>
</tr>
<tr>
<td>O-P</td>
<td>Oliver-Phar</td>
</tr>
<tr>
<td>OPA</td>
<td>Ocular Pulse Amplitude</td>
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<tr>
<td>OPD</td>
<td>Optical Path Difference</td>
</tr>
<tr>
<td>Relax.</td>
<td>Relaxation</td>
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<tr>
<td>RMS</td>
<td>Root Mean Square</td>
</tr>
<tr>
<td>SS-OCT</td>
<td>Swept Source-Optical Coherence Tomography</td>
</tr>
<tr>
<td>STD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>TD-OCT</td>
<td>Time Domain-Optical Coherence Tomography</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$A$</td>
<td>Contact area</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Indenter’s shape factor</td>
</tr>
<tr>
<td>$C(t)$</td>
<td>Creep function</td>
</tr>
<tr>
<td>$E(x,y,z)$</td>
<td>Elastic modulus in Cartesian system</td>
</tr>
<tr>
<td>$E(r,\theta)$</td>
<td>Elastic modulus in polar system</td>
</tr>
<tr>
<td>$E_r$</td>
<td>Reduced modulus</td>
</tr>
<tr>
<td>$E_\infty$</td>
<td>Long-term elastic modulus</td>
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<tr>
<td>$e$</td>
<td>Strain</td>
</tr>
<tr>
<td>$e^*$</td>
<td>Strain rate</td>
</tr>
<tr>
<td>$e_0$</td>
<td>Constant strain</td>
</tr>
<tr>
<td>$\varepsilon_{ij}$</td>
<td>Strain components</td>
</tr>
<tr>
<td>$F$</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>$G_0$</td>
<td>Instantaneous shear modulus</td>
</tr>
<tr>
<td>$G(t)$</td>
<td>Shear relaxation modulus with respect to time</td>
</tr>
<tr>
<td>$g_i$</td>
<td>Normalized shear relaxation moduli</td>
</tr>
<tr>
<td>$H$</td>
<td>Indentation depth</td>
</tr>
<tr>
<td>$I$</td>
<td>Identity matrix</td>
</tr>
<tr>
<td>$J(t)$</td>
<td>Creep function</td>
</tr>
<tr>
<td>$J$</td>
<td>Jacobean matrix</td>
</tr>
<tr>
<td>$M(t)$</td>
<td>Relaxation function</td>
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<tr>
<td>$m(c)$</td>
<td>Cross-correlation function</td>
</tr>
<tr>
<td>$P$</td>
<td>Indentation load</td>
</tr>
<tr>
<td>$P$</td>
<td>Pressure</td>
</tr>
<tr>
<td>$v$</td>
<td>Poisson’s ratio</td>
</tr>
<tr>
<td>$S$</td>
<td>Stiffness</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Stress</td>
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</table>
\( \eta \)  
Viscosity

\( \tau \)  
Relaxation time

\( \Gamma \)  
Shear stress

\( \theta_0 \)  
Incident angle

\( \gamma \)  
Shear strain

\( U(t) \)  
Magnitude of displacement with respect to time

\( U_{j,i} \)  
Magnitude of displacement components

\( u_{j,i} \)  
Displacement components

\( \Omega \)  
Sub-volume

\( \nabla f(x_i) \)  
Gradient of the function \( f(x_i) \)
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Chapter 1: Introduction

1.1 Background

It is often of great clinical importance to predict the behaviour of the cornea, e.g. when affected by various diseases and during post-surgical recovery. To do this, a numerical model that is able to simulate the corneal behaviour while at the same time is easily modified for individuals are highly desirable. This model should represent the geometry, boundary conditions and material properties of the cornea from clinical measurements. The literature describes numerous studies aimed at developing finite element models that replicates the cornea in situ, under normal physiological and various diseased conditions. Some aspects of these models, such as geometry and boundary conditions, can be generated from clinical measurements that typically measure the cornea interior and posterior curvatures, thickness and the intraocular pressure. Amongst the various input parameters required in a numerical model, those related to the corneal material properties are the most challenging to determine. A true representative of the corneal properties should account for corneal inhomogeneity as well as for its time-dependent characteristics. A number of analytical rheological models have been developed that attempt to incorporate these aspects, either by attributing them to the corneal microstructural constituents, i.e. collagen fibres and proteoglycan matrix, separately or to the cornea as a whole.

To date, and to the best of our knowledge, none of the developed analytical rheological models, which allow for full description of corneal properties, have been implemented into a numerical model. The available numerical models, however, have considered various time-independent rheological models such as hyperelastic models rather than the time-dependent ones and have accommodated the inhomogeneity by dividing the cornea to various homogeneous regions. The parameters of the time-independent models were then evaluated by either the classical method of curve fitting to the experimental data or the more recent method of inverse numerical updating that has been conducted by a small number of research groups.
1.2 Original contribution of this work

In this work for the first time, a method is developed that is able to determine the local parameters of the corneal rheological model in microstructural level and the spatial distribution of the properties with the help of two sets of experiments. The method proposed combines two distinct experimental methods with a numerical method of inverse finite element analysis in order to characterize the cornea based on different structural responses. Point wise data obtained from nano-indentation experiment was used in combination with the inverse finite element method to determine the parameters of a pre-existing time-dependent rheological model. In doing so, the microstructure of the cornea became of primary importance. To accommodate the fibre-matrix composite-like structure of the cornea, a method using two numerical models in sequence was developed. To validate the method, the obtained parameters were compared and contrasted with those obtained from a classical stress relaxation experiment.

A combination of full field data obtained from an inflation experiment and the inverse finite element method was then used to determine the spatial variation of the corneal properties. In this approach, the long-term behaviour of the cornea, which is time-independent, was targeted. In order to solve this problem efficiently, the spatial variation of the parameters were first studied through the radial direction and then through the circumferential direction utilizing two numerical models. In the models, the cornea was partitioned into a number of isotropic homogeneous regions using different partitioning schemes. In the first stage, a radially partitioned model was used to recover the mean variation of the parameters along the radial direction. Having obtained a pattern for the variation of the parameters along the radial direction then the variation of the parameters along the circumference (which was assumed previously to be uniform) was examined using a circumferentially partitioned model.

It was the main concern of our work to obtain the properties via the experimental methods that are non-invasive and have the potential to be implemented in vivo. Both of the experiments used in this project, can be modified to replace the existing clinical measurement such as those used for measurement of the intra ocular pressure and the corneal thickness. In addition, the characterization method
developed in this work paves the way for determining the corneal inhomogeneous and time-dependent properties collectively, using an appropriate experimental method that can provide the full field and the time-dependent response, simultaneously.

1.3 Arrangement of the thesis

Chapter 2 provides a background of previous relevant work on the cornea. Chapters 4, 5 and 6 are dedicated to describing the three main experimental procedures used in this work and providing their results.

Chapter 4 provides an overview of the corneal rheological model and the procedure for the stress relaxation experiments (used for validation) is described.

Chapter 5 describes the indentation experimental procedure and presents the results from the experiments.

Chapter 6 describes the inflation experiment and explains how the full-field data were evaluated. The full-field cross-sectional data for the middle of the cornea is shown.

Chapter 7, 8 and 9 describe the numerical methods developed in this work as follows:

Chapter 7 describes the finite element modelling used in the inverse method. The two models of nano-indentation, i.e. isotropic and transversely isotropic, are used in chapter 8 in order to develop the inverse method for characterizing the corneal time-dependent behaviour. The two inflation models, with different partitioning schemes, are used to determine the spatial distribution of the properties.

Chapter 8 introduces a background to the inverse method. It also provides a general flowchart for the developed algorithm and the variation for each case.

Chapter 9 provides solutions from the inverse method and discusses them. It is divided into two sections. One section provides the inverse solution for the time-dependent parameters and the other one presents the solution for distribution of the parameters.
Chapter 2: Literature Review

2.1 Anatomy of the Cornea

The cornea is a transparent front part of the eye that covers the iris, pupil, and anterior chamber. Figure 2-1 shows a cross-sectional front view of an eyeball demonstrating the location of the cornea.

![Figure 2-1 Schematic of front part of eyeball](image)

The cornea is joined to the white of the eye, known as the sclera, via the limbus. The limbus is a transitional zone around the cornea with approximately 1.5–2.0 mm width and is positioned between the cornea and sclera [1]. The sclera is a membrane made of rugged and robust tendon with thickness of about 760 μm. The cornea is a transparent tissue due to its lattice microstructural arrangement. However, in the sclera, a random arrangement of the same microstructural components results in opaqueness [1, 2]. About 30mm away from the cornea, six straight muscles, called ciliary muscles, are attached to the eyeball. Four of them control the movement of the eye, known as rectus muscles, and the other two controls the eye contractions, known as oblique muscles. The net forces of the oblique muscles on the eye were studied by Bell et al. (1982) but the magnitude of their effect on the cornea is not currently fully understood [2].
The cornea refracts light and is responsible for approximately two-thirds of the eye's total optical power [3]. While the cornea contributes the most to the eye's focusing power, its focus is fixed. The curvature of the lens, on the other hand, can be adjusted to tune the focus. Because transparency is of prime importance, the cornea does not have blood vessels. It receives nutrients via diffusion from the tear fluid through the outside surface (epithelium), diffusion from the aqueous humour through the inside surface (endothelium) and from some chemicals supplied by nerve fibres [4]. As the cornea does not have a blood supply, it obtains oxygen directly through the air. The oxygen first dissolves in the tear fluid and then diffuses throughout the cornea [4]. The human cornea, like those of other primates, is composed of five layers. However, the cornea of pig and other herbivores only have four layers [5]. The five layers of the human cornea from the anterior to posterior are shown in Figure 2-2.

![Figure 2-2 Schematic of human corneal layers](image)

1- Epithelium
This is a thin layer, 30-35 µm thick, consisting of easily regenerated cells. It resists a free flow of tear fluids, and prevents bacteria from entering the corneal stroma.
2- Bowman's membrane
This is a tough and condensed layer, 8-14 µm thick, comprised of irregularly
arranged collagen fibres. It protects the corneal stroma and is absent or very thin
in non-primates [5].

3- Stroma
This is a thick layer composing approximately 90% of the corneal overall
thickness. It consists of regularly arranged collagen fibres (type one) embedded
in a matrix of proteoglycan. There are two theories regarding the transparency of
the cornea:

a. Corneal transparency is due to the lattice arrangement of the collagen fibres
in the stroma, where the light scatter by individual fibres is cancelled by
destructive interference of the light from other fibres [7].

b. The spacing of the neighbouring collagen fibres in the stroma must be less
than 200 nm for the cornea to be transparent [8].

4- Descemet's membrane
This is a thin acellular layer, around 5-20 µm thick, composed of collagen fibres
(type two) less rigid than stromal collagen fibres. It serves as a modified
basement membrane for the stroma to grow.

5- Endothelium
This is a simple monolayer, approximately 5 µm thick. It is responsible for
regulating fluid transport between the aqueous humor and corneal stroma.
Unlike the corneal epithelium, the endothelium cells do not regenerate. Instead,
they stretch to compensate for dead cells. If the endothelium can no longer
maintain a proper fluid balance, stromal swelling occurs and subsequently the
cornea loses its transparency [9].

2.1.1 Geometry

Corneal geometry is part of an ellipsoid (the reason it is not a sphere is because of
the scleral overlap above and below). A normal cornea is a prolate ellipsoid, i.e.,
steeper in the center and flatter in the periphery. An abnormal cornea is, however, an
oblate ellipsoid, i.e., flatter in the centre and steeper in the periphery, as shown in
Figure 2-3. The oblated cornea focuses the light rays reflected from an object at two
points rather than just one, so that the object will seem distorted or stretched, a condition known as astigmatism [10].

![Figure 2-3 Different types of corneal ellipsoid geometry, a) prolate ellipsoid (normal cornea) and b) oblate ellipsoid (abnormal cornea) [10]](image)

The cornea has two perpendicular diameters when projected on a surface. The horizontal diameter, which is along the nasal-temporal (N-T) direction, is longer than the vertical diameter, which is along the inferior-superior (I-S) direction. The average horizontal and vertical diameters are 11.7 mm and 10.6 mm for the human cornea [11] and 14.9 mm and 12.4 mm for the porcine cornea [12] respectively.

The corneal thickness for humans varies from limbus to limbus, with a thinner centre, 515-540 µm, and thicker periphery, 750-790 µm, according to a number of studies shown in Table 2-1. In general, corneal thickness for humans varies up to 150 µm from the centre to the periphery [11]. Porcine cornea is, however, on average 70 µm thicker than human cornea, showing a less regular variation from centre to periphery [12]. A detailed study conducted by Elsheikh et al. (2007) showed that corneal thickness for a normal human can be influenced by parameters such as age, gender, and ethnicity [13]. A statistical study by Doughty et al. (2006) showed that young male adults between ages of 20 to 30 have thicker cornea, whereas, children of up to the age of six have the thinnest cornea. In addition, some diseases, such as keratoconus, affect the thickness of the corneal stroma [14]. Accurate measurement
of the thickness is important because it can mask an accurate reading of intraocular pressure (IOP) and can lead to incorrect predictions of glaucoma [15]. For instance, a thin cornea with less than 535µm thickness will show an artificially low IOP reading.

Figure 2-4 Dimensions of human cornea between the age of 20 to 30, a) diameters, b and c) radii of curvature, d) anterior depth and e) thickness [11]
Other crucial corneal dimensions are the interior and posterior radii of curvature. An accurate measurement of the corneal curvature is important for the study of optical focus and diagnosing astigmatism [20]. Table 2-2 presents a comparison of the value of radii of curvature for human cornea obtained by various researchers. The study carried out by Fernandez et al. (2002) is often taken as the main reference [20].

Table 2-1 Corneal thickness for humans measured by various researchers

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Central Thickness (μm)</th>
<th>Peripheral Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maurice et al. (1978)</td>
<td>526±5</td>
<td>758±5</td>
</tr>
<tr>
<td>Ashwin et al. (2009)</td>
<td>537±5</td>
<td>763±5</td>
</tr>
<tr>
<td>Dubbelman et al. (2006)</td>
<td>530 ± 5</td>
<td>755 ± 5</td>
</tr>
<tr>
<td>Boote et al. (2003)</td>
<td>527 ± 8</td>
<td>765 ± 5</td>
</tr>
</tbody>
</table>

Table 2-2, Corneal radii of curvature for humans measured by various researchers

<table>
<thead>
<tr>
<th>Research group</th>
<th>$R_{Posterior}$ (mm)</th>
<th>$R_{Anterior}$ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fernandez et al. (2002)</td>
<td>6.50±0.2</td>
<td>7.80±0.2</td>
</tr>
<tr>
<td>Elsheikh et al. (2007)</td>
<td>6.49±0.05</td>
<td>7.82±0.20</td>
</tr>
<tr>
<td>Dubbelman et al. (2006)</td>
<td>6.53±0.3</td>
<td>7.79±0.27</td>
</tr>
<tr>
<td>Kwok et al. (1984)</td>
<td>6.52±0.27</td>
<td>7.75±0.28</td>
</tr>
</tbody>
</table>
2.1.1 Microstructure

The mechanical properties of the cornea are derived primarily from a particular architecture of the collagen fibres, which reveal a high degree of spatial anisotropy. Several research groups have investigated the arrangement of the stromal collagen fibres for human cornea using synchrotron x-ray scanning [24], [1]. They mapped the orientation of collagen fibres across and through the thickness of the cornea and limbus [1, 24, 25]. An early study by Aghamohammadzadeh et al. (2004) obtained an x-ray scattering intensity contour plot of the fibre orientation across human cornea and limbus. The plot also indicated the number of fibres and the preferential orientation through the thickness [24]. Later, Pinsky et al. (2005) used Aghamohammadzadeh’s data to develop a mathematical model for the corneal anisotropy based on the angular probability of the strain energy density function [26].

The scattered intensity data obtained by Aghamohammadzadeh et al. (2004) revealed that collagen fibres exhibit a preferred orientation that varies from point to point throughout the cornea and limbus. In general, corneal collagen fibres are organized into dome-like concentric oriented sheets (lamellae) embedded in a proteoglycan matrix, as shown in Figure 2-5, with approximately 300 lamellae through the thickness in the centre and approximately 500 lamellae through the limbus [26].

Figure 2-5 Arrangement of concentric collagen lamellae throughout corneal thickness [19]
Collagen lamellae have a preferred orthogonal orientation along the nasal-temporal and the inferior-superior directions in the centre, showing a gradual transition to tangential orientation toward the periphery [26, 27]. The preferred orientation is more pronounced in the posterior stroma than the anterior stroma [28]. The same pattern of fibre orientation is observed in the porcine cornea, with a difference in the size of the area with the preferred orientation [29]. Figure 2-6 illustrates a point-based orientation of the collagen fibres across the human cornea and limbus.

Figure 2-6 Diagram of preferred orientation of collagen fibres across human cornea and limbus showing orthogonal orientation in centre and tangential orientation at the circumference. The central and circumferential regions are divided with lines [24]

2.2 Corneal Diseases and Disorders

Understanding corneal biomechanical response in the case of various diseases and post-surgeries is of great clinical importance. Knowing that the cornea acts as the eye's outermost lens with 65%-75% of the total focusing power, any change in its curvature can cause focusing problems such as, myopia, hyperopia and
astigmatism; conditions generally known as refractive problems. Myopia or near-sightedness can occur due to high corneal curvature. Hyperopia or farsightedness can occur due to low corneal curvature [5]. It should be mentioned, however, that myopia and hyperopia can also result from lens malfunction. Astigmatism, or blurred vision, is another refractive disorder that can arise from abnormal or uneven corneal curvature [10]. Glasses, contact lenses and refractive surgery can correct these problems.

Some diseases, such as corneal dystrophies, alter the shape and thickness of the cornea permanently. This can cause focusing problems, or in severe cases can damage general vision and cause blindness. A corneal dystrophy is a condition in which one or more part of the cornea loses its thickness gradually [30][30, 23]. The most common corneal dystrophies include Fuchs' dystrophy, keratoconus and lattice dystrophy [30].

Fuchs' dystrophy occurs when the endothelial layer gradually deteriorates. The endothelial layer then becomes less efficient at pumping aqueous humour out of the stroma, causing corneal swell and a change of curvature, which leads to severe pain and visual impairment [31].

Keratoconus is the most common corneal dystrophy. It arises when the centre of the cornea thins and gradually bulges outward, forming a rounded cone shape. This abnormal curvature changes the cornea's refractive power, producing moderate to severe distortion (astigmatism), myopia, light sensitivity, and consequently pain [32].

Lattice dystrophy occurs with accumulation of abnormal fibres deposited throughout the stroma. These deposits are viewed as comma-shaped overlapping dots and cause fibres to branch and create a lattice effect. Over the course of time, the lattice lines will grow opaque and give the cornea cloudiness [33].

Early stage treatment for dystrophies may involve frequent visits and prescription of ointments, drops or bandage contact lenses but advanced cases may call for surgical interventions, sometimes in the form of corneal transplantation [34].

Refractive eye surgery is a common term for all types of eye surgeries that are used to reduce or cure vision disorders such as myopia, hyperopia and astigmatism, as
well as degenerative disorders, such as keratoconus. In general, all the refractive surgeries involve an incision through the cornea or the sclera. They can be classified based on whether the induced incision changes the curvature of the corneal anterior surface. In cataract extraction surgery for instance, the scleral incision is designed to minimize changes in the corneal surface, whereas in Laser-Assisted In-situ keratomileusis (LASIK), modifying the corneal anterior surface is the primary objective [18]. However, in both classes of surgery, the achieved outcome can differ from the planned outcome due to the mechanical deformation of the cornea. Occasionally, the refractive surgeries result in different levels of postoperative astigmatism [17]. In the case of cataract surgery, the incision causes local load redistribution and consequently changes the curvature of the cornea (astigmatism) [19]. The direction and whereabouts of the scleral incision plays an important role in the occurrence of postoperative astigmatism [25, 35]. In general, a detailed knowledge of the relative local fibre orientation and density will allow the surgeon to decide on the most appropriate incision site and direction [36]. If the mechanical response of the cornea could be modelled in such a way as to indicate the likely outcome of a surgical procedure, it could be possible to avoid any post-operative complexities. This is one of the driving forces for the current work.

2.3 Clinical Measurement

Regular ophthalmic measurement becomes important when there is a risk of eye diseases, in particular glaucoma. Glaucoma is an eye disease, in which the optic nerves are damaged and can lead to blindness if left untreated. It is normally associated with increased fluid pressure in the eye. A suspicion of glaucoma arises when a more frequent fluctuation of the intraocular pressure is detected. However, this can be controlled by monitoring the corneal intraocular pressure and taking appropriate action [15]. Other corneal measurements, such as curvature and surface measurements become significant in the case of refractive disorders and astigmatism.
2.3.1 Corneal Thickness Measurement

The measurement of corneal thickness is known as pachymetry. Pachymetry is an important corneal measurement, especially prior to ophthalmic surgeries to ensure sufficient corneal thickness to prevent corneal abnormal bulging, a side effect known as ectasia, [37]. It is essential for the early detection of glaucoma if it is to be treated successfully [38]. Pachymetry involves various techniques such as ultrasonic [28, 31], confocal microscopy [39], and Optical Coherence Tomography (OCT) [40].

A common approach is ultrasonic pachymetry, in which the thickness is measured from a delay of a reflected ultrasound from anterior and posterior corneal surfaces. It is an efficient and accurate way to measure corneal thickness [31]. However, its accuracy and reproducibility relies heavily on the perpendicularity of the ultrasound probe to the corneal centre [31, 32].

Confocal microscopy measures the corneal thickness from a delay of a reflected light from two focusing points on the anterior and posterior surfaces [33]. Confocal microscopy is different to conventional microscopy in the way that it limits the reflection of the light scattered from outside the focal point [130]. The precision of confocal microscopy is limited by corneal motion [131].

A more recently developed technique is optical coherence tomography (OCT), which is based on light interferometry principles [43, 133, 143]. OCT is a noncontact cross-sectional imaging method with high resolution. It produces rapid and reproducible thickness mapping over a wide area of the cornea, unlike the ultrasonic and the confocal microscopy technique [34].

Several studies have compared the corneal thickness measurements obtained via ultrasonic and optical techniques [16, 35-37]. They found that the thickness readings obtained from the optical techniques were slightly smaller than those from the ultrasonic technique due to limitations when measuring the corneal refractive index [41, 42].
2.3.2 Corneal Curvature/Power Measurement

Mapping the corneal anterior surface and measuring its radius of curvature are essential measurements for intraocular lens calculations, refractive surgeries, contact lens fitting, and detecting astigmatism [43]. The corneal dioptre power can, furthermore, be calculated from a formula knowing the anterior radius of curvature [44]. The anterior surface of the cornea is obtained using various topography methods, such as computerized videokeratography [45], elevation-based keratography [46] and interferometry-based keratography [47].

In general, corneal topography, also known as keratometry, is an imaging technique, in which the anterior radius of curvature is measured from the size of an image reflected from two para-central points on the cornea based on the reflection principle [45, 46]. The reflection principle defines a relationship between object size, \( o \), image size, \( I \), distance between reflective surface and object, \( d \), and radius of curvature, \( R \).

If three of these variables are known, the fourth can be calculated using the following formula:

\[
R = \frac{2dl}{o} \tag{2-1}
\]

Corneal topography has a number of limitations. For instance, it only measures a small region of the cornea and is susceptible to focus and misalignment errors [48].

To measure the corneal posterior surface, a more elaborate technique is required because imaging of the posterior surface must be performed through the anterior surface, which acts as a magnifying glass and distorts the perceived shape of the posterior surface. Therefore, it is necessary to take into account the imaging distortion and the refraction of the anterior surface [18].

2.3.3 Measurement of Intra Ocular Pressure

Intra ocular pressure (IOP) is a term used for the pressure applied to the cornea by the aqueous humor. It is determined by coupling the production and drainage of the
aqueous humor through the eye’s anterior chamber [4]. The human’s IOP varies between 1.4-2.8 kPa based on age, sex and ethnicity [49] with the average for a healthy adult of 2.13±0.2 kPa [14, 50]. The IOP fluctuates within about ±0.05 kPa/sec. This characteristic of the IOP becomes important in studying the time-dependent behaviour of the cornea [50]. The porcine IOP is measured to be between 2-4 kPa [51, 51, 52]. In general, there are two methods to measure the IOP; a) contact and b) non-contact tonometry.

a. Contact tonometry

i. Goldman applanation tonometry (GAT) is the most common method to measure the IOP. A schematic of the Goldman applanation tonometer is shown in Figure 2-7. In GAT, the IOP is inferred from a force required to flatten a constant area of the cornea based on the Imbert-Fick law [8]. Various studies have shown that its accuracy depends on factors such as corneal thickness, curvature, structural rigidity, and the presence of some corneal disorders [49, 55, 56, 58, 82].

![Figure 2-7 Schematic of Goldman applanation tonometer (GAT) highlighting measurement of the intra ocular pressure (IOP)](image-url)
ii. Dynamic contour tonometry (DCT) is a new method based on the principle of contour matching, in which the IOP reading is less dependent on corneal parameters, because it avoids any corneal deformation [53]. In DCT, a probe is placed on the cornea that applies a small change of pressure without deforming it. An integrated pressure sensor measures the IOP hundreds of times per second [54]. DCT also measures variation in pressure due to the cardiac cycle, known as the ocular pulse amplitude (OPA) [55].

b. Non-contact tonometry
i. Air-puff tonometry uses a rapid air pulse to flatten the cornea. The IOP is then estimated by detecting the force of the air jet via an electro-optical system. This method is not considered an accurate method to measure the IOP but provides a fast and simple way to screen for high IOPs [56].

ii. Ultrasonic tonometry is a later, more advanced method to measure the IOP. In this method, the cornea is flattened by an ultrasonic beam. The IOP is then measured, based on the Imbert-Fick law, by determining the force generated from the ultrasound transducer [57]. The advantages and disadvantages of this method have not been comprehensively yet studied, however, an empirical study showed that the IOP measured with this method was more constant across the cornea [58].

Various factors influence the IOP measurement. Many studies have shown that corneal thickness has an impact on IOP measurement so that increased corneal thickness leads to an artificially high estimation of the IOP [53, 54]. This is more noticeable in GAT [14, 59, 60]. In addition, a corneal ex-vivo study showed that the viscoelastic nature of the cornea has a role in damping the IOP. In other words, the corneal ability to relax under pressure is a buffering mechanism protecting against small IOP changes [61].

2.4 Corneal Optical Imaging Techniques

Early imaging techniques were performed ex vivo and were invasive. In the early 1970s, confocal microscopy was developed to acquire high-resolution corneal images at cellular level, ex vivo [65]. Later, the advances in confocal microscopy led to the
observation of the cornea at the subcellular level [66, 67]. In vivo images of the cornea were first acquired in 1976 using a specular microscope that only enabled the visualization of the corneal endothelium [68]. After the development of optical coherence tomography (OCT) in the early 1990s, it became a powerful technique for imaging the internal structure of biological tissues including the cornea [69]. Generally, the OCT is an interferometry technique that provides high resolution, in vivo cross-sectional images of biological tissues at the microstructural level. It can achieve image resolutions of 10–15µm, which is 10 to 100 times finer than standard ultrasonic technique or confocal microscopy [70]. Moreover, it is an imaging techniques that can acquire real time in situ images without a need for excision and pre-treatment of the tissue. One drawback of the OCT is its limited maximum imaging depth of tissue to approximately 2–3 mm due to light weakening and scattering effects [71]. The first OCT tomogram of the human cornea in vitro was obtainable by Huang et al. (1991) [72]. The early OCT technique was based on time domain interferometry, known as TD-OCT, which acquired one-dimensional measurements [73-75]. Later, a faster variant of OCT based on Fourier domain interferometry, known as swept-source OCT (SS-OCT) was developed, which employs a spectral discrimination approach. This method uses a rapidly tuned narrowband source, which can achieve a superior sensitivity with better signal-to-noise ratio [76, 77]. SS-OCT has been used to acquire two dimensional [78, 79] and three dimensional [80] images of the cornea with an axial resolution of about 10µm.

In general, the axial resolution of the SS-OCT technique is governed by the bandwidth of its light source [81]. For an ultra-high resolution image, with axial resolution of about 2 µm, an ultra-broadband laser was used in the system developed by Akiba et al. (2007) [82]. The development of high-speed SS-OCT by Wojtkowski et al. (2002) allowed for real-time acquisition of corneal images, which made it possible to produce video-rate tomograms. This can display dynamic processes such as fluctuation of the intraocular pressure and pulsation of blood vessels [83].

2.5 Corneal Elastography Techniques

In general, elastography is a non-invasive method, in which a real time strain map or (under some assumptions) stiffness map of a soft tissue is obtained via various
imaging techniques such as, ultrasonics, magnetic resonance and tomography techniques. Ultrasound has the advantages of being cheaper, faster and more portable than other techniques. To determine the mechanical properties of the tissue directly, several simplifying mathematical assumptions are typically made, such as local homogeneity and the absence of boundary effects [84]. In 1991, Ophir et al. first reported the use of ultrasound image speckle cross correlation for the estimation of elasticity in biological tissue [85]. Over a decade later, Hollman et al. presented strain maps from an ex vivo cornea using high-frequency ultrasound speckle tracking [86]. Schmitt extended the general concept of elastography to OCT, and developed an imaging technique with superior spatial resolution to ultrasound and with a simplified procedure [87]. Spectral domain OCT elastography was described by Ruiz et al. (2005) to measure 1D (axial) corneal displacements [88]. Recently, Ford et al. (2011) implemented this technique to study corneal mechanical behaviour by measuring 2D subsurface displacement [89]. The feasibility of using magnetic resonance elastography (MRE) to assess the mechanical properties of the intact cornea (as part of the eye globe) was evaluated by Litwiller et al. (2010). They performed MRE by introducing mechanical vibrations of a known frequency and encoding the resulting tissue motion into the phase of the MR images with a synchronous gradient field applied at the same frequency.

2.6 Swept-source optical coherence tomography

Optical coherence tomography (OCT) is a non-invasive technique that allows in-situ and real time imaging of semi-transparent scattering materials. It emerged in the early nineties as an extension of low coherence interferometry. Its spatial resolution is between 10 to 100 times finer than conventional ultrasound imaging and magnetic resonance imaging, which makes it of great clinical and surgical importance [76]. Using a state-of-the-art Ti-Sapphire laser, ultrahigh-resolution imaging, with axial resolutions as fine as 1–2 μm, has been demonstrated [164]. The maximum imaging depth in most tissues is limited, by optical attenuation and scattering, to approximately 2–3 mm [70, 71]. Depending on the properties of the light source and the bandwidth of the emitting spectrum, OCT can achieve sub-micron resolution [69].
The principles of OCT have been described in detail in the literature [165, 166]. Figure 6-8 shows a schematic of a typical OCT (Michelson interferometer).

Figure 6-8 Schematic of an OCT system, based on a Michelson interferometer [167]

As shown in Figure 6-8, the illuminated light is divided into two arms; a sample arm (containing the item of interest) and a reference arm (usually a mirror). Interference between the back scattered light from the sample and the reference gives rise to an interference pattern (fringes) that can be expressed in terms of intensity, $I$. Sharp refractive index variations between layers in the sample medium manifest themselves as corresponding intensity peaks in the interference pattern. The interference occurs only when the distance travelled by the light in both paths matches to within the coherence length of the light. Therefore, the depth (axial) resolution of an OCT system is determined by the temporal coherence of the light source [69]. Depth information can be obtained from time-domain (TD) measurements, in which the interference pattern is obtained by translating the reference mirror to change the reference path length and match multiple optical paths due to layer reflections within the sample. Depth information can also be derived from frequency domain (FD) measurements by Fourier transformation of the output spectrum. In such an arrangement, the reference optical path length remains fixed and component frequencies of the OCT output are detected using a
spectrometer [166]. The main advantage of FD-OCT over TD-OCT can be found in the increased sensitivity, which enables high data acquisition rates.

In OCT, a two- or three-dimensional image is obtained by making multiple depth scans (A-scans). These scans are performed whilst laterally scanning the beam in either one or two orthogonal directions.

In general, there are two types of FD-OCT, one being spectral-domain OCT (SD-OCT), based on a spectrometer with a multi-channel analyser. The other one, uses a rapidly tuned narrow-band laser source, and is known as swept-source OCT (SS-OCT) [69]. In principle, the A-scan rate is only limited by the read-out rate of the linear detector-array of the applied spectrometer in SD-OCT or by the tuning speed of the swept source in SS-OCT [69].

The system used in this work is a SS-OCT configuration (Thorlabs OCS1300-SS) which employs a rapidly tuned narrowband source with central wavelength, $\lambda=1310$ nm and spectral bandwidth, $\Delta\lambda=100$ nm to illuminate the interferometer and records the interference signal with a single photo detector. At each lateral position, depth information encoded in the frequency of the interference signal is efficiently extracted by calculating the Fourier transform of the latter, which results in the reconstruction of a depth profile (A-scan). The measurement point is moved laterally by means of galvo-scanning mirrors to record adjacent A-scans, which are then combined to create a 2D image in the $xy$ plane (B-scan). Finally, multiple adjacent 2D images are concatenated in the $z$ axis to form a 3D data volume.

### 2.7 Mechanical Characterization Methods

In order to characterize the mechanical behaviour of biological tissues, several important factors have to be considered. It is of high importance to characterize the biological tissues in situ using non-invasive methods if possible. Moreover, as biological materials are often inhomogeneous and their properties vary from individual to individual, the characterization methods should not comprise any assumptions or simplifications and should be able to account for any small variations between individuals [91]. These methods can be divided into two main categories;
standard experimental methods and combinations of numerical and experimental methods.

2.7.1 Standard Experimental Methods

The most common approach to characterize the mechanical properties of biological tissue is to perform common experiments such as tensile, compressive, stress relaxation, creep tests. These experimental methods are usually invasive as the sample tissue needs to be extracted from the living body. Furthermore, it is not easy to design an experiment that fully replicates the complex physiological conditions of the tissue. Another disadvantage lies in the simplifications and assumptions made for complex biological tissues. To characterize the cornea using the tensile test, the cornea is simplified as a homogeneous material. Many research groups have studied the corneal mechanical behaviour using standard experiments such as tensile, creep and stress relaxation experiments. The study by Zeng et al. (2001) compares the mechanical properties of human and porcine cornea using uniaxial tensile creep and stress relaxation tests [92]. They concluded that if tensile strength and stress–strain relations are the only mechanical factors to be investigated, the porcine cornea can be used as a substitute for the human cornea. However, when stress relaxation is the case, porcine cornea cannot be used as an appropriate substitute for human cornea because the porcine cornea relaxes more rapidly than the human cornea.

Boyce et al. (2007) studied the effect of pre-conditioning on the response of bovine cornea using similar tests [93]. In addition, they compared the corneal behaviour along the NT and IS axis. They found that pre-conditioning causes the cornea to become stiffer and that the bovine cornea is stiffer along the IS axis. However, the studies of Boote et al. (2003) on the human cornea, found no difference between the responses in the Nasal-Temporal (NT) and Inferior-superior (IS) directions [19]. Bryant et al. (1994) and (1996) investigated the effect of surgical incisions and wounds on the tensile strength of the human cornea [94, 95]. They observed that there is no significant difference in the tensile strength of the incised or wounded cornea in comparison to a healthy one, with only a difference in the mode of failure.

Elsheikh et al. (2005) investigated various deficiencies in the tensile test and examined how these should be corrected by means of mathematical analysis in
order to correlate with the results of an inflation test [73]. According to their study, three sources of inaccuracy exist in performing the tensile test. These originated from variation in thickness along the length of the tensile strips and flattening of the corneal original curvature. The correctional mathematical procedure involved a numerical integration of stress over the length, addition of stress distribution across the anterior and posterior surface and deriviation of longitudinal strain [96]. Although they proposed the mathematical correction for the tensile test, some inconsistency between the tensile and inflation tests still remained.

An early research conducted by Nyquist et al. (1968) studied the effect of a tensile test on the alignment of corneal fibres [97]. They showed that the tensile test engaged, in particular, the on-axis load-bearing fibres and reoriented the off-axis load-bearing fibres towards the loading direction. Later, this was confirmed by the studies of Elsheikh et al. (2007) and Anderson et al. (2004) showing that the stress-strain response measured by the tensile tests is stiffer than the one measured by the inflation tests [96]. In general, the inherent limitations and inaccuracies of the tensile test in respect to the corneal real physiological behaviour makes other experimental methods such as the inflation test more desirable [96, 98, 99].

The inflation technique is more suitable than the tensile test for corneal study because of its ability to simulate corneal in vivo conditions [21, 32, 100] and to address the corneas inhomogeneity and anisotropy [13, 101]. In an inflation test, the cornea, often with part of the surrounding sclera is mounted on an artificial anterior chamber (AAC) and is subjected to a hydrostatic pressure change. In the work of Anderson et al. (2004), the corneal mechanical behaviour was studied by measuring its displacement on the apex only [102], whereas Boyce et al. (2008) measured the full field displacement of the anterior surface [100]. The surface full field measurement enabled them to study a pattern of deformation that reflects the corneal spatially varying properties. Boyce et al. found that central cornea deformed less than the peripheral/limbal region. This was atributed to the spatially varying corneal properties as a result of different fibre orientation. Their deduction was consistent with the analytical measurements of Hjortdal et al. (1996) [99]. The study of Boyce et al. (2008), however, did not include any information through the thickness of the cornea.
Another type of experiment that can be used for small scale corneal study is the indentation method. Recently, indentation has become a popular technique in studying the mechanical properties of biological tissues. However, it poses some difficulties on very soft tissues, such as cornea [103]. This will be explained in detail in Section 5.3.2. In general, the standard methods of analysing the indentation data, Oliver–Pharr (OP) method [104], cannot be applied for biological tissues exhibiting viscoelastic (time-dependant) behaviour. The OP method is originally developed to analyse the behaviour of hard materials such as metals that have an elastic-plastic (time-independent) response [104]. Some research groups, however, have implemented the OP method to characterize the biological tissues by eliminating their time-dependent behaviour [105]. However, a direct measurement of the time-dependent behaviour of these materials is far more useful. Three methods are implemented in the literature to analyse the indentation data of a viscoelastic material [103]: empirical, analytical and inverse finite element method.

In the empirical solution, a trend for the load-depth data is obtained by fitting the indentation data to different curve types, e.g. polynomial, power law, etc. [106]. The curve fitting parameters obtained via this method do not usually describe the parameters of a material constitutive model. In analytical solutions, the trend for the load-depth data is obtained by incorporating a viscoelastic rheological model into the fundamental elastic Herzian contact equation using the Boltzmann superposition law [105, 107, 108]. The principle of Boltzmann superposition allows the analytical solutions to be found for multiple stage experimental loading conditions as shown in the study by Oyen (2005) [109]. The main drawback for the analytical method exists within the choice of the viscoelastic creep function, which is based on a tensile mode of stress not a complex indentation one [108]. A study of Ahearne et al. (2007) investigated the corneal time-dependant behaviour using a micro indentation technique [103]. They analysed the data based on Fung's material model, which describes a uniaxial relaxation mode of stress. An alternative is to use the inverse finite element method (IFEM) which is able to account for the simplifying assumptions made in the analytical solutions. The IFEM has been used by researchers to study various biological tissues, such as human bones [110] and skin [111], assuming a simple elastic model. Namani et al. (2009) implemented the IFEM to determine the hyperelastic behaviour of several soft biological tissues [112].
However, most of biological tissues exhibit a viscoelastic behaviour and there has been no comprehensive study of their time-dependent properties using a combination of the IFEM and the indentation technique to date. However, the study of Liu et al. (2009) on the viscoelastic characterization of soft gels indicated that such analysis are suitable for biological tissues [113].

2.7.2 Combinations of Numerical and Experimental Methods

A more recent approach for mechanical characterization of biological tissues is to combine numerical and experimental techniques such as the inverse finite element method (IFEM) [94] or virtual fields method (VFM) [66]. In this approach, a relevant mechanical response of the tissue obtained by an experimental method is used as an input to a numerical algorithm and the parameters of the constitutive model are recovered, iteratively in the case of IFEM [114], or non-iteratively in the case of VFM [115]. This approach was initially developed to study inhomogeneity in engineering materials [116-119] and was also used to detect damage in the field of fracture mechanics [120-122]. The IFEM has become popular in the field of biomechanics due to being non-invasive, therefore, it has the ability to be implemented in vivo. A potential drawback of this method is that it does still require in vitro measurements to inform the material constitutive model. However, once this has been validated, no further in vitro testing is required. Previously, the IFEM has been implemented to characterize various biological tissues, such as cartilage [123], aneurysmal arterial tissue [123, 124] and skin [111], assuming linear elastic behaviour and homogeneity. More sophisticated studies have characterized breast tissue [125], aneurysmal cerebral tissue [126] and foot heel tissue [127] with an assumption of nonlinear elastic (hyperelastic) behaviour. The fact that these tissues have viscoelastic behaviour and are inhomogeneous is usually ignored [118]. The viscoelasticity was addressed in the study of Kauer et al. (2002) and Kim et al. (2005) on aspirational and abdominal tissues, respectively [128]. The study of Seshaiyer et al. (2001) addressed the issue of inhomogeneity in arterial membranes by considering a number of homogeneous regions, a method known as subdomain IFEM [129]. The study of Kroon et al. (2008), elaborated further and estimated anisotropic inhomogeneity of atherosclerotic vascular tissue by implementing various anisotropic
models to the homogeneous regions [124]. Regarding the cornea, Nguyen et al. (2011) employed the IFEM on the inflated cornea to determine the anisotropic hyperelastic properties [130]. So far, few studies have combined all the aspects of inhomogeneity, anisotropy and viscoelasticity together to fully characterize a biological tissue.

2.8 Corneal Constitutive Models

Early studies investigated corneal behaviour using a simple homogeneous, isotropic material model with linear elastic behaviour for small strains (less than 5%) or a hyperelastic behaviour for larger strains [98, 99, 131]. Later studies, however, have developed a more complex model by taking account of the corneal microstructure and modelling it as an anisotropic hyperelastic material [98, 132, 133]. The anisotropy has been incorporated into the hyperelastic model by employing a homogenization scheme, in which the strain energy density of the entire cornea is related to the weighted strain energy density of the fibres, based on the probability of the fibres preferred orientation [134-136].

The studies of Boot et al. (2003) and Hukins et al. (1998) investigated the effect of fibre density on corneal biomechanical behaviour [19, 137]. They found that since fibres are the stiffer constituents of the corneal microstructure, the density of their preferred orientation or degree of anisotropy, determined the corneal response. In the literature, two models were proposed for corneal anisotropy; discrete fibre model and distributed fibre model. The discrete fibre model, which has been solved both analytically and numerically, simplified the fibre structure into two distinctive regions; a central region having an transversely isotropic fibre orientation and limbus region having a tangential fibre orientation [136, 138, 139]. Nguyen et al. (2011) determined the parameters of a discrete anisotropic hyperelastic model [130]. The distributed fibre model represents the fibre structure as a continuous model with a gradual change from the centre to the limbus [26, 139, 140].

A number of studies revealed that the overall mechanical behaviour of the cornea is viscoelastic and highly non-linear [32, 93, 97, 99, 141, 142]. The studies of Anderson et al. (2004) on porcine and Boyce et al. (2008) on bovine cornea suggested that
their stress–strain curve can be divided into two distinctive phases, a matrix regulated phase with low stiffness followed by a fibre regulated phase with much higher stiffness [100, 102]. For the porcine cornea, the physiological rage of pressures located close to the end of the matrix regulated phase and for the bovine cornea it is located near the beginning of the fibre regulated phase, as shown in Figure 2-9.

![Stress vs. strain curve](image)

Figure 2-9 Stress vs. strain curve, showing location of physiological range of pressure for bovine and porcine cornea [135]

Earlier studies, such as that of Thornton et al. (2001), assumed a linear viscoelastic model for tendons and ligaments and incorporated a one dimensional microstructure [143]. Lanir et al. (1983) integrated a two dimensional microstructure into a quasi-linear viscoelastic model for fibrous connective tissues [134]. Later, Bischoff et al. (2006) incorporated a three dimensional transversely isotropic microstructure into Lanir’s model [144]. The study of Boyce et al. (2008) showed that within the physiological pressure range, corneal behaviour can be approximated to linear viscoelasticity [78]. A general non-linear viscoelastic model was developed by Gerhard et al. (2006) at tissue level without considering anisotropy [91]. Their model served as a base for further development. Nguyen et al. (2008) called into question the ability of a quasi-linear viscoelastic model to represent the overall behaviour of soft fibrous tissues, particularly the cornea [140]. They developed a full anisotropic
nonlinear viscoelastic model for the cornea, which was fundamentally different to other developed anisotropic models. Typically, a nonlinear viscoelastic model is based on incorporation of the principle of continuum deformation into various combinations of viscous and elastic components [145]. The anisotropy is then applied to only the viscous deformation. Nguyen et al. (2008), however, specified the anisotropy for both the viscous and the elastic deformation. It can be seen, therefore, that generally in the literature, despite a strong evidence for corneal viscoelastic behaviour and available models, only a few studies have measured the value of their parameters.

2.9 Numerical Modelling

In the literature, it can be seen that the cornea has been numerically modeled using the finite element method in order to study its mechanical response to various factors, such as surgical procedures [122]. The degree of success in predicting the corneal response depends strongly on how close to reality the model is. In early studies, a series of assumptions and simplifications were made in order to generate a finite element model that could be solved using the techniques and computers available at the time. Later, following enhancements in the finite element modelling software increases in computational power and more accurate measurement of corneal properties, more realistic models that included anisotropy, inhomogeneity and time dependancy were introduced [144].

Early studies, such as those of Buzard et al. (1992) and Bryant et al. (1996), used a 2D axisymmetric elastic finite element model for the cornea [146, 147]. Their work confirmed the effectiveness of numerical modelling in corneal biomechanics but was unable to represent the asymmetrical effect of diseases and surgeries. A more detailed 3D model was produced by Pinsky et al. (1991) to predict the immediate change of the corneal anterior surface after refractive surgeries. However, this was still based on assumption of linear elastic behaviour [132]. Similar assumptions were adopted by Velinsky et al. (1992) to produce a model to determine the effect of cuts in keratoconus surgery [133]. Velinsky et al. found that a simple elastic material model is inadequate to study the long term corneal behaviour. Anderson et al. (2004)
used corneal topographic data to create surface geometry and implemented a hyperelastic model to simulate an asymmetric effect of the keratoconus disease [102]. Their work showed how the finite element modelling could be customized with clinical measurements. Elsheikh et al. (2007) investigated the effect of structural parameters on the accuracy of the finite element model [148]. They found that parameters such as, thickness variation, boundary conditions and material properties have a significant effect on the simulated response, thus, they should be incorporated in the model. Counter intuitively, they proposed that corneal topography has a negligible influence so that the cornea can be safely approximated to a spherical object.

In the published literature, corneal numerical models vary from a simple homogeneous isotropy to a more complex inhomogeneous anisotropy. Pandolfi et al. (2006) initially developed a homogeneous transversely isotropic model [136] and later enhanced this by dividing it into two sections: a central transversely isotropic region and a limbus circumferential region [139]. Similarly, Nguyen et al. (2011) created a model with two anisotropic regions and performed a parametric study based on inflation results to find the optimum anisotropic model for the central region [130]. They considered a fixed degree of anisotropy for the limbal region and varied the degree of anisotropy for the central region into five cases of fibre orientations: inferior-superior (IS), nasal-temporal (NT), orthogonal (ORTH), circumferential (CIRC) and transversely isotropic (Trans-ISO). They found that the inflation response of the ORTH model was indistinguishable from that of the Trans-ISO model. Therefore, the ORTH and Trans-ISO models of the central cornea can best simulate the experimental response.

Summary

In this chapter, a detailed review of the previous studies about the subject of this work is presented. Initially, corneal anatomy, including geometry and microstructure is explained, in order to familiarize with the subject material of this research. To specify the importance of this study, a review of various corneal diseases and disorders (relevant to the corneal biomechanics) followed by their potential treatments is provided. In addition, clinical measurements of various corneal parameters are introduced, with emphasis on optical measurement techniques. To
direct the chapter toward the methodology of this research, two main characterization methods for biological materials are discussed and specified for the cornea. Finally, an assessment of various corneal analytical and numerical models is provided.
Chapter 3: Aim, Objectives and Methodology

3.1 Aim and objectives

In this work, we aim to develop a numerical-experimental method that is able to characterize the time-dependant properties of the cornea, as well as to determine the spatial distribution of the properties in a robust and computationally efficient manner. This method should have the potential to be implemented in vivo and be modified individually based on the clinical measurements. The method used the numerical method of inverse finite element analysis (IFEA) and combined it with two distinct experimental methods. The combination of IFEA with the nano-indentation experiment determined the local parameters of the time-dependent rheological model at microstructural level. The performance of this method was validated with obtained parameters from a simple stress relaxation test.

In determining the spatial distribution of the properties, the IFEA was combined with the full field data obtained from the inflation experiment. To solve the problem efficiently, a simplifying assumption was made and two numerical models were used sequentially. The assumption was examined and validated and also the obtained spatial distribution agreed with the finding from the literature.

3.2 Introduction to methodology

In solid mechanics, there exists a variety of methods dedicated to solving inverse identification problems. Such problems may arise when there is a lack of knowledge of either the material properties or the boundary conditions. In the case of material properties, the inverse method on its own is not adequate. The reason is that some initial methods (usually experimental methods) are still required to inform the material’s constitutive model, which fully describes the stress-strain relationship. Stress-strain plots, obtained from standard tests, will reveal the material’s time-dependency or independency, linearity or nonlinearity, isotropy or anisotropy, deformation recoverability or non-recoverability and stress-state dependency etc. The combinations of these characteristics lead to a definition of the material’s constitutive model.
The standard tests, when utilized on their own, become no longer viable in obtaining the parameters of the constitutive model if the material under test exhibits spatial variation of the properties. However, they are still useful for identifying the general constitutive model. Typically, in standard tests such as tensile, compression etc., the material is assumed to be homogeneous and its overall behaviour is targeted. On the other hand, an adaptation of full field measurement techniques such as elastography or fast optical coherence tomography in combination with inverse methods can lead to determination of variation of material properties spatially.

The combination of the inverse solutions with the experimental methods (either full field or point wise measurement) can fall into two main categories; analytical and numerical approaches. A significant amount of research has been carried out to solve inverse problems analytically. For example, Barbone et al. (2004) used displacement fields that were obtained from elastography to determine the distribution of shear moduli by solving the momentum equation for an incompressible elastic material. Their method was restricted, however, to linear elastic incompressible materials and became unsuitable even for nearly incompressible materials [84]. The numerical approaches, however, can be applied to a wide range of material properties. The numerical methods consist of two main types: virtual fields’ method (VFM) and inverse finite element method. The VFM is a non-iterative method that is based on the selection of virtual fields. In the early literature, the virtual fields were selected in the spatial domain as polynomials of a spatial variable for homogeneous materials [149]. In recent developments, this method has been implemented in the frequency domain and the selected virtual fields are based on a set of cosine and sine functions of different spatial frequencies, which can be used for non-homogeneous materials, a method known as Fourier-series-based VFM [149]. The Fourier-series-based VFM is a reliable inverse numerical technique that recovers the distribution of the material properties using the full-field measurement data. It is a non-iterative method, thus, is fast in comparison to other inverse solutions. This method also tackles those problems where the boundary conditions are not well defined. The drawback of this method is that at its current development stage, it only applies to materials showing a time-independent behaviour. Additionally, it is very sensitive to the quality of the experimental data as well as noise.
The inverse finite element method, however, can recover the time-dependant properties of a material from any experimentally determined data (either full-field measurements or point wise). The study of Nguyen et al. (2013) showed that this method is less sensitive to the experimental noise than Fourier-series-based VFM [149]. However, due to the iterative nature of this method, it is relatively time consuming and computationally costly.

In this research, a combination of the inverse finite element method with two distinctive in vivo measurements is used to recover point wise time-dependent properties and to determine the spatial distribution of long-term elastic properties.

In order to achieve the corneal time-dependent properties and its distribution collectively, an experimental technique should be used in which the full field data are provided based on time, so that any deformation resulting from an applied load can be monitored based on time. In general, the full field data can be obtained via various imaging techniques that provide 2D or 3D images of deformed and undeformed states of the cornea. These imaging techniques should be fast enough to account for the fluctuation of the corneal intra ocular pressure. Therefore, they should acquire the images within a small fraction of time in order to prevent any corneal deformation within the acquisition time. In this research due to the relative slowness of the utilized imaging technique (swept source-optical coherence tomography), a time-dependent full field deformation data can not be achieved. Therefore, the spatial variation of the properties can not be investigated together with the time-dependency. Therefore, these two aspects were considered by means of two distinct experimental techniques.

The well established constitutive model of the cornea [122] describes the cornea with a recoverable and time-dependent deformation when subjected to the physiological range of pressure (IOP). This characterization of the cornea was used to inversely obtain the point-wise time-dependent parameters described as,

$$E = E^*[ (x, y, z)_{t=0}, E(t)]$$  \hspace{1cm} (3-1)

where $E = E^* (x, y, z)$ are three constant parameters at $t = 0$, referring to the instantaneous elastic moduli at the microstructural level, i.e. $x$, $y$ and $z$ of the
Cartesian coordinate system. $E(t)$ denotes the time-dependent elastic moduli. The cornea also shows a time-independent behaviour when subjected to a long term, constant pressure. This response of the cornea was used to study the distribution of the properties in 2D, i.e.

$$E_{\infty} = E(r, \theta)_{t \to \infty}$$

(3-2)

where $E(r, \theta)$ when $t \to \infty$ is the long-term elastic moduli that varies along $r$ and $\theta$ directions in the polar coordinate system. Figure 3-1 shows a diagram, summarizing the overall methodology used in this research (each of the boxes are expanded in the following sections).
Figure 3-1 A general diagram showing the methodology used in this research.
3.2.1 Development of a method to characterize corneal time-dependent behaviour

Figure 3-2 illustrates the methodology used in this work to obtain the corneal time-dependent parameters.
Figure 3-2 Diagram showing the procedure used to characterize corneal time-dependent behaviour.
Initially, a simple stress relaxation test was performed to determine the parameters of the corneal rheological model using curve fitting. These parameters were later, used in a validation experiment using nano-indentation. The validation test showed that an isotropic model could only partially describe the corneal indentation response.

In the first part of our research, a new technique was developed to find the point wise (local) parameters of the corneal rheological model. This combines the IFEM with a nano-indentation experiment on both isotropic and transversely isotropic material models. Any spatial variation of these parameters across the cornea is not the subject of this study, as these parameters were only evaluated in the centre of the cornea. However, the technique leads itself to the evaluation of the spatially varying properties given appropriate spatially varying experimental data. In the first stage, the time-dependant parameters and the corresponding out-of-plane instantaneous elastic parameters were recovered using an isotropic finite element model. In the second stage, the time-dependent parameters were considered to remain unchanged by the choice of the finite element model. Therefore, for simplicity and cost efficiency, only the remaining in-plane instantaneous elastic parameter was recovered with the help of a transversely isotropic finite element model. Finally, the recovered parameters were compared and contrasted with the determined parameters from the stress relaxation test. To enable a valid comparison the parameters were obtained from one pair of porcine cornea.

3.2.2 Development of a method to characterize the spatial distribution of corneal properties

Figure 3-3 shows a diagram that summarizes the methodology used in obtaining the distribution of the corneal properties.
In the second part of the research, a method was developed to determine the spatial distribution of the corneal properties. The long-term response of the cornea, i.e. when $t \to \infty$, was targeted due to the current limitation for determining high quality, time-dependant, full-field displacement data. The technique combines the IFEM with full-field measurements obtained from an inflation experiment to obtain the distribution of the elastic parameters along the radial and circumferential directions, i.e. $E(r, \theta)$. Two volume images of the cornea, before and after the inflation, were acquired using swept source-optical coherence tomography (SS-OCT) and the full field data were evaluated by processing the volume images using digital volume correlation (DVC). In this research, although the distribution of the corneal
parameters through the thickness from a 2D image (middle slice) was of interest, the reason for acquiring and processing the volume image (not a single 2D image) was to take into account any possible out-of-plane deformation of the middle slice of the cornea during the inflation. This is explained in detail in Chapter 6. In the first stage of the inverse analysis, the corneal parameters through the thickness, $E(r)$, were recovered with the help of an in-depth partitioned finite element model and an assumption of uniform averaged properties along the circumference. In the second stage, the parameters along the circumference, $E(\theta)$, were recovered with the help of a circumferentially partitioned finite element model. The partitioning method enables a more computationally efficient and numerically robust solution to the inverse problem, avoiding local optima with solutions not consistent with the known physiology of the cornea.
Chapter 4: Stress Relaxation Experiments

4.1 Introduction

A classical approach to obtain the material properties and derive a constitutive model for biological tissues is to conduct standard experiments such as bulk tensile, stress relaxation, creep or compression tests. A number of studies have used this approach to characterize the cornea and have shown that the corneal rheology can be approximated to linear viscoelasticity within the physiological range of pressure, i.e. 2-4 kPa for porcine cornea [46].

In this chapter, the rheological model of the cornea is first explained by providing a background in both linear and nonlinear viscoelasticity. Then, the parameters of the model are obtained for a porcine cornea by performing a stress relaxation experiment and employing a curve fitting method.

4.1.1 Theory of Viscoelasticity

A distinctive feature for viscoelastic materials is the way that they respond to applied stress or strain in a time-dependent manner, exhibiting the characteristics of both viscous fluids and elastic solids. For a viscoelastic material, the stress-strain relation is described as a function ($F$) of time ($t$) such that

$$ e = F_1(\sigma, t) $$

or

$$ \sigma = F_2(e, t) $$

where $e$ and $\sigma$ are strain, stress respectively. For viscoelastic materials, nonlinearity occurs when the deformation is large or when the material changes its properties as a function of the deformation, i.e. either the elastic component is non-Hookean or the viscous component non-Newtonian. Linear viscoelasticity, however, is generally applicable for small deformations. For linear viscoelastic materials, Equation 4-1 can be simplified to
\[ e = \sigma C(t) \]  
\[ \text{or} \]  
\[ \sigma = e M(t) \]

where \( C(t) \) is the creep function at a constant stress \( \sigma_0 \),

\[ C(t) = \frac{e(t)}{\sigma_0} \]  
(4-3)

and \( M(t) \) is the relaxation function at a constant strain \( e_0 \),

\[ M(t) = \frac{\sigma(t)}{e_0} \]  
(4-4)

Equation 4-2 is used to study the variation of strain or stress with respect to time under the condition of constant stress or strain. They also show that the strain and stress are directly proportional to each other, e.g. when the stress is doubled the strain is also doubled for a given time, as shown in Figure 4-1. This behaviour is observed in linear viscoelastic materials.

Figure 4-1 Schematic of stress relaxation and creep response of a linear viscoelastic material, a) stress relaxation response at three strain levels and b) creep response at three stress levels
4.1.2 Linear Viscoelastic Model

The time dependant stress-strain relation of a linear viscoelastic material is usually described in models with various combinations of elastic springs and viscous dashpots [147]. The elastic component (spring) obeys Hooke’s law for small strain as

\[ \sigma = Ee. \]  

(4-5)

This component responds to an applied load instantaneously by deforming and can be considered time-independent. The viscous component (dashpot), however, does not respond immediately and dissipates the applied energy over time. It behaves as a Newtonian fluid with the stress-strain relationship of the form,

\[ \sigma = \eta \frac{de}{dt} \]  

(4-6)

where \( \eta \) and \( \frac{de}{dt} \) are the viscosity and the strain rate respectively. The latter term indicate the time dependency of the deformation. There are various models for the arrangement of springs and dashpots of linear viscoelastic materials such as Maxwell, standard linear solid and generalized Maxwell model [149]. The simple form of the Maxwell model (Maxwell element) is represented by a dashpot and a spring connected together in series.

The approach described above can be expanded to the Generalized Maxwell (GM) model, which uses a series of springs and dashpots aligned parallel to a single spring, Figure 4-10 to describe more complex viscoelastic behaviour.
Figure 4-10 Schematic of the Generalized Maxwell model of linear viscoelasticity

The GM model can better characterize the stress relaxation behaviour observed in many polymers as it assumes that the material does not relax at a single time, but at a distribution of times. From a morphological point of view, polymeric materials often have different lengths of molecular segments; some segments are shorter and relax much more quickly than the long ones and this can be represented by different values of $E$ and $\eta$ in the constitutive Maxwell element shown in Figure 4-10. It should also be noted that, unlike the simple Maxwell model, the deformation in the GM model is totally recoverable (in time) on the total removal of the load due to the spring in parallel to the Maxwell elements. While this model is appropriate for some materials, such as cornea, some polymers undergo irreversible time-dependant deformation in which case a dashpot should be added in series. Non-linear viscoelasticity can be incorporated by introducing non-Newtonian and/or non-Hookean elements into the model [145]. However, in our case a linear GM model, as illustrated in Figure 4-10, is sufficient to describe the viscoelastic behaviour.

In the GM model, the strain on each parallel element is equal to the total imposed strain, while the total stress is the sum of the stresses in the spring and each of the Maxwell elements so that,
\[ e = e_s = e_M \quad (4-7) \]

\[ \sigma = \sigma_s + \sigma_{M_1} + \cdots + \sigma_{M_i} = \sigma_s + \sum_{i=1}^{N} \sigma_{M_i} \quad (4-8) \]

The subscripts \( s \) and \( M \) denote the spring and Maxwell element, respectively. The term \( N \) denotes the number of Maxwell elements.

Substituting Equation 4-5 and 4-6 into Equation 4-8 gives:

\[ \sigma(t) = e_0 \left( E + \sum_{i=1}^{N} E_i \exp\left( -\frac{t}{\tau} \right) \right). \quad (4-9) \]

Equation 4-9 is described for a uniaxial mode of stress. To make it independent from the mode of stress, it is often defined in terms of shear [150] such that,

\[ \Gamma(t) = \gamma_0 \left( G + \sum_{i=1}^{N} G_i \exp\left( -\frac{t}{\tau} \right) \right) \quad (4-10) \]

\[ G(t) = G + \sum_{i=1}^{N} G_i \exp\left( -\frac{t}{\tau} \right) \quad (4-11) \]

where \( \Gamma \) and \( \gamma \) are shear stress and shear strain and \( G \) is the long-term shear modulus. The instantaneous shear modulus, \( G_0 \), is related to the long-term shear modulus by,

\[ G(t = 0) = G_0 = G + \sum_{i=1}^{N} G_i. \quad (4-12) \]

Therefore, Equation 4-20 becomes,
\[ G(t) = G_0 - \sum_{i=1}^{N} G_i \left( 1 - \exp \left( -\frac{t}{\tau_i} \right) \right) \]  

(4-13)

where \( G(t) \) is the shear relaxation modulus with respect to time. Equation 4-13 can be written in dimensionless form by normalizing with respect to \( G_0 \),

\[ g(t) = 1 - \sum_{i=1}^{N} g_i \left( 1 - \exp \left( -\frac{t}{\tau_i} \right) \right). \]  

(4-14)

The terms \( g_i \) and \( \tau_i \) are material constants. \( g_i \) is the normalized shear relaxation modulus of element \( i \) at time \( t \) and \( \tau_i \) is the relaxation time corresponding to the relevant \( g_i \). Equation 4-14 is a Prony series expansion of time-dependant shear relaxation moduli with \( N \) of terms. Commercially available computational packages often use the Prony series expansion of the shear relaxation modulus to define the linear viscoelastic behaviour of a material. There are three advantages to the Prony series formulation of the GM model,

1- It is derived directly from the GM model to explain stress relaxation behaviour.
2- It separates the instantaneous elastic behaviour from the long-term viscoelastic behaviour. The instantaneous elastic behaviour can be described by means of a simple Hookean linear elastic model and the long-term viscoelastic behaviour by a Prony series, Equation 4-14.
3- It is independent of the mode of the experimental load, i.e. uniaxial or multi-axial.

**4.1.3 Optimum Stress Relaxation Curve for a Linear Viscoelastic Material**

Stress relaxation describes how viscoelastic materials relieve stress under constant strain. During stress relaxation experiments, the material is initially loaded with a constant strain rate, i.e. \( \dot{\varepsilon} \), or deformation rate and then kept at a constant value of strain while recording stress as a function of time. A schematic graph of a stress relaxation experiment is provided in Figure 11-4.
In general, an ideal stress relaxation graph consists of two parts, an instantaneous elastic deformation and a time-dependent stress decay such that,

- The instantaneous elastic part is the initial linear part of the stress-strain curve, which is described by Hooke’s law. It refers to the characteristic of the single spring in the GM model. It is obtained from an experiment with the highest achievable strain rate.
- The stress decay is seen in the second part of the stress-time curve. It is defined by the Prony series expansion of the dimensionless shear relaxation moduli, Equation 4-14. This part characterizes the viscous behaviour of the material and refers to the Maxwell elements in the GM model.

In general, the ideal stress relaxation curve should exhibit the material’s viscous behaviour within the stress decay part and its instantaneous elastic behaviour within the loading part. In other words, the material should not exhibit any viscous behaviour during the loading part. Figure 4-5 shows the stress relaxation response of a linear viscoelastic material at various strain rates, marking the ideal curve at the highest strain rate.
Figure 4-5 Stress relaxation responses of a linear viscoelastic material at various strain rates, a) stress vs. time curves showing that at the strain rates of $\dot{\varepsilon}_3$, the material's viscous effect is eliminated within the loading part and b) stress vs. strain curves showing that at the strain rate of $\dot{\varepsilon}_3$, the material exhibits a linear viscoelastic behaviour.

4.2 Sample Preparation

The relaxation tests required careful preparation of samples to ensure reliability and stability. The samples were prepared from porcine eyeballs that were obtained from a local butcher within 24 hours of slaughter. All experiments were then performed within 24 hours of receipt. During this time, the cornea was stored in cotton cloth sheets saturated with saline solution (Bausch & Lomb Eyes Saline Solution, Tetronic@1304) at $5^\circ$C. Prior to the experiments, the corneal epithelium layer was removed, because this layer is damaged after the slaughter of the animal. The endothelium layer is responsible for regulation and transportation of the fluid between the aqueous and corneal stroma [151]. When an animal dies, this layer dies too and can no longer maintain a proper fluid balance. Therefore, stromal swelling due to excess fluids will occur and cause damage to the epithelium layer.

Several rectangular nasal-temporal corneal strips were cut from the porcine cornea while retaining some of surrounding sclera to provide a gripping area, as shown in Figure 4-6. As the cornea has an elliptical shape with a longer diameter along the
nasal-temporal axis compared to the inferior-superior axis (by about 3 mm), the nasal-temporal strip provides a better sample for the tensile machine.

Figure 4-6 Nasal-Temporal (N-T) strip of porcine cornea with the surrounding sclera cut for stress relaxation experiment, a) schematical view [18] and b) the left porcine cornea of a pair

For the stress relaxation test, two batches of corneal strips were prepared. In the first batch, four strips were cut from two pairs of eyeballs to perform the experiment at various strain rates and obtain the optimum strain rate for further use. In the second batch, one strip was cut from a left cornea of a pig to perform the experiment at the optimum strain rate. The right cornea of the same pig was used for the nano-indentation experiments. After the corneal strip (from the second batch) was gripped
in the scleral region (up to the border of the cornea), the dimensions were measured at various points along the length with a digital calliper. Thickness and width measurements were taken at 5 points along the length of the strip. Their mean values and standard deviation are provided in Table 4-3. The length was measured once to be 13 mm.

Table 4-3 Average dimension of corneal strip used in stress relaxation test

<table>
<thead>
<tr>
<th>Thickness mm</th>
<th>Width mm</th>
<th>Gauge Length mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.40± 0.13</td>
<td>2.30± 0.07</td>
<td>13</td>
</tr>
</tbody>
</table>

4.3 Experimental Methodology

The experimental tests were undertaken in the following order:

- Four stress relaxation experiments were performed at different strain rates in order to find the optimum strain rate.
- One stress relaxation experiment was performed on the left cornea of the pair at the optimum strain rate, the results of which were used to obtain the parameters of the GM model.

The stress relaxation tests were performed using an Instron 4858 Micro-Tester machine and 5 mN load cell, as shown in Figure 4-7. The experiment was performed at room temperature (because of the limitation of the tensile machine in order to perform the experiment at normal physiological temperature) and the corneal strip was sprayed with the saline solution at regular intervals in order to maintain its moisture.
Figure 4-7 Setup of stress relaxation experiment performed on the nasal-temporal strip of the left porcine cornea

The protocol for the stress relaxation experiments was to pull the corneal strip up to 5.3% strain, hold at this strain and then allow 2000 s for the stress to relax. Since the compliance of the cornea is significantly larger than that of the machine, the strain was measured from the displacement of the grips. Initially, four tensile tests were performed under four different true strain rates of 0.001, 0.01, 0.05, and 0.1 /s in order to find the minimum strain rate required for the material to perform in an approximately linear viscoelastic manner. Achieving data at strain rates above 0.1 /s was not possible due to limitation of the machine. This was found to be 0.1 /s. Having found the ideal strain rate, one experiment was performed on the left corneal strip to obtain the stress-strain and stress-time curves in order to fit into the material’s rheological model and obtain its parameters.
4.4 Results and Analysis

Initially, a range of experiments with various strain rates were performed to find the optimum loading strain rate, at which the initial part of the stress-strain curve shows a linear behaviour. Figure 4-8 shows the stress-time curves obtained at various strain rates. The initial (loading) regions of the curves are magnified in the stress-strain plots, shown in Figure 4-8.
Figure 4-8 Stress relaxation results at various strain rates showing standard deviations. Loading part is highlighted as a box, a) stress vs. time curves and b) stress vs. strain curves for the loading part
It can be observed from the Figure 4-8 (b) that the cornea shows linear elastic behaviour within the loading part of the curve with a strain rate of 0.1 /s. Therefore, a relaxation experiment at a strain rate of 0.1 /s will best reveal the viscoelastic behaviour of the cornea.

In order to obtain the viscoelastic parameters of the cornea, a stress relaxation experiment was then performed on the left corneal strip of a pair of porcine cornea at the optimum strain rate of 0.1 /s. The instantaneous elastic modulus, $E$, was inferred from the initial part of the resulting stress-strain curve, where a linear line with correlation coefficient of $R^2 = 0.98$, was fitted to the data (based on Hookean elastic model), as shown in Figure 4-9 (a). The decay part of the stress-time curve was fitted to Equation 4-14. Prior to the fitting, the experimental stress vs. time data had to be converted to the time-dependant normalized shear relaxation moduli such that,

$$E(t) = \frac{\sigma(t)}{e_0} \tag{4-15}$$

where $E(t)$ is the time-dependant relaxation moduli. $e_0$ is the constant strain level. The shear relaxation moduli is then calculated as,

$$G(t) = \frac{E(t)}{2(1 + v)} \tag{4-16}$$

where $G(t)$ is the time-dependant shear relaxation moduli and $v$ is the the Poisson’s ratio. The shear relaxation modulus can then be written in dimensionless form as,

$$g(t) = \frac{G(t)}{G_0} \tag{4-17}$$

where $g(t)$ is the normalized time-dependant shear relaxation moduli and $G_0$ is the instantaneous shear modulus obtained from the instantaneous elastic modulus $E$. 


After converting the experimental data to the time-dependant shear relaxation moduli, Matlab’s “lsqnonlin” intrinsic optimization algorithm was used to fit the data to Equation 4-14. The parameters were then obtained by minimizing the root mean square (RMS) difference between the experimental data and the fitted value as, 

\[ \text{Min: } \Delta = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (U(t)_{FEAl} - U(t)_{Exp})^2}. \]  

(4-18)

The best fit was obtained when using two terms in the Prony series, i.e. \( N = 2 \), shown in Figure 4-9 (b). Hence, in total, five parameters were obtained from the curve fitting and their values are presented in Table 4-4.
Figure 4-9 Stress relaxation result for the left porcine cornea at a strain rate of 0.1 /s, a) loading part of the stress vs. strain data. The linear elastic model is fitted to the data to find the instantaneous elastic modulus and b) decay part of the normalized shear relaxation moduli, $g$, vs. time data. The Prony series is fitted to the data to find the viscoelastic parameters.
Table 4-4 Corneal linear viscoelastic parameters obtained from stress relaxation experiment using curve fitting

<table>
<thead>
<tr>
<th>$E$ (MPa)</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>$\tau_1$ (s)</th>
<th>$\tau_2$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.250</td>
<td>0.351</td>
<td>0.437</td>
<td>410.5</td>
<td>1605.3</td>
</tr>
</tbody>
</table>

The elastic behaviour of the cornea refers to the parameter $E$, which is obtained from the initial part of the curve. The viscous behaviour refers to the parameters $g_1$, $g_2$, $\tau_1$, and $\tau_2$, which are obtained from fitting the Prony series to the stress decay part of the curve.

From a microstructural point of view, parameters $g_1$, $g_2$, $\tau_1$, and $\tau_2$ can be associated with the characteristics of the corneal matrix, which exhibit the viscous behaviour [152-154], while parameter $E$ can be used to describe the combined response of the corneal fibres and matrix [153, 155].

**Summary**

In this chapter, initially, the theory of viscoelasticity is discussed and then a detailed review of the corneal constitutive model is presented. The Generalized Maxwell model of linear viscoelasticity, which is well established for the cornea in the literature, is described in detail. Later, the sample preparation is explained thoroughly and is followed by the experimental procedure. Three relaxation tests are performed to obtain the optimum testing conditions (strain rate) and then a relaxation test is performed on a left cornea of a pair using the optimum strain rate. The stress-strain and stress-time relationship are obtained. Finally, the parameters of the GM model were determined using curve fitting.
Chapter 5: Nano-Indentation Experiments

5.1 Introduction

Nano-indentation technique was originally developed to investigate the elastic-plastic behaviour of materials using a sharp conical or pyramidal indenter. Typically, for elastic-plastic materials, the indentation test consists of a loading and unloading part, as shown in Figure 5-1(a), and the material properties are usually characterized using the Oliver-Pharr (OP) method [185]. In the OP method, the stiffness and modulus of the material are determined from the initial part of the unloading curve based on the Herzian contact principle such that

\[
S = \frac{dP}{dH} = \frac{2\beta E_r \sqrt{A}}{\sqrt{\eta}}
\]  

(5-1)

where \(S\), \(P\) and \(H\) are stiffness, load and depth respectively. \(A\) is the projected contact area and \(\beta\) is the indenter’s shape factor. \(E_r\) is reduced modulus determined from,

\[
\frac{1}{E_r} = \frac{(1 + v_s^2)}{E_s} + \frac{(1 + v_i^2)}{E_i}
\]  

(5-2)

where \(E\) and \(v\) are the elastic modulus and Poisson’s ratio, respectively. The subscript \(s\) refers to the specimen while \(i\) refers to the indenter.

As the nano-indentation technique became increasingly popular, the OP method was implemented to characterize the behaviour of viscoelastic materials such as polymers and biological tissues. The OP method measures hardness and modulus of the viscoelastic material by eliminating the time-dependant behaviour. The time-dependant behaviour is removed by applying a long holding period, i.e. deformation (creep response) of the material becomes independent of time and the material behaves like an elastic material [156]. A schematic application of the OP method for elastic-plastic and viscoelastic materials is illustrated in Figure 5-1.
Figure 5-1 Schematic of nano-indentation curves for various materials, showing the implementation of the Oliver-Pharr method for elastic-plastic and viscoelastic materials in order to obtain the stiffness, $S$, a) load vs. depth curve for elastic–plastic material, b) load vs. depth curve for viscoelastic material without removal of the time-dependant behaviour, c) load vs. depth curve for viscoelastic material after removal of the time-dependant behaviour and d) depth vs. time curve for viscoelastic material, showing the holding time and the removal of the time-dependant behaviour (marked in dashed line).

To fully characterize the behaviour of viscoelastic materials, however, a direct measurement of the time-dependant response is necessary [105]. For viscoelastic materials such as soft tissues, it is inappropriate to use a sharp indenter. The sharp indenter masks their time-dependant behaviour by inducing a sudden change from an elastic response to an unrecoverable plastic one. This might also damage the
material and induce excessive indentation depth. Therefore, for soft viscoelastic materials, spherical or flat indenters are recommended.

An ideal indentation curve for a viscoelastic material is obtained when elastic deformation occurs during loading, elastic recovery during unloading, viscoelastic deformation during holding at maximum load, and viscoelastic recovery during holding after removal of load. However, in reality, this ideal process is difficult to achieve, because the loading and unloading processes will include some effects of viscous behaviour. To minimize these effects, therefore, loading/unloading rate should be adjusted such that the material shows approximately linear elastic behaviour within these regions [108].

In general, there are three approaches to characterize the time-dependant behaviour of materials based on indentation data: empirical method, analytical method and the inverse finite element method.

1- Empirical Method

In empirical methods, a trend for the depth-time experimental data is found using various equations such as polynomial, power law, etc. The obtained parameters may serve as a means of characterizing the material but often are not general and may not bear any relationship to the material’s rheological model.

2- Analytical Method

In analytical methods, a trend for the load-depth indentation data is obtained from an analysis of the mechanics, e.g. using an elastic Herzian contact and the incorporation of linear viscoelastic rheological model [105]. The elastic Herzian contact depth for a spherical indenter is given by,

\[
3h^2 = \frac{3}{4\sqrt{R}} P \left(1 - \nu^2\right) \frac{1}{E} \tag{5-3}
\]
where \( P \) and \( h \) are load and displacement and \( R \) is radius of the spherical indenter. \( E \) and \( \nu \) are the elastic modulus and the Poisson’s ratio. Equation 5-3 can be rewritten in terms of the shear modulus, \( G \), for an incompressible material, i.e. \( \nu = 0.5 \), as,

\[
\frac{h^2}{3} = \frac{3}{8\sqrt{R}} \left( \frac{P}{2G} \right).
\] (5-4)

The term \( P/2G \) can be replaced by a general form of creep function, \( J(t) \), for viscoelastic rheological models [157] such that

\[
\frac{P}{2G} = \int_0^t J(t-u) \frac{dp}{du} du
\] (5-5)

where \( u \) is the dummy variable of integral for time. Substituting Equation 5-5 into Equation 5-4 (principle of Boltzmann superposition) [105][105] gives,

\[
\frac{h^2}{3} (t) = \frac{3}{8\sqrt{R}} \int_0^t J(t-u) \frac{dP}{dh} dh
\] (5-6)

Additionally, the principle of Boltzmann superposition allows the analytical solutions to be found for multiple stage experimental loading, in which the load is applied in several steps.

One main assumption made in analytical solutions is within the choice of the creep function. Typically, the creep function is derived based on uniaxial tensile creep not indentation. There are two fundamental differences between the tensile (macro-scale) creep and the indentation (micro-scale) creep [108]. Therefore, analytical methods can describe average properties of the material.

a- The macro-scale creep function is obtained under the condition of constant stress, whereas the indentation creep is under the condition of constant force. Although, the assumption of constant stress may hold true with some approximation for macro-scale creep, it introduces considerable error for the micro-scale creep, knowing that during the creep, the stress decreases as the contact area increases [106, 108][106, 108].

b- The tensile creep function is derived based on a uniform stress mode. In indentation, however, the material is subjected to a complex localised stress mode.
3- Inverse Finite Element Method

The inverse finite element method is an alternative way to characterize viscoelastic materials using indentation data. It takes into account the shortcomings of the analytical methods in deriving the creep function. Unlike the analytical solutions, it is regardless of the stress mode and can take into account the condition of constant load rather than constant stress. In the inverse method, the indentation depth with respect to time is an input to an optimization algorithm and the parameters of the corneal rheological model are recovered iteratively, as explained in the Section 4.1.2. In this research, the inverse finite element method is utilized to characterize corneal properties and obtain the parameters of the corneal rheological model, as explained in Section 4.4.

5.2 Sample preparation

5.2.1 Artificial corneal trephinate

The initial stages of this project had drawn together many of the known dimensions and mechanical properties of the porcine cornea. Therefore, an artificial corneal trephinate (ACT), as shown in Figure 5-2, was made to practice the experimental procedure and gain confidence in the methodology. The material for the ACT was selected to have close bulk properties to a porcine cornea in terms of Young’s modulus and refractive index. The specification of the material is provided in Appendix 1. The material was composed of a mixture of silicon resin and polyamine formaldehyde as hardener with a weight ratio of 10 to 1. The mixture cured inside a custom designed mould within 24 hours at the room temperature of 25°C. The mould was designed and prepared in an early stage of this project. The specifications of the mould are provided in Appendix 2. The process of blending the hardener into the resin caused air bubbles to be trapped in the viscous mixtures. The air bubbles were removed by spreading the mixture into a thin layer on a transparent glass sheet in order to allow enough surface area for the bubbles to burst open. Afterward, the mixture was collected carefully with a spatula to poor into the mould and cure. After curing, the ACT sample was mounted on a bulge of hard elastic synthetic putty (blue tack) that accounted for the ACT curvature and was glued around the edges.
5.2.2 Cornea

For the nano-indentation test, one whole porcine cornea (right cornea of a pair) with a small portion of sclera was extracted from the right eyeball (the left cornea of the pair was used in the stress relaxation test as explained in Section 4.4). The test was performed within 12 hours of receipt. During this time, the cornea was stored in cotton cloth sheets saturated with ophthalmic saline solution at 5°C. The cornea was then mounted on a bulge of blue tack and glued around the scleral region, as shown in Figure 5-3, to help maintain its original curvature and provide a support.
5.3 Experimental methodology

5.3.1 Nano-indentation procedure for artificial corneal trephinate

A number of indentations were performed on the artificial corneal trephinate (ACT) using several spherical steel indenters with radii of 50, 100 and 600 µm. The 50 and 100 µm radius indenters did not provide sufficient detectable contact force for the experiment to begin. This is due to the soft nature of the ACT sample. Therefore, a larger radius indenter was made using a 600±0.5 µm radius stainless steel ball attached to an aluminium stub. The objectives of the nano-indentation experiment on the ACT sample were:

1- To examine the possibility of performing the nano-indentation on a very soft material such as cornea
2- To establish a suitable experimental procedure for testing soft viscoelastic materials
3- To obtain the calibration parameters

One of the first steps was to study the existence of thermal drift and if so apply an appropriate correction time. Thermal drift can be the cause of an observed change of depth during a constant load (in addition to creep) [158][159]. The thermal drift occurs due to changes in the dimensions of the instrument, in particular the indenter, as a matter of thermal expansion or contraction. To correct for this effect, most of the nano-indentation machines allow for a holding period at 80% of the load removal. The reason that this is done at a low load value is that at this load, the material is less likely to creep thus any change in its depth is due to the thermal drift. A linear regression to the load-displacement response within the hold period can be used to obtain the thermal drift rate. The thermal drift rate is then applied to all the depth reading according to the time they were logged. A good test of the effectiveness of the correction is that the thermal drift rate, when applied to the thermal drift data, should collapse into a single point or very close to it. Therefore, it is important to apply a proper holding period. First, in order to find if significant thermal drift exists in our case, two indentation tests were performed on the ACT sample at different loading/unloading rates of 0.05 mN/s and 5 mN/s. Note that the ACT material is hyperelastic (as specified in the manufacturer’s data sheet) and should exhibit time-
independent mechanical behaviour. Thus, any change in the load vs. depth curve at different rates could be attributed to the effect of thermal drift. A depth-control method was used, in which the load was applied until 10 µm depth was reached then kept constant for 300 seconds. This was followed by 80% removal of the maximum load. Having found the presence of thermal drift, an appropriate correction time was implemented and the same experimental procedure (with the two loading/unloading rates) was performed on another point, at 50 µm horizontal distance from the first point.

5.3.2 Nano-indentation procedure for cornea

Having found the calibration parameters (explained in Section 5.3.4) and the thermal drift correction time, three nano-indentation tests were performed near the centre of the right cornea, with 50 µm horizontal intervals, as shown in Figure 5-4. The indentation points were adjusted via a microscope to be at the centre of the cornea and perpendicular to the indenter.

![Figure 5-4](image)

Figure 5-4 Right porcine cornea placed along temporal-nasal meridian (TN) showing locations of three indentations with 50 µm distance from each other

The experiments were performed at room temperature and at 70% RH to keep the cornea moist. The weight lost during the experiment was measured to be less than 1%. After moisture equilibrium and during the experiment, a thin layer of water was
observed on the surface of the cornea via the machine’s on-board microscope. This feature was important for the description of contact mechanics, as described in Section 7.3.2. Figure 5-5 shows the setup of the nano-indentation experiment.

Figure 5-5 Nano-indentation experiment on right porcine cornea using spherical indenter. The arrow indicates the direction of loading.

As the cornea is a very compliant material, the key factor to achieve stable and repeatable experimental data is to use appropriate experimental conditions, i.e. loading/unloading rate, holding time and maximum depth at load holding. In this experiment, a trapezoidal loading scheme (loading, holding and unloading) was employed with a depth control method (maximum depth sensitivity of 0.1 μN), as shown in Figure 5-6. The load was applied at a rate of 0.05 mN/s until a 5 μm depth was reached then kept constant for 200 seconds, followed by 80% removal of the maximum load at the same rate. The loading rate was selected to be 0.05 mN/s in order to minimize the viscous effect on the elastic deformation over the loading period, as explained in Section 5.1. During the 200 seconds holding time, the cornea
reached a steady state in which its deformation became independent of time. The profile of the loading scheme for the corneal sample is provided in Figure 5-6.

Figure 5-6 Schematic of nano-indentation loading schemes, a) load, $F$, vs. depth, $u$, and b) depth, $u$, vs. time, $t$

5.3.3 Nano-indentation machine

The nano-indentation experiments were performed using a Nano-Tester 600 system manufactured by Micro Materials, Wrexham, UK. A schematic view of the system is shown in Figure 5-7.
At the heart of the system is a pendulum that can rotate on a frictionless pivot. A coil is mounted at the top of the pendulum. With a coil current present, the coil is attracted towards a permanent magnet, producing motion of the indenter towards the sample and into the sample surface. The displacement of the indenter is measured by means of a parallel plate capacitor, of which one plate is attached to the indenter holder. When the indenter moves toward the sample, the capacitance changes, and this is measured by means of a capacitance bridge. The capacitance bridge unit is located close to the capacitor in order to minimise stray capacitance effects. By tuning the capacitance bridge so that the bridge is close to its balance condition, it is possible to measure the change in signal and the capacitance that corresponds to changes in depth. When the system is at full sensitivity, the distance between the capacitor's plates is about 0.3-0.5 mm with maximum measurement depth of about 1.5 mm. This can be adjusted through the “depth calibration” process.

Below the plates there is a counter-balance weight, necessary to counter the mass of the coil and the indenter. Under balance conditions the pendulum, with no voltage across the coil, has the tendency to gently fall clockwise. Through a process called “zero load calibration”, enough coil current is provided to bring the pendulum to a vertical position. A precise DC motor with displacement resolution of 17.3 nm in x, y, and z directions is used to adjust the sample stage. A high-resolution microscope is integrated into the machine in order to define the exact position of any indentation.

5.3.4 Calibration procedure

Prior to each experiment, two kinds of calibrations have to be performed; capacitance bridge (or bridge box) calibration and contact surface detection. As explained in Section 5.3.3, the machine uses a capacitive bridge circuit for displacement measurement. It is important to balance the capacitance bridge accurately to ensure maximum sensitivity to small displacements. In our experiment with 70% RH, the capacitance bridge calibration was challenging because the moisture influenced the conductivity of the electric circuit. This was overcome by
performing the calibration quickly at 50% RH and allowing the system to reach 70% RH and stabilize.

An important issue in the nano-indentation test was to identify the corneal contact surface prior to the experiment as part of the calibration procedure. This is important for the machine’s accurate measurement of the indentation depth, i.e. acting as a reference. To detect the surface, a small voltage is applied to move the indenter with a very low speed of 3.8μm/s toward the sample surface. At the point of contact, a change of voltage is detected when the force registers in excess of 0.01 mN [160]. In our experiment, due to the large compliance of the cornea, a speed of 0.1μm/s was required to make the surface contact detection possible.
5.4 Results

5.4.1 Results for artificial corneal trephinate

Two nano-indentation tests were performed on the ACT sample at 0.05 mN/s and 5 mN/s loading/unloading rates. The results are shown in Figure 5-8.

Figure 5-8 Nano-indentation results (before application of thermal drift holding time) for the artificial corneal trephinate at two different loading/unloading rates, a) load vs. depth curve and b) depth vs. time curve
As shown in Figure 5-8 (b), the ACT does not creep during the 300 seconds holding period. This agrees with the assumed hyper-elastic (time-independent) behaviour of the ACT material. Figure 5-8 (a) shows a shift between the two curves with the different loading rates. As the ACT material is hyperplastic and has a rate-independent manner, the different loading rates should not influence the depth-time response. Therefore, the shift is attributed to the effect of thermal drift such that at the higher loading rate, heat was generated between the indenter and the sample around the contact surface and caused an additional depth increase. To correct the effect of thermal drift, a holding period of 80 seconds at 80% of maximum load removal was applied (typically, a holding time of 60 seconds is recommended for polymeric materials) and the experiments were repeated. The results (after the application of the thermal drift holding period) are shown in Figure 5-9.

Figure 5-9 Nano-indentation results (after application of the thermal drift holding time) for corneal trephinate at two different loading/unloading rates

It can be observed from Figure 5-9 that the shift between the curves with different loading/unloading rates is nearly removed by the application of 80 seconds thermal drift holding time. As the ACT had very close material properties to the cornea, this correction time was applied to the experiments on the cornea.
5.4.2 Results for cornea

Three indentations were performed in the centre region of the right cornea. The reason that the number of indentations was limited was due to the difficulty to achieve consistent curves from two types of experiments, i.e. stress relaxation and nano-indentation that are obtained for a pair of cornea (from one animal). The results are shown in Figure 5-10.
Figure 5-10 Three nano-indentation results obtained from central porcine cornea (right cornea of the pair), a) load vs. depth curve and b) depth vs. time curve

Figure 5-10(a) shows an approximately linear behaviour for the loading part, creep response for the holding part and an elastic recovery for the unloading part. This primarily indicates that the cornea has time-dependant behaviour, unlike the ACT. It also shows that the experimental curves are close to the ideal indentation curve for a viscoelastic material, as explained in Section 5.1. Figure 5-10 (b) shows the creep response, where the cornea reaches an equilibrium state, i.e. when the penetration depth becomes independent of time, after about 150 seconds.

**Summary**

In this chapter, initially, a background of the nano-indentation is provided followed by a description of the approaches to characterize the time-dependant behaviour of soft tissue. Then, the preparation method of two types of sample (phantom and cornea) and the experimental procedures are described. A description of the calibration procedure became important due to the soft nature of the samples. Finally, the indentation results for each of the samples are provided and analysed.
Chapter 6: Inflation Experiments

6.1 Introduction

The “Inflation test” is a technique used to replicate the hydrostatic physiological condition of the cornea outside a living organism. The cornea is constrained in an Artificial Anterior Chamber (AAC) where it is subjected to a controlled stepwise (one or more steps) hydrostatic pressure change. By studying the incurred deformation in relation to the applied pressure, the mechanical behaviour of the cornea has been investigated by various researchers. Elsheikh et al. performed an inflation experiment and studied the corneal mechanical response by monitoring apical deformation via a laser displacement sensor [13]. The more elaborate work of Boyce et al. performed the inflation experiment and obtained spatially resolved deformation of the epithelium surface (2D) via digital imaging and image correlation [100]. Both groups observed a viscoelastic response for the cornea in the form of rate-dependent hysteresis, creep and relaxation, showing linear viscoelastic behaviour at physiological pressures, 2-5 kPa, and a non-linear one at large pressure range, up to 250 kPa. However, despite numerous research studies dedicated to corneal mechanical behaviour, only a few have characterized the corneal properties using the in vitro inflation experiment. The most notable of which, used the result obtained by Boyce et al. and evaluated the hyper-elastic constants by approximating the corneal behaviour to elastic [130].

In this work, we develop a method to characterize the spatially varying properties of the cornea through the thickness and along the circumference. We targeted the long-term elastic behaviour of the cornea and our goal was to investigate whether the long-term elastic modulus varies through the thickness and across the corneal stroma. This was achieved by measuring the 3D deformation field within the corneal tissue using a combination of Optical Coherence Tomography (OCT) and Digital Volume Correlation (DVC). OCT provides 3D volume reconstructions of the corneal tissue for a reference and a deformed state (after a change in pressure in the AAC), DVC is used to compare the reference and deformed datasets and construct a 3D displacement field within the stroma. Owing to the curvature of the cornea, OCT reconstructions require a refraction correction prior to the evaluation of the displacement field with DVC. An algorithm developed in our research group was
used for this purpose. Furthermore, the displacement fields obtained with DVC from refraction corrected OCT reconstructions were analysed to obtain through-the-thickness and along-the-circumference material properties of the corneal stroma along the nasal-temporal direction, as explained in Chapter 7.

In addition, we studied the effect that noise in the OCT reconstructions has on the strain levels that can be reliably measured. This was done by performing a stationary test and introducing rigid body translation on a flat phantom. In the first case, DVC was performed on two successive reconstructed volumes without any loads applied. In the second case, DVC was performed on several OCT reconstructions of the phantom, which was moved in a controlled way using translation stages. These tests were used to determine the displacement and strain ‘noise floor’ by evaluating the resulting mean and standard deviation values. Finally, the full-field corneal displacements obtained from the inflation test were compared against the DVC noise floor. The developed method has the potential to be used for characterizing the viscoelastic properties of the cornea given an instrument capable of determining accurate, time resolved, full-field displacement data.

Using the inflation test in combination with fast imaging techniques such as MRI or ultrasonic elastography techniques can lead to non-destructive estimation of corneal time-dependant (viscoelastic) properties [12, 84, 161]. In this study, however, the scanning time of the OCT system used is of the order of several minutes and therefore the time-independent response of the cornea was targeted.

### 6.2 Sample preparation

A right porcine eyeball was received from an abattoir within 12 hours after slaughter. The experiment was then carried out within 12 hours of receipt of the eyeballs. A scalpel was used to excise the cornea, including a ring of the surrounding sclera to clamp it in the AAC. This is known as corneal trephinate that was stored at 5°C in a cotton pad saturated with ophthalmic saline solution. The epithelium layer, damaged before receipt of the eye, was gently scraped off and washed away with the saline solution. The dimensions of the cornea were measured using a coordinate measuring machine. The corneal thickness was measured at several points along
the nasal-temporal and inferior-superior meridians. The measurements were taken three times for each point and averaged. Figure 6-1 shows the locations of the points where the thickness measurements were taken and shows the horizontal and vertical diameters. In addition, the weight of the cornea was measured before and after the experiment as water loss/gain may affect the tissue’s properties (optical and mechanical). During a two-hour test, we observed a weight gain of 0.6%.

Figure 6-1 Corneal dimensions, indicating nasal (N), temporal (T), inferior (I) and superior (S) directions, a) inferior-superior and nasal-temporal diameters, b) thickness measurement points showing average values and standard deviations
6.3 Artificial anterior chamber

An artificial anterior chamber, designed and built prior to this work (by Samantha Kings), was used to hold a human cornea in accordance with the average dimensions and boundary conditions as obtained from the Arizona eye model [162]. The Arizona eye model is one of the recently developed theoretical eye model and far the most comprehensive comparatively with the previously ones.

The AAC consists of three sections: 1) main chamber, 2) retaining ring and 3) locking ring, shown in Figure 6-2.

![Figure 6-2 Artificial anterior chamber showing, a) exploded view, b) retaining ring (top view), c) retaining ring (bottom view) and d) cross section of chamber](image)

The retaining ring provides a secure clamp of the sclera to the chamber. It prevents the cornea to twist and tear as the locking ring is screwed onto the chamber. The key feature of the retaining ring is its inner contact angle, at which the cornea is clamped.
A 10° angle was designed based on the Arizona eye model, where the edge of the cornea meets the globe of the eye [163]. This also avoids corneal distortion due to bending around the edges, thus eliminating spurious corneal flexural stress at normal levels of IOP. The main chamber provides a fluid reservoir and three inlet ports; one for connection of a pressure transducer and two for connection of fluid inlet and outlet tubes. In order to eliminate any air bubbles, the chamber includes a sloping face toward the bottom of the internal cavity to purge them.

### 6.4 Inflation procedure

An inflation experiment was performed to determine the long-term properties of the cornea, \( E_\infty \). The inflation test rig used in this research is shown in Figure 6-3.

![Inflation test rig](image)

**Figure 6-3 Inflation test rig**

The inflation experiment was carried out at room temperature (T=\( \sim 23°C \)). The cornea was placed in the AAC and secured with the locking ring. A 1 ml micro-syringe was used to control the AAC’s pressure by injecting small amounts of solution in the main chamber in a controlled way. The pressure was measured with a calibrated pressure sensor. The cornea was initially subjected to a pressure of 2 kPa (Reference state) and kept at this pressure for 120 seconds to stabilize. The cornea regained its original curvature at this pressure without additional stress. A ‘reference’ volume reconstruction of the cornea was then recorded using a swept-source OCT.
system. The pressure was then increased to 2.5 kPa (Deformed state), which took 10 seconds, and kept at that pressure for 600 seconds until the deformation (creep) response of the cornea reached an equilibrium state then a ‘deformed’ volume reconstruction was recorded.

6.4.1 Pressure sensor calibration

The pressure sensor was calibrated against a benchmark manometer. Figure 6-4 shows the voltage/pressure response such that,

\[ V = a + bP \]  

where \( a = 0.05 \) mV and \( b = 2.49 \) mV/kPa.

![Figure 6-4 Calibration graph for pressure sensor](image)

6.4.2 Pressure control scheme

As stated above, during the inflation test the cornea was scanned before and after the pressure was increased. Each full scan takes ~3 minutes, during which the cornea should ideally remain static, i.e. without creep. At \( P = 2 \) kPa, where the cornea is at the physiological reference condition, the hydrostatic pressure was stable over time. After the pressure rise, however, the pressure tends to drop slowly.
This could be attributed to either leakage from the AAC or corneal creep due to the viscoelastic nature of the cornea. Fluid leakage was confirmed and to reduce this, solid paraffin was added to the edges of the retaining and the locking rings, which were tightly adjusted. As pressure drop still persisted, a controlled pressure scheme was implemented to limit the corneal deflection during the OCT scan of the ‘deformed’ state. The pressure scheme is illustrated in Figure 6-5. The arrows indicate the pressure drop, which happens quickly at the beginning and then gradually slows down. The 0.04 kPa drop corresponds to the 0.1 mV resolution of the digital voltmeter used.

Figure 6-5 Pressure is maintained in the AAC by injecting fluid with a microsyringe. When the pressure gradient reaches a value of ~0.012 kPa/minute, the ‘deformed’ state is scanned with OCT.

After approximately 600 seconds, the pressure drops at a rate of ~0.012 kPa/minute, which corresponds to ~0.036 kPa during a full OCT scan, i.e. approximately 7% of the 0.5 kPa pressure change. In this research, the deformation of the cornea is analysed only for the area around the superior-inferior meridian, which is scanned in a fraction of the time required for a full scan, i.e. time it takes to record a number of
slices equal to the size of the sub-volume used in DVC. The effective pressure drop during the scan of this small portion of the cornea is ~0.2% of the 0.5 kPa pressure change.

6.5 Inflation test methodology

The measurement procedure consists of several steps that can be summarized as follows:

1- The porcine cornea is prepared and placed in the AAC.
2- The hydrostatic pressure is set to a reference value $P = 2$ kPa and maintained for 120 seconds to stabilize.
3- An OCT scan is performed to record the corneal ‘reference’ state.
4- The pressure is increased to 2.5 kPa during a 10-second ramp, and maintained for 600 seconds so that the corneal creep response reaches an equilibrium state and stabilizes.
5- During this time, any pressure drop is compensated by adjusting the pressure back to 2.5 kPa with a micro-syringe connected to the AAC.
6- An OCT scan is performed to record the corneal ‘deformed’ state.
7- The OCT corneal structure reconstructions are transformed to remove distortions due to light refraction at the cornea-air interface due to corneal curvature.
8- Digital volume correlation is used to evaluate full-field displacement and strain fields within the stroma.

6.5.1 Tomographic imaging of cornea with SS-OCT

A swept-source OCT system was used to scan the cornea along the nasal-temporal meridian and acquire multiple adjacent 2D cross sections of the cornea that comprised a data volume, as explained in Section 6.5.1.2. The data volume was stored in an “.IMG” file, which is a type of processed binary file with reduced noise level of up to 8 bits per pixel. To transfer the volume data to the DVC software, multiple “.bmp” images were extracted from the “.IMG” file.
6.5.2 Spatial resolution and field of view

The axial (depth) resolution of the system in air (water) is 12 (9) µm and the transverse resolution is 15 µm. 1024 B-scans were acquired at a rate of 7 fps (frames per second) and stacked along the $z$ axis to generate the volume. Figure 6-7 shows how the data volume is constructed and the $xy$, $xz$ and $yz$ 2D views.

Figure 6-7 Corneal OCT reconstruction: a) data volume and b) 2D cross sections
The reconstructed data volume contains $1024 \times 1024 \times 512$ voxels corresponding to a sample volume of $10 \times 10 \times 3$ mm$^3$ (lateral x x lateral z x axial y in air) with a grey-scale colour depth of 8 bits (256 intensity values). The x and z axes encode lateral position while the y axis encodes optical path length or optical distance, i.e. the product of the physical length of the light path and the refractive index, $n$, of the medium through which light propagates. The voxel size is $10.7 \times 10.7 \times 4.3$ µm$^3$ (lateral x x lateral z x axial in cornea, y). The voxel size along the y axis depends on the refractive index of the medium and it can be calculated simply by dividing the measured corneal thickness over the number of voxels throughout the thickness. Note that this approximation is made under a constant hydration level, where the cornea is assumed to have a constant refractive index through the corneal thickness [168].

To reduce the effect of electronic noise in the OCT data volumes, a 4-frame averaging scheme was implemented, in which four axial scans (A-scans) were performed and averaged for each (x, z) point in the field of view. Frame averaging does not affect the spatial resolution. However, it increases the acquisition time allowing for other sources of error to affect the results, e.g. environmental disturbances (vibration, air currents) and sample motion.

### 6.5.3 Factors affecting the quality of corneal cross-sectional images

The contrast of an OCT image depends on the intensity of modulation of the interference signal, which is influenced by factors such as the size of the scattering particles and the difference between the refractive indices of the particles and the matrix within which they are embedded [169]. One common factor that influences the quality of the OCT image is the effect of photo-detector saturation. When this happens, the interference signal is clipped at the maximum intensity level. In the frequency domain, this leads to higher frequency harmonics and appears in the reconstructed images as vertical bright lines or wider stripes. This effect is more remarked at the air/cornea interface, especially where the incident beam meets the cornea at $90^\circ$ angle and is retro reflected. Figure 6-7 illustrates a schematic of the light saturation effect.
Figure 6-6 At normal incidence at the corneal apex, the high intensity of the reflected beam can cause saturation of the photodetector, leading to reconstruction artifacts. This effect, however, can be reduced by minimizing the difference between the refractive indices of cornea and the medium surrounding it. In this research, initially, the cornea was immersed in water, refractive index $n = 1.33$. The air/water interface showed a convex shape that could result in optical distortion of the reconstructed corneal geometry [170]. An optical glass window was therefore used over the water, effectively creating an index-matching chamber on top of the cornea. The use of the glass window together with water, however, reduced the intensity of the scattered light and degraded image contrast (required to evaluate displacement fields with digital volume correlation) due to introduced dispersion. For this reason, it was decided that the index-matching chamber, even if it reduced refraction distortion effects, would not be used, favouring instead high contrast corneal reconstructions. Figure 6-7 shows OCT cross sections of the central cornea with and without the presence of water and the optical glass window.
Figure 6-7 OCT cross sections of central cornea, a) immersed in water, b) using a flat optical glass over the water and c) in air.

Figure 6-7 (c) shows the highest contrast for the same penetration depth (notice the scattering level around the endothelium. Another factor that can affect the quality of the OCT images is vibration, which leads to artifacts in the images due to motion of the object during the scan. To minimize this effect, the experiment was carried out on an optical table with passive vibration isolation. No evidence of vibration artefacts were later found.
6.5.4 Effect of refraction distortion and the correction method

In general, OCT images are subjected to two types of distortion: fan and optical distortions [170, 171]. Fan distortion is associated with the architecture of the scanning system and is caused when the scanning beam forms a curved or angular interface with the plane of the scanning object. For the system used in this work, the scanning beam moves parallel to the optical axis of the objective lens (y axis), so fan distortion is not present. Optical distortion, or refraction-induced distortion, occurs when imaging a curved sample whose refractive index does not match the refractive index of the medium in which it is embedded. Therefore, light beams are bent at the sample/medium interface. This can produce a significant distortion of geometrical features in the reconstructed sample so that some geometrical parameters, such as curvature (of inner structures) and thickness cannot be retrieved directly from the OCT images. Imaging the inner layers (beneath the epithelium) of the cornea is therefore subject to this type of distortion, as each layer differs in shape and structure [171]. This also causes an error in the actual position of the microstructural features inside the cornea. To explain the effect of refractive distortion, a schematic view is presented in Figure 6-8.

![Figure 6-8 Refraction of rays at the cornea epithelium and generation of an image point, I, for each object point, B, in the stroma](image)

Figure 6-8 Refraction of rays at the cornea epithelium and generation of an image point, $I$, for each object point, $B$, in the stroma
As shown in Figure 6-8, the scanning beam enters the cornea from the top. The ray reaches point $A$ on the epithelium surface at an incident angle $\theta_0$. After crossing the epithelium, the ray is refracted along a direction set by Snell’s law of refraction. The refracted ray from $A$ then reaches a scattering point $B$ in the stroma. In the OCT image, the object point $B$ (in the object space) appears at a point in the image space, $I$, known as the image point. The OCT system records this refracted optical path in the image as a vertical A-scan line, denoted as $OI$. As the optical path difference (OPD) is equal to the physical distance in the medium multiplied by the refractive index, therefore the length of $OI$ in the image space is calculated as,

$$\text{OPD} = |OI| = n_0|OA| + n_1|AB| \tag{6-2}$$

Therefore, the reconstructed image is actually expanded and deformed outwards for the ray illustrated in Figure 6-8, compared to the real configuration of the object. The distorted volume data will then introduce errors in the displacement and strain fields, leading to spurious values. In general, in order to infer the distortion free image (in the object space), the inverse transformation should be performed using a correction algorithm and a parametric representation of the shape of the air/cornea interface. In this work, we used an approach based on Fermat’s principle to correct for refraction distortions [172]. This principle states that the path taken between two points by a ray of light is the path that can be reached in the least time. Thus, point $B$ is reached by a unique ray refracted at point $A$.

To correct the OCT data volume for the cornea, initially, a third order polynomial function $f(x_A, z_A)$ was fitted to the top interface of the cornea. Then for the object point $B$ with the coordinates $(x_B, y_B, z_B)$, the coordinates of the corresponding point $A$, $(x_A, f(x_A, z_A), z_A)$, where the ray enters the cornea was calculated from Equation 6-2 such that,

$$\text{OPD} = n_0f(x_A, z_A) + n_1[(x_B - x_A)^2 + (y_B - f(x_A, z_A))^2 + (z_B - z_A)^2]^{1/2} \tag{6-3}$$

where $x_B, y_B, z_B$ were known, as they are defined in a 3D Cartesian grid in the object space, therefore, the unknowns were $x_A$ and $z_A$.  


By minimizing the OPD, the coordinates of point $A$, $(x_A, f(x_A, z_A), z_A)$, can be found for any given object point $B$. The coordinates of the corresponding image point $I$, $(x'_I, y'_I, z'_I)$, are then determined as:

$$\begin{align*}
    x'_I &= x_A \\
    y'_I &= n_0 f(x_A, z_A) - n_1 AB \\
    z'_I &= z_A
\end{align*}$$

(6-4)

where $AB$ is,

$$AB = [(x_B - x_A)^2 + (y_B - f(x_A, z_A))^2 + (z_B - z_A)^2]^{1/2}$$

(6-5)

Once the coordinates of the image point $I$ are established, the intensity value at that point is calculated using 3D bilinear interpolation and associated to object point $B$. This process is repeated for every point in the object space that lies under the cornea/air interface.

### 6.5.5 Evaluation of displacement fields within the cornea

In order to obtain the displacement field inside the stroma, volume reconstructions of the cornea in the reference and deformed states were processed using Digital Volume Correlation (DVC). A commercial package from LaVision was utilized, based on the Fast Fourier Transform algorithm [173]. The determination of tomographic displacement fields involves four main steps:

1- Reconstruction of the corneal volume for reference (P=2.0 kPa) and deformed (P=2.5 kPa) states.
2- Refraction correction of both data volumes recorded in (1).
3- Discretization of the volume into a set of sub-volumes that are centred on points of interest at which a displacement vector will be evaluated
4- Measurement of displacement vector field using volume correlation methods
In this work, DVC was initially implemented on the whole corneal volume data, i.e. \(1024 \times 1024 \times 512\) voxels from 1024 nasal-temporal cross-sectional images, but the poor speckle contrast near the cornea/sclera interface lead to erroneous estimates of displacement in that area. Thus, only the middle 600 cross-sectional images (aligned in the inferior-superior direction) were eventually used in the material property determination.

### 6.5.5.1 Background of digital volume correlation

Digital Volume Correlation is an extension Digital Image Correlation (DIC), a technique used to measure surface deformation [174]. The technique provides a discrete displacement vector field by correlating volume data from a deformed sample with that of a sample in a reference state, as illustrated in Figure 6-9. DIC is based on the principle of pattern matching [175].

![Figure 6-9 Schematic of reference and deformed volume data, a) reference data volume showing reference sub-volume and displacement vector (c) and b) corresponding deformed sub-volume in deformed data volume](image-url)
The data volume is subdivided into a set of sub-volumes that are centred on points that belong to a regular grid (usually Cartesian, cubic). The displacement vector is determined by tracking and matching the spatial variations in the intensity within the sub-volumes in the reference and deformed states. In principle, this is performed by maximizing a correlation coefficient, which measures the degree of similarity of the grey level distributions in the sub-volumes between the reference and deformed states. The best prediction of the displacement leads to the highest degree of similarity of the grey level distributions thus the maximal correlation coefficient. As shown in Figure 6-9, consider two scalar signals of \( f \) and \( g \) as continuous functions of \( x, y \) and \( z \), which represent a pair of intensity patterns in a sub-volume, \( \Omega \), before and after a continuous mapping. Correlation matching of the sub-volumes can be obtained by finding an optimal mapping that maximizes the cross-correlation functional, \( m(c) \) as,

\[
m(c) = (f * g)(x,y,z) = \int f(x,y,z)g(x,y,z)d\Omega(x,y,z)
\]  

(6-6)

where \( * \) represents cross-correlation. The term \( c \) is the displacement vector. The cross-correlation function can also be written using Fourier transforms such that

\[
m(c) = F(f * g) = F\{f\}^* F\{g\}
\]  

(6-7)

where \( F \) denotes the Fourier transform and \( * \) denotes the complex conjugate [176].

The Fourier transform for a general function, \( \alpha \), is defined as,

\[
F\{\alpha\} = \int n \alpha(x,y,z)e^{-i(k_x x + k_y y + k_z z)} dk_x dk_y dk_z
\]  

(6-8)

where \( k_i = \frac{2\pi N_i}{T} \) \( (i = x, y, z) \) and \( k = \frac{1}{T} \) is the wave number and \( N \) is the number of sampling points.

The Fourier transformed cross-correlation function is employed in most DVC software packages, as well as the one used in this work, for efficient numerical computation. The applicability of this algorithm, however, is limited to small strain (~5%) and small rotation (~5°) problems [176].
6.5.5.2 Influence of sub-volume size

The choice of sub-volume size used in DVC is important as it determines the computation time and the spatial resolution of the displacement field. While small sub-volumes may lead to a dense spatial distribution of displacement vectors, if they are too small compared with the size of the 3D speckle or texture features then the matching problem may not reach the optimum solution. Large sub-volumes, however, lead to good estimates of displacements at the expense of low spatial resolution. It is worth noting that in the case of cornea, we require a high spatial resolution in order to obtain independent displacement values through the thickness of the cornea, as these will be used to infer the corneal modulus as a function of position within the cornea. Four different sub-volume sizes were tested using two reconstructed volumes from the stationary test: $12^3$, $24^3$, $36^3$ and $48^3$ voxels. Each sub-volume had 50% overlap with its six adjacent neighbours. A sub-volume size of $24^3$ was considered a good compromise in terms of strain resolution and the spatial resolution. The spatial resolution becomes especially important for thin specimens having curved or irregular geometry.

6.5.5.3 Validation tests

We cannot have any confidence in displacement measurements obtained with OCT and DVC unless the results obtained are validated in controlled experiments. Therefore, in order to evaluate any errors caused by noise and reconstruction uncertainties, DVC was performed on two subsequent reconstructed volumes of the stationary phantom specimen using the $24^3$ voxel sub-volume with 50% overlap (courtesy of Mr. Jiawei Fu). We performed two validation tests: In the first one, a phantom (silicone rubber seeded with scattering particles) was kept stationary and two OCT volumes were scanned sequentially. The expected displacement field inside the volume of the phantom should therefore be zero throughout the phantom’s volume. However, electronic noise in the OCT system, digitization errors, refraction distortions, interpolation techniques used in the DVC algorithm, can lead to displacement and strain errors with spatial fluctuations. In the second test, the phantom was placed on a translation stage and moved 10 μm along the $y$ axis, in
order to validate the absolute value of the measured displacement, average strain (zero) and also the corresponding standard deviation (STD). The mean value of the strain field is used to indicate the average strain of the sample and the STD of the 3D strain field indicates the ‘spatial fluctuations’ of the strain field.

6.5.5.3.1 Phantom preparation

A Flat phantom was used in the validation tests to eliminate refraction distortion, as shown in Figure 6-10. It was made of silicone rubber, as described in Chapter 4, and seeded with talcum particles with 5 µm average diameter. The silicone and hardener mixture was spun at 2000 rpm for 5 minutes to reduce the amount of bubbles and obtain uniform thickness, and cured at room temperature for 24 hours. Dimensions were measured using a coordinate measuring machine to be 5.2×5.5×1.2 mm, width × length × thickness.

Figure 6-10 Flat phantom used in validation experiments
6.5.5.3.2 Stationary test

The strain mean and standard deviation were evaluated over a reconstructed volume with 1024×1024×512 voxels, corresponding to a physical size of 11×11×3 mm³. The mean value of all strain components $\varepsilon_{xx}$, $\varepsilon_{yy}$, $\varepsilon_{zz}$, $\varepsilon_{xy}$, $\varepsilon_{xz}$ and $\varepsilon_{yz}$ for the middle slice of the phantom (along the length) were between $-1\times10^{-4}$ to $1\times10^{-4}$ and the standard deviations were between $4\times10^{-4}$ to $6\times10^{-4}$. These values indicate the strain ‘noise floor’, i.e. a strain level under which signal cannot be distinguished from noise. It can be seen that the noise is lower than the strain levels introduced by the inflation test, described in Section 6.6.

6.5.5.3.3 Rigid body translation test

A rigid body translation of 10 µm along the y axis was introduced between the acquisition of the reference and deformed data volumes. The average of all the strain components were between $-1.2\times10^{-4}$ to $1\times10^{-4}$ (consistent with a strain-free situation) and the standard deviation were between $7\times10^{-4}$ to $9\times10^{-4}$. Compared with the stationary test, the average strain components were very close to the expected values introduced with the translation stages, while the standard deviation values were slightly higher. This is because in addition to all the error sources present in the stationary test (mainly due to noise in the OCT data), the rigid body translation introduces additional errors as the speckle field changes and moves between the reference and displaced states, which needs to be accommodated by interpolation in the DVC algorithm.

6.6 Evaluation of corneal displacement and strain distributions

A porcine cornea was inflated from a reference state at 2 kPa to a deformed state at 2.5 kPa within 10 seconds. The SS-OCT system scanned the corneal volume along the temporal to nasal meridian. The reference and deformed states of the cornea were scanned after 120 and 600 seconds, respectively, in order to stabilize the corneal viscoelastic deformation. Each reconstructed volume was corrected for
refraction distortions as described in Section 6.5.1.4. Figure 6-11 shows the middle slice OCT image of the cornea before and after the refraction correction.

![Uncorrected](image1)
![Corrected](image2)

Figure 6-11 Middle slice OCT image of the cornea, a) before the refractive correction and b) after the refractive correction

After correcting the OCT volume data, they were processed using digital volume correlation to measure the resultant displacement and strain fields. Figure 6-12(a) illustrates the data volume used for these measurements, with 1024×512×600 voxels (x, y and z axis, respectively). The use of the 24³ voxels sub-volume with 50% overlap leads to a displacement field volume with 84×42×50 points, as shown in Figure 6-12(b) and Figure 6-13.
Figure 6-12 Specifications of the OCT volume reconstruction of porcine cornea and the corresponding displacement data volume, a) volume size and orientation of the OCT data and b) dimensions of the displacement data volume.
Figure 6-13 Specifications of the displacement data volume, a) dimensions of the displacement data volume showing sub-volume size obtained with $24^3$ voxels sub-volumes and 50% overlap and b) two adjacent sub-volumes with 50% overlap.

Figure 6-14 illustrates a schematic view of the DVC displacement map as a stack of temporal-nasal 2D slices. The middle slice corresponds to the middle section of the cornea along the temporal-nasal meridian.
Figure 6-14 Schematic of DVC results as a stack of nasal-temporal 2D slices, the middle slice corresponds to the middle of cornea

Figure 6-15 shows the output of the DVC algorithm before and after the implementation of refraction correction for the nasal-temporal middle slice of the cornea. Results, designated here as ‘displacements’ in both cases are provided along the x, y and z axes, i.e. $U_x$, $U_y$ and $U_z$. Note that the $U_y$ map for the uncorrected data indicates changes in the optical path difference (OPD) rather than the displacement.
Figure 6-15 Displacement maps in a porcine cornea (nasal-temporal middle slice) from an increase in the pressure from 2.0 kPa to 2.5 kPa. The left column shows the DVC output obtained from OCT reconstructions that have not been corrected for refraction at the air/cornea interface. The right column shows displacements after refraction correction.

From the displacement maps, it can be seen that the horizontal displacement, $U_x$, is close to zero near the central region and increases symmetrically towards the peripheral region, resulting in a half positive (motion to the right) half negative (motion to the left) distribution. The vertical displacement, $U_y$, is more significant than the horizontal one, as expected from the case of inflating a thin shell spherical structure. The maximum $U_y$ is located near the centre of the corneal inner surface and gradually decreases toward the outer surface. $U_z$, i.e. the displacement of the middle slice along the z axis (out of the plane of the figure), is spatially uniform and close to zero, as expected.
The strain maps were calculated from the centred finite difference of the displacement data such that:

\[
\varepsilon_{ij} = \frac{1}{2}(U_{i,j} + U_{j,i})
\]  

(6-9)

The strain fields (before and after the implementation of the refraction correction algorithm) for the nasal-temporal middle slice of the cornea in the xy plane are shown in Figure 6-16.

Figure 6-16 Strain map inside the porcine cornea (nasal-temporal middle slice) from an increase in the pressure from 2.0 kPa to 2.5 kPa. The left column shows strain obtained from OCT reconstructions that have not been corrected for refraction. The right column shows strain after refraction correction.

It can be seen from the strain maps in Figure 6-16 that the distribution of the normal strain components, i.e. \( \varepsilon_{xx} \) and \( \varepsilon_{yy} \), indicate a tensile state along the horizontal x
axis and a compressive state along the vertical $y$ axis, which is greatest in the central region of the cornea. The in-plane shear strain, $\varepsilon_{xy}$, is slightly positive in the peripheral regions close to the retaining ring. In all cases, the higher values in the peripheral regions are due to the low contrast in the OCT reconstructed data, which represent spurious values of strain, as shown in Figure 6-17. As this data was not reliable it was not used in further analysis.

![Figure 6-17](image)

Figure 6-17 Low speckle contrast in the OCT reconstructions leading to spurious values of strain, a) in-plane shear strain, $\varepsilon_{xy}$, for the middle slice cornea and b) corresponding corrected OCT image

Interestingly, Figure 6-18 shows a region within the $\varepsilon_{yy}$ strain map indicating positive values located close to the endothelium layer, in the centre of the cornea. Typically, the strain value at this region is expected to be negative, as the cornea is subjected to a compressive stress in the $y$ direction. It was assumed that the positive strain is due to the water absorption of the bottom of the cornea. The assumption was based on the literature mentioning that endothelial cells (after the death of the animal) can no longer maintain fluid balance and causes stromal swelling due to excess fluids [8]. This effect is worthy of further investigation. In this research the positive strain data and the vicinity of it are discarded.
Summary

In this chapter, initially, the sample preparation, experimental setup and procedure are described. Further, a background about the OCT volume data reconstruction and displacement evaluation with DVC is provided. Prior to explaining the evaluation of the displacement field, the effect of refraction distortion, which is an issue in the volume data reconstruction, is discussed. A series of validation tests are described, which are performed to evaluate any errors caused by noise and reconstruction uncertainties. Finally, the evaluated displacement and strain maps of the central slice cornea is provided and discussed.
Chapter 7: Finite Element Modelling

7.1 Introduction

In this research, four different finite element models (FEMs) of the cornea were created as follows:

1- A model of the stress relaxation test to validate the experimental results
2- Two models (isotropic and transversely isotropic) of the nano-indentation test to be employed in an inverse updating method
3- A model of the inflation test to be used in an inverse algorithm

For each corneal model, boundary conditions and geometry differed based on the type of experiment. Also, based on the corneal mechanical responses, in each finite element model (time-dependant or time-independent), an appropriate rheological model was considered. The stress relaxation and nano-indentation models were used to study the corneal time-dependant behaviour considering a linear viscoelastic rheological model. The inflation model, on the other hand, was used to obtain a spatial distribution of the corneal time-independent properties, thus, a linear elastic rheological model was employed. In order to compromise between our two objectives, i.e. an effective representation of the experiments and a sensible computational cost (which becomes especially important in the inverse updating calculations), various simplifications such as symmetry about the $y$-axis were employed in the FEMs, as shown in Figure 7-1.

Figure 7-1 Schematic of cornea, showing $y$-axis as axis of symmetry and meridians, i.e. nasal-temporal (N-T) and inferior-superior (I-S)
7.2 Finite element model of stress relaxation experiment

A 3D finite element model was produced in order to validate the stress relaxation experiment. The corneal strip was modeled using Abaqus Standard 6.11 with the measured geometry, as described in Section 4.2. The generalized Maxwell (GM) model of linear viscoelasticity, as described in Section 4.1.2, was used as the rheological model and the experimentally obtained parameters, presented in Section 4.4, were input to the FEM. The cornea was assumed to be almost incompressible with a Poisson's ratio of 0.49 [130]. The model had 2500 quadratic hexahedral elements, with a reduced-integration scheme used to reduce the computational cost. The strip was fixed in rotation and translation at one end and was loaded from the other end, as shown in Figure 7-2.

![Figure 7-2 Finite element model of the cornea in the stress-relaxation test, showing the boundary conditions](image)

Two deformation schemes were applied based on the experimental data; a loading step of 8 seconds with true strain rate of 0.1 s\(^{-1}\) and a holding period of 2000 seconds at 5% true strain. The model was solved using the Abaqus implicit solver. The results of the modeling are compared with the experimental data in Section 9.2.

7.3 Finite element model of nano-indentation experiment

Initially, an isotropic GM model of the nano-indentation experiment was generated to validate the experimental data. Then, the same model was used to develop an inverse updating algorithm that recovered the viscoelastic parameters of the cornea in tissue level. Finally, in order to account for the fibre-matrix, composite-like structure of the cornea, a transversely isotropic model was generated that was
implemented at the developed inverse algorithm to recover the sub-tissue level parameters.

**7.3.1 Effect of corneal domain and boundary conditions**

Initially, a comparative numerical study was performed in order to determine a corneal domain and ascertain the degree of influence of the blue tack on the simulated results. Two axisymmetric models were created using Abaqus 6.11, one with full corneal thickness and a supporting base layer of blue tack and one with partial thickness.

A comparative numerical study was performed in order to determine a corneal domain and ascertain the degree of influence of the blue tack on the simulated results. Two axisymmetric models were created using Abaqus 6.11, one with full corneal thickness and a supporting base layer of blue tack and one with partial thickness:

1. Corneal domain of 1.4×1.2 mm², full thickness, and partial geometry of blue tack
   The properties of the blue tack were obtained from the literature, Young's modulus of 0.7 MPa and Poisson's ratio of 0.49 [177].

2. Corneal domain of 0.8×1.2 mm², partial thickness

In order to compare results of the two models, other modelling parameters such as boundary conditions, element type and contact properties were the same, as described in Section 7.3.2. The nano-indentation experiment was simulated, inputting the corneal viscoelastic parameters, as described in Section 4.1.2. Figure 7-3 illustrates the two FEMs with different corneal domains.
Figure 7-3 Finite element models with different corneal domains, a) showing cornea and blue tack sections and refined mesh area, b) full corneal thickness and c) partial corneal thickness

Depth vs. time plots (obtained for cornea’s apical node) from both models are compared in Figure 7-4.

Figure 7-4 Comparison of simulated apical depth vs. time results obtained from two models; one with full corneal thickness and blue tack and one with partial corneal thickness, error< 1%
The result obtained from the two models showed a negligible difference, i.e. less than 1% error. Therefore, for further analysis, the model with partial domain of $1.2 \times 0.8 \text{ mm}^2$ with 4500, quadratic quadrilateral elements were used.

### 7.3.2 Isotropic model

An isotropic finite element model of the nano-indentation experiment was created using Abaqus Explicit 6.11. The model was generated in 2D using the axisymmetric condition for a corneal domain of $1.2 \times 0.8 \text{ mm}^2$. The corneal domain was assumed flat, as the indentation area was very small in comparison to the total corneal curvature. The radius of curvature of the porcine cornea is reported in the literature as $\sim8 \text{ mm}$, compared to a maximum indentation depth of $\sim9 \times 10^{-3} \text{ mm}$ in the indentation model.

The water layer, which was observed at the interface of the cornea and the indenter, was not implemented in the model because it had a negligible effect on the recorded force values and consequently the measured force/displacement/creep profile. To study this effect, the magnitude of force due to the formation of a meniscus was approximately determined based on the Young–Laplace equation for a spherical indenter. The adhesion force was estimated as being of the order of 10nN, which is much smaller than that of the max force applied to the cornea in the indentation test.

Approximately, 4500 quadratic quadrilateral elements with reduced-integration were used in the indentation model. An adaptive meshing scheme based on Arbitrary Lagrangian-Eulerian (ALE) analysis was implemented for the region of the cornea under the largest deformation [178]. The adaptive meshing was applied to maintain a high-quality mesh throughout the analysis. The indenter was modelled as a rigid body with a radius of 0.6 mm. The cornea was fixed along the axis of symmetry, $y$, for translation in $x$ direction and along the base line for $x$ and $y$ rotation and translation. The contact was assumed frictionless based on the observation of a thin layer of water on the surface of the cornea. Figure 7-5 illustrates the isotropic finite element model of the corneal nano-indentation, showing the boundary conditions and the mesh.
A linear viscoelastic rheological model was used for the cornea in the form of a Generalized Maxwell (GM) model. In Abaqus, the linear viscoelasticity is separated into two terms:

1- An elastic modulus, $E$, that defines the instantaneous elastic behaviour

2- Parameters of the Prony series: $g_i$ that refers to the $i$th normalized shear relaxation modulus and $\tau_i$ that refers to the corresponding relaxation time.

An advantage of this approach, as explained in Section 4.1.2, is that the linear viscoelastic parameters of the cornea, i.e. $E$, $g_1$, $g_2$, $\tau_1$ and $\tau_2$, can be attributed individually to different microstructural components, i.e. fibres and matrix. Parameter $E$ can be used to describe the combined response of the corneal fibres and matrix [153, 153, 155] whereas, when appropriately transformed, $g_i$ and $\tau_i$ can describe the corneal matrix behaviour [152, 154].
7.3.3 Transversely isotropic model

To extend the isotropic model, a 3D transversely isotropic transversely isotropic model was created to account for the composite microstructure of the cornea and to allow for fibre orientations. Since the instantaneous elastic behaviour of the cornea could be separated from the viscoelastic part, as explained in Section 4.1.2, the transversely isotropic model was restricted to the elastic part only. A corneal domain of $1.2 \times 0.8 \times 0.8$ mm$^3$ was meshed with 30000 quadratic brick elements using the mesh refinement region for the area under indentation. The boundary conditions and the contact specifications were defined in the same way as for the isotropic model, as explained in Section 7.3.2.

The central region of the cornea, around the $y$-axis, has a transversely isotropic fibre orientation with fibres aligned orthogonally in the $xz$ plane. It was assumed that the properties in the orthogonal directions are equal. Figure 7-6 shows the transversely isotropic model, showing the fibre orientation.

Figure 7-6 Transversely isotropic Transversely isotropic model of the central cornea in nano-indentation. Fibres are aligned along the $x$ and $z$ axes (Cartesian coordinate system).
The elastic behaviour of the cornea can be written in the form of Hooke’s law as,

$$\boldsymbol{\varepsilon} = \mathbf{S} \boldsymbol{\sigma}$$  \hspace{1cm} (7-1)

where $\boldsymbol{\varepsilon}$, $\boldsymbol{\sigma}$ and $\mathbf{S}$ are the strain, stress and compliance tensors, respectively. The expansion of Hooke’s law for a transversely isotropic material is given by,

$$
\begin{bmatrix}
\varepsilon_{xx} \\
\varepsilon_{yy} \\
\varepsilon_{zz} \\
\varepsilon_{xy} \\
\varepsilon_{yz} \\
\varepsilon_{zx}
\end{bmatrix}
= 
\begin{bmatrix}
\frac{1}{E_x} - \frac{v_{yx}}{E_y} & -\frac{v_{zx}}{E_z} & 0 & 0 & 0 \\
\frac{v_{xy}}{E_x} & \frac{1}{E_y} - \frac{v_{xy}}{E_y} & 0 & 0 & 0 \\
\frac{v_{xz}}{E_x} & \frac{v_{yz}}{E_y} & \frac{1}{E_z} & 0 & 0 \\
0 & 0 & 0 & \frac{1}{G_{xy}} & 0 \\
0 & 0 & 0 & 0 & \frac{1}{G_{yz}} \\
0 & 0 & 0 & 0 & 0
\end{bmatrix}
\begin{bmatrix}
\sigma_{xx} \\
\sigma_{yy} \\
\sigma_{zz} \\
\sigma_{xy} \\
\sigma_{yz} \\
\sigma_{zx}
\end{bmatrix}
$$  \hspace{1cm} (7-2)

An transversely isotropic, Hookean solid is characterised by nine constants, $E_x$, $E_y$, $E_z$, $G_{xy}$, $G_{yz}$, $G_{xz}$, $v_{xy}$, $v_{yz}$ and $v_{xz}$. For the transversely isotropic cornea, $E_x$ and $E_z$ are the moduli along the fibre directions, $x$ and $z$. $E_y$ is the modulus along the loading direction and can be considered normal to the direction of fibres embedded in the matrix, as shown in Figure 7-6. Parameter $E_y$ is deduced from the isotropic instantaneous elastic constant, i.e. $E_y = E_{\text{isotropic}}$. The following symmetric conditions are then assumed:

$$E_x = E_z \hspace{1cm} (7-3)$$

$$G_{xy} = G_{yz}$$

$$v_{yx} = v_{yz}, \ v_{xz} = v_{zx}, \ v_{xy} = v_{xy}$$

where $E_i$, $G_{ij}$ and $v_{ij}$ are elastic modulus, shear modulus and Poisson’s ratio corresponding to the planes of symmetry, i.e. $xy$ and $zy$ planes. Further, using an
assumption of corneal incompressibility i.e., \( v_{xy} = v_{zy} = 0.5 \), Poisson’s ratio and shear modulus on the plane of orthotropy, \( v_{xz} \) and \( G_{xz} \) can be obtained from [179],
\[
v_{xz} = 1 - v_{xy}
\]

\[
G_{xz} = \frac{E_x}{2(1 + v_{xz})}.
\]

Thus, the transversely isotropic elastic cornea is characterized by four independent parameters: \( E_x \), \( E_z \), \( E_y \) and \( G_{xy} \). Parameter \( G_{xy} \) was recently measured by Nickerson [180], who suggested a value of \( G_{xy} = 3 \) Pa. Parameter \( E_y \) is obtained from the isotropic model, as will be explained in Section 9.2.2, leaving only \( E_x \) and \( E_z \) to be obtained from the transversely isotropic model.

### 7.4 Finite element model of inflation experiment

In order to study the distribution of the corneal properties via the inverse finite element method, the long-term inflation response of the cornea was simulated. In this study, the distributions along the radial and circumferential directions were examined, in a sequential manner. In the first stage, spatial variation of the properties along the radial direction (through the corneal thickness) was studied. It was assumed that the cornea has uniform average properties along the circumferential direction. A finite element model was produced, in which the cornea was partitioned into six isotropic and homogeneous sections along the radial direction. The model was then implemented in the inverse finite element algorithm to recover the distribution of the properties along the radial direction.

In the second stage, variation of the properties along the circumferential direction was investigated, having considered uniform properties through the radial direction. A finite element model was generated, in which the cornea was divided into ten isotropic homogeneous sections along the circumferential direction. This model was used to recover the circumferentially varying properties.
7.4.1 Geometry

Initially, two identical axisymmetric 2D models were produced. Geometry was partially reconstructed from the middle slice OCT image and partially from the geometrical measurements. Initially, the anterior and posterior curvature was created by using coordinates of the points extracted from the OCT image, as shown in Figure 7-7(a). Since the OCT image does not illustrate the corneal geometry at the clamped bottom region due to the low speckle contrast, as explained in Section 6.6, the remaining geometrical features such as, the angle of clamp and the in-plane radius were generated from the corneal measurements. The reconstructed geometry was then verified against the thickness measurements, provided in Section 6.2, to be the same, as shown in Figure 7-7(b).

Figure 7-7 Geometry of the corneal model, a) reconstruction of radii of curvature based on the OCT middle image and b) verification of the reconstructed geometry with experimental measurements (thickness measurements)
7.4.2 Boundary conditions

The cornea was fixed along the axis of symmetry, $y$, for translation in the $x$ direction and along the base line for $x$ and $y$ rotation and translation. A uniform pressure was applied to the endothelium layer, which increased from 0 to 0.5 kPa in 10 seconds. The boundary conditions are shown in Figure 7-8.

![Figure 7-8 Boundary conditions](image)

7.4.3 Finite element model with radial partitions

To study the distribution of the properties through the radial direction, $r$, (in polar coordinate system), the average of the properties along the circumferential direction was assumed uniform. An FE model was generated, in which the cornea was partitioned along the radial direction into six elastic homogeneous regions (with equal thickness throughout), as shown in Figure 7-9. The domain was then meshed with 5200 triangular linear reduced integration elements with element size of 0.7 mm$^2$. 
After studying the distribution of the properties along the radial direction, the variation of the properties along the circumferential direction, $\theta$, was studied. An FE model was produced, in which the cornea was divided into ten elastic homogeneous regions along the circumference (assuming a uniform property through the thickness) as shown in Figure 7-10. The domain was then meshed with the same number, type and size of the elements as described in Section 7.4.3.
Chapter 8: Inverse Finite Element Analysis

8.1 Introduction

Most finite element textbooks have described the solution of forward problems, in which the material properties are known and one only needs to obtain the kinematic field. Another class of problem, however, consists of finding material properties when the kinematic field is known. A powerful method of solving such problems is the inverse finite element method (IFEM). The IFEM is a numerical-experimental approach that allows the characterization of materials, where standard experimental methods fall short. For instance, the characterization of an inhomogeneous material via a tensile test only results in obtaining the average properties, whereas, by utilizing the IFEM the spatial varying properties can be determined. Another advantage of this method is that it can be used to determine properties of a material while under a complex stress mode. Furthermore, the combination of the inverse finite element method and non-destructive full field measurement methods, such as optical coherence tomography, could eventually combine to provide an in situ technique to characterize biological tissues.

In the inverse finite element method, an optimization algorithm is coupled with a finite element analysis in order to determine the optimal values for a set of target parameters. A user defined objective function, e.g. least square difference between experimental data and simulation, serves to measure the optimality of the parameters. A finite element model is required to simulate the structural response with the target parameters as input. The target parameters can be various physical quantities, such as the dimensions of a component, which is the case in topology and shape optimization, or the material parameters, which is the case in inverse material parameter estimation related to experimental testing. In the latter, initially, one needs to select an appropriate rheological model for the material and define a set of material parameters as variable in the optimization. The optimization algorithm searches for an optimal fit of the simulation result to the experimental data. The algorithm computes the structural response using finite element analysis and updates the values of the parameters until the computed response matches the experimentally measured ones according to a user defined convergence criterion.
The quality of the inverse solution depends strongly on the formulation of the objective function and the quality of the simulation [117]. In addition, the experiment has to be performed in a way to reveal information relevant for the estimation of the target parameters in the inverse method. It is preferable to keep the number of target parameters (variables) as small as possible. Models with a large number of variables tend to produce parameter values that are not reasonable from a physical point of view [181]. The larger the number of target parameters, the smaller is the influence of any single parameter on the structural response of the system and thus also on the objective function [121].

It is the scope of this research to develop an inverse finite element method in combination with two different experimental methods (inflation and nano-indentation) to determine the time-dependant and spatially varying properties of the porcine cornea. The inverse algorithm combined with the nano-indentation technique determines the time-dependant anisotropic properties and its combination with the inflation full-field measurement technique reveals the distribution of the properties. This is more fully described in Sections 8.4 and 8.5.

### 8.1.1 Theory of local vs. global optima

Generally, optimization is the process of finding the point that optimizes a function. More specifically:

- A local minimum of a function is a point, where the function value is smaller than or equal to the value at nearby points, but possibly greater than at a distant point. A local optimization method performs optimal steps locally and may lead to only locally optimal parameters value.

- A global minimum is a point where the function value is smaller than or equal to the value at all other feasible points. The global method finds a set of parameter values that corresponds to a global optimality of the function. Figure 8-1 illustrates local and global minima of an objective function.
Generally, local optimization strategies, commonly known as gradient-based algorithms, find a local optimum (the local optimum can be a global optimum) within the valley (basin of attraction) of the starting point. In contrast, global optimization strategies are designed to search through more than one basin of attraction [182]. In the literature, the inverse finite element method is reported to be a type of local optimization strategy [183, 184]

### 8.1.2 Theory of gradient-based algorithms

In gradient-based algorithms, also known as steepest descent algorithms, one takes steps proportional to minus the gradient of the objective function, $f(x)$, at a current point, $x_i$, as,

$$ \Delta x = x_{i+1} - x_i = -\lambda \nabla f(x_i) $$

(8-6)

where $\Delta x$ is the size of the steps, $i \geq 0$ and $\nabla f(x_i)$ is the gradient of the function. For $\lambda \to 0$ a small enough number, then $f(x_0) \geq f(x_1) \geq f(x_2) \geq \cdots$ and the sequence $x_i$ converges to the local minimum, as shown in Figure 8-2.
Figure 8-2 illustration of an objective function with two one-dimensional minima, showing the basins of attraction (in different line styles) and the direction of steepest descent. The steepest descent path, starting at a point, \( x_0 \), goes to the minima in the basin containing \( x_0 \).

Any starting point, \( x_0 \), that is selected within one valley leads the direction of the steepest descent toward one minimum point. Therefore, it is important to choose a reasonable initial value. A wrong initial value can lead the steepest decent path toward a different minimum in another valley as shown in Figure 8-2. For a function with several variables, a priori knowledge of the reasonable initial value for the variables and avoidance of interdependency between them is therefore essential [185].

In gradient-based algorithm, the basic idea is to approximate \( f(x) \) at the point \( x_i \) by the second order Taylor expansion as,

\[
f(x) = f(x_i) + \nabla f(x_i)(x - x_i)
\]  

(8-7)

which reasonably reflects the behaviour of function \( f \) in the basin of attraction around the point \( x_i \).

To find the min/max of a function, the first condition is that the gradient of the function should be zero such that,
\[ J = \nabla f(x) = 0 \]  

(8-8)

where \( J \) is the Jacobean matrix. The second condition is that the eigenvalues of the Hessian matrix (square matrix of second-order derivatives) should be positive, i.e.

\[ H = \nabla^2 f(x), \text{ Eigenvalue of } H \geq 0. \]  

(8-9)

The gradient-based algorithm used in this research is the robust deterministic method of Levenberg-Marquardt (LM). The LM algorithm also known as the damped least-squares method, is a more general form of the gradient-based algorithms that provides a numerical solution to minimize an objective function, \( S(x) \), in the form of a least squares difference between the vector of experimental data, \( y_i \), and the function \( f(x) \),

\[ S(x) = \sum_{i=1}^{n} [(y_i - f(x_i))^2] \]  

(8-10)

where \( y_i \) is a vector with \( i \) components. In each iteration, the parameter vector \( x_i \) is replaced by a new estimate, \( x_i + \Delta x \). To determine \( \Delta x \) the function \( f(x) \) at the new point \( x_i + \Delta x \) is approximated as,

\[ f(x_i + \Delta x) \approx f(x_i) + \nabla f(x_i) \Delta \]  

(8-11)

Therefore, Equation 8-10 becomes:

\[ S(x + \Delta x) \approx \sum_{i=1}^{n} [y_i - f(x_i) - \nabla f(x_i) \Delta x]^2 \]  

(8-12)

Or in vector notation,

\[ S(x + \Delta x) \approx ||y - f(x) - J\Delta x||^2 \]  

(8-13)

To find the minimum of the objective function, \( S \), the gradient of \( S \) with respect to \( \Delta x \) should be zero such that,
\[(J^T)\Delta x = J^T[y - f(x)]\]  

(8-14)

In the case of Levenberg–Marquardt algorithm, Equation 8-14 is modified by a damping factor \(\lambda\) so that,

\[(J^T + \lambda I)\Delta x = J^T[y - f(x)]\]  

(8-15)

where \(I\) is the identity matrix. At each iteration, \(\lambda\) is adjusted according to the speed of reduction in the objective function, \(S\). If the reduction of \(S\) is rapid, a smaller value \(\lambda\) is used (bringing the algorithm closer to the Gauss–Newton algorithm [184]), whereas if the reduction is insufficient, the value of \(\lambda\) is increased (bringing the algorithm closer to other types of the gradient-based algorithm) [184]. This characterization of the LM algorithm makes it a relatively robust algorithm in finding a solution even if the starting points (initial values) are far off from the minimum. However, the LM algorithm tends to be slower than other gradient-based algorithms. In this research in order to increase solution speed, the variables are constrained using a priori knowledge obtained from the stress relaxation experiments.

### 8.3 Flow of the inverse finite element algorithm

As mentioned in Section 8.1 the inverse finite element method (IFEM) is a mixed numerical-experimental method to recover material properties by optimizing an appropriate objective function. The objective function describes the difference between the responses of a finite element model, which incorporates adjustable parameters describing the material properties, and the related experimental data. Amongst a family of potential gradient-based optimization algorithms, Levenberg–Marquardt algorithm (LM) is efficient when dealing with a moderate number of parameters, particularly when prior knowledge of the parameters target values is not available. In the case of a large number of parameters, the LM algorithm is relatively slow to converge in comparison with other types of gradient-based algorithms. However, the performance of the LA algorithm can be increased by applying constraints on the parameters search space.
Another factor to consider is the type of kinematic response for defining the objective function. The majority of publications in this field have used displacement data in the IFEM for small-scale deformation since the measurement procedure for displacement is straightforward [186, 187]. The use of strain data in defining the objective function is more attractive than displacement data in large-scale testing, as it does not require any frame of reference [188].

In an optimization problem, there are occasions when convergence criteria are not met. One of the causes of this is instability of the analysis due to a large number of variables, usually seen in the cases of shape and topology optimization. Thus, a sensitivity analysis helps to narrow down the optimization parameters. In our case, a sensitivity analysis was not necessary because the number of variables (material parameters) are small and of similar importance. A general flow chart of the inverse algorithm developed in this research is presented in Figure 8-3.

Figure 8-3 Flowchart of the inverse method used to determine corneal material properties
Firstly, a numerical model of the experiment should be set up, as described in Sections 7.3 and 7.4. The model should contain information on the boundary conditions and the parameters that describe the material properties as optimization variables. The optimization procedure was driven and controlled in Matlab R2012a, as described in following sequences:

1- An input file for the FEM was generated and initial values for the material parameters were assigned.
2- The file was sent to the numerical solver, Abaqus 6.11, to obtain the structural response (displacement).
3- After the analysis was finished, the FE output file was read and nodal displacements were extracted.
4- An objective function was defined based on a least squares difference between the numerical results and experimental data. The experimental data (displacement) forms a reference for an iterative adjustment of the variables in the numerical model.
5- If the convergence criteria, i.e. value of the objective function less was than $10^{-8}$, the optimization was terminated and the variables values taken as the material parameters.

In the case where the convergence criteria was not met, the variables were updated with a tolerance of less than $10^{-6}$. The function "Fmincon" of the Matlab gradient-based optimization toolbox was selected that uses the LM variable updating scheme. The function requires constraining the variables to upper and lower values in order to reduce the optimization time. The variable updating was continued until the stopping criteria were met.

Two main issues were encountered during the course of the optimization as described below:

1- In each iteration, the LM algorithm updated the variables by a small amount, eight decimal places, in order to calculate the gradient of the objective function. In order to reflect the effect of such a small change in material parameters, high precision (16 decimal digits) output data should be produced. Therefore, the FE analysis had to be performed with a high precision solver, which increases the total time of the inverse analysis.
The time elapsed for the output file to be generated had to be considered in the Matlab code.

The general procedure of the IFEM was implemented in two different scenarios: a) to determine the viscoelastic properties of the cornea and b) to study the spatial distribution of the properties. The procedure was modified for each case.

8.4 Inverse finite element method in finding viscoelastic properties

The inverse finite element method was employed in combination with the nano-indentation experiment to obtain the parameters of the corneal rheological model (GM model of linear viscoelasticity) in the centre. The IFEM was used to first obtain the corneal viscoelastic parameters $E_y, g_1, g_2, \tau_1$ and $\tau_2$ from the isotropic model and then to find the instantaneous elastic parameters in the orthogonal directions along the fibres, i.e. $E_x$ and $E_z$, from the transversely isotropic model.

8.4.1 Isotropic parameters

The IFEM was used to determine the corneal viscoelastic parameters from the nano-indentation isotropic model. The five parameters $E_y, g_1, g_2, \tau_1$ and $\tau_2$, as explained in Section 7.3.2, were considered as the variables to be recovered iteratively. The variables were constrained to be within upper and lower values. The constraint values were selected based on the results from the stress relaxation experiments.

The objective was to minimize the root mean square (rms) difference between the experimental indentation depth, $u_{Exp}$, and the numerical nodal (apical node) displacement in y axis, $u_{y\,FEA}$, with respect to time (total time of the experiment) as,

$$Min: \Delta = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left( u_{y\,(i)\,FEA} - u_{(i)\,Exp} \right)^2} \quad (8-16)$$
where $i$ represents the $i^{th}$ time step and $N$ is the total number of time steps. The term $u$ denotes the numerical displacement of the apical node along the $y$ axis, or the experimental depth.

### 8.4.2 Transversely isotropic parameters

After obtaining the viscoelastic parameters, the IFEM was used to find the in-plane elastic parameters along the orthogonal fibre directions, i.e. $E_x$ and $E_z$, as explained in Section 7.3.3, from the transversely isotropic model. This time only the loading part of the nano-indentation was analysed. The two parameters were limited to upper and lower bounds. The objective was similar to Equation 2-1, i.e. minimization of the root mean square (rms) difference between the loading part of the experimental indentation depth and the numerical $y$ displacement of the apical node with respect to time (loading time of the experiment).

### 8.5 Inverse finite element method to determine spatial distribution of properties

In another scenario, the inverse finite element method was employed in combination with the inflation model to obtain the spatially varying properties of the cornea. In this study, the long-term elastic response of the cornea was targeted, in which the time-dependant behaviour of the cornea (creep response) was eliminated by applying a hydrostatic pressure for a long time, as discribed in Section 6.4.2. The IFEM was used to first study the mean variation of the properties along the radial direction with the help of the radially partitioned model and then to investigate the mean variation of the properties along the circumferential direction with the help of the circumferentially partitioned model.

#### 8.5.1 Distribution of properties in radial direction

In order to study the distribution of the corneal properties in the radial direction, the IFEM was employed in combination with the model described in Section 7.4.3. In the model, the cornea was discretized into six homogeneous regions with equal
thickness. An elastic modulus, $E_r$, and a Poisson’s ratio were assigned to each region. The six elastic moduli, $E(r_1)-E(r_6)$, were considered as the optimization variables and were constrained to upper and lower values. The value of Poisson’s ratio, for all the regions was assumed constant with a value of 0.49.

The objective function was defined to minimize the root mean square difference between the displacement magnitude of FE nodes of interest and the displacement magnitude of the corresponding experimental data points as,

$$
\text{Min}: \Delta = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (U(i)_{FEM} - U(i)_{Exp})^2}
$$

(8-17)

where $N$ is the number of the FE nodes or the experimental data points. The term $U$ is the magnitude of displacement described as,

$$
U = \sqrt{u_x^2 + u_y^2}
$$

(8-18)

where $u_x$ and $u_y$ denotes the displacement in $x$ and $y$ directions, respectively.

In order to minimize the analysis time, the objective function was defined for only a selection of data points rather than all of the data. The data-points were selected in three sets of nodes from the FE model in a way that they represent an extended region in the cornea, as shown in Figure 8-4.
Figure 8-4 Finite element model with six radial partitions, showing three sets of nodes that were selected to define the objective function. Apical node (top left on the outer edge) is at (0,0) of the coordinate system. Set 1 (marked in black) is the group of nodes located close to the epithelium layer. Set 2 (marked in white) is the group of nodes located along the stromal middle layer. Set 3 (marked in blue) is the group of nodes located through the thickness, along the optical axis.

The corresponding location and the displacement values of the selected nodes were found through interpolation within the experimental displacement maps, x and y displacement, as shown in Figure 8-5. This was done because the mesh grid in the finite element model was different to the data grid of the experimental displacement maps.
Figure 8-5 Experimental data points that were correlated with nodes from the radially partitioned model, a) correlated data-points within $x$-displacement map, $u_x$ and b) correlated data-points within $y$-displacement map, $u_y$

As can be observed from Figure 8-5, the data-points are not selected from the bottom region of the cornea as the displacement field in this region was evaluated from a low contrast OCT data, as described in Section 6.6.

### 8.5.2 Distribution of properties in circumferential direction

Having found the variation of the corneal properties through the thickness, the IFEM was used again to study the distribution in the circumferential direction from the model with circumferential partitions as explained in Section 7.4.4. In this model the properties in the radial direction were considered uniform and the cornea was divided into ten homogeneous sections with random thickness. An elastic modulus, $E_\theta$, and a Poisson’s ratio were assigned to each section. The ten Moduli, $E(\theta_1)$-$E(\theta_{10})$, were considered as the optimization variables. The variables were subjected to upper and lower constraints. The value of Poisson’s ratio was kept constant at
0.49 for all the sections. The objective function was similar to Equation 4-1 for a selection of data-points. The points were selected in three sets of nodes from the model, as shown in Figure 8-6. Please note that the nodes in the radially partitioned model are different to those in the circumferentially partitioned one as they have different mesh grids.

Figure 8-6 Finite element model with ten circumferential partitions, showing three sets of nodes selected to define the objective function. The apical node is at (0,0) of the coordinate system. Set 1 (marked in black) is the group of nodes located close to the epithelium layer. Set 2 (marked in white) is the group of nodes located along the stromal middle layer. Set 3 (marked in blue) is the group of nodes located through the thickness, along the optical axis.

The procedure to correlate the selected FE nodes to the experimental data was explained in Section 8.5.1. Figure 8-7 shows the experimental data points that were correlated to the FE nodes within the x and y displacement maps.
Figure 8-7 Experimental data points that were correlated with nodes from the circumferentially partitioned model, a) correlated data-points within $x$-displacement map, $u_x$ and b) correlated data-points within $y$-displacement map, $u_y$. 
Chapter 9: Numerical Results

9.1 Introduction

The inverse finite element method (IFEM) was combined with two types of experiments to evaluate time dependent and time-independent properties of the cornea based on its structural response. First, a validation experiment was performed using the isotropic nano-indentation model and the viscoelastic parameters (obtained from the stress relaxation test). The validation experiment was undertaken to investigate if an isotropic model is sufficient in describing corneal material behaviour when exposed to a complex stress state. The IFEM was then employed with the nano-indentation experimental data to obtain the viscoelastic parameters of the cornea at sub-tissue level, utilizing the isotropic and transversely isotropic models. The IFEM was also used with the inflation experiment to determine the spatial distribution of the corneal mechanical properties. In the inflation test, the time-independent behaviour of the cornea was targeted; the cornea was considered as heterogeneous, elastic material with properties that could vary spatially through the thickness and across the circumference. To determine the spatial variation of the properties via the IFEM, two finite element models with different partitioning schemes, i.e. radial and circumferential partitions, were utilized. In each model, the cornea was considered to have a number of homogeneous regions. First, the cornea was assumed to have uniform mean properties along the circumference and the radial distribution of the properties was determined. Then the circumferential distribution was examined. In chapters 7 and 8, the full descriptions of the models and the details of the inverse approach were presented. In this chapter, solutions from the inverse analyses are provided and discussed.

9.2 Evaluation of the viscoelastic properties

Three nano-indentations were performed near the centre of the right cornea of a pair of porcine eyeballs. The results are shown in Section 5.4.2. Initially, a validation experiment was performed using the isotropic model developed in Section 7.3.2 to predict the time dependent depth penetration for the nano-indentation. The material parameters were input from the stress relaxation test, as described in Section 4.5.
Figure 9-1 shows the depth vs. time experimental data in comparison with the simulation results.

![Graph showing depth vs. time for nano-indentation data in comparison with simulation results](image)

**Figure 9-1 Depth vs. time nano-indentation data in comparison with the simulation results obtained from the isotropic model**

It can be seen from Figure 9-1 that there is reasonable correspondence in the trends for the creep, but the loading part shows a significant discrepancy. One potential reason for this can be found in the reported orthotropy of the cornea with the fibres orientated within the plane of the cornea. Schematics of the fibre and load directions in the nano-indentation and relaxation tests are provided in Figure 9-2. As the tensile relaxation test is performed along the fibre direction in the centre, and considering that other fibres tend to reorient themselves toward the load direction [101] the characterisation is perhaps not representative of a deformation during nano-indentation, which will have a large component within the plane of the cornea.
Figure 9-2 Alignment of the corneal fibres to the load direction during the stress relaxation and the nano-indentation test, a) schematic of the stress relaxation test, showing parallel loading direction to fibres in the centre of the cornea and b) schematic of the nano-indentation in the centre of the cornea, showing loading normal to the direction of the fibres.
An alternative to the determination of the material properties via the stress relaxation test is to use nano-indentation combined with the inverse finite element method using a sequential application of the isotropic and transversely isotropic models. First, the isotropic model was used to recover the isotropic viscoelastic parameters. Later, since the viscous time-dependent parameters would be unchanged by the choice of orthotropy, a more computationally efficient inverse analysis was performed to recover transversely isotropic elastic parameters from the transversely isotropic model.

### 9.2.1 Isotropic parameters

In the first stage, the IFEM was applied to the three indentation data (loading, holding, and unloading) in order to recover the isotropic viscoelastic parameters, as explained in Section 7.3.2. The profile obtained once the inverse algorithm had converged is shown in Figure 9-3. The recovered parameters are provided in Table 9-5. The convergence plots are provided in Appendix 3.

![Figure 9-3 Converged profiles obtained from the inverse method using the isotropic model in comparison with the nano-indentation data](image.png)
Table 9-5 Isotropic parameters of the corneal rheological model obtained from the nano-indentation experiment using the inverse finite element method and the isotropic model

<table>
<thead>
<tr>
<th></th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>$\tau_1 (s)$</th>
<th>$\tau_2 (s)$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indent 1</strong></td>
<td>0.153</td>
<td>0.332</td>
<td>0.454</td>
<td>399.1</td>
</tr>
<tr>
<td><strong>Indent 2</strong></td>
<td>0.147</td>
<td>0.335</td>
<td>0.449</td>
<td>400.1</td>
</tr>
<tr>
<td><strong>Indent 3</strong></td>
<td>0.159</td>
<td>0.329</td>
<td>0.459</td>
<td>398.0</td>
</tr>
<tr>
<td><strong>Average, STD</strong></td>
<td><strong>0.153±0.03</strong></td>
<td><strong>0.332±0.03</strong></td>
<td><strong>0.454±0.05</strong></td>
<td><strong>399.0±1.0</strong></td>
</tr>
</tbody>
</table>

9.2.2 Transversely isotropic parameters

The second stage was limited to the loading phase of the indentation only, which enabled characterization of the two in plane (along the fibre directions) transversely isotropic elastic constants, as explained in Section 7.3.3. The out-of-plane (in the loading direction) transversely isotropic constants, having previously been recovered from the isotropic model, i.e. $E_{iso} = E_y = 0.153±0.03$ MPa, as provided in section 9.2.3. The profiles obtained once the inverse algorithm had converged are shown in Figure 9-4 and the recovered parameters are provided in Table 9-6. The convergence plots are provided in Appendix 4.
Figure 9-4 Converged profiles obtained from the inverse method using the transversely isotropic model in comparison with the nano-indentation data (loading part).

Table 9-6 Transversely isotropic parameters of the corneal rheological model obtained from the nano-indentation experiment using the inverse finite element method and the transversely isotropic model

<table>
<thead>
<tr>
<th>Transversely isotropic-IFEM</th>
<th>$E_x \text{ (MPa)}$</th>
<th>$E_z \text{ (MPa)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-plane</td>
<td>In-plane</td>
<td></td>
</tr>
<tr>
<td>Indent 1</td>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>Indent 2</td>
<td>0.29</td>
<td>0.20</td>
</tr>
<tr>
<td>Indent 3</td>
<td>0.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Average, STD</td>
<td>0.25±0.04</td>
<td>0.23±0.03</td>
</tr>
</tbody>
</table>
9.3 Evaluation of the spatial distribution of the properties

In order to evaluate the spatial variation of the corneal properties, the IFEM was combined with full field displacement data obtained from the inflation test. The evaluated displacement maps are provided in Section 6.6 for the central slice of the cornea along the nasal-temporal meridian. In the inflation test, the long-term elastic response was targeted and the cornea was considered as an inhomogeneous elastic material, with spatially varying elastic modulus. The inverse method was used in a sequential application of the radially and circumferentially partitioned models, as explained in Sections 7.4.3 and 7.4.4. First, the mean variation of the properties along the circumference was assumed to be uniform and the corneal long-term elastic parameters along the radial direction, $r$, were recovered. Second, the variation of the moduli across the circumference, $\theta$, was examined considering a uniform spatial variation of the properties through the radial direction.

9.3.1 Distribution of the properties in the radial direction

Firstly, the inverse method was used to recover the six elastic parameters of the cornea in the radial direction, $E(r_1) - E(r_6)$. The radial partitioned model was chosen first in order to reduce the number of variables; hence, the computational cost. This was done based on a priori knowledge that cornea has a uniform average fibre orientation through the thickness (radial direction) [24]. It was assumed that each section had uniform modulus in the circumferential direction. Three sets of data selected from the experimental displacement maps were considered as reference points for the optimization and the objective function was formulated to minimize the root mean square difference between the reference data and the corresponding numerical results, as explained in Section 8.6.1.

The experimental displacement profiles for the three data sets are compared with those from the inverse algorithm once the objective function had converged in Figure 9-5. The convergence plots are provided in Appendix 5. The profiles show a comparison between the displacement components in the $xy$ plane, i.e. $u_y$ and $u_x$, for each data set. There is excellent agreement between the experimental and numerical results, indicating a successfully converged solution to the inverse problem. In order to further check the performance of the inverse algorithm, the
whole field experimental displacement maps for $u_y$ and $u_x$ were compared with the numerical results. The numerical displacement fields were determined using the recovered elastic moduli and the radially partitioned finite element model, as shown in Figure 9-6.

Figure 9-5 Profiles obtained from the inverse method using the inflation model with radial partitions. The profiles show the numerical and experimental displacement components, $u_y$ and $u_x$, for the three data sets. The apex of the cornea is at (0,0) of the coordinate system.
Figure 9-6 Comparison between the experimental displacement maps and the numerical results using the radially partitioned model, a) numerical displacement in $x$, $u_x$, b) experimental displacement in $x$, $u_x$, c) numerical displacement in $y$, $u_y$ and d) experimental displacement in $y$, $u_y$

It can be observed from Figure 9-6 that the numerical displacement from the numerical analysis in the $x$ direction, $u_x$, is in a good agreement with the experimental data. The numerically determined displacement map in the $y$ direction, $u_y$, however, shows a slightly larger tensile dominated area in the central region of the cornea, as shown in Figure 9-6(c). A potential reason for this can be found in the assumption of the uniform average properties in the circumferential direction. In the next stage, the validity of this assumption is examined by determining the spatial distribution of the elastic properties in the circumferential direction.

The inverse method was performed twice (each time with a different initial values of the variables) to check the repeatability of the algorithm. The recovered values of the elastic moduli from the two runs were very similar with a standard deviation of ±0.03 MPa, as shown in Table 9-7. The profiles of the recovered moduli in the radial
direction are shown in Figure 9-7. It can be seen that there is no significant spatial variation in properties through the corneal thickness.

Table 9-7 Long-term elastic parameters of the cornea distributed along the radial direction recovered from the inverse method using the inflation test

<table>
<thead>
<tr>
<th>Parameter (MPa)</th>
<th>$E(r_1)$</th>
<th>$E(r_2)$</th>
<th>$E(r_3)$</th>
<th>$E(r_4)$</th>
<th>$E(r_5)$</th>
<th>$E(r_6)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>0.492</td>
<td>0.435</td>
<td>0.508</td>
<td>0.500</td>
<td>0.449</td>
<td>0.448</td>
</tr>
<tr>
<td>Run 2</td>
<td>0.447</td>
<td>0.485</td>
<td>0.458</td>
<td>0.450</td>
<td>0.499</td>
<td>0.498</td>
</tr>
<tr>
<td>Average max ±0.03 STD</td>
<td>0.469</td>
<td>0.460</td>
<td>0.483</td>
<td>0.475</td>
<td>0.474</td>
<td>0.473</td>
</tr>
</tbody>
</table>

Figure 9-7 Profile of the six recovered elastic moduli (averaged over two runs) distributed in the radial sections, $r_1$-$r_6$.
9.3.2 Distribution of the properties in the circumferential direction

In the second stage, the IFEM was implemented to examine the assumption made in Section 9.5.2 and find the distribution of the elastic parameters in the circumferential direction. From previous analysis, it can be assumed that modulus is constant in the radial direction. Ten elastic moduli, i.e. \( E(\theta_1) - E(\theta_{10}) \), were recovered from the circumferentially partitioned model. The objective function was defined for three sets of data points, as explained in Section 8.6.2.

Figure 9-8 shows displacement profiles from the experimental data and from the inverse algorithm once the objective function had converged. The convergence plots are provided in Appendix 6. The profiles show the displacement components, \( u_y \) and \( u_x \), for the three data sets. It can be seen that there is good agreement between the experimental and numerical results. In addition, a comparison between the experimental displacement maps, \( u_y \) and \( u_x \), and the numerical results determined from the circumferentially partitioned model using the recovered elastic moduli is shown in Figure 9-9. It can be seen that there is a good correlation between the experimental and numerical displacement maps, with the \( u_y \) displacement map being better represented in the circumferentially partitioned model than the radially partitioned one.
Figure 9-8 Profiles obtained from the inverse method using the circumferentially partitioned inflation model. The profiles show the displacement components, \( u_y \) and \( u_x \), for three data sets. The apex of the cornea is at (0,0) of the coordinate system.
Figure 9-9 Comparison between the experimental displacement maps and the numerical results using the circumferentially partitioned model, a) numerical displacement in $x$, $u_x$, b) experimental displacement in $x$, $u_x$, c) numerical displacement in $y$, $u_y$ and d) experimental displacement in $y$, $u_y$.

The recovered elastic parameters over two runs had 0.04 MPa standard deviations, as provided in Table 9-8. A profile of the recovered moduli in the ten circumferential sections is illustrated in Figure 9-10. In can be seen that there is a uniform section in the central area of the cornea and a reducing modulus toward the edge. This will be discussed in the next section.
Table 9-8 Long-term elastic parameters of the cornea distributed along the circumferential direction recovered from the inverse method using the inflation test

<table>
<thead>
<tr>
<th>Parameter (MPa)</th>
<th>$E(\theta_1)$</th>
<th>$E(\theta_2)$</th>
<th>$E(\theta_3)$</th>
<th>$E(\theta_4)$</th>
<th>$E(\theta_5)$</th>
<th>$E(\theta_6)$</th>
<th>$E(\theta_7)$</th>
<th>$E(\theta_8)$</th>
<th>$E(\theta_9)$</th>
<th>$E(\theta_{10})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>0.488</td>
<td>0.500</td>
<td>0.499</td>
<td>0.448</td>
<td>0.448</td>
<td>0.422</td>
<td>0.380</td>
<td>0.349</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td>Run 2</td>
<td>0.460</td>
<td>0.440</td>
<td>0.459</td>
<td>0.498</td>
<td>0.510</td>
<td>0.460</td>
<td>0.415</td>
<td>0.365</td>
<td>0.360</td>
<td>0.356</td>
</tr>
<tr>
<td>Average</td>
<td>max ±0.07 STD</td>
<td>0.474</td>
<td>0.470</td>
<td>0.479</td>
<td>0.473</td>
<td>0.480</td>
<td>0.454</td>
<td>0.418</td>
<td>0.372</td>
<td>0.354</td>
</tr>
</tbody>
</table>

Figure 9-10 Profile of the ten recovered elastic moduli (averaged over two runs) distributed in the circumferential sections, $\theta_1$-$\theta_{10}$. 
9.4 Discussion

In the first part of the research, the parameters of the corneal viscoelastic model were obtained via two main methods, i.e. the experimental method (stress relaxation experiment) and the numerical/experimental method (inverse finite element method/nano-indentation), and the obtained values are compared in Table 9-9.

The inversely obtained parameters were determined over three nano–indentation experiments. The initial combination of the inverse algorithm with the isotropic model was used to recover the out-of-plane viscoelastic parameters. Using the recovered out–of-plane parameter, the later combination of the inverse algorithm with the transversely isotropic model revealed the in-plane elastic parameters.

Table 9-9 Parameters of the corneal rheological model obtained from the stress relaxation experiment, using curve fitting and the nano-indentation experiment using the inverse finite element method (NI IFEM). $E_y$ and viscoelastic parameters are recovered from the isotropic model. $E_x$ and $E_z$ are recovered from the transversely isotropic model.

<table>
<thead>
<tr>
<th>Method</th>
<th>$E_x$ (MPa)</th>
<th>$E_z$ (MPa)</th>
<th>$E_y$ (MPa)</th>
<th>$g_1$</th>
<th>$g_2$</th>
<th>$\tau_1$ (s)</th>
<th>$\tau_2$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relax. experiment</td>
<td>0.250</td>
<td>0.351</td>
<td>0.437</td>
<td></td>
<td></td>
<td>410.5</td>
<td>1605.3</td>
</tr>
<tr>
<td>NI IFEM</td>
<td>±0.04</td>
<td>±0.08</td>
<td>±0.06</td>
<td>±0.03</td>
<td>±0.05</td>
<td>±1.00</td>
<td>±7.50</td>
</tr>
<tr>
<td>±STD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Transversely isotropic model

Isotropic model
It can be seen from Table 9-9 that there is good agreement of parameters, $g_1$, $g_2$, $\tau_1$, and $\tau_2$ between those obtained using the relaxation experiment and that recovered from the nano-indentation experiment employing the isotropic model. In addition, the recovered in-plane elastic moduli which are along the fibre directions, $E_x$ and $E_z$, are close to the values obtained from the relaxation test, but the out-of-plane modulus which is normal to the fibre directions, $E_y$, is significantly smaller. This offers support for the explanation that the discrepancies lie in the composite nature of the material, since it suggests that the normal direction is more compliant than the in-plane directions, as one might expect when fibres are orientated within the plane of the cornea. These results suggest that an transversely isotropic linear viscoelastic model is an appropriate description of corneal behaviour, since the composite-like structure leads to direction dependent properties.

Interestingly, the values of the instantaneous elastic moduli are of the same order of magnitude as those obtained in the study of Kampmeier et al. (2000) and Ahearne et al. (2007), i.e. between 0.1 to 0.9 MPa [103,142]. The values for viscoelastic time constants, however, are different, suggesting that the time-dependent properties may be more susceptible to conditioning than the elastic parts.

In the second part of this research, the distribution of the long-term elastic parameters of the cornea was determined via the numerical/experimental method developed. The parameters of the cornea were considered to vary in two directions, i.e. radial and circumferential. The inversely obtained distribution of the parameters was determined over one set of full field inflation data with the help of two models. The initial combination of the inverse algorithm with the radially partitioned model was used to recover the radial variation of the parameters, having assumed no circumferential variation. A slight discrepancy was observed between the numerical and the experimental $u_y$ displacement map, as shown in Figure 9-6 that could be attributed to this assumption. This lead to the examination of the assumption using a combination of the inverse algorithm with the circumferentially partitioned model to determine the circumferential variation of the corneal properties.

It can be seen from Figure 9-9 that there is good agreement between the displacement maps obtained from the numerical simulation and the experiment. In addition, as shown in Figure 9-7 and Figure 9-10, the values of the recovered elastic
moduli, which are distributed in the radial direction, \( E(r_i) \), are close to the values of those distributed in the circumferential directions, \( E(\theta_i) \) (where \( i \) is the number of sections in the relevant directions). This observation offers support for explaining the discrepancy and suggests that the assumption of average uniform variation along \( \theta \) direction is appropriate in order to recover the distribution of the corneal parameters based on the sequential two-model approach.

The uniform distribution of the elastic moduli through the thickness, as shown in Figure 9-7, can be interpreted from the microstructural point of view as the stromal fibres (in the absence of the epithelium layer) are organized into a number of lamella with an average uniform orientation through the thickness. Within individual lamellae, the collagen fibres form a regularly packed parallel array that follows the direction of the lamellae. The stroma, therefore, can be thought of as being formed of dome-like concentric sheets of uniformly oriented lamellae in-depth (on average), with each sheet one lamellae thick [26].

The profile of the elastic moduli across the circumference, as depicted in Figure 9-10, shows a gradual decrease (about 25%) from the centre toward the periphery. The non-uniform distribution of the elastic moduli across the circumference indicates that the cornea is stiffer in the centre than the peripheral region. This is consistent with the deformation pattern of the cornea during the inflation test reported by Boyce et al. They showed that within the physiological range of pressure (IOP), the majority of the deformation was localized in the limbus and peripheral regions, which left the central cornea largely un-deformed [100]. From a microstructural point of view, the variation of the properties along the circumferential direction can be interpreted by the preferred orientation of the stromal lamella across the cornea showing that the tangential alignment of the lamella in the limbal region is more compliant than the orthogonally aligned central lamella. It is also possible that the circumferential variation of the properties can be caused by the influence of the clamped boundary conditions by reorienting the limbal lamella, which do not fully replicate the physiological boundary condition. Future efforts to model the cornea in three-dimension with a spatially varying fibre orientation distribution could yield further insight into this effect.

The developed hybrid method is restricted to 2D determination of the corneal properties. A full (3D) characterization is more computationally costly. Future work,
However, can focus on lowering the cost of the 3D analysis by implementing a number of simplifications such as reducing the number of partitioning sections through and across the cornea (in the case of evaluating the distribution) and reducing the domain and the number of elements (in the case of evaluating the time-dependent properties).

In addition, an investigation of the corneal swelling can be performed by comparing the results with those obtained for an artificial corneal trephinate. This can provide an insight to the causes and the possible prevention methods.

Chapter 10: Conclusions and future work

10.1 Conclusions

In this work, a method is proposed and examined to determine corneal mechanical properties from two points of views: time dependency and spatial variation. This method is based on a combination of experimental techniques (nano-indentation and inflation) and inverse finite element analysis. Even though in this thesis the method relies on in vitro measurements, it has the potential to be expanded to in vivo measurement of the corneal properties.

- The methods developed in this thesis combine a nano-indentation experiment and inverse finite element analysis to measure the local time-dependent properties of the cornea. The corneal time-dependant response, as an input to the inverse algorithm, was obtained from the nano indentation experiment and the parameters of the corneal model were recovered using the inverse finite element method. To investigate the performance of the inverse method, the recovered parameters were checked against the fitted parameters from a stress relaxation experiment.

- The use of a GM rheological model for the cornea enables the instantaneous elastic behaviour to be separated from the long-term viscoelastic behaviour. It was shown that the instantaneous elastic behaviour is dependent on the type of experiment and the corneal composite-like microstructure. Hence, an
transversely isotropic rheological model is required to fully characterize the time-dependent behaviour of the cornea.

- The proposed method was able to determine the properties of the cornea in a complex stress state when a point-wise structural response is in hand (as is the case of nano-indentation). In this case, the microstructure of the cornea plays an important role so that the determined time-dependent parameters describe the properties at sub-tissue level.

- In order to characterize the corneal local time-dependent properties at a microstructural level, the method avoids high computational cost by utilizing two separate finite element models sequentially. The use of an isotropic and an transversely isotropic model in a separate manner allows for the measurement of the out-of-plane viscoelastic and in-plane instantaneous elastic parameters in a way that is much faster and more reliable than when a transversely isotropic viscoelastic model is used.

- The time-dependent properties are measure in 2D for half of the middle cross-section of the cornea (axisymmetric model) in an efficient computational manner. However, to evaluate the properties for the whole cornea in 3D, the computational cost will increase significantly. To compensate the cost involved, it is possible to reduce the number of elements and to reduce the corneal domain of the FEM.

- Optical coherence tomography and inverse finite element analysis were used to determine the distribution of the time-independent parameters for the middle cross-section of the cornea. The full field long-term elastic response, as an input to the inverse algorithm, was obtained from a corneal inflation experiment using swept-source optical coherence tomography to image the stromal microstructure before and after inflation. Digital volume correlation was used to evaluate the resulting 3-D displacement field, which was in turn used to find a distribution of elastic modulus along the circumference and through the thickness of the cornea, recovered using an inverse finite element analysis.
• The long-term elastic parameters recovered via this method are a generic measure of the corneal properties and do not fully represent the parameters of the corneal rheological model, as the cornea is a viscoelastic material. However, the spatial variation of these parameters can indicate variation in the parameters of the rheological model, i.e. parameters of the GM model.

• A simple assumption of uniform properties in the circumferential direction enables determination of the distribution of the properties in a computationally effective way. However, reducing the number of partitioning sections based on a priori knowledge from the literature can further reduce the computational cost.

• It was shown that initial assumption of uniform properties in the circumferential direction was a valid assumption to determine the spatial distribution in the radial direction.

• In these experiments, the distribution of the parameters could not be determined within two regions in the cornea: the clamped peripheral edge and the endothelium middle section. This was due to low contrast of the OCT stromal reconstructions in the clamped peripheral edge, which leads to spurious values of displacement and strain that cannot be included in the analysis. A more powerful OCT technique can overcome this limitation and provide a better image contrast.

• In addition, a positive through thickness strain indicated the possibility of stromal swelling in the middle endothelial section. As this has not been confirmed by an independent method and as the mechanical model used did not include stromal swelling, this region had to be excluded from the analysis. Further investigation using an artificial corneal trephinate can verify this effect.

10.2 Future work

Further work should first focus on implementing the developed technique on full field 3D data and determining the distribution of the properties throughout the whole
cornea. The increased computational cost can be counterbalanced by reducing the number of partitioning sections.

By improving the experimental method (using a digital pressure applier/control or a test rig that holds the whole eye globe) and using faster image acquisition, it would be possible to measure a time resolved full-field inflation response of the cornea. This could be used in the inverse method to determine the spatial variation and the time-dependant properties, collectively.

The time-dependent properties are determined for only a small part of the cornea using a 2D axisymmetric model, to reduce the computational cost. However, in order to determine the time-dependent properties for the whole cornea, a 3D analysis should be performed, which increases the computational cost. Further work can investigate the effect of various simplifications and assumptions in the FEM to increase the computational efficiency.

The time-dependent properties are determined in microstructural level using an isotropic and a transverse isotropic model sequentially. In order to evaluate the properties for the whole cornea (in 3D) it might be more computational efficient to combine the two models and analyse a single one.

In addition, indentation tests could be performed across the cornea to map the spatial distribution of local time-dependent parameters. By using a non-contact indentation technique (in the case of air-puff tonometry) and obtaining the time resolved indentation response, the developed method can be applied in vivo to characterize the corneal properties.

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