OPTICS OF THE HUMAN EYE IN DIABETES

Adnan
(MSc, BS Optom, BSc, DipMT, Cert Ophth)

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Supervisors:
Prof David A. Atchison
Prof Nathan Efron
Dr Marwan Suheimat

School of Optometry & Vision Science, Faculty of Health and Institute of Health & Biomedical Innovation
Queensland University of Technology
Brisbane, Australia
2014
Keywords

Aberrations, accommodation, decentration, diabetes, flicker photometry, hyperglycaemia, lens tilt, magnetic resonance imaging, phakometry, refractive index, straylight, type 1 diabetes.
Abstract

Aim: People with diabetes have visual problems and often these are the first symptoms at the time of diagnosis. In many respects the optics of diabetic eyes make them appear as older eyes than those of people of the same age without diabetes. The overall aim of this thesis was to test this as a hypothesis. While there have been studies that have considered larger numbers of people, this thesis builds on previous work by way of a comprehensive study of a large range of biometric and optical parameters in diabetes.

Methods: Biometric and other parameters of 74 people with type 1 diabetes and an age matched control group were assessed. Most of the people were part of the Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic Markers (LANDMark) study at the Institute of Health and Biomedical Innovation. In order to facilitate a longitudinal evaluation of the progression of severity of neuropathy with type 1 diabetes with no or mild diabetic neuropathy, nearly all of these participants had low levels of neuropathy, retinopathy and nephropathy.

Some testing involved common clinical tests, but novel methods were developed to measure lens parameters such as yellowing, surface radii of curvature, diameter, refraction index distribution and equivalent refractive index.

Results: Marginal or no significant differences were found between groups for corneal shape, corneal thickness, pupil size, pupil decentrations and most higher-order aberrations (total, corneal and internal) for 4.5 mm pupils. Relative to the control group, the diabetes group demonstrated smaller anterior chamber depths, more curved lenses, thicker lenses, lower lens
equivalent refractive index, greater straylight, lower amplitudes of accommodation, greater lens yellowing, and different ocular aberration coefficients for horizontal coma and vertical coma. While the hypothesis has been supported, most of these differences did not increase significantly with age. A novel finding was the lower lens diameter in diabetic eyes than in non-diabetic eyes.

**Conclusion:** As nearly all of the diabetes participants had low levels of neuropathy, retinopathy and nephropathy, it is concluded that age-related changes in the optics of the eyes of people with diabetes need not be accelerated if the diabetes is well controlled.
Table of Contents

Keywords..............................................................................................................i
Abstract ...............................................................................................................ii
Table of Contents ..............................................................................................iv
List of Figures ....................................................................................................vii
List of Tables .......................................................................................................xii
List of Abbreviations ...........................................................................................xv
Statement of Original Authorship .....................................................................xvi
Acknowledgements ............................................................................................xvii

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

Chapter 2: Literature Review ...............................................................................8
2.1 Diabetes: Overview .........................................................................................8
  2.1.1 Definition and Classification .................................................................8
  2.1.2 Criteria of Diagnosis ............................................................................9
  2.1.3 Prevalence of Diabetes .......................................................................10
  2.1.4 Complications of Diabetes .................................................................12
2.2 Overview of the Eye ......................................................................................13
2.3 Influence of Diabetes on Biometry and Optics of the Human Eye .............17
  2.3.1 Cornea .................................................................................................18
  2.3.2 Aqueous Humour ................................................................................19
  2.3.3 Pupil ....................................................................................................20
  2.3.4 Lens ....................................................................................................20
  2.3.5 Vitreous ..............................................................................................22
  2.3.6 Retina and Choroid ............................................................................22
  2.3.7 Axial Length .......................................................................................23
  2.3.8 Refraction ............................................................................................23
  2.3.9 Higher-order Aberrations .................................................................29
  2.3.10 Straylight ..........................................................................................30
2.4 Influence of Diabetes on Retinal Functions and Amplitude of Accommodation ....32
2.5 Techniques ..................................................................................................33
2.6 Summary .....................................................................................................34

Chapter 3: Methodology .....................................................................................37
3.1 Participants ...................................................................................................38
3.2 Statistical Justification of Participant Numbers ...........................................42
3.3 Data Analysis ...............................................................................................43
Chapter 4: Results ........................................................................................................... 108
4.1 Characteristics of Participants ................................................................................. 109
4.2 Ocular Biometry ........................................................................................................ 113
  4.2.1 Spherical Equivalent Refraction ........................................................................ 114
  4.2.2 Anterior Corneal Radius of Curvature ............................................................... 117
  4.2.3 Anterior Corneal Asphericity ............................................................................ 120
  4.2.4 Corneal Central Thickness .............................................................................. 123
  4.2.5 Posterior Corneal Radius of Curvature ............................................................ 127
  4.2.6 Anterior Chamber Depth ................................................................................ 130
  4.2.7 Pupil Diameter ................................................................................................ 134
  4.2.8 Pupil Decentration .......................................................................................... 137
  4.2.9 Anterior Lens Radius of Curvature .................................................................. 140
  4.2.10 Posterior Lens Radius of Curvature ................................................................. 144
  4.2.11 Lens Central Thickness .................................................................................. 147
  4.2.12 Lens Equivalent Refractive Index ................................................................... 150
  4.2.13 Lens Equivalent Power .................................................................................. 153

4.3 Straylight .................................................................................................................... 156
4.4 Amplitude of Accommodation ................................................................................. 159
4.5 Ocular Aberrations and their Components ................................................................. 167
4.6 Lens Yellowing ........................................................................................................... 177
4.7 Lens Dimensions and Refractive Index Distribution .................................................. 181
4.8 Summary .................................................................................................................... 188

Chapter 5: Discussion ...................................................................................................... 192
5.1 Summary ..................................................................................................................... 192
5.2 Do People with Diabetes have Greater Higher-order Aberrations than Age-matched Controls? ................................................................. 194

5.3 Can Loss of Accommodation with Diabetes be Attributed to Changes in the Lens Including Refractive Index Distribution? ................................................................. 195

5.4 Do Diabetic Lenses Become Yellower at Greater Rates with Age than Age-matched Normal Lenses? ................................................................. 197

5.5 Is the Refractive Index Distribution of the Diabetic Lens Different from that of the Non-diabetic Lens? ................................................................. 197

5.6 Are Corrections to Assumed Refractive Index Needed in Biometric Measurements of Diabetic Eyes? ................................................................. 198

5.7 Do People with Diabetes have More Straylight than People without Diabetes? ........... 199

5.8 Limitation of the Study ......................................................................................... 199

5.9 Further Work ........................................................................................................ 200

References 202

Appendices 228

Appendix A: Effect of Phakometer Positioning Errors on Refraction, Eye Rotation and Purkinje Image Sizes ........................................................................ 228

Appendix B: Manual for using Phakometry Software ........................................ 235

Appendix C: Publications and Conference Publications ........................................ 242
List of Figures

Figure 2:1 The horizontal section of the right eye as seen from above. [Adapted from Atchison & Smith (2000d)].......................... 14

Figure 2:2 Typical accommodative stimulus-response function of a young adult. ........................................................................ 15

Figure 3:1 Scheimpflug principle. a) tilted image plane; b) tilted lens plane [Adapted from Dubbelman thesis, (2002)]. ................. 49

Figure 3:2 Layout of stimulus in compensation comparison method for C-Quant................................................................. 54

Figure 3:3 The operator screen of the C-Quant instrument with upper graph showing the straylight value and age (red dot) of the participant compared with the range of normal values at different ages................................................. 55

Figure 3:4 Hartmann-Shack principle. ................................................................. 58

Figure 3:5 Re-referencing the cornea. ............................................................. 61

Figure 3:6 Slider positions for different refractions and the quadratic regression fit .......................................................... 64

Figure 3:7 Accommodative response/stimulus curves of a non-diabetic participant ............................................................. 65

Figure 3:8 Accommodative responses (left) and pupil sizes (right) of four participants......................................................... 67

Figure 3:9 Three graphs show the accommodative response of participants when the luminance of the internal target was 0.1................................................................. 68

Figure 3:10 Flicker photometry system.......................................................... 70

Figure 3:11 Flicker generation and pulse width.......................................... 71

Figure 3:12 Thresholds of two participants (P1, P2) with and without a yellow filter.......................................................... 73

Figure 3:13 Thresholds of two participants (P1, P2) with 2 Hz and 5 Hz flicker frequencies......................................................... 74

Figure 3:14 Threshold of three participants (P1, P2, P3) when the pulse width modulation of rotating knob was kept at 25, 50 and 100 per rotation .................................................. 75
Figure 3:15 Phakometer. Purkinje images are formed of the illumination ring source. ........................................... 77
Figure 3:16 Ellipse fitting mode of the software........................................... 79
Figure 3:17 PR calibration curve of a participant ........................................... 80
Figure 3:18 Target position and angle relative to the centre-of-rotation of the eye. ........................................... 82
Figure 3:19 Data and linear fits of Purkinje images positions, with respect to pupil centre, versus vertical and horizontal components of eye rotations. ........................................... 82
Figure 3:20 Refraction error, as a function of refraction given on Optometer scale on the phakometer ........................................... 84
Figure 3:21 Ratio of eye rotation angle to the angle subtended by image at entrance pupil of eye ........................................... 85
Figure 3:22 Equivalent mirror method for determining anterior radius of curvature of lens. ........................................... 88
Figure 3:23 Determining posterior radius of curvature of lens. ........................................... 89
Figure 3:24 Three-surface schematic eye for paraxial ray tracing ........................................... 91
Figure 3:25 Parameters involved in determining height of PI ........................................... 92
Figure 3:26 Parameters involved in determining height of PIII ........................................... 92
Figure 3:27 Schematic diagram of fixation target during MR imaging procedure ........................................... 98
Figure 3:28 Right eye MSE image of a 28 years old non diabetes participant at different stages of eye rotation procedure for analysis ........................................... 105
Figure 3:29 Schematic representation of the refractive index extraction procedure from the MSE images and schematic diagram of lens dimensions measurement ........................................... 106
Figure 3:30 Lens dimensions for refractive index profiles ........................................... 107
Figure 4:1 Relationships between age and diabetes duration ........................................... 111
Figure 4:2 Relationships between age and spherical equivalent refraction for people with and without diabetes ........................................... 115
Figure 4:3 Relationships between age and anterior corneal radius of curvature (using Pentacam) for people with and without diabetes ........................................... 118
Figure 4:4 Relationships between age and anterior corneal radius of curvature (using Medmont) for people with and without diabetes. .................................................................118

Figure 4:5 Relationships between age and anterior corneal asphericity for people with and without diabetes. ......................121

Figure 4:6 Relationships between age and corneal central thickness (Pentacam) for people with and without diabetes. ..............124

Figure 4:7 Relationships between age and corneal central thickness (Lenstar) for people with and without diabetes. ..............125

Figure 4:8 Relationships between age and posterior corneal radius of curvature for people with and without diabetes. ..............128

Figure 4:9 Relationships between age and anterior chamber depth (using Pentacam) for people with and without diabetes. .........131

Figure 4:10 Relationships between age and anterior chamber depth (using Lenstar) for people with and without diabetes. .............132

Figure 4:11 Relationships between age and pupil diameter for people with and without diabetes. ........................................135

Figure 4:12 Relationships between age and pupil decentrations along horizontal and vertical axes for people with and without diabetes. ........................................................................138

Figure 4:13 Relationships between age and anterior lens radius of curvature for people with and without diabetes. ..............142

Figure 4:14 Anterior lens radius of curvature as a function of age from Wiemer et al. (2008d). .........................................................143

Figure 4:15 Relationships between age and posterior lens radius of curvature for people with and without diabetes. ..............145

Figure 4:16 Relationships between age and lens central thickness for people with and without diabetes. .................................148

Figure 4:17 Lens thickness as a function of age from Wiemer et al. (2008d). ..............................................................................149

Figure 4:18 Relationships between age and lens equivalent refractive index for people with and without diabetes. .................151

Figure 4:19 Lens equivalent refractive index as a function of age from Wiemer et al. (2008d). .........................................................152

Figure 4:20 Relationships between age and lens equivalent power for people with and without diabetes. .................................154
Figure 4:21 Relationships between age and strayline for people with and without diabetes. .............................................................158

Figure 4:22 Bland-Altman plot comparing the objective and subjective methods of amplitude of accommodation. ..........160

Figure 4:23 Relationships between age and objective amplitude of accommodation for people with and without diabetes. ........162

Figure 4:24 Relationships between age subjective amplitude of accommodation for people with and without diabetes. ....163

Figure 4:25 Relationships between age and diabetes duration for the people who had accommodation measurements. ..............165

Figure 4:26 Comparison of subjective amplitude of accommodation between previous studies (Braun, et al., 1995; Moss, et al., 1987) and present study.................................................................166

Figure 4:27 Relationships between age and corneal aberration components for people with and without diabetes. ................169

Figure 4:28 Relationships between age and total ocular aberration components for people with and without diabetes. ........172

Figure 4:29 Relationships between age and internal aberration components for people with and without diabetes. ..........175

Figure 4:30 Relationships between age and lens yellowing for people with and without diabetes. ...........................................179

Figure 4:31 Comparison of lens yellowing from Lutze & Bresnick (1991) and Davies & Morland (2002) with the current study.................................................................180

Figure 4:32 Characteristic MSE images from each of the groups:.......183

Figure 4:33 Normalised refractive index profiles for diabetes and non-diabetes groups: .................................................................186

Figure 4:34 Normalised refractive index profiles for diabetes and non-diabetes groups, together with fits: .................................187

Figure A: 1 Determining errors with phakometer system. ......................231

Figure A: 2 Refraction error, as a function of refraction given on the Optometer scale.................................................................233

Figure A: 3 Change in image size (%), as a function of refraction given on the Optometer scale. ......................................................234

OPTICS OF THE HUMAN EYE IN DIABETES
Figure B: 1 A screen capture of the Purkinje software interface. ..................237
Figure B: 2 Interface of the Phakometry software ......................................238
Figure B: 3 Biometry and Purkinje height information. .................................239
Figure B: 4 Snap shot of the optimization result in Notepad. ............................240
List of Tables

Table 2:1 Summary of short-term refractive changes in people with diabetes .................................................................................................................. 25
Table 2:2 Summary of the refractive changes after induction of hyperglycaemia or hypoglycaemia in people with and without diabetes .......................................................................................................................... 29
Table 3:1 Biometric parameters and their determination ............................................. 39
Table 3:2 Different parameters affecting the Signal to Noise Ratio in MR images ................................................................................................................................. 99
Table 3:3 Protocol for the MR imaging .........................................................................101
Table 4:1 Participants recruited for different tests and numbers after exclusion .................................................................................................................................111
Table 4:2 Characteristics of participants ......................................................................113
Table 4:3 Pearson correlations of spherical equivalent refraction with different ocular and systemic factors ............................................................114
Table 4:4 Pearson correlations of anterior corneal radius of curvature (Pentacam, Medmont) with different ocular and systemic factors .................................................................................................................................117
Table 4:5 Pearson correlations of anterior corneal asphericity with different ocular and systemic factors .................................................................................................................................120
Table 4:6 Pearson correlations of corneal central thickness (Pentacam, Lenstar) with different ocular and systemic factors .................................................................................................................................123
Table 4:7 Pearson correlations of posterior corneal radius of curvature with different ocular and systemic factors ...............................................................127
Table 4:8 Pearson correlations of anterior chamber depth with different ocular and systemic factors .................................................................................................................................130
Table 4:9 Pearson correlations of pupil diameter with different ocular and systemic factors .................................................................................................................................134
Table 4:10 Pearson correlations of pupil decentration with different ocular and systemic factors .................................................................................................................................137
Table 4:11 Characteristics of participants for lens anterior radius of curvature, posterior radius of curvature, and equivalent refractive index .................................................................................................................................140
Table 4:12 Pearson correlations of anterior radius of curvature with different ocular and systemic parameters..........................141

Table 4:13 Pearson correlations of posterior lens radius with different ocular and systemic parameters .....................................144

Table 4:14 Pearson correlations of lens central thickness with different ocular and systemic parameters.............................147

Table 4:15 Pearson correlations of lens equivalent refractive index with different ocular and systemic parameters .......................150

Table 4:16 Pearson correlations of lens equivalent power with different ocular and systemic parameters.............................153

Table 4:17 Characteristics of participants for straylight testing .................156

Table 4:18 Pearson correlations of straylight with different ocular and systemic factors ..........................................................157

Table 4:19 Characteristics of participants for amplitude of accommodation testing ............................................................159

Table 4:20 Pearson correlations of objective amplitude of accommodation with different ocular and systemic factors ..................161

Table 4:21 Pearson correlations of subjective amplitude of accommodation with different ocular and systemic factors .....................161

Table 4:22 Characteristics of participants for ocular aberrations testing .................................................................167

Table 4:23 Pearson correlations of corneal aberrations components with different ocular and systemic factors .........................168

Table 4:24 Pearson correlations of total ocular aberrations components with different ocular and systemic factors.............170

Table 4:25 Pearson correlations of internal aberrations components with different ocular and systemic factors.......................173

Table 4:26 Characteristics of participants for lens yellowing .......................177

Table 4:27 Pearson correlations of log lens yellowing with different ocular and systemic parameters .................................178

Table 4:28 Characteristics of participants for lens dimensions and refractive index distribution ..................................182
Table 4:29 Co-efficients of fit $n(r) = C_o + C_pr^p$ to refractive index data along equatorial diameter line and optical axis of different groups

Table 4:30 Co-efficients of fit $n(r) = C_o + C_pr^p$ to anterior axial and posterior axial refractive index data of different groups using the second approach to normalisation along the optical axis.

Table 4:31 Multiple regression fits in the whole group and in the diabetes group

Table 4:32 Multiple regression fits in the whole group with either diabetes duration or diabetes status as factors, and where diabetes duration was a significant factor.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DM1</td>
<td>Type 1 Diabetes Mellitus</td>
</tr>
<tr>
<td>DM2</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>D</td>
<td>Dioptre</td>
</tr>
<tr>
<td>HOA</td>
<td>Higher order aberrations</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated haemoglobin</td>
</tr>
<tr>
<td>LogMAR</td>
<td>log minutes of arc resolution</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>RMS</td>
<td>Root-mean-squared</td>
</tr>
</tbody>
</table>
Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature:  

18-12-2014  

Date:
Acknowledgements

First and foremost, I will thank almighty Allah who kindly helped me to complete my thesis.

The success of this thesis is attributed to my principal supervisor Professor David Atchison for giving me the opportunity to pursue this PhD. This thesis would not have been possible without his invaluable support, advice, guidance and patience over the last three years. Thank you so much for forcing me to look at research and my work in different ways and for opening my mind. Your support was essential to my success over here.

I thank my associate supervisor Prof Nathan Efron for his support, encouragement and an opportunity to work with him. In addition, I would like to pay thanks to my associate supervisor Dr Marwan Suheimat for providing indispensable advice and support on different aspects of my project and for cooperation in difficult time of my study.

Furthermore, immeasurable appreciation and deepest gratitude are extended to the following people who helped with development of techniques and procedures. Visiting Research Professor Edward Mallen helped build the phakometer and the dark adaptation chamber for flicker photometry. Dr Ankit Mathur developed the objective method for amplitude of accommodation and the software to estimate corneal tilt, decenteration, and aberrations. Dr Sanjeev Kasthurirangan explained the use of his program for the phakometer and helped with further development. Associate Prof Dr Andrew Zele gave advice on the flicker photometry technique. John Stephens built the electronic control box for flicker photometry. I thank the people involved with magnetic resonance imaging including Professor Jim
Pope who developed the imaging protocols, radiographers Aiman Al Najjar and Anita Burns for running sessions, engineering graduate student Farshid Sepehrband for developing imaging analysis routines, and fellow graduate student Pavan Verkicharla for advising on participant management.

I am grateful to members of Professor Efron’s team in the Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic Markers (LANDMark) study. Drs Nicola Pritchard and Katie Edwards gave considerable advice about participant recruitment and investigation protocols. Fellow graduate student Cirous Dehghani helped screened participants and Kath Macintosh helped recruit participants.

I extend my gratitude to my study participants, IHBI reception for facilitating participant management, Diabetes Australia for advertising my study in their newsletter, Ophthalmologist Dr Junaid for checking gradings of lens and fundus images, and the Research Methods group at IHBI, in particular Dimitrios Vagenas and Jules Hernández-Sánchez, for statistical advice on linear regressions and power analysis.

My sincere gratitude goes to my friends Beryl Hsu, Laura Delgado Mercedes Reyes and Majid Memony for their moral support, help and encouragement during my stay in Brisbane.

Finally I thank the Faculty of Health at QUT and my supervisor Prof Atchison for scholarships to support my study.
Dedicated to

Pakistani Children
Chapter 1: Introduction

Diabetes mellitus is a group of metabolic diseases characterised by chronic hyperglycaemia. Its main classifications are Type 1 diabetes mellitus (DM1), which is characterised by auto-immune destruction of pancreatic beta-cells that leads to loss of insulin secretion, and Type 2 diabetes mellitus (DM2) which is the most common form. Diabetes affects all parts of the human eye with its most important ocular complication being diabetic retinopathy. In addition, it affects the eye’s optics and biometry of the human eye. In many optical respects, diabetic eyes act like older normal eyes.

Greater central corneal thickness has been reported in people with diabetes than in people without diabetes in some studies (Busted, Olsen, & Schmitz, 1981; Lee, Oum, Choi, Lee, & Cho, 2005) but not in others (Inoue, Kato, Inoue, Amano, & Oshika, 2002; Keoleian, Pach, Hodge, Trocme, & Bourne, 1992; Wiemer, Dubbelman, Kostense, Ringens, & Polak, 2007; Ziadi et al., 2002).

Wiemer et al. (2007) found smaller posterior cornea radii of curvature in people with diabetes than in people without diabetes without significant differences in the anterior cornea radii of curvature, but this did not affect the overall corneal power.

Age-related lenticular changes are more pronounced in people with diabetes than in people without diabetes (Bron, Sparrow, Brown, Harding, & Blakytny, 1993; Brown & Hungerford, 1982; Fledelius & Miyamoto, 1987; Løgstrup, Sjølie, Kyvik, & Green, 1996; Løgstrup, Sjølie, Kyvik, & Green, 1997; Pierro, Brancato, Zaganelli, Guarisco, & Calori, 1996; Saw, Wong, Ting,

With increasing age anterior chamber depth decreases, lens thickness increases, lens surface curvatures increases, and lens equivalent refractive index decreases (Atchison et al., 2008). The intraocular distance changes are greater in people with diabetes than in people without diabetes (Braun, Benson, Remaley, Chew, & Ferris III, 1995; Pierro, et al., 1996; Saw, et al., 2007; Wiemer, et al., 2008d). People with diabetes, at least for DM1, have greater lens surface curvatures than people without diabetes.

Similarly, nearly twice the rate of change in equivalent refractive index for DM1 (0.0007/year) occurs than for people without diabetes (Wiemer, et al., 2008d). Equatorial diameter increases with ageing according to Kasthurirangan et al. (2011), but not Strenk et al. (1999); no information is available on diameters in diabetes.

Wiemer et al. (2008c) found different impacts of DM1 and DM2 on lens biometry. They found thicker lens nuclei, anterior cortices and posterior cortices in DM1 than in non-diabetic people. In DM2, they found significant thicker nuclei than non-diabetic people but thinner posterior cortices than in non-diabetic people.

Lenticular refractive index distribution changes with ageing and accommodation. Kasthurirangan et al. (2008) found alterations of gradient refractive index, but with the central and edge refractive indices remaining
unaffected. Refractive index variation occurred over a large portion of the lens during accommodation, but with ageing the variation was restricted to the peripheral part of the lens. These changes with ageing explain the lens paradox, which is that despite the lens curvatures increasing with age, most eyes do not become more myopic. The study showed a contribution of the gradient index distribution to accommodation as proposed by Gullstrand and incorporated in his No. 1 schematic eye.

Diabetic people have lower lens light transmission than non-diabetic people (Davies & Morland, 2002; Lutze & Bresnick, 1991; Van Best, Vrij, & Oosterhuis, 1985; Zagers, Pot, & van Norren, 2005). *In vivo* (Lutze & Bresnick, 1991) and *in vitro* (Kessel, Lundeman, Herbst, Andersen, & Larsen, 2010) studies have found that diabetic lenses are yellower than non-diabetic lenses.

Lower subjective amplitudes of accommodation have been found for people with diabetes than for age-matched controls (Cavallerano, 1990; Moss, Klein, & Klein, 1987; Yamamoto, Adachi-Usami, & Kuroda, 1989). Moss et al. (1987) and Braun et al. (1995) found that duration of diabetes was approximately 60% and 40% as important, respectively, as age in reducing amplitude. Most studies used a narrow age range for which decline in the amplitude of accommodation with age was not investigated. Also, subjective techniques are unreliable due to depth-of-focus. There appears to have been only one objective measurement of amplitude of accommodation for people with diabetes, an electrophysiological study by Yamamoto et al. (1989) who found reduced amplitudes in people with diabetes.

The decrease in equivalent gradient index in diabetes (Wiemer, et al., 2008d) might be due to a change in refractive index distribution such as occurs in ageing or to an overall decrease in refractive index throughout the lens. If the
change in lens shape during accommodation is not affected then either of these could explain the reduction in accommodation with age. Should the change in lens shape be reduced during accommodation, it is possible that there may be a neural component, although there may also be contributions due to the changed architecture of the lens and its supporting structures.

One aspect that is not known well is how the lens refractive index distribution differs between people with and without diabetes. It is important to know this to understand the accommodation mechanism and to predict effects of acute hyperglycaemic attacks on refraction and visual acuity.

With increasing age, higher order aberrations increase. This is attributable to the lens as there is little age-related change in corneal optics. When monochromatic higher order aberrations are corrected with large pupils, visual acuity improves by approximately 0.1 to 0.15 log minutes of arc resolution (1.2-1.4X) (Guo, Atchison, & Birt, 2008) and contrast sensitivity improves up to 6 times (Liang, Williams, & Miller, 1997). Therefore, it is expected that any increased higher order aberrations in people with diabetes could reduce visual acuity and other visual functions.

Shahidi et al. (2004) found approximately 33% greater higher-order aberrations with 6 mm pupils in people with diabetic retinopathy than in non-diabetic people. Wiemer et al. (2009) examined higher-order aberrations with 5 mm pupils in 25 people with diabetes presenting with acute hyperglycaemia and blurred vision. They found 18% lesser higher-order aberrations in four of their people with diabetes when they brought hyperglycaemia under control, but no changes in the others. There has been
no similar study between non-diabetic people and people with diabetes but without retinopathy.

Several studies have shown that long term diabetes is associated with increased retinal thickness (Goebel & Kretzchmar-Gross, 2002) but some studies have found thinner retinas in people with diabetes or without minimal retinopathy (Biallosterski et al., 2007; Van Dijk et al., 2010). Choroids are thinner in people with diabetes than in age-matched controls (Esmaeelpour et al., 2011). Lima et al. (2010) compared age matched controls with DM2 and found that macular pigment density decreased as HbA1c (glycated haemoglobin) levels increased.

It is thought that hyperglycaemia results in myopia (Fledelius & Miyamoto, 1987; Furushima, Imaizumi, & Nakatsuka, 1999; Mäntyjärvi, 1988) and hyperopia has been reported during reduction of hyperglycaemia hyperglycaemia (Giusti, 2003; Lin, Lin, Chang, & Tsai, 2009; Okamoto, Sone, Nonoyama, & Hommura, 2000; Saito et al., 1993). In contrast to the above studies, some investigators have observed both myopic and hyperopic shifts during hyperglycaemia (Sonmez et al., 2005; Wiemer, et al., 2009). The mechanisms of long term and short term hyperglycaemic changes on refractive status of the eye are poorly understood (Cavallerano, 1990).

Wiemer et al. (2008b) studied refractive changes in 5 healthy young participants during acute hyperglycaemia. For one participant, they observed a hyperopic shift with increased anterior lens curvature and decreased lens equivalent refractive index. They concluded that refraction changes during hyperglycaemia may be explained on the basis of change in lens shape and refractive index.
In previous studies involving intraocular distance measurements by techniques such as ultrasonography, optical pachymetry, and optical coherence tomography, a fixed refractive index was assumed within one or more elements. In partial coherence interferometry, a fixed index has been assumed to be adequate for the whole eye (e.g. IOLMaster) or fixed indices are assigned to each medium (e.g. Lenstar). A non-optical technique of value here is magnetic resonance imaging as no such assumptions are made.

Investigation of the optics of the diabetic eye is a relatively new area of research, especially for objective measurements of amplitude of accommodation, intra-ocular aberrations, straylight, lens diameter and the lenticular refractive index distribution. The causes of amplitude of accommodative loss are not known. This research will utilise state-of-the-art ocular measurement techniques to examine the changes in optics of diabetic eyes, and investigate a range of potential ocular factors (such as lens thickness, shape and refractive index distribution) that may contribute to loss of amplitude of accommodation.

The findings from this research will add to our understanding of the underlying causes of optical changes in diabetic ocular components, but will not provide information regarding histological or biochemical changes. This research will provide insights into the visual problems faced by people with diabetes.

The over-riding hypothesis of this study is that eyes of people with diabetes act as older eyes than those of people of the same age without diabetes. The aims are as follows:
1) To determine whether people with diabetes have greater higher-order aberrations than people without diabetes of the same age.
2) To identify whether loss of accommodation with diabetes can be attributed to changes in the lens to which the refractive index distribution contributes.

3) To determine whether diabetic lenses become yellower at greater rates than aged-matched normal lenses.

4) To determine whether refractive index distribution of the diabetic lens is different from that of the non-diabetic lens.

5) To determine whether corrections to assumed refractive index are needed in biometric measurements of diabetic eyes.

6) To determine whether people with diabetes have more straylight than people without diabetes.

The contents of this thesis are as follows. Chapter 2 is a literature review about diabetes, what is known about the relationship between diabetes and the optics of the human eye, and how visual functions are affected by diabetes. Chapter 3 describes the participants and techniques used in the study. The latter include both standard clinical and specialised techniques. The background of the techniques is included, and as appropriate, full details of development of some specialised techniques such as phakometry are included. Chapter 4 gives results of the various optics and biometric measurements for people with DM1 and for age-matched controls. Discussion and summary of the study are given in Chapter 5.
Chapter 2: Literature Review

2.1 Diabetes: Overview

Diabetes mellitus is a group of metabolic diseases characterised by chronic hyperglycaemia. It has multiple complications and premature morbidity and mortality. It is reaching epidemic levels throughout the world, producing cardiovascular complications, eye and kidney diseases and limb amputations. Reducing morbidity and mortality, and improving quality of life are major public health goals, which can be achieved by earlier disease diagnosis and improved screening for associated complications. Diabetes affects the refractive components of the eye, so blurriness of vision is often the first sign.

2.1.1 Definition and Classification

Diabetes results from defects in insulin secretion, insulin action or both. It has been classified on the basis of the level of hyperglycaemia (Gries, 2003).

Type 1 diabetes mellitus (DM1), which was known previously as insulin-dependent diabetes, is juvenile in its onset and is characterised by autoimmune destruction of pancreatic beta-cells, leading to loss of insulin secretion. Its cause is not well known and it is not preventable with current knowledge. Excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, vision changes and fatigue are symptoms of DM1 which may occur suddenly (WHO, 2011a).
Type 2 diabetes mellitus (DM2) is the most common form of the disease, being responsible for over 90% of diabetics in the world. It includes insulin resistance and impaired insulin secretion conditions. Excess body weight and physical inactivity are contributing factors for DM2. It has similar but not as severe symptoms as DM1. It may be diagnosed several years after its onset when the complications have already occurred. It was thought that DM2 occurs only in adults, but it has also been found in children (WHO, 2011a).

Gestational diabetes is hyperglycaemia of variable severity due to carbohydrate intolerance with onset or first recognition during pregnancy. It affects approximately 7% of all pregnant women and is considered to be a major risk for later development of diabetes mellitus (American Diabetes Association, 2004).

Latent Autoimmune Diabetes in Adults comprises up to 10 – 15% adults diagnosed with DM2. It is a form of diabetes which is diagnosed in individuals who are older than the usual age of onset of type 1 diabetes. Maturity onset diabetes of the young is a monogenic form of diabetes with an autosomal dominance inheritance characterised by non-ketotic hyperglycaemia in adolescents of young adults in conjunction with a family history of diabetes (Patel & Macerollo, 2010).

2.1.2 Criteria of Diagnosis

According to the World Health Organization (WHO, 2011b), glycosylated haemoglobin (HbA1c) can be used as a diagnostic test with HbA1c of 6.5% as the cut off point for diagnosing diabetes, with the normal range being 4-6%. The previous WHO diagnostic criterion for diabetes was fasting plasma glucose \( \geq 7.0 \) mmol/l or 2 - h plasma glucose \( \geq 11.1 \) mmol/l (National Health
and Medical Research Council, 2008). The diagnosis should be confirmed with a repeat HbA1c test, unless clinical symptoms and plasma glucose levels ≥ 11.1 mmol/l are present (WHO, 2011b).

Haemoglobin is found in red blood cells, which carry oxygen from the lungs to the body cells and remove waste carbon dioxide from them. A small percentage of haemoglobin has glucose attached to it, forming haemoglobin A. High levels of serum glucose sustained over a long period of time will increase haemoglobin A. The red blood cells circulate for 60-120 days in the body, so the blood level of haemoglobin A provides an average of blood glucose levels for a period of 2-3 months (Cavallerano, 1990). It is a useful measure as, unlike plasma glucose levels, it is not subject to within hour fluctuations but indicates the chronic elevated blood glucose level.

2.1.3 Prevalence of Diabetes

Diabetes is known as the epidemic of the 21st century. It is estimated that 366 million of the world’s population suffer from diabetes and predicted that it will increase to 552 million by 2030 (Whiting, Guariguata, Weil, & Shaw, 2011). The prevalence of diabetes increases with age and it is high in certain racial groups.

Approximately 80% of people with diabetes are in developing countries. Prevalence estimates of diabetes and impaired glucose tolerance are high in Asian countries, and are expected to increase during the next two decades. It is expected that diabetes in adults in developing countries and developed countries will increase by 69% and 20%, respectively, between 2010 and 2030 (Shaw & Chisholm, 2003). Diabetes develops at younger ages in Asian populations than in white populations, and hence the morbidity and
mortality associated with the disease and its complications are more common in young Asian people than in young white people (Ramachandran, Wan Ma, & Snehalatha, 2010).

Around one million Australians (7.5% aged 25 years or over) have diabetes and the number is expected to increase over the coming decades. Approximately 275 adults develop diabetes each day (Barr et al., 2006). Its prevalence increases with age, and more than 20% of the population aged over 60 years have DM2. The prevalence of DM2 has more than doubled in Australia since 1981 (Shaw & Chisholm, 2003). In some Aboriginal and Torres Strait islander communities as many as one-third of the people have diabetes, and DM2 may be among the highest in the world. For this reason, Federal state and territory governments have included diabetes as one of the National Health Priority Areas (Dunstan et al., 2000).

By world standards, Australia provides excellent opportunities for healthy lifestyles (WHO, 2000). However with the modernisation and industrialisation of society, diseases such as diabetes and heart disease have great impact. Lifestyle changes have also had an unfavourable influence on diet, as the consumption of energy-rich foods has increased. Together with a reduction in physical activity, this is contributing to an increase in obesity which in return is contributing to the increase in diabetes (Barr, et al., 2006).

Measures such as avoiding tobacco usage, doing physical activity, maintaining healthy body weight, and eating vegetables and fruits are helpful in preventing or delaying DM2 (WHO, 2011a).
2.1.4 Complications of Diabetes

Diabetes can cause a range of complications that lead to disabilities, shortened life expectancy and reduced quality of life. As well as personal health costs, the disease inflicts a large public health burden (Barr, et al., 2006). People with diabetes often develop diverse microvascular, macrovascular, and neuropathic complications. It has a number of systemic effects, including skin disorders, peripheral vascular disease, increased bone fragility, diabetic nephropathy, diabetic cardiomyopathy, coronary artery disease, cerebrovascular disease and diabetic neuropathy. As raised blood glucose concentrations diminish the ability of the body to combat infection, infections of the skin are particularly common (Ariffin, Hill, & Leigh, 1992; Williams & Pickup, 2004).

Mortality caused by diabetes is predominantly due to cardiovascular disease. Long term microvascular complications lead to increase in morbidity: diabetic retinopathy is the leading cause of premature blindness worldwide (Centres for Disease Control and Prevention, 1993), diabetic nephropathy is the most common cause of end stage renal failure (Bergrem & Leivestad, 2001) and diabetic neuropathy is the leading cause of non-traumatic lower limb amputation (Patout, Birke, Horswell, Williams, & Cerise, 2000). These changes are related to age of the patients and to duration of the disease.

Diabetes affects all parts of the eye. It has important ocular complications, including diabetic keratopathy, diabetic retinopathy, glaucoma, diabetic cataract, anterior ischemic optic neuropathy, iridopathy, recurrent styes and ocular motor nerve palsies (Ariffin, et al., 1992; Howells, 1953; Williams & Pickup, 2004). Variations in blood glucose level change refractive components and thus lead to unstable refraction (Okamoto, et al., 2000). Diabetic keratopathy includes recurrent erosions, delayed wound healing,
ulcers and oedema of the cornea. The cornea suffers from neuropathy with loss of corneal sensation and innervation (Lutty, 2013). Diabetic retinopathy is one of the leading causes of blindness in the world. It is generally regarded as a vascular disease. Classically, it presents with micro-aneurysms and small haemorrhages in the early stages. Several studies have indicated that neural loss may occur before any sign of vasculopathy can be observed (Barber et al., 1998; Bearse, 2006; Biallosterski, et al., 2007; Van Dijk, et al., 2010).

2.2 Overview of the Eye

The human eye consists of outer, middle and inner layers (Figure 2:1). The outer layer consists of the anterior cornea and posterior sclera. The cornea is transparent and approximately spherical with a radius of curvature of about 8 mm. It has a tear film in front of its outer surface, and has distinctive parts from the outer surface: the epithelium, Bowman’s membrane, the stroma, Dua’s layer, Descemet’s membrane and endothelium. The middle layer is the uveal tract composed of anteriorly iris, posteriorly choroid, and intermediately ciliary body which has the ciliary muscle. Behind the iris is a transparent, biconvex structure covered with a capsule called the lens. The inner layer is the light-sensitive retina which is connected to the brain by the optic nerve. The thickness of the retina varies from about 166 µm at the foveal centre to about 277 µm in the nasal area (Grover, Murthy, Brar, & Chalam, 2010). The retina consists of a number of cellular and pigmented layers and a nerve fibre layer. The light-sensitive cells (rods and cones) are at the back of the retina and the light must pass through the rest of the retina to reach them.
The inside of the eye is divided into three chambers: the anterior chamber between cornea and iris which contains the fluid known as the aqueous humour, the posterior chamber between iris, ciliary body and lens, and the vitreous chamber between lens and retina which contains the vitreous humour.

Image forming light enters the eye through the cornea, and is refracted by the cornea and the lens to be focused at the retina. The diameter of the incoming beam of light is controlled by the iris, which forms the aperture stop of the eye. The image of the aperture stop in the cornea is the entrance pupil, usually called the pupil.

![Diagram of the eye](image)

**Figure 2:1** The horizontal section of the right eye as seen from above. [Adapted from Atchison & Smith (2000d)].

The power of the lens can be changed when the eye needs to focus at different distances. This process is called accommodation. Various theories have been proposed for the mechanism of accommodation but Helmholtz’s theory is widely accepted (Atchison, 1995). According to Helmholtz’s theory, during accommodation when the eye changes focus from a far to near object,
the multiunit ciliary muscle contracts, and the tension on the zonules is released. The crystalline lens takes a more rounded shape because of the elastic properties of the lens capsule. In the unaccommodated form, when the focus of the eye at its far point, the ciliary muscle is relaxed and the lens is flattened due to tension of the zonules (Atchison & Smith, 2000a).

![Figure 2:2 Typical accommodative stimulus-response function of a young adult.](image)

Usually an accommodation stimulus-response curve shows a lead of accommodation response for distance vision, a linear portion with a slope of less than one and a saturation point corresponding to the amplitude of accommodation (Figure 2:2).

The age related optical changes in the human eye increase monochromatic aberrations (Artal, Berrio, Guirao, & Piers, 2002), increase intraocular light scattering and reduce light transmission (Cavallotti & Cerulli, 2010). These
contribute to losses in visual acuity and contrast sensitivity with age, although neural factors also play a role (Elliott et al., 2009).

Some studies have found increase in anterior corneal radius of curvature with increasing age (Hayashi, Hayashi, & Hayashi, 1995) but other have not found this (Atchison, et al., 2008; Douthwaite, Hough, Edwards, & Notay, 1999). The average anterior cornea is slightly prolate (negative asphericity) (Atchison & Smith, 2000a). Dubbelman et al. (2006) found a decrease in negative asphericity with age but Atchison et al. (2008) found no change.

The human lens grows throughout life with considerable changes in size, shape, mass and stiffness (loss of elasticity). The young lens is soft and easy to deform, while the old lens is stiffer and unable to be deformed (Heys, Cram, & Truscott, 2004). The equivalent refractive index, the constant index that gives the same lens power as the gradient index distribution, decreases with age (Atchison, et al., 2008). In the unaccommodated state, lens thickness increases (Brown, 1974; Dubbelman & Van der Heijde, 2001a) and anterior chamber depth decreases (Brown, 1974; Dubbelman & Van der Heijde, 2001b; Koretz, Strenk, Strenk, & Semmlow, 2004) with age at rates of approximately 0.024 mm/year and 0.011 mm/year, respectively (Atchison, et al., 2008).

The lens has a non-uniform refractive index distribution that increases from the lens surface to the nucleus (Atchison & Smith, 2000b). The refractive index contributes to the optical power of the lens so that the power of the lens is greater than it would be if the lens were homogeneous and had a uniform refractive index equal to its peak value in the nucleus. Kasthurirangan et al. (2008) showed changes in refractive index distribution with age and accommodation such that the gradient is altered while the central and edge refractive indices are unaffected. With increasing age,
refractive index change is restricted to smaller regions of the peripheral lens while with accommodation the change of index occurs over a larger part of the lens. These changes explain the lens paradox, which is that despite the lens curvatures increasing with age, most eyes do not become more myopic. The study showed a contribution of the gradient index distribution to accommodation as proposed by Gullstrand and incorporated in his No. 1 schematic eye (Gullstrand, 1909).

The human lens gradually loses its ability to accommodate with age. Presbyopia results when the loss is sufficient to interfere with close tasks such as reading. Total loss is complete around 55 years of age. Ramsdale and Charman (1989) found linear decrease of accommodation in individuals with age. Several studies have measured loss of accommodation with age (Atchison & Smith, 2000a) been proposed to explain loss of accommodation with age. These can be divided into lenticular and extralenticular theories. In the former, the loss is due to decreasing ability of the lens and/or capsule to deform or to the lens’ changing geometrical relationship with the zonules and ciliary body. In the latter there are changes in the supporting structures, such as weakening ciliary muscle, increase in connective tissue preventing the muscle from moving, or loss of tissue elasticity. The preferred theory remains the lenticular theory of the lens becoming less deformable with age (Atchison, 1995).

### 2.3 Influence of Diabetes on Biometry and Optics of the Human Eye

Diabetes mellitus has considerable consequences for the biometry of the eye. In patients with DM, the lenses are more convex, thicker and have decreased equivalent refractive index as compared to the lenses of non-diabetic eyes. Amplitude of accommodation is reduced in diabetic eyes.
Some people with diabetes complain of blurred vision during hyperglycaemia; the mechanism of these symptoms is unclear. Some authors (Huggert, 1953; Saito, et al., 1993; Wiemer, et al., 2008b), but not others (Giusti, 2003; Okamoto, et al., 2000; Planten, Kooymen, Vries, & Wolderingh, 1979), have found biometrical changes during hyperglycaemia. Some authors have suggested that transient refractive changes occur due to changes in lenticular refractive index including its gradient (Duke-Elder, 1925; Giusti, 2003; Mäntyjärvi, 1988; Planten, 1981). Transient changes might result from some combination of change in lenticular refractive index, change in aqueous and/or vitreous indices, choroidal thickness changes, and tonus of ciliary muscle.

An accurate knowledge about the influence of long-term diabetes mellitus on the refractive components of the eye is very important. Furthermore, the mechanisms underlying blurred vision, refractive changes and reduced accommodation may be clarified with an accurate description of the changes in the refractive properties of the eye and refractive index distribution in the lens during hyperglycaemia in people with diabetes.

2.3.1 Cornea

Diabetes mellitus has considerable effects on morphology, physiology and clinical appearance of the cornea (Sánchez-Thorin, 1998), leading to increased corneal fluorescence (Ishiko et al., 2000; Van Schaik, Coppens, van den Berg, & Van Best, 1999), impaired epithelial barrier function (Gekka et al., 2004), decreased corneal sensitivity (Cousen, Cackett, Bennett, Swa, & Dhillon, 2007), and altered corneal endothelial morphology (Roszkowska, Tringali, Colosi, Squeri, & Ferreri, 2000; Schultz, Matsuda, Yee, Edelhauser, & Schultz,

Several studies have reported greater central corneal thickness in adults and children with diabetes than in non-diabetic adults and children (Busted, et al., 1981; Larsson, Bourne, Pach, & Brubaker, 1996; Lee, et al., 2005; Ozdamar et al., 2010; Roszkowska, et al., 2000; Storr-Paulsen, Singh, Jeppesen, Norregaard, & Thulesen, 2014; Su et al., 2008) but some have not (Inoue, et al., 2002; Keoleian, et al., 1992; Wiemer, et al., 2007; Ziadi, et al., 2002). Some authors (Su, et al., 2008; Zengin, Özbek, Arikan, Durak, & Saatci, 2010), but not all (Pierro, Brancato, & Zaganelli, 1993) have found a positive correlation between corneal thickness and HbA1c levels in people with diabetes. Zengin et al. (2010) found people with diabetes having HbA1c levels > 7% had higher central corneal thickness than those with with HbA1c levels < 7%.

Wiemer et al. (2007) found smaller posterior radii of curvature in people with diabetes than in people without diabetes without significant differences in the anterior radius of curvature, but this did not affect the overall corneal power.

2.3.2 Aqueous Humour

With ageing, there is no change in spectral transmission and scattering in aqueous humour (Atchison & Smith, 2000c). Aqueous humour flow
decreases with age (Toris, Yablonski, Wang, & Camras, 1999) and this is more pronounced in people with diabetes than in people without diabetes (Lane et al., 2010; Lane et al., 2001).

2.3.3 Pupil

The natural pupil becomes smaller with increase in age (Watson & Yellott, 2012; Winn, Whitaker, Elliott, & Phillips, 1994), and is smaller in people with diabetes than those without diabetes, particularly at low light levels (Bahrami & Goncharov, 2014; Cahill, Eustace, & de Jesus, 2001; Hreidarsson, 1982; Lei et al., 2011; Yang, Yu, & Yao, 2006; Zaczek & Zetterström, 1998).

2.3.4 Lens

Age related changes in the lens and to the depth of the anterior chamber are more pronounced in people with diabetes than in non-diabetic people (Bron, et al., 1993; Brown & Hungerford, 1982; Fledelius & Miyamoto, 1987; Løgstrup, et al., 1996; Løgstrup, et al., 1997; Pierro, et al., 1996; Saw, et al., 2007; Sparrow, et al., 1992; Wiemer, et al., 2008d). These changes are dependent on diabetes duration (Løgstrup, et al., 1996; Sparrow, et al., 1990; Wiemer, et al., 2008c).

With increasing age, the lens becomes thicker and the anterior chamber depth decreases (Atchison, et al., 2008; Dubbelman & Van der Heijde, 2001b). Wiemer et al. (2008c) found different impacts of DM1 and DM2 on lens biometry. They found significantly thicker nuclei, anterior cortices and posterior cortices in DM1 than in non-diabetic people. In DM2, they found significant thicker nuclei than non-diabetic people but thinner posterior cortices than in non-diabetic people.

Chapter 2: Literature Review
Surface curvatures are greater in people with diabetes than in people without diabetes (Sparrow, et al., 1992; Wiemer, et al., 2008d) although Wiemer et al. found that this occurred in DM1 only. Equivalent refractive index of the lens is lower in DM1 than without diabetes, and compensates for the steeper surface curvatures so that there is no significant change in lens power (Wiemer, et al., 2008d). For DM1, the age related change in equivalent refractive index is nearly twice that of controls at −0.0007/year (Wiemer, et al., 2008d), but no information is available about changes in refractive index distribution that cause the reduction in equivalent refractive index. In this case, the magnetic resonance imaging techniques employed by Jones et al. (2005) and Kasthurirangan et al. (2008) can be useful. Also, equatorial diameter increases with ageing according to Kasthurirangan et al. (2011), but not by Strenk et al. (1999); no information is available on diameters in diabetes.

People with diabetes have lower lens light transmission than people without diabetes (Davies & Morland, 2002; Sparrow, et al., 1990; Van Best, et al., 1985; Zagers, et al., 2005). Van Best et al. (1985) reported an average 0.5% decrease of lens light transmission in the people with diabetes for each year of diabetes measured with fluorophotometry. The decrease occurred 15 years earlier in diabetic people with more than 10 years of diabetic duration than in non-diabetic people.

Both in vivo (Davies & Morland, 2002; Lutze & Bresnick, 1991) and in vitro (Kessel, et al., 2010) studies have reported that lenses of people with diabetes are yellower than those of people without diabetes.

People with diabetes have lower amplitudes of accommodation than non-diabetic people (Moss, et al., 1987; Yamamoto, et al., 1989). This might be due

Chapter 2: Literature Review
to a change in refractive index distribution such as occurs in ageing, or to an overall decrease in refractive index throughout the lens. Either could explain the reduction in accommodation with age if the change in lens shape upon accommodation is not affected.

2.3.5 Vitreous

In an in vitro study Sebag (1993) found accelerated age related changes in diabetic vitreous compared with that of aged-matched non-diabetic vitreous, with more fibrous tissue and liquefaction in the former. A nine years old vitreous with five years of diabetes duration was morphologically similar to that of fifty six years old non-diabetic vitreous.

2.3.6 Retina and Choroid

Generally, an increase in retinal thickness has been reported in people with long-term DM and advanced stages of retinopathy (Esmaeelpour, et al., 2011; Goebel & Kretzchmar-Gross, 2002). Esmaeelpour et al. (2011), but not Asefzadeh et al. (2008), observed retinal thinning in people with diabetes without diabetic retinopathy.

Hernández et al. (2010) found an increase in macular thickness during reduction of hyperglycaemia in 18 out of 24 eyes of people with diabetes and blurred vision. However, Prakash et al. (2011) found macular thinning during reduction of hyperglycaemia. Jeppesen et al. (2007) and Wiemer et al. (2008a) induced hyperglycaemia in non-diabetic people and found no difference in retinal thickness between hyperglycaemic and euglycaemic states.

2.3.7 Axial Length

Pierro et al., (1999) found no difference in axial lengths of people with diabetes, but without retinopathy, and of non-diabetic people. They found a negative correlation between diabetic retinopathy and axial length. To reduce the effect of myopia, they selected non-myopic diabetic and non-myopic non-diabetic people with axial lengths less than 24 mm. As there was no information about the refractive status, this study provides little useful information.

2.3.8 Refraction

People with diabetes have both long term (chronic) and short term (acute) alterations in their refractive status, but the mechanisms are poorly understood (Cavallerano, 1990). Long term refractive changes occur over years and short term changes occur from a few minutes to months.

2.3.8.1 Long Term Refractive Changes

Higher prevalence of myopia has been observed in people with diabetes than in people without diabetes (Fledelius, 1983, 1986; Jacobsen, Jensen, Lund-Andersen, & Goldschmidt, 2008; Mäntyjärvi, 1988). Fledelius (1983) found 38% myopia in 381 people with diabetes and 27.5% myopia in 1035 non-
diabetic people. Higher prevalence of low order late onset (≤ 1D, ≥ 20 years) myopia has been reported in people with diabetes than in people without diabetes (Fledelius, 1986). Poor glycaemic control has been considered a risk factor for higher prevalence of myopia in people with diabetes (Jacobsen, et al., 2008; Morgan, Ohno-Matsui, & Saw, 2012).

Many etiological factors are involved in myopia development (Morgan, et al., 2012). Age related lens changes are more pronounced in people with diabetes than in people without diabetes, which may also contribute to development of myopia. These biometrical changes are more evident in DM1 people (Wiemer, et al., 2008c) than in DM2 people and this might be the reason for more myopic refractions in the former group (Jacobsen, et al., 2008).

2.3.8.2 Short Term Refractive Changes

It is thought that in people with diabetes, hyperglycaemia results in myopia (Fledelius & Miyamoto, 1987; Mäntyjärvi, 1988) and hyperopia has been reported during reduction of hyperglycaemia (Giusti, 2003; Lin, et al., 2009; Okamoto, et al., 2000; Saito, et al., 1993), but some investigators have observed both myopic and hyperopic shift during hyperglycaemia (Sonmez, et al., 2005; Wiemer, et al., 2009). Studies and case reports showing these short term refractive changes have been summarised in Table 2:1.
Most of these studies showed positive correlation between the maximum hyperopic change and HbA1c level on admission. The hyperopic change started within few days or weeks of hyperglycaemia treatment and reverted to the baseline within months (Giusti, 2003; Li, et al., 2010; Lin, et al., 2009;
Okamoto, et al., 2000). Okamoto et al. (2000) found that time taken by the hyperopic change to reach the peak and recover during hyperglycaemia depends upon the maximum hyperopic change (and initial plasma glucose concentration). Saito et al. (1993) found that these effects are similar in both eyes of the same individual at high and low fasting plasma glucose levels. Larger refractive changes have been observed in people with diabetes having considerable myopia (Lin, et al., 2009; Okamoto, et al., 2000) with Lin et al. (2009) reporting a participant with high myopia showing a hyperopic change of 6.25 D in both eyes.

Small but significant biometrical changes have been found by some authors during hyperglycaemia (Fledelius, et al., 1990; Saito, et al., 1993; Tai, et al., 2006) and during reduction of hyperglycaemia (Sonmez, et al., 2005). Sonmez et al. (2005) found increase in power of the flatter corneal meridian through c-scan corneal topography. They also observed a negative correlation between anterior chamber depth and change in refraction. Saito et al. (1993) observed an increase in lens thickness through photography and ultrasonography, and decrease in anterior chamber depth through photography but not through ultrasonography. Tai et al. (2006) found a decrease in anterior chamber depth with Orbscan II but not through ultrasonography and Wiemer et al. (2009) found a decrease in anterior chamber depth with Scheimpflug camera only in eight of their 25 participants. Fledelius et al. (1990) observed an increase in lens thickness and a decrease in anterior chamber depth through ultrasonography. Contrary to these findings, others found no biometrical changes (Giusti, 2003; Li, et al., 2010; Lin, et al., 2009; Okamoto, et al., 2000). Several of the authors inferred that changes in refractive index, particularly lens refractive index, might be responsible for refractive changes in people with diabetes.
Huntjens et al. (2012) investigated how short-term changes in blood glucose levels affected refractive and ocular biometry (central corneal thickness, anterior chamber depth, lens thickness, axial length and ocular aberrations) during the day. While “minor changes of marginal statistical or optical significance were observed for some parameters”, they concluded that the normal short-term fluctuations in blood glucose level of people with diabetes are not usually associated with acute changes in refraction or ocular aberrations.

In a theoretical study, Charman et al. (2012) considered the possible lenticular origin of transient hyperopic refractive shifts. Refractive shifts of up to a few diopters could be explained by reduction in refractive index near the lens centre and alteration in the rate of change between centre and surface, so that most of the index change occurred closer to the lens surface. Restoration of the original refraction depended on further change in the refractive index distribution with more gradual changes in refractive index from the lens center to its surface.

Table 2:2 is a summary of studies in which hypoglycaemia or hyperglycaemia were induced. The studies were conducted on people with different types of diabetes, with large variations of glucose level, variable durations of diabetes, different methods of treatment and different systemic diseases which affect the refractive components. They did not consider very short term variations (e.g. within a day or from day to day) in blood glucose level.

To find the effects of glucose fluctuations on refraction in people with diabetes, hyperglycaemia and hypoglycaemia were induced in people with diabetes (Gwinup & Villarreal, 1976; Steffes, 1999)(Table 2:2). Gwinup &
Villarreal (1976) induced hyperglycaemia in ten people with diabetes, four having monocular aphakia. The aphakic eyes showed hyperopic shifts and phakic eyes showed myopic shifts (maximum 0.65 D) within 15 minutes. They supposed that retinal swelling reducing the distance between cornea and retina may be responsible for hyperopic shift, and lens swelling may be responsible for myopic shift. Steffes (1999) induced hypoglycaemia in a diabetic participant from 5.3 mmol/l (95 mg/dl) to 2.8 mmol/l (50 mg/dl), and observed maximum reduction in myopia of 0.25 D myopia within 40 mins from initiation of hypoglycaemia. This participant took a similar time to regain the base line refraction when glucose was administered. It was supposed that decrease of glucose in aqueous caused this refractive shift.

Furushima et al. (1999) and Wiemer et al. (2008b) investigated the short term effects of hyperglycaemia on biometry of healthy individuals. Myopic shift was found by Furushima et al. (1999) with increase in lens thickness and decrease in anterior chamber depth while Wiemer et al. (2008b) found hyperopic shift in one participant and no changes for four other participants. The participant with hyperopic shift showed 1.96 mm decrease in anterior lens radius of curvature and 0.014 decrease in lens equivalent refractive index. This participant received twice the oral glucose dose (150 mg glucose) compared with the others because of a delayed rise in glucose level.

Wiemer et al. (2008b) proposed that equivalent refractive index and radius of curvature changes are responsible for hyperopic shift while Furushima et al. (1999) argued that lens thickness is responsible for myopic shift.

**Summary**

The above studies do not show a clear picture about the mechanism of the short term refractive changes in people with diabetes. The sensitivity of the
instruments is important in these measurements. Many of the authors believed that change in refractive index, probably alteration in lens gradient refractive index, plays a major role.

Table 2.2 Summary of the refractive changes after induction of hyperglycaemia or hypoglycaemia in people with and without diabetes.

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Number of participants, age range (years)</th>
<th>Glucose changes</th>
<th>Mean change in refraction Participants</th>
<th>Biometrical changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gwinup &amp; Villarreal (1976)</td>
<td>10, not reported</td>
<td>Hyperglycaemia</td>
<td>Four aphakic diabetic participants showed hyperopic shifts, six phakic diabetic participants showed myopic shifts</td>
<td>not reported</td>
</tr>
<tr>
<td>Furushima et al. (1999)</td>
<td>7, 18-29</td>
<td>Hyperglycaemia</td>
<td>−1.9 D (1.9 ± 0.39 D) Non-diabetic participants</td>
<td>Increase in lens thickness (1.14 ± 0.41 mm) and decrease in anterior chamber depth (1.21 ± 0.50 mm)</td>
</tr>
<tr>
<td>Steffs (1999)</td>
<td>1, not reported</td>
<td>Hypoglycaemia</td>
<td>0.25 D less myopic Diabetic participant</td>
<td>not reported</td>
</tr>
<tr>
<td>Wiemer et al. (2008b)</td>
<td>5, 21-33</td>
<td>Hyperglycaemia</td>
<td>One participant showed 0.4 D hyperopic shift Non-diabetic participants</td>
<td>Decrease in anterior lens radius of curvature (1.96 mm) and decrease in equivalent refractive index (0.014)</td>
</tr>
</tbody>
</table>

2.3.9 Higher-order Aberrations

With increasing age, higher-order aberrations increase. This is attributable to the lens as there is little age-related change in corneal optics. Shahidi et al. (2004) found approximately 33% greater higher-order aberrations with 6 mm pupils in people with diabetes mellitus than in non-diabetic people. Valeshabad et al. (2014) found greater higher order root-mean square aberrations and overall 4th-order aberrations in a group of people with diabetes than in a control group for 5 mm pupils. Wiemer et al. (2009) examined higher-order aberrations with 5 mm pupils during hyperglycaemia and the normal glucose state in 25 people with diabetes. In four people (18%), they found an increase in higher-order aberrations during hyperglycaemia. In only one of five normal participants, hyperglycaemia
induction increased spherical aberration (change +0.08 µm, 5.7 mm pupils) (Wiemer, et al., 2008b).

Calvo-Maroto et al. (2014) reported total ocular, corneal and internal higher-order aberrations in small groups of type 1 and type 2 diabetes, but without a control group. Internal aberrations were determined as the differences between ocular and corneal aberrations. There were no significant differences between the groups. The aberrations were very high for 5mm pupils, with ocular higher-order root-mean-squared aberrations of 0.63 ± 0.23 µm and 0.53 ± 0.25 µm for DM1 and DM2, but there was no control group. In addition, it is not clear whether the ocular and corneal aberrations used the same reference axis.

When monochromatic higher-order aberrations are corrected with large pupils, visual acuity improves by approximately 0.1 to 0.15 log minutes of arc resolution (1.2-1.4x)(Guo, et al., 2008) and contrast sensitivity can improve up to 6 times (Liang, et al., 1997). If there are greater higher-order aberrations in people with diabetes than in people without diabetes, it is expected that correcting higher-order aberrations would improve visual performance more in the former than in the latter.

2.3.10 Straylight

People with diabetes complain of visual disturbances but the mechanisms are not well understood (Huntjens, et al., 2012; Wiemer, et al., 2009). Having visual acuity of 6/6 or better is not enough to ensure good vision (De Wit, Franssen, Coppens, & van den Berg, 2006; Michael et al., 2009; Van Der Meulen et al., 2012). Real-world visual scenarios with bright distant light sources and low contrast surroundings cannot be represented by visual
acuity and contrast sensitivity measurements. These assess vision due to small details of point spread function (from 1 to 10 min arc) and do not account for point spread function information beyond 60 min arc produced by straylight (van den Berg, Franssen, & Coppens, 2010). Straylight is responsible for glare while driving at night, perception of halos around bright lights, facial recognition problems, hazy vision and lowered contrast (Elliott & Bullimore, 1993).

Straylight in healthy young people is due to physiological imperfections in ocular structures. The cornea and lens contribute 2/3 of the straylight with the other 1/3 contributed by the iris, sclera, vitreous and fundus (van den Berg, 1995). Straylight is approximately constant until 45 years of age and then increases. According to van den Berg (1995), it can be described according to the formula

\[ \log(s) = P + \log \left[ 1 + \frac{(\text{age}/65)^4}{...} \right] \]  

where \( P = 0.90 \) is the asymptomatic value for \( \log(s) \) at lower ages, and 65 years is the age at which the straylight doubles relative to that of healthy young people. Greater straylight occurs when cataract is present than when it is not (Bal, Coeckelbergh, Van Looveren, Rozema, & Tassignon, 2010; Van Der Meulen, et al., 2012) and straylight reduces after cataract surgery (Rozema, Coeckelbergh, Caals, Bila, & Tassignon, 2013). Conditions such as corneal oedema, keratoconus, Fuchs’ corneal dystrophy, and vitreous floaters are also responsible for increasing straylight (Jinabhai, O’Donnell, Radhakrishnan, & Nourrit, 2012; Mura et al., 2011).

Morishige et al. (2001) and Takahashi et al. (2007) found greater light scattering in the corneal epithelial basement membrane in people with
diabetes than in people without diabetes when measured objectively through confocal microscopy. As greater age related optical changes seem to be found in people with diabetes than in people without diabetes, we expect greater straylight in people with diabetes than in people without diabetes.

2.4 Influence of Diabetes on Retinal Functions and Amplitude of Accommodation

Abnormal retinal functions have been found in people with diabetes (Fortune, Schneck, & Adams, 1999; Holm & Adrian, 2012; Lecleire-Collet et al., 2011; Shimada, Li, Bearse, Sutter, & Fung, 2001; Tyrberg, Pönjavic, & Lövestam-Adrian, 2005). Multifocal electroretinograms have delays in implicit time (Fortune, et al., 1999; Holm & Adrian, 2012) and absence of the 3rd position waveform in the second order component (Palmowski, Sutter, Bearse, & Fung, 1997; Tyrberg, et al., 2005).

Amplitude of Accommodation

Several studies have reported lower amplitudes of accommodation in people with diabetes than in age matched controls (Cavallerano, 1990; Moss, et al., 1987; Spafford & Lovasik, 1986; Yamamoto, et al., 1989). Most of these studies used narrow age ranges, and thus did not investigate the rate of change with age. Apart from the study of Yamamoto et al. (1989), these were all subjective techniques and were thus affected by depth-of-focus which overestimates amplitude.

Spafford & Lovasik (1986) used a Lovasik-Woodruff dynamic accommodation meter to determine the monocular amplitude of accommodation subjectively via a push-up method in thirty DM1 and thirty
age-matched non-diabetics and found significant lower amplitudes of accommodation in the former. Using a monocular push-up method, Mantyaryi & Nousiainen (1988) found 9.9 D mean amplitude of accommodation in children with diabetes, approximately 1.9 D less than for non-diabetic children. Moss et al. (1987) indicated that subjective amplitude loss in diabetes could be accounted for by adding the duration of diabetes to the age of the participants. Pawelski & Gliem (1971), and Braun et al. (1995) found that duration of diabetes was approximately 60%, 46%, and 40% as important, respectively, as age in reducing amplitude. Yamamoto et al. (1989) found smaller accommodative amplitudes in people with diabetes than in non-diabetics using an indirect method based on pattern reversal visually evoked cortical potentials.

Related to reduction in amplitude of accommodation with diabetes, Leffler et al. (2008) found that the preferred reading addition in a 43-71 year population was significantly related to the duration of diabetes, although not the presence of diabetes, such that the addition was predicted to increase by 0.06 D/year of diabetes duration.

Accommodative spasm has been reported during hyperglycaemia in people with diabetes, and it disappeared after recovery (Marmor, 1973; Melani & Battistini, 1963; Rosen, 1956; Turtz & Turtz, 1958).

2.5 Techniques

A variety of techniques has been used for measuring ocular biometry in association with diabetes. These include ultrasound, Scheimpflug imaging and (recently) partial coherence interferometry for intra-ocular distances,
Placido disk imaging and Scheimpflug imaging for anterior corneal topography and Scheimpflug imaging for posterior corneal topography, and Scheimpflug imaging for lenticular shape and equivalent refractive index. Scheimpflug imaging is versatile in its application and will be described in section 3.4.5.2. I will use the method of phakometry instead of Scheimpflug imaging to determine lenticular shape and equivalent refractive index (section 3.5.5).

There are assumptions involved in the different techniques, including the refractive indices of different media and particularly that of the lens. This suggests that determination of the refractive index distribution of the diabetic lens is of considerable importance, and a magnetic resonance imaging method of doing this will be described in section 3.5.6.

Lens yellowing has been determined by fluorophotometry and flicker photometry, and an application of the latter will be described in section 3.5.4. Aberrometry has been used recently in people with diabetes (section 2.3.9). In combination with corneal topography, it will be used to determine corneal and internal aberration components (section 3.5.2). The aberrometer will also be adapted to providing objective amplitude of accommodation measurement (section 3.5.3). An easy-to-use instrument, the C-Quant, has recently become available for straylight estimates (section 3.5.1).

**2.6 Summary**

Most of the previous studies on the influence of diabetes on the optics of the eye included participants with a considerable range of severity of complications including diabetic retinopathy. Aberrations were measured
with dilated pupils, and there is no information about intra-ocular contributions to these aberrations. Subjective measures of amplitude of accommodation in previous studies were affected by depth of focus and relative distance magnification, and there is need for objective measurements of amplitude of accommodation. Ocular scattering has been studied in the cornea with confocal microscopy, but no information is available about whole eye straylight. There is no information about lens diameter and the lens refractive index distribution in people with diabetes.

Lower amplitudes of accommodation in people with diabetes might be due to a change in refractive index distribution such as occurs in ageing, or to an overall decrease in refractive index throughout the lens. Either could explain the reduction in accommodation with age if the change in lens shape upon accommodation is not affected.

The hypothesis of this thesis is that eyes of people with diabetes act as older eyes than those of people of the same ages without diabetes. For this purpose, the thesis has the following aims, as already presented in chapter 1:

1) To determine whether people with diabetes have greater higher-order aberrations than people without diabetes of the same age.
2) To identify whether loss of accommodation with diabetes can be attributed to changes in the lens to which the refractive index distribution contributes.
3) To determine whether diabetic lenses become yellower at greater rates than aged-matched normal lenses.
4) To determine whether refractive index distribution of the diabetic lens is different from that of the non-diabetic lens.
5) To determine whether corrections to assumed refractive index are needed in biometric measurements of diabetic eyes.

6) To determine whether people with diabetes have more straylight than people without diabetes.

Investigating a range of potential ocular factors (such as lens thickness, shape, diameter and refractive index distribution) that may contribute to loss of amplitude of accommodation will add to our understanding of the underlying causes of optical changes in diabetic ocular components. This research will also provide insights into the visual problems faced by people with diabetes.
Chapter 3: **Methodology**

Section 3.1 covers participant recruitment, including inclusion and exclusion criteria. Section 3.2 gives a statistical justification of the number of participants. Section 3.3 presents the data analysis methods. Section 3.4 includes the standard clinical techniques that were used including visual function assessments of colour vision (visual acuity, letter contrast sensitivity and subjective amplitude of accommodation), refraction, pupil diameter, slit-lamp and fundus photography, corneal topography, and partial coherence interferometry. Section 3.5 includes specialised techniques of straylight, ocular aberrations and their component aberrations, objective amplitude of accommodation, flicker photometry for lens yellowing, phakometry, and magnetic resonance imaging. Table 3:1 is a list of biometric parameters and the techniques by which they were investigated.

This was a controlled, cross sectional observational study. Magnetic resonance imaging procedures were conducted by radiographers Aiman Al Najjar and Anita Burns at the Centre for Advanced Imaging of the University of Queensland. Blood was collected from participants by an experienced nurse at the Institute of Health and Biomedical Innovation. Fundus photography screening was conducted by fellow graduate student Cirous Dehghani. All other procedures were performed by the author at the Ophthalmic & Visual Optics and Anterior Eye laboratories at the Institute of Health & Biomedical Innovation of Queensland University of Technology.
Ethical approval was obtained from the Queensland University of Technology and the University of Queensland before the commencement of the study (QUT ethics number 1100001182, UQ ethics number 2012000172), and written informed consent was obtained from participants after explanation of the nature and possible consequences of the study. All participants were treated in accordance with the Declaration of Helsinki.

3.1 Participants

The majority of participants were recruited from the Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic Markers (LANDMark) study at the Institute of Health and Biomedical Innovation (54/74 diabetes, 19/64 non diabetes), and others were recruited through advertisement in media, advertisement in university websites and from the QUT health clinics. A key strategy of the LANDMark study was to recruit participants with type 1 diabetes with no or mild diabetic neuropathy, in order to facilitate a longitudinal evaluation of the progression of severity of neuropathy. This is essential for the establishment of the predictive validity of novel ophthalmic markers of diabetic neuropathy – especially corneal confocal microscopy. Consequently, nearly all of the participants recruited through LANDMark had low levels of the classic triad of diabetic complications – neuropathy, retinopathy and nephropathy.
Table 3.1 Biometric parameters and their determination

<table>
<thead>
<tr>
<th>Biometric parameter</th>
<th>Method or instrument, section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical equivalent refraction</td>
<td>Aberrometer (for screening), 3.4.2</td>
</tr>
<tr>
<td>Anterior corneal radius of curvature</td>
<td>Medmont E-300 corneal topographer, 3.4.5.1</td>
</tr>
<tr>
<td></td>
<td>Pentacam, 3.4.5.2</td>
</tr>
<tr>
<td>Anterior corneal asphericity</td>
<td>Medmont E-300 topographer, 3.4.5.1</td>
</tr>
<tr>
<td>Corneal decentration and tilt</td>
<td>Medmont E-300 corneal topographer, COAS aberrometer, 3.5.2</td>
</tr>
<tr>
<td>Cornea central thickness</td>
<td>Lenstar, 3.4.6</td>
</tr>
<tr>
<td></td>
<td>Pentacam, 3.4.5.2</td>
</tr>
<tr>
<td>Posterior corneal radius of curvature</td>
<td>Pentacam, 3.4.5.2</td>
</tr>
<tr>
<td>Anterior chamber depth</td>
<td>Lenstar, 3.4.6</td>
</tr>
<tr>
<td></td>
<td>Pentacam, 3.4.5.2</td>
</tr>
<tr>
<td>Pupil diameter</td>
<td>COAS aberrometer, 3.4.3</td>
</tr>
<tr>
<td>Anterior lens radius of curvature</td>
<td>Phakometry, 3.5.5.4</td>
</tr>
<tr>
<td>Lens equivalent refractive index</td>
<td>Phakometry, 3.5.5.4</td>
</tr>
<tr>
<td>Posterior lens radius of curvature</td>
<td>Phakometry, 3.5.5.4</td>
</tr>
<tr>
<td>Lens central thickness</td>
<td>Lenstar, 3.4.6</td>
</tr>
<tr>
<td>Lens refractive index distribution</td>
<td>Magnetic resonance imaging, 3.5.6</td>
</tr>
<tr>
<td>Lens diameter</td>
<td>Magnetic resonance imaging, 3.5.6</td>
</tr>
<tr>
<td>Lens equivalent power</td>
<td>Phakometry, 3.5.5.4</td>
</tr>
<tr>
<td>Anterior segment length, Lenstar</td>
<td>Lenstar, 3.4.6</td>
</tr>
<tr>
<td>Vitreous chamber depth</td>
<td>Lenstar, 3.4.6</td>
</tr>
<tr>
<td>Axial length</td>
<td>Lenstar, 3.4.6</td>
</tr>
</tbody>
</table>

Participants with a physician diagnosis of type 1 diabetes and using insulin were recruited with ages between 19 and 63 years, with mean ages 41 ± 13 and 42 ± 13 years in people without diabetes and with DM1, respectively. Visual functions testing and ocular health assessment included case history (including self-reported duration of diabetes), slit lamp biomicroscopy, intraocular pressure (I-Care) and colour vision assessment (Lanthony desaturated 15). Blood was collected from participants and was sent to
laboratory for a HbA1c assay. HbA1c (glycated haemoglobin) provides average plasma glucose for the preceding two to three months. Capillary blood glucose was measured with an Accu-Chek glucometer through finger prick in people with DM1 only.

Participants with corrected visual acuities ≤ 0.1 log minimum angle of resolution (logMAR), Pelli-Robson contrast sensitivity scores ≥ 1.65, equivalent spherical refraction ≤ ±3.5 D, and normal colour vision were included. Participants with mild diabetic retinopathy were included e.g. micro-aneurysms, hard exudates, cotton-wool spots, and/or mild retinal haemorrhages. For the MRI component of the study, participants were also screened according to the standard MRI clinical checklist. The stringent criteria were adopted to ensure exclusion of people with cataract and moderate to severe diabetic retinopathies, and to minimise the influence of refraction on ocular parameters.

For participants recruited directly by me, the right eye was selected where it fulfilled the criteria; otherwise the left eye was selected. In the LANDMark study, people were examined for the eye on the side of hand dominance; as this testing involved contact with the cornea, the other eye was examined on the same day where possible. If this eye did not fulfil the criteria and the participant was able to return on another day, I tested the better eye.

Participants were excluded from the study with more than mild diabetic retinopathy (e.g. soft exudates, venous beading and/or severe retinal haemorrhage), retinal diseases, glaucoma, uveitis, ocular trauma or surgery, epilepsy, endocrine disorders (except DM1), hypertension, neurological or psychiatric disorders, anaemia, contact lens wear and cataract (posterior subcapsular, cortical and nuclear of grades higher than 1). Slit lamp photographs
and C-Quant values (straylight > 1.60 log(s) was excluded) were used to classify participants with and without cataract. For amplitude of accommodation measurements (section 3.5.3.), participants using systemic medications with known accommodation effects or central nervous system effects were excluded.

The anticipated time for standard clinical and specialised testing was 2 - 4 hours, with an extra hour (and transport time) required for those undergoing magnetic resonance imaging.

There were different numbers of participants for different tests due to participant time availability, financial considerations and criteria for some tests (e.g. amplitude of accommodation). Some participants were recruited on the day of participation in the LANDMark study, but were not able to devote the full 2 - 4 hours to our study on the same day and were not also able to come again.

Magnetic resonance imaging was performed in a limited number of participants due to financial limitations. An additional twenty people, mainly young adults, performed most tests (including magnetic resonance imaging). While it is most likely that they did not have diabetes, they were excluded because HbA1c assays were not able to be performed.

All potential participants performing MRI underwent a checklist. This checklist included pacemaker or artificial heart valve, syringe driver, brain or aortic clip or neurostimulators, metal mesh implants/clips/wire sutures, medicated skin patches, hearing aid/implant, dental bridge or dentures with wires, glass eye, joint replacement, bullet/shrapnel wound, metal fragments in eye/head/skin, artificial limb, people working with metals, suffering from
claustrophobia, using mascara, pregnant, intra-uterine device, fractures bones treated with metal, major surgery, tattoos and history of kidney disease/disorder. People having any of these conditions were excluded.

3.2 Statistical Justification of Participant Numbers

Statistical justification of the number of participants was based on power analysis using the freeware program G*Power version 3.1.7 and using prior data. The calculations were done for \( \alpha \) (type 1 error) of 0.05 and power \( (1 - \text{type 2 error}) \) of 0.80, and equal numbers of participants were considered for control (no diabetes) and experimental (DM1) groups. For regressions against age, inputs required were difference in regression slopes \( (| \Delta \text{ slope} |) \) standard deviations \( \sigma_{x1} \) and \( \sigma_{x2} \) of the independent variable of age for the two groups, standard deviations \( \sigma_{y1} \) and \( \sigma_{y2} \) of the dependent variable, and correlation coefficients \( \rho_1 \) and \( \rho_2 \). For comparisons of means of two groups, inputs were differences in means \( (| \Delta \text{ mean} |) \) and standard deviations \( \sigma_{x1} \) and \( \sigma_{x2} \).

Estimate of numbers for lens shape and equivalent lens refractive index were based on Wiemer et al. (2008d). For example, their Figure 3 provided equivalent refractive index data. Inputs were \( | \Delta \text{ slope} | 0.000365/\text{year}, \sigma_{x1} 12.225 \text{ years}, \sigma_{x2} 9.352 \text{ years}, \sigma_{y1} 0.007444, \sigma_{y2} 0.009234, \rho_1 -0.5775 \) and \( \rho_2 -0.7255 \) to give an estimated 34 participants/group. Other results from Wiemer et al. were 78 participants/group and 71 participants/group for anterior radius of curvature and lens thickness, respectively. Lens yellowing numbers were based on data from figure 2 of Lutze & Bresnick (1991) for groups of under 51 years age (control 35 \( \pm \) 9 years and DM1 32 \( \pm \) 10 years). Inputs were \( | \Delta \text{ slope} | 0.013484/\text{year}, \sigma_{x1} 10.0 \text{ years}, \sigma_{x2} 9.5 \text{ years}, \sigma_{y1} 0.30, \sigma_{y2} 0.16, \rho_1 0.82 \) and \( \rho_2 0.66 \) to give an estimated 17 participants/group.
Root-mean squared higher order aberrations were estimated from figure 4 of Shahidi et al. (2004) for groups of similar age (control 47 ± 9 years and DM1 52 ± 12 years). Inputs were |Δ mean| 0.11 μm, σ x1 0.14 μm, and σ x2 0.18 μm to give an estimate of 28 participants in each group.

On the basis of these power analyses, I considered that 70 participants (eyes) in each of the diabetes and non-diabetes groups should give sufficient statistical power to provide significant age-related effects of diabetes for most, if not all, ocular parameters. Accordingly, I aimed to get this number (see section 4.1 for numbers of participants and reasons for non-inclusion in each test).

3.3 Data Analysis

Analyses were performed with IBM SPSS Statistics 21 version. Statistical significance was set at p < 0.05 for all tests. The normality of data was checked by both mathematical and graphical methods. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to determine the significance level of Standardised residuals while Standardised residual plots were used to judge the normality of the data by visual inspection (Section 4.1).

The Student unpaired t-test and the chi-square test determined significance of differences between the two groups for non-categorical data (e.g. lens thickness) and categorical data (e.g. proportions of males and females), respectively. Analysis of variance (ANOVA) was performed to determine the difference in lens dimensions and refractive index between different participant groups.
Simple linear regressions were applied to find the effects of ocular (spherical equivalent refractive error, lens thickness, axial length) and systemic parameters (age, diabetes duration, HbA1c, gender) on various parameters e.g. straylight. Multiple regression analysis was used to determine the combined effects of age and diabetes duration on parameters. We note that different approaches have been used previously. Sparrow et al. (1990) determined the linear regression for the non-diabetic group, superimposed this fit upon their diabetes group and then determined the additional effect due to the diabetes duration. Wiemer et al. (2008d) made a multiple regression analysis in type 1 diabetes participants with diabetes duration and age as factors. Our multiple regression analysis was performed in “Enter” fashion with age and diabetes duration as factors, but with gender and/or axial length as additional factors where these were significant according to simple linear regression. This analysis was done both for the whole group, with duration for the participants without diabetes given as zero, and for only the participants with diabetes.

ANCOVA analysis [between-participants factor: group (diabetes, non-diabetes); covariate: age] was performed to determine the interaction between group (diabetes, non-diabetes) and age. Also, it showed the significance of difference in slopes for each parameter (e.g. lens thickness) with age between people with and without diabetes.

3.4 Clinical Techniques

This section includes the standard clinical techniques performed on all participants. Visual function assessment, slit lamp and fundus photography were performed to screen the participants.
3.4.1 Visual Function Assessment

All measurements were undertaken monocularly without use of dilating or cycloplegic drugs.

Colour vision (Lanthony desaturated D15) was performed in a dark room with a custom built white illumination box; the source was a 20 W, 600 mm Duro-test True-Lite fluorescent tube (correlated colour temperature 5500°K, colour rendering index > 90) to give illuminance at the centre of the floor of approximately 1600 lux. Corrected distant visual acuity at 6 metres was measured using the Early Treatment Diabetic Retinopathy Study chart (ETDRS) with a log-MAR scale using a termination rule of four mistakes (or more) on a line (Carkeet, 2001); chart luminance was 150 cd/m² as measured with a Topcon BM-7A luminance-colorimeter. Photopic letter contrast sensitivity was tested using the Pelli-Robson contrast sensitivity chart (Clement Clark, UK) at one metre and 134 cd/m² luminance. This consists of letters of constant size, with lines of 6 characters, 3 of a particular contrast. Contrast reduces in 0.15 log steps per triplet from left to right and downwards. The participant was rested in the room for at least 5 minutes to adapt to room luminance, and encouraged to identify and guess each character in the chart for up to 20 seconds when the letters were difficult to identify. Contrast sensitivity was scored by characters and noted as log (CS). When “C” was called “O” it was marked to be correct (Elliott, Bullimore, & Bailey, 1991). The measurement was finalised when the participant was unable to identify any of the three letters of the same contrast.

Subjective amplitude of accommodation was measured with a hand held Redenstock Badal optometer (Schober, 1958) at approximately 500 cd/m².
luminance. The lens power is approximately +12.0 D. The target was placed at the far end of the optometer. The participant was instructed to move the target towards the eye and stop where the bottom line of 6/12 characters first became clear. This was noted as the far point. The participant was instructed to bring the target towards eye, and when the bottom line became blurred and could not be cleared this point was noted as near point of accommodation. Three sets of measurements were taken for each participant. The amplitude of accommodation was taken as the difference between the mean scale readings for the near and far points.

3.4.2 Refraction

Refraction is usually done using standard clinical techniques of subjective refraction, retinoscopy and autorefraction. However, for this study objective refraction was determined from aberrometry (section 3.5.3.1). At least three aberrometer readings, taken with the COAS-HD aberrometer under the unaccommodated state with room lights turned off, were averaged. This has been found to be a reliable, accurate instrument for determining refraction (Salmon, West, Gasser, & Kenmore, 2003). All readings were taken with reference to the corneal plane and refraction was measured using 2nd and 4th order Zernike polynomial coefficients for a 4 mm pupil at 555 nm wavelength.

3.4.3 Pupil Diameter

Pupil diameter can be determined with pupillometers of varying sophistication, but in this study it was determined from at least three aberrometer readings taken with the COAS-HD aberrometer under the unaccommodated state with room lighting turned off (section 3.5.3.1).
3.4.4 Slit-lamp and Fundus Photography

A Topcon SL-D digital slit lamp was used to take lens images. The images were graded for cataract classification using the LOCS III (lens opacity classification system) protocol. Fundus photographs were taken through a non-mydriatic camera (Visucampro-Carl Zeiss) for diabetic retinopathy detection according to NHMRC guidelines (NHMRC, 2008). The images were graded for cataract and diabetic retinopathy by two Optometrists and the gradings were confirmed by an Ophthalmologist.

3.4.5 Corneal Topography

Computer-aided videokeratoscopes provide comprehensive descriptions of corneal surface shape, either for the anterior surface or for both surfaces. There are several types of videokeratoscopes including Placido videokeratoscopes, Scheimpflug instruments, Raster-stereogrammetry, Slit-profiling and Moire fringing (Seitz, Behrens, & Langenbuchar, 1997).

3.4.5.1 Corneal Topographer

The Medmont E-300 Corneal topographer is an example of a Placido videokeratoscope. It contains 32 dark and light Placido rings fitted into a cone and illuminated by red light-emitting diodes (Medmont, 2010). The anterior cornea acts as a convex mirror and images are captured by a camera. Positions, sizes and spacings of these rings in the images are used to determine the corneal shape. Closer rings indicate steeper corneas, and hence larger refractive power. An arc-step algorithm is used for image reconstruction to allow accurate corneal height interpretation (Medmont, 2010).
There are four different maps of corneal topography, axial, tangential, elevation and refractive, that provide a comprehensive description of the anterior corneal surface (Roberts, 1998; Salmon & Horner, 1995). The axial maps display corneal curvature and power of the surface with respect to the keratoscope axis. The tangential maps display the local curvature and power of the surface. The elevation maps display the distance from a specified best-fit sphere to the surface in microns. The refractive map displays the true refractive power of the surfaces in dioptres (Medmont, 2010).

The accuracy of the Medmont instrument is good for spherical and aspheric test surfaces (Tang, Collins, Carney, & Davis, 2000) and it exhibits highly repeatable results (Cho, Lam, Mountford, & Ng, 2002; Chui & Cho, 2005). It has been used to derive corneal shape (Atchison, et al., 2008) and anterior corneal contribution to aberrations (Mathur, Atchison, & Tabernero, 2012) and was used for this purpose in this study (section 3.5.2). At least two anterior corneal topography images with the instrument’s quality specification of 95% for alignment or better were analysed.

To estimate anterior corneal radius of curvature \( R \) and asphericity \( Q \) in the conicoid equation

\[
X^2 + Y^2 + (1 + Q)Z^2 - 2ZR = 0 \ldots (3.1)
\]

for 6 mm diameter corneas, files with suffixes .axl, .dst, .hgt, .slp, and .tgl. were exported from the topographer into a Matlab program which gave a least squares solution. To do this, the data were referenced from the corneal topographic centre to the position on the cornea corresponding to the centre of the pupil during aberration measurements (section 3.5.2.2).

Files with the suffix .mxf (having anterior height data) and .ptd were exported into the custom built Matlab program and then into the optical...
design package Zemax so that the corneal tilt and decentration, and corneal contribution to aberrations could be determined (section 3.5.2.2).

3.4.5.2 Scheimpflug Camera

The Scheimpflug camera is a modified form of a slit-lamp camera to improve depth of focus.

![Scheimpflug principle](image)

**Figure 3:1** Scheimpflug principle. a) tilted image plane; b) tilted lens plane [Adapted from Dubbelman thesis, (2002)].

In a Scheimpflug camera, the slit beam, camera lens plane and CCD sensor plane intersect at one point and the image of an obliquely positioned object is captured. This can be performed by tilting the lens plane, image plane or a combination, so that the plane of focus becomes parallel with the slit beam (Figure 3:1).

The high depth-of-focus and the high resolution of the Scheimpflug image make it possible to detect small changes in the shape of the cornea and the lens (Rosales & Marcos, 2009). There are two types of image distortions in the Scheimpflug imaging system. The first type of distortion arises from the geometry of the camera, which introduces a variation of the magnification along the image plane, since the image and object planes are not parallel. The second type of distortion arises because the posterior corneal, anterior lens
and posterior lens surfaces are viewed through the ocular surfaces in front of them.

Dubbelman et al. (2001b; 2005; 2003) corrected geometrical distortions and retrieved real coordinates of the anterior cornea surface image captured on the CCD camera chip by ray tracing backwards from the image plane (i.e., CCD chip) through the optics of the camera, to the object plane (slit beam). Similarly, the posterior corneal surface was traced through the optics of the camera and anterior corneal surface to get the real coordinates of the posterior corneal surface. The anterior surface of the lens was then traced through the optics of the camera and the cornea. Finally, the posterior surface of the lens was traced through the optics of the camera, the cornea and the anterior lens.

The Oculus Pentacam is a 2-dimensional imaging system using the Scheimpflug principle. It does not correct any distortion on the crystalline lens surfaces but it gives optical distortions-corrected data of the posterior corneal surface (Rosales & Marcos, 2009).

By using a rotating Scheimpflug camera system, the Pentacam performs 12 to 50 single captures of the anterior segment of the eye, which can be converted to a three-dimensional model for analysis. It can perform five types of evaluation: Scheimpflug tomography, three-dimensional chamber analysis (volume, angle and depth), pachymetry, densitometry of the lens, and corneal topography.

The Pentacam has excellent repeatability for measuring central corneal thickness (Barkana et al., 2005; Lackner, Schmидinger, Pieh, Funovics, & Skorpik, 2005; O’Donnell & Maldonado-Codina, 2005), corneal curvature...
Chapter 3: Methodology

(Shankar, Taranath, Santhirathelagan, & Pesudovs, 2008), lens densitometry (Kirkwood, Hendicott, Read, & Pesudovs, 2009) and anterior chamber depth (Rabsilber, Khoramnia, & Auffarth, 2006; Shankar, et al., 2008). In this study, the Pentacam was used to measure the cornea anterior and posterior radii of curvature, which were used in calculation of lens radii of curvature and lens equivalent refractive index (section 3.5.5.4).

The Pentacam indicates the position of the pupil centre with respect to the corneal apex. There were insufficient information to determine the geometric centre of the cornea limbus, and thus I was unable to reference the corneal data to the aberrometer pupil centre. Therefore, I used the Medmont E300 corneal topography for determining anterior corneal asphericity and corneal contribution to aberrations. However the Pentacam was preferred for Phakometry because it provides radii of curvature for both corneal surfaces (section 3.5.2.2).

3.4.6 Partial Coherence Interferometry

Partial coherence interferometry is a non-invasive optical technique to measure distances. It depends on a laser Doppler to measure the echo delay and intensity of infrared light reflected back from tissue interfaces (Rosenfield & Logan, 2009). It removes any influence of longitudinal eye motions during measurement by using the cornea as a reference surface. It emits radiation from a low power diode laser. A short coherence length beam splits into two parallel beams. When these beams are reflected from cornea and retina, they produce interference patterns from which the optical path length can be determined (Rabbetts & Mallen, 2007a). The instrument provides longitudinal resolution of 0.3 to 10 µm (Drexler, Baumgartner, Findl, Hitzenberger, & Fercher, 1997).
The Zeiss IOLMaster 500 measures axial length (cornea to retinal pigment epithelium) to a precision of 0.01 mm (Rabbetts & Mallen, 2007a). It has been used for axial length measurements with good repeatability (Hussin, Spry, Majid, & Gouws, 2005; Lam, Chan, & Pang, 2001). It does not recognise individual interfaces and applies an average group index of 1.3549 to the eye at 780 nm.

The Haag-Streit Lenstar LS 900 uses a broadband source. It can measure central corneal thickness, anterior chamber depth, lens thickness, axial thickness, corneal diameter, pupil size, eccentricity of the visual line (visual axis with respect to the pupil centre), retinal thickness and choroidal thickness. Several studies have reported excellent repeatability (Liampa, Kynigopoulos, Pallas, & Gerding, 2010; Rabsilber, Jepsen, Auffarth, & Holzer, 2010; Rohrer et al., 2009). Read et al. (2011) compared the Lenstar (LS 900) and spectral domain optical coherence tomographer (Copernicus SOCT HR) for choroidal and retinal thickness, and found excellent agreement between the two instruments.

The Lenstar was used in the present study for measurements of corneal thickness, anterior chamber depth, lens thickness and axial length. Because the Lenstar gives all the intraocular distances in the eye, but the Pentacam does not, the former was used for phakometer calculations of lens radii of curvature and lens equivalent refractive index (section 3.5.5.4) which need all the distances.
3.5 Specialised Techniques

This section describes the specialised techniques used to find the straylight, amplitude of accommodation, aberrations, lens yellowing, lens radii of curvatures, lens equivalent refractive index and lens refractive index distribution.

3.5.1 Straylight

3.5.1.1 Introduction

Retinal image quality depends upon ocular scattering, aberrations and diffraction. There are two types of scattering: forward scattering (retinal straylight), which is responsible for a veiling light over retina and reduction of retinal contrast, and backward scattering, responsible for reduction in the amount of light reaching the retina. There is more forward scattering than backward scattering (Atchison & Smith, 2000a).

Retinal straylight is responsible for the outer skirt of the point spread function beyond approximately 60 min arc, and affects visual function tests such as contrast sensitivity, visual field and the pattern electro-retinogram (van den Berg, Franssen, Kruijt, & Coppens, 2013).

Both psychophysical and optical methods are used to measure forward scattering, but psychophysical methods are more meaningful because of their dependency on participant perception (Piñero, Ortiz, & Alio, 2010).

3.5.1.2 Instrument Principle

The C-Quant (Oculus Optikgeräte, Wetzler, Germany) is a commercial instrument using a compensation comparison to measure straylight. It has a
central test field which is divided into two halves (right and left half fields) and an outer circular ring (acting as a straylight source) at an average 7 degree eccentricity (Figure 3:2). When the outer ring flashes ON, due to eye imperfections some of the light reaches the fovea. This light is perceived as a weak flickering in the central test field. In only one of the two half fields a variable counterphase compensated flicker is introduced. This results in two types of flicker with different depth modulation in the central test field. One flicker is a combination of straylight and compensated light in one half field, and the other flicker is due to straylight in other half field. The amount of light in the compensated half field can be varied. In a series of trials the participant decides which half field has the stronger flicker. The instrument determines the amount of light at which no flickering is observed in the compensated field, and this is the measure of ocular straylight.

Figure 3:2 Layout of stimulus in compensation comparison method for C-Quant. The outer ring flashes, the left half field has compensated flicker and right half field is without compensation. The participant has to decide which of the two half fields has the stronger flicker.

To give some more detail, in measuring straylight a series of 1 or 2 seconds straylight stimuli are introduced and variable amount of compensated light is presented. The instrument has a two-alternative-force-choice
psychophysical measurement algorithm to decide the field with stronger flicker. The responses are recorded as either 0 or 1 by two push buttons representing the two half fields. The selection of the compensated half field is scored as 1 while the non-compensated half field is scored as 0. A psychometric curve is fitted to the participant’s responses using a maximum likelihood technique. The logarithm of straylight parameter log(s) and two reliability parameters, expected standard deviation ESD and quality parameter Q, are deduced from the psychometric curve (Figure 3:3). A measurement is considered reliable when ESD ≤ 0.08 and Q > 1.0 (Coppens, Franssen, & van den Berg, 2006).

Figure 3:3 The operator screen of the C-Quant instrument with upper graph showing the straylight value and age (red dot) of the participant compared with the range of normal values at different ages. The lower graph shows the participant responses as a function of straylight compensation level. The red curve is the psychometric curve fitted to the participant responses with the red dot being the straylight value. The grey bands show the normal range for the participant’s age. The blue filled circles represent the initial phase of stimuli while the red filled circles show the final phase of stimuli.
To reduce the number of stimuli presentations and to obtain accurate measurements there is an option for selecting age ranges (A, B, C, D, E) and there is an option (F, G) for cataract or corneal disturbances. In order to familiarise the participant with the instrument, five stimuli with obvious flicker differences between the two halves are presented, and then 25 more stimuli (12 stimuli in initial phase and 13 stimuli in final phase) are presented to obtain the straylight parameters. In initial phase stimuli, the outer ring flicker increases from low intensity to high intensity while the compensation flicker is kept constant, while in the final phase the outer ring flicker is constant and amount of light in the compensation flicker varies as described above. Changing the compensation light alters the luminance balance of the two half fields, but this is prevented by additional light being added to the non-compensated half field (C-Quant Guide; Coppens, Franssen, van Rijn, & van den Berg, 2006; Franssen & Coppens, 2007; Franssen, Coppens, & van den Berg, 2006, 2007; Van Den Berg, Coppens, & Franssen, 2005).

3.5.1.3 Procedure

All participants were briefed about the experiment and a flicker stimulus was shown on the screen using “simulate” option before starting the actual experiment. Room lights were turn off except light from the computer monitor. One eye was tested for each participant and the non-tested eye was patched. Spherical equivalent refractive correction was provided, and five measurements were taken and averaged.

3.5.2 Ocular Aberrations and their Component Aberrations

Objective aberrometers use light propagating out of the eye, after reflection at a point on the retina, to measure aberration. Objective sequential aberrometers include laser ray-tracers (Navarro & Losada, 1997) and
objective simultaneous aberrometers include the Hartmann-Shack aberrometer (Liang, Grimm, Goelz, & Bille, 1994), the objective Tscherning aberroscope and the objective crossed-cylinder aberroscope (Walsh, Charman, & Howland, 1984).

There are three major parts of a Hartmann-Shack aberrometer: a light detector, monochromatic light source, and a wavefront sensor consisting of a micro-lens array and a CCD detector. A narrow beam of collimated light radiation projects a spot onto the retina and a part of this light is scattered back from the retina. The wavefront exiting the eye is captured by the micro-lens array and is broken into multiple small beams. Each micro-lens forms a spot of the wavefront or light on the camera. The micro-lens array is conjugate with the pupil and its focal plane is at the detector. For a perfect eye, the wavefront at the sensor would be a plane wave. The transverse ray aberration (slope of the wavefront) associated with each micro-lens can be determined in terms of vectors by subtracting the positions of reference ($X, Y$) centroids from that of aberrated centroids ($X_a, Y_a$) of the image spots (Figure 3:4).

Hartmann-Shack aberrometers are more popular than most other types of aberrometers because they are faster and are less affected by scattering of light (Dai, 2008). They have been extensively used for measuring aberrations because they are reliable and accurate (Cheng, Himebaugh, Kollbaum, & Thibos, 2003; Thibos & Hong, 1999). It is difficult to measure aberrations accurately with subjective techniques because of the dependence on subjective judgement.

In this study, a COAS-HD (Complete Ophthalmic Analysis System – High Definition, Wavefront Sciences) Hartmann-Shack aberrometer was used to
determine refraction, measure ocular aberrations and its components, and measure amplitude of accommodation (section 3.5.3.1). The COAS-HD uses an 840 nm super luminescent diode source and a charged-coupled device camera (CCD) camera. It contains a micro-lens array with $83 \times 62$ lenslets. Each lenslet has a 2.48 mm focal length and is 0.108 mm in diameter, and transverse aberrations are sampled at 0.159 µm across the pupil (Mathur, 2009). A 4.5 mm diameter pupil was used in this study, corresponding to approximately 490 positions.

![Figure 3:4 Hartmann-Shack principle. Left: Side view shows an aberrated wavefront focused on the CCD camera by the micro-lens array. The micro-lens array is conjugate with the pupil of the eye. Right: distorted lattice of spots produced by an aberrated wavefront on the CCD camera. [Adapted from Atchison (2005)].](image)

### 3.5.2.1 Aberration Terminology

Ocular aberrations were determined according to the OSA/ISO system that uses Zernike aberration polynomials (ISO, 2008). It uses a two index system with polynomials written as $Z_n^m$ where index $n$ indicates the radial order of the polynomial in the pupil and index $m$ indicates the angular dependence of the polynomial in the pupil. Co-efficients of the polynomials are written as
$C_n^m$. An alternative to the above is to write polynomials and co-efficients as $Z(n, m)$ and $C(n, m)$.

Co-efficients were determined to the 6th order for 4.5 mm pupils. The major terms of interest were spherical aberration ($C_n^0$ co-efficient), vertical coma ($C_n^{-1}$ co-efficient), and horizontal coma ($C_n^1$ co-efficient), as well as the root-mean-square higher-order aberration (HO RMS) determined from the 3rd to 6th aberration orders. These terms were selected because their co-efficients are usually greater than co-efficients of other higher-order aberrations.

As recommended by the standard, to allow for mirror symmetry when combining right and left eye data, signs were changed for left eye co-efficients of Zernike polynomials with positive, odd $m$ indices (e.g. $C_3^1$) or with negative, even $m$ indices (e.g. $C_4^{-2}$).

3.5.2.2 Component Aberrations

Ocular higher-order axial aberrations increase with age. In young eyes, the anterior corneal and internal ocular aberration components usually compensate each other, but this balance is gradually lost with increasing age (Atchison & Markwell, 2008; Berrio, Tabernero, & Artal, 2010; Kelly, Mihashi, & Howland, 2004). Berrio et al. (2010) quantified the extent to which internal aberrations balance the corneal aberrations as:

$$\text{Compensation factor (CF)} = 1 - (\frac{\vert C_i - \text{internal} \vert}{\vert C_i - \text{corneal} \vert}). \quad (3.2)$$

where $C_i$ represents any Zernike coefficient. Compensation factor = 1 indicates perfect compensation and compensation factor = 0 indicates no compensation.
Internal ocular aberrations (posterior cornea and lens aberrations) can be estimated by subtracting the anterior corneal aberrations, estimated from videokeratoscope data, from the total ocular aberrations measured with the aberrometer. However, these two instruments work under different illumination conditions and have different origins of the aberrations measurements. In the videokeratoscope, corneal aberrations are measured under photopic conditions due to high luminance of the Placido ring target near the eye and the reference of corneal aberration is the corneal topography centre, while in the aberrometer the ocular aberrations are usually measured under mesopic conditions (room lighting is kept low) and the reference of total aberration is the entrance pupil centre.

This problem is overcome by referencing the corneal topography to the entrance pupil under the wave aberration measurements conditions. This requires finding the positions of the corneal topography centre and pupil centre of the wave aberration relative to the limbus centre (Figure 3:5a). This was done in a Matlab program. The program has components to deal with topographer and aberrometer information. One component reads the topographer .mxf files and iris image files (.ptd) and the other reads the aberrometer’s iris image file (.IX). The mean corneal radius of curvature \( R \), the average of the flattest and steepest meridians, is determined from the .mxf file. The keratometric centre is automatically identified as the centre of the smallest placido ring of the .ptd image file and the limbus centre was obtained by fitting a draggable ellipse to the limbus. This gives the corneal topographic centre relative to the limbus centre in the plane of the entrance pupil. For a second component, the IX images from the COAS-HD aberrometer are manually fitted with two draggable ellipses, one for obtaining the limbus centre and one for obtaining the pupil centre. This gives the aberrometer pupil centre relative to the limbus centre in the plane of the
entrance pupil. The corneal topographic centre relative to the aberration pupil centre $TCPC (x, y)$ is given by (Figure 3:5a):

$$TCPC (x, y) = [\text{corneal topographic centre} (x, y) - \text{limbus centre} (x, y)] -$$

$$[\text{aberrometry pupil centre} (x, y) - \text{limbus centre} (x, y)] ......(3.3)$$

Positive $x$ and positive $y$ correspond to the topographic centre being to the right and above the aberrometry pupil centre from the reference of an observer looking at the front of an eye. The anterior chamber depth $ACD$ measured by the Lenstar is entered by the user and $TCPC (x, y)$, $R$ and $ACD$ are saved into a .dat file.

Figure 3:5 Re-referencing the cornea. a) TCPC determination from aberrometry and corneal topography [adapted from Tabernero et al. (2009)]; b) Horizontal components of corneal decentration and tilt $(x, \theta_x)$ [adapted from Mathur et al. (2012)].

Using into-the-eye ray-tracing, anterior corneal aberrations at 555 nm up to sixth order were estimated with Zemax optical design software (Radiant Zemax, Redmond, USA). A macro program was written in Zemax programming language to read the .mxf files incorporating the anterior height data (section 3.4.5.1) and to convert them to 8 mm diameter “grid sag” surfaces. This macro read the .dat file mentioned above in order to estimate the position of the entrance pupil $EP$ relative to the cornea, tilt about the horizontal and vertical axes $(\theta_x, \theta_y)$, and the anterior corneal decentration $(x, y)$ (Mathur, et al., 2012). The equations required are (Figure 3:5b):
\[ EP = \frac{1}{n \left[ \frac{ACD}{R} - n - 1 \right]} \quad \text{......... (3.4)} \]

\[
(\theta_x, \theta_y) = (\tan^{-1}\left[ \frac{TCPC_x}{WD + EP} \right], \tan^{-1}\left[ \frac{TCPC_y}{WD + EP} \right]) \quad \text{......... (3.5)}
\]

\[
(x, y) = (WD \tan \theta_x, WD \tan \theta_y) \quad \text{......... (3.6)}
\]

where \( n \) is the refractive index of the cornea and is taken as 1.3375, and \( WD \) is working distance of the corneal topographer (60 mm) (Mathur, et al., 2012). Positive \( \theta_x \) and positive \( \theta_y \) correspond to the inferior and right sides of the participant’s cornea moving away from an observer looking at the front of the eye.

Under the control of the macro, the corneal system consisted of a distant object, a stop to coincide with the entrance pupil, the anterior cornea surface at \(-EP\) from the stop with decentration and tilt relative to the line-of-sight, and an image plane. The stop diameter was 4.5 mm. The refractive index inside the anterior corneal surface was taken as 1.3375, rather than a more realistic index of 1.376, to compensate for the lack of a posterior corneal surface. The image plane was placed to minimise RMS wavefront error.

The macro created anterior corneal aberration co-efficients as .txt files. These were imported into an Excel program, and internal aberration co-efficients were determined by subtracting the corneal aberration coefficients from ocular aberration co-efficients. To allow for mirror symmetry between right and left eyes, signs of some co-efficients of left eyes were changed (section 3.5.2.1).

A pilot study was performed on five participants to determine the repeatability of the corneal decentration and tilt procedure. Measurements
were performed on two different occasions for each participant, and two images were analysed on each occasion. The standard deviations for decentration along horizontal and vertical axes were similar at ± 0.11 mm and ± 0.12 mm, respectively. The standard deviations for tilts about the horizontal and vertical axes were ± 0.47° and ± 0.45°, respectively.

3.5.3 Calibration of Aberrometer for Amplitude of Accommodation

Objective amplitude of accommodation was determined with the COAS-HD aberrometer (section 3.5.2).

The COAS-HD slider position was calibrated for different accommodation stimuli. An observer (the author) focused a distance telescope at infinity by adjusting its eye piece. The telescope was placed in front of the COAS-HD and the instrument was moved so that front of the telescope objective was focused by the pupil camera of the instrument and thus was at the correct eye position. Ophthalmic trial lenses (−6 D to +8 D in 1 D intervals) were placed against the objective lens of the telescope. The observer looked through the telescope at the fixation target with lights turned off in the room and moved the slider until the fixation target was in focus for each trial lens. Powers of the trial lenses were verified with a vertometer.

Three readings of slider position were taken for each trial lens and averaged. Trial lens power sign was reversed to give the refraction and a quadratic regression was calculated for the slider position (Figure 3:6):

\[ y = -0.0165r^2 + 1.0085r - 1.4696 \]  

where \( y \) indicates COAS slider position and \( r \) indicates refraction. To calculate the slider position for different accommodative stimuli, “\( r \)” was
replaced by “\(r - a\)” in equation (3.7), where \(r\) is base line refraction and \(a\) is accommodative stimulus:

\[
y = -0.0165^\ast (r-a)^2 + 1.0085^\ast (r-a) - 1.4696 \quad \text{..... (3.8)}
\]

3.5.3.1 Procedure to Determine Amplitude of Accommodation

Room lights were turned off. The brightness of the COAS internal target was set to a setting of 0.1 in the control software (see next section for explanation of selecting this setting). A participant placed his or her chin on the chin rest with the non-tested eye patched. The COAS was moved so that the pupil of the tested eye was in focus and aligned according to the image of the pupil camera. The participant was instructed to relax the eyes while looking at the target. Three readings were taken with “auto-acquire mode”, using 2nd and 4th order Zernike polynomial coefficients for a 4 mm pupil. The average spherical equivalent was calculated from these readings and referred to as the baseline spherical equivalent.
The slider control was changed in the software to “acquire (single) mode” so that the slider did not move in response to a refraction/accommodation combination. The slider position was adjusted using the computer mouse so 1 D stimulus was provided as calculated from equation (3.8). The participant was instructed to keep the target in focus as well as possible and the average spherical equivalent was calculated from three measurements. The same procedure was adapted for other accommodation stimuli until it was clear that a maximum accommodation response had been achieved. Accommodative response was calculated by subtracting the average spherical equivalent from the baseline spherical equivalent:

$$\text{Accommodative response} = \text{baseline spherical equivalent} - \text{spherical equivalent} \ldots \ldots (3.9)$$

Figure 3:7 shows an accommodative response/stimulus curve for a non-diabetic participant. The maximum accommodation response is 6.3 D.

![Accommodative response/stimulus curve](image)

Figure 3:7 Accommodative response/stimulus curves of a non-diabetic participant. The error bars indicate the standard deviations from three measurements.
3.5.3.2 Pilot Work

To determine the effect of COAS internal target brightness on the pupil size and accommodative response, four young non-diabetic participants were recruited from students of the Queensland University of Technology. Their ages ranged from 24 – 34 years. The brightness level of the COAS was set at 0.1, 1, 5 and 10 in the control software for participants 1 and 2, and an additional level of 5 was used for participants 3 and 4. Maximum accommodative response was determined at different brightness levels according to the procedure described in the previous section.

For participant 1 maximum accommodative response (5.3 ± 1.3 D) was observed at the highest brightness level (10), but for the other three participants the maximum response occurred at the lowest brightness level (0.1). The maximum pupil diameter for all participants occurred at the lowest brightness (0.1) and size decreased with increase of accommodative response, although the changes were small for participant 3 (Figure 3:8).

As there was generally little influence of brightness level on accommodation response and as three out of four participants showed maximum accommodative response at the lowest brightness level (0.1), we decided to use this level in the main experiments. Measurements on further three participants indicate good accommodation response at this level (Figure 3:9).
Figure 3:8 Accommodative responses (left) and pupil sizes (right) of four participants. For participants 1 and 2, internal target brightness levels were at 0.1, 1 and 10. For participants 3 and 4 an additional brightness level of 5 was added. The error bars indicate standard deviations. Ages of participants were 24 years (1), 25 years (2), 25 years (3) and 34 years (4).
Figure 3.9 Three graphs show the accommodative response of participants when the luminance of the internal target was 0.1. The error bars indicate the standard deviations. Ages of participants were 30 years (5), 27 years (6), and 25 years (7).

### 3.5.4 Flicker Photometry – Lens Yellowing

Flicker photometry is a technique first used by Abney and Festing (1886) to establish the additivity of luminances. It can be adapted to measure lens optical density (Delori & Burns, 1996; Lutze & Bresnick, 1991; Wooten, Hammond, & Renzi, 2007; Xu, Pokorny, & Smith, 1997), spectral sensitivity of ocular media (Kraft & Werner, 1994) and spectral luminous efficiency of the eye (Sagawa & Takahashi, 2001).

In heterochromic flicker photometry, two sources of different colours illuminate the same area in the visual field alternatively. The intensity of one of these sources is adjusted until the perception of flicker is eliminated or
minimised. The lens is relatively non-absorbing for 550 nm light, so an elevated threshold for a 420 nm stimulus relative to that of a 550 nm stimulus is a measure of 420 nm light absorbed by the lens. The rods are equally sensitive to these wavelengths (Lutze & Bresnick, 1991). Therefore, lens density could be directly measured by comparing absolute scotopic thresholds to rhodopsin curves (Norren & Vos, 1974).

With increasing age, lens absorption increases at short wavelengths, resulting in lens yellowing. The objective of this test is to measure and compare lens yellowing in people with and without diabetes.

3.5.4.1 Apparatus and General Procedure

A flicker photometer was enclosed in a 140 cm long x 100 cm wide x 200 cm high room covered with a black cloth to allow dark adaptation. The photometer consisted of a 565 nm green LED (60 mcd, 10 mm diffused, 10° viewing angle, Kingbright L-813GD) and a 430 nm blue LED (60 mcd, 10 mm diffused, 10° viewing angle, Kingbright RT1017BUW) with 550±10 nm (Thorlabs, 25 mm FB420-10) and 420 ± 10 nm (Thorlabs, 25 mm FB550-10) narrow band interference filters, respectively (Figure 3:10). The light from the two sources was combined through a fifty-fifty 20 mm cube beam splitter (Melles-Griot 03BSC007). The light was limited by an 8 mm diameter aperture having a diffuser at a distance of 48 cm from the eye and thus subtending 1.0°. A 1.2 log unit neutral density filter was placed in front of it. The fixation target was a red LED at 7° angle on the temporal side which had been dimmed by four pieces of 1.2 log unit neutral density filter. The system has the limitation that it was not possible to measure the light output of the system, and hence the lens yellowing obtained is a relative measure.
Chapter 3: Methodology

Figure 3:10 Flicker photometry system.

The system was controlled by a Delphi software program called “Bg LED”, a control box with a microprocessor, control knob and a push button. The program sent commands and parameters to the microprocessor control unit. It set the flicker frequency (setting the number of pulses) of LEDs, and it set the initial setting of pulse width modulation (luminance) of green and blue LEDs. It also received the luminance of the blue LED when the participant pushed the button. The control knob varied the luminance of the blue LED by varying the pulse width modulation between 0 and 255.

To produce 5 Hz frequency (see next section), we set 245 pulses per 100 ms in the program (Figure 3:11).
At the start of an experiment, each participant was shown the apparatus and a brief description of the experiment was given. The non-tested eye was covered with an eye-patch. Each participant was dark-adapted for 25 minutes. The participant put his/her chin on the chin rest with the eye level with the light sources, and looked at the red fixating target while observing the flickering of the alternating LEDs. The pulse width modulation of both the green and blue LEDs was set initially to 10. The participant rotated the control knob to alter the luminance (pulse width modulation) of the blue LED. To avoid Troxler’s phenomenon, participants were advised to look at the roof of the room for short intervals of time during testing.

Participants located the non-flicker range by clicking the push button at the middle of the range, when no flickering was observed. Later on the technique was changed, with participants determining the upper end of the lower flicker range and the lower end of the upper flicker range, and we calculated
the middle of the range from the average values of these two readings. Ten such readings were taken on each participant, and the average of these 10 values was taken as the threshold.

3.5.4.2 Pilot Work

To test the system and choose the best parameters, we conducted several pilot experiments. Seven participants, consisting of six myopes and one emmetrope aged 28 to 57 years and all with 6/6 visual acuity, took part. The participants found the upper and lower end of the non-flicker range by rotating the knob, and clicked the push button at the middle to get the threshold. Ten such readings were taken for each individual threshold.

Experiment 1

To show that threshold increases with lens yellowing an experiment was conducted with and without a yellow filter (Lee filter, 013-straw tint) on two participants. The flicker frequency was set at 5 Hz and 25 pulse width modulation change per rotation of the control knob. The mean threshold doubled with the yellow filter, slightly less than expected (Figure 3:12).

The expected change in threshold with the filter was calculated by measuring the average transmittances of the yellow filter for 400 nm to 440 nm and for 530 nm to 570 nm by weighting spectral transmittances $t_\lambda$ with their scotopic sensitivity factors $k_\lambda$. The ratio of these average transmittances was given by

$$\frac{\sum_{400}^{570} t_\lambda k_\lambda \Delta \lambda}{\sum_{440}^{530} k_\lambda \Delta \lambda} = 2.25 \ldots \ldots (3.10)$$
Figure 3:12 Thresholds of two participants (P1, P2) with and without a yellow filter. Error bars are standard deviations. Dashed lines predict thresholds with the filter.

Experiment 2
To find the most suitable flicker frequency, I performed experiments on two participants with 2 Hz and 5 Hz frequencies having 50 pulse width modulations per rotation of the control knob. Initially both green and blue LEDs were set to 10 pulse width. The thresholds of participant 1 and participant 2 were more variable with 5 Hz frequencies having standard deviation 8.1 and 5.8, respectively as compared to 2 Hz frequencies with 2.9 and 4.9 standard deviations, respectively (Figure 3:13). However, the participants felt that they had difficulty in detecting the flicker with 2 Hz while they felt comfortable with 5 Hz. Hence, 5 Hz was selected as a flicker frequency for the study.
Figure 3:13 Thresholds of two participants (P1, P2) with 2 Hz and 5 Hz flicker frequencies.

Experiment 3
To find the most suitable pulse width modulation per rotation of the control knob an experiment was conducted in three steps. The initial luminance of the green and blue LEDs were set to 10 pulse width modulation.

In the first step, the frequency of the flicker was set to 5 Hz and with 25 pulse width modulation change per rotation of control knob. There were three participants (1, 2 and 3). Participants had very low mean threshold (Figure 3:14). Participants experienced difficulty in finding the non-flicker range because of the large number of rotations this involved.
In the second step, the flicker frequency was set to 5 Hz with 50 pulse width modulation change per rotation of the control knob. There were 4 participants (1, 2, 4 and 5). The mean thresholds and standard deviations increased for participants 1 and 2 compared with their results for 25 pulse width modulation change per rotation (Figure 3:14). The participants still felt difficulty in finding the non-flicker range.

In the third step, the flicker frequency was set to 5 Hz with 100 pulse width modulation change per rotation of the control knob. There were 4 participants (1, 2, 3 and 5) (Figure 3:14). The participants felt more comfortable in finding the non-flicker position with 100 pulse width modulation change per rotation of the control knob than with lower rates because they did not have to turn it as far (fewer rotations) to bring greater changes in luminance. **Hence, the 100 pulse width modulation per rotation was selected for the study.**
3.5.5 Phakometry

Purkinje images have been used to assess the properties of cornea and lens since their description by Purkinje (Rabbetts & Mallen, 2007b). When an eye is illuminated with a light source, four main Purkinje images are formed by reflection at anterior (PI) and posterior (PII) corneal surfaces and at anterior (PIII) and posterior (PIV) lens surfaces. PIII is approximately twice the size of PI in an unaccommodated eye, while PIV is inverted and slightly smaller than PI. PI, PII and PIV are formed near the pupil plane while PIII lies in the vitreous in the unaccommodated state. In phakometry, the sizes of PIII and PIV relative to that of PI are used to determine lens surface radii of curvature and equivalent index.

3.5.5.1 Phakometer

A phakometer was built on a 450 mm x 300 mm movable optical breadboard over a base with a forehead and chin rest for easy alignment (Figure 3:15). It contained a semicircular ring of thirteen 890 nm LEDs (Osram, SFH 487) angled 20° inwards. The ring arrangement was used rather than single sources to make images more easily distinguishable (PIII and PIV are inverted with each other) and locatable when they are partially obscured by the pupil.

An OLED (viewing area 12.78 mm x 9 mm, dimensions 19.8 mm x 15.2 mm x 5.1 mm, pixel pitch 15 µm) controlled by computer, presented a fixation target across ±2.68° horizontal and ±1.99° vertical range at nine positions. A beam splitter reflected the target to the participant. Images were captured by an IR-enhanced CCD camera (PixeLINK) provided with a 55 mm focal length telecentric lens (Edmund optics) focused at a distance of 260 mm. The Badal lens (100 mm focal length) presented the target displayed on the OLED. Its
anterior focal point coincided with the camera’s focus point. It corrected spherical refraction from $-8$ to $+3$ D and could be used to provide accommodative stimuli.

![Figure 3:15 Phakometer. Purkinje images are formed of the illumination ring source. The OLED displays the fixation targets through the beam splitter. During accommodation calibration the cross-hair fixation target, illuminated uniformly by a white LED through a diffuser, is collimated by a focusing lens (100 mm) for the left eye while the photorefractor LEDs illuminate the right eye.](image)

The photorefractor consisted of fourteen 890 nm LEDs mounted in a custom-built knife-edge pattern in front of the lower half of the camera. Its intensity was controlled manually by a custom built electronic box. The focusing lens and the fixation target were placed on a translation stage for adjustment of inter-pupillary distance.
The optical axis of the camera-lens was aligned to the central target (5th) on the OLED screen. Participants were aligned when they fixated the central fixation target of the OLED and the pupil of the eye was imaged in the centre of the camera as seen on the computer screen. PI was clearly visible at each focussing plane and PIII was more difficult to see clearly than PIV. The camera was first focused at PIII and images of the eye were taken after the best possible combination of PI, PIII and PIV was obtained.

All measurements were taken with the room light turned off. Custom built software in Matlab (MathWorks Inc., Natick, MA, version R2011) was written with three modes of fitting ellipses, a merit function to calculate lens radii of curvature, and photorefraction to measure refraction. The fitting ellipse mode fitted ellipses to Purkinje images PI, PIII, PIV, the pupil and the limbus. It included an option to take the log of the image to enhance PIII detection (see Appendix B). The fittings give the sizes and centres of the Purkinje images, pupil diameter, and limbus diameter (Figure 3:16). The Purkinje image positions can be referred to the pupil centre or to the cornea limbus centre. One millimetre on the image taken by the camera corresponds to 66.2 pixels on the CCD camera.
Photorefraction was used to measure accommodative responses. The participant was asked to view the cross-hair fixation target through his/her left eye while the ophthalmic trial lens (+6 to −6 in 2 D steps) was placed in front of the right eye in a trial frame along with an infrared pass filter (Kodak Wratten 89B, low cut-off 700 nm). This filter prevented the right eye from looking at the left eye cross-hair fixation target during refraction. The back vertex distance of the trial lens was also measured.

To measure photorefraction for different accommodative stimuli, the OLED was moved along the dioptre scale. The photorefraction images of the right eye were taken when it was looking at the central fixation target (5th) of the OLED and the left eye was patched. The infrared filter was placed between the beam splitter and the camera.

Pupil centration with respect to instrument was ensured through the pupil camera displayed on the computer monitor. Pupillary images with vertical
luminance gradient were taken and analysed using photorefraction mode of the software written in Matlab. The photorefraction slope generated by the trial lens was compensated for the back vertex distance, and the induced refraction generated was plotted against pupil luminance profile (as shown in figure below).

![Figure 3:17 PR calibration curve of a participant.](image)

3.5.5.3 Angle Kappa, Lens Tilt and Decentration

Purkinje image locations (PI, PIII and PIV), with respect to pupil centre, can be used to estimate angle kappa, lens tilt, and lens decentration through rotating the eye with respect to a source. Measurements were taken but not used in the study because of lack of time to process all the images, but the process is given for completeness of the Phakometry procedure. I assume a linear relationship between rotation angle and its factors (Tabernero, Benito, Nourrit, & Artal, 2006) to obtain:

\[
\beta_{align\ PIII} \quad and \quad PIV = \beta_{global\ rotation} + \beta_{lens\ tilt} + \beta_{lens\ decentration} \quad (3.11)
\]

\(\beta_{align\ PIII}\) and \(PIV\) is the eye rotation required to align PIII and PIV, \(\beta_{global\ rotation}\) is a global rotation of the eye due to angle kappa (\(\kappa\)) which is obtained by
aligning the PI image with the entrance pupil centre, $\beta_{\text{ens dec}}$ is the rotation required to align PIII and PIV that compensates for the lens decentration with respect to the pupil, and $\beta_{\text{ens tilt}}$ compensates for the tilt of the lens. Tabernero et al. showed that the equation was equivalent to

$$\beta_{\text{align PIII and PIV}} = K + A_1Tilt + A_2Dec \quad \text{......... (3.12)}$$

where $Tilt$ is the lens tilt, $dec$ is the lens decentration, and $A_1$ and $A_2$ are constants determined by ray tracing with model eyes to be $A_1 = -1.1$ and $A_2 = 2.0$ deg/mm.

**Procedure**

After longitudinal and transverse alignment was made to give the best possible combination of PI, PIII and PIV images along the line of sight for the central target (5th), the participant was asked to fixate at the nine targets in turn. Horizontally the targets were 5.80 mm apart and vertically they were 4.30 mm apart, making angles of approximately $\pm 2.68^\circ$ and $\pm 1.99^\circ$ at the eye’s centre-of-rotation, respectively.
Figure 3:18 Target position and angle relative to the centre-of-rotation of the eye. The target 5.80 mm from the central fixation point on the OLED screen made 2.68° and 3.32° angles at the pupillary plane, respectively. Similarly, the 4.30 mm target made 1.99° and 2.58° angles.

The position of each Purkinje image with respect to the pupil centre was plotted against horizontal and vertical components of eye rotation, and regression fits were made (Figure 3:19).

Figure 3:19 Data and linear fits of Purkinje images positions, with respect to pupil centre, versus vertical and horizontal components of eye rotations.

The linear regression fits for the horizontal eye rotation components are

\[ PI_h = a_{h1}x + b_{h1} \quad PIII_h = a_{h3}x + b_{h3} \quad Plv_h = a_{h4}x + b_{h4} \quad (3.13a-c) \]

and the regression fits for the vertical eye rotation components are
\[ P_{Iv} = a_{v1}x + b_{v1} \quad P_{IIIv} = a_{v3}x + b_{v3} \quad P_{IVv} = a_{v4}x + b_{v4} \quad \ldots \quad (3.14a-c) \]

Angle kappa components are where PI fits cross the x-axes:

\[ K_h = -b_{h1}/a_{h1} \quad \ldots \quad (3.15a) \]
\[ K_v = -b_{v1}/a_{v1} \quad \ldots \quad (3.15b) \]

PIII and PIV overlap at \((X_h, Y_h)\) for the horizontal eye rotation component and at \((X_v, Y_v)\) for the vertical eye rotation component. \(X_h\) and \(X_v\) are the estimates of the rotation angle components at which this occurs and \(Y_h\) and \(Y_v\) are the estimates of the components of lens decentration at which this occurs. The \((X_h, Y_h)\) co-ordinates of the PIII and PIV vertical components of the overlap point are

\[ X_h = -(b_{h3} - b_{h4})/(a_{ah3} - ah_{ah4}) \quad \ldots \quad (3.16a) \]
\[ Y_h = a_{ah3}X_h + b_{h3} \quad \ldots \quad (3.16b) \]

Similarly the \((X_v, Y_v)\) co-ordinates of the PIII and PIV vertical components of the overlap point are

\[ X_v = -(b_{v3} - b_{v4})/(a_{av3} - av_{av4}) \quad \ldots \quad (3.17a) \]
\[ Y_v = a_{av3}X_v + b_{v3} \quad \ldots \quad (3.17b) \]

Lens tilt components are obtained by rearranging the second of Tabernero et al.’s equations:

\[ Tilt_h = -(X_h - k_h - 2.0Y_h)/1.1 \quad \ldots \quad (3.18a) \]
\[ Tilt_v = -(X_v - k_v - 2.0Y_v)/1.1 \quad \ldots \quad (3.18a) \]

The setup was designed so that the camera was imaged at the pupil and hence was close to the image PI. In order to get good PIII and PIV images, particularly the former, sometimes it was necessary to move the camera slightly closer to the eye. The error \(\Delta R_x\) associated with the refraction stimulus \(R_x\) for such a positioning error \(x\) is shown in Appendix A to be

\[ \Delta R_x = xR_x^2/(1 - xR_x) \quad \ldots \quad (3.19) \]
Errors are shown in Figure 3:20 for positioning errors of 5 mm and 10 mm. In practice, I expect that the positioning error would not be greater than (−) 5 mm, which is likely to result in errors of refraction or accommodation no greater than 0.5 D.

![Figure 3:20 Refraction error, as a function of refraction given on Optometer scale on the phakometer, caused by the phakometer being too close to the eye by (−)5 mm and (−)10 mm.](image)

The centre-of-rotation is behind the entrance pupil by about 12 mm, which means that the angles of rotation are not those subtended at the entrance pupil by a stationary eye. As given in Appendix A, the angle of rotation $u'$ was related to the angle $\overline{u}_{Rx}$ subtended by the entrance pupil, through a separation $x$ by

$$\frac{u'}{u'_{Rx}} = 1 - xRx \quad \text{......... (3.20)}$$

This is shown in Figure 3:21 for $x = +12$ mm used throughout.
Figure 3.21 Ratio of eye rotation angle to the angle subtended by image at entrance pupil of eye, when the centre-of-rotation is 12 mm behind the entrance pupil.

This ratio is considerably different from 1.0 for large refractions, and thus the eye rotation angle was determined according to

\[
\vec{u} = \frac{\bar{h}F}{(1 - xR_x)} = \ldots \ldots \ldots (3.21)
\]

where \(x\) is 12 mm, \(\bar{h}\) is the height on the display stimulus and \(F\) is the lens power (+10 D) (see Appendix A). Of course, positioning errors will affect the eye rotation angle up to an estimated 4%.
3.5.5.4 Lens Radii of Curvature and Equivalent Refractive Index

A merit function was used to calculate lens radii of curvature and equivalent refractive index from Purkinje image heights together with the refraction determined from the optometer setting, corneal radii of curvatures obtained from Pentacam, and corneal thickness, anterior chamber depth, lens thickness, vitreous and axial length obtained from Lenstar.

Heights of PI, PIII and PIV were estimated from the image which was taken when the participant was looking at the central (5th) fixation target. These heights were the averages of horizontal and vertical components of the ellipse fitted to the Purkinje images. Three images were analysed for each participant and the heights were averaged.

Corneal radii of curvatures were obtained from Pentacam, and corneal thickness, anterior chamber depth, lens thickness and axial length were obtained from Lenstar. Three readings were taken for each parameter and The merit function was set to terminate when further improvement was not possible or after a specified number of cycles; 2000 cycles was set as the number of cycles and ensured that the function was not terminated without reaching its optimum value.

Two types of algorithms have been used to estimate the lens radii of curvature from the Purkinje image sizes: the equivalent mirror theorem and the merit function. The merit function is more accurate than the equivalent mirror theorem because the latter overestimates the posterior radius of curvature (Rosales, Dubbelman, Marcos, & Van der Heijde, 2006). For this study I used the merit function (Atchison, et al., 2008), but here I provide an explanation of both algorithms.
3.5.5.4.1 The Equivalent Mirror Theorem

The equivalent mirror theorem states that an optical system comprising one or more refracting surfaces followed by a plane or spherical mirror can be replaced by an “equivalent” spherical mirror. The vertex and centre of curvature of the equivalent mirror are the images of the vertex and centre of curvature of the mirror as formed by the refracting elements.

For the posterior cornea the refracting surface is the anterior cornea. As the posterior cornea image is usually not visible we usually ignore it and a three Purkinje image eye model is used. For the anterior lens surface, the refracting element is the cornea. For the posterior lens surface, the refracting elements are the cornea and the anterior lens surface.

**Lens Anterior Surface**

The radius of curvature \( r'_{2c} \) of the equivalent mirror for the anterior lens surface is given by

\[
r'_{2c} = r_i \left( \frac{h'_3}{h'_1} \right) \quad \text{......... (3.22)}
\]

where \( h'_1 \) and \( h'_3 \) are the heights of first and third Purkinje images, and \( r_i \) is the radius of curvature of the anterior cornea.
Figure 3.22 Equivalent mirror method for determining anterior radius of curvature of lens.

In Figure 3.22, $A_{2e}$ is the vertex and $C_{2e}$ is the centre of curvature of the anterior surface of equivalent mirror, $A_2$ is the vertex and $C_2$ is the centre of curvature of anterior lens surface, $d_{1a}$ is the apparent anterior chamber depth, $d_1$ is the real anterior chamber depth, $r_{2e}$ is the radius of curvature of the equivalent mirror, $r_2$ is the radius of curvature of the anterior lens surface, and $n_1$ and $n_2$ are the refractive indices of air and aqueous humour.

Now

$$A_1C_2 = d_1 + r_2 \text{ and } A_1C_{2e} = d_{1a} + r_{2e} \quad ... \quad (3.23, 3.24)$$

Since $C_2$ and $C_{2e}$ are conjugate by refraction at the cornea

$$L'-L = n_2/(d_1 + r_2) - 1/(d_{1a} + r_{2e}) \quad ... \quad (3.25)$$

The only unknown, $r_2$ can be determined.

**Lens Posterior Surface**

The radius of curvature of the equivalent mirror corresponding to the posterior surface is given by

$$r'_{3e} = r_{1}(h'_{4}/H_{1}) \quad ... \quad (3.26)$$
where \( h'1 \) and \( h'4 \) are the heights of first and fourth Purkinje images, and \( r_i \) is the radius of curvature of the cornea. The method described for the anterior lens surface can be modified for the posterior lens surface (Figure 3:23).

\[
L'_3 = \frac{n'_4}{d_3} \text{ and } F_3 = L'_3 - L_3 \quad (3.27), (3.28)
\]

and the posterior lens surface radius of curvature is given by

\[
r'_5 = \frac{F_3}{n'_4 - n_3} \quad (3.29)
\]

**3.5.5.2 Merit Function**

Several studies have used merit functions to calculate lens radii of curvature (Atchison, et al., 2008; Garner, 1997; Rosales, Wendt, Marcos, & Glasser, 2008). The merit function used here is a recursive technique which is a combination of three components. One component (MF1) is the square of the difference between actual and predicted vitreous length obtained from ray tracing into the eye to the retina. Two other components (MF2 & MF3) are the
squares of the difference between measured and predicted Purkinje image separations obtained from ray tracing into- and then out-of-the-eye after reflection from the lens front or back surfaces. MF can be expressed as

\[ MF = MF_1 + MF_2 + MF_3 = (V_{the} - V_{exp})^2 + (h_{3the} - h_{3exp})^2 + (h_{4the} - h_{4exp})^2 \ldots (3.30) \]

The input values \( V_{exp}, h_{3exp} \) and \( h_{4exp} \) are the experimental vitreous length and the experimental heights of PIII and PIV, respectively. \( V_{the}, h_{3the} \) and \( h_{4the} \) are the vitreous length and the theoretical heights of PIII and PIV, respectively, which are obtained recursively by ray tracing. Lens radii of curvature and equivalent refractive index are estimated by varying them to minimize the merit function. The anterior radius of curvature affects all components of the merit function, and the posterior radius of curvature and equivalent refractive index affect the first and third components. \( V_{the} \) is determined by ray tracing into the eye to the retina after refraction at the cornea and lens.

The paraxial ray tracing involved with determining \( MF_2 \) will be described. While four surface eye models are used in this study, to simplify the explanation a three surface eye model omitting the posterior cornea will be assumed. An image formed by refraction at a surface is given by:

\[ l' = \frac{lm'}{m + l(n' - n)} \ldots (3.31) \]

where \( n \) and \( n' \) are the refractive indices in the object and image spaces, respectively, and \( l \) and \( l' \) are the object space and image space distances, respectively. By putting \( n' = -n \), we can get the reflected image position in a surface:

\[ l' = \frac{lr}{2l - r} \ldots (3.32) \]

The transverse magnification is given by:
In Figure 3:24, $r_1$ is corneal radius of curvature, $r_2$ is anterior lens radii of curvature, $r_3$ is posterior lens radius of curvature, $d_1$ is anterior chamber depth, $d_2$ is lens thickness, and $n_1$, $n_2$, $n_3$ and $n_4$ are the refractive indices of air, aqueous humour, lens and vitreous, respectively.

**PI height:**

The position of PI relative to the cornea is given by (Figure 3:25):

$$l_{11} = \frac{l_{10}r_1}{2l_{10} - r_1} \ldots (3.34)$$

where $l_{10}$ and $l_{11}$ are the distances of the object and PI from the cornea, respectively, and $r_1$ is the corneal radius of curvature. Magnification is given by:

$$M_i = \frac{l_{11}}{l_{10}} \ldots (3.35)$$

The height of PI is given by:

$$h'_1 = M_i h_1 \ldots (3.36)$$
where $h_1$ is the object height and $h'_1$ is the first Purkinje image height.

![Diagram](image1.png)

**Figure 3:25 Parameters involved in determining height of PI.**

**PIII height:**

Ray tracing is performed. In figure 3:26, $h_3$ is the object height and $h'_3$ is PIII image height, $l_{30}$ is the object position from cornea, $l_{31}$ is the image distance from cornea, $l_{32}$ is the object position from lens, $l_{33}$ is the image position with respect to lens, $l_{34}$ is the object position from cornea and $l_{35}$ is the image position with respect to cornea.

![Diagram](image2.png)

**Figure 3:26 Parameters involved in determining height of PIII.**

Chapter 3: Methodology
Refraction at the lens:

\[ l_{31} = \frac{l_{30}r_1n_2}{r_1n_1 + l_{30}(n_2 - n_1)} \] \hspace{1cm} (3.37)

Referred to the anterior lens surface:

\[ l_{32} = l_{31} - d_1 \] \hspace{1cm} (3.38)

where \( d_1 \) is the anterior chamber depth and \( l_{32} \) is the object distance referred to the lens. Reflection at the anterior lens surface gives

\[ l_{33} = \frac{l_{32}r_2}{2l_{32} - r_2} \] \hspace{1cm} (3.39)

where \( r_2 \) is one of the variables evaluated by the merit function.

Referred to the cornea:

\[ l_{34} = l_{33} + d_1 \] \hspace{1cm} (3.40)

where \( l_{33} \) is the object position with respect to cornea.

Refraction at the cornea:

\[ l_{35} = \frac{l_{34}r_1n_1}{r_1n_2 + l_{34}(n_1 - n_2)} \] \hspace{1cm} (3.41)

where \( l_{35} \) is the image position after refracting from the cornea. PIII magnification is

\[ M_3 = \frac{l_{35}l_{33}}{l_{34}l_{32}l_{31}} \] \hspace{1cm} (3.42)

Heights of PIII and PI are

\[ h'_1 = h_1M_1 \quad h'_3 = h_1M_3 \] \hspace{1cm} (3.43)
\[ \frac{h'_3}{h'_1} = \frac{M_3}{M_1} \text{ ...... (3.44)} \]

**Determining \(MF_3\):**

To evaluate the \(MF_3\) component of the merit function in equation 3.30 involving the height of PIV, a similar procedure is followed as for the \(MF_2\) component involving the height of PIII, considering the refraction in the anterior lens and reflection in the posterior lens surface.

**Modelling for phakometry:**

For the Phakometry procedure, a four refracting surface model eye was used. The Badal setting for the phakometer provided the refraction. The Lenstar was used to determine intraocular ocular distances (see next paragraph for an explanation of how vitreous depth was obtained). The averages of anterior and posterior principal meridians radii of curvature of the cornea were obtained from the Pentacam. Refractive indices of the cornea, aqueous and vitreous at 555 nm were taken as those of the Gullstrand number 1 eye: 1.376, 1.336 and 1.336, respectively. Refractive indices for the source of wavelength 890 nm were determined from the dispersion equations provided by Atchison & Smith (2005) (their Table 5): 1.36822, 1.32829 and 1.32855, respectively. Atchison & Smith also provided a correction to the refraction (their equation (5a)):

\[ R_{x890} = R_{x555} + 0.839 \text{ ...... (3.45)} \]

After the lens radii of curvature and lens equivalent index were determined, an estimate of lens refractive index at 555 nm was made. From the equations for the different media given by Atchison & Smith, a linear relationship between the lens indices at the two wavelengths is
\[ n_{L555} = 1.0262n_{L890} - 0.0273 \]  

Lens equivalent power \( F_e \) at 555 nm was calculated from

\[ F_e = F_{L1} + F_{L2} - (t_l/n_L)F_{L1}F_{L2} \]  

where \( n_L \) is lens refractive index at 555 nm, \( t_l \) is lens thickness, and \( F_{L1} \) and \( F_{L2} \) are the front and front surface powers determined from

\[ F_{L1} = (n_L - n_a)/r_{L1}, \quad F_{L2} = (n_v - n_L)/r_{L2} \]

with \( n_a \) and \( n_v \) being refractive indices of aqueous and vitreous at 555 nm, and \( r_{L1} \) and \( r_{L2} \) being radii of curvature of the lens front and back surfaces.

A note is added here about determining vitreous length. The Lenstar determines the position of the retinal epithelium, and its default axial length is determined by subtracting 200 \( \mu m \) assumed to be the distance from the retinal pigment epithelium to the internal limiting membrane. For the purpose of phakometry only, I have reinstated the 200 \( \mu m \) because the position of the photoreceptors, rather than the internal limiting membrane, is relevant for refraction. The vitreous length, which is not given by the Lenstar, was calculated by subtracting the sum of the corneal thickness, anterior chamber depth and lens thickness from the axial length.

### 3.5.6 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) visualises detailed internal structure and soft tissues of the body with great contrast. It is a non-optical and non-invasive technique that can take images of the whole eye \textit{in vivo} with multiple slices of any desired plane or planes.

MRI is based on the principles of nuclear magnetic resonance (NMR). It uses a powerful magnetic field to align the nuclear magnetisation of hydrogen nuclei in the body and radio frequency (RF) fields to systematically alter the
alignment of the magnetization. This causes the nuclei to produce a rotating magnetic field detectable by the scanner. To gather enough information to construct an image, this signal is manipulated to encode spatial information by the application of magnetic field gradients (Novelline & Squire, 2004).

**Procedures**

MRI was used to measure the lens refractive index distribution and lens diameter using a 3 Tesla (Siemens Trio) whole body scanner in the Centre for Advanced Imaging at the University of Queensland.

Participants with and without diabetes were sub-divided into young (18 – 30 years) and older (47 – 60 years) age groups. As well as the selection criteria given in section 3.1 and clinical MRI scanning criteria, participants with diabetes in the young group had at least 2 D of amplitude accommodation (section 3.5.3.1), and participants in the older group had at least ten years of diabetes duration. Female participants were advised not to use eye makeup (including mascara) on the day of experiment to avoid artefacts that arise from the high magnetic susceptibility properties of mascara.

Special considerations were taken for the participants with diabetes. After they gave written consent, their insulin pumps were removed from their bodies. Blood glucose levels were measured, and participants with high blood levels were advised to inject insulin before scans and were rested for at least 15 minutes.

During the MRI procedure, participants were positioned supine on the table and heads were stabilised with appropriate padding. An adjustable mirror able to move vertically was mounted at approximately 45° angle to the
vertical in the magnetic bore. Participants were asked to focus (through the mirror) on a white Maltese cross fixation target on a black background presented on a translucent screen at the end of the magnet bore at approximately 0.93 m from the eye (Figure 3:27).

A standard 4.0 cm (Siemens) receiver coil was taped over the examined eye so that the target was visible through the coil hole. A thin spacer made from self-adhesive felt glued to the surface of the coil body was used to minimise skin contact with the coil, in order to protect against localised RF heating. The non-examined eye was occluded using a patch. Participants were instructed to focus and fixate on the target, and minimize blinking during data acquisition. They were advised to blink and/or close their eyes between data acquisitions to avoid eye dryness.

For the young participants, measurements were performed with and without accommodation stimulation, whereas for the older participants measurements were performed only without accommodation stimulation. All images were taken with best corrected refraction in place by attaching a suitable lens to the 20 mm thick surface coil on the opposite side from the participant’s eye. In the young diabetic group 4 D of accommodation was stimulated, while in the young non-diabetic group 5 D of accommodation was stimulated with negative lenses. The maximum amplitude of accommodation was measured in each participant before the MRI procedure (section 3.5.3.1). Participants were advised to focus on the target without excessive effort and to avoid any head movement.
**Imaging protocol**

MRI has relatively slow acquisition rates, limited signal-to-noise ratio (S/N), is prone to artefacts and has limited resolution. Several factors influence the signal-to-noise ratio (Table 3:2).

Pilot experiments were performed to test the effects of different parameters on the image quality, in order to minimise image artefacts and to optimise the Signal-to-Noise ratio (Table 3:2). High resolution images were obtained with the 4.0 cm surface coil and were compared with a 32-channel phased-array head coil. The surface coil showed superior results in respect of signal-to-noise ratio and image resolution, although the latter had the advantage of imaging both eyes together.
Table 3.2 Different parameters affecting the Signal to Noise Ratio in MR images

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SNR</th>
<th>Resolution</th>
<th>Scan time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing slice thickness</td>
<td>Increases</td>
<td>Decreases</td>
<td>Decreases</td>
</tr>
<tr>
<td>Increasing FOV</td>
<td>Increases</td>
<td>Decreases</td>
<td>Increases</td>
</tr>
<tr>
<td>Increasing matrix size</td>
<td>Increases</td>
<td>Increases</td>
<td>Increases</td>
</tr>
<tr>
<td>Increasing TR</td>
<td>Increases</td>
<td></td>
<td>Increases</td>
</tr>
<tr>
<td>Increasing TE</td>
<td>Increases</td>
<td></td>
<td>Increases</td>
</tr>
<tr>
<td>Increasing NEX</td>
<td>Increases</td>
<td></td>
<td>Increases</td>
</tr>
<tr>
<td>Increasing magnetic field strength</td>
<td>Increases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employing local coils</td>
<td>Increases</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The imaging protocol is given in Table 3.3. The first scan was a localiser scan to locate the position of the eye in the centre of the field of view (FOV). Multi-slice fast spin echo (FSE) images (64 mm FOV; 256 x 256 matrix; 2 mm slice thickness (no gaps); TR = 4000; TE=16; echo train length 12, imaging time 128 s) were obtained in both axial and sagittal planes. A T2-weighted half-Fourier acquisition single shot turbo spin echo sequence (HASTE) generated 3D isotropic images of the eye with 0.5 mm cubic voxels (128 x 128 x 64 matrix; TR = 2500; TE = 56; imaging time 4 min.). A single slice multi-echo spin (MSE) sequence (64 mm FOV; 256 x 256 matrix; 2 mm slice thickness; TR = 2000; 4 echos: TE = 12.5 / 25 / 37.5 / 50; imaging time 4.5 mins.) was used to acquire data for calculating the refractive index distribution through the lens. For this purpose, a single slice was placed through the symmetry axis of the lens, using the centre slice from the sagittal FSE image to identify this axis. For young participants, after placing negative lenses over the eye to stimulate accommodation, the protocol was repeated except for the FSE axial and HASTE 3D imaging. Images in the table having serial numbers “6” and “9” were used to calculate lens refractive index distribution.
The transverse or spin-spin relaxation time ($T_2$) is inversely proportional to the concentration of macro-molecules (notably crystallin proteins), which in the crystalline lens is related to refractive index (Jones & Pope, 2004). Consequently, a multi-spin echo (MSE) sequence can be used to obtain a map of the $T_2$-distribution through the lens, which is then converted to a refractive index map using a calibration equation (see below). Lens refractive index distribution can be determined (Jones, et al., 2005; Jones & Pope, 2004; Kasthurirangan, et al., 2008) using the decay of pixel signal intensity $S$ fitted to the single exponential decay equation:

$$ S = S_0 e^{-R_2 T_E} \quad \ldots \quad (3.49) $$

where $T_E$ is the delay between 180 pulses known as the echo time, $S_0$ is the pixel intensity extrapolated to $T_E = 0$ (the signal corresponding to the equilibrium or steady state magnetisation), and $R_2$ is the inverse of $T_2$ and is the relaxation rate for the lens location (voxel) corresponding to the pixel.
Table 3.3 Protocol for the MR imaging.

<table>
<thead>
<tr>
<th>Series No</th>
<th>Type</th>
<th>Orient’n</th>
<th>Slice Thick</th>
<th>No. Slices</th>
<th>Slice Space</th>
<th>FOV</th>
<th>Matrix</th>
<th>TR</th>
<th>TE</th>
<th>FAT</th>
<th>SAT</th>
<th>No. Avgs</th>
<th>Image Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-accommodated images</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Localisers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FSE (ETL=12)</td>
<td>Axial</td>
<td>2 mm</td>
<td>15</td>
<td>Nil</td>
<td>64 mm</td>
<td>256x256</td>
<td>4000</td>
<td>16</td>
<td>Y</td>
<td>1</td>
<td>2min 8s</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FSE (ETL=12)</td>
<td>Sagittal</td>
<td>2 mm</td>
<td>15</td>
<td>Nil</td>
<td>64 mm</td>
<td>256x256</td>
<td>4000</td>
<td>16</td>
<td>Y</td>
<td>1</td>
<td>2min 8s</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3D HASTE</td>
<td>Axial</td>
<td>0.5 mm</td>
<td>64</td>
<td>Nil</td>
<td>64 mm</td>
<td>128x128</td>
<td>2500</td>
<td>56</td>
<td>Y</td>
<td>2</td>
<td>4min 2s</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>MSE</td>
<td>Axial</td>
<td>2 mm</td>
<td>1</td>
<td>N/A</td>
<td>64 mm</td>
<td>256x256</td>
<td>2000</td>
<td>12.5/25/37.5/50 ms</td>
<td>Y</td>
<td>1</td>
<td>4min 32s</td>
<td></td>
</tr>
<tr>
<td>Accommodated images</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Localiser</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>FSE (ETL=12)</td>
<td>Sagittal</td>
<td>2 mm</td>
<td>15</td>
<td>Nil</td>
<td>64 mm</td>
<td>256x256</td>
<td>4000</td>
<td>16</td>
<td>Y</td>
<td>1</td>
<td>2min 8s</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>MSE</td>
<td>Axial</td>
<td>2 mm</td>
<td>1</td>
<td>N/A</td>
<td>64 mm</td>
<td>256x256</td>
<td>2000</td>
<td>12.5/25/37.5/50 ms</td>
<td>Y</td>
<td>1</td>
<td>4min 32s</td>
<td></td>
</tr>
</tbody>
</table>
The transverse relaxation rate $R_2$ was determined for each lens pixel using equation (3.49). The $R_2$ map was transformed to a refractive index map at 589 nm wavelength of light using Jones et al.’s (2005) calibration equation:

$$n = 1.3554 + 0.001549R_2 - 6.34 \times 10^{-6}R_2^2 \ldots (3.50)$$

where $n$ is refractive index.

A normalised refractive index distribution can be defined along the axis and equator of the crystalline lens according to (Jones, et al., 2005; Kasthurirangan, et al., 2008; Smith, Atchison, & Pierscionek, 1992):

$$n(r) = c_0 + c_pr^p \ldots (3.51)$$

where $r$ is the normalised distance from the lens centre ($r = 0$ at the centre and $r = 1$ at the periphery), $C_0$ is the index at the lens centre, $C_p$ is the difference in refractive index between the lens centre and periphery, and the exponent $p$ characterises the GRIN rate of change. Along the optical axis, the normalised optical path [OP] from the lens centre to the surface is

$$[OP] = \int_0^1 n(r)dr = \int_0^1 (c_0 + c_pr^p)dr = \left[ c_0r + c_pr^{p+1} \right]_0^1 = c_0 + c_p / (p + 1) \ldots (3.52)$$

which is the average index $n_{av}$ since the normalised true path is 1.0.

If uncertainties are known in the individual parameters, such as might be given by standard errors when fitting to equation (3.51), the uncertainty in $\Delta n_{av}$ is given by

$$\Delta n_{av} = \Delta C_0 + \frac{\Delta C_p}{p + 1} - \frac{\Delta p C_p}{(p + 1)^2} \ldots (3.53)$$

**Data Processing**

Imaging analysis was performed using custom built software written in Matlab to measure refractive index distribution. There were four steps
involved in analysis of the image to measure refractive index a) rotation of
the image to a common axis for image analysis purposes for all participants,
b) segmentation of the lens from the whole MSE image, c) extracting the
whole lens refractive index map, and d) selecting axial and equatorial lens
refractive index profiles.

For rotation, a straight line was drawn by the user from the left edge to the
right edge of the lens using the mouse (Figure 3:28b). The software calculates
the angle of line between the line drawn by the user relative to the horizontal
and the image is rotated according to the angle. After rotation a visual check
is performed to confirm that appropriate rotation has been obtained to align
the symmetry axis of the lens with the vertical; otherwise the user repeats the
process until a satisfactory result is obtained.

Next, the user draws (with a mouse) a rectangular box around the lens which
defines the region of interest. The analysis software identifies the pixel
intensity from each of the four MSE images and computes the refractive
index value for each pixel using the procedure outlined in the previous
section (Figure 3:29a). The software then automatically segments out the lens
from the rest of the image using a thresholding algorithm (Figure 3:29b).
Although the iris touched the anterior lens (Figure 3:28a), this did not affect
the process because the signal from the iris decayed much more slowly in the
later echo images. Pixels corresponding to the aqueous and vitreous
humours were artificially assigned a refractive index of 1.336
(Kasthurirangan, et al., 2008).

Due to motion and blinking artefacts in some participants, MSE images and
hence refractive index maps suffered from noise. Therefore, and in order to
make comparisons between different subject groups, lens refractive index

Chapter 3: Methodology
profiles were computed using the line of pixels closest to the lens axis or equatorial diameter, and also from a 3-pixel-wide band centred on these axes (Figure 3:29c). For this purpose, the segmented lens was used, and the rows and columns of data in the refractive index maps that corresponded most closely to the equator and axis of the lens respectively were identified. For example, in Figure 3:29c equatorial profiles were computed from the centre row and by averaging the three pixel wide band of refractive indices perpendicular to the equatorial direction. As MSE images had in-plane resolution of 0.25 mm and slice thickness of 2 mm, this gave an effective voxel size of 0.375 mm$^3$ (3 * 0.25 * 0.25 * 2). The central refractive index was calculated as the mean refractive index over nine pixels at the lens centre.
Figure 3:28 Right eye MSE image of a 28 years old non diabetes participant at different stages of eye rotation procedure for analysis. a) First stage, before applying the rotation algorithm, b) second stage, when a line was drawn through the equatorial diameter line of the lens c) and finally when the desired eye rotation was achieved.
Figure 3:29 Schematic representation of the refractive index extraction procedure from the MSE images and schematic diagram of lens dimensions measurement. (a) Customised software identified pixels within the image and generated a refractive index distribution map of the lens (b) A segmentation algorithm segmented the lens from the rest of the refractive index map. The vertical and horizontal axes of the figure represent pixels. (c) Profiles of refractive index over a central single pixel row and averaged over 3 rows of pixels, plotted against pixel number: Left) in the equatorial direction closest to the equator, with pixels from left to right indicating nasal to temporal refractive index data; Right) in the axial direction closest to the lens axis, with pixels from left to right indicating anterior to posterior refractive index data. Each pixel represents 0.25 mm.

The first MSE image (TE = 12.5 ms) with the best S/N and contrast was selected to determine lens diameter and axial thickness manually using ImageJ software (developed by Wayne Rasband, National Institutes of Health, available in public domain at http://rsbweb.nih.gov/ij/index.html). The equatorial diameter was measured along the equatorial diameter line between nasal and temporal edges of the lens and the axial thickness was measured along the optical axis between the anterior and posterior edges of the lens. An anterior axial thickness was measured from the anterior edge of the lens to the centre of the equatorial diameter line. Similarly, a posterior...
axial thickness was measured from the posterior edge to the centre of the equatorial diameter line.

To analyse refractive index data, lens dimensions were normalised for each person. For the equatorial diameter line, the normalised dimension extended from $-1$ to $+1$. The data were folded about the optical axis to give a normalised dimension 0 to $+1$, and group data were fitted according to equation (3.51).

The optical axis dimension was normalised in two different approaches. In the first approach, normalisation extended from $-1$ to $+1$ relative to the midpoint between the anterior and posterior vertices of the lens for each person. The data were folded about the midpoint (red dot in Figure 3:30) to give a normalised dimension 0 to $+1$, and group data were fitted according to equation (3.51). In the second approach, separate analyses were done for the portions anterior and posterior to the equatorial diameter line (blue dot Figure 3:30) with normalised dimension for each portion of 0 to $+1$.

The normalisation and fitting were similar to those used by Kasthurirangan et al. (2008), except that they normalised each person’s dimensions to the average equatorial diameter or axial thickness of the relevant group and they did not use the second normalisation approach for the axial data.

![Figure 3:30 Lens dimensions for refractive index profiles](image)
Chapter 4: **Results**

This chapter presents all results except for the pilot data of Chapter 3. It has eight main sections. Section 4.1 covers general characteristics of participants in the study and reasons why some participants did not do particular tests. Section 4.2 contains ocular biometry results of spherical equivalent refraction, anterior corneal radius of curvature, anterior corneal asphericity, corneal central thickness, posterior corneal radius of curvature, anterior chamber depth, pupil diameter, pupil decentration, anterior lens radius of curvature, posterior lens radius of curvature, lens central thickness, lens equivalent refractive index and lens equivalent power. Sections 4.3, 4.4, 4.5, 4.6 and 4.7 cover straylight, amplitude of accommodation, ocular aberration and its components, lens yellowing, and lens dimensions and refractive index distribution, respectively. A summary section 4.8 lists the significant multiple regression equations found in previous sections.

For each parameter in sections 4.2 to 4.6, simple regression correlations are given with the systemic and ocular factors of age, diabetes duration, HBA1c level, spherical equivalent refraction, axial length and gender. This is followed by multiple regressions, both to the whole group of participants (with duration for the participants without diabetes given as zero) and to only the diabetes group, to investigate the importance of age and duration of diabetes to the parameter. Where factors of gender or axial length were significant according to the simple regressions (spherical equivalent refraction), these were included in the multiple regression i.e. the analyses were “adjusted” for the influence of these factors.
T-test results comparing the groups are for the mean of the non-diabetes group subtracted from the diabetes group, together with 95% confidence intervals.

Figures show dependence of parameters on age for both diabetes and non-diabetes groups, and ANCOVA analysis is presented determining the significance of the group differences in rate of change with age. For lens dimensions and refractive index (section 4.7), ANOVAs and unpaired t-tests are shown that consider the influence of age group and diabetes status. For each parameter in sections 4.2 - 4.7, there is a discussion of results with reference to previous studies.

4.1 Characteristics of Participants

There were 138 participants, consisting of 74 people with diabetes and 64 people without diabetes. As mentioned at the end of section 3.1, twenty people had already been excluded in the analyses because HbA1c assays were not able to be performed. Different numbers of participants were available for different tests. Reasons included limited time available with the participants, the C-Quant instrument for straylight analysis not being available from the start of experimentation, eligibility of participants for particular tests e.g. amplitude of accommodation was measured only in participants less than 47 years of age, and limited funds available for magnetic resonance imaging (Table 4:1).

Participant groups were well balanced for age. The mean age and standard deviation of the group with diabetes were 42 ± 13 years (range 19 – 63 years),
with a duration of diabetes of 20 ± 11 years (3 – 52 years). The mean age and standard deviation of the group without diabetes were 41 ± 13 years (20 – 62 years).

There was a significant gender difference between the two groups, with fewer females than males in the diabetes group (30/44) and fewer females than males in the non-diabetes group (44/20). Many more females than males without diabetes volunteered in response to advertisements, while more males than females already in the LANDMark study agreed to take part. Normality testing found that only straylight and corneal and total ocular spherical aberration coefficients were not normally distributed. However, the residuals of the straylight were normal distributed. Transformations were tried unsuccessfully to normalise the data, and it was decided to ignore this deficiency and treat these parameters as if the data were normally distributed.

There was no significant difference in visual acuity between the groups with and without diabetes, but log contrast sensitivity was significantly higher in the non-diabetes group (1.89 ± 0.10) than in the diabetes group (1.81 ± 0.14).
### Table 4.1 Participants recruited for different tests and numbers after exclusion

<table>
<thead>
<tr>
<th>Test Description</th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>Reasons for not participating, except for lack of time availability, and exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total participants</td>
<td>74</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Anterior corneal radius of curvature and asphericity (Medmont)</td>
<td>50/47</td>
<td>49/47</td>
<td>Five participants’ images were too poor for analysis</td>
</tr>
<tr>
<td>Phakometry</td>
<td>72/67</td>
<td>62</td>
<td>In 5 participants, PIII quality was poor</td>
</tr>
<tr>
<td>Pupil decentration</td>
<td>50/47</td>
<td>49/47</td>
<td>As for corneal r-o-c, five participants’ images were too poor for analysis</td>
</tr>
<tr>
<td>Straylight (C-Quant)</td>
<td>63</td>
<td>57</td>
<td>Instrument not available at start of study</td>
</tr>
<tr>
<td>Amplitude of accommodation</td>
<td>42</td>
<td>32</td>
<td>Limited to people &lt; 47 years</td>
</tr>
<tr>
<td>Aberrations</td>
<td>50/46</td>
<td>49/47</td>
<td>As for corneal r-o-c, five participants’ corneal images were too poor for analysis and one participant with pupil diameter less than 4.5 mm was excluded from the diabetes group</td>
</tr>
<tr>
<td>Flicker photometry – lens yellowing</td>
<td>38/30</td>
<td>45/41</td>
<td>Eight participants in diabetes group and four participants in non-diabetes group were not able to perform the test</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>Expense. One participant blinked excessively and image quality was poor, and another was not completed because of claustrophobia.</td>
</tr>
<tr>
<td><strong>MRI young group</strong> (18 – 30 years)</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td><strong>MRI older group</strong> (47 – 60 years)</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 4.1](image1.png)

Figure 4.1 Relationships between age and diabetes duration. Regression fit is $Y = +0.385(±0.097)\text{Age} + 3.74(±4.24)$, $R^2 = 0.18$, $p < 0.001$. Values in brackets are standard errors.
Figure 4:1 compares diabetes duration with age. While there was a significant linear relationship, the correlation was low. Some of the older participants had diabetes for only a short time e.g. see the cluster of 3 people with ages 58 - 62 years and durations less than 5 years.
4.2 Ocular Biometry

Table 4.2 shows the characteristics of the subject who took part in the ocular biometry tests.

**Table 4.2 Characteristics of participants**

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>74</td>
<td>64</td>
<td>0.48</td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>40 ± 12, 19 – 63</td>
<td>43 ± 12, 20 – 62</td>
<td>0.77</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>48/26</td>
<td>54/10</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>30/44</td>
<td>44/20</td>
<td>0.001*</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>-0.02 ± 0.21</td>
<td>-0.04 ± 0.20</td>
<td>0.55</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.81 ± 0.14</td>
<td>1.89 ± 0.10</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (D), aberrometer</td>
<td>-0.58 ± 1.13</td>
<td>-0.44 ± 0.94</td>
<td>0.44</td>
</tr>
<tr>
<td>Anterior corneal radius of curvature (mm), Pentacam</td>
<td>+7.76 ± 0.22</td>
<td>+7.82 ± 0.27</td>
<td>0.15</td>
</tr>
<tr>
<td>Anterior corneal radius of curvature (mm), Medmont</td>
<td>+7.73 ± 0.20</td>
<td>+7.78 ± 0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>Anterior corneal asphericity Q</td>
<td>-0.06 ± 0.13</td>
<td>-0.07 ± 0.12</td>
<td>0.86</td>
</tr>
<tr>
<td>Corneal centre thickness (mm), Pentacam</td>
<td>0.545 ± 0.032</td>
<td>0.537 ± 0.043</td>
<td>0.26</td>
</tr>
<tr>
<td>Corneal centre thickness (mm), Lenstar</td>
<td>0.542 ± 0.031</td>
<td>0.537 ± 0.037</td>
<td>0.37</td>
</tr>
<tr>
<td>Posterior corneal radius of curvature (mm)</td>
<td>+6.36 ± 0.24</td>
<td>+6.43 ± 0.26</td>
<td>0.07</td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Pentacam</td>
<td>2.75 ± 0.40</td>
<td>2.86 ± 0.36</td>
<td>0.09</td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Lenstar</td>
<td>2.74 ± 0.40</td>
<td>2.89 ± 0.34</td>
<td>0.03*</td>
</tr>
<tr>
<td>Pupil diameter (mm)</td>
<td>5.96 ± 0.90</td>
<td>6.22 ± 0.84</td>
<td>0.08</td>
</tr>
<tr>
<td>Pupil decentration along x-axis (mm)</td>
<td>+0.17 ± 0.50</td>
<td>+0.30 ± 0.42</td>
<td>0.19</td>
</tr>
<tr>
<td>Pupil decentration along y-axis (mm)</td>
<td>+0.11 ± 0.33</td>
<td>+0.16 ± 0.32</td>
<td>0.43</td>
</tr>
<tr>
<td>Anterior lens radius of curvature (mm)</td>
<td>+9.53 ± 1.08</td>
<td>+10.62 ± 1.14</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Posterior lens radius of curvature (mm)</td>
<td>-5.89 ± 0.72</td>
<td>-6.32 ± 0.74</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Lens equivalent refractive index, 555nm</td>
<td>1.426 ± 0.011</td>
<td>1.431 ± 0.012</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Lens central thickness, Lenstar</td>
<td>4.31 ± 0.49</td>
<td>4.01 ± 0.36</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Lens equivalent power (D), 555 nm</td>
<td>25.06 ± 3.27</td>
<td>24.28 ± 2.33</td>
<td>0.13</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.80 ± 1.11</td>
<td>5.03 ± 0.31</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Blood glucose level (m mol)</td>
<td>9.30 ± 3.55</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>19 ± 9</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* indicates significant difference between groups. Data are expressed as the mean ± standard deviation
4.2.1 Spherical Equivalent Refraction

Spherical equivalent refraction was calculated in all participants using the aberrometer (section 3.5.3.1). Table 4.3 shows the Pearson correlations of spherical equivalent refraction with various ocular and systemic factors for the whole group. Spherical equivalent refraction was correlated significantly only with age, lens thickness and axial length.

Table 4.3 Pearson correlations of spherical equivalent refraction with different ocular and systemic factors

<table>
<thead>
<tr>
<th>Correlation co-efficient p</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19</td>
<td>0.03*</td>
<td>0.76</td>
<td>0.50</td>
<td>0.03*</td>
<td>&lt; 0.001*</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjustment for axial length AL. Age and axial length contributed significantly to the fit:

\[ y = +0.020(\pm0.007)\text{Age} - 0.538(\pm0.090)\text{AL} + 11.45(\pm2.14), \ R^2 = 0.24 \]

When the multiple regression analysis was restricted to the diabetes group, again age and axial length contributed significantly to the fit:

\[ y = +0.025(\pm0.010)\text{Age} - 0.660(\pm0.134)\text{AL} + 14.11(\pm3.14), \ R^2 = 0.29 \]
Figure 4.2 Relationships between age and spherical equivalent refraction for people with and without diabetes. Fit for diabetes group: \( Y = +0.016(\pm0.010)\text{Age} – 1.23(\pm0.45), R^2 0.03, p 0.14 \); fit for non-diabetes group: \( Y = +0.016(\pm0.009)\text{Age} – 1.10(\pm0.40), R^2 0.05, p 0.10 \). Values in brackets are standard errors.

Figure 4.2 shows the mean spherical equivalent refraction as a function of age for the two groups. ANCOVA did not find a significant difference in regression slopes between the groups (\( F_{1, 133} = 0.00, p 0.99 \)). The difference in refraction between the groups was \(-0.16 \pm 0.49 \) D (mean ± 95% CI, p 0.44).

**Discussion**

The multiple regression analyses show significant association of age and axial length, but not of diabetes, with spherical equivalent refraction. It is well known that axial length is the major determinant of spherical refraction e.g. Atchison et al. (2008) and that refraction in adults moves in the hyperopic direction as age increases e.g. Atchison and Smith (2000a). The
simple linear fits in Figure 4:2 do not confirm the age trend with spherical refraction, but taking the analyses together indicates that the participant group is reasonably representative of the general population. I note that Wiemer et al.’s (2008d) study including 114 DM1 participants and 74 non-diabetes participants did not find a significant difference between groups.

To conclude, diabetes does not appear to have influenced spherical equivalent refraction.
4.2.2 Anterior Corneal Radius of Curvature

Table 4.4 shows the Pearson correlations of anterior corneal radius of curvature with various ocular and systemic factors for the whole group for both Pentacam and Medmont instruments. In the case of the Pentacam, anterior radius of curvature was correlated significantly with axial length and gender, while in the case of the Medmont measurement anterior radius of curvature was only correlated significantly with axial length.

Table 4.4 Pearson correlations of anterior corneal radius of curvature (Pentacam, Medmont) with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient (Pentacam) p</td>
<td>-0.07</td>
<td>-0.13</td>
<td>-0.11</td>
<td>-0.10</td>
<td>-0.07</td>
<td>0.65</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.14</td>
<td>0.23</td>
<td>0.26</td>
<td>0.40</td>
<td>&lt; 0.001*</td>
<td>0.02*</td>
</tr>
<tr>
<td>Correlation co-efficient (Medmont) p</td>
<td>0.02</td>
<td>-0.10</td>
<td>-0.14</td>
<td>-0.04</td>
<td>0.04</td>
<td>0.46</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.89</td>
<td>0.32</td>
<td>0.20</td>
<td>0.69</td>
<td>0.71</td>
<td>&lt; 0.001*</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* indicates significance

For both Pentacam and Medmont, multiple regression analysis was performed with age and diabetes duration as predictors after adjusting for gender and axial length using the whole subject group. For the whole group or for the diabetes group, neither factor contributed significantly to the fit.

Chapter 4: Results
Figure 4:3 Relationships between age and anterior corneal radius of curvature (using Pentacam) for people with and without diabetes. Fit for diabetes group: $Y = -0.001(\pm0.002)\text{Age} + 7.78(\pm0.09)$, $R^2 = 0.00$, $p = 0.77$; fit for non-diabetes group: $Y = -0.002(\pm0.003)\text{Age} + 7.90(\pm0.12)$, $R^2 = 0.01$, $p = 0.45$. Values in brackets are standard errors.

Figure 4:4 Relationships between age and anterior corneal radius of curvature (using Medmont) for people with and without diabetes. Fit for diabetes group: $Y = -0.001(\pm0.002)\text{Age} + 7.70(\pm0.11)$, $R^2 = 0.00$, $p = 0.75$; fit for non-diabetes group: $Y = -0.001(\pm0.003)\text{Age} + 7.81(\pm0.13)$, $R^2 = 0.00$, $p = 0.81$. Values in brackets are standard errors.
Figure 4:3 and Figure 4:4 show corneal anterior radius of curvature as a function of age for the two groups. There was no significant age trend in either group. The mean differences between the groups measured with Pentacam and Medmont of $-0.06 \pm 0.09$ mm (mean ± 95% CI, p 0.15) and $-0.05 \pm 0.09$ mm (mean ± 95% CI, p 0.24), respectively, were not statistically significant. ANCOVA did not find significant difference in regression slopes between the groups for either Pentacam or Medmont ($F_{1,133} = 0.20$, p 0.66 and $F_{1,90} = 0.01$, p 0.94, respectively).

**Discussion**

The analyses show significant associations of axial length and gender (at least for the Pentacam) with anterior corneal radii of curvature as has been found previously (Atchison, 2006; Atchison, et al., 2008), but not of diabetes, with spherical equivalent refraction. The Pentacam and Medmont instrument gave similar results. I note that Wiemer et al. (2007) also did not find any significant difference in the anterior corneal radius of curvature between DM1 and non-diabetes groups.

To conclude, neither diabetes nor age appears to have influenced anterior corneal radius of curvature.
4.2.3 Anterior Corneal Asphericity

Table 4:5 shows the Pearson correlations of anterior corneal asphericity with various ocular and systemic factors for the whole group. Anterior corneal asphericity was correlated significantly only with age and axial length.

Table 4:5 Pearson correlations of anterior corneal asphericity with different ocular and systemic factors

<table>
<thead>
<tr>
<th>Correlation co-efficient p</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04*</td>
<td>0.21</td>
<td>0.07</td>
<td>-0.01</td>
<td>0.12</td>
<td>-0.10</td>
<td>0.34</td>
<td>0.03</td>
</tr>
<tr>
<td>0.49</td>
<td>0.95</td>
<td>0.26</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for axial length. Neither age nor diabetes contributed significantly to the fit. When the multiple regression analysis was restricted to the diabetes group, age alone contributed significantly to the fit:

\[ y = +0.004(\pm0.002) \text{Age} - 0.68(\pm0.55), \ R^2 = 0.15 \]
Figure 4:5 Relationships between age and anterior corneal asphericity for people with and without diabetes. Fit for diabetes group: \( Y = +0.004(\pm0.001)\text{Age} – 0.22(\pm 0.06), R^2 0.14, p 0.010; \) fit for non-diabetes group: \( Y = 0.000(\pm 0.001)\text{Age} – 0.08(\pm 0.07), R^2 0.00, p 0.85. \) Values in brackets are standard errors.

Figure 4:5 shows the anterior corneal asphericity as a function of age for the two groups. The mean difference of 0.01 ± 0.01 mm (mean ± 95% CI) between the groups was not statistically significant. ANCOVA did not find a significant difference in regression slopes between the groups \( (F_{1, 90} = 3.20, p 0.08). \)

**Discussion**

The simple linear regression analyses showed significant associations of age and axial length with anterior corneal asphericity; the former does not match a previous study for 101 emmetropes without diabetes using the Medmont instrument (Atchison, et al., 2008) which found no effect of age. In the subsequent multiple regression analysis, only age was a significant predictor and then only for the diabetes group. The mean differences between the
groups were not significantly different, as found previously by Wiemer et al. (2007).

To conclude, diabetes does not appear to have influenced anterior corneal asphericity.
4.2.4 Corneal Central Thickness

Table 4.6 shows the Pearson correlations of corneal central thickness with various ocular and systemic factors for the whole group for both Pentacam and Lenstar instruments. Corneal central thickness was not correlated significantly with any factor in either case.

Table 4.6 Pearson correlations of corneal central thickness (Pentacam, Lenstar) with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient (Pentacam)</td>
<td>−0.10</td>
<td>0.16</td>
<td>0.03</td>
<td>0.10</td>
<td>0.01</td>
<td>−0.04</td>
<td>−0.01</td>
</tr>
<tr>
<td>P</td>
<td>0.29</td>
<td>0.07</td>
<td>0.76</td>
<td>0.27</td>
<td>0.90</td>
<td>0.62</td>
<td>0.87</td>
</tr>
<tr>
<td>Correlation co-efficient (Lenstar)</td>
<td>−0.05</td>
<td>0.12</td>
<td>0.01</td>
<td>0.07</td>
<td>0.00</td>
<td>0.02</td>
<td>−0.03</td>
</tr>
<tr>
<td>P</td>
<td>0.58</td>
<td>0.16</td>
<td>0.91</td>
<td>0.41</td>
<td>1.00</td>
<td>0.84</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* indicates significance

For the Pentacam, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Only diabetes duration contributed significantly to the fit:

\[ y = 0.0005(\pm 0.0003)\text{DiaDur} + 0.552(\pm 0.011), \text{ } R^2 = 0.04 \]

When the multiple regression analysis was restricted to the diabetes group, neither factor contributed significantly to the fit.
For the Lenstar, multiple regression analysis was performed with age and diabetes duration as predictors using the whole subject group and then only the diabetes group. Neither predictor had significant influence in either case.

Figure 4:6 Relationships between age and corneal central thickness (Pentacam) for people with and without diabetes. Fit for diabetes group: $Y = 0.0000(\pm0.0003)\text{Age} + 0.545(\pm0.013)$, $R^2$ 0.00, $p$ 0.97; fit for non-diabetes group: $Y = -0.0006(\pm0.0004)\text{Age} + 0.560(\pm0.018)$, $R^2$ 0.03, $p$ 0.19. Values in brackets are standard errors.
Chapter 4: Results

Figure 4:7 Relationships between age and corneal central thickness (Lenstar) for people with and without diabetes. Fit for diabetes group: $Y = 0.0003(\pm 0.0003) \times \text{Age} + 0.530(\pm 0.013)$, $R^2 0.01$, $p 0.35$; fit for non-diabetes group: $Y = -0.0005(\pm 0.0004) \times \text{Age} + 0.561(\pm 0.016)$, $R^2 0.04$, $p 0.11$. Values in brackets are standard errors.

Figure 4:6 and Figure 4:7 show corneal central thickness as a function of age for the two instruments. There were no significant age trends in either age group. The differences between groups with Pentacam and Lenstar were not significant (mean ± 95% CI 0.008 ± 0.012 mm Pentacam and 0.005 ± 0.013 mm Lenstar). ANCOVA did not find significant difference in regression slopes between the groups for either Pentacam or Lenstar ($F_{1,132} = 1.20$, $p 0.28$ and $F_{1,132} = 3.54$, $p 0.07$), respectively.

Discussion

The multiple regression analysis for the whole group with the Pentacam found that central corneal thickness increases with diabetes duration at a rate of 0.5 $\mu$m/year. However, none of the other analyses showed an association of diabetes with thickness. Age did not predict thickness, in agreement with
other studies e.g. Atchison et al. (2008). The Pentacam and Lenstar instruments gave similar estimates of thickness.

As mentioned in section 2.3.1, several previous studies have reported greater corneal thickness in people with than in people without diabetes. As an example, Lee et al. (2005) found a mean difference of 20 μm between DM1 and non-diabetes groups as compared with the non-significant differences in this study of 5 - 8 μm, and Lee et al. also found an increase in thickness with duration. However as for this study, Wiemer et al. (2007) did not find difference in corneal central thickness between the two groups.

In conclusion, age did not influence central corneal thickness and the balance of analysis did not find influence of diabetes on corneal thickness, although the latter is not supported by most of the literature.
4.2.5 Posterior Corneal Radius of Curvature

Table 4.7 shows the Pearson correlations of posterior corneal radius of curvature with various ocular and systemic factors for the whole group. Posterior corneal radius of curvature was correlated significantly with diabetes duration, axial length and gender.

Table 4.7 Pearson correlations of posterior corneal radius of curvature with different ocular and systemic factors

<table>
<thead>
<tr>
<th>Correlation co-efficient (Pentacam)</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.22</td>
<td>0.02*</td>
<td>0.22</td>
<td>0.48</td>
<td>0.65</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors after adjusting for gender and axial length using the whole subject group and then only the diabetes group. In neither case did age or diabetes duration contribute significantly to the fit.
Figure 4:8 Relationships between age and posterior corneal radius of curvature for people with and without diabetes. Fit for diabetes group: $Y = +0.003(\pm 0.002) \text{Age} + 6.22(\pm 0.10)$, $R^2 = 0.03$, $p = 0.14$; fit for non-diabetes group: $Y = +0.001(\pm 0.003) \text{Age} + 6.40(\pm 0.11)$, $R^2 = 0.00$, $p = 0.74$. Values in brackets are standard errors.

Figure 4:8 shows posterior corneal radius of curvature as a function of age for the two groups. The difference of $-0.08 \pm 0.08$ (mean ± 95% CI, $p = 0.07$) mm between the groups was not statistically significant. There was no significant age trend in either group. ANCOVA did not find significant difference in regression slopes between the groups ($F_{1,133} = 0.52$, $p = 0.47$).

**Discussion**

As for the anterior cornea, the simple linear regressions found that the posterior cornea’s radius of curvature was associated significantly with axial length and gender, but it was also associated significantly with diabetes duration (in the negative direction). This was not supported by multiple
regressions, but the mean difference of −0.08 mm between groups was close to being significant (p 0.07). Wiemer et al. (2007) found significantly smaller radius of curvature in their DM1 group than in their control group (mean difference ± 95% CI −0.14 ± 0.08 mm, p < 0.05). A power analysis on our results (G*power 3.1.7, α 0.05, power 0.8) indicates that 159 participants per group would be needed to show significance.

There are several differences between this study and that of Wiemer et al. (2007, 2008d) which may account for differences in results for posterior radius of curvature and for other parameters in section 4.2. The techniques were different, with Wiemer et al. using Scheimpflug imaging for all of their parameters. Importantly, Wiemer at al. included participants with advanced stage retinopathy and had a large range of refractions for both diabetes and non-diabetes groups (−10 to +6 D), whereas this study included participants with no more than minimal diabetic retinopathy and had a restricted range of refraction for both diabetes and non-diabetes groups (−3 to +2 D).

In conclusion, age does not appear to have influenced posterior corneal radius of curvature, but there is some indication that diabetes affected it.
4.2.6 Anterior Chamber Depth

Pearson correlation was performed on combined data of people with and without diabetes for the whole group for both Pentacam and Lenstar instruments (Table 4:8). In case of Pentacam, anterior chamber depth was correlated significantly with all factors except for HbA1c, while in case of the Lenstar measurements anterior chamber depth was correlated significantly with all factors except HbA1c and gender.

Table 4:8 Pearson correlations of anterior chamber depth with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient, Pentacam</td>
<td>$-0.36^*$</td>
<td>$-0.42^*$</td>
<td>$-0.13$</td>
<td>$-0.63^*$</td>
<td>$-0.42$</td>
<td>$0.42$</td>
<td>$0.20$</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt; 0.001^*$</td>
<td>$&lt; 0.001^*$</td>
<td>$0.19$</td>
<td>$&lt; 0.001^*$</td>
<td>$&lt; 0.001^*$</td>
<td>$&lt; 0.001^*$</td>
<td>$0.02^*$</td>
</tr>
<tr>
<td>Correlation coefficient, Lenstar</td>
<td>$-0.40^*$</td>
<td>$-0.44^*$</td>
<td>$-0.16$</td>
<td>$-0.71^*$</td>
<td>$-0.22$</td>
<td>$0.46$</td>
<td>$0.15$</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt; 0.001^*$</td>
<td>$&lt; 0.001^*$</td>
<td>$0.11$</td>
<td>$&lt; 0.001^*$</td>
<td>$0.01^*$</td>
<td>$&lt; 0.001^*$</td>
<td>$0.09$</td>
</tr>
</tbody>
</table>

* indicates significance

For Pentacam, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for gender and axial length. Diabetes duration, age and axial length contributed significantly to the fit:

$$y = -0.009(\pm 0.002)\text{DiaDur} - 0.010(\pm 0.002)\text{Age} + 0.147(\pm 0.056)\text{AL} - 0.21(\pm 0.72), R^2 = 0.43$$

When the multiple regression analysis was restricted to the diabetes group, again diabetes duration, age and axial length contributed significantly to the fit:

$$y = -0.016(\pm 0.003)\text{DiaDur} - 0.007(\pm 0.003)\text{Age} + 0.146(\pm 0.041)\text{AL} - 0.12(\pm 0.94), R^2 = 0.54$$
For Lenstar, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for axial length. Diabetes duration, age and axial length contributed significantly to the fit:

\[ y = -0.008(\pm 0.002) \text{DiaDur} - 0.011(\pm 0.002) \text{Age} + 0.179(\pm 0.027) \text{AL} - 0.87(\pm 0.64), \ R^2 = 0.46 \]

When the multiple regression analysis was restricted to the diabetes group, again diabetes duration, age and axial length factors contributed significantly to the fit:

\[ y = -0.013(\pm 0.003) \text{DiaDur} - 0.010(\pm 0.003) \text{Age} + 0.187(\pm 0.037) \text{AL} - 1.00(\pm 0.87), \ R^2 = 0.56 \]

\[ \text{Figure 4:9 Relationships between age and anterior chamber depth (using Pentacam) for people with and without diabetes. Fit for diabetes group: } Y = -0.012(\pm 0.003) \text{Age} + 3.24(\pm 0.15), \ R^2 = 0.13, \ p < 0.001; \text{ fit for non-diabetes group: } Y = -0.010(\pm 0.003) \text{Age} + 3.27(\pm 0.15), \ R^2 = 0.13, \ p < 0.01. \text{ Values in brackets are standard errors.} \]

\[ \text{Figure 4:9 shows anterior chamber depth as a function of age with the Pentacam. There was significant decrease of 0.012 mm/year and 0.010 mm/year in people with and without diabetes, respectively. ANCOVA did not find significant difference in regression slopes between the groups (F}_{1, 133} \]

Chapter 4: Results
People with diabetes had non significantly shallower anterior chamber depths than people without diabetes (mean ± 95% CI –0.11 ± 0.14 mm, p 0.09).

Figure 4:10 Relationships between age and anterior chamber depth (using Lenstar) for people with and without diabetes. Fit for diabetes group: $Y = -0.013(±0.003)Age + 3.29(±0.15)$, $R^2 = 0.118$, p < 0.001; fit for non-diabetes group: $Y = -0.010(±0.003)Age + 3.30(±0.14)$, $R^2 = 0.14$, p < 0.01. Values in brackets are standard errors.

Figure 4:10 shows anterior chamber depth as a function of age with the Lenstar. There was a significant decrease of 0.013 mm/year and 0.010 mm/year in people with and without diabetes, respectively. ANCOVA did not find significant difference in regression slopes between the groups ($F_{1,133} = 0.37$, p 0.55). People with diabetes had significantly shallower anterior chamber depth than people without diabetes (mean ± 95% CI –0.14 ± 0.13 mm, p 0.03).
Discussion

All the multiple regression analyses showed that increases in both age and diabetes duration and decrease in axial length were associated with decreased anterior chamber depth. However, the ANCOVA did not find difference in slopes between the diabetes and non-diabetes group for the plots of age against anterior chamber depth. While the Pentacam and Lenstar instruments gave similar results, the difference between groups was significant only for the latter.

The results support other studies in finding decreases in anterior chamber depth with age and with diabetes duration (section 2.3.4). The mean differences in anterior chamber depth between the diabetes and non-diabetes groups were similar to that reported by Wiemer et al. (2008d): mean ± 95% CI 0.13 ± 0.11 mm. Contributions of diabetes duration to the multiple regressions were similar to those of previous studies for the diabetes group (–0.008 mm/year and –0.013 mm/year for the two instruments here as compared with –0.012 mm/year and –0.014 mm/year reported by Wiemer et al. (2008d) and Sparrow et al. (1990), respectively. However, unlike the other two studies, the differences in rate of anterior chamber depth changes with age between the groups were small and were not significant: –0.002 mm/year and –0.003 mm/year here compared with –0.009 and –0.007 mm/year for the Wiemer et al. and Sparrow et al. studies, respectively.)

In conclusion, anterior chamber depth decreases with age as reported in several previous studies. Diabetes appears to accelerate this process, but not to the same extent as in other studies.
4.2.7 Pupil Diameter

Pearson correlation was performed on combined data of people with and without diabetes (Table 4.9). Pupil diameter was correlated significantly with all factors except for lens thickness, spherical equivalent refraction and gender.

<table>
<thead>
<tr>
<th>Pupil diameter</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA₁c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>−0.40</td>
<td>−0.25</td>
<td>−0.17</td>
<td>−0.16</td>
<td>−0.07</td>
<td>0.29</td>
<td>−0.04</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001*</td>
<td>0.01*</td>
<td>0.04*</td>
<td>0.07</td>
<td>0.38</td>
<td>&lt; 0.01*</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for axial length. Age and axial length contributed significantly to the fit:

\[
y = -0.029(±0.005)\text{Age} + 0.280(±0.072)\text{AL} + 0.76(±1.71), \ R^2 = 0.29
\]

When multiple regression analysis was restricted to the diabetes group, again age and axial length contributed significantly to the fit:

\[
y = -0.025(±0.008)\text{Age} + 0.305(±0.108)\text{AL} − 0.07(±2.54), \ R^2 = 0.24
\]
Figure 4:11 Relationships between age and pupil diameter for people with and without diabetes. Fit for diabetes group: $Y = -0.024(\pm 0.008)Age + 6.99(\pm 0.33)$, $R^2 = 0.12$, $p < 0.01$; fit for non-diabetes group: $Y = -0.029(\pm 0.007)Age + 7.43(\pm 0.31)$, $R^2 = 0.21$, $p < 0.001$. Values in brackets are standard errors.

Figure 4:11 shows the relationship of pupil diameter to age. People with and without diabetes had a non-significant difference of $-0.26 \pm 0.30$ mm (mean $\pm$ 95% CI, $p 0.08$). There were significant decreases of $-0.024$ mm/year and $-0.029$ mm/year in pupil diameter in people with and without diabetes, respectively. ANCOVA did not find significant difference in regression slopes between the groups ($F_{1,133} = 0.64$, $p 0.42$).

**Discussion**

Diabetes duration had a significant association with pupil diameter according to the simple linear regression analysis, but disappeared as a significant factor in multiple regression analyses which showed that increases in age and axial length decreased and increased pupil size, respectively. Also, the mean pupil sizes were not significantly different in diabetes and non-diabetes groups, contrary to previous findings (section...
2.3.3). The age effect is well known (Atchison & Smith, 2000a; Watson & Yellott, 2012). A power analysis (G*Power, \( \alpha = 0.05 \), power 0.8) indicates that 100 participants would be needed to show significance of the mean differences between diabetes and non-diabetes groups.
4.2.8 Pupil Decentration

Table 4:10 shows the Pearson correlations of pupil decentration along the horizontal axis (Dx) and pupil decentration along the vertical axis (Dy) with various ocular and systemic factors for the whole group. Pupil decentration components were not correlated significantly with any factor.

Table 4:10 Pearson correlations of pupil decentration with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dx</td>
<td>-1.66</td>
<td>-0.09</td>
<td>-0.13</td>
<td>-0.07</td>
<td>-0.12</td>
<td>-0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>p</td>
<td>0.11</td>
<td>0.40</td>
<td>0.20</td>
<td>0.48</td>
<td>0.28</td>
<td>0.79</td>
<td>0.86</td>
</tr>
<tr>
<td>Dy</td>
<td>0.03</td>
<td>-0.08</td>
<td>-0.15</td>
<td>-0.07</td>
<td>0.05</td>
<td>0.07</td>
<td>-0.02</td>
</tr>
<tr>
<td>p</td>
<td>0.76</td>
<td>0.47</td>
<td>0.20</td>
<td>0.50</td>
<td>0.66</td>
<td>0.51</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole subject group and only the diabetes group. Neither factor had significant influence in either case, for either the vertical or the horizontal axis.
Figure 4.12: Relationships between age and pupil decentrations along horizontal and vertical axes for people with and without diabetes. Linear fits for pupil decentration along horizontal axis in people with and without diabetes were $Y = -0.003 \pm 0.006 \text{Age} + 0.32 \pm 0.27$, $R^2 = 0.01$, $p = 0.64$ and $Y = -0.011 \pm 0.006 \text{Age} + 0.74 \pm 0.26$, $R^2 = 0.08$, $p = 0.05$, respectively. Linear fits for pupil decentration along vertical axis in people with and without diabetes were $Y = 0.000 \pm 0.004 \text{Age} - 0.11 \pm 0.17$, $R^2 = 0.00$, $p = 0.99$ and $Y = +0.002 \pm 0.004 \text{Age} + 0.07 \pm 0.20$, $R^2 = 0.00$, $p = 0.71$, respectively.

Figure 4.12 shows pupil decentration along horizontal and vertical axes as a function of age for the two groups. ANCOVA did not find significant difference in regression slopes between the groups for either horizontal or vertical decentrations ($F_{1, 90} = 0.87$, $p = 0.35$ and $F_{1, 90} = 0.50$, $p = 0.48$, respectively). The differences in pupil decentrations between the groups were DX: $-0.13 \pm 0.19 \text{mm}$ (mean $\pm 95\% \ CI$, $p = 0.19$); DY: $-0.05 \pm 0.13 \text{mm}$ (mean $\pm 95\% \ CI$, $p = 0.43$).

**Discussion**

As pupils become smaller, at least due to the effects of luminance, they move nasally e.g. Mathur et al. (2014). Because pupils become smaller with age e.g. Atchison and Smith (2000a), I considered that pupil centre shifts at least in the horizontal direction might occur as a function of age and be accentuated by the presence of diabetes. Such shifts could be correlated with aberration differences between diabetes and non-diabetes groups (section 4.5).
However, the linear and multiple regression analyses showed no significant association of any factor with pupil decentration.

To conclude, neither diabetes nor age influenced pupil decentration.
4.2.9 Anterior Lens Radius of Curvature

The characteristics of the sub-groups for anterior lens radius of curvature, posterior lens radius of curvature and equivalent refractive index are summarised in Table 4:11. There were 67 participants with diabetes and 62 age-matched participants without diabetes.

Table 4:11 Characteristics of participants for lens anterior radius of curvature, posterior radius of curvature, and equivalent refractive index

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>67</td>
<td>62</td>
<td>0.46</td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>41 ± 13, 19 – 63</td>
<td>42 ± 13, 20 – 62</td>
<td>0.78</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>44/23</td>
<td>52/10</td>
<td>0.02*</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>24/43</td>
<td>41/21</td>
<td>0.001*</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>−0.04 ± 0.18</td>
<td>−0.06 ± 0.15</td>
<td>0.48</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.81 ± 0.14</td>
<td>1.90 ± 0.10</td>
<td>0.001*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (dioptres)</td>
<td>−0.64 ± 1.01</td>
<td>−0.38 ± 0.95</td>
<td>0.15</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>2.78 ± 0.36</td>
<td>2.89 ± 0.35</td>
<td>0.11</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>4.30 ± 0.49</td>
<td>4.01 ± 0.35</td>
<td>0.001*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.59 ± 0.92</td>
<td>23.73 ± 0.90</td>
<td>0.36</td>
</tr>
<tr>
<td>Anterior lens radius of curvature (mm)</td>
<td>+9.53 ± 1.08</td>
<td>+10.62 ± 1.14</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Posterior lens radius of curvature (mm)</td>
<td>−5.89 ± 0.72</td>
<td>−6.32 ± 0.74</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Equivalent refractive index (555 nm)</td>
<td>1.426 ± 0.011</td>
<td>1.431 ± 0.012</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.83 ± 1.11</td>
<td>5.05 ± 0.32</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Lens equivalent power (D), (555 nm)</td>
<td>25.06 ± 3.27</td>
<td>24.28 ± 2.33</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>19 ± 11</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* significant difference between groups

Table 4:12 shows the Pearson correlations of anterior lens radii of curvature with various ocular and systemic factors for the whole group. Anterior lens
radius of curvature was correlated significantly with all parameters except for spherical equivalent refraction and gender.

Table 4:12 Pearson correlations of anterior radius of curvature with different ocular and systemic parameters

<table>
<thead>
<tr>
<th>Anterior lens radii of curvature</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Gender</th>
<th>Axial length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>−0.33</td>
<td>−0.56</td>
<td>−0.41</td>
<td>−0.61</td>
<td>−0.17</td>
<td>0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.001*</td>
<td>&lt; 0.001*</td>
<td>0.06</td>
<td>0.57</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed, with age and diabetes duration as predictors using the whole subject group after adjusting for axial length. Age, duration of diabetes and axial length contributed significantly to the fit significant:

\[
y = -0.045(±0.007)DiabDur - 0.026(±0.007)Age + 0.460(±0.090)AL + 0.72(±2.12), R^2 = 0.47
\]

When the multiple regression analysis was restricted to the diabetes group, diabetes duration and axial length were the only factors contributing significantly to the fit:

\[
y = -0.040(±0.012)DiabDur + 0.418(±0.123)AL + 0.98(±2.87), R^2 = 0.40
\]

The fits for this group indicates that age does not contribute to the variation in the anterior lens radius of curvature beyond that explained by diabetes duration.
Figure 4:13 Relationships between age and anterior lens radius of curvature for people with and without diabetes. Fit for diabetes group: $Y = -0.024(\pm 0.010)\text{Age} + 10.52(\pm 0.44)$, $R^2 = 0.08$, $p = 0.02$; fit for non-diabetes group: $Y = -0.043(\pm 0.010)\text{Age} + 12.39(\pm 0.44)$, $R^2 = 0.23$, $p < 0.001$. Values in brackets are standard errors.

Figure 4:13 shows anterior lens radius of curvature as a function of age for the two groups. People with diabetes had a significantly smaller radii of curvature than people without diabetes, with a difference of $-1.09 \pm 0.40$ mm (mean ± 95% CI) between the two groups. There were significant decreases of 0.024 mm/year and 0.043 mm/year in people with and without diabetes, respectively. ANCOVA did not find significant difference in regression slopes between the groups ($F_{1,125} = 1.63$, $p = 0.20$).

**Discussion**

There was a marked difference in the mean anterior radius of curvature for the two groups, and diabetes duration contributed to variation in the diabetes group. These results were similar to those of Wiemer et al. (2008d), who had a mean difference of 1.11 mm between groups and a diabetes
duration slope of $-0.056 \text{ mm/year}$. As age increased, radii of curvature decreased significantly in both studies. However, the slopes were not significantly different between groups in this study, while the slope was greater for the diabetes group than for the non-diabetes group in Wiemer et al.’s study (mean difference $\pm 95\% \text{ CI} -0.037 \pm 0.027 \text{ mm/year}$) (see Figure 4:14).

In conclusion, the diabetes group had smaller radii of curvatures than the non-diabetes group, but the reductions with increase in age were not significantly different.

Figure 4:14 Anterior lens radius of curvature as a function of age from Wiemer et al. (2008d).
4.2.10 Posterior Lens Radius of Curvature

Pearson correlation was performed on combined data of people with and without diabetes general characteristics given in Table 4:13. Posterior lens radius of curvature was correlated significantly with all parameters except gender.

Table 4:13 Pearson correlations of posterior lens radius with different ocular and systemic parameters

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA$_1c$</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Gender</th>
<th>Axial length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient (Pentacam)</td>
<td>0.22</td>
<td>0.36</td>
<td>0.25</td>
<td>0.43</td>
<td>0.21</td>
<td>-0.10</td>
<td>-0.35</td>
</tr>
<tr>
<td>$P$</td>
<td>0.01*</td>
<td>&lt; 0.001*</td>
<td>0.01*</td>
<td>&lt; 0.001*</td>
<td>0.02*</td>
<td>0.27</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed, with age and diabetes duration as predictors using the whole subject group after adjusting for axial length. Age, diabetes duration and axial length contributed significantly to the fit:

$$y = +0.017(\pm0.005)\text{DiaDur} + 0.012(\pm0.005)\text{Age} - 0.270(\pm0.066)\text{AL} - 0.35(\pm1.55), R^2 = 0.25$$

When multiple regression analysis was restricted to the diabetes group, neither diabetes duration nor age contributed significantly to the fit.
Figure 4:15 Relationships between age and posterior lens radius of curvature for people with and without diabetes. Fit for diabetes group: \( Y = +0.005(\pm0.007)\text{Age} - 6.08(\pm0.30), R^2 = 0.01, p < 0.50 \); fit for non-diabetes group: \( Y = +0.023(\pm0.007)\text{Age} - 7.26(\pm0.30), R^2 = 0.15, p < 0.01 \). Values in brackets are standard errors.

Figure 4:15 shows lens posterior radius of curvature as a function of age for the two groups. People with diabetes had significantly smaller radii of curvature than people without diabetes, with a difference of \( 0.43 \pm 0.26 \text{ mm} \) (mean ± 95% CI) between the two groups. There were absolute decreases of 0.005 mm/year and 0.023 mm/year in people with and without diabetes, respectively but this was only significant in the latter. ANCOVA did not find significant difference in regression slopes between the groups (\( F_{1,125} = 3.36, p = 0.07 \)).

**Discussion**

There was a marked difference in the mean posterior radius of curvature for the two groups, and both diabetes duration and age contributed to variation in the whole group. These results were similar to those of Wiemer et al. (2008d), who had a mean difference of 0.37 mm between groups and a
diabetes duration slope of \(-0.031\) mm/year. As age increased, radii of curvature decreased in both studies. However, in this study the slope for the diabetes group was not significant and the slopes were not significantly different between groups, while the slope was greater for the diabetes group in Wiemer et al.’s study (mean difference ± 95% CI +0.023 ± 0.021 mm/year).

In conclusion, the diabetes group had smaller radii of curvatures than the non-diabetes group, but the reductions with increase in age were not significantly different.
4.2.11 Lens Central Thickness

Pearson correlation was performed on combined data of people with and without diabetes (Table 4:11). Lens central thickness was correlated significantly with all parameters except spherical equivalent refraction and gender.

Table 4:14 Pearson correlations of lens central thickness with different ocular and systemic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Spherical equivalent refraction</th>
<th>Gender</th>
<th>Axial length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>0.68</td>
<td>0.62</td>
<td>0.33</td>
<td>0.14</td>
<td>0.07</td>
<td>-0.22</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.11</td>
<td>0.40</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjustment for axial length. The independent effects of diabetes duration, age and axial length were significant:

\[ y = +0.016(\pm0.002)\text{DiaDur} + 0.021(\pm0.002)\text{Age} - 0.091(\pm0.024)\text{AL} + 5.35(\pm0.56), \quad R^2 = 0.73 \]

When the multiple regression analysis was restricted to the diabetes group, again the three factors contributed significantly to the fit:

\[ y = +0.020(\pm0.003)\text{DiaDur} + 0.021(\pm0.003)\text{Age} - 0.113(\pm0.033)\text{AL} + 5.69(\pm0.77), \quad R^2 = 0.77 \]

The fits indicate similar importance of both diabetes duration and age.
Figure 4:16 Relationships between age and lens central thickness for people with and without diabetes. Fit for diabetes group: $Y = +0.027(\pm0.003) \text{Age} + 3.16(\pm0.14)$, $R^2 = 0.60$, $p < 0.001$; fit for non-diabetes group: $Y = +0.021(\pm0.002) \text{Age} + 3.16(\pm0.11)$, $R^2 = 0.54$, $p < 0.001$. Values in brackets are standard errors.

Figure 4:16 shows lens central thickness as a function of age for the two groups. People with diabetes had significantly greater lens thickness than people without diabetes, with a difference of $+0.29 \pm 0.15$ mm (mean ± 95% CI) between the two groups. There were significant increases of 0.027 mm/year and 0.021 mm/year in people with diabetes and without diabetes, respectively. People with diabetes had thicker lenses than people without diabetes with mean difference $0.29 \pm 0.08$ mm. ANCOVA did not find significant difference in regression slopes between the groups ($F_{1, 133} = 2.23$, $p = 0.11$).

**Discussion**

Lens thickness was considerable greater in the diabetes group than in the non-diabetes group (mean 0.29 mm), with both age and diabetes duration contributing to variation. Lens thickness increased with age more quickly for
the diabetes group than for the non-diabetes group, but the difference in rate of 0.006 mm/year was not significant. The difference between groups was greater than that of Wiemer et al. (2008d) (mean ± 95% CI 0.20 ± 0.04 mm). The importance of diabetes duration was similar to those obtained by Wiemer et al. of +0.020 ± 0.008 mm/year and by Sparrow et al. (1990) of +0.017 mm/year, but the rates of change with age were significantly different between the groups in these studies e.g. +0.009 ± 0.008 95% CIs in Wiemer et al.’s study (Figure 4:17).

In conclusion, lens thickness was considerably greater in the diabetes group than in the non-diabetes group, but the greater rate of increase with age in the former was not quite significant.

Figure 4:17 Lens thickness as a function of age from Wiemer et al. (2008d).
4.2.12 Lens Equivalent Refractive Index

Pearson correlation was performed on combined data of people with and without diabetes general characteristics given in table (Table 4:15). Lens equivalent refractive index was correlated significantly with all parameters except spherical equivalent refraction.

Table 4:15 Pearson correlations of lens equivalent refractive index with different ocular and systemic parameters

<table>
<thead>
<tr>
<th>Correlation co-efficient</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Gender</th>
<th>Axial length</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>-0.48</td>
<td>-0.31</td>
<td>-0.21</td>
<td>-0.37</td>
<td>-0.13</td>
<td>-0.18</td>
<td>-0.26</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for axial length. Diabetes duration, age and axial length contributed significantly to the fit:

\[
y = -0.0003(\pm 0.0001)DiaDur - 0.0004(\pm 0.0001)Age - 0.0033(\pm 0.0010)AL + 1.525(\pm 0.024),
R^2 = 0.35
\]

When the multiple regression analysis was restricted to the diabetes group, diabetes duration and axial length were significant factors:

\[
y = -0.0003(\pm 0.0001) DiaDur - 0.0041(\pm 0.0015)AL + 1.537(\pm 0.034), R^2 = 0.28
\]

The fits for the group with diabetes indicate that diabetes duration contributed to the variation in the equivalent lens refractive index beyond that explained by age.
Figure 4:18 Relationships between age and lens equivalent refractive index for people with and without diabetes. Fit for diabetes group: \( Y = -0.00033(\pm 0.00010) \text{Age} + 1.439(\pm 0.004), R^2 = 0.14, p < 0.001; \) fit for non-diabetes group: \( Y = -0.00056(\pm 0.00009) \text{Age} + 1.455(\pm 0.004), R^2 = 0.38, p < 0.001. \) Values in brackets are standard errors.

Figure 4:18 shows lens equivalent refractive index as a function of age. There were significant decreases of \(-0.00033/\text{year}\) and \(-0.00056/\text{year}\) in people with diabetes and without diabetes, respectively. People with diabetes had lower refractive index than people without diabetes with a difference \(-0.006 \pm 0.004\) (mean ± 95% CI). ANCOVA did not find significant difference in regression slopes between the groups (\(F_{1,125} = 3.07, p = 0.08\)).

**Discussion**

Equivalent refractive index was considerably lower in the diabetes group than in the non-diabetes group, with only diabetes duration contributing to variation in the diabetes group. The index decreased more slowly with age...
for the diabetes group than for the non-diabetes group, but the difference in rate of 0.00023/year was not significant. The difference between groups was similar to that of Wiemer et al. (2008d) (mean ± 95% CI –0.004 ± 0.002). Diabetes duration was also important to variation in the diabetic group in Wiemer et al.’s (2008d) study at –0.00018 ± 0.00017/year (mean ± 95% CI). The rate of change of index with age was much higher in Wiemer’s study for the diabetes group at –0.0007/year compared with the –0.0003/year found here (Figure 4:19), and unlike this study the rate of change in index with age was greater for the diabetes than for the non-diabetes group.

In conclusion, equivalent refractive index was considerably lower in the diabetes group than in the non-diabetes group, but the rates of decrease with age were not quite significantly different.

Figure 4:19 Lens equivalent refractive index as a function of age from Wiemer et al. (2008d).
4.2.13 Lens Equivalent Power

Table 4:16 shows the Pearson correlations of lens equivalent power with various ocular and systemic factors for the whole group. Lens equivalent power was calculated using refractive indices at 555 nm. Lens equivalent power was correlated significantly with age, axial length and gender.

Table 4:16 Pearson correlations of lens equivalent power with different ocular and systemic parameters

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>-0.26</td>
<td>0.16</td>
<td>0.13</td>
<td>0.15</td>
<td>0.08</td>
<td>-0.74</td>
<td>-0.30</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.01*</td>
<td>0.07</td>
<td>0.14</td>
<td>0.08</td>
<td>0.38</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group after adjusting for gender and axial length. Only age and axial length contributed significantly to the fit:

\[ y = -0.046(\pm0.013)\text{Age} - 1.997(\pm0.190)\text{AL} + 73.70(\pm4.44), \ R^2 = 0.59 \]

When the multiple regression analysis was restricted to the diabetes group, neither age nor diabetes duration contributed significantly to the fit.
Figure 4:20 shows lens equivalent power as a function of age. There was a significant reduction in power with age for the non-diabetic group (p 0.02), but not for the diabetes group (p 0.05). The difference of 0.69 ± 0.94 D (mean ± 95% CI) between the groups was not statistically significant. ANCOVA did not find significant difference in regression slopes between the groups (F1, 125 = 0.01, p 0.92).

**Discussion**

The mean equivalent lens powers of the diabetes and non-diabetes groups were not significantly different. Power reduced with age for both groups; it was significant for the non-diabetes group and nearly significantly for the diabetes group (p 0.05). Wiemer et al. (2008d) did not find effects of age or
diabetes on lens power with their Scheimpflug technique. However, Atchison et al. (2008) found a significant decrease of 0.033 D/year in a non-diabetic group using the Phakometry technique, with a similar mean power (23.9 ± 0.6 D) (mean ± 95% CI) to that found here (24.3 ± 0.6 D) (mean ± 95% CI).
4.3 Straylight

The characteristics of the sub-groups for straylight testing are summarised in Table 4:17. There were 63 participants with diabetes and 57 age-matched participants without diabetes. Twelve participants with diabetes and seven participants without diabetes (12% of total) did not do this test because of unavailability of the C-Quant instrument at the beginning of the study. Two older participants with straylight higher than 1.60 logs were excluded according to the study criteria.

Table 4:17 Characteristics of participants for straylight testing

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>63</td>
<td>57</td>
<td>0.48</td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>41 ± 12, 19 − 63</td>
<td>41 ± 12, 20 − 62</td>
<td>0.83</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>41/22</td>
<td>50/7</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>26/37</td>
<td>39/18</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>−0.03 ± 0.22</td>
<td>−0.04 ± 0.21</td>
<td>0.76</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.82 ± 0.13</td>
<td>1.91 ± 0.09</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (dioptres)</td>
<td>−0.59 ± 1.16</td>
<td>−0.51 ± 0.95</td>
<td>0.72</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>2.76 ± 0.36</td>
<td>2.88 ± 0.36</td>
<td>0.09</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>4.25 ± 0.45</td>
<td>4.00 ± 0.34</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.59 ± 0.94</td>
<td>23.69 ± 0.91</td>
<td>0.58</td>
</tr>
<tr>
<td>Straylight (log(s))</td>
<td>0.95 ± 0.19</td>
<td>0.83 ± 0.15</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.78 ± 1.14</td>
<td>5.03 ± 0.32</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>19 ± 11</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* significant difference

Table 4:18 shows the Pearson correlations of straylight with various ocular and systemic factors for the whole group. Straylight was correlated significantly with age, duration of diabetes, HbA1c and lens thickness. The correlations were similar for diabetes duration and age.
Table 4: Pearson correlations of straylight with different ocular and systemic factors

<table>
<thead>
<tr>
<th>Correlation co-efficient</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>0.46</td>
<td>0.50</td>
<td>0.29</td>
<td>0.49</td>
<td>-0.01</td>
<td>-0.08</td>
<td>-0.08</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001*</td>
<td>&lt;0.001*</td>
<td>&lt; 0.01*</td>
<td>&lt; 0.001*</td>
<td>0.88</td>
<td>0.36</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Both factors contributed significantly to the fit:

\[ y = 0.006 \pm 0.001 \text{DiaDur} + 0.005 \pm 0.001 \text{Age} + 0.61 \pm 0.05, R^2 = 0.38 \]

When the multiple regression analysis was restricted to the diabetes group, again both factors contributed significantly to the fit:

\[ y = 0.007 \pm 0.002 \text{DiaDur} + 0.004 \pm 0.002 \text{Age} + 0.67 \pm 0.07, R^2 = 0.30 \]

The fit for the whole group indicates similar importance of the two factors, but the fit for the group with diabetes indicated a greater importance of duration of diabetes than of age.
Figure 4.21 Relationships between age and strayline for people with and without diabetes. Fit for diabetes group: \( Y = +0.006 \pm 0.002 \times \text{Age} + 0.70 \pm 0.08 \), \( R^2 = 0.17 \), \( p < 0.01 \); fit for non-diabetes group: \( Y = +0.007 \pm 0.001 \times \text{Age} + 0.53 \pm 0.06 \), \( R^2 = 0.36 \), \( p < 0.01 \). Values in brackets are standard errors.

Figure 4.21 shows straylight as a function of age for the two groups. There was significant increase of 0.006 mm/year and 0.007 mm/year in people with diabetes and without diabetes, respectively. People with diabetes had higher straylight than people without diabetes with difference +0.12 ± 0.06 logs (mean ± 95% CI). ANCOVA did not find significant difference in regression slopes between the groups (\( F_{1,116} = 0.67 \), \( p = 0.41 \)).

**Discussion**

Straylight was greater in the diabetes than in the non-diabetes group. It increased with both diabetes duration and age according to the multiple regressions, but the rates of change with increase in age were similar for both groups.
4.4 Amplitude of Accommodation

The characteristics of the sub-group for amplitude of accommodation testing are summarised in Table 4:19. There were 43 participants with diabetes and 32 age-matched participants without diabetes.

Table 4:19 Characteristics of participants for amplitude of accommodation testing

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>43</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>33 ± 8, 19 – 46</td>
<td>34 ± 8, 20 – 46</td>
<td>0.43</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>30/13</td>
<td>29/3</td>
<td>0.03*</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>17/26</td>
<td>19/13</td>
<td>0.09</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>−0.05 ± 0.07</td>
<td>−0.01 ± 0.27</td>
<td>0.41</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.83 ± 0.14</td>
<td>1.91 ± 0.08</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (dioptres)</td>
<td>−0.83 ± 1.04</td>
<td>−0.62 ± 0.83</td>
<td>0.35</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>2.94 ± 0.29</td>
<td>3.03 ± 0.28</td>
<td>0.18</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>3.97 ± 0.31</td>
<td>3.86 ± 0.25</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.52 ± 0.90</td>
<td>23.79 ± 0.86</td>
<td>0.19</td>
</tr>
<tr>
<td>Objective amplitude of accommodation (D)</td>
<td>2.7 ± 1.6</td>
<td>4.1 ± 2.1</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Subjective amplitude of accommodation (D)</td>
<td>4.0 ± 1.7</td>
<td>5.6 ± 2.1</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.96 ± 1.23</td>
<td>5.01 ± 0.36</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>16 ± 8</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* significant difference between groups
Figure 4: Bland-Altman plot comparing the objective and subjective methods of amplitude of accommodation. The mean difference and the prediction limits are represented by the straight lines.

Figure 4:22 is a Bland-Altman plot of agreement between the objective and subjective methods for the combined groups. The objective amplitudes were smaller than the subjective amplitudes by $1.39 \pm 1.21$ D (mean $\pm$ 95% CI). People with diabetes had lower objective (mean $\pm$ SD, 2.70 ± 1.59 D) and subjective (mean $\pm$ SD, 3.98 ± 1.72 D) amplitudes of accommodation than people without diabetes (objective: mean $\pm$ SD 4.07 ± 2.10 D), subjective: mean $\pm$ SD 5.60 ± 2.12 D).

Table 4:20 shows the Pearson correlations of objective amplitude of accommodation with various ocular and systemic factors for the whole group. Accommodation was correlated significantly with age, duration of diabetes, HbA1c, lens thickness and spherical equivalent refraction but not
with axial length and gender. The correlation was higher for age than for diabetes duration.

Table 4:20 Pearson correlations of objective amplitude of accommodation with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>-0.64</td>
<td>-0.48</td>
<td>-0.37</td>
<td>-0.69</td>
<td>0.24</td>
<td>0.14</td>
<td>-0.04</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.04*</td>
<td>0.23</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* indicates significance

Table 4:21 shows the Pearson correlations of subjective amplitude of accommodation with various ocular and systemic factors for the whole group. Accommodation was correlated significantly with age, duration of diabetes, HbA1c, lens thickness but not with spherical equivalent refraction, axial length and gender. The correlation was higher for age than for diabetes duration.

Table 4:21 Pearson correlations of subjective amplitude of accommodation with different ocular and systemic factors

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation co-efficient</td>
<td>-0.65</td>
<td>-0.57</td>
<td>-0.42</td>
<td>-0.72</td>
<td>0.15</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>&lt; 0.001*</td>
<td>0.21</td>
<td>0.22</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole subject group. The independent effects of both of these on objective amplitude of accommodation were significant (Table 4:20), and both factors contributed significantly to the fit:

\[
y = -0.083(\pm 0.015) \text{DiaDur} - 0.145(\pm 0.018) \text{Age} + 8.92(\pm 0.65), \ R^2 = 0.59
\]
When the multiple regression analysis was restricted to the diabetes group, again both factors contributed significantly to the fit:

\[ y = -0.076(\pm 0.023)\text{DiaDur} - 0.097(\pm 0.022)\text{Age} + 7.13(\pm 0.74), \quad R^2 = 0.51 \]

Similarly, the independent effects of diabetes duration and age on subjective amplitude of accommodation were both significant (Table 4:21), and both factors contributed significantly to the fit:

\[ y = -0.105(\pm 0.014)\text{DiaDur} - 0.154(\pm 0.017)\text{Age} + 10.67(\pm 0.61), \quad R^2 = 0.68 \]

When the multiple regression analysis was restricted to the diabetes group, again both factors contributed significantly to the fit:

\[ y = -0.106(\pm 0.022)\text{DiaDur} - 0.103(\pm 0.021)\text{Age} + 9.08(\pm 0.70), \quad R^2 = 0.63 \]

Figure 4:23 Relationships between age and objective amplitude of accommodation for people with and without diabetes. Fit for diabetes group: \( Y = -0.117(\pm 0.023)\text{Age} + 6.62(\pm 0.81), \quad R^2 0.38, \quad p < 0.01; \) fit for non-diabetes group: \( Y = -0.226(\pm 0.027)\text{Age} + 11.85(\pm 0.96), \quad R^2 0.70, \quad p < 0.01. \) Values in brackets are standard errors.
Figure 4:23 shows objective amplitude of accommodation as a function of age. There were significant decreases of $-0.117$ D/year and $-0.226$ D/year in people with diabetes and without diabetes, respectively. ANCOVA showed significant differences in regression slopes between the groups ($F_{1,71} = 8.90$, $p < 0.01$).

Figure 4:24 shows subjective amplitude of accommodation as a function of age. There were significant decreases of $-0.131$ D/year and $-0.232$ D/year in people with diabetes and without diabetes, respectively. ANCOVA showed significant differences in regression slopes between the groups ($F_{1,71} = 7.52$, $p < 0.01$).
Discussion

In support of a previous study (Moss, et al., 1987), we have found lowered amplitude of accommodation in participants with diabetes when compared with age-matched controls. We have estimated the importance of duration of diabetes relative to that of age to be 0.6 to 1.0, which overall indicates greater importance of age than of diabetes duration. These estimates are a little higher than previous estimates using subjective amplitudes of 0.4 to 0.6 (Braun, et al., 1995; Moss, et al., 1987; Pawelski & Gliem, 1971).

Subjective amplitudes of accommodation were greater than objective amplitudes by a mean 1.4 D. The trends for the two measurements were similar. All the multiple regressions indicated that both increasing diabetes duration and age were associated with reduction in amplitude, with estimates of the importance of diabetes duration relative to age varying from 0.57 to 1.03. Despite this, the rates of change of amplitude loss with age were greater in people without diabetes than in people with diabetes; this result was unexpected, but may be related to the considerable number of older participants who had short diabetes durations (Figure 4:25).

Figure 4:26 shows subjective amplitudes of accommodation from different studies (Braun, et al., 1995; Moss, et al., 1987). Comparisons for both diabetic and non-diabetic groups are possible only with the study of Moss et al. For Moss et al. rates of change with age were similar for groups both with and without diabetes (about −0.25 D/year) and were similar for my group without diabetes, but considerably higher than for my diabetes group (0.13 D/year). The results for Moss et al appear high with the trends indicating that their group with diabetes would have mean amplitude of more than 3 D at the age of 50 years, such that presbyopia would not occur until at least 50
years rather than more commonly in the mid-40s. The slope in Braun et al.’s study in people with diabetic retinopathy was low at 0.071 D/year.

I believe that the low subjective amplitudes in my study are due to the small detail of the target, giving small influence of depth-of-focus on judgements and hence giving a critical estimate of amplitude. In the Badal hand optometer, the angular size of the target does not change with target position. The acuity of the target is specified as 0.5 (0.3 logMAR), but measurements of the targets show that the letter detail is fine relative to the size of the letter and the acuity is actually about 0.0 logMAR.

Two of the people with diabetes had zero objective accommodation response. This biased the results as it was not known at what age they would have ceased to have a response, but we believed it was better to include them than bias the results even more by not including them.

Figure 4.25 Relationships between age and diabetes duration for the people who had accommodation measurements. Regression fit is $Y = +0.272(\pm0.143)\text{Age} + 6.75(\pm4.93)$, $R^2 = 0.08$, $p = 0.06$. Values in brackets are standard errors.
Figure 4.26 Comparison of subjective amplitude of accommodation between previous studies (Braun, et al., 1995; Moss, et al., 1987) and present study. For the present study, only fits have been shown.
4.5 Ocular Aberrations and their Components

The characteristics of the sub-groups for ocular aberrations testing at 4.5 mm pupil diameter are summarised in Table 4:22. There were 46 participants with diabetes and 47 age-matched participants without diabetes. Five participants’ corneal topography images were too poor to perform analysis and were excluded.

Table 4:22 Characteristics of participants for ocular aberrations testing

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>46</td>
<td>47</td>
<td>0.34</td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>41 ± 8, 20–63</td>
<td>43 ± 11, 20–62</td>
<td>0.43</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>32/14</td>
<td>38/09</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>18/28</td>
<td>29/18</td>
<td>0.04*</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>−0.03 ± 0.18</td>
<td>−0.05 ± 0.16</td>
<td>0.53</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.81 ± 0.14</td>
<td>1.88 ± 0.10</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (dioptres)</td>
<td>−0.70 ± 1.08</td>
<td>−0.35 ± 0.95</td>
<td>0.11</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>2.74 ± 0.40</td>
<td>2.86 ± 0.34</td>
<td>0.14</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>4.27 ± 0.50</td>
<td>4.08 ± 0.37</td>
<td>0.04*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.51 ± 0.83</td>
<td>23.75 ± 0.86</td>
<td>0.44</td>
</tr>
<tr>
<td>Corneal horizontal coma coeff (µm)</td>
<td>−0.104 ± 0.117</td>
<td>−0.113 ± 0.118</td>
<td>0.70</td>
</tr>
<tr>
<td>Corneal vertical coma coeff (µm)</td>
<td>−0.070 ± 0.119</td>
<td>−0.031 ± 0.114</td>
<td>0.11</td>
</tr>
<tr>
<td>Corneal spherical aberration coeff (µm)</td>
<td>+0.050 ± 0.034</td>
<td>+0.047 ± 0.032</td>
<td>0.59</td>
</tr>
<tr>
<td>Corneal higher order RMS (µm)</td>
<td>0.201 ± 0.123</td>
<td>0.201 ± 0.115</td>
<td>1.00</td>
</tr>
<tr>
<td>Total horizontal coma coeff (µm)</td>
<td>−0.022 ± 0.084</td>
<td>+0.017 ± 0.094</td>
<td>0.04*</td>
</tr>
<tr>
<td>Total vertical coma coeff (µm)</td>
<td>−0.013 ± 0.086</td>
<td>+0.027 ± 0.058</td>
<td>0.01*</td>
</tr>
<tr>
<td>Total spherical aberration coeff (µm)</td>
<td>+0.042 ± 0.075</td>
<td>+0.025 ± 0.051</td>
<td>0.21</td>
</tr>
<tr>
<td>Total higher order RMS (µm)</td>
<td>0.180 ± 0.092</td>
<td>0.170 ± 0.072</td>
<td>0.57</td>
</tr>
<tr>
<td>Internal horizontal coma coeff (µm)</td>
<td>+0.082 ± 0.145</td>
<td>+0.131 ± 0.158</td>
<td>0.13</td>
</tr>
<tr>
<td>Internal vertical coma coeff (µm)</td>
<td>+0.053 ± 0.132</td>
<td>+0.058 ± 0.122</td>
<td>0.98</td>
</tr>
<tr>
<td>Internal spherical aberration coeff (µm)</td>
<td>−0.008 ± 0.090</td>
<td>−0.021 ± 0.053</td>
<td>0.39</td>
</tr>
<tr>
<td>Internal higher order RMS (µm)</td>
<td>0.268 ± 0.111</td>
<td>0.274 ± 0.114</td>
<td>0.80</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.97 ± 1.15</td>
<td>5.01 ± 0.31</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>19 ± 11</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± standard deviation
* Indicates significant difference between groups.
Table 4:23 shows the Pearson correlations of corneal aberrations components with various ocular and systemic factors for the whole group. The only significant correlation was found for vertical coma with HbA1c.

Table 4:23 Pearson correlations of corneal aberrations components with different ocular and systemic factors

<table>
<thead>
<tr>
<th>Corneal aberrations components</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal coma correlation co-efficient</td>
<td>0.11</td>
<td>0.06</td>
<td>0.05</td>
<td>0.09</td>
<td>0.08</td>
<td>-0.09</td>
<td>-0.18</td>
</tr>
<tr>
<td>p</td>
<td>0.31</td>
<td>0.59</td>
<td>0.67</td>
<td>0.40</td>
<td>0.43</td>
<td>0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>Vertical coma correlation co-efficient</td>
<td>-0.18</td>
<td>-0.16</td>
<td>-0.22</td>
<td>-0.11</td>
<td>-0.17</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>p</td>
<td>0.09</td>
<td>0.12</td>
<td>0.04*</td>
<td>0.32</td>
<td>0.10</td>
<td>0.10</td>
<td>0.42</td>
</tr>
<tr>
<td>Spherical aberration correlation co-efficient</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0.07</td>
<td>-0.15</td>
<td>-0.12</td>
<td>0.11</td>
<td>-0.03</td>
</tr>
<tr>
<td>p</td>
<td>0.78</td>
<td>0.79</td>
<td>0.49</td>
<td>0.16</td>
<td>0.25</td>
<td>0.31</td>
<td>0.77</td>
</tr>
<tr>
<td>HORMS correlation co-efficient</td>
<td>0.17</td>
<td>0.01</td>
<td>0.06</td>
<td>0.04</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>p</td>
<td>0.11</td>
<td>0.92</td>
<td>0.56</td>
<td>0.74</td>
<td>0.75</td>
<td>0.77</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* indicates significance

For all aberrations components, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group and then only the diabetes group. For each component, neither factor contributed significantly to the fit in either case.
Chapter 4: Results

Figure 4:27 Relationships between age and corneal aberration components for people with and without diabetes. a) Horizontal coma linear fit, diabetes group: $Y = +0.001(\pm0.001)\text{Age} - 0.13(\pm0.06)$, $R^2 = 0.00$, $p = 0.66$; non-diabetes group: $Y = +0.001(\pm0.001)\text{Age} - 0.18(\pm0.06)$, $R^2 = 0.02$, $p = 0.31$. b) Vertical coma linear fit, diabetes group: $Y = -0.003(\pm0.001)\text{Age} + 0.04(\pm0.06)$, $R^2 = 0.08$, $p = 0.06$; non-diabetes group: $Y = -0.001(\pm0.001)\text{Age} + 0.01(\pm0.06)$, $R^2 = 0.01$, $p = 0.46$. c) Spherical aberration linear fit: diabetes group: $Y = +0.000(\pm0.000)\text{Age} + 0.04(\pm0.02)$, $R^2 = 0.00$, $p = 0.67$; non-diabetes group: $Y = +0.000(\pm0.000)\text{Age} + 0.06(\pm0.02)$, $R^2 = 0.01$, $p = 0.44$. d) HORMS linear fit, diabetes group: $Y = +0.001(\pm0.002)\text{Age} + 0.17(\pm0.06)$, $R^2 = 0.01$, $p = 0.64$; non-diabetes group: $Y = +0.002(\pm0.001)\text{Age} + 0.10(\pm0.06)$, $R^2 = 0.07$, $p = 0.07$. Values in brackets are standard errors.

Figure 4:27 shows corneal aberration components as functions of age. There was no significant difference between the groups for any aberration component. There was no significant rates of change with age for any combination of group and aberration component, and ANCOVA did not find any significant differences in regression slopes between groups.
**Total Ocular Aberrations**

Table 4:24 shows the Pearson correlations of total ocular aberrations components with various ocular and systemic factors for the whole group. Significant correlations were for horizontal coma with diabetes duration and HbA1c, for vertical coma with all factors except gender, for spherical aberration with diabetes duration, and for HORMS with diabetes duration.

**Table 4:24 Pearson correlations of total ocular aberrations components with different ocular and systemic factors**

<table>
<thead>
<tr>
<th>Total Ocular aberration components</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal coma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>correlation co-efficient p</td>
<td>-0.06</td>
<td>-0.23</td>
<td>-0.24</td>
<td>-0.12</td>
<td>-0.01</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Vertical coma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>correlation co-efficient p</td>
<td>-0.39</td>
<td>-0.52</td>
<td>-0.33</td>
<td>-0.62</td>
<td>-0.21</td>
<td>0.41</td>
<td>0.04</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>correlation co-efficient p</td>
<td>-0.02</td>
<td>0.31</td>
<td>0.11</td>
<td>0.19</td>
<td>0.16</td>
<td>-0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>HORMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>co-efficient p</td>
<td>0.04</td>
<td>0.27</td>
<td>0.19</td>
<td>0.21</td>
<td>-0.01</td>
<td>-0.20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

For horizontal coma, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Only diabetes duration contributed significantly to the fit:

\[
y = -0.0017(\pm0.0008)\text{DiaDur} + 0.025(\pm0.033), R^2 = 0.05
\]

When the multiple regression analysis was restricted to the diabetes group, neither factor contributed significantly to the fit.
For vertical coma, multiple regression analysis was performed with age and diabetes duration as predictors using the combined groups. Both factors contributed significantly to the fit:

\[ y = -0.0030(\pm 0.0005)Diadur - 0.0021(\pm 0.0005)Age + 0.121(\pm 0.022), \, R^2 = 0.38 \]

When the multiple regression analysis was restricted to the diabetes group, diabetes duration alone contributed significantly to the fit:

\[ y = -0.0045(\pm 0.0011)Diadur + 0.123(\pm 0.043), \, R^2 = 0.42 \]

For spherical aberration, multiple regression analysis was performed with age and diabetes duration as predictors using the combined groups. Only diabetes duration contributed significantly to the fit:

\[ y = +0.0017(\pm 0.0005)Diadur + 0.031(\pm 0.023), \, R^2 = 0.10 \]

When the multiple regression analysis was restricted to the diabetes group, again diabetes duration contributed significantly to the fit:

\[ y = +0.0036(\pm 0.0011)Diadur + 0.022(\pm 0.036), \, R^2 = 0.20 \]

For HORMS, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Only diabetes duration contributed significantly to the fit in either case.

\[ y = +0.0018(\pm 0.0007)Diadur + 0.155(\pm 0.030), \, R^2 = 0.07 \]

When the multiple regression analysis was restricted to the diabetes group, again diabetes duration contributed significantly to the fit:

\[ y = +0.0044(\pm 0.0013)Diadur + 0.127(\pm 0.044), \, R^2 = 0.22 \]
Figure 4:28 Relationships between age and total ocular aberration components for people with and without diabetes. a) Horizontal coma linear fit, diabetes group: $Y = +0.001(\pm0.001)\text{Age} - 0.05(\pm0.04)$, $R^2$ 0.01, $p$ 0.48; non-diabetes group: $Y = -0.002(\pm0.001)\text{Age} + 0.10(\pm0.05)$, $R^2$ 0.06, $p$ 0.09. b) Vertical coma linear fit, diabetes group: $Y = -0.003(\pm0.001)\text{Age} + 0.11(\pm0.04)$, $R^2$ 0.18, $p$ < 0.01; non-diabetes group: $Y = -0.002(\pm0.001)\text{Age} + 0.12(\pm0.03)$, $R^2$ 0.22, $p$ 0.001. c) Spherical aberration linear fit, diabetes group: $Y = +0.000(\pm0.001)\text{Age} + 0.03(\pm0.04)$, $R^2$ 0.00, $p$ 0.84; non-diabetes group: $Y = +0.000(\pm0.001)\text{Age} + 0.04(\pm0.03)$, $R^2$ 0.01, $p$ 0.64. d) HORMS linear fit, diabetes group: $Y = +0.001(\pm0.001)\text{Age} + 0.14(\pm0.05)$, $R^2$ 0.02, $p$ 0.41; non-diabetes group: HORMS linear fit $Y = +0.000(\pm0.001)\text{Age} + 0.18(\pm0.04)$, $R^2$ 0.00, $p$ 0.78. Values in brackets are standard errors.

Figure 4:28 shows the total ocular aberration components as functions of age. Significant differences between the groups were $-0.039 \pm 0.037$ (mean $\pm$ 95% CI) $\mu$m for horizontal coma and $-0.040 \pm 0.030$ (mean $\pm$ 95% CI) $\mu$m for vertical coma. There were significant decreases of $-0.003$ $\mu$m/year and $-0.002$ $\mu$m/year for vertical coma in people with and without diabetes, respectively. ANCOVA did not find significant differences in regression slopes between
the groups for horizontal coma, vertical coma, spherical aberration or HORMS but it was close to be significant for horizontal coma \((F_{1,89} = 3.04, \ p = 0.09)\).

**Internal Aberrations**

Table 4:25 shows the Pearson correlations of internal aberration components with various ocular and systemic factors for the whole group. The only significant correlations were for vertical coma with lens thickness and for spherical aberration with diabetes duration and lens thickness.

**Table 4:25 Pearson correlations of internal aberrations components with different ocular and systemic factors**

<table>
<thead>
<tr>
<th>Internal aberration components</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal coma correlation co-efficient</td>
<td>-0.12</td>
<td>-0.18</td>
<td>-0.18</td>
<td>-0.14</td>
<td>-0.07</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>p values</td>
<td>0.26</td>
<td>0.09</td>
<td>0.09</td>
<td>0.18</td>
<td>0.51</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Vertical coma correlation co-efficient</td>
<td>-0.07</td>
<td>-0.16</td>
<td>0.04</td>
<td>-0.27</td>
<td>0.03</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>p values</td>
<td>0.52</td>
<td>0.14</td>
<td>0.97</td>
<td>&lt; 0.01*</td>
<td>0.76</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td>Spherical aberration correlation co-efficient</td>
<td>-0.01</td>
<td>0.29</td>
<td>0.07</td>
<td>0.23</td>
<td>0.19</td>
<td>-0.22</td>
<td>0.05</td>
</tr>
<tr>
<td>p values</td>
<td>0.96</td>
<td>0.01*</td>
<td>0.52</td>
<td>0.03*</td>
<td>0.07</td>
<td>0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>HORMS correlation co-efficient</td>
<td>0.13</td>
<td>0.06</td>
<td>0.06</td>
<td>0.12</td>
<td>-0.08</td>
<td>0.023</td>
<td>0.19</td>
</tr>
<tr>
<td>p values</td>
<td>0.22</td>
<td>0.55</td>
<td>0.55</td>
<td>0.25</td>
<td>0.47</td>
<td>0.83</td>
<td>0.07</td>
</tr>
</tbody>
</table>

For horizontal coma, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group and then for only the diabetes group. Neither factor contributed significantly to the fit in either case.
For vertical coma, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Neither factor contributed significantly to the fit. When the multiple regression analysis was restricted to the diabetes group, diabetes duration alone contributed significantly to the fit:

\[ y = -0.0052(\pm 0.0020)\text{DiaDur} + 0.084(\pm 0.065), R^2 = 0.14 \]

For spherical aberration, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Only diabetes duration factor contributed significantly to the fit.

\[ y = +0.0018(\pm 0.0006)\text{DiaDur} - 0.021(\pm 0.038), R^2 = 0.08 \]

When the multiple regression analysis was restricted to the diabetes group, again diabetes duration contributed significantly to the fit:

\[ y = +0.0043(\pm 0.0013)\text{DiaDur} - 0.023(\pm 0.042), R^2 = 0.21 \]

For HORMS, multiple regression analysis was performed with age and diabetes duration as predictors using the whole group and then only the diabetes group. Neither diabetes duration nor age contributed significantly to the fit in either case.
Figure 4.29 Relationships between age and internal aberration components for people with and without diabetes. a) Horizontal coma linear fit, diabetes group: \( Y = +0.000(\pm0.002)\text{Age} + 0.08(\pm0.08), R^2 0.00, p 0.96 \); non-diabetes group: \( Y = -0.003(\pm0.002)\text{Age} + 0.27(\pm0.08), R^2 0.07, p 0.08 \). b) Vertical coma linear fit, diabetes group: \( Y = +0.000(\pm0.002)\text{Age} + 0.07 (\pm0.07), R^2 0.00, p 0.89 \); non-diabetes group: \( Y = -0.001(\pm0.001)\text{Age} + 0.11(\pm0.07), R^2 0.01, p 0.43 \). c) Spherical aberration linear fit, diabetes group: \( Y = -0.000(\pm0.001)\text{Age} - 0.01(\pm0.05), R^2 0.00, p 0.99 \); non-diabetes group: \( Y = +0.000(\pm0.001)\text{Age} - 0.02(\pm0.03), R^2 0.00, p 0.99 \). d) HORMS linear fit, diabetes group: \( Y = +0.002(\pm0.001)\text{Age} + 0.20(\pm0.06), R^2 0.03, p 0.22 \); non-diabetes group: \( Y = +0.001(\pm0.001)\text{Age} + 0.25(\pm0.06), R^2 0.01, p 0.62 \). Values in brackets are standard errors.

Figure 4.29 shows the internal ocular aberrations as a function of age. There was no significant difference between the groups for any aberration component. There was no significant rates of change with age for any
combination of group and aberration component, and ANCOVA did not find any significant differences in regression slopes between groups.

**Discussion**

For only two coefficient/component combinations, total ocular horizontal coma and total ocular vertical coma, were there significant differences between diabetes and non-diabetes groups (Table 4:22), and there were no significant differences of higher-order RMS between the groups.

Across the various aberration component and whole group/diabetes group combinations, only a few were associated significantly with diabetes: horizontal coma (total), vertical coma (internal, total), spherical aberration (internal, total) and HORMS (total). For the diabetes group alone, both internal and total ocular vertical coma and spherical aberration changed significantly as a function of diabetes duration.

Shahidi et al. (2004) and Valeshabad et al. (2014) reported greater higher-order RMS aberrations in diabetic groups than in control groups for 6 mm and 5 mm pupils, respectively. Diabetic type was not specified, but all had diabetic retinopathy. The smaller pupil size of 4.5 mm was used for this study as it was large enough to include assessment of all but one participant.

In conclusion, in this study diabetes appears to have had minor effects on aberrations.
4.6 Lens Yellowing

The characteristics of the sub-groups for flicker photometry testing are summarised in Table 4:26. There were 30 participants with diabetes and 41 age-matched participants without diabetes. Eight participants in the diabetes group and four participants in the non-diabetes group were not able to perform the testing.

Table 4:26 Characteristics of participants for lens yellowing

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>30</td>
<td>41</td>
<td>0.48</td>
</tr>
<tr>
<td>Age (mean ± SD, age range), years</td>
<td>39 ± 12, 19 – 63</td>
<td>40 ± 11, 20 – 62</td>
<td>0.64</td>
</tr>
<tr>
<td>Number of eyes (R/L)</td>
<td>24/6</td>
<td>35/6</td>
<td>0.55</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>11/19</td>
<td>29/12</td>
<td>0.01*</td>
</tr>
<tr>
<td>Visual acuity (Log-MAR)</td>
<td>-0.05 ± 0.26</td>
<td>-0.03 ± 0.24</td>
<td>0.74</td>
</tr>
<tr>
<td>Log contrast sensitivity</td>
<td>1.84 ± 0.12</td>
<td>1.91 ± 0.08</td>
<td>0.02*</td>
</tr>
<tr>
<td>Spherical equivalent refraction (dioptres)</td>
<td>-0.61 ± 1.17</td>
<td>-0.55 ± 0.83</td>
<td>0.43</td>
</tr>
<tr>
<td>Anterior chamber depth (mm)</td>
<td>2.82 ± 0.35</td>
<td>2.90 ± 0.36</td>
<td>0.39</td>
</tr>
<tr>
<td>Lens thickness (mm)</td>
<td>4.13 ± 0.44</td>
<td>3.98 ± 0.33</td>
<td>0.12</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>23.66 ± 0.90</td>
<td>23.66 ± 0.78</td>
<td>0.99</td>
</tr>
<tr>
<td>Log lens yellowing</td>
<td>1.72 ± 0.34</td>
<td>1.49 ± 0.28</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>HbA1c (m mol)</td>
<td>7.77 ± 1.20</td>
<td>5.03 ± 0.35</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>18 ± 11</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Table 4:27 shows the Pearson correlations of lens yellowing with various ocular and systemic factors for the whole group. Lens yellowing was significantly correlated with age, duration of diabetes, HbA1c and lens thickness but not with spherical equivalent refraction, axial length and gender. The correlations was higher for age than for diabetes duration.
Table 4: Pearson correlations of log lens yellowing with different ocular and systemic parameters

<table>
<thead>
<tr>
<th>Correlation co-efficient</th>
<th>Age</th>
<th>Diabetes duration</th>
<th>HbA1c</th>
<th>Lens thickness</th>
<th>Spherical equivalent refraction</th>
<th>Axial length</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.63</td>
<td>0.48</td>
<td>0.40</td>
<td>0.62</td>
<td>0.11</td>
<td>~0.15</td>
<td>0.68</td>
</tr>
</tbody>
</table>

* indicates significance

Multiple regression analysis was performed with age and diabetes duration as predictors using the whole group. Both factors contributed significantly to the fit:

\[ y = 0.011(\pm0.002)\text{DiaDur} + 0.016(\pm0.002)\text{Age} + 0.87(\pm0.09), R^2 = 0.56 \]

When the multiple regression analysis was restricted to the diabetes group, only age contributed significantly to the lens yellowing fit:

\[ y = 0.008(\pm0.004)\text{Age} + 0.97(\pm0.15), R^2 = 0.53 \]
Figure 4.30 Relationships between age and lens yellowing for people with and without diabetes. Fit for diabetes group: $Y = +0.019(\pm 0.004)\text{Age} + 1.00(\pm 0.15)$, $R^2 0.47$, $p < 0.001$; fit for non-diabetes group: $Y = +0.018(\pm 0.003)\text{Age} + 0.78(\pm 0.12)$, $R^2 0.49$, $p < 0.001$. Values in brackets are standard errors.

Figure 4.30 shows lens yellowing as a function of age. People with diabetes had significantly higher lens yellowing than people without diabetes with mean difference $0.23 \pm 0.15$ logs (mean $\pm 95\%$ CI). There were significant, similar increases of $0.019$ log yellowing/year and $0.018$ log yellowing/year in people with and without diabetes, respectively.

**Discussion**

Lens yellowing was higher in the diabetes group than in the non-diabetes group, but the rates of change with age were similar.

Figure 4.31 compares my results with those of previous studies (Davies & Morland, 2002; Lutze & Bresnick, 1991). When comparing the studies, attention can be paid only to the slopes as I have no absolute scale. All
studies have greater lens yellowing in the diabetes group. Similar to this study, Davies and Morland (2002) found a significant effect of age but not diabetes duration on lens yellowing in their diabetes group (10 DM1 and 24 DM2 participants). However, both studies (Davies & Morland, 2002; Lutze & Bresnick, 1991) found greater rates of increase of lens yellowing with age in DM1 groups than in non-diabetes groups.

Figure 4:31 Comparison of lens yellowing from Lutze & Bresnick (1991) and Davies & Morland (2002) with the current study. For clarity the fits only have been shown.
4.7 Lens Dimensions and Refractive Index Distribution

The characteristics of the sub-groups for the MRI investigation are summarised in Table 4:28. There were 17 participants with diabetes (7 young, 10 older) and 23 age-matched participants without diabetes (13 young, 10 older). Selection of the participants has been described in sections 3.1 and 3.5.6.

Two-way ANOVAs were performed to consider the effects of diabetes and age on each of lens equatorial diameter, axial thickness and centre refractive indices. The diabetes group had significantly lower equatorial diameters than the non-diabetes group ($F_{1,36} = 24.5$, $p < 0.01$; diabetes group mean ± 95% CI: $8.65 \pm 0.26$ mm; non-diabetes group $9.42 \pm 0.18$ mm), and significantly greater axial thickness than the non-diabetes group ($F_{1,36} = 11.9$, $p < 0.01$; diabetes group mean ± 95% CI: $4.33 \pm 0.30$ mm; non-diabetes group $3.80 \pm 0.14$ mm). The centre refractive indices of the two groups were not significantly different ($F_{1,36} = 3.0$, $p = 0.09$).

Age group did not affect equatorial diameter significantly ($F_{1,36} = 0.10$, $p = 0.20$). The older group had significantly greater axial thickness than the young group, as was expected from section 4.2.11 ($F_{1,36} = 22.4$, $p < 0.01$; older group mean ± 95% CI: $4.35 \pm 0.26$ mm; young group $3.70 \pm 0.25$ mm) (Figure 4:32). The older group had significantly lower centre refractive index than the young group ($F_{1,36} = 4.14$, $p = 0.04$; older group mean ± 95% CI: $1.398 \pm 0.003$; younger group $1.401 \pm 0.004$).
Table 4.28 Characteristics of participants for lens dimensions and refractive index distribution

<table>
<thead>
<tr>
<th></th>
<th>People with diabetes</th>
<th>People without diabetes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of young participants</td>
<td>7</td>
<td>13</td>
<td>0.40</td>
</tr>
<tr>
<td>Number of older participants</td>
<td>10</td>
<td>10</td>
<td>1.00</td>
</tr>
<tr>
<td>Young group (mean ± SD, age range), years</td>
<td>23 ± 4, 20 − 30</td>
<td>24 ± 4, 20 − 29</td>
<td>0.32</td>
</tr>
<tr>
<td>Older group (mean ± SD, age range), years</td>
<td>54 ± 4, 48 − 59</td>
<td>55 ± 4, 50 − 61</td>
<td>0.31</td>
</tr>
<tr>
<td>Young group, number of eyes (R/L)</td>
<td>6/1</td>
<td>13/0</td>
<td>0.16</td>
</tr>
<tr>
<td>Older group, number of eyes (R/L)</td>
<td>9/1</td>
<td>9/1</td>
<td>1.00</td>
</tr>
<tr>
<td>Young group, gender (F/M)</td>
<td>2/5</td>
<td>7/6</td>
<td>0.28</td>
</tr>
<tr>
<td>Older group, gender (F/M)</td>
<td>6/4</td>
<td>6/4</td>
<td>1.00</td>
</tr>
<tr>
<td>Young group, visual acuity (Log-MAR)</td>
<td>-0.03 ± 0.07</td>
<td>0.00 ± 0.30</td>
<td>0.83</td>
</tr>
<tr>
<td>Older group, visual acuity (Log-MAR)</td>
<td>-0.05 ± 0.05</td>
<td>-0.07 ± 0.30</td>
<td>0.23</td>
</tr>
<tr>
<td>Young group, log contrast sensitivity</td>
<td>1.85 ± 0.12</td>
<td>1.91 ± 0.09</td>
<td>0.05*</td>
</tr>
<tr>
<td>Older group, log contrast sensitivity</td>
<td>1.88 ± 0.09</td>
<td>1.87 ± 0.10</td>
<td>0.82</td>
</tr>
<tr>
<td>Young group, spherical equivalent refractive (dioptres)</td>
<td>-0.39 ± 0.84</td>
<td>-0.72 ± 1.07</td>
<td>0.32</td>
</tr>
<tr>
<td>Older group, spherical equivalent refractive (dioptres)</td>
<td>-0.06 ± 1.21</td>
<td>-0.36 ± 1.11</td>
<td>0.62</td>
</tr>
<tr>
<td>Young group, MRI lens equatorial diameter (mm)</td>
<td>8.68 ± 0.51</td>
<td>9.53 ± 0.35</td>
<td>0.00*</td>
</tr>
<tr>
<td>Young group, MRI axial lens thickness (mm)</td>
<td>3.93 ± 0.29</td>
<td>3.58 ± 0.11</td>
<td>0.02*</td>
</tr>
<tr>
<td>Young group, MRI lens centre refractive index</td>
<td>1.400 ± 0.007</td>
<td>1.403 ± 0.008</td>
<td>0.42</td>
</tr>
<tr>
<td>Young group, MRI lens anterior axial thickness (mm)</td>
<td>1.22 ± 0.29</td>
<td>1.13 ± 0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>Young group, MRI lens posterior axial thickness (mm)</td>
<td>2.71 ± 0.12</td>
<td>2.45 ± 0.18</td>
<td>0.00*</td>
</tr>
<tr>
<td>Older group, MRI lens equatorial diameter (mm)</td>
<td>8.63 ± 0.56</td>
<td>9.27 ± 0.47</td>
<td>0.01*</td>
</tr>
<tr>
<td>Older group, MRI lens axial thickness (mm)</td>
<td>4.61 ± 0.65</td>
<td>4.10 ± 0.33</td>
<td>0.04*</td>
</tr>
<tr>
<td>Older group, MRI lens centre refractive index</td>
<td>1.397 ± 0.005</td>
<td>1.398 ± 0.005</td>
<td>0.55</td>
</tr>
<tr>
<td>Older group, MRI lens anterior axial thickness (mm)</td>
<td>1.67 ± 0.27</td>
<td>1.37 ± 0.28</td>
<td>0.03*</td>
</tr>
<tr>
<td>Older group, MRI lens posterior axial thickness (mm)</td>
<td>2.94 ± 0.50</td>
<td>2.72 ± 0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>Young group, HbA1c (m mol)</td>
<td>7.60 ± 0.92</td>
<td>4.95 ± 0.26</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Older group, HbA1c (m mol)</td>
<td>7.92 ± 0.99</td>
<td>5.10 ± 0.28</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Young group, diabetes duration (years)</td>
<td>17 ± 4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Older group, diabetes duration (years)</td>
<td>30 ± 12</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± standard deviation
Figure 4:32 Characteristic MSE images from each of the groups: a) 30 year young female with 24 year diabetes duration, b) 58 year older male with 35 year diabetes duration, c) 28 years young male non-diabetes, and d) 52 years older male non-diabetes. These demonstrate the main findings in this section of greater axial thickness for older than for young lenses (b versus a and d versus c) and smaller equatorial diameter and greater axial thickness for diabetes than for non-diabetes lenses (a versus c and b versus d).

The combined normalised refractive index profile data of each group (young with DM1, young without DM1, older DM1, older without DM1) were fitted by the power equation (3.51) for different dimensions, and the average refractive index along the axis of the lens was determined with equation (3.52).

Table 4:29 shows fitted refractive index co-efficients for the equatorial diameter line, and for the optical axis, together with the average axial refractive index. In this table, the first normalisation approach described in section 3.5.6 was used for fitting the axial data, in which the profiles are folded about the mid-point of the lens axis and no distinction is made between the anterior and posterior segments. Table 4:30 shows the fitted refractive index co-efficients for the optical axis obtained using the second
normalisation approach for fitting the axial data described in section 3.5.6, in which the anterior and posterior segments are fitted separately, together with the corresponding average axial refractive index. Figure 4:33 shows refractive index data along the normalised equatorial diameter line and the optical axis (first normalisation approach). Figure 4:34 shows the data for the equatorial diameter line and optical axis, together with fits according to equation (3.51).

Table 4:29 Co-efficients of fit $n(r) = C_o + C_p r^p$ to refractive index data along equatorial diameter line and optical axis of different groups, together with the average refractive index along the axial direction. Numbers in brackets are standard errors. * significantly different from older group with diabetes; # significantly different from older group without diabetes.

<table>
<thead>
<tr>
<th>Participant group</th>
<th>$C_o$ equator</th>
<th>$C_p$ equator</th>
<th>$p$ equator</th>
<th>$C_o$ axial</th>
<th>$C_p$ axial</th>
<th>$p$ axial</th>
<th>$n_0$ axial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young group with diabetes</td>
<td>1.4004* (0.0010)</td>
<td>−0.0309 (0.0026)</td>
<td>2.8022* (0.4522)</td>
<td>1.4016* (0.0017)</td>
<td>−0.0448* (0.0061)</td>
<td>2.5541 (0.5621)</td>
<td>1.3890 (0.0054)</td>
</tr>
<tr>
<td>Young group no diabetes</td>
<td>1.4015* (0.0006)</td>
<td>−0.0432 (0.0040)</td>
<td>4.4812* (0.5228)</td>
<td>1.3988 (0.0012)</td>
<td>−0.0297 (0.0027)</td>
<td>3.6039 (0.6518)</td>
<td>1.3923 (0.0027)</td>
</tr>
<tr>
<td>Older group with diabetes</td>
<td>1.3974 (0.0006)</td>
<td>−0.0318 (0.0043)</td>
<td>8.6327 (1.5302)</td>
<td>1.3967 (0.0010)</td>
<td>−0.0281 (0.0028)</td>
<td>3.5286 (0.6390)</td>
<td>1.3905 (0.0025)</td>
</tr>
<tr>
<td>Older group no diabetes</td>
<td>1.3978 (0.0006)</td>
<td>−0.0608 (0.0123)</td>
<td>9.5572 (1.5638)</td>
<td>1.3961 (0.0010)</td>
<td>−0.0235 (0.0025)</td>
<td>4.9400 (1.0944)</td>
<td>1.3921 (0.0021)</td>
</tr>
</tbody>
</table>

Table 4:30 Co-efficients of fit $n(r) = C_o + C_p r^p$ to anterior axial and posterior axial refractive index data of different groups using the second approach to normalisation along the optical axis. Numbers in brackets are standard errors. * significantly different from older group without diabetes.

<table>
<thead>
<tr>
<th>Participant group</th>
<th>$C_o$ anterior axial</th>
<th>$C_p$ anterior axial</th>
<th>$p$ anterior axial</th>
<th>$C_o$ posterior axial</th>
<th>$C_p$ posterior axial</th>
<th>$p$ posterior axial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young group with diabetes</td>
<td>1.3993 (0.0040)</td>
<td>−0.0501 (0.0111)</td>
<td>2.0717 (0.8565)</td>
<td>1.4008 (0.0013)</td>
<td>−0.0391* (0.0085)</td>
<td>5.2397 (1.5146)</td>
</tr>
<tr>
<td>Young group no diabetes</td>
<td>1.3995 (0.0030)</td>
<td>−0.0524 (0.0095)</td>
<td>2.3139 (0.7401)</td>
<td>1.3991 (0.0011)</td>
<td>−0.0219 (0.0031)</td>
<td>4.2919 (1.1320)</td>
</tr>
<tr>
<td>Older group with diabetes</td>
<td>1.3951 (0.0018)</td>
<td>−0.0443 (0.0137)</td>
<td>4.5203 (1.6944)</td>
<td>1.3967 (0.0001)</td>
<td>−0.0237 (0.0026)</td>
<td>4.3872 (0.9786)</td>
</tr>
<tr>
<td>Older group no diabetes</td>
<td>1.4014 (0.0022)</td>
<td>−0.0498 (0.0088)</td>
<td>2.8434 (0.7970)</td>
<td>1.3959 (0.0010)</td>
<td>−0.0190 (0.0028)</td>
<td>5.0866 (1.4774)</td>
</tr>
</tbody>
</table>

There was considerable variation within groups. The central plateaus appeared wider for the older than for the younger groups, particularly along the equatorial diameter line.

Chapter 4: Results
Unpaired t-tests were used to compare groups for each of the directions, in which the standard deviations were not assumed to be equal and Bonferroni correction was applied as there were 6 pair-wise comparisons per direction. There were a few significant differences only (Table 4:29 and Table 4:30). These involved the following: both young groups compared with both older groups - $p$ equatorially; both young groups compared with the older diabetes group - $C_o$ equatorially; young diabetes group compared with both older groups – $C_P$ axially; young diabetes group compared with older group without diabetes - $C_o$ axially, $C_P$ posterior axially and $p$ posterior axially. There were no significant differences between diabetes and non-diabetes groups of the same age.

**Discussion**

The interesting finding was the smaller equatorial diameters in the group with diabetes than in the control group. The refractive index data within groups were highly variable. A few statistically significant differences were found between young and older groups, although not between diabetes and non-diabetes groups of the same age.

Comparing the non-diabetic lenses in this study with the findings of Kasthurirangan et al. (2008) for unaccommodated lenses, there was agreement in that there were similar axial thickness changes with age, there was no change in centre refractive index with age, and the rate of decline in refractive index from centre to the periphery was greater along the equatorial diameter line than along the optical axis. However, there were some differences: Kasthurirangan et al.’s mean diameter was 0.23 mm smaller than found here, their central indices were about 0.007 higher, differences between centre and edge refractive indices were about 0.005 greater, and
they found significantly larger lens diameters in the older group than in the younger group whereas there was no difference in the current study.

Figure 4: Normalised refractive index profiles for diabetes and non-diabetes groups: (a) young groups, equatorial diameter line, (b) older groups, equatorial diameter line, (c) young groups, axial, and (d) older groups, axial. Positive distances correspond to nasal/anterior parts of the lens.
Figure 4: Normalised refractive index profiles for diabetes and non-diabetes groups, together with fits: (a) young groups, equatorial diameter line, (b) older groups, equatorial diameter line, (c) young groups, axial, (d) older groups, axial, (e) young groups, anterior axial, (f) older groups, anterior axial, (g) young groups, posterior axial, and (h) older groups, posterior axial.


4.8 Summary

Table 4:31 shows multiple regression fits for biometric and other parameters, both for the whole group and for the diabetes group. The emphasis is on diabetes duration and age as factors, but axial length is included where it has significant effects in regression fits that includes diabetes duration and/or age; otherwise no factors are shown. These equations have been presented in earlier sections, but for simplicity here, standard errors are omitted and aberration components are listed only when there are significant effects of diabetes duration and/or age.

In the multiple regression fits shown in Table 4:31, duration of diabetes was an occasional significant contributor to the variation in a parameter – ignoring the aberration parameters for which effects were small, it was significant for 11/21 parameters for the whole group and 8/21 parameters for the diabetes group. For the whole group, this may because it was nothing more than a proxy for having diabetes. To investigate this, diabetes duration was replaced by diabetes status in regressions. Diabetes status refers to the presence or absence of diabetes status, and values of 1 and 0 were assigned to these states, respectively. Table 4:32 shows results, and includes parameters from Table 4:31 for which diabetic duration was a significant factor (there were no parameters for which diabetes status and not diabetes duration was a significant factor).

For six parameters, swapping from diabetes duration to diabetes status meant that that diabetes was lost as a significant factor and its contribution was replaced to some extent by changes in the age co-efficient. For seven parameters, diabetes was retained as a significant factor, but with some lowering of the regression fit (i.e. $R^2$ reduced) and compensatory increase in
the age co-efficient of up to 40%. These findings indicate that diabetes duration provided more information than the presence of diabetes alone, and was worthwhile including in regression analyses.

Table 4:31 Multiple regression fits in the whole group and in the diabetes group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Dur’n (yrs)</th>
<th>Age (years)</th>
<th>Axial length (mm)</th>
<th>constant (D/mm/-/logs/μm)</th>
<th>R-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical equivalent refraction (D)</td>
<td>Whole Group</td>
<td>+0.020</td>
<td>−0.538</td>
<td>+11.45</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.025</td>
<td>−0.660</td>
<td>+14.11</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Anterior corneal radius of curvature (mm), Pentacam</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior corneal radius of curvature (mm), Medmont</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior corneal asphericity Q</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.004</td>
<td>−0.68</td>
<td></td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Corneal centre thickness (mm), Pentacam</td>
<td>Whole Group</td>
<td>+0.0005</td>
<td>+0.552</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corneal centre thickness (mm), Lenstar</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior corneal radius of curvature (mm)</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Pentacam</td>
<td>Whole Group</td>
<td>−0.009</td>
<td>−0.010</td>
<td>+0.147</td>
<td>−0.21</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.016</td>
<td>−0.007</td>
<td>+0.146</td>
<td>−0.12</td>
<td>0.54</td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Lenstar</td>
<td>Whole Group</td>
<td>−0.008</td>
<td>−0.011</td>
<td>+0.179</td>
<td>−0.87</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.013</td>
<td>−0.010</td>
<td>+0.187</td>
<td>−1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Pupil diameter (mm)</td>
<td>Whole Group</td>
<td>−0.029</td>
<td>+0.280</td>
<td>+0.76</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.025</td>
<td>+0.305</td>
<td>−0.07</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Pupil decentration along x-axis (mm)</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupil decentration along y-axis (mm)</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior lens radius of curvature (mm)</td>
<td>Whole Group</td>
<td>−0.045</td>
<td>−0.026</td>
<td>+0.460</td>
<td>+0.72</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.040</td>
<td></td>
<td>+0.418</td>
<td>+0.98</td>
<td>0.40</td>
</tr>
<tr>
<td>Posterior lens radius of curvature (mm)</td>
<td>Whole Group</td>
<td>+0.017</td>
<td>+0.012</td>
<td>−0.270</td>
<td>−0.35</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lens central thickness, Lenstar (mm)</td>
<td>Whole Group</td>
<td>+0.016</td>
<td>+0.021</td>
<td>−0.091</td>
<td>+5.35</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.020</td>
<td>+0.021</td>
<td>−0.113</td>
<td>+5.69</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Chapter 4: Results
Table 4:31 (cont.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Dur’n (yrs)</th>
<th>Age (years)</th>
<th>Axial length (mm)</th>
<th>constant (D/mm/-/logs/µm)</th>
<th>R-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens equivalent refractive index</td>
<td>Whole Group</td>
<td>−0.0003</td>
<td>−0.0004</td>
<td>−0.0033</td>
<td>+1.525</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.0003</td>
<td>−0.0041</td>
<td>−1.997</td>
<td>+1.537</td>
<td>0.28</td>
</tr>
<tr>
<td>Lens equivalent power (D)</td>
<td>Whole Group</td>
<td>−0.046</td>
<td>−1.997</td>
<td>73.70</td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.046</td>
<td>−1.997</td>
<td>73.70</td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>Straylight (logs)</td>
<td>Whole Group</td>
<td>+0.006</td>
<td>+0.005</td>
<td>+0.61</td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.007</td>
<td>+0.004</td>
<td>+0.67</td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Objective amplitude of accommodation (D)</td>
<td>Whole Group</td>
<td>−0.083</td>
<td>−0.145</td>
<td>8.92</td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.076</td>
<td>−0.097</td>
<td>7.13</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>Subjective amplitude of accommodation (D)</td>
<td>Whole Group</td>
<td>−0.105</td>
<td>−0.154</td>
<td>10.67</td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.106</td>
<td>−0.103</td>
<td>9.08</td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Total horizontal coma (µm)</td>
<td>Whole Group</td>
<td>−0.0017</td>
<td>+0.025</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.0017</td>
<td>+0.025</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Total vertical coma (µm)</td>
<td>Whole Group</td>
<td>−0.0030</td>
<td>−0.0021</td>
<td>+0.121</td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.0045</td>
<td>−0.0021</td>
<td>+0.123</td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Total spherical aberration (µm)</td>
<td>Whole Group</td>
<td>+0.0017</td>
<td></td>
<td>+0.031</td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.0036</td>
<td></td>
<td>+0.022</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Total HORMS (µm)</td>
<td>Whole Group</td>
<td>+0.0018</td>
<td></td>
<td>+0.155</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.0044</td>
<td></td>
<td>+0.127</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Internal vertical coma (µm)</td>
<td>Whole Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>−0.0052</td>
<td></td>
<td>+0.084</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Internal spherical aberration (µm)</td>
<td>Whole Group</td>
<td>+0.0018</td>
<td>−0.021</td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.0043</td>
<td>−0.021</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Log lens yellowing</td>
<td>Whole Group</td>
<td>+0.011</td>
<td>+0.016</td>
<td>+0.87</td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Diabetes group</td>
<td>+0.008</td>
<td>+0.97</td>
<td></td>
<td></td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 4: Multiple regression fits in the whole group with either diabetes duration or diabetes status as factors, and where diabetes duration was a significant factor.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Dur’n(yrs)/Diabetes status</th>
<th>Age (years)</th>
<th>Axial length (mm)</th>
<th>constant (D/mm/-/logs/μm)</th>
<th>R-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal centre thickness (mm), Pentacam</td>
<td>Diabetes dur’n</td>
<td>+0.0005</td>
<td></td>
<td></td>
<td>+0.552</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Pentacam</td>
<td>Diabetes dur’n</td>
<td>−0.009</td>
<td>−0.010</td>
<td>+0.147</td>
<td>−0.21</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior chamber depth (mm), Lenstar</td>
<td>Diabetes dur’n</td>
<td>−0.008</td>
<td>−0.011</td>
<td>+0.179</td>
<td>−0.87</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior lens radius of curvature (mm)</td>
<td>Diabetes dur’n</td>
<td>−0.045</td>
<td>−0.026</td>
<td>+0.460</td>
<td>+0.72</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−1.031</td>
<td>−0.036</td>
<td>+0.524</td>
<td>−1.01</td>
<td>0.45</td>
</tr>
<tr>
<td>Posterior lens radius of curvature (mm)</td>
<td>Diabetes dur’n</td>
<td>+0.017</td>
<td>+0.012</td>
<td>−0.270</td>
<td>−0.35</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>+0.394</td>
<td>+0.015</td>
<td>−0.293</td>
<td>+0.01</td>
<td>0.25</td>
</tr>
<tr>
<td>Lens central thickness, Lenstar (mm)</td>
<td>Diabetes dur’n</td>
<td>+0.016</td>
<td>+0.021</td>
<td>−0.091</td>
<td>+5.35</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>+0.249</td>
<td>+0.025</td>
<td>−0.127</td>
<td>+6.01</td>
<td>0.61</td>
</tr>
<tr>
<td>Lens equivalent refractive index</td>
<td>Diabetes dur’n</td>
<td>−0.0003</td>
<td>−0.0004</td>
<td>−0.0033</td>
<td>+1.525</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−0.0063</td>
<td>−0.0004</td>
<td>−0.0031</td>
<td>+1.522</td>
<td>0.35</td>
</tr>
<tr>
<td>Straylight (logs)</td>
<td>Diabetes dur’n</td>
<td>+0.006</td>
<td>+0.005</td>
<td></td>
<td>+0.61</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>+0.113</td>
<td>+0.007</td>
<td></td>
<td>+0.85</td>
<td>0.30</td>
</tr>
<tr>
<td>Objective amplitude of accommodation (D)</td>
<td>Diabetes dur’n</td>
<td>−0.083</td>
<td>−0.145</td>
<td></td>
<td>8.92</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−1.531</td>
<td>−0.160</td>
<td></td>
<td>9.57</td>
<td>0.56</td>
</tr>
<tr>
<td>Subjective amplitude of accommodation (D)</td>
<td>Diabetes dur’n</td>
<td>−0.105</td>
<td>−0.154</td>
<td></td>
<td>10.67</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−1.783</td>
<td>−0.171</td>
<td></td>
<td>11.47</td>
<td>0.61</td>
</tr>
<tr>
<td>Total horizontal coma (µm)</td>
<td>Diabetes dur’n</td>
<td>−0.0017</td>
<td></td>
<td></td>
<td>+0.025</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−0.0400</td>
<td></td>
<td></td>
<td>+0.043</td>
<td>0.05</td>
</tr>
<tr>
<td>Total vertical coma (µm)</td>
<td>Diabetes dur’n</td>
<td>−0.0030</td>
<td>−0.0021</td>
<td></td>
<td>+0.121</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>−0.0451</td>
<td>−0.0026</td>
<td></td>
<td>+0.136</td>
<td>0.24</td>
</tr>
<tr>
<td>Total spherical aberration (µm)</td>
<td>Diabetes dur’n</td>
<td>+0.0017</td>
<td></td>
<td></td>
<td>+0.031</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total HORMS (µm)</td>
<td>Diabetes dur’n</td>
<td>+0.0018</td>
<td></td>
<td></td>
<td>+0.155</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal spherical aberration (µm)</td>
<td>Diabetes dur’n</td>
<td>+0.0018</td>
<td></td>
<td>−0.021</td>
<td>+0.87</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td></td>
<td></td>
<td></td>
<td>+0.021</td>
<td>0.08</td>
</tr>
<tr>
<td>Log lens yellowing</td>
<td>Diabetes dur’n</td>
<td>+0.011</td>
<td>+0.016</td>
<td></td>
<td>+0.87</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Diabetes status</td>
<td>+0.256</td>
<td>+0.018</td>
<td></td>
<td>+0.76</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Chapter 4: Results
Chapter 5: Discussion

5.1 Summary

This study follows other studies of optics of diabetic eyes in recent decades, most notably that of Wiemer and colleagues (Wiemer, et al., 2008c; Wiemer, et al., 2007, 2008d; Wiemer, et al., 2009; Wiemer, et al., 2008a, 2008b). While there have been studies that have considered larger numbers of people, this is the only comprehensive study of a large range of biometric and optical parameters.

The hypothesis of this thesis was that eyes of people with diabetes act as older eyes than those of people of the same age without diabetes. An associated list of aims was presented in chapter 1; these are addressed below. A group of up to 74 people with diabetes type 1 were compared with an age matched control group. Based on the results of various biometric and optical parameters that have been presented and discussed in Chapter 4, it is easy to conclude that the hypothesis has been supported. Relative to the control group, the diabetes group demonstrated smaller anterior chamber depths, more curved lenses, lower lens equivalent refractive index, greater straylight, lower amplitudes of accommodation, greater lens yellowing and different coefficients for two total ocular higher-order aberrations. Marginal or no significant differences were found between groups for corneal shape, corneal thickness, pupil size, pupil decentrations and other higher-order aberrations (total, corneal and internal).

Not explicitly stated in the hypothesis, but given as part of the aim about lens yellowing (section 5.8), was the expectation that differences between diabetic
eyes and non-diabetic eyes would increase with age. Increased differences with age had been found previously for central lens thickness (Sparrow, et al., 1990; Wiemer, et al., 2008d), for lens yellowing (Davies & Morland, 2002; Lutze & Bresnick, 1991) and for the radii of curvature of the lens and equivalent lens index (Wiemer, et al., 2008d). In the multiple regression fits, duration of diabetes was only an occasional significant contributor to the variation in a parameter, and in the case of combining diabetic and non-diabetic eyes, it was suspected that this may have been only because it was really a stand-in for having diabetes; however the analysis in section 4.8 indicated that diabetes duration gives more information than the presence or absence of diabetes. The ANCOVA analyses showed that the slopes of a parameter against age were significantly different for the two groups only for amplitude of accommodation (section 4.4). They were nearly significant for anterior corneal asphericity (4.2.3), central corneal thickness (4.2.4), anterior chamber depth (4.2.6), lens thickness (4.2.11), posterior lens radius of curvature, lens equivalent refractive index (4.2.10), and total ocular horizontal coma co-efficient (4.5), with p values ranging between p 0.07 and 0.11 suggesting insufficient power of testing. The differences in slope were opposite to the expected direction for amplitude of accommodation (4.4, 5.3), posterior lens radius of curvature (4.2.10), and lens equivalent refractive index (4.2.12).

The conclusion to be drawn is that the changes in this type 1 diabetes group, relative to the control group, were small. While this is unfortunate in the context of testing my hypothesis and addressing the thesis aims, it indicates that people with well-controlled diabetes relative to neuropathy (section 3.1) (and who are striving especially hard to remain compliant with health/lifestyle/medications given that they were under close scrutiny in the LANDMark study) need not have accelerated changes in the optics of the eye.
with ageing, and presumably the serious consequences of diabetes can be slowed.

One very interesting finding of the study is that, in the MRI investigation (section 4.8), lens diameters were smaller in the diabetes group than non-diabetic group. This may affect functions such as accommodation where the zonules may be exerting greater pressure on the lens. This has implications concerning accommodation restoration with scleral expansion surgery in people with diabetes. The numbers for the investigation were small in this study (40 across two ages) and further investigation is warranted to study changes in the lens diameter, movements of the zonule and ciliary muscle during accommodation.

5.2 Do People with Diabetes have Greater Higher-order Aberrations than Age-matched Controls?

I did not find evidence that ocular aberrations, nor the corneal and internal components of these aberrations, were higher in people with diabetes than people without diabetes. Two higher-order aberrations, horizontal and vertical coma, had significantly different coefficients for the two groups.

It is generally understood that the aberrations of the eye increase with age, although such changes were not marked in the few studies in which there was control for the refraction distribution (Atchison & Markwell, 2008; Plainis & Pallikaris, 2008). Thus, if diabetic eyes behave like older eyes, it might be expected that ocular aberrations would be higher in the diabetes group than in the control group. In particular, the internal contribution to aberrations, which will be determined by the lens, might also increase.
Shahidi et al. (2004) and Valeshabad et al. (2014) reported greater higher-order RMS aberrations in diabetic groups (with diabetic retinopathy) than in control groups for 6 mm and 5 mm pupils, respectively. The pupils were dilated in these studies. An unresolved question is whether I would have obtained significant differences at a larger pupil than the 4.5 mm pupil selected because it was large enough to include all but one participant. I did not dilate pupils because I thought that there might be interactions between natural pupil size, decentration and aberrations. In fact I found that the differences in pupil size between the groups were not significant, although there was a tendency for diabetic pupils to be smaller (section 4.2.7), and that there was no tendency for different pupil decentrations (relative to the limbus) in the groups (section 4.2.8).

5.3 Can Loss of Accommodation with Diabetes be Attributed to Changes in the Lens Including Refractive Index Distribution?

Optical and neurological contributions to loss of accommodation were not able to be distinguished in this study. While there were biometric differences between diabetes and non-diabetes participants – lens surface curvatures, lens thickness, equivalent refractive index and amplitude of accommodation – no picture emerged of the contribution to loss of accommodation with diabetes. Refractive index distributions were different between young people and older people equatorially, but there were no significant differences between the diabetes and non-diabetes groups of the same age (section 4.7). Unfortunately, biometric and optical changes with accommodation were not investigated.
Previous studies of amplitude of accommodation in diabetes have used subjective methods (Braun, et al., 1995; Moss, et al., 1987). These overestimate amplitude because of depth-of-focus effects (Hamasaki, Ong, & Marg, 1956; Sun et al., 1988). Accordingly they will interact with pupil size, which had been expected to be smaller with diabetes. Therefore, I used the aberrometer to measure amplitude objectively (section 4.4). While as expected, objective amplitudes were smaller than subjective amplitudes, trends and dependencies were similar for the two methods.

The Moss et al. (1987) study found that subjective amplitudes were smaller in a diabetic group than in a control group by about 1.8 D, but the rates of loss with age were similar for both at about 0.25 D/year. This suggests that the onset of presbyopia, the loss of adequate near vision focus due to loss of accommodation, would occur a few years earlier in people with diabetes.

The current study found that, while people with diabetes had lower amplitudes than those without diabetes, the rate of loss for the former with increase in age were actually smaller (0.12 vs. 0.23 D/year objectively and 0.13 vs. 0.23 D/year subjectively). The relative loss of accommodation with diabetes appears to be a proportional effect in this group. If the subjective amplitude at which presbyopia occurs is taken to be the fitted value for the control group at 45 years (3.2 D), presbyopia is predicted to occur at 40 years for people with diabetes. Of course, in an individual, this will be affected by the age of onset of diabetes.

Surprisingly, I could find nothing in the clinical literature to compare with this estimate of presbyopia onset in diabetes patients. Leffler et al. (2008) predicted that the preferred reading addition in presbyopes was significantly
related to the duration of diabetes (although not the presence of diabetes) at a rate of 0.06 D per year.

5.4 Do Diabetic Lenses Become Yellower at Greater Rates with Age than Age-matched Normal Lenses?

Lens yellowing was higher in the diabetes group than in the non-diabetes group, but the rates of change with age were similar contrary to previous studies (Davies & Morland, 2002; Lutze & Bresnick, 1991). This is possibly related to the mild levels of diabetic retinopathy of participants in the current study.

5.5 Is the Refractive Index Distribution of the Diabetic Lens Different from that of the Non-diabetic Lens?

I could not find any evidence that this was the case.

The magnetic resonance imaging component of the study included young (20 – 29 years) and older (48 – 61 years) diabetes and non-diabetes groups with approximately 10 subjects in each. Power fits of refractive index (equation (3.51)) were made to the combined data of the participants along the equatorial diameter line and optical axis in each of the groups. There were a few significant differences involving the young groups compared with the older groups, but there were no significant differences within either age group for the people with and without diabetes. The age-related effects in refractive index distribution support the study by Kasthurirangan et al. (2008).
Recently 3-D optical coherence tomography has been developed by the Marcos group to measure lens parameters including the lens gradient index e.g. Siedlecki et al. (2012). With the much higher resolution of OCT than MRI, it will be interesting if subtleties in refractive index distribution in different conditions such as diabetes can be distinguished, although like our method their technique relies on approximations and assumptions.

5.6 Are Corrections to Assumed Refractive Index Needed in Biometric Measurements of Diabetic Eyes?

The answer is no, as argued below.

As mentioned in section 5.5, the magnetic resonance imaging component of the study included young (20 – 29 years) and older (48 – 61 years) diabetes and non-diabetes groups. The average axial refractive index had a 0.003 range between the groups, and the variation within groups (standard deviations 0.008 to 0.014) was much greater than the between-group variation. While the differences between groups were not significant, let us assume that they were. Further, assume that an instrument like the Lenstar is calibrated for a lens refractive index \( n_{\text{ref}} \). The associated optical path length is

\[
OPL = d_L n_{\text{ref}}
\]

where \( d_L \) is the lens central thickness. If this optical path length had been determined for another lens with a refractive index \( n \) and thickness \( (d_L - \Delta d_L) \) the relationship would be

\[
OPL = (d_L - \Delta d_L) n
\]

\( \Delta d_L \), the error in central thickness given by the instrument, can be found by equating the right hand sides of the two equations:

\[
\Delta d_L = d_L (n_{\text{ref}}/n - 1)
\]
If it is assumed that $n_{\text{ref}}$ is 1.3921 and corresponds to the average axial refractive index of the older, non-diabetes group, the average errors for the other groups range between $-0.0001d_L$ and $+0.0022d_L$. If the measured thickness was 5.00 mm, errors would range between $-0.001$ mm and $+0.012$ mm. The precision of the Lenstar (although not necessarily the accuracy) is 0.01 mm, which is similar to the range of these errors.

### 5.7 Do People with Diabetes have More Straylight than People without Diabetes?

Straylight increased with both diabetes duration and age according to the multiple regressions reported in section 4.3, but the rate of change with increase in age was not significantly different than those of the control group.

### 5.8 Limitation of the Study

The participant group has been described in section 3.1, with a summary in section 5.1. The diabetes group may be considered to be “well controlled”. This selection was both a strength and a weakness: a strength in that I was dealing with a relatively homogenous group, and a weakness in that differences between people with and without diabetes were not as marked as would have been otherwise expected. Power tests to determine the necessary size of groups (section 3.2), particularly as regards rate of change of parameters with age, were based on studies including people with more severe ocular complications of diabetes than in this study. As discussed in some sections in chapter 4, the group sizes were not sufficient for some tests and new estimates were given e.g. 159 and 100 participants per group for corneal posterior radius of curvature and for pupil size in sections 4.2.5 and

Chapter 5: Discussion
4.2.7, respectively. Unfortunately, there were not sufficient resources nor time to obtain the additional participants.

5.9 Further Work

This research relates to long term changes in people with diabetes with minimal diabetic retinopathy. It would be interesting to conduct a similar study with people having severe diabetic retinopathy using high resolution optical coherence tomography to study the lens. It would also be interesting use a provocative glucose clamping study to investigate the effect of short term fluctuations of blood glucose on biometrical and optical changes in diabetes. In particular, determination could be made as to whether short term changes mimic the effects of longer term diabetes in regards to changes in equivalent index and surfaces, and loss of accommodation. As reported in section 2.3.8.2, refraction changes during hyperglycaemia may be in either the myopic or hyperopic directions, with Wiemer et al. (2009) suggesting that this may depend upon whether increased lens curvature or reduced equivalent refractive index is dominant. In such a study, young diabetes participants could have blood glucose levels clamped at 5 mmol/l (euglycaemic) and 15 mmol/l (hyperglycaemic) for 2 hours each. Tests could include uncorrected distance visual acuity, corneal topography and Lenstar for the unaccommodated state, and aberrometry and phakometry at a range of accommodation levels.

In this study, I was unable to determine likely neural and contributions to the loss of amplitude of accommodation in diabetes (section 5.3). Investigating changes in lens shape (surface curvatures and diameters) and refractive
index distribution during accommodation may provide understanding of this.
References


References


References
References


Charman, W. N., Adnan, & Atchison, D. A. (2012). Gradients of refractive index in the crystalline lens and transient changes in refraction among patients with
diabetes. *Biomedical Optics Express, 3*(12), 3033-3042. doi:10.1364/BOE.3.003033


References


References
References


References


Grover, S., Murthy, R. K., Brar, V. S., & Chalam, K. V. (2010). Comparison of retinal thickness in normal eyes using Stratus and Spectralis optical...


References


References


References


References


References


References


References


References
http://www.scopus.com/inward/record.url?eid=2-s2.0-17344388483&partnerID=40&md5=feede74771e65ad000f7ee236932544.


References


References


References


References


References


References


References


References


References


References
Appendices

Appendix A: Effect of Phakometer Positioning Errors on Refraction, Eye Rotation and Purkinje Image Sizes

The phakometer is designed so that the entrance pupil of the eye is at the focal point of the phakometer (Badal) lens and is focused by the camera. However, sometimes the phakometer position must be altered to improve quality of Purkinje images, particularly that of PIII. Also, the eye rotates at a point behind the eye entrance pupil. Furthermore, changes in phakometer position will change the size of the Purkinje images. This appendix considers errors in refraction, eye rotation and Purkinje image sizes associated with positioning errors.

For this investigation, ray tracing is performed using heights and angles with refraction and transfer equations having the form

$$u' = u + hF \quad h_0 = h - ud$$

where $u$ is incident angle at a surface, $u'$ is refracted angle at the surface, $h$ is height at the surface, $F$ is lens power and $d$ is distance to the next surface for which $h_0$ is the ray height. Here anticlockwise angles from a ray to the axis are taken as positive and heights above the axis are taken as positive.

Refraction

In Figure A: 1, the display plane passes through O, a (negative) distance $l$ from the first principal plane of the Badal lens of power $F$. The display’s image plane passes through O’. The eye entrance pupil at EP is a distance $1/F$
+ x from the second principal point of the Badal lens, where x is the positioning error.

We trace a paraxial marginal ray from O to O', starting with the marginal ray angle u. Transfer to the lens with height \( h_L \) is given by

\[ h_L = 0 - (-lu) = ul \]  \hspace{1cm} (A1)

which can be re-arranged to

\[ u = h_L/l \]  \hspace{1cm} (A2)

Refraction at the lens gives refracted angle

\[ u' = u + h_L F \]  \hspace{1cm} (A3)

Substituting the right hand side of equation (A2) for u in equation (A3) gives

\[ u' = h_L/l + h_L F = h_L[(1 + Fl)/l] \]  \hspace{1cm} (A4)

which can be re-arranged to

\[ h_L = u'l/(1 + Fl) \]  \hspace{1cm} (A5)

The transfer to the pupil with height \( h_{eye} \) is given by

\[ h_{eye} = h_L - u'(1/F + x) \]  \hspace{1cm} (A6)

Substituting the right hand side of equation (A5) for \( h_L \) in equation (A6) gives

\[ h_{eye} = -u'[1 + xF(1 + Fl)]/[F(1 + Fl)] \]  \hspace{1cm} (A7)

The ray is then transferred to O’ a distance \( l/L_{eye} \) from the entrance pupil, and for which height is zero, to give

\[ 0 = h_{eye} - u'/L_{eye} \]  \hspace{1cm} (A8)

from which

\[ L_{eye} = h_{eye}/u' \]  \hspace{1cm} (A9)

Substituting the right hand side of equation (A7) for \( h_{eye} \) in equation (A9) gives
Appendices

\[ L_{\text{eye}} = -F(1 + Fl)/[1 + xF(1 + lF)] \]  \hfill (A10)

In equation (A10), setting \( x \) to zero gives the refraction \( R_x \) as

\[ R_x = -F - F^2l \]  \hfill (A11)

Substituting \( R_x \) for the right hand side of equation (A11) in equation (A10) gives

\[ L_{\text{eye}} = R_x/(1 - xR_x) \]  \hfill (A12)

The refraction error \( \Delta R_x \) associated with positioning error \( x \) is given by subtracting equation (A11) from equation (A12) to give

\[ \Delta R_x = xR_x^2/(1 - xR_x) \]  \hfill (A13)

**Eye Rotation**

The object is a point of height \( \bar{h} \) on the display plane (Figure A: 1). Raytracing refraction and transfer equations are performed from this point to the centre of the entrance pupil centre using heights and angles. The initial angle of the ray is \( \bar{u} \) and the height \( \bar{h}_L \) of the ray at the lens is given by

\[ \bar{h}_L = \bar{h} - (-\bar{u}l) = \bar{h} + \bar{u}l \]  \hfill (A14)

which can be re-arranged to

\[ \bar{u} = (\bar{h}_L - \bar{h})/l \]  \hfill (A15)

Refraction at the lens gives

\[ \bar{u}' = \bar{u} + \bar{h}_L F \]  \hfill (A16)

Substituting the right hand side of equation (A15) for \( \bar{u} \) in equation (A16) gives

\[ \bar{u}' = [(\bar{h}_L (Fl + 1) - \bar{h})]/l \]  \hfill (A17)

which can be re-arranged to

\[ \bar{h}_L = (\bar{u}'l + \bar{h})/(1 + Fl) \]  \hfill (A18)
Transfer to the centre of the entrance pupil gives

\[ 0 = \bar{h}_L - \bar{u}'(1/F + x) \]  
(A19)

Substituting the right hand side of equation (A18) for \( \bar{h}_L \) in equation (A19) gives

\[ \bar{u}' = \bar{h}F/[ 1 - x(-F - F^2l)] \]  
(A20)

Substituting \( R_x \) for the right hand side of equation (A11) in equation (A20) gives

\[ \bar{u}' = \bar{h}F/(1 - xR_x) \]  
(A21)

In equation (A21), setting \( x \) to zero gives the angle \( \bar{u}'_{RX} \) as

\[ \bar{u}'_{RX} = \bar{h}F \]  
(A22)

Comparing equations (A21) and (A22) gives

\[ u'/\bar{u}'_{RX} = 1 - xR_x \]  
(A23)

Figure A: 1 Determining errors with phakometer system.
Purkinje Image Sizes

The design distance for the infrared ring target from the entrance pupil is 75 mm. Using an appropriate schematic eye, we can determine the changes in the ratios of the Purkinje image sizes when this distance is altered. We used the four-surface Navarro schematic eye with refractive indices for 890 nm determined from the dispersion equations given by Atchison & Smith (2005). The equations for ray tracing can be the paraxial refraction equations as given at the start of the appendix in which reflections are treated by putting \( n' = -n \) and having refractive indices and distances following reflection as being negative. Object and image distances in each surface are monitored and magnification for the Purkinje images is given using equations similar to those given in section 3.5.5.4.2. Alternatively a raytracing procedure may be used similar to that given in the section for a three-surface schematic eye.

Results

Refraction

The scale for the target display assumes that the Badal condition holds according to equation (A11). Should the condition not hold there are errors in the refraction given by equation (A13). There are similar errors when the accommodation stimulus is changed. Errors in refraction are shown in Figure A: 2. when the instrument is brought too close to the eye by 5 and 10 mm (positioning errors \( x = -5 \text{ mm and } -10 \text{ mm}, \text{ respectively} \)). The refraction error is negative for both positive and negative refractions, extending to approximately \(-1.0 \text{ D at } \pm 10 \text{ D refractions for } -10 \text{ mm displacements and about half this for } -5 \text{ mm displacement} \). If the display is moved from a position corresponding to a scale reading of 0 D to one corresponding to that for \(-8 \text{ D}, \text{ the accommodation stimulus increases by } 7.3 \text{ D rather than by } 8.0\)
D. We expect positioning errors of the order of 5 mm, and errors in refraction and accommodation associated with this do not seem particularly high.

![Figure A: 2 Refraction error, as a function of refraction given on the Optometer scale, caused by the phakometer being too close to the eye by (-) 5 mm and (-)10 mm.]

_Eye rotation_

If the phakometer is brought closer to the eye (negative values of \( x \)), the image subtense increases for negative refractions and increases for positive refractions. The errors amount to 10 % for 10 mm displacement errors. However, the important issue is not the angular subtense, but the rotation of the eye. Calculations of the eye rotation for the different target location on the display were derived using equation (A21) on the basis of the rotation centre being 12 mm behind the entrance pupil (Figure A: 3). Obviously angular errors will also be affected by positioning errors, amounting to a maximum of about 5% for a 5 mm positioning error.
Appendices

Figure A: Change in image size (%), as a function of refraction given on the Optometer scale, caused by the phakometer being too close to the eye by (–) 5 mm and (–) 10 mm.

**Purkinje Image Sizes**

For an object distance of –75 mm, Purkinje images PI, PIII and PIV are 3.67 mm, 10.06 mm and 3.76 mm inside the schematic eye, respectively, and ratios PIII/PI and PIV/PI are 1.834 and –0.769, respectively. The ratios, rather than the absolute image sizes, are important in phakometry. If the object distance changes to –70 mm, changes in these ratios are –0.54% and –0.04%, respectively. If the object distance changes to –65 mm, proportional changes in these ratios are –1.15 % and –0.09 %, respectively. These seem to be small errors.

**Other**

Because the camera has a telecentric lens, an incorrect distance from the eye because of positioning errors is not important.
Appendix B: Manual for using Phakometry Software

Targets for Fixation

A slide with black background is created in Microsoft PowerPoint. Nine circles (each filled with white colour and having a black number from 1 to 9) are created at nine different positions in this slide. One circle is presented in the centre while others (eight circles) are represented on the top and bottom, and both sides of the slide. The magnification ratio between OLED monitor and primary monitor is 1:21.1. On primary monitor, the horizontal inter central circles distance is 122.5 mm while the vertical inter central circles distance is 90.0 mm which on OLED monitor results in 5.8 mm horizontal inter central circles distance and 4.3 mm vertical inter central circles distance. When a participant looks through the beam splitter a mirror image effect is observed. To cancel this effect, PowerPoint slides are flipped horizontally on OLED monitor. This first slide is used during alignment of the participant.

Nine more slides are created. Each of these slides has one only single circle at one of the nine positions on the first slide.

The OLED is connected to the computer through the VGA card (graphic card). Every computer has its primary monitor in the form of a window. It can detect the OLED monitor, which can be seen when the “display” icon is clicked in the control panel. The display interface has the tab “settings” which has monitor setting options. The extended monitor option keeps the two monitors (one is computer primary monitor and other is OLED monitor) side by side.

Whenever another monitor is connected with a computer, the “slide show” tab in the PowerPoint is active with “show presentation on” for choosing
different monitors. In this way, the OLED monitor is chosen. The slides on it can be changed by clicking on the tab “From beginning” and then keep on changing by arrow keys on the keyboard. In this way, alternatively the circles on the OLED monitor can be changed.

Capturing Images

To capture images, the following steps are performed

- The participant’s head is placed on the head rest. He/she looks through the Badal lens to the OLED screen. The OLED screen is moved longitudinally along the Badal setup to correct refractive error.
- When the participant looks at the central fixation target (central circle) on the OLED screen and he/she can observe all the nine targets and the pupil centre is in the centre of the primary monitor, the eye is considered to be aligned with the camera.
- Double click on the “Pixel Link” camera icon to open the camera user interface. When the eye is aligned with the camera, Purkinje images PI and PIV are easily visible. To get Purkinje image PIII clearly on screen and in images, one needs to move the instrument back and forth and manipulate the gain, exposure time, and gamma correction in the camera interface. Click the “capture button” in the interface to capture an image. This image can be saved in the location indicated in the browsing path.

Purkinje Image Heights

Purkinje images heights are calculated with the Purkinje software using Matlab. To open the software, double click on “Purkinje” file. Write “Purkinje” in the command window, and press enter key on keyboard. A window will open as shown below (without the image).
Figure B: 1 A screen capture of the Purkinje software interface. PI, PIII, PIV, Pupil, and Lim are the buttons for the calculation of the Purkinje images heights (PI, PIII, PIV), pupil diameter and limbus diameter, respectively. The Log button is used to take the log of the image to make the Purkinje images clearer and the Edge button is used to perform edge detection in the image.

To get an image, click on the “load image” button and one will get the path for browsing where images have been saved. Click on the image you want to analyse and it will come to the software window (Figure B: 1). One can take the log of the image each time before measuring the height of each Purkinje image.

To calculate the PI Purkinje image height, push the PI button, stretch the cursor anywhere on the image and one will get the ellipse to fit on PI. Double click inside the ellipse and software will record the PI height (radius).

To calculate the PIII image height, push the log button again and press the PIII button. Stretch the cursor on the image and fit the ellipse over the PIII.
Double click inside the ellipse and software will record the PIII height (radius).

The same procedure will be used for PIV, Pupil and Limbus in this order. When all these parameters are calculated press the “Show PI, PIII, and PIV radius” button to see the results and after that press “Save Results”. The results are displayed in the command window as well as saved in the Excel file “Results” in the current folder of the Matlab window (where Phakometry m files are saved). Open the “Results” Excel file with “Open with Matlab” option.

**Optimization to Determine Lens Parameters**

We have “Phakometry” software which contains a merit function routine to estimate lens radii of curvature and lens equivalent refractive index.

Open “PR.m file” in Matlab from the Phakometry software. Write “PR” in command window and press the enter key in the keyboard. A dialog box “Pixels per millimetre” will appear. In this box, enter the number of pixels per millimetre in the images. Click “OK” and the following interface appears (Figure B: 2).

![Figure B: 2 Interface of the Phakometry software](image)

Press the “calculate radii” button in the interface (Figure B: 2) which gives an interface with defaults values (Figure B: 3). Biometry data derived from different instruments is entered along with Purkinje image heights. Note that
refractive error, radii and image heights are averaged between the principal meridians.

![Biometry and Purkinje height information](image)

**Figure B: 3 Biometry and Purkinje height information.**

Enter the number of cycles in the “# of cycles” push the “Submit” button. 2000 cycles is recommended; this is slow but means that the optimisation should be complete in nearly all cases. Lens radii of curvature and equivalent refractive index results are obtained and saved in a Notepad text file (Figure B: 4). The Notepad file can be named according to participant ID by writing the participant ID in the “Biometry and Purkinje information window” and the ID is displayed on top of the file e.g. AM(96).txt-Notepad (Figure B: 4).
Figure B: 4 Snap shot of the optimization result in Notepad. The green rectangle shows the equivalent refractive index at 890 nm, while the blue rectangle shows equivalent refractive index at 555 nm (correction factor 1.006712214). The red rectangle shows lens anterior radius of curvature and the pink rectangle shows lens posterior radius of curvature.
Changes we can make in Software

Purkinje

In “Purkinje.m” file we can change the number of pixels per millimetre at line 65 calculated from the image we need to analyse.

\[
\text{handles.pixel
diameter} = \frac{1}{62.7}; \text{ (mm) from ruler}
\]

Means 62.7 pixels per millimetre in a image

Phakometry

In “subject_read.m” file, we can change refractive indices for different wavelength of LEDs at lines 330 to 335.

In “Double_Radii.m” file, we can change the distance of the eye and source of light at line 24. In the same file we can change the “radius of the source ring” at line 25.

In “PR.m” file, we can change the default value “pixels per millimetre” to our own default value at line 7.
Appendix C: Publications and Conference Publications


Adnan & Atchison, D.A. Changes in straylight and corneal light scattering in a newly diagnosed case of Type 2 diabetes. *Clinical and Experimental Optometry (accepted).*

