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Assessment of Ocular Accommodation in Humans

Nicola Szostek

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Szostek, Nicola

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ASSESSMENT OF OCULAR ACCOMMODATION IN HUMANS

By

Nicola Szostek

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Copyright statement

Abstract

Assessment of ocular accommodation in humans

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Accommodation is the change in the dioptric power of the eye altering the focus from distance to near. Presbyopia is the loss of accommodative function that occurs with age. There are many techniques used to measure accommodation, however, there is little consensus as to how clinical data should be collected and analysed. The overarching theme of this thesis is the *in vivo* examination of accommodation and how lifestyle can affect the onset of presbyopia.

An open-field autorefractor with badal adaption was used to examine accommodative dynamic profiles under varying demands of vergence. From this data a new metric for assessing the time for accommodative change was derived. Furthermore this thesis describes a bespoke automated accommodative facility instrument that was developed to provide further assessment of accommodative speeds.

Defocus curves are used for assessing accommodation and depth-of-focus; the work presented explores the use of non-linear regression models to define the most appropriate method of assessing defocus curves in phakic subjects, and pseudophakic subjects implanted with an extended depth-of-focus intraocular lens. Using an absolute cut-off criteria of +0.30logMAR improved the repeatability and reliability of the depth-of-focus metrics over a cut-off criteria relative to the best corrected visual acuity.

A swept-source anterior segment optical coherence tomographer (AS-OCT) was used to image the morphology of the ciliary muscle during accommodation. The accuracy of ciliary muscle measurements was improved when using reference points on the sclera

to align the AS-OCT scan. The use of a ciliary muscle area metric demonstrated poor repeatability and reliability when compared to the traditional assessment of muscle morphology via thickness measurements.

Physiological ageing in the crystalline lens occurs in line with ageing in other structures in the body. The methods for assessing accommodative function examined in previous chapters, were used to examine whether lifestyle factors which affect the rate of systemic ageing, such as smoking, also affect accommodative function. Although being a current smoker and having greater central adiposity was associated with a slower time for accommodative change, further research is required before these findings can be applied to the target population.

For my sister Charlotte, who has always guided me through

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Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Sub-Committee. Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment. Relevant scientific seminars and conferences were regularly attended at which work was often presented and papers prepared for publication.

Publications and Presentations:

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List of Abbreviations

0.04_{area}	Area-of-focus with a relative best-corrected visual acuity±004logMAR
0.04_{dist}	Range-of-focus with a relative best-corrected visual acuity±004logMAR
0.03_{area}	Area-of-focus with an absolute criteria of +0.30logMAR
0.30_{dist}	Range-of-focus with an absolute criteria of +0.30logMAR
AD	Accommodative Dynamics
AF	Accommodative Facility
AoA	Amplitude of Accommodation
AREDS	Age-Related Eye Disease Study
ARMD	Age-related Macular Degeneration
AS-OCT	Anterior Segment Optical Coherence Tomography
BCVA	Best Corrected Visual Acuity
BMES	Blue Mountains Eye Study
BMI	Body-Mass Index
Binoc.	Binocularly
C	Cycle
CLAS	Crystalline Lens Ageing Study
CMA_1	The cross-sectional area of the portion of the ciliary muscle that lies between the scleral spur, and 1mm from the scleral spur.
CMA_2	The cross-sectional area of the portion of ciliary muscle that lies between 1mm and 2mm from the scleral spur.
CMA_3	The cross-sectional area of the portion of ciliary muscle that lies between 1mm and 2mm from the scleral spur.
CMA_{total}	The cross-sectional area of the portion of ciliary muscle that lies between the scleral spur, and 3mm from the scleral spur.
CMT_1	Ciliary Muscle Thickness at 1mm from the scleral spur

CMT_2	Ciliary Muscle Thickness at 2mm from the scleral spur
CMT_3	Ciliary Muscle Thickness at 3mm from the scleral spur
CMT_{Max}	Ciliary Muscle Thickness at the point of maximum thickness
CoV	Coefficient of Variance
CPM	Cycles Per Minute
CPS	Critical Print Size
CVD	Cardiovascular Disease
D	Dioptre, Dioptric power
DC	Dioptre Cylinder
DoF	Depth of Focus
DR	Dynamic Retinoscopy
DS	Dioptre Sphere
EDoF IOL	Extended Depth of Focus Intra-ocular lens
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food Frequency Questionnaire
GSH	Reduced glutathione
HEPA	Health Enhancing Physical Activity
ICC	Intraclass Correlation Coefficient
IOL	Intraocular lens
IPAQ	International Physical Activity Questionnaire
LoA	Latency of Accommodation
LoD	Latency of Disaccommodation
LE	Left Eye
MEM	Monocular Estimate Method
MIOL	Multifocal Intraocular lens
Monoc.	Monocularly
OCT	Optical Coherence Tomography
oToAC	Objective Time for Accommodative Change

PcAF	Patient Controlled Accommodation Facility
pLoA	Latency of accommodation (previously used method)
pLoD	Latency of disaccommodation (previously used method)
RE	Right Eye
RI	Refractive Index
ROS	Reactive Oxygen Species
RP	Reference Point
SAF	Standardised Accommodative Facility
SD	Standard Deviation
SMES	Singapore-Malay Eye Study
TAF	Accommodative Facility (via the traditional method recommended by Zellers et al. (1984))
sToAC	Subjective Time for Accommodative Change
TD-ASOCT	Time Domain Anterior Segment Optical Coherence Tomography
ToA	Time for Accommodation
ToD	Time for Disaccommodation
ToAC	Time for Accommodative Change
VA	Visual Acuity
WHO	World Health Organisation

Chapter 1: Introduction

Accommodation can be defined as the change in the dioptric power of the eye, altering the eye's focus from distance to near (Millodot and Laby, 2002). This is achieved by a change in surface curvature and equatorial diameter of the crystalline lens.

The structures involved in the mechanism of accommodation are shown in Figure 1.1; these include the crystalline lens, the ciliary body and the lens zonules.

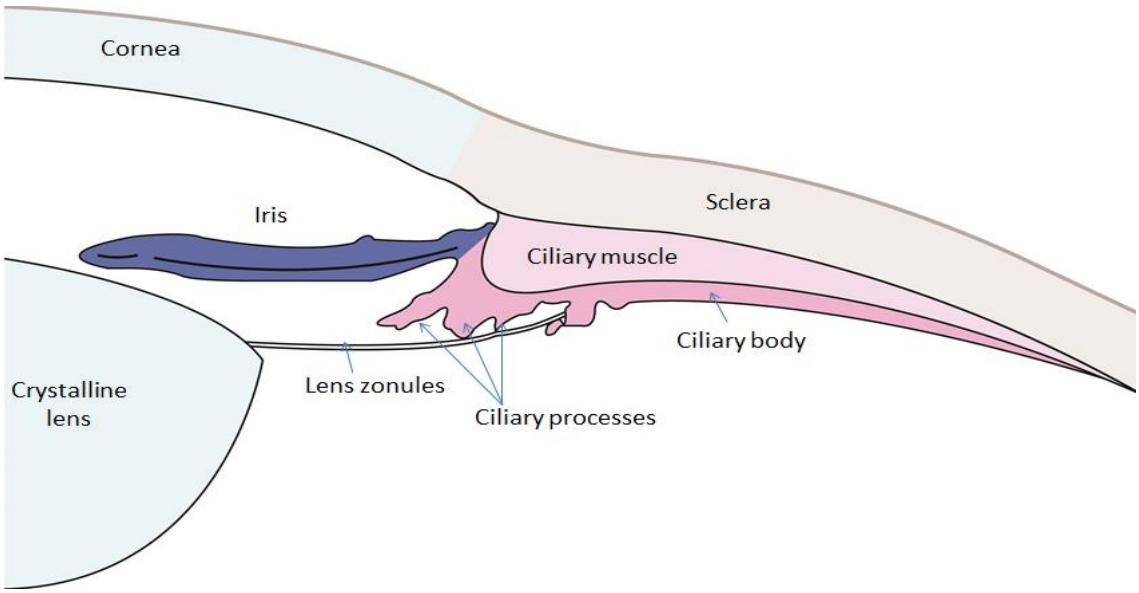

Figure 1.1: A schematic diagram of the structures of the anterior eye. Reproduced from Oyster (1999c)

1.1 The crystalline lens

The crystalline lens is a transparent, flexible structure, which changes shape and dioptric power to focus light onto the retina. The lens is held in suspension behind the pupil and posterior surface of the iris, and in front of the anterior surface of the vitreous (Millodot and Laby, 2002).

Figure 1.2 shows the structure of the crystalline lens. Surrounding the crystalline lens is the lens capsule; an elastic, transparent extracellular matrix which is secreted by the epithelial and superficial fibre cells (Lens et al., 2008). The lens capsule is largely composed of collagen type IV (Barraquer et al., 2006). The outer most anterior surface of the lens consists of cuboid-shaped cells and comprises the lens epithelium.

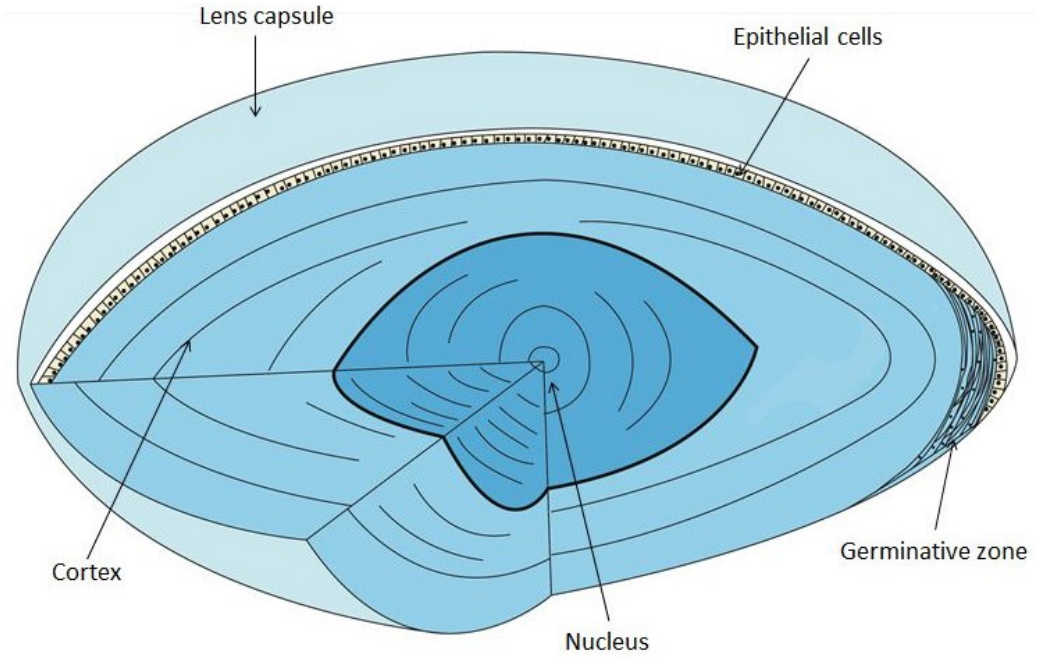

Figure 1.2: A cross-sectional diagram displaying the structure of the crystalline lens. Reproduced from Oyster (1999a)

The majority of the lens' body is formed of elongated lens fibre cells arranged in concentric layers, where the outer cells, constituting the lens cortex, extend from the anterior epithelium to the posterior lens surface (Glasser, 2011). Only lens epithelial cells in the germination zone near the equatorial lens margin undergo mitosis (Rafferty and Rafferty, 1981). The newer cells migrate posteriorly and differentiate into lens fibre cells at the lens equator (Atchison, 1995). The lens fibre cells then elongated to allow the apical end of the cell to meet the lens epithelium, and the basal end of the cell to meet the posterior lens surface and the inner lens capsule (Charman, 2008, Augusteyn, 2010). At this point the newly differentiated lens fibre cells synthesize and accrue large quantities of crystallin proteins. The cells' migration from the germinative zone towards the lens nucleus continues; the two ends of the fibre cells move along their corresponding surfaces until they meet equivalent elongating fibre cells at the anterior and posterior midline, forming junctions called sutures (Glasser, 2011). The basal cell end then detaches from the lens capsule. Once the lens fibre cells reach this stage, their membrane-bound intracellular organelles degrade and are removed (Maisel et al., 1981, Atchison, 1995, Augusteyn, 2010). As newer cells are formed and migrate towards the lens nucleus, the more mature fibre cells become compacted deeper towards the lens nucleus (Dubbelman et al., 2003). These cells and the intra-cellular proteins remain in the lens throughout life, meaning that the most central cells comprising the lens nucleus are the eldest and are present from embryonic development. As newer cells are formed,

both the number of lens fibre cells and the size of the lens increase over lifetime (Scammon and Hesdorffer, 1937).

The refractive properties of the lens are attributed to the curvature of both the anterior and posterior lens surfaces, as well as the higher refractive index (RI) of the crystalline lens (approximately 1.4), relative to the aqueous and the vitreous humour (approximately 1.336) (Oyster, 1999c). The RI of the crystalline lens is not homogenous throughout its structure; the RI is greatest at the central plateau, at approximately 1.41 (Jones et al., 2005, Kasthurirangan et al., 2008) and decreases with increasing distance into the peripheral zones, to approximately 1.38 (Jones et al., 2005, Kasthurirangan et al., 2008). Whilst the lens is in an accommodative state, this change in distribution throughout the lens, is thought to add to the power and affect optical aberrations of the eye (Garner and Smith, 1997). During accommodation the diameter of the central plateau region decreases, although not significantly (Garner and Smith, 1997, Kasthurirangan et al., 2008).

In an emmetropic eye, when looking at a distance object, the crystalline lens adopts its flattest shape with its least refractive power (Glasser, 2011). As focus changes to a near object, to compensate for the negative vergence of light entering the eye, the crystalline lens increases in power by increasing its central thickness as well as increasing its anterior and posterior lens surface curvature (Kasthurirangan et al., 2011). This is a result of contraction of the ciliary body releasing tension in the lens zonules, allowing the elastic lens to return to its more spherical shape.

The posterior surface of the lens meets with the patellar fossa, a depression on the anterior surface of the vitreous. Due to the high water content of the vitreous, it remains rigid and cannot be compressed; therefore, during accommodation there is a relatively small change in curvature of the posterior lens surface comparative to the change in curvature in the anterior lens surface (Oyster, 1999c).

The lens fibre cells and proteins are aligned with high regularity within the crystalline lens in order to achieve transparency (Glasser, 2011). There is an abundance of soluble and insoluble protein within the lens itself; the three most commonly found soluble proteins within the lens are: α-crystallins, β-crystallins and γ-crystallins. Each lens fibre cell has a protein concentration approximately three times greater than the cytoplasm of other typical cells (Glasser, 2011). Transparency of the lens is also aided by small and uniformed extracellular spaces between fibre cells and the absence of membrane-bound intracellular organelles.

1.2 The ciliary body

The ciliary body, as shown in Figure 1.1, begins anteriorly at the scleral spur in the anterior chamber angle and extends posteriorly to the ora serrata of the retina (Oyster, 1999c). The ciliary body is thought to be an anterior extension of the retina and choroid. The full length of the ciliary body has been found to be longer temporally, and shorter nasally *in vitro* (Aiello et al., 1992), although *in vivo* studies have contested these

observations (Sheppard and Davies, 2010, Sheppard and Davies 2011). The inner surface of the ciliary body consists of two epithelial layers which are continuous with the retinal layers; the non-pigmented ciliary epithelium which runs continuously with the sensory retina, and the pigmented ciliary epithelium, which runs continuously with the retinal pigmented epithelium (Tamm and Lutjen-Drecoll, 1996). The ciliary body consists of two sections: the pars plicata anteriorly and the pars plana posteriorly. The pars plana forms the smooth portion of the ciliary body, the outer most surface of the pars plana meets the vitreous, this layer is covered in zonular fibres orientated longitudinally (Rohen, 1979, Glasser et al., 2001). The base of the iris runs continuously into the pars plicata, which is formed of the ciliary processes. The ciliary processes are involved in producing aqueous humour and form the site at which the lens zonules are inserted (Oyster, 1999b). The aqueous humour, which is produced in the ciliary processes, is secreted into the anterior chamber.

Within the ciliary body, lies the ciliary muscle; the anterior ciliary muscle originates at the scleral spur, where its tendons insert through to the trabecular meshwork, and anchor the muscle during contraction (Oyster, 1999c). There are three types of anterior muscle tendons forming the anterior attachment of the ciliary muscle: Type I tendons consisting of collagen and elastic fibres attaching the scleral spur to the longitudinal ciliary muscle fibres. Type II tendons consist of elastic fibres and extend to the trabecular meshwork. Type III extend through the trabecular meshwork into the corneal stroma (Tamm and Lutjen-Drecoll, 1996). The posterior attachment of the ciliary muscle is via

elastic tendons originating from longitudinal and radial fibres, inserting into the Bruch's membrane of the choroid.

The outer surface of the ciliary muscle is loosely attached to the sclera, this thin outer surface consists of; collagen fibres, fibroblasts and melanocytes and form the supra-choroidal lamina (Tamm and Lutjen-Drecoll, 1996). In this region the ciliary muscle fibres are orientated so that as the muscle contracts, the mass of the ciliary body moves forwards and inwards and there is a narrowing of the ciliary ring diameter, leading to the anterior choroid also being pulled forwards (Strenk et al., 1999, Pardue and Sivak, 2000, Glasser et al., 2001, Charman, 2008, Richdale et al., 2013). There are three main groups of muscle fibres forming the smooth muscle of the ciliary body; the longitudinal (or peripheral meridional) fibres, the circular fibres and the radial fibres (Tamm and Lutjen-Drecoll, 1996). The main group is the longitudinal fibres, which extend longitudinally from the scleral spur to the choroid, parallel to the sclera (Tamm and Lutjen-Drecoll, 1996, Glasser, 2011). The circular fibres lie closest to the ciliary processes and lens. The radial fibres attach anteriorly to the scleral spur and posteriorly to the choroid, these fibres are branched into 'Y'- or 'V'- shapes (Glasser, 2011).

The main role of the ciliary muscle is to initiate a change in shape and dioptric power (D) of the crystalline lens to allow light to focus on the retina. Stimulation of the M3 muscarinic receptors on the ciliary body occurs via the Edinger-Westphal nucleus (Ostrin and Glasser, 2007). During contraction, the inwards and anterior movement of the ciliary muscle releases tension on the lens zonules, causing the acting elastic force to fall

to below that of the opposing elastic force of the lens capsule, allowing the lens to assume it's more spherical and powerful state (Charman, 2008).

1.3 The lens zonules

The lens zonules suspend the crystalline lens between the posterior surface of the iris and the patellar fossa of the vitreous. There are two types of zonules; the main fibres (which include the anterior and vitreous fibres) and the tension fibres (Rohen, 1979).

The anterior lens zonules originate from the non-pigmented layer of the ciliary processes of the anterior ciliary body and attach to the lens capsule near the lens equator, some of which penetrate the capsule attaching to the main body of the lens. A number of the zonules cross over one another as they extend from the ciliary body to the lens (Glasser and Campbell, 1999). The vitreous zonules extend from the ora serrata to the ciliary processes (Glasser, 2008, Lutjen-Drecoll et al., 2010). Rohen (1979) states that intermediate tension fibres link the anterior and vitreous fibres and insert into the ciliary epithelium. Charman (2008) and Gilmartin (1995) suggest the function of these tension fibres is to support the main fibres allowing fast accurate adjustments of accommodation.

Lens zonules are composed of protein fibrillins; these fibrillins are composed of many fibrils grouped together in bundle fibres of approximately 4-6 to 40-50 micrometers (Davanger, 1975). The components of the zonules are secreted by the ciliary epithelium,

these are non-collagenous carbohydrate-protein mucopolysaccharides and glycoproteins (Glasser, 2011).

1.4 The mechanism of accommodation

The exact mechanism of accommodation is still debated (Glasser, 2006), although Helmholtz's theory of accommodation (Helmholtz, 1855) is widely accepted.

Accommodation is activated by the parasympathetic pathway stimulating contraction of the ciliary smooth muscle. The pre-ganglionic para-sympathetic signals originate in the Edinger-Westphal nucleus and travel through the ciliary ganglion via the oculomotor nerve (cranial nerve III). Contraction is mediated by M3 muscarinic receptors (Ostrin and Glasser, 2004a). Helmholtz's theory states that as the ciliary smooth muscle contracts, the apex of the muscle and the main muscle mass moves both anteriorly and inwards towards the optical axis of the eye, decreasing the diameter of the ciliary muscle ring (Strenk et al., 1999, Pardue and Sivak, 2000, Glasser et al., 2001, Charman, 2008, Richdale et al., 2013). This action releases tension in the lens zonules, so that their acting elastic force falls to below that of the opposing elastic force of the lens capsule, allowing the lens to assume it's more spherical and powerful state (Charman, 2008). As the lens assumes its more spherical shape there is an increase in the curvature of the anterior lens surface, with a comparatively smaller increase in the posterior lens surface curvature (Ni et al., 2011). This difference in curvature change is thought to be due to

three reasons. Firstly, a thicker anterior capsule compared to the posterior capsule, secondly, a greater change in tension in the anterior lens zonules, and finally the vitreous resisting movement at the posterior lens surface (Charman, 2008). Furthermore, as accommodation occurs, there is a forward movement of the lens (Strenk et al., 2004b) that decreases the anterior chamber volume (Kasthurirangan et al., 2011). Fincham (1937) noted that the decrease in anterior chamber volume was less than the accompanying increase in axial thickness of the lens, suggesting that there is a backwards movement in the posterior lens surface with accommodation.

Lens volume remains the same during accommodation, therefore as the axial thickness of the lens increases, there is a decrease in lens equatorial diameter (Strenk et al., 1999, Glasser, 2006, Jones et al., 2007, Ostrin and Glasser, 2007).

There is some debate as to whether the iris may also contribute to the accommodative mechanism; Crawford et al. (1990) suggested that the iris may pull the ciliary body forwards and inwards to aid the lens to assume a more spherical shape, and yet Fincham (1937) observed successful accommodation in aniridic subjects.

The mechanism of dis-accommodation is innervated by the sympathetic pathway, mediated by the β2-adrenergic receptors causing a relaxation of the ciliary body, facilitated by the restoring force of the choroid. As the ciliary body relaxes, there is an increase in tension in the lens zonules, as well as the elastic nature of the choroid pulling the ciliary body posteriorly and outwards (Strenk et al., 1999). The increase in tension in the lens zonules pulls the lens capsule and therefore the lens nucleus into a much

thinner and flatter shape, so that the power of the eye coincides with the eye's far point (Glasser, 2006, Glasser, 2008). The anterior and posterior lens surfaces reduce in curvature; increasing the volume of the anterior and posterior chambers (Glasser, 2006). Coleman has proposed a theory of accommodation which stated that changes in the pressure in the vitreous, in comparison to the anterior chamber during accommodation, may increase the forward movement of the anterior lens surface, increasing accommodation (Coleman, 1970, Koretz and Handelman, 1982, Coleman, 1986, Coleman and Fish, 2001). However, Fisher observed successful accommodation in patients who did not have a vitreous (Fisher, 1983).

Schachar has proposed an alternative theory for the mechanism of accommodation, based on observations made when the application of equatorial pressure to other encapsulated biconvex objects resulted in these objects assuming the same shape changes observed in the lens during accommodation (Schachar and Fygenson, 2007). Schachar's theory states that as the ciliary muscle contracts, equatorial zonular tension increases, with a decrease in anterior and posterior zonular tension (Schachar, 2006). The force from the zonules results in lenticular changes including; an increase in axial lens thickness, the central portion of the anterior and posterior lens surfaces increase in curvature, with a decrease in curvature of the peripheral anterior and posterior lens surfaces. This is thought to result in an increase in the dioptric power of the lens, and a decrease in spherical aberrations (Abolmaali et al., 2007). Using this alternative mechanism, Schachar proposed a different theory for the mechanism of presbyopia;

that as equatorial diameter increases with age there is an associated decrease in zonular tension resulting in a reduction in the ciliary muscle length. This reduction in ciliary muscle length is thought to cause a reduction in maximum ciliary muscle strength, resulting in reduced accommodative function with age (Schachar et al., 2006). However, an overwheming volume of literature supports Helmholtz theory of accommodation over Schachar's theory (Strenk et al., 1999, Strenk et al., 2005, Glasser, 2006, Charman, 2008, Richdale et al., 2008, Sheppard and Davies, 2010, Sheppard and Davies, 2011, Richdale et al., 2013, Richdale et al., 2016).

1.5 The near vision triad and components of accommodation

The 'near vision triad' is the response observed in pre-presbyopes to a near vision stimulus. The response consists of accommodation stimulated by retinal blur, convergence stimulated by fixation disparity and pupil constriction. There are four components of accommodation: tonic, reflex, convergence, and proximal.

Tonic accommodation can be described as the 'resting state' of accommodation, it is the accommodation that is present in the absence of a stimulus e.g. in darkness. This is due to a constant tonus in the ciliary muscle, resulting from a balance between parasympathetic and sympathetic innervation, when no stimulus is present (Zadnik et

al., 1999). Millodot and Laby (2002) state this to be approximately 1.25D, but with a variable range.

Reflex accommodation is the automated response to a blur circle at the retina, induced to maintain a clear and sharp image, achieved by a change in dioptric power of the eye (Millodot and Laby, 2002).

Convergence accommodation is the component of accommodation which is induced by a change in convergence, a part of the near vision triad (Millodot and Laby, 2002). Convergence is driven by fixation disparity, and the response is dictated by an individual's accommodation/convergence: accommodation ratio (AC/A ratio).

Proximal accommodation is induced by the awareness or belief of a near vision target within a subject's viewing plane (Rosenfield et al., 1990).

In phakic eyes the accommodative response consists of both 'true' accommodation: the dioptric power change in the eye, and 'pseudo' accommodation: an increase in depth-of-focus resulting from pupil constriction. In presbyopes where no 'true' accommodation remains, pupil constriction from the near vision triad still occurs, increasing depth-of-focus, and allowing some pseudo-accommodation to remain.

1.6 Measuring accommodative function

In order to conduct near vision tasks efficiently the accommodative change must occur quickly and smoothly, whilst the response must be accurate and sustained. The

efficiency of the accommodative change is often referred to as the accommodative function or accommodative ability.

Accommodative dysfunction and presbyopia are characterised by reduced accommodative function, leading to asthenopic symptoms (Hennessey et al., 1984a, Levine et al., 1985, Sterner et al., 2006). Measuring accommodation in practice is important to examine a subject's accommodative function and assess the need and suitability for near vision correction. Traditional methods of correcting presbyopia including reading spectacles, monovision, and either contact lens or spectacle multifocal correction, provide functional options for most activities (Charman, 2014). However, each modality of optical correction has their own limitations leading to compromises in visual function, which has subsequently driven current research into novel methods of restoring accommodation after presbyopia or cataract surgery (Glasser, 2008). In an attempt to address this need numerous accommodative and extended depth of focus intraocular lens designs have been developed (Wolffsohn et al., 2006, Schmidinger et al., 2006, Maxwell et al., 2009, Sheppard and Davies, 2010, Lichtinger and Rootman, 2012, Pepose et al., 2017). In order to assess the effectiveness of novel intraocular lens (IOL) designs, tests which can accurately assess accommodative function are essential (Sheppard et al., 2010).

Each of the methods for measuring accommodative components are categorised as either subjective or objective. Subjective methods rely on the patient's perception of blur as its end point criterion; therefore these measurements are a combined value of

both true- and pseudo- accommodation. Objective methods measure the refractive status of the eye whilst viewing a target at a set distance. The end-point criterion is entirely objective, there is no subjective input from either the subject or examiner, removing the component of pseudo-accommodation. Therefore, measurements from objective methods are of the true-accommodative response only. This can lead to subjective methods giving higher readings of accommodation than objective methods (Glasser, 2006). In an absolute presbyope, where no true-accommodation is present, pseudo-accommodation may remain; leading to subjective methods over-estimating accommodative function, and indicating that true-accommodation may be present, where there is none. Due to these limitations Wold et al. (2003) concluded that a combination of subjective and objective methods should be used when analysing the effectiveness of novel methods for restoring accommodation.

The different subjective and objective tests to assess the accommodative function quantify one or more different parameters of the accommodative response; these parameters are listed alongside their corresponding subjective and objective accommodation tests in Table 1.1.

The accommodative response or amplitude of accommodation (AoA) is the maximum accommodative power change of the eye in response to an accommodative target. Accommodative lag or lead is the difference between the accommodative demand of the near target and the accommodative response of the eye. The most common

subjective and objective methods for assessing accommodative function both clinically and in a research setting are displayed in Table 1.1 and reviewed below.

Parameters	Subjective Tests	Objective Tests
Time for the accommodative response	Accommodative facility	Accommodative dynamics
Accuracy of response (Accommodative lag)	None applicable	Accommodative dynamics Dynamic retinoscopy
Absolute response or Amplitude of accommodation	Push-Up/Pull-Down test Minus-to-blur / Defocus curves	Accommodative dynamics
Sustainment of response	None applicable	Accommodative dynamics

Table 1.1: The comparable subjective and objective tests for assessing the different parameters of accommodative function.

1.6.1 The Push-Up Test

As the Royal Air Force rule (RAF rule, Richmond Products, USA.) is relatively quick to use and widely available, the Push-Up test is often used in clinical practice to measure AoA (Elliott and Flanagan, 2014). This is a subjective technique and therefore it is often found to over-estimate AoA and to be the least repeatable in comparison to other accommodative function tests (Rosenfield and Cohen, 1996, Ostrin and Glasser, 2004a, Kasthurirangan and Glasser, 2006b, Win-Hall et al., 2007, Anderson et al., 2008, Gupta et al., 2008, Leon et al., 2012). The low repeatability of the Push-Up test has been attributed to;

- Practitioner skill: if the test is performed too quickly the measured AoA is over-estimated (Rosenfield and Cohen, 1996, Anderson et al., 2008)
- Proximal cues and depth-of-focus cues (Atchison et al., 1997)
- The subjectivity of the end criterion relying on the subject's perception (Glasser, 2006).
- The compressed scale on the upper section the RAF rule can lead to over-estimations and reduce the repeatability of AoA in younger patients. (Anderson et al., 2008).
- As the target is moved towards the patient the minutes-of-arc subtended on the retina will increase, leading to over-estimations of AoA.
- Using the same target for the patient to assess blur can lead to over-estimations of the response and repeatability, from a learning effect.

Some attempts to reduce the effects of these errors have been made. A variation of this method is the Pull-down test; this removes the learning effect of the target, improving repeatability; Antona et al. (2009) demonstrated that the Pull-down test gives lower values of AoA in comparison to the Push-Up test. In clinical practice a modified version of the Push-Up test is often used, which combines both the Push-Up to find a break-point and the Pull-Down to find a recovery-point, to calculate an average AoA.

1.6.2 The Minus-to-blur/Defocus curves

The minus-to-blur method has been shown to assess the AoA; by introducing increasingly powered concave lenses in front of the eye, whilst fixating on a target. The highest powered concave lens through which the best corrected visual acuity (BCVA) can be maintained is recorded as the AoA. The AoA measured via the minus-to-blur method is often lower than when measured via the Push-Up test. This has been attributed to a number of factors including: the minimization effects from the increase in negative lens power, and the use of a distance target removing pupil miosis and depth-of-focus cues (Rosenfield and Cohen, 1996, Wold et al., 2003, Ostrin and Glasser, 2004a, Gupta et al., 2008), and the end-point criteria relying on subjective blur. Minus-to-blur has been shown to over-estimate measurements of AoA when compared to dynamic retinoscopy (DR) (Leon et al., 2012), and objective measures of AoA from optometers.

If the power of lens used to induce defocus is plotted against the visual acuity achieved, a defocus curve can be plotted. Defocus curves have been used extensively in research to assess the depth-of-focus, specifically to evaluate the effectiveness of novel accommodating or extended depth-of-focus IOL designs (Schmidinger et al., 2006, Gupta et al., 2007, Gupta et al., 2008, Alfonso et al., 2009, Antona et al., 2009, Buckhurst et al., 2012, Leon et al., 2012, Wolffsohn et al., 2013). Despite the popularity of defocus curves there is a lack of standardisation for both the methods used to construct defocus curves, and the methods used to derive information (Gupta et al., 2007, Gupta et al.,

2008, Buckhurst et al., 2012, Wolffsohn et al., 2013). This can make it difficult to compare studies that have looked at how successful different accommodating and extended depth-of-focus IOLs designs are at restoring accommodation.

1.6.3 Dynamic retinoscopy

Dynamic Retinoscopy (DR) measures accommodative lag or lead. Accommodative lag is the difference between the accommodative response and the dioptric stimulus (Millodot and Laby, 2002). DR relies on a subjective end-point criteria decided upon by the practitioner; therefore removing the subjective component from the patient. Studies have shown that a skilled and experienced practitioner, can produce repeatable measurements of accommodative lag (Leon et al., 2012) which are comparable to corresponding measurements obtained using an auto-refractor (McClelland and Saunders, 2003). Generally, accuracy and repeatability of DR techniques relies on practitioner skill, the quality of the observed reflex, the co-operation of the subject in fixating on the near target, and involuntary accommodative spasm. There are two widely used methods for DR: the Monocular Estimate Method (MEM) and Nott's method. Both examine the monocular response under binocular viewing conditions to a near target. The MEM DR uses lenses presented briefly in front of the eye to neutralise the lag or lead of accommodation. Nott's method involves the practitioner physically moving the retinoscope forwards or backwards until neutralization of the lag or lead is achieved. Numerous studies have compared the validity and repeatability of these

methods. Locke and Somers (1989) found that these two techniques produced interchangeable results. However, further work by Rosenfield et al. (1996) found that measurements via Nott's DR gave the closest agreement to those obtained with an objective auto-refractor than the MEM. It was concluded that Nott's DR was more appropriate for measuring accommodative response because the addition of powered lenses can lead to the subject adapting to these lenses before the accommodative response is fully assessed, leading to inaccuracies (Rosenfield et al., 1996, del Pilar Cacho et al., 1999).

1.6.4 Accommodative facility

Accommodative facility (AF) or lens rock is a subjective technique which can be used to assess the eyes' ability to change focus quickly and accurately (Eperjesi, 2004). Clinically, the AF test is used to investigate symptomatic accommodative dysfunction, (Levine et al., 1985, Goss, 1992, Gall and Wick, 2003) and is predominantly used in a paediatric setting (Hennessey et al., 1984b, Wick et al., 2002b). Studies have found that in both children and adults with symptomatic accommodative dysfunction, AF is often reduced, even when all other measures of accommodative metrics such as AoA and accommodative lag are within a normal range (Levine et al., 1985, Wick and Hall, 1987, Wick et al., 2002a, Gall and Wick, 2003).

There is significant variation in the methodology used to assess AF in the literature (Wick et al., 2002b) which has hindered attempts to establish normative values. Currently, the

method set out by Zellers et al (1984) is the one most commonly recommended for use in clinical practice (Eperjesi, 2004, Elliott and Flanagan, 2014). This method assesses the number of cycles of ±2.00D (an accommodative change of 4D) that a subject can adjust their focus to view a target clearly, within one minute. One cycle is the subject 'clearing' both the positive and negative lenses. From the cycles per minute achieved, the time taken for each cycle can be calculated. This time period is equivalent to the time for accommodative change, (ToAC) i.e. the combined time taken for the eye to accommodate and dis-accommodate. The test is normally conducted monocularly, and binocularly using a stereogram target to monitor suppression.

The normative values for binocular AF using a stereogram target are >7 CPM, or >11 CPM monocularly (Zellers et al., 1984), for individuals within 18 to 30 years of age. When applying these values to subjects outside this age range problems arise: when Wick and Hall (1987) applied this binocular criterion to a group of schoolchildren, they found an artificially high failure rate. They postulated that either a lower pass/fail criteria should be used for school screening, and/or AF results should be considered in conjunction with other measures of accommodation, such as lag and amplitude, before a diagnosis of binocular accommodative dysfunction is made. As such, in school-children Scheiman et al. (1988) recommended normative values for binocular AF using a stereogram target should be >5 CPM, or >7 CPM monocularly. The variations in these values have been attributed to the variation in methods and subjects utilised in these studies (Wick et al., 2002b).

1.6.5 Objective and dynamic assessment of accommodation

Objective optometers such as open-field auto-refractors and power-refractors, can be used to measure the absolute accommodative response to a target, or to assess the accommodative dynamic profile. Autorefractors measure the refractive status of an eye whilst viewing an accommodative target. Power-refractors use the same principles as DR to measure the accommodative response: analysing the light reflex reflecting from the fundus. By removing the need of the subject or clinician's judgement of the end-point, no pseudo-accommodation is measured when using an objective optometer, therefore lower measurements of absolute accommodative response are found in comparison to subjective methods.

For both an auto-refractor and a power-refractor accommodation can be stimulated by either altering the distance of a real target or by using concave lenses. Technical features provided by such devices, which are desirable for accommodation evaluation include: the use of infra-red light to control pupil constriction (Wolffsohn et al., 2002), and the availability of an open-field viewing platform to minimise proximal accommodation (Hennessy, 1975). The accuracy of objective optometers are limited by pupil size, and from Purkinje image reflections that can make measurements difficult to obtain. Win-Hall and Glasser (2009) also found that dry eyes, precipitated by repeated measurements with an auto-refractor can lead to unexpected astigmatic components to be found.

Power-refractors are often used to measure refractive error in infants (Braddick et al., 1979) and during eye-screening in school-children. This is often attributed to various factors including the ease of training non-clinical staff to take measurements, the fact that two eyes can be examined at once, and because the procedure is conducted at a greater distance (1m) than an auto-refractor. Some studies have shown that power-refractors can give more hyperopic readings of refractive error when compared to auto-refractors and subjective refraction suggesting that there may be a need for individual calibration to ensure accurate readings are obtained (Jainta et al., 2004, Blade and Candy, 2006). Gabriel and Mutti (2009) found comparable results of accommodative response in infants measured with the MEM DR and power-refraction. Power-refractors can generally obtain measurements of accommodation through smaller pupils, than other optometers, and are therefore the optometer of choice for use with older subjects (Ostrin and Glasser, 2004a). However, a study by Richdale et al. (2013) found that whilst an auto-refractor gave more consistent readings of the accommodative response when compared with a power-refractor, the corresponding readings from the two instruments were not significantly different to each other. Open-field auto-refractors have been validated to measure accommodative response in both phakic populations (Win-Hall et al., 2007) and pseudophakic populations (Davies et al., 2003, Win-Hall and Glasser, 2009)

Dynamic assessment of accommodation can be performed using both auto-refraction and power-refractors. Using these accommodative profiles, numerous objective

metrics can be derived to describe the parameters shown in Table 1.1. These metrics can include: absolute accommodative response, accommodative lag, time constants, peak velocity of accommodation, response times, and micro-fluctuations (Beers and van der Heijde, 1996, Kasthurirangan et al., 2003, Kasthurirangan and Glasser, 2006b, Radhakrishnan et al., 2007, Win-Hall et al., 2007, Allen et al., 2010, Anderson et al., 2010). Many studies have compared the metrics used to describe the accommodative parameters of absolute response and accuracy to other available subjective and objective tests (McClelland and Saunders, 2003, Ostrin and Glasser, 2004b, Kasthurirangan and Glasser, 2006b, Win-Hall et al., 2007, Gupta et al., 2008). Comparatively fewer have examined the relationship between the metrics used to describe the time taken for the accommodative change (i.e. accommodative lag, time constants, peak velocity of accommodation, response times) and the corresponding subjective test of AF (Radhakrishnan et al., 2007, Allen et al., 2010). Furthermore, there is also little concensus between the studies on the exact definitions and methods used, to derive each of the accommodative metrics from the accommodative dynamic profile.

1.7 Presbyopia

Presbyopia is the natural loss of accommodative function with increasing age (Anderson et al., 2008). Although presbyopia begins in early infancy, the onset of asthenopic symptoms, including eyestrain whilst attempting near tasks, occurs around the age of

40-45 years (Holden et al., 2008). By the age of 50-55 years all accommodation is effectively lost (Hamasaki et al., 1956), although some pseudo-accommodation may still remain contributing to a subjective amplitude of accommodation of 1.00D in this age group (Ostrin and Glasser, 2004a).

The exact mechanism of presbyopia is still debated; there are numerous age-related structural changes in the accommodative apparatus, which may contribute to the loss of accommodative ability. However, not all of the age-related structural changes are thought to contribute equally to the loss of accommodation. Presbyopia in humans is mainly thought to be a lensocentric phenomenon, and attributed to an increase in stiffness and density of the crystalline lens, as new cells are formed and deposited, thus decreasing the elasticity of the crystalline lens (Strenk et al., 2005, Glasser, 2006, Charman, 2008, Glasser, 2008, Van de Sompel et al., 2010, Sheppard and Davies, 2011, Richdale et al., 2013).

1.7.1 Age –related changes in the crystalline lens

The thickness of the crystalline lens capsule changes with increasing age (Krag et al., 1997); Barraquer et al. (2006) noted the anterior lens capsule increased with age, the central posterior lens capsule thickness did not change with age, whilst the peripheral posterior lens capsule thickness decreased with age. It has been found that there is a significant decrease in mechanical strength in both the anterior lens capsule (Krag et al.,

1997) and the posterior lens capsule with age (Krag and Andreassen, 2003), and that this decrease starts earlier in the posterior lens capsule than the anterior lens capsule.

The crystalline lens grows continuously throughout life which results in an increase in:

- Crystalline lens mass with age (Glasser and Campbell, 1999).
- The curvature of the anterior lens surface (Dubbelman et al., 2005, Atchison et al., 2008, Kasthurirangan et al., 2011).
- Axial lens thickness (Sorsby et al., 1963, Koretz et al., 1989, Strenk et al., 1999, Dubbelman et al., 2001, Jones et al., 2007, Atchison et al., 2008, Richdale et al., 2008, Kasthurirangan et al., 2011).

As a result of the increase in axial lens thickness, anterior chamber depth decreases (Koretz et al., 1989, Dubbelman et al., 2001, Atchison et al., 2008, Kasthurirangan et al., 2011) and anterior segment length increases. There is some debate as to whether equatorial diameter of the crystalline lens also becomes wider; Kasthurirangan et al. (2011) and Atchison et al. (2008) found an increase in equatorial diameter, however many other studies have found no significant change in equatorial diameter with progressing age (Strenk et al., 1999, Jones et al., 2007). Since the age-related changes in shape are localised to the inner portion of the crystalline lens, the external shape of the older unaccommodated lens, resembles that of a younger accommodating lens (Strenk et al., 1999, Jones et al., 2007) .

In addition to the above structural shape changes of the crystalline lens, the RI of the lens nucleus reduces with age (Dubbelman et al., 2001, Moffat et al., 2002), although the magnitude of this change has not always been shown to be significant (Jones et al., 2005, Jones et al., 2007). Due to the increase in the curvature of the crystalline lens, there should be a myopic shift in the eye with age, however it is commonly observed that the ageing eye shows a hyperopic shift (Attebo et al., 1999, Wong et al., 2001, Wickremasinghe et al., 2004, Shufelt et al., 2005). This is known as the lens paradox, for which there are two possible explanations (Moffat et al., 2002, Koretz and Cook, 2001). Firstly, a flattening of the central lens surface, and an increase in steepness towards the lens periphery resulting in a reduction in the gradient index power. An alternative explanation is that either a decrease in RI in the inner lens nucleus, or an increase in RI in the outer lens cortex could lead to a decrease in the variation of RI through the lens (Smith et al., 1992, Jones et al., 2005, Jones et al., 2007, Kasthurirangan et al., 2008). Both the lens stiffness and the lens stiffness gradient increases with age; in younger lenses the lens nucleus is softer than the lens cortex, however this stiffness differential reverses with age (Heys et al., 2004, Weeber et al., 2007). Consequently, these changes reduce the ability of the lens cortex to mould and increase the thickness of the lens nucleus during accommodation, resulting in the overall loss of accommodative ability. As well as the above changes there is a reduction in circumlental space with progressing age (Strenk et al., 2000, Kasthurirangan et al., 2011).

1.7.2 Age-related changes in the ciliary body

Physiologically age changes in the ciliary body include a decrease in: the total area and length of the ciliary muscle (Tamm et al., 1992b, Pardue and Sivak, 2000), the density of longitudinal, meridional and radial fibres of the ciliary muscle (Tamm et al., 1992b), and the ciliary muscle ring diameter (Strenk et al., 1999, Strenk et al., 2006, Kasthurirangan et al., 2011, Richdale et al., 2016). In addition to these changes, with increasing age the inner apex of the ciliary muscle has been shown to displace forwards and inwards towards the axis of the eye (Strenk et al., 1999, Pardue and Sivak, 2000, Strenk et al., 2004, Strenk et al., 2010, Sheppard and Davies, 2011). It is unclear as to whether this is a cause or effect of the ciliary muscle being pulled inwards by the anterior zonules (Tamm et al., 1992b). As a consequence of these changes, it has been suggested that the ciliary muscle is impaired in its ability to reshape the crystalline lens into its flatter unaccommodated state (Glasser, 2011). Indeed studies have shown that with ageing there is a loss of muscle fibres from the ciliary muscle, and an increase in connective tissue (Nishida and Mizutani, 1992, Tamm et al., 1992b, Pardue and Sivak, 2000). Sheppard and Davies (2011) and Richdale et al. (2013) reported conflicted observations on whether the cross-sectional thickness of the ciliary muscle significantly decreases with age. Ambiguity in the findings may be related to differences in the methodologies employed in these studies; nevertheless the age-related changes observed in these studies have been relatively small, and thought to be insignificant in comparison to the overall cross-sectional thickness, with accommodative effort (Richdale et al., 2013).

Therefore, it has been concluded that the overall accommodative function of the ciliary muscle does not significantly decline with age (Strenk et al., 1999, Pardue and Sivak, 2000, Strenk et al., 2005, Strenk et al., 2006, Strenk et al., 2010, Sheppard and Davies, 2011, Richdale et al., 2013, Shao et al., 2015, Richdale et al., 2016). Furthermore, the age-related changes observed in the physiological structure and function of the ciliary muscle are not thought to be significant enough to contribute to the development of presbyopia in humans.

1.7.3 Age-related changes in the lens zonules

Due to continual growth of the lens with age; the distance between the site of zonular insertion into the lens capsule, and the lens equator increases (Farnsworth and Shyne, 1979) such that the zonules-free region decreases (Sakabe et al., 1998). Farnsworth and Shyne (1979) noted that the rate of increase in this distance is relatively constant until the fifth decade of life, when it increases more rapidly. This should result in a change in zonular tension with age, however Glasser (2011) stated that this does not occur for two reasons. Firstly because the distance between the ciliary body and the site of the lens zonules insertion into the lens capsule remains constant with age, and secondly because the elasticity of the lens zonules remains constant with age.

1.7.4 Factors affecting the onset of presbyopia

As discussed by Hickenbotham et al. (2012) there are a number of primary, secondary and tertiary factors which can affect the onset of presbyopia. These are summarised in Table 1.2. There are many interactions between these factors, for instance exposure to ultra-violet light (UV) may be dependent on occupation and ethnicity; gender may impact on occupation and arm length (Millodot and Millodot, 1989). Age is likely to influence several of these factors, given that with advancing age the pupil size and focusing ability decreases, lens density increases, and there are changes in refractive error. Studies on the impact of some of the tertiary factors stated in Table 1.2 have been discussed.

Primary Factors	Secondary Factors	Tertiary Factors
Focusing ability Habitual reading distance Depth of focus	Refractive error/ocular aberrations Occupation/ near vision requirements Arm length Pupil size Lens optical density	UV exposure Indoor lighting levels Complexity of near task Gender Ethnicity Social economic status

Table 1.2: Factors that affect the onset of presbyopia

Numerous studies have noted that females are prescribed higher near corrections than age-matched males (Pointer, 1995, Duarte et al., 2003, Burke et al., 2006, Nirmalan et al., 2006, Patel et al., 2006, Hashemi et al., 2017), although Hunter and Shipp (1997) and

(Castagno et al., 2017) failed to identify a difference. Conversely, several studies have reported that female subjects demonstrate higher AoA than age-matched males, although these findings are ambiguous (Koretz et al., 1989, Carnevali and Southaphanh, 2005, Kragha, 1986, Millodot and Millodot, 1989). It has therefore been suggested that the apparent need for females to have a higher near correction is not due to physiological differences in accommodation, but rather due to differences based on gender; that is, females generally have shorter arms (and therefore shorter working distances) (Millodot and Millodot, 1989) tending to do more detailed near work (Hickenbotham et al., 2012).

Studies into the differences in ethnicity and the onset of presbyopia have been numerous with conflicting results. A study which compared Hispanic populations and non-Hispanic populations in the USA found no significant difference between the age of onset and progression of presbyopia (Carnevali and Southaphanh, 2005). Hunter and Shipp (1997) compared the reading additions of Caucasians and black African-American patients attending for sight tests at the University of Alabama, Birmingham School of Optometry and found no significant difference in the age of onset or progression of presbyopia. However other studies conducted in South Africa (Hofstetter, 1949, Hofstetter, 1963, Hofstetter, 1968), Nigeria (Olurin, 1973), and Southeast Asia (Ong, 1981) that did similar record reviews found that black African patients were prescribed higher reading additions at a significantly younger age. The reasons for the apparent differences in these findings could indicate that there are other influential factors which

affect the onset of presbyopia, possibly relating to: social economic status (Hunter and Shipp, 1997) and ambient temperature of the living environment (Weale, 2003). Hunter and Shipp (1997) discussed how previous studies found that socio-economic status, such as low levels of education and low levels of income were associated with presbyopia; however their study failed to confirm this link when comparing reading additions prescribed to patients, to the average incomes of their zip codes. Weale (2003) discussed how numerous studies have linked ambient temperature to age of onset and development of presbyopia: suggesting that, the nearer an individual lives to the equator (and therefore generally experiences higher ambient temperatures), the earlier and faster they are likely to develop presbyopia. In support of this supposition, Truscott and Zhu (2010) reported an association between heat and the biomechanical changes which occur in the crystalline lens during presbyopia and cataract formation.

1.8 *In vivo* imaging of the accommodative apparatus

Previously, it has been difficult to image the ciliary muscle *in vivo* due to the position of the iris (Strenk et al., 2006). High resolution imaging of both the crystalline lens and the ciliary body *in vivo* during accommodation has only been possible since more recent advances in imaging with Anterior-Segment Optical Coherent Tomography (AS-OCT) (and to a lesser extent MRI scanners, (Strenk et al., 1999, Kasthurirangan et al., 2011, Sheppard et al., 2011)).

Previous to the advancement of imaging instruments, studies observing the ciliary body were conducted either *in vitro*, in patients with aniridia, or on Rhesus monkeys. Rhesus monkeys were thought to provide a sound model for accommodation and presbyopia research because their accommodative apparatus (Koretz et al., 1987), mechanisms (Glasser and Kaufman, 1999, Croft et al., 2006), and development of presbyopia is similar to humans (Kaufman et al., 1982, Neider et al., 1990). Numerous studies have observed that the primary underlying mechanism of presbyopia in Rhesus monkeys is the same as in humans, i.e. an increase in stiffness of the crystalline lens with age (Glasser and Campbell, 1999, Glasser and Kaufman, 1999, Heys et al., 2004, Richdale et al., 2008, Richdale et al., 2013). Despite these similarities there are some key structural developmental differences between the human and Rhesus monkey crystalline lens, ciliary muscle and choroid (Koretz et al., 1987, Koretz et al., 1988, Lutjen-Drecoll et al., 1988). In contrast to humans, one of the factors that significantly contribute to presbyopia in Rhesus monkeys is a reduction in the contractility of the ciliary muscle with increasing age (Lutjen-Drecoll et al., 1988), as a result of age-related changes in the choroid (Tamm et al., 1991, Tamm et al., 1992a). Furthermore, the ciliary muscle in a Rhesus monkey moves posteriorly with age, in contrast to the anterior movement observed in ageing humans (Strenk et al., 1999, Pardue and Sivak, 2000, Strenk et al., 2004a, Strenk et al., 2010, Sheppard and Davies, 2011). Due to these differences in the ageing of the ciliary muscle and other accommodative apparatus between humans and Rhesus monkeys, further study of the human ciliary muscle *in vivo* is required.

The motivation for developing an improved understanding of the structure and function of the human ciliary muscle has numerous reasons. The current drive in research to develop novel methods to restore accommodation (Glasser, 2008) has led to the development of accommodative and extended depth of focus IOL designs (Sheppard and Davies, 2010, Lichtinger and Rootman, 2012). By establishing the per dioptre changes in the biometric measurements of the ciliary muscle and crystalline lens with accommodation, an improved understanding of the interactions between an accommodating IOL and the ciliary muscle, could be obtained. It is envisaged that this would better inform accommodating IOL design and optimum placement within the eye (Sheppard et al., 2010, Richdale et al., 2013, Richdale et al., 2016).

Recent research has suggested that the ciliary muscle morphology may also have a role in myopigenis or accommodative dysfunction. Studies have shown that ciliary muscle thickness varies with refractive error in children and young adults; the ciliary muscle in myopic eyes has been found to be longer (Sheppard and Davies, 2010) and thicker in the posterior region (Bailey et al., 2008, Schultz et al., 2009, Oliveira et al., 2005, Kuchem et al., 2013, Buckhurst et al., 2013, Pucker et al., 2013). However, the relationship between the change in morphology or physiology of the ciliary muscle and the development of reactive error is unknown, and requires further research (Bailey et al., 2008, Sheppard and Davies, 2010, Buckhurst et al., 2013, Pucker et al., 2013).

Although AS-OCT imaging provides numerous research opportunities, there are some limitations with this technique. One challenge lies within image analysis; trying to

identify anatomically corresponding points along the ciliary muscle for each individual subject, at which to compare changes in thickness in the ciliary muscle as it contracts is problematic (Bailey, 2011). Bailey et al. (2008) and Oliveira et al. (2005) measured the ciliary muscle thicknesses at points set posteriorly from the scleral spur. Sheppard and Davies (2010) argued that this does not consider the fact that ciliary muscle thickness and length varies with refractive error (Oliveira et al., 2005), therefore Sheppard and Davies (2010) attempted to identify the length of the ciliary muscle and then split the body and considered the thickness at proportional locations of 25%, 50% and 75% relative to the length. Despite its benefit, this technique has been suggested to be difficult to replicate due to the limited resolution of the OCT images making it difficult to identify the end of the ciliary muscle (Bailey, 2011).

Images produced by AS-OCT include some inherent optical distortions, which need to be corrected by applying an appropriate refractive index (RI) to the image. The AS-OCT Zeiss Visante (Carl Zeiss Meditec Inc., Dublin, CA, USA), which is often employed in ciliary muscle studies, apply n= 1.000 (RI of air) to the structures before the anterior corneal surface, n=1.388 (RI of the cornea) to all of the corneal layers, and 1.343 to all of the structures behind the posterior corneal surface (Richdale et al., 2008). However, the RI of the ciliary muscle and sclera are estimated to be 1.382 and 1.48, respectively (Tearney et al., 1995, Dirckx et al., 2005). This has led to disparities between the exact RI, and way the RI is applied to the image data during image analysis (Bailey et al., 2008,

Sheppard and Davies, 2010, Kao et al., 2011, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Laughton et al., 2015).

In studies that have examined the ciliary muscle *in vivo* during accommodation, accommodative lag should be considered (Charman, 2008, Lossing et al., 2012, Richdale et al., 2013). To accurately assess the ciliary muscle shape change per dioptre of accommodation, the accommodative response of the eye should be measured simultaneously, rather than assumed to be accurate to the accommodative demand of the target (Lossing et al., 2012). In view of this recommendation, several investigators have implemented the use of power refractors to monitor accommodative response whilst the images are captured with the Visante OCT (Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016). However due to technical restrictions relating to the size and design of OCTs, many studies have been unable to implement this facility to track the accommodative response simultaneously (Sheppard and Davies, 2010).

AS-OCTs have in built software to allow the analysis of images and biometric measurements of structures. To do this, the software features movable straight-line calliper tools. However, utilising these straight-line callipers to measure the ciliary muscle does not account for scleral curvature and could therefore lead to increased variability in the thickness measurements, particularly across the posterior portion (Kao et al., 2011).

Solutions to these limitations have varied across the literature, however, to allow comparisons between studies, standardisation in the methods utilised to capture and analyse images is essential (Bailey, 2011). Furthermore, much of the literature which has used an AS-OCT to study the ciliary muscle *in vivo*, has utilised the time domain AS-OCT Zeiss Visante (Carl Zeiss Meditec Inc., Dublin, CA, USA), this instrument not allow the selection of a specific reference point for scan analysis (Bailey et al., 2008, Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lewis et al., 2012, Lossing et al., 2012, Richdale et al., 2012, Buckhurst et al., 2013, Pucker et al., 2013, Richdale et al., 2013, Richdale et al., 2016). This could therefore limit the repeatability and validity of any results obtained from repeated measurements.

The development of anterior segment swept source spectral OCT, has advanced the resolution and speed of image acquisition. As yet, to the author's knowledge there have been no studies examining the ciliary muscle utilising a swept-source AS-OCT, such as the Tomey CASIA SS-1000 AS-OCT (Tomey, Nagoya, Japan).

1.9 Ageing

Ageing in any organism is inevitable. In biological papers, ageing is often discussed as a chronic disease; a simplified overview states that as this disease progresses there is reduced resilience to respond and adapt to environmental stresses, due to reduced

physiological capacity, which leads to greater susceptibility to age-related health conditions (Troen, 2003).

There are numerous ageing theories or processes used to explain the mechanisms of biological ageing; these processes are broadly split across two (not mutually exclusive) categories: pre-programmed/developmental-genetic theories, and stochastic/damage-error theories (Troen, 2003, Jin, 2010, Bao et al., 2014).

Ageing is an extremely complex process affected by both physiological and environmental factors, from cellular to organismic level. The interspecies and individual rate of ageing results from the exposure to physiological and environmental factors, and interactions between the ageing theories (Troen, 2003, Davidovic et al., 2010). Whilst some studies debate that one ageing theory has a more profound effect on the rate of ageing than others, it is likely that one single ageing theory could not solely explain the process (Troen, 2003, Jin, 2010, Bao et al., 2014, Goldsmith, 2015, Libertini, 2015, Goldsmith, 2016).

An overview of the possible interactions between the ageing theories is shown in Figure 1.3. These interactions are not only between the individual theories, but also across two main categories of ageing. For instance, UV exposure can increase oxidative damage via the free-radical theory, which could also affect the rate of somatic DNA damage, the DNA damage could lead to genetic mutations, affecting protein and hormone production, impacting on the Endocrine and Immunological theories. A brief overview of these theories is given below.

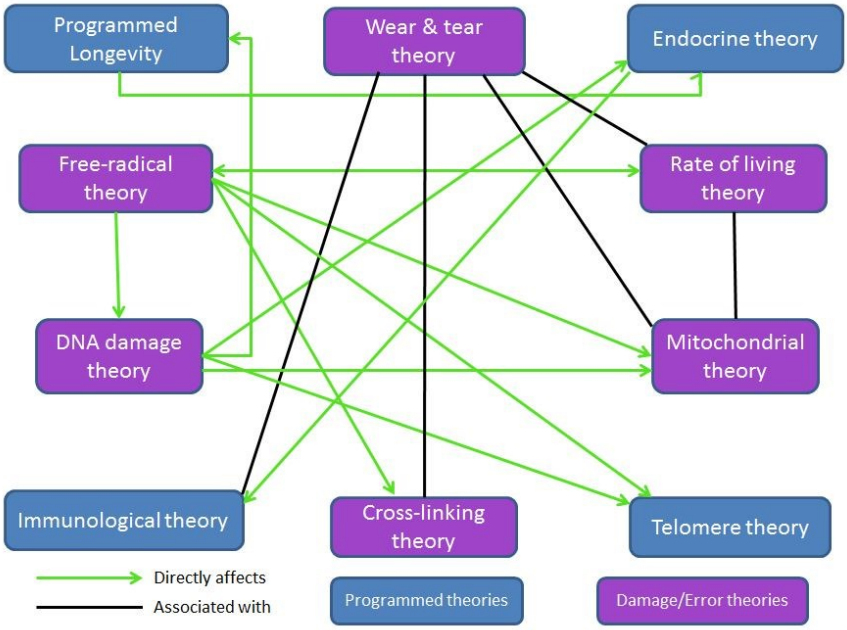

Figure 1.3: The interactions and relationships between the different ageing theories.

1.9.1 Programmed or Developmental-genetic Theories

1.9.1.1 Programmed longevity

Programmed longevity theory states that ageing is a result of different genes being expressed at different points over a lifetime and that genetic instability can switch certain genes 'on' or 'off' (Davidovic et al., 2010). Troen (2003) has discussed that this is possibly an evolutionary development, and that it can lead to the different biological systems within one organism, ageing at different rates.

1.9.1.2 Endocrine theory

The Endocrine theory suggests that ageing is controlled by 'pre-set' biological clocks regulated by hormones from the endocrine system. This theory has been linked to the menopause in women, and insulin/IGF-1 signalling (that has a role in the regulation of ageing) (Bao et al., 2014).

1.9.1.3 Immunological theory

The Immunological theory states that the development of the immune system is dynamic throughout a human's life. In childhood the immune system is in a stage of development, which peaks at, or just after puberty, and then begins a programmed decline. This leaves the body more vulnerable and susceptible to infections, and chronic diseases such as Alzheimer's disease (Rozemuller et al., 2005), and cardiovascular disease (CVD) (Hearps et al., 2014).

1.9.1.4 Telomere theory

The telomere theory states that the lifespan of an organism is dictated by the lifespan of its composing cells and the number of times those cells can divide. Replication of DNA during cell division is terminated at the sequence of an end telomere (TTAGGG). Telomeres shorten after each cell division due to intrinsic molecular factors; this can lead to DNA damage and cell death (Pathai et al., 2013). Therefore the number of times

a human cell can divide is thought to be pre-programmed and is affected by the length of the telomeres at the end of chromosomes (Park and Yeo, 2013). This process is known to be accelerated by oxidative stress.

1.9.2 Stochastic or Damage/Error Theories

1.9.2.1 Wear and tear theory

Initially suggested by Dr August Weismann in 1882, this theory states that as cells and tissues age, they wear out due to continual use (Jin, 2010). Aided by exposure to 'stress' environments such as UV exposure, smoking, and toxins in our diet, this therefore relates to the free-radical theory (Salvi et al., 2006).

1.9.2.2 Cross-linking theories

This theory states that ageing is due to the accumulation of cross-linked proteins (specifically collagen) causing damage to cells and tissues via two mechanisms; firstly, enzyme-controlled processes and secondly, via glycation which involves oxidation and glucose (Bailey et al., 1998). Glycation is often associated with the development of both presbyopia and cataracts (Truscott and Zhu, 2010).

1.9.2.3 Free radical theory

The free radical theory is one of the stochastic ageing theories, that can be affected by physiological and environmental factors. The free radical theory links the oxidative damage caused by reactive oxygen species (ROS) to ageing (Harman, 1956). ROS are unstable molecules with at least one unpaired electron and are therefore highly reactive. ROS and free radicals acquire an electron from a surrounding molecule; leading to further electron transport chain reactions and production of further ROS. If the chain of reaction is not halted, damage occurs to surrounding biological structures including lipids, nucleic acids, and proteins, as well as cellular structures such as cell membranes and DNA (Jacob and Mason, 2005). There are numerous anti-oxidant molecules including ascorbic acid (vitamin C), vitamin E, reduced glutathione (GSH), various enzymes and proteins, which provide redox reactions to stabilise ROS and free-radicals. If the levels of ROS exceeds the levels of anti-oxidant defence molecules, systemic oxidative stress occurs. Prolonged periods of systemic oxidative stress can lead to increased levels of systemic chronic inflammation in humans. There is a complex interlinking relationship between chronic inflammation and oxidative stress, creating a vicious cycle, whereby chronic inflammation can trigger the release of more ROS, further exacerbating oxidative stress (Bryan et al., 2013). This is known to accelerate the ageing process (Sohal, 2002, Harper et al., 2004, Touyz and Briones, 2011, Vitale et al., 2013) and directly affect the cardio-vascular system, causing CVD such as hypertension, which are both caused by, and affects the levels of chronic inflammation (Touyz and Briones,

2011). This theory is often linked to the formation of cataracts (Truscott, 2005, Lobo et al., 2010).

1.9.2.4 DNA damage theory

DNA damage theory states that ageing occurs due to the accumulation of damage to the genetic material of cells. Damage to cellular DNA occurs naturally and continually in living cells (often from ROS); whilst most of this damage is repaired, if the rate of damage exceeds the rate of repair by DNA polymerases and other repair mechanisms, the damage can accumulate resulting in genetic mutations. These genetic mutations can lead to cell malfunction, specifically in non-dividing cells, resulting in cell death, leading to tissue damage (Park and Yeo, 2013).

1.9.2.5 Rate of living theory

Rate of Living theory states that the greater the metabolic rate of an organism, the shorter its life span, i.e. the length of an individual's life is inversely proportional to the rate of energy expenditure (Sohal, 2002). This could be linked to the free-radical theory, since the greater the energy expenditure, the higher the metabolic rate which consequently increases the rate of ROS production.

1.9.2.6 Mitochondrial theory

The mitochondrial theory links with both the free radical and the DNA damage theories of ageing. Park and Yeo (2012) describes how the high rate of metabolic reactions (including aerobic respiration) occurring within mitochondria results in the mitochondrial-DNA being more susceptible to damage from ROS and free radicals (Pak et al., 2003, Harper et al., 2004). Within the mitochondria as levels of oxidative stress increase damage occurs, this is compounded by anti-oxidants and DNA repair enzymes being external to the organelle. Damage to mitochondrial DNA can lead to reduced energy production and an increased production of free radicals (Balaban et al., 2005). Dysfunction of mitochondria has been linked to cataract formation along with an imbalance of ROS (Brennan and Kantorow, 2009).

1.9.3 Anti-ageing theories

Many theories on lifestyle modifications that can increase lifespan exist (Sohal, 2002, Jin, 2010, Park and Yeo, 2013). Increasing anti-oxidant intake and calorie-restricted diets, have both resulted in increased longevity (Simpson et al., 2017); however, for ethical and practical reasons, this latter modification has not been tested in humans and any dietary changes need to be made with caution, as the full effects of this may still be unknown (Lobo et al., 2010). Exercise is known to have advantageous effects on the body due to increased muscle and skeletal tissue, reduction in body weight and reduction in risk of CVD, which could increase lifespan (Park and Yeo, 2013).

1.10 Physiology and ageing of the crystalline lens

The crystalline lens requires energy (in the form of ATP) for growth and to maintain transparency, this is mostly derived from glucose via aerobic respiration and anaerobic glycolysis (Mathias et al., 2010). This, along with other homeostasis reactions exposes the lens cells and organelles to a constant supply of endogenous ROS and free radicals, increasing the risk of oxidative damage. Other exogenesis ROS i.e. from smoking and UV radiation can further increase the levels of oxidative stress. It is known that vitamin E and GSH located in cell membranes, along with ascorbic acid (vitamin C), cysteine and many other enzymes act as repair systems; providing redox reactions to stabilise ROS and free-radicals, reducing oxidative stress (Lou, 2003, Michael and Bron, 2011).

GSH has been stated as the chief anti-oxidant in the lens (Giblin, 2000), Truscott (2005) suggested that concentrations of 2mM (millimolars) of GSH is sufficient to protect against oxidative damage causing cataracts. GSH not only directly reduces ROS and free radicals; it acts synergistically with other anti-oxidants such as ascorbic acid. With increasing age, the production, activity and recycling of GSH falls (Zhang and Augusteyn, 1994, Spector, 1995), leaving the lens nucleus cells more susceptible to damage from oxidative stress.

As discussed in Section 1.1, the crystalline lens contains an abundance of structural protein types: α-crystallin, β-crystallin and γ-crystallins. Lens α-crystallins have a

principal role in the stress response and cell survival (Andley et al., 1998, Andley, 2008), and they have two main functions:

1. Act as heat shock proteins (HSP) and as molecular chaperones, responding to potential stresses from heat, inflammation and hypoxia (Lindquist and Craig, 1988).
2. Molecular chaperone function (Graw, 2009).

Although the crystalline proteins are relatively stable, through-out their life they undergo numerous non-enzymatic modifications.

Deamidation affects α- and β- crystallins, the process involves the unfolding of their tertiary structure, resulting in insolubility, and leaving them more vulnerable to oxidation. This has been related to cataract formation, and is known to occur naturally during embryogenesis, and throughout life (Michael and Bron, 2011).

Glycation occurs via the Maillard reaction in the lens (Nagaraj et al., 2012); glycation involves covalent modification of the crystallins by sugar aldehyde groups such as glucose, fructose, glyoxal, pentoses and resultant products of ascorbic acid degradation (Ortwerth and Olesen, 1988). The end products of glycation are termed advanced glycation end products (AGEs). AGEs formulated in the lens nucleus can contribute to the accumulation of chromophores, which increase absorption of blue-light and lead to nuclear opacities. AGEs can also form cross-links with proteins, and bind with receptors forming receptor advanced glycation end products (RAGE), causing an inflammatory

response and increasing the state of oxidative stress (Pathai et al., 2013). The resulting modifications of the α- and β- crystallins decreases chaperone function, which is further affected by oxidative damage and truncation of α- and β- crystallins.

Protein cross-linking, the formation of high-molecular weight aggregates, and insolubility of crystallins occur as a result of oxidation of modified or unfolded crystallins. Each of these can lead to changes in the highly-ordered protein structure, leading to loss of lens transparency, light scatter and hardening of the lens. This results in cataract formation and the loss of accommodative function. The changes in the lens nucleus can contribute to the formation of chromophores leading to lens nucleus opacities (Michael and Bron, 2011).

Loss of lens transparency, increased light scatter and protein insolubility also occurs as a direct result of loss of α-crystallin chaperone activity with advancing age (Graw, 2009). These changes are accelerated as anti-oxidant defence levels drop, leaving proteins susceptible to oxidative damage (Giblin, 2000, Harding, 1970).

Truscott and Zhu (2010) and Pathai et al. (2013) have suggested that the underlying ageing mechanisms for the development of both presbyopia and cataracts are similar. Both of these ocular conditions have been associated with the ageing theories of cross-linking, free-radical, mitochondrial, DNA-damage, and telomere (Hegde and Varma, 2005, Truscott, 2005, Brennan and Kantorow, 2009, Truscott, 2009, Lobo et al., 2010, Truscott and Zhu, 2010, Babizhayev et al., 2011). Numerous physiological factors such as obesity, conditions related to metabolic syndrome, and nutritional status (Paunksnis

et al., 2007, Lindblad et al., 2008, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013), as well as environmental factors such as smoking, (Cumming and Mitchell, 1997, Klein et al., 1999, Klein et al., 2003, Raju et al., 2006, Xu et al., 2006, Navarro Esteban et al., 2007, Tan et al., 2008c, Wu et al., 2010, Ye et al., 2012), promote these ageing processes and are associated with an increased risk of, or the earlier development of cataracts (Robman and Taylor, 2005).

1.10.1 Obesity

Both an increased Body-Mass Index (BMI) and greater central adiposity, as predictors of obesity, have been associated with cataract formation (Glynn et al., 1995, Schaumberg et al., 2000, Paunksnis et al., 2007, Lindblad et al., 2008, Lim et al., 2009, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013). Furthermore, Glynn et al. (1995) found a dose-response relationship for BMI and cataract, with a 2-point increase on the BMI scale, increasing the risk of cataract by 12%.

There are some discrepancies between studies as to which types of cataracts are associated with an increased BMI. The Blue Mountains Eye Study (BMES) concluded that a BMI >30 was associated with a higher incidence of cortical cataracts after 5 years (Ghaem Maralani et al., 2013), in agreement with the Singapore-Malay Eye Study (SMES) (BMI >25) (Sabanayagam et al., 2011), and another study which examined this link in an Asian population (BMI >30) (Lim et al., 2009); however this latter study also found an association between higher BMI (>30) and posterior subcapsular cataracts.

The mechanism underlying the relationship between raised BMI and cataracts is thought to be due to oxidative stress. Excessive visceral fat is known to increase chronic inflammation; increasing the levels of oxidative stress (Fernández-Sánchez et al., 2011, Lumeng and Saltiel, 2011, Savini et al., 2013). Furthermore, both adults and children with excessive central adiposity are known to have reduced levels of anti-oxidants (Canoy et al., 2005, Andersen et al., 2006, Kaidar-Person et al., 2008); potentially further increasing the oxidative stress levels and chronic inflammation. Systemic chronic inflammation in humans increases the risk of the metabolic syndrome disorders such as hypertension, hyperlipidaemia, and type 2 diabetes (Dandona et al., 2005, Jacob and Mason, 2005, Kyselova et al., 2005, Cheung and Wong, 2007, Self-Medlin et al., 2009, Agarwal et al., 2016, Kim et al., 2016), which have been found to be associated with increased rates of biological ageing (Tzanetakou et al., 2012, Babizhayev et al., 2014)

1.10.2 Metabolic syndrome

Metabolic syndrome is the term given to a group of systemic conditions; dyslipidaemia, hypertension (HBP), central obesity and diabetes mellitus. These conditions are known to both raise and be caused by raised levels of systemic inflammation, which can directly and indirectly lead to a greater risk of other conditions within the metabolic syndrome group (Savini et al., 2013).

Acute levels of inflammation in the body are known to increase the oxidative state of tissues. In the eye this can lead to an increased risk of ocular pathology such as diabetic

retinopathy (Li et al., 2017), age-related macular degeneration (ARMD) (Venza et al., 2012) and cataracts (Spector, 1995). The association between metabolic syndrome and cataracts is well established. Two cross-sectional European studies have found a positive relationship with metabolic syndrome and the risk of cataract (Lindblad et al., 2008, Paunksnis et al., 2007). The first stage of the BMES was a cross-sectional study, which examined the incidence of cataracts in metabolic syndrome and its individual component conditions: diabetics, CVD, dyslipidaemia and BMI. This study found that metabolic syndrome was associated with a higher incidence of all three types of cataracts (Tan et al., 2008b). Further follow-up for a longitudinal study as part of the BMES concluded that metabolic syndrome was associated with an increased 5-year incidence of both cortical and posterior sub-capsular cataracts (Ghaem Maralani et al., 2013). The SMES was a cross-sectional study with a South Asian-based population; it found that metabolic syndrome was associated with a higher prevalence of cortical cataracts, but not nuclear sclerotic or posterior subcapsular cataracts (Sabanayagam et al., 2011).

1.10.3 Hypertension (HBP)

Associations between HBP and cataract development have been equivocal. Several studies have found a general association between HBP and with either the development of cataracts or cataract extraction (Paunksnis et al., 2007, Lindblad et al., 2008, Tan et al., 2008b, Sabanayagam et al., 2011). Other studies have found associations between

HBP and certain types of cataract, specifically nuclear sclerotic (Na et al., 2014), and posterior subcapsular (Richter et al., 2012).

1.10.4 Dyslipidaemia

A relationship between raised levels of serum triglycerides and cataracts was noted in a cross-sectional study in Europe (Paunksnis et al., 2007). In contrast, the cross-sectional portion of the BMES did not suggest a relationship between low-HDL cholesterol and cataract formation (Tan et al., 2008b). However, the longitudinal follow-up did find an association between low-HDL and cortical cataract formation (Ghaem Maralani et al., 2013). The authors concluded that the 10-year time period was needed to observe this relationship and suggested that oxidative stress was the underlying mechanism behind this association between dyslipidaemia and cataract formation (Varma et al., 1984, Klimov et al., 1993, Ghaem Maralani et al., 2013).

1.10.5 Diabetes

A higher incidence of cataracts in diabetics compared to non-diabetics is well established (Janghorbani et al., 2000, Lindblad et al., 2008, Kang et al., 2016). The underlying mechanism behind this observation is thought to be due to poor glycaemic control and increased levels of oxidative stress (Larsen et al., 1989, Biswas et al., 2004, Hegde and Varma, 2005, Hashim and Zarina, 2012). Sorbitol is a sugar derived from

glucose, and if glycaemic control is poor, sorbitol can accumulate in the crystalline lens, causing osmotic stress, resulting in the lens swelling. This can lead to transient changes in refractive error (Skarbez et al., 2010) and transient accommodative paralysis (Marmor, 1973). Moreover, the Maillard reaction, resulting in accumulation of AGEs occurs at a faster rate in diabetics (Garlick et al., 1984, Pathai et al., 2013). Diabetics are also known to have lower levels of accommodation compared to non-diabetics (Moss et al., 1987, Kergoat and Lovasik, 1991).

There are some discrepancies over which types of cataracts are associated with diabetes; the BMES confirmed a link between diabetes and a higher risk of nuclear sclerotic cataracts (Tan et al., 2008b). Further analysis of the data showed diabetes to have an increased 5-year and 10-year incidence of cortical and posterior subcapsular cataracts (Ghaem Maralani et al., 2013). Diabetes has also been identified as a risk factor for nuclear sclerotic cataracts in a Korean population (Na et al., 2014). Interestingly this study did not find this association for cortical cataracts, which is in contrast with the AREDS study that found diabetes to be a significant risk factor for cortical cataracts in a North American population (Chang et al., 2011).

1.10.6 Nutritional status

As previously discussed numerous anti-oxidants are found in the crystalline lens and are known to contribute towards the anti-oxidant defence mechanism (Dagnelie et al., 2000, Lou, 2003, Lien and Hammond, 2011, Koushan et al., 2013, Kang et al., 2016).

Therefore, both observational studies and interventional studies have examined whether anti-oxidant intake has any effect on the incidence of cataract formation in different populations.

1.10.6.1 Observational Studies

Increased plasma levels of vitamin C & E have been found to be inversely proportional to nuclear cataracts in Indian (Dherani et al., 2008) and US populations (Jacques et al., 2001).

The Beaver Dam Eye Study (BDES) found a significant inverse relationship between lutein and zeaxanthin intake and the risk of cataracts. This study also assessed the intake of other vitamins and the risk of cataracts; there were no associations found with the intake of vitamin C or E (Lyle et al., 1999). However, where a subject was found to have other risk factors predisposing them to cataracts, e.g. a positive smoking status, increasing the intake of these two vitamins, decreased the risk of cataracts (Lyle et al., 1999). Although, it should be noted, that smokers have been observed to have diets with fewer nutrients and a reduced vitamin C status, compared to non-smokers (Dallongeville et al., 1998).

Whereas many studies have found an association between an increased intake of micronutrients and a reduced risk of cataracts; trying to identify the specific micro-nutrients involved in this association is difficult due to the contrasting findings. The

cross-sectional stage of the BMES associated a higher intake (greater than the recommend daily intake) of protein, vitamin A, niacin, thiamin, and riboflavin, with a lower risk of developing nuclear sclerotic cataracts (Cumming et al., 2000). Another investigation reported that the use of vitamin supplements containing vitamins A, vitamin B or multivitamins to be associated with reduced risk of nuclear and cortical cataracts (Kuzniarz et al., 2001). However, the longitudinal portion of the BMES suggested that an increased long-term intake of vitamin C in isolation or in combination with vitamins E, beta-carotene and zinc reduced the risk of nuclear cataracts (Tan et al., 2008a).

The Nurses' Health Study in the USA found that high plasma levels of vitamin C, vitamin E, and carotenoids were associated with a lower risk of cataract extraction. They associated higher levels of vitamin A, vitamin C, vitamin E, lutein or zeaxanthin intake with a reduced risk of cataract extraction. The study also found that supplementation of either vitamins C or E over a period of 10 years or more was associated with an approximately a 20% to 30% lower risk of cataract extraction (Kang et al., 2016).

Generally these studies (along with many other observational studies), concluded that further research was required to confirm and fully investigate the mechanisms for any associations found (Leske et al., 1991, Seddon et al., 1994, Leske et al., 1998, Lyle et al., 1999, Cumming et al., 2000, Mares-Perlman et al., 2000, Jacques et al., 2001, Kuzniarz et al., 2001, Tan et al., 2008a).

1.10.6.2 Interventional studies

There have been numerous interventional studies where the incidents of cataracts have been compared between groups of subjects given either supplements or placebos. Similarly to the observational studies, these interventional studies have provided ambiguous findings.

The Linxian Cataract Trials in China gave a nutritional deprived population either a multivitamin or one of 4 vitamin mixes (containing: riboflavin and niacin, or retinol and zinc, or vitamin C and molybdenum, or vitamin E with beta-carotene and selenium) or a placebo. The study reported that the multivitamins reduced the incidence of nuclear cataracts by 36%; the mix of riboflavin and niacin also reduced the risk of nuclear sclerotic cataracts (by 44%), but increased the risk of posterior sub-capsular cataracts (to a smaller degree) (Sperduto et al., 1993).

Two small interventional studies using lutein and a combination of lutein and zeaxathin found an improvement in the visual function of patients with cataract (Olmedilla et al., 2003), and in patients with no ocular pathology (Kvansakul et al., 2006)

The Age-Related Eye Disease Study (AREDS) -1 concluded that supplementation with a combination of vitamin C, vitamin E, and beta-carotene did not affect the incidence of cataract (AREDS I, 2001). The follow-up AREDS-2 study trialled lutein and zeaxanthin supplementations as a treatment for cataract, but found no significant difference between the supplemented and placebo group in progression to cataract surgery.

However, this study did find that lutein and zeaxanthin supplementation was beneficial to those who had a low dietary intake of the two nutrients (Chew et al., 2013).

Two studies of male physicians investigated the effects of vitamin E supplementation vs. placebo, vitamin C supplementation vs. placebo, and beta-carotene vs. placebo and associations with cataracts, over an 8 and 12-year period. No significant increases or decreases in the incidence of cataracts or progression to cataract surgery was found for any of the supplementation groups in comparison to the placebo groups (Christen et al., 2003, Christen et al., 2010). Similar findings for the vitamin E supplementation and beta-carotene supplementation were found in The Women's Health Study (Christen et al., 2004, Christen et al., 2008).

A clinical trial examining the use of a multivitamin vs. placebo in subjects with, or without early cataracts found a reduction in incidence of nuclear sclerotic cataracts, but an increased incidence of posterior subcapsular cataracts in the supplemented group (Maraini et al., 2008).

Two recent reviews of the available literature from both the observational and interventional studies suggest that more data is needed to draw firm conclusions (Chew, 2013, Weikel et al., 2014). Despite these recommendations, Weikel (2014) commented that the limited data from the interventional studies supported the observational studies in suggesting that some nutrients may be beneficial in preventing nuclear sclerotic cataracts.

1.10.7 Smoking status

Galor and Lee (2011) have discussed the effects of smoking on overall ocular health: they state that smoking heightens the levels of general ocular inflammation, increasing oxidative stress whilst decreasing the level of endogenous anti-oxidants in the crystalline lens. In a study examining the effect of smoking on the density of the crystalline lens nucleus Pekel et al. (2014) found that heavy smokers had a denser lens nucleus than non-smokers, however these differences were not found to be statistically significant, possibly due to the small sample size examined.

Numerous studies on populations of different ethnicities have found smoking to be a risk factor for cataract formation, both in isolation but also in association with other factors (such as obesity, reduced anti-oxidant intake, and CVD) (Cumming and Mitchell, 1997, Klein et al., 1999, Klein et al., 2003, Raju et al., 2006, Xu et al., 2006, Navarro Esteban et al., 2007, Tan et al., 2008c, Wu et al., 2010, Ye et al., 2012, Kang et al., 2016). Three large cross-sectional studies have also examined the association between smoking and cataracts; the BDES found a significant correlation between smoking and an increased risk of developing nuclear sclerotic cataracts, which further increased with the number of years and packs of cigarettes smoked (Klein et al., 1999). This association was confirmed after a 10-year incidence follow-up (Klein et al., 2003).

The BMES found that smoking was associated with a higher prevalence of nuclear sclerotic and posterior subcapsular cataracts (Cumming, 1997). However, during the

long-term follow-up of the BMES the association between smoking and posterior subcapsular cataract was not found (Tan et al., 2008c).

Results from the SMES also associated current smokers with developing nuclear cataracts at a younger age, however this study found that smoking increased the risk of all 3 major types of cataracts (nuclear, cortical and posterior subcapsular). Furthermore, a border-line (but non-significant) dose-response trend was found for prevalence of any type of cataract and subjects who smoke >5 packs per day, compared to non-smokers. This trend was found to be stronger and significant for nuclear-sclerotic cataracts and subjects who smoked > 5 packs of cigarettes per week, compared to non-smokers. Interestingly the study found that past smokers showed no increased risk of cataract development (Wu et al., 2010), suggesting a possible recovery system.

1.10.8 Alcohol intake

Several studies have investigated whether alcohol intake affects the risk of developing cataracts with ambiguous findings.

The BDES found a positive relationship between alcohol intake and the incidence of cataract (Klein et al., 1999); these findings have been supported by other studies (Clayton et al., 1982, Harding and Van Heyningen, 1988). In comparison, the cross-sectional BMES found an increased risk of cataracts in heavy drinkers only if they were also heavy smokers (Cumming and Mitchell, 1997). The long-term follow-up of the BMES found that both heavy drinkers (> 2 standard drinks per day) and non-drinkers

were at a significantly higher risk of cataract surgery, in comparison to moderate drinkers (1-2 standard drinks per day) (Kanthan et al., 2010). Contrary to these observations, however some investigators have failed to find any association between alcohol consumption and cataract formation (Italian-American Cataract Study Group, 1991, Leske et al., 1991, Wu et al., 2010).

1.10.9 UV exposure

Increased exposure to UV-A and UV-B can increase the levels of oxidative stress in the crystalline lens and is therefore known to elevate the risk of cataracts (Robman and Taylor, 2005, Roberts, 2011). Most recently, Na et al. (2014) showed that daily exposure of >6 hours of sunlight, significantly increased the risk of both nuclear sclerotic and cortical cataracts. Both decreased cloud cover (Mohan et al., 1989) and increased outdoor activities (Italian-American Cataract Study Group, 1991) have also been associated an increased risk of cataract.

1.10.10 Socio-economic status

It has been concluded that low socio-economic status is a risk factor for numerous eye pathologies such as glaucoma, diabetic retinopathy, ARMD and cataracts (Cackett et al., 2008). Several studies have examined the relationship between socio-economic status in isolation and in conjunction with other modifiable factors whilst assessing the

prevalence of cataracts in different populations around the globe. Generally these studies have observed that a lower level of education (Mohan et al., 1989, Italian-American Cataract Study Group, 1991), a lower income, or a lower-entry job level are associated with a higher risk of cataracts (Reidy et al., 1998, Foster et al., 2003, Klein et al., 2003, Krishnaiah et al., 2005, Athanasiov et al., 2008).

1.11 Conclusions

With the current demand for developing novel solutions of restoring accommodation after presbyopia or cataract removal, it is critical to have an improved understanding of the structure and physiology of the accommodative apparatus including the crystalline lens, ciliary muscle, and the lens zonules. Additionally, it is vital that the age-related changes that affect these structures, and factors that can influence the rate of these changes are identified and mechanisms understood.

In view of these objectives, it is evident that accurate and repeatable methods of assessing accommodation are required to determine the success of methods in restoring accommodation, and to clinically investigate and manage accommodative dysfunction and presbyopia. All subjective and objective methods of measuring accommodation provide useful assessments of the different parameters of the accommodative function. However, standardisation of these methods is imperative to

allow comparisons of findings between studies, and allow further understanding of the relationships between objective and subjective tests.

In research, the dynamic profile of accommodation is often examined; however there is often a lack of standardisation between which accommodative metrics are utilised, and how these metrics are derived, this is particularly important when considering the speed of the accommodative change. As such, there is a need to further examine which metrics most accurately describe this parameter, how these are derived, and the relationship between these metrics and the subjective AF test. Attempts to standardise the technique used to assess AF have been made, however the current method still has some inherent limitations which could reduce the accuracy of the results obtained.

Defocus curves are commonly used to derive information about depth-of-focus, assessing the effectiveness of multifocal and extended depth-of-focus IOL designs in restoring accommodative function in pseudophakic eyes. Yet further standardisation between the methods used to construct defocus curves and derive the depth-of-focus is needed to allow closer comparisons between IOL-designs.

Historically, the structure and physiology of the crystalline lens has been easier to study *in vivo* in humans, due to ease of access; whereas *in vivo* studies of the human ciliary muscle have been hindered by the position of the iris, and until more recent years, a lack of a high-resolution, non-invasive imaging system. The introduction of AS-OCTs have since provided the opportunity to investigate the human ciliary muscle in greater detail, *in vivo*. However, in order to accurately compare the results of such studies, there

is a need to standardise how images are acquired and which metrics are the most appropriate to accurately describe the ciliary muscle physiology and change in morphology during accommodation, and with increasing age.

Both presbyopia and cataracts result from age-related changes to the structure of the crystalline lens. Many physiological and environmental factors affect the rate of ageing within different structures of the body. Truscott and Zhu (2010) have described how the underlying biological ageing mechanisms of both presbyopia and cataract are similar. Therefore, it is possible that the development of both conditions could be influenced by the same physiological and environmental factors. Much of the current literature has concentrated on identifying lifestyle factors that can increase the risk of cataract development (Athanasiov et al., 2008, Lim et al., 2009, Lindblad et al., 2008, Robman and Taylor, 2005, Nita and Grzybowski, 2017). As yet, no studies have examined whether the lifestyle factors which have been associated with the risk of developing cataracts including; smoking status, obesity, anti-oxidant intake, or alcohol intake, also affect the rate at of development of presbyopia.

1.12 Aims of this thesis

The overarching aim of this thesis was to scrutinise the methods used to assess accommodative function, and examine the suitability of these methods in pre-presbyopes, presbyopes and pseudophakes. Additionally one chapter has focused on

investigating the associations between lifestyle and the loss of accommodative function. In an attempt to address some of the issues raised above further aims of this thesis were:

- To investigate a novel method for deriving metrics to describe the accommodative dynamic profile and to assess their relationship to AF.
- To validate a new instrument for measuring accommodative facility, to improve accuracy.
- To examine the influence of a binocular stereogram target during the binocular AF test.
- To assess the validity and repeatability of a novel defocus curve metric in a phakic and a pseudophakic population.
- To explore the use of a swept source spectral AS-OCT (Tomey CASIA 1000 AS-OCT) for the assessment of the ciliary muscle during accommodation.
- To investigate if segments of the ciliary muscle cross-sectional area can be used to assess ciliary muscle morphology.
- To identify if lifestyle factors (associated with increased risk of cataracts), are associated with reduced accommodative function.

Chapter 2: Validation of novel metrics from the accommodative dynamic profile

2.1 Introduction

As discussed in section 1.6.4 the accommodative dynamic profile can be assessed using an auto-refractor, and used to derive different accommodative metrics to describe the parameters of accommodation. Numerous metrics have been derived to quantify the time taken for the accommodative change to occur, including the latency of accommodation, response times, time constants and peak velocity. Across the literature there is significant variability in the methods used to define, measure, and calculate metrics from the accommodative dynamic profile. A brief summary of how each is broadly defined, and the most common methods used to calculate each metric are discussed below.

Latency of accommodation is defined as the time delay between the onset of the accommodative stimulus and the initiation of the accommodative response, which is illustrated by the red arrow in Figure 2.1. The starting point of the latency of accommodation is well defined and established across the literature, and is considered as the introduction of the near stimulus. However, the methods for identifying the initiation of the accommodative response as the end point, differs. One point proposed by Anderson et al. (2010) was the start of the first of five consecutive data points to

increase in accommodation. Another proposed by Schor et al. (1999) and utilised by Kasthurirangan et al. (2003), and Kasthurirangan and Glasser (2006b) was the first point at the beginning of a sequence where three consecutive data points increased in accommodation, followed by a further four data points where no two consecutive points decreased in accommodation. Both of these end-points require visual inspection of the data which is time consuming.

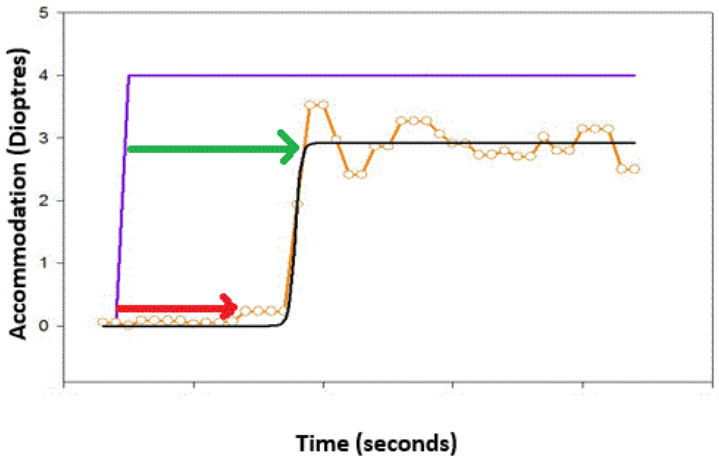

Figure 2.1 An accommodative dynamic profile of a pre-presbyope.
The purple line represents the onset of the accommodative stimulus, the yellow line represents the accommodative response and the black line is the curve fitted to smooth the data. The red arrow demonstrates the latency of accommodation and the green arrow represents the time for accommodation.

Response times are generally defined as the time interval between the change in the accommodative stimulus, and reaching the maximum accommodative (or disaccommodative) response, as illustrated by the green arrow in Figure 2.1. Studies that have examined response times have not specified the exact methodology used to

identify the precise start and end-points to calculate this time interval (Heron et al., 2001, Radhakrishnan et al., 2007, Allen et al., 2010).

Time constants are defined as the time to reach a set percentage of the total accommodative response. Many studies have used 63% as this set point (Beers and Van Der Heijde, 1994, Kasthurirangan et al., 2003, Anderson et al., 2010). However, Radhakrishnan et al. (2007) and Allen et al. (2010) defined the time interval as the period between reaching 10% and 90% of the total accommodative response.

Peak velocity is the maximum speed of the accommodative change reached at a set point of the accommodative response. The peak velocity is calculated using the following formula:

$$Vmax = \frac{a}{\tau}$$

Where Vmax is peak velocity, a is the accommodative response, and τ is the time constant. Although studies tend to agree on this formula for calculation, the variation in methods used to derive the time constant, will ultimately lead to variations in the peak velocity calculated (Beers and Van Der Heijde, 1994, Kasthurirangan et al., 2003, Radhakrishnan et al., 2007, Allen et al., 2010, Anderson et al., 2010).

Before any of these metrics are calculated the accommodative dynamic profile data is smoothed to remove erroneous results. Various methods to achieve this have been applied, including averaging three consecutive points and re-plotting the profile (Anderson et al., 2010). Others have fitted smoothing curves (Beers and Van Der Heijde,

1994, Kasthurirangan et al., 2003, Kasthurirangan and Glasser, 2006b, Radhakrishnan et al., 2007, Allen et al., 2010).

The apparent lack of standardisation in the methodology used to derive these metrics from the accommodative profile makes it difficult to compare results across studies, and draw firm conclusions. Therefore, this study aims to investigate a novel method for deriving the latency of accommodation and the time for accommodative change, from the accommodative dynamic profile and their correlation with AF.

2.2 Methods

The abbreviations used in this chapter are stated in Table 2.1.

Abbreviation	Metric
AoA	Amplitude of accommodation
CPM	Cycles Per Minute
LoA	Latency of accommodation (novel)
LoD	Latency of disaccommodation (novel)
oToAC	Objective time for accommodative change
pLoA	Latency of accommodation (As derived in previous studies)
pLoD	Latency of disaccommodation (As derived in previous studies)
sToAC	Subjective time for accommodative change
ToA	Time for accommodative change
ToD	Time for accommodation
ToAC	Time for disaccommodation

Table 2.1 The abbreviations of the accommodative metrics used in this chapter

2.2.1 Subjects

Forty-three subjects (18 males, 25 females) of mean age 31, standard deviation (SD) ± 8 years (range 19 – 48) were recruited from the student and staff population at Plymouth University through convenience sampling. Exclusion criteria included current or previous ocular pathologies or trauma, binocular vision abnormalities and diabetes mellitus (Skarbez et al., 2010). Subjects whose best corrected visual acuity (BCVA) was worse than 0.0 LogMAR were also excluded. All subjects gave informed consent to participate in the study, following explanation of the procedures and the risks involved. The study adhered to the tenets of the Declaration of Helsinki and was approved by Plymouth University's Research Ethics Committee.

An initial objective and subjective refraction was performed to establish any habitual refractive error. Any refractive error >±0.50DS and/or >0.75DC was corrected with soft contact lenses. The mean spherical equivalent refractive error of the subjects included in this study was RE: -0.90DS ±2.10 and LE: -0.88DS ±2.00.

2.2.2 Subject visits

Each subject was assessed on three separate visits separated by at least 24 hours, to assess intra-observer and inter-observer. At visit one and two, a single examiner assessed each subject. At the third visit a second examiner who was blind to the previous

results examined each subject. The measurements of accommodation were conducted in a random order.

2.2.3 The accommodative dynamic profile

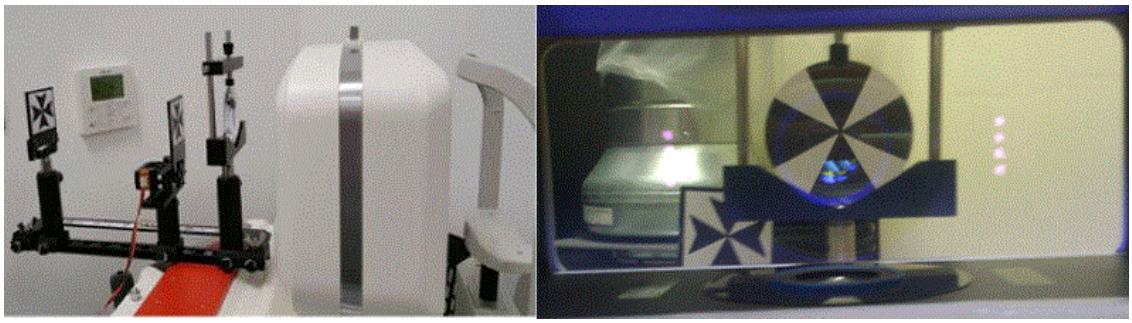

Figure 2.2. The set-up of the auto-refractor and Badal lens system (left), and the subject's view of the Maltese targets whilst measuring accommodative dynamics (right).

The accommodative dynamic profile was assessed monocularly on each eye using the Grand Seiko Auto-refractor WAM-5500 (Grand Seiko Co. Ltd., Hiroshima, Japan) with a motorised Badal adaption (Figure 2.2, left). The contralateral eye was occluded in all cases. Subjects viewed a Maltese cross target within the Badal lens system (Figure 2.2, right). The system consisted of a +5.00 D full aperture convex 2 inch lens, with a Maltese cross target placed at the focal point of the lens (20 cm); thus allowing a measure of the refractive error at 0 D of accommodation (simulating distance vision). A second Maltese cross was attached to a motorized flipper system to provide an accommodative stimulus of 4 Dioptres on demand (simulating near vision). The badal system and auto-refractor were then activated to capture real time measurements of refractive status whilst the accommodative demand was alternated between 0 and 4 D. The auto-refractor was set

to take measurements at a rate of 8Hz for six full cycles. An individual cycle consisted of a near and distance target presentation, each lasting for five seconds.

2.2.4 Accommodative facility (AF)

AF was assessed monocularly with the contralateral eye occluded. The subject was presented with a near vision target at a viewing distance of 40cm, and instructed to look at a four-letter N5 word. Using confirmation flippers a +2.00D lens was presented in front of the ipsilateral eye and the subject was asked to report when the target first became “clear”. Once a positive response was given, the flippers were rotated so that a -2.00D lens was placed in front of the eye, the subject was again asked to report verbally when the target was “clear”. Presentation of the +2.00DS lens followed by -2.00DS lens provided a 4D change in accommodation and was classed as one cycle. After an initial ‘practice’ with two cycles or until the test was understood, a timer was started and the number of full cycles (presentation of +2.00DS and -2.00DS) achieved within one minute was recorded.

2.2.5 Data Analysis

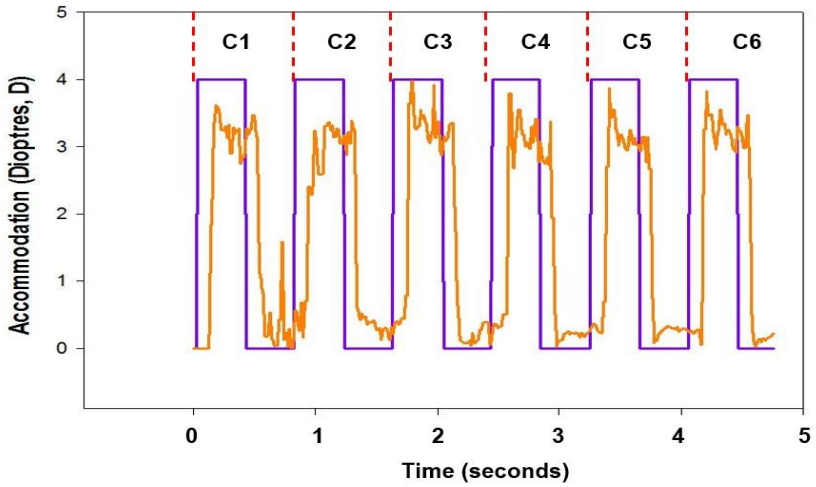

Figure 2.3: The accommodative response of a 27-year-old participant (yellow line), over six cycles of accommodative demand flipping between 0D and 4D (purple line)

For each subject six full cycles of accommodative and disaccommodative dynamics were collected. Each accommodative dynamic profile (as shown in Figure 2.3) was split into individual cycles (labelled C1 to C6.); the start of a cycle was identified as the first data point following the 4D target presentation; the end of a cycle was identified as the last data point of the 0D target presentation. At the start of C1 there was a short time delay between the onset of the stimulus and the start of the auto-refractor recording accommodative response. The end point of C6 was determined by the observer terminating the auto-refractor and DynaWAM software. Therefore, both C1 and C6 were deemed unreliable, so only C2 to C5 were used for analysis. Cycles assessed were further split into accommodation and disaccommodation; accommodation was defined as the phase between the first to the last data point with the 4D stimulus. The

disaccommodation phase was defined as the first to the last data point with the 0D stimulus.

Matlab software (R2014a, The MathWorks Inc., Massachusetts, USA) was utilised to fit a 4-parameter non-linear sigmoidal regression curve (Equation 1) to the accommodative response data points in each cycle (Appendix 1).

$$y = a + \left\{ \frac{b}{1 + e\left(-\left[\frac{x-d}{c}\right]\right)} \right\}$$

Equation 1

A 4-parameter non-linear regression where α is the minimum accommodative response (diopters), b = the asymptote, c is the mid-point between the minimum and maximum accommodative response (diopters) and y is accommodative response (dioptres) and x is the time (seconds)

Figure 2.4 shows C4 from Figure 2.3 with a 4-parameter non-linear regression curve fitted to smooth the data.

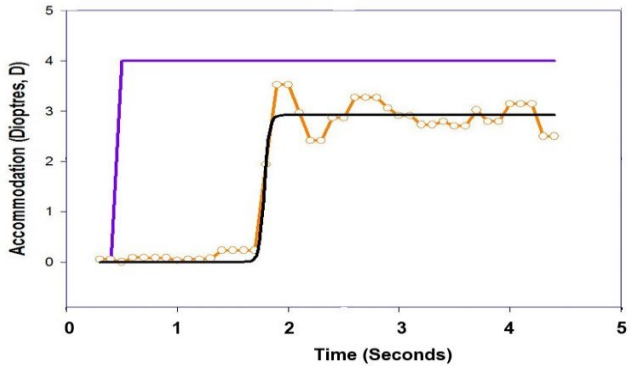

Figure 2.4: The accommodative portion of the C4 from the accommodative profile in Figure 2.3.
The accommodative demand (purple line), the accommodative response (yellow line) and the 4-parameter non-linear regression curve fitted to smooth the accommodative response (black line).

During a blink, the auto-refractor would continue to take measurements resulting in brief erroneous measurements. These were identified and removed before data analysis. If these blinks occurred during accommodation or disaccommodation, the data from that curve was excluded from analysis.

Only three of the four cycles with the most significant linear fits of r^2 were used for data analysis. A mean of these three cycles was found for all of the calculated metrics. From each cycle the following metrics were calculated using MATLAB software.

2.2.5.1 Latency of accommodation (LoA) and disaccomodation (LoD)

Latency of accommodation and disaccommodation were calculated using a method similar to that used in previous studies (pLoA and pLoD) (Schor et al., 1999, Kasthurirangan and Glasser, 2006a, Anderson et al., 2010). The data was smoothed by calculating a mean of three consecutive data points, and this averaged data was re-plotted against time (Anderson et al., 2010). The data was then visually inspected to identify the first data point corresponding to the initial accommodative response. The identification of the initial accommodative response was confirmed once it met the following two criteria: (Schor et al., 1999, Kasthurirangan et al., 2003, Kasthurirangan and Glasser, 2006a):

1. The response was followed by three data points with consecutive increases in accommodation (or decreases in disaccommodation)
2. The response was followed by seven data points where no two consecutive data points had a decrease in accommodation (or increase in disaccommodation).

For the purposes of this study a novel method for deriving LoA and LoD was assessed:

- LoA was defined as the time taken to achieve 1% of the full accommodative response to the 4D near target.
- LoD was defined as the time taken to achieve 1% of the full disaccommodative response once the 4D target was removed and 0D stimulus introduced.

Equation 2 was used to calculate LoA and LoD

$$y = a + \left\{ \frac{(b * 0.01 - a)}{1 + e\left(-\left[\frac{x - d}{c}\right]\right)} \right\}$$

Equation 2

Calculation of LoA and LoD where α is the minimum accommodative response (diopters), b = the asymptote, c is the mid-point between the minimum and maximum accommodative response (diopters) and y is accommodative response (dioptres) and x is the time (seconds)

2.2.5.2 Time for accommodation (ToA) and disaccommodation (ToD), and objective time for accommodative change (oToAC)

- ToA was defined as the time taken to achieve 99% of the full accommodative response to the 4D near target.
- ToD was defined as the time taken to achieve 99% of the full disaccommodative response once the 4D target was removed and 0D stimulus introduced.
- ToAC was defined as the sum of ToA and ToD.

Equation 3 was used to calculate ToA and ToD.

$$y = a + \left\{ \frac{(b * 0.99 - a)}{1 + e\ \left(-\left[\frac{x - d}{c}\right]\right)} \right\}$$

Equation 3

Calculation of ToA and ToD where α is the minimum accommodative response (diopters), b = the asymptote, c is the mid-point between the minimum and maximum accommodative response (diopters) and y is accommodative response (dioptres) and x is the time (seconds)

2.2.5.3 Subjective time accommodative change (ToAC)

AF is a measure of the number of times a participant can accommodate and dis-accommodate to a 4D vergence stimulus in 60 seconds. In order to compare AF with ToAC, the time taken (in seconds) to complete a single cycle needed to be calculated.

This time was termed sToAC and was achieved by dividing 60 by the total number of cycles.

2.2.6 Statistical Analysis

2.2.6.1 Assumptions of normality

After visual inspection of descriptive statistics, histograms, box-plots and Sharpiro-Wilks tests all of the accommodation metrics were found to have a non-normal distribution.

2.2.6.2 Comparisons between the right and left eye

Wilcoxon's Signed Rank tests were used to determine if there were any significant differences between the right and left eye. Where no significant difference was found, the right eye only was then used for further analysis.

2.2.6.3 Repeatability

Intra- and inter-observer repeatability was examined by assessing Intraclass Correlation Coefficients (ICC) for visits 1 - 2 and visits 1-3, respectively. This was calculated using two-way mixed single measures (absolute agreement);

$$ICC\ (absolute, 2) = \frac{subject\ variability}{(subject\ variability + measurement\ error) \div 2}$$

2.2.6.4 Latency analysis

To examine the agreement between LoA-pLoA, and LoD-pLoD, Bland and Altman plots were constructed. Using these plots the proportional bias of the data was assessed. To assess the associations between LoA-pLoA and LoD-pLoD, Spearmans correlation coefficient and Wilcoxons signed-rank tests were also evaluated.

2.2.6.5 Relationship between the objective and subjective measurements of time to accommodation

Spearman's Rho two-tailed tests were conducted to investigate the correlation between the LoA, LoD, pLoA, pLoD, ToA, ToD and ToAC and CPM achieved during AF.

2.2.6.6 Relationship between the accommodative measurements and age

Spearman's Rho two-tailed tests were conducted to investigate the correlation between the LoA, LoD, pLoA, pLoD, ToA, ToD, ToAC, and CPM achieved during AF, with age.

2.2.6.7 Comparisons of the time for accommodation metrics

Bland and Altman plots of sToAC and oToAC were constructed to determine if any proportional bias was present. Wilcoxons signed rank tests were conducted to investigate whether sToAC was significantly faster or slower than oSoAC, and Spearman's correlation coefficients were also assessed.

2.2.6.8 Comparisons of accommodation and disaccommodation metrics

Wilcoxon's signed rank tests were conducted comparing LoA-LoD, pLoA-pLoD, and ToA-ToD to investigate whether the accommodation or disaccommodation metric was faster.

2.2.6.9 Regression Analysis

Both a forward stepwise and backward regression analysis was completed to find which metric was the best predictor AF.

2.3 Results

The box plots of all the accommodative parameters measured are shown in Figure 2.5.

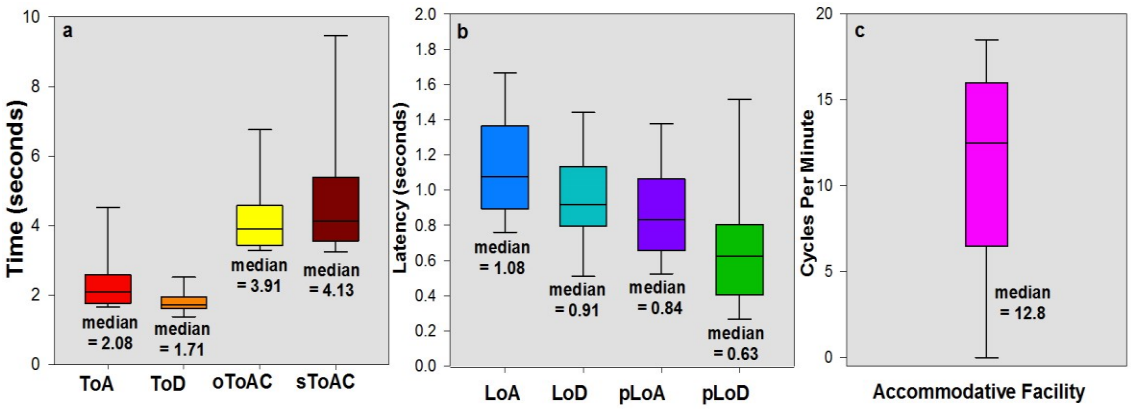

Figure 2.5: Box plots displaying the median, 10th, 25th, 75th and 90th percentiles of data for all accommodative metrics derived from the accommodative dynamic profile and AF

2.3.1 Comparison of the right and left eye

Wilcoxon's signed rank 2-tailed analysis showed that there was no significant difference between the right and left eye at the 0.05 significance level for any of accommodative metrics measured (Appendix 2). Therefore only right eye data from each participant was used for further analysis.

2.3.2 Comparison of latency calculation methods

Latency values obtained using the metric LoA were greater than the values from pLoA (Z= -3.212, p= 0.001) and demonstrated a moderate correlation (r_s= 0.342, p= 0.032). In addition LoD was greater than pLoD (z = -2.920, p = 0.004) and achieved a moderate correlation (r_s = 0.427, p = 0.004).

Bland and Altman plots comparing LoD-pLoD and LoA-pLoA (Figure 2.6) show a mean difference of 0.28 and 0.24 seconds respectively, with relatively large limits of agreement on both plots.

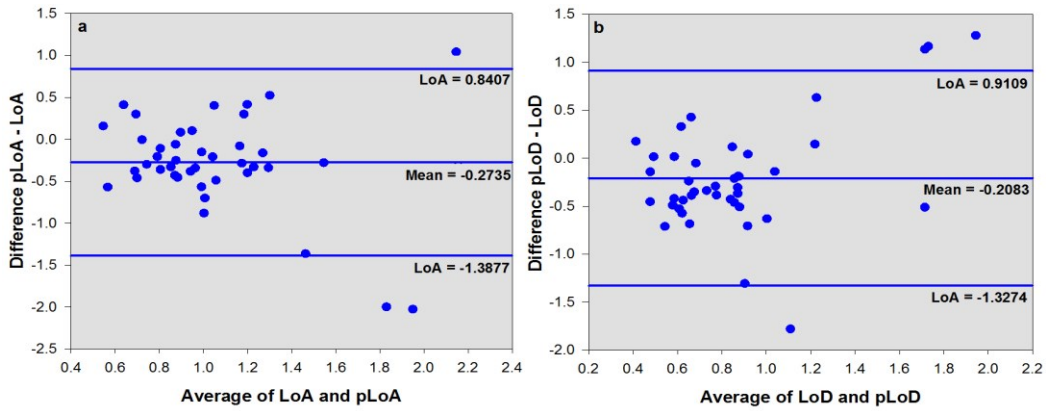

Figure 2.6: Bland and Altman plots of a. LoA-pLoA and b. LoD-pLoD

2.3.3 Correlations between accommodative metrics and AF

Figure 2.7 shows the correlations coefficients of the accommodative metrics when compared with AF. AF demonstrated a moderate, inverse correlation with ToA, ToD, and oToAC (r_s= -0.407, p <0.007), but failed to demonstrate any significant association with LoA, LoD, pLoA, and pLoD.

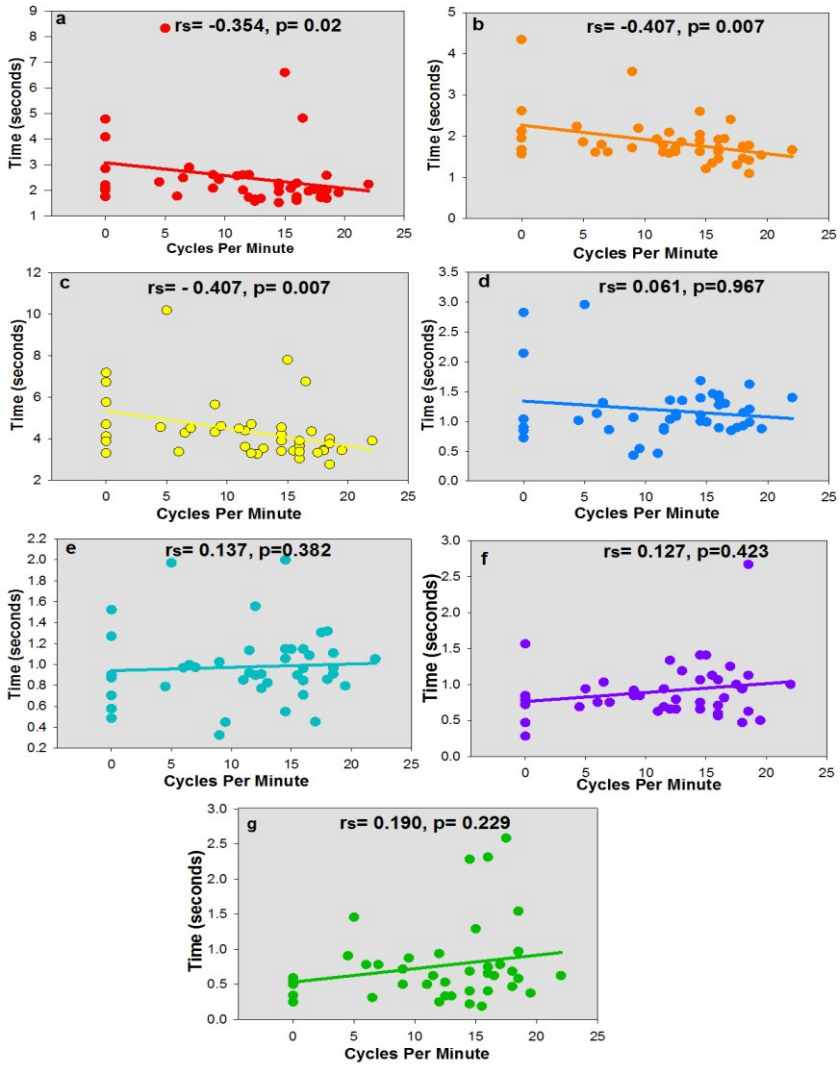

Figure 2.7: The correlation coefficient between AF and a. ToA, b. ToD, c. oToAC, d. LoA, e. LoD, f. pLoA, and g. pLoD

2.3.4 Correlations between accommodative metrics and age

Figure 2.8 shows the correlation coefficients of the accommodative metrics with age. ToA, ToD and oToAC displayed a moderate positive correlation with age, whereas AF showed a strong inverse correlation with age. However, none of the latency of accommodation or disaccommodation metrics demonstrated a correlation with age.

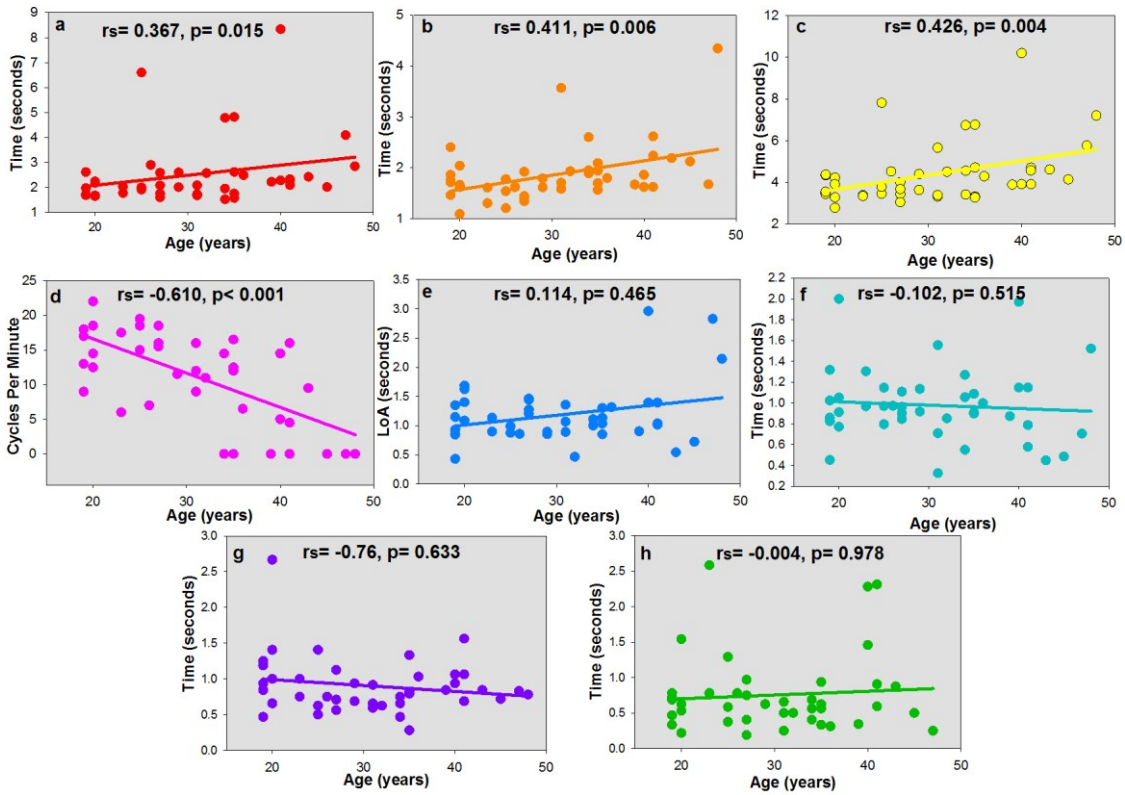

Figure 2.8: The correlation coefficient between age and a. ToA, b. ToD, c. oToAC, d. AF, e. LoA, f. LoD, g. pLoA, and h. pLoD.

2.3.5 Comparison of oToAC and sToAC

sToAC was significantly greater than oToAC (z=-2.498, p=0.012) and the two metrics showed a moderate correlation (r_s=0.371, p=0.026). The Bland and Altman plotsrevealed a mean difference of 0.80s between the two metrics and significant proportional bias: as mean oToAC and sToAC times increased, the values for sToAC increased disproportionally to those for oToAC.

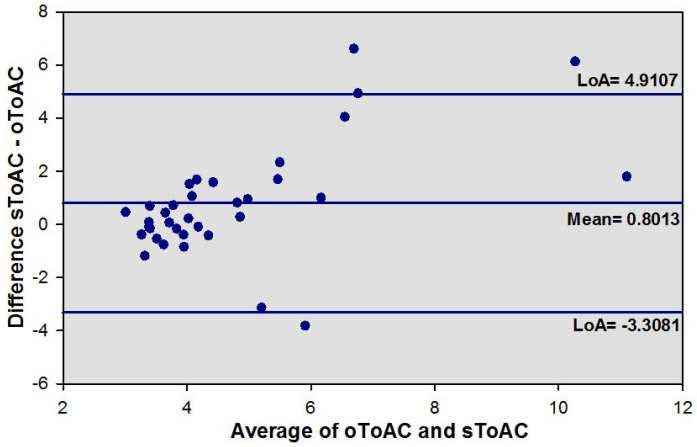

Figure 2.9: Bland and Altman plot of sToAC and oToAC

2.3.6 Repeatability

Table 2.2 shows the intra- and inter-observer repeatability of each metric measured. AF demonstrated high levels of both intra-observer and inter-observer repeatability. The lowest level of intra-observer repeatability was found with ToA, and the lowest level of inter-observer repeatability was observed with pLoA.

	Intra-observer Repeatability	Inter-observer Repeatability
AF	0.843	0.889
ToA	0.258	0.645
ToD	0.811	0.568
oToAC	0.491	0.575
sToAC	0.843	0.889
LoA	0.937	0.384
LoD	0.342	0.519
pLoA	0.475	0.295
pLoD	0.610	0.375

Table 2.2: The Intraclass correlation coefficients of each measured metric, showing both intra-observer repeatability and inter-repeatability.

2.3.7 Comparisons of accommodation and disaccommodation metrics

Wilcoxon's signed ranks analysis revealed that ToD was significantly faster than ToA (Z= -3.357, p< 0.001), LoD was significantly faster than LoA (Z= -3.236, p<0.001) and that similarly pLoD was significantly faster than pLoA (Z= -2.683, p= 0.007).

2.3.8 Regression Analysis

Both a forward stepwise and backward regression analysis was used as an exploratory test to determine which metric was the best predictor of AF. ToA, ToD and ToAC were

the independent variables included, and it was found that ToD (t= -2.673, p= 0.011) was the single best predictor of AF. However when age was added into the model, age (t= -5.422, p <0.001) was found to be the single best predictor of AF, this was also found to be true in the backwards model.

2.4 Discussion

Numerous metrics have been used to describe the speed of accommodation from the accommodative dynamic profile. Different studies have used a variety of methods to derive these metrics, so that it is difficult to compare the results across the literature. Therefore, the present investigation utilised subjective measures of accommodative facility to validate both current and novel metrics from the accommodative dynamic profile.

2.4.1 Latency

Previous studies have found average latency times for 19-40 years of approximately 0.3s (Sun et al., 1988, Heron et al., 2001, Heron et al., 2002, Heron and Charman, 2004, Kasthurirangan and Glasser, 2006a, Anderson et al., 2010). Although in this study medians (rather than means) have been reported, the median latencies were found to be slower (LoA 1.07s, LoD 0.91, pLoA 0.84s and pLoD 0.63s) than previously reported, due to the different equipment used to measure accommodative dynamics. In this study

all of the latency calculations have an accuracy limited to ±0.125s, the interval at which each data set was recorded by the auto-refractor. This, for example could have accounted for up to 19.8% of the total of the median of pLoD (0.63s).

The medians of LoA and LoD (1.07s and 0.91s, respectively) were slightly higher than the medians of the previously used metric pLoA and pLoD (0.84s and 0.63s respectively). The higher values for LoA and LoD could be attributed to the difference in methods of calculating these latencies from the data. Firstly, data smoothing for LoA and LoD was achieved by fitting a 4-paramenter non-linear regression curve; whereas for pLoA and pLoD the data smoothing was achieved by averaging three consecutive values. Secondly, LoA and LoD were calculated from the onset of the stimulus until 1% of the accommodative response was reached, this would generally be a longer time period than pLoA and pLoD, which are calculated until the initiation of the accommodative response. The measures for pLoA and pLoD are not interchangeable with the results for LoA and LoD respectively: LoA and LoD results are longer as they assess the time taken to the 1% growth point rather than the initial point of change. Furthermore the novel metrics utilise curve fitting as opposed to visual inspection of the data and as such demonstrate greater repeatability.

The relationship between age and latency in adults has been examined in numerous studies, with equivocal findings. Many studies have agreed with the findings of this study; that there is no significant correlation between age and latency (Sun et al., 1988, Heron et al., 2001, Heron et al., 2002, Heron and Charman, 2004). Conversely Anderson

et al. (2010) found a significant decrease in both accommodative and disaccomodative latencies with age (Anderson et al., 2010). Furthermore, some studies have found a significant increase in the disaccommodative latency in adults (Kasthurirangan and Glasser, 2006a, Heron et al., 2002). The ambiguity in the relationship between age and latency from these studies could be due to whether the accommodative demand used whilst measuring the accommodative dynamics was proportional to the maximum effort for each age group. In older participants a step change accommodative demand would represent a significantly larger proportion of their overall accommodative response ability compared to younger participants, and would therefore require greater effort (Heron et al., 2002, Kasthurirangan and Glasser, 2006a).

2.4.2 Correlations with accommodative facility (AF)

AF provides a combined assessment of both accommodation and disaccommodation. As expected no association was found between measures of AF and the LoA, LoD, pLoA and pLoD metrics. This is likely to be due to a number of factors; primarily all of these metrics define the accommodative or disacommodative response individually rather than the gross accommodative/disaccommodative behaviour as assessed by AF. Furthermore, the latency metrics only evaluate the time taken to initiate the accommodative/disaccomodative response rather than the time taken to complete the response, which is more analogous to the AF measures.

Unlike the latency metrics, ToA, ToD, and oToAC all assess the time taken to complete the accommodative/disaccommodative response. Therefore, it is unsurprising that these metrics were correlated with AF. ToD was found to be the best predictor of AF when age was excluded as a factor. This latter finding was unexpected as it would be predicted that the oToAC relates better to AF as it provides a measure of the gross accommodative/disaccommodative response. This discrepancy may be explained by examining the Bland and Altman comparisons of the AF (expressed as sToAC) and oToAC. The results of sToAC were slower than that of oToAC. A likely reason for these differences may be due to the objective measures of ToA, ToD, and oToAC accounting only for the time of response, whereas sToAC calculated from AF would account for both time and accuracy of the accommodative response. This could also explain why the median oToAC (3.91s) was found to be significantly faster than the median sToAC (4.80s). Another reason for this slower subjective time could be the combined reaction times of the participant reporting when the target is clear and the practitioner reacting to this, which would not be accounted for with the objective measures. Furthermore the subjectivity of the endpoint for sToAC and the influence of depth-of-focus, could also have contributed to the slower sToAC compared to oToAC.

A significant proportional bias was found on the Bland and Altman plot, resulting from the upper ceiling effect of the sToAC. In order for a cycle to be registered using AF the subject is required to accommodate to a sufficient level to resolve the target. In comparison the WAM flipper system is pre-programmed and changes accommodative

demand independent of the subjects accommodative effort. In cases where a subject is unable to accommodate sufficiently it is likely that using a lower accommodative stimulus may have produced more concurrent results.

As expected, age was found to be the single best predictor of AF demonstrating that the technique is a valid assessment of accuracy and amplitude of accommodative response which is known to decline with age (Duane, 1912, Hamasaki et al., 1956, Strenk et al., 2005, Glasser, 2006, Lockhart and Shi, 2010). ToA, ToD and oToAC took into account the time taken to reach 99% of the amplitude of accommodative response. However, latency accounts only for time until initiation of the response. This may explain why latency failed to change significantly with age.

To summarise, ToD was the metric found to be most closely associated with AF. In addition it demonstrated the highest repeatability of all of the metrics, and was observed to be the most valid and repeatable metric in relation to AF.

2.4.3 Objective time of accommodation

The median ToA (2.09s) and ToD (1.71s) found in the present study are in accordance with the times observed by Radhakrishnan et al. (2007) (2.39s and 2.04s respectively) and Allen et al. (2010) (1.89s and 2.64s, respectively). In both of these studies accommodative dynamics were measured on the ipsilateral eye whilst AF was performed on the contralateral eye; thus, directly measuring subjective and objective reaction times simultaneously (Radhakrishnan et al., 2007, Allen et al., 2010).

Other studies, which have measured accommodative response times have reported shorter times of between 0.53s and 0.9s (Tucker and Charman, 1979, Ibi, 1997, Heron et al., 2002). The slower ToA and ToD found in this study may be due to inherent differences in the methodology and analysis. It has also been noted that both latencies and response times are more difficult to measure accurately if the magnitude of response is small (Heron et al., 2002).

Contrary to the findings of Heron et al. (2002) the present results demonstrated that ToA, ToD and oToAC significantly increased with age. Heron et al. (2002) proposed that the apparent maintenance of reaction time, whilst the AoA reduced with age, could be due to an age-related decrease in the diameter of the ciliary ring in the unaccommodated state. The equatorial diameter of the lens remains constant in presbyopia, yet, the distance between the unaccommodated ciliary ring and lens equator decreases (Strenk et al., 1999). These physiological changes could reduce the maximum possible zonular lens tension achievable, resulting in a reduction in the maximum AoA without a change in the response time (Heron et al., 2002). However, the findings of this study fail to support this supposition, and indicate that the reduction in elasticity of the crystalline lens or capsule with age reduces ToA, ToD, oToAC, sToAC and AoA.

The accommodative latency is attributed to the time taken for the neurological processing involved in the recognition of a blurred target, to the innervation of the ciliary muscle. Results from this study would suggest that age does not impact on this

neurological phase as readily as the time phase where the change in shape of the crystalline lens occurs, leading to the corresponding change in accommodation. Furthermore, when Shao et al. (2015) imaged the accommodative apparatus during accommodation, they noted a time delay between the ciliary muscle contraction and the resultant change in shape of the crystalline lens; this time delay would further contribute to the latency period of accommodation. Contraction of the ciliary muscle is unaffected by increasing age (Richdale et al., 2013, Richdale et al., 2016); this would support the findings of this study in demonstrating that, unlike the time for accommodative change the latency period showed no significant change with increasing age, these differences are likely to be due to presbyopic changes in the crystalline lens structure.

2.4.4 Difference between time to accommodate and disaccommodate

In accordance with previous studies, all objective metrics examining accommodation were found to be faster than those assessing disaccommodation (Vilupuru and Glasser, 2002, Beers and van der Heijde, 1996).

In view of the present findings, the rate of lenticular shape change during accommodation and disaccommodation appear to be asymmetric; with a more rapid increase in radius of curvature during disaccommodation. This difference in time for

accommodation and disaccommodation may be related to the mechanism of accommodation. It has been suggested that disaccommodation occurs more rapidly due to the passive nature of increasing zonular tension, in contrast to accommodation which requires a decrease in zonular tension (Beers and van der Heijde, 1996, Vilupuru and Glasser, 2002). Alternatively, the nature of the accommodative response may be explained by the properties of the elastic capsule surrounding the lens. It is conceivable that the capsule has a viscoelastic nature rather than being purely elastic, causing a significant delay when generating a convex shape.

2.4.5 Considerations of this study

The Grand Seiko WAM auto-refractor took measurements of accommodation every 0.125 seconds. This would have limited all time and latency metrics measured in this study to ±0.125s and accounted for up to 19.8% of the total of the median of pLoD (0.63s). This interval time is significantly greater than other studies that have examined the accommodative dynamic profile using Power refractors, which take measurements at frequencies of 25Hz (0.04s) (Kasthurirangan et al., 2003, Kasthurirangan and Glasser, 2006b, Radhakrishnan et al., 2007, Allen et al., 2010), or 30Hz (0.03s) (Anderson et al., 2010). The difference in frequencies of measurement acquisition in this study, compared to previous investigations may partly explain the longer latency periods found in this study, compared to those found previously (Sun et al., 1988, Heron et al., 2001,

Heron et al., 2002, Heron and Charman, 2004, Kasthurirangan and Glasser, 2006b, Anderson et al., 2010).

To further examine the relationship between the accommodative time metrics derived from the accommodative dynamic profile and AF, a novel instrument could be developed to allow separate logging of the subjective times for accommodation and disaccommodation from the AF test. An instrument which allows the subject to initiate the flip of the lenses during the AF test would allow for more accurate measurements of sToAC to be calculated. This would remove the practitioner reaction time, and the time error added to the average sToAC which is determined by the point at which the AF cycle is terminated.

2.4.6 Conclusions

In summary the key findings of this study was:

- The novel metrics for latency examined were more repeatable than the metrics used in previous studies.
- The novel method of calculating latency were not interchangeable with the method used in previous studies.
- Time for disaccommodation was the metric most associated with accommodative facility, and demonstrated the highest repeatability of all of the metrics

Chapter 3: The validation of a Patient-controlled Accommodative Facility Instrument

3.1 Introduction

In Chapter 2 the objective metrics describing the time for accommodative change, derived from the accommodative dynamic profile, demonstrated a strong relationship with accommodative facility (AF). It became apparent that the development of a novel instrument could improve the accuracy of these measurements, and allow closer examination of the relationship between the time metrics from the accommodative dynamic profile and AF. This chapter describes the development of this novel instrument and employs it in scrutinising the traditional method of AF used in clinical practice.

For an efficient change in focus of the eye to occur, accommodation must be rapid, accurate and stable. AF is a subjective test for assessing the time taken for the accommodative change whilst accounting for the accuracy of the response (Eperjesi, 2004).

The AF test has been shown to be both valid and repeatable (Zellers et al., 1984). Clinically, the AF test is used to investigate symptomatic accommodative dysfunction, (Levine et al., 1985, Goss, 1992, Gall and Wick, 2003) and is predominantly used in the paediatric setting (Hennessey et al., 1984b, Wick et al., 2002b). Studies have found that

in both children and adults with symptomatic accommodative dysfunction, AF is often reduced, even when all other measures of accommodation, such as amplitude of accommodation and dynamic retinoscopy, are within a normal range (Levine et al., 1985, Wick and Hall, 1987, Wick et al., 2002a, Gall and Wick, 2003).

The standardised traditional method for AF uses ±2.00DS flippers to induce a 4D accommodative change. The flippers are alternated by the practitioner each time the subject reports a near target as 'clear', suggesting that sufficient accommodative effort has been achieved; the number of full cycles (changing from positive-to-negative lenses and changing back from negative-to-positive lenses) 'cleared' within one minute are recorded in cycles per minute (CPM) (Zellers et al., 1984, Wick et al., 2002b). Normative values of AF are typically quoted to be >7 CPM binocularly using a stereogram target, or >11 CPM monocularly (Zellers et al., 1984).

There are several limitations in the AF test (Kedzia et al., 1999), including:

1. The optical magnification (by the +2.00DS lens) and minification (by the -2.00DS) of the target, is likely to artificially increase the time taken to accommodate and decrease the time taken to disaccommodate.
2. The subjective nature of the technique is likely is to lead to inherent variability in the test results.
3. Saccadic eye movements are required to move between symbols on the fixed target.

4. Having a discrete magnitude for accommodative change (4D) may disproportionally disadvantage patients with a lower amplitude of accommodation.
5. The technique is based on the combined reaction time of the practitioner and patient and thus further increasing test variability.
6. Application of the normative values for AF to different age ranges.

Solutions have been sought to reduce the impact of these limitations on the results of the AF test. The subjective nature of the AF method can lead to uncertainty relating to the accuracy and repeatability of the test. Such issues are more pronounced when assessing AF in the paediatric population; studies have found children often achieve fewer CPM during AF compared to adults, despite having greater amplitudes of accommodation (Scheiman et al., 1988, Kedzia et al., 1999). Furthermore the cognitive demand of the task affects the time taken by the subject to appreciate the target as clear and relaying this to the practitioner. Kedzia et al. (1999) suggested reducing the cognitive demand of the task from saying aloud the letter or number on the target, to instead asking the subject to say 'clear' or 'now' when the target is focused, or allowing the patient to initiate the 'flip' of the lenses. The authors found that by compensating for the child's recall and reaction times in this manner, measures of binocular AF CPM were comparable to Zeller's normative values for AF.

It has been proposed that the discrete magnitude for accommodative change (4D) reduces the sensitivity of the traditional AF method, when utilised to differentiate between symptomatic and asymptomatic accommodative dysfunction in adults (Siderov and DiGuglielmo, 1991, Wick et al., 2002a, Yothers et al., 2002). Therefore, Yothers et al. (2002) and Scheiman and Wick (2014) recommended that when assessing symptomatic adults, the accommodative change value should be relative to the individual's amplitude of accommodation. However, not all studies agreed with the reduced sensitivity findings in early presbyopes (Levine et al., 1985, Gall and Wick, 2003), and the proposed solution would be impractical to implement in a clinical setting. The AF technique is significantly affected by the reaction times of both the subject and practitioner thus artificially limiting the CPM achieved. In an attempt to remove this error in a paediatric population, Kedzia et al. (1999) firstly examined the response time of subjects by performing the AF through plano lenses, calculating the average time taken for each cycle. AF was then performed through ±2.00 lenses using the traditional method, and the average time taken for each cycle was calculated. This former calculated time was then used to compensate the latter; this effectively removed the combined reaction times of the subject and practitioner reaction time from the final measurements.

As discussed above, Kedzia et al. (1999) proposed allowing the subject to initiate the 'flip' of the lenses. A motorised system could minimise (and regulate) the time delay caused by the practitioner reaction time, and part of a subject's neuro-processing time

involved in calling aloud the target. Furthermore, this would allow improved comparisons of assessments of the time for accommodation (ToA), time for disaccommodation (ToD), and time for accommodative change (ToAC) derived from AF, to the corresponding objective measurements derived from the accommodative dynamic profile. In Chapter 2, the subjective method of AF was correlated with an objective ToAC (oToAC) metric derived from the accommodative dynamic profile. oToAC was calculated by summating the ToA and ToD measured from the same cycle of changing focus from distance-to-near and near-to-distance. When considering the traditional method of assessing AF it is possible to calculate a subjective ToAC (sToAC) if the time period over which the test is completed (sixty seconds) is divided by the number of cycles achieved. However, the sToAC is likely to be longer than the oSoAC due to reaction times, and at which point in final the cycle, the test is terminated. This error would be proportionally greater the fewer CPM achieved.

To overcome such errors, it can be anticipated that a modified technique for assessing AF, where the time interval for each lens flip could be recorded automatically, and it would be possible to record separate measurements for subjective ToA and ToD, allowing sToAC to be calculated as oToAC.

Another difficulty with AF testing is the application of the normative values for AF (Zellers et al., 1984). Findings of ≥7 CPM binocularly using a stereogram target and ≥11 CPM monocularly, were noted in a study of subjects between the ages of 18 and 30 years, who had previously passed a binocular vision-screening exam, and were classed

as having 'normal' binocular function. Applying these normative values to children, adults older than 30 years, or subjects with binocular dysfunction could lead to an artificially high false positive rate when investigating accommodative dysfunction. Wick and Hall (1987) found an artificially high failure rate when they applied this binocular criterion to a group of schoolchildren. They postulated that either a lower pass/fail criteria should be used for school screening, and/or AF results should be considered in conjunction with other measures of accommodation, such as lag and amplitude, before a diagnosis of binocular accommodative dysfunction is made. Scheiman et al. (1988) recommended normative values of AF for schoolchildren to be monocularly ≥7 CPM and ≥5 CPM binocularly if a stereogram target is used. Siderov and DiGuglielmo (1991) found much lower CPM of 1.2 with the AF test, or 8.9 CPM when using ±1.00DS lenses. Other studies that have attempted to either set normative values of AF or apply normative values across different populations (Table 3.1). The contrasting results from these studies have been attributed to the range of ages of the subjects included, variations in the screening methods used to define 'normal', and/or the different methods used to measure AF (Wick et al., 2002b).

The traditional method for assessing binocular AF recommends the use of a stereogram target to monitor for suppression (Zellers et al., 1984, Wick et al., 2002b). Bifoveal fixation is mandatory to achieve binocular fusion. Assessment of AF requires performance of a visually demanding task, during which the visual stress it imposes could lead to suppression of one eye, breaking down bifoveal fixation. If such

Study	Age range (years)	Inclusion/ exclusion	Test procedure	Results (CPM)			Comments
Burge (1979)	6 to 39 n= 30	6/6 & N5 Monoc. AoA of >5D Pre-presbyope Non-strabismic	Traditional	 RE LE Binoc F* Binoc S**	Mean 12.5 11.5 9 7	Median 13.5 12 9.5 6	
Griffin et al. (1977)	20 to 30 n= 27	Not stated	Traditional Verbally reported numbers	 Dominant eye Binoc F* Binoc S**	'Norms' (11) 18 12.5 9	'Suspect' (16) 15 9 4	Classed as 'suspect' if during AF they supressed or scored <9CPM.
(Hoffman and Rouse, 1980)	Literature review						Recommended monoc or binoc of 12CPM 6/9 line at 40cm. Diff of 2 or more CPM between eyes
Hennessey et al. (1984b)	8 to 14 n= 60	40 secs of arc. Normal AoA (Hofstetters)	Used target of 6/12 monoc. and a 6/9 stereogram	 RE LE Binoc	Asymp (30) 14±6 15.2±6.7 10.7±8	Symp (20) 8.6±5.5 9.2±6.7 4±6	Used Zellers et al., (1984) criteria as normative values
Zellers et al. (1984)	18 to 30 n= 100	Pass screening, BCVA of 6/9, 40 secs of arc	Stereogram 6/9	 Monoc Binoc	RE 11.6±5 7.7±5	LE 11.1±5.3	
Levine et al. (1985)	16 to 32 n= 105	Significant anisometropia	20/25	 Monoc	Asymptomatic 14.6	Symptomatic 10.1	Concluded that cut-off between symptomatic and asymptomatic was 11CPM
Siderov and DiGuglielmo (1991)	30 to 42 n= 45	Stereoacuity of 40 sec, 6/6 binocularly	6/9 stereogram	Standard binoc. only & ±1.00DS	±2.00 1.2±2.1	±1.00 8.9±5.2	

Table 3.1 The variation in methods and findings in accommodative facility testing across the current literature
**** Binocular measurements without suppression monitored, ** Binocular measurements with suppression monitored***

occurs during binocular AF testing, erroneous measurements will be recorded (Griffin et al., 1977, Burge, 1979). Due to the increased visual demands of the task, the CPM achieved when a stereogram target is used is expected to be significantly less than if suppression is not monitored. This reduction is expected to be between 3 and 3.5CPM (Burge, 1979, Griffin et al., 1977). However, the studies that have explored this relationship have numerous limitations that need to be considered. Burge (1979) mixed child and adult subjects; accommodative ability is known to reduce through-out life (Glasser, 2006, Charman, 2008), and children are known to achieve fewer CPM ompared to adults with the traditional method of AF (Hoffman and Rouse, 1980, Hennessey et al., 1984a, Wick and Hall, 1987, Scheiman et al., 1988, Wick et al., 2002b). By including subjects with a large age range this would have over-estimated the reduction in binocular CPM achieved using a stereogram target, in adults. Griffin et al. (1977) did not state the inclusion or exclusion criteria of their subjects or whether screening for binocular dysfunction was conducted, therefore their study could have included both patients with 'normal' accommodative function and binocular abnormalities. Again, this could have led to an over-estimation of the reduction in binocular CPM achieved using a stereogram target. Therefore, further work is required to understand the true effect of monitoring suppression during the binocular AF test, in adults. Such an investigation with an instrument which allows subjects to initiate the 'flip' of the lenses, and automated logging of the time interval at each lens flips would increase the accuracy

of the times recorded, and allow improved comparisons between subjective and objective ToA, ToD and ToAC to be made.

3.1.1 Aims

The primary aim of this study is to validate a novel Patient-controlled motorised Accommodative Facility System (PcAF). The second aim of this study is to examine the influence of a binocular stereogram target and to determine which type of target is the most appropriate for binocular AF assessment.

3.2 Methods

3.2.1 Subjects

Thirty subjects (8 male, 22 female) of mean age 25, SD ± 7 years (range 18 – 34) were included in this study, from the student and staff population of Plymouth University through convenience sampling. The inclusion criteria for the study were as follows:

- BCVA in soft contact lenses equal to or better than 0.0 LogMAR monocularly and binocularly (Thomson Test Chart).
- Normal stereo acuity (defined as 40 secs of arc) with the Titmus Fly test (Stereo Optical, Chicago USA).
- The absence of binocular vision abnormalities (compensated phorias, measured at 40cm, were acceptable up to 4^{Δ} on cover test).

- At least one cycle per minute (CPM) could be achieved monocularly, by each eye and binocularly within one minute during AF.

The exclusion criteria for this study included current or previous ocular pathologies or trauma, and/ or diabetes mellitus.

Ethical approval was obtained from the Plymouth University Ethics committee and the study was performed in accordance to the tenets of the Declaration of Helsinki. All subjects gave informed consent to participate in the study, following an explanation of the procedures and the risks involved.

The subjects underwent an objective and subjective refraction, any refractive error >±0.50DS and/or >0.75DC was corrected with soft contact lenses. A screening exam was conducted to ensure each subject met the inclusion/exclusion criteria. The mean spherical equivalent refractive error for the subjects was -1.59DS (range -6.38DS to plano; myopic n=17, emmetropic n=13), with the mean BCVA of -0.04logMAR (0.00LogMAR to -0.16logMAR).

3.2.2 Part 1: Validation of the Patient-controlled Accommodative facility Instrument

3.2.2.1 Accommodative Dynamics (AD)

Monocular AD was assessed using the Grand Seiko Auto REF WAM-5500 with a motorised Badal adaption. The methods used to measure the AD and derive the metrics of ToA, ToD and ToAC are as described in section 2.2.3 and section 2.2.5

3.2.2.2 Traditional Accommodative facility (TAF)

Figure 3.1: The Bernell BC29 mini variable vectogram used during AF testing

A Bernell BC29 mini variable stereogram (Figure 3.1) set at 40cm from the subject was used as the test target for the AF test. The BC29 stereogram displays three lines of letters equivalent to a reduced Snellen near acuity of 6/9. When polarising filters are

used to view the stereogram, the top and middle line of letters are viewed by one eye, whilst the fellow eye views the lower and middle line. The subject was asked to confirm if all three lines of letters were present and clear. The subject was presented with a +2.00D lens using confirmation test flippers, and asked to report when 'all three lines of letters were present and clear', at which point the lens presented was flipped by the practitioner to a -2.00D lens and held in place until the subject once again reported that 'all three lines were present and clear'; this was classed as one cycle. After an initial 'practice', with at least two cycles or until the subject understood the task, a timer was started and the number of full cycles achieved within one minute was recorded. TAF was tested under the following conditions:

- Right eye with no polarising filters (left eye occluded)
- Left eye with no polarising filters (right eye occluded)
- Binocularly viewing a polarised version of the Bernell BC29 card with polarising filters

Between each AF test subjects were allowed a break of up to two minutes to minimise fatigue effects.

3.2.2.3 Patient-controlled Accommodative Facility

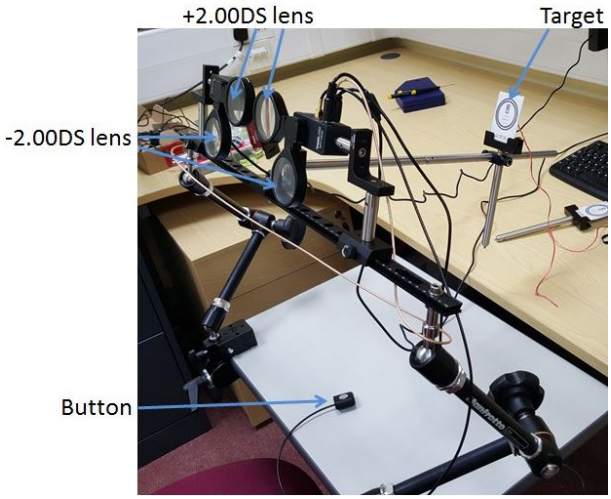

Figure 3.2: Patient-controlled accommodative facility instrument

A motorised flipper system (Figure 3.2) was built to allow assessment of the patient-controlled accommodative facility (PcAF). Subjects viewed the Bernell BC29 card through either the positive or negative 2.00D lenses and were required to press a button every time all three lines, on the card, were present and clear. The button response initiated the motorised flippers (Thor Labs Inc., USA) and the lenses were switched at a speed of 300ms. LabVIEW Software (National Instruments, USA) was used to control the motorised system and to record the time interval at which the lenses were switched, to the nearest 0.1s. From the time outputs both time for accommodation (ToA) and time for disaccommodation (ToD) were recorded separately. Assessment of the AF with the PcAF was repeated under the same three conditions as TAF. Between each AF test subjects were allowed a break of up to two minutes to minimise fatigue effects.

3.2.3 Part 2: Investigating the effects of using a stereogram target on binocular accommodative facility

The same subjects were asked to perform binocular TAF and PcAF under the same instructions as described above under the following viewing conditions:

- Binocularly viewing a polarised version of the Bernell BC29 card with polarising filters
- Binocularly viewing a non-polarised ('flat') version of the Bernell BC29 card with polarising filters.

Between each AF test subjects were allowed a break of up to two minutes to minimise fatigue effects.

3.2.4 Repeatability

Repeatability of the AF tests in both part one and part two were assessed. To examine the intra-observer repeatability of the TAF and PcAF, all AF tests were repeated on 10 separate visits on a single subject, with at least 48 hours between each visit.

To test inter-examiner repeatability, ten subjects returned for a second visit with a different examiner (PB) where the PcAF was repeated under the three conditions.

3.2.5 Data Analysis

3.2.5.1 TAF

Measures of TAF ToAC were calculated by dividing 60 seconds by the number of CPM achieved.

3.2.5.2 PcAF

The PcAF system recorded the time intervals between the presentation of the lenses controlled by the patient. The timings were exported into Excel 2016 Software (Microsoft, USA) and used to calculate ToA and ToD in seconds. PcAF ToAC was derived by summating the values for ToA and ToD. CPM was calculated by dividing the number of lens presentations, within one minute, by two (given that a cycle represents two lens changes).

3.2.5.3 Assumptions of normality

Visual inspection of descriptive statistics, histograms, box-plots and Sharpiro-Wilks tests were used to determine whether the accommodation metrics were found to have a normal or non-normal distribution. For each statistical investigation, where a normal distribution was found then parametric statistical analysis was performed and the appropriate non-parametric statistical test was performed where a non-normally distributed data was found, as detailed below.

3.2.5.4 Part 1: Validating the PcAF

Pearson's correlations coefficients or Spearman's tests were conducted using SPSS 23 software (IBM, USA), and scatterplots were constructed to examine the correlations

between the AF CPM achieved with both TAF and PcAF under both monocular and binocular viewing conditions, using the stereogram target.

Further correlations tests and scatterplots were examined to explore the correlation between measures of ToAC obtained with TAF, PcAF, and AD. Associations between measures of ToA and ToD, via PcAF and AD were also examined using Spearman's Rho correlation coefficents.

Two-tailed paired t-tests and Wilcoxon's signed rank tests were conducted to investigate if there were significant differences between each of the paired metrics. A Friedman's two-way analysis of variance test was conducted to investigate the agreement between measures of monocular ToAC derived from the TAF, PcAF and AD. Bland and Altman plots were constructed to examine the mean difference and limits of agreement between the paired metrics.

3.2.5.5 Part 2: The effect of a stereopsis target with binocular viewing conditions

Pearson's and Spearman's correlation co-efficient tests were used to investigate correlation between the CPM achieved when using either a 'flat' or stereogram target with both TAF and PcAF. Wilcoxon's signed rank tests and two tailed paired t-tests were used to determine if using a stereogram target significantly affected the CPM achieved binocularly, with both the TAF and PcAF. Bland and Altman plots were constructed to examine the mean difference and limits of agreement between the paired metrics.

3.2.5.6 Repeatability

Intra-observer repeatability for each accommodative metric was assessed by calculating the co-efficient of variance (CoV):

$$CoV = \frac{\sigma}{\mu}$$

Where σ is the standard deviation and μ is the mean.

Interobserver variability was evaluated by using ICC; two-way mixed single measures (absolute agreement);

$$ICC\ (absolute, 2) = \frac{subject\ variability}{(subject\ variability + measurement\ error) \div 2}$$

3.3 Results

3.3.1 PART 1: Validation of the PcAF

Table 3.2, displays the means, standard deviations, medians and CoV for intra-observer repeatability. The metrics denoted with * were found to be non-normally distributed. The means or medians, and spread of the all of the metrics are shown in Figure 3.3.

			Mean (SD)	Median	CoV
TAF	Monoc.	CPM	13.7 ±3.9	14.8	7%
		ToAC*	5.01 ±2.5	4.1	8%
	Binoc. (stereogram)	CPM	9.8 ±4.1	10.0	16%
PcAF	Monoc.	CPM	15.4 ±5.2	14.8	8%
		ToA*	1.99 ±1.84	1.84	14%
		ToD*	2.71 ±2.94	1.81	8%
		ToAC*	4.65 ±3.04	3.87	9%
	Binoc. (stereogram)	CPM	10.1 ±4.6	10.0	16%
Accommodative Dynamics	Monoc.	ToA*	1.96 ±0.63	1.90	8%
		ToD	1.59 ±0.47	1.61	8%
		ToAC	3.58 ±0.92	3.67	5%

Table 3.2, The means, medians and intra-observer repeatability (CoV) of the accommodative metrics measured

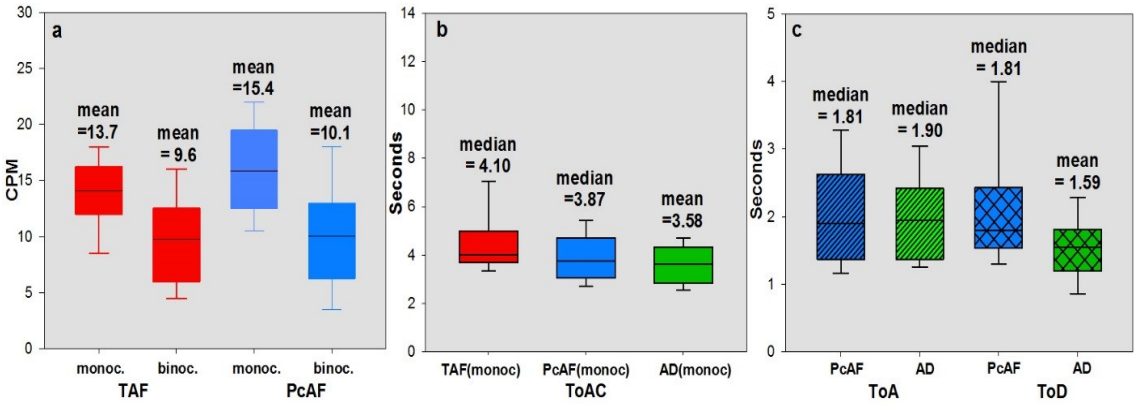

Figure 3.3, The box plots display the mean or median, 10th, 25th, 75th and 90th percentiles of data for each AF metric measured: a, CPM achieved monocularly and binocularly for TAF, and PcAF. b, ToAC measured monocularly for TAF, PcAF, and AD. c, The ToA and ToD, measured monocularly for PcAF and AD.

There was a strong positive correlation between the monocular CPM achieved with the TAF and PcAF ($r = 0.626$, $p < 0.001$) (Figure 3.4), however the subjects achieved more CPM

with the PcAF when compared to the TAF (t= -2.531, p= 0.017). The Bland and Altman plot revealed relatively wide limits of agreement.

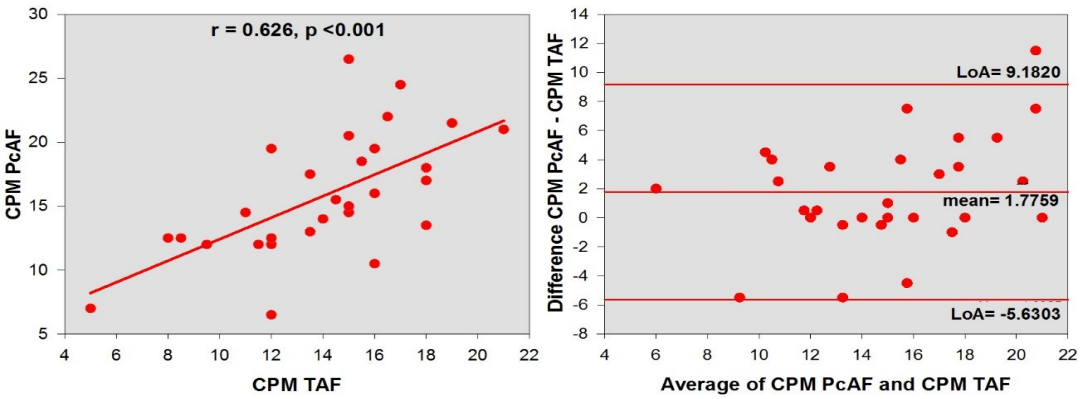

Figure 3.4 The correlation coefficient (left), and Bland and Altman agreement (right) between monocular measurements of CPM achieved during TAF and PcAF

In comparison, the binocular measures of CPM with the TAF and the PcAF using a stereopsis target correlated significantly (r = 0.776, p < 0.001) (Figure 3.5), and the CPM achieved were similar (t= -1.094, p = 0.284), and with smaller limits of agreement on the Bland and Altman plot.

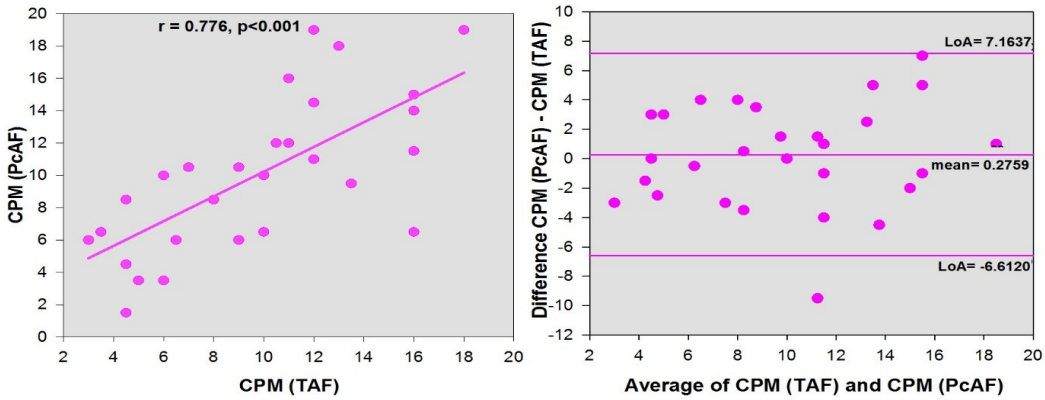

Figure 3.5 The correlation coefficient (left), and Bland and Altman agreement (right) between binocular measures of CPM achieved with the TAF and PcAF

Measures of ToA and ToD derived from the PcAF and AD demonstrated strong positive correlations ($r_s = 0.879$, $p < 0.001$, and $r_s = 0.867$, $p < 0.001$, respectively). ToA measured with PcAF and AD were similar ($Z = -1.049$, $p = 0.294$), and good agreement was observed between the two methods on the Bland and Altman plot (Figure 3.6). In comparison, the ToD was found to be slower with PcAF when compared with the value achieved with AD ($Z = -4.722$, $p < 0.001$). The Bland and Altman plot (Figure 3.7) reveals significant proportional bias and the agreement between the two methods decreased ($r = -0.849$, $p < 0.001$) as ToD values increased (Figure 3.7).

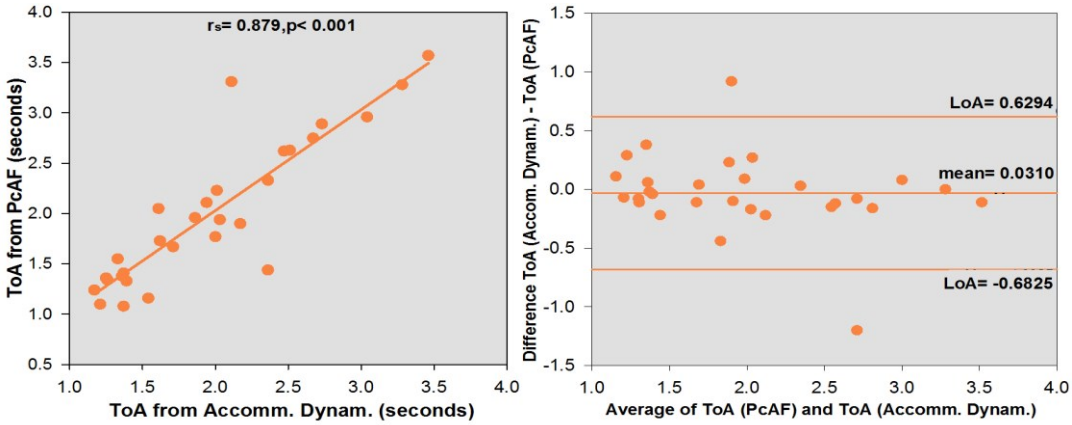

Figure 3.6: The correlation coefficient (left) and Bland and Altman agreement (right) between measures of ToA derived from PcAF and AD

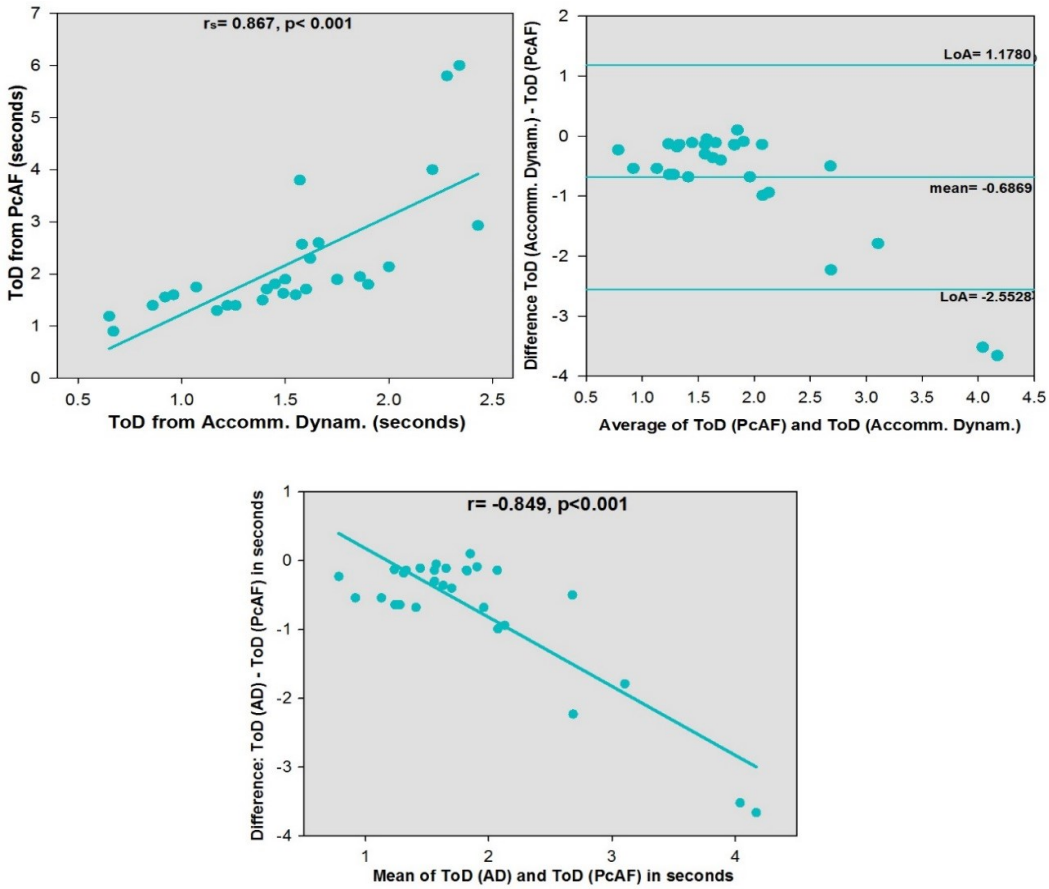

Figure 3.7 The correlation coefficient (top left), Bland and Altman agreement (top right), and the correlation coefficient of the proportional bias between between ToD (PcAF) and ToD (AD) (bottom centre)

Figures 3.8 and 3.9 show that measures of monocular ToAC derived from PcAF correlated significantly with both the TAF ToAC (r_s= 0.666, p< 0.001) and AD ToAC (r_s= 0.775, p< 0.001). A Friedman's two-way analysis of variance test demonstrated a significant difference between measures of monocular ToAC with the TAF, PcAF and AD ($X_{(2)}$= 20.581 p< 0.001). Post-hoc analysis, with Wilcoxon's signed rank tests showed that AD ToAC was significantly faster than both the PcAF ToAC (Z= -3.633, p < 0.001), and the TAF ToAC (Z= -3.568, p< 0.001). Measures of ToAC via PcAF were significantly

faster than TAF ToAC (Z= -1.979 p= 0.048). The Bland and Altman plots (Figures 3.8 and 3.9) demonstrated proportional bias as ToAC values increased, agreement between the methods decreased.

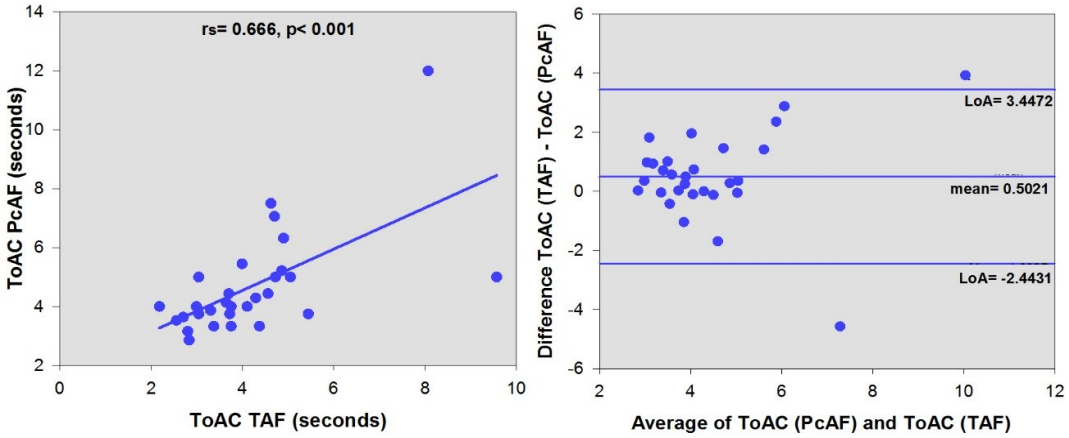

Figure 3.8 The correlation coefficient and Bland and Altman agreement between ToAC derived via PcAF and TAF

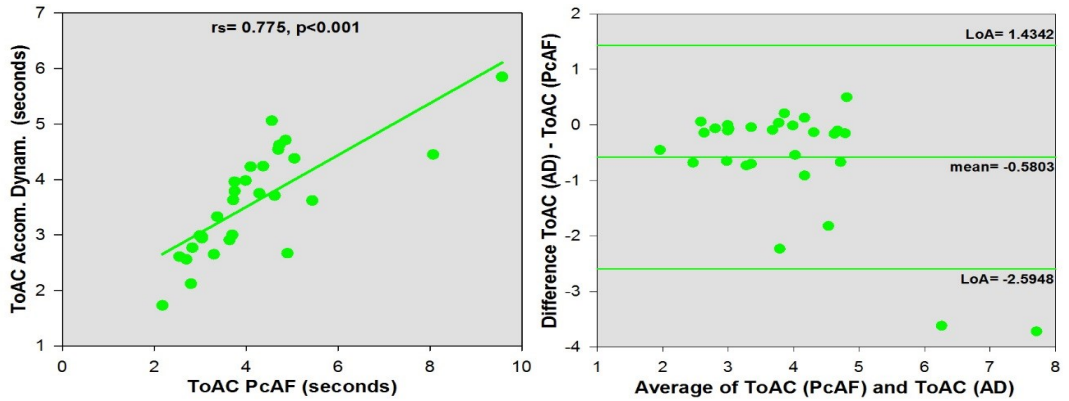

Figure 3.9: The correlation coefficient and Bland and Altman agreement between ToAC (PcAF) and ToAC (AD)

3.3.1.1 Repeatability

Good intra-observer repeatability was shown for all accommodative metrics as displayed in Table 3.2. Binocular AF measurements displayed greater levels of variability and poorer repeatability than monocular AF measurements.

Good inter-observer repeatability was shown for all of the PcAF as displayed by the ICC values in Table 3.3; the lowest levels of ICC were observed for ToD. Good agreement is confirmed by the Bland and Altman plots, as shown in Figure 3.10.

PcAF Metric	ICC
CPM monocular	0.936
ToA	0.942
ToD	0.724
ToAC	0.942
CPM binocular flat	0.976
CPM binocular stereo	0.937

Table 3.3: The Intraclass correlation co-efficient for inter-observer repeatability for all the accommodative metrics measured using the PcAF.

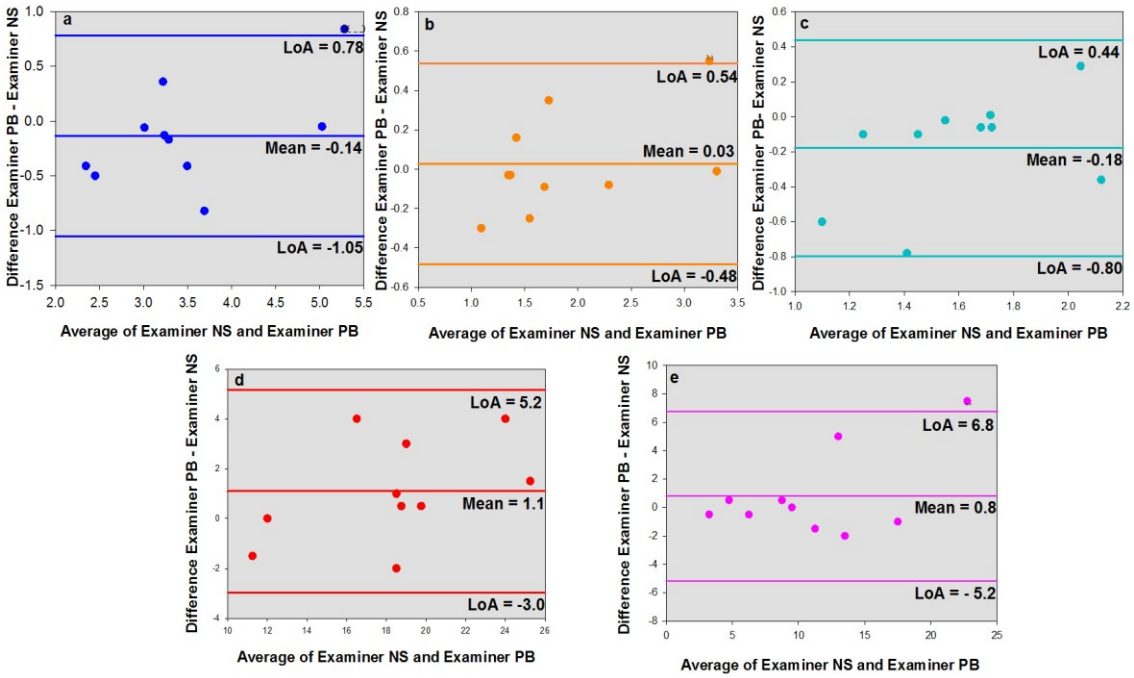

Figure 3.10 Bland and Altman plots demonstrating the inter-observer repeatability for PcAF metrics measured: a. ToAC measured monocularly, b. ToA measured monocularly, c, ToD measured monocularly, d. CPM measured monocularly, e. CPM measured monocularly with a stereogram

3.3.2 PART 2: Investigating the effects of using a stereogram target on binocular accommodative facility.

The means and data spread of CPM achieved binocularly with the TAF and PcAF, with both the stereogram and 'flat' target are shown in Figure 3.11. Binocular measures of CPM were compared for both the TAF and PcAF when using a 'flat' and stereogram target (Table 3.4). There was a positive correlation between the CPM results achieved with a 'flat' and stereogram target both with TAF ($r = 0.896$, $p < 0.001$) (Figure 3.12), and PcAF ($r_s = 0.886$, $p < 0.001$) (Figure 3.13).

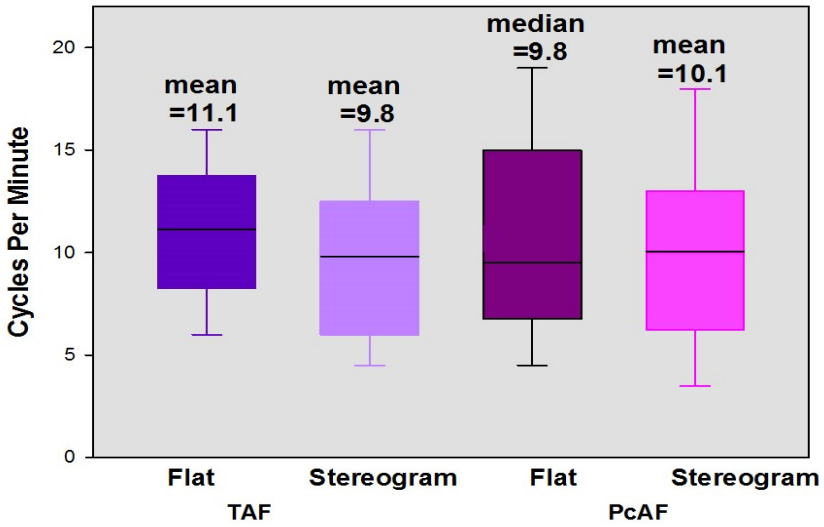

Figure 3.11: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data spread of data for all of the binocular AF measurements

The stereogram target significantly reduced the mean number of CPM achieved with TAF by 1.3 CPM (t= -1.742, p= 0.004). In comparison, similar CPM were observed between the stereo and flat targets with PcAF (Z=-1.171 p= 0.241). Intra-repeatability was reduced for both types of target when using the PcAF.

		Mean (SD)	Median	CoV
TAF	'Flat' target	11.1 ±3.7	11.0	8%
	Stereogram	9.8 ±4.3	10.0	16%
PcAF	'Flat' target*	10.9 ±5.2	9.8	12%
	Stereogram	10.1 ±4.7	10.0	15%

Table 3.4: The mean, medians and CoV of binocular AF, *non-normally distributed

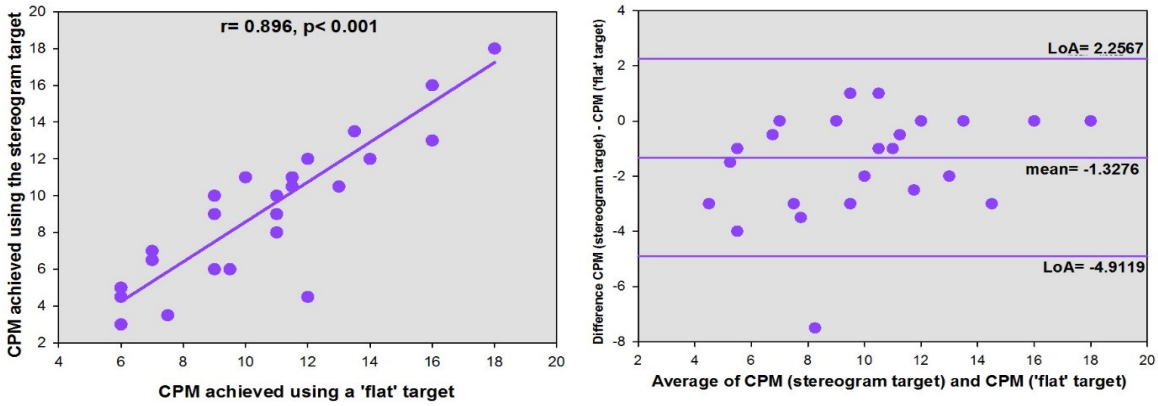

Figure 3.12 The correlation coefficient and Bland and Altman agreement between binocular TAF using different targets

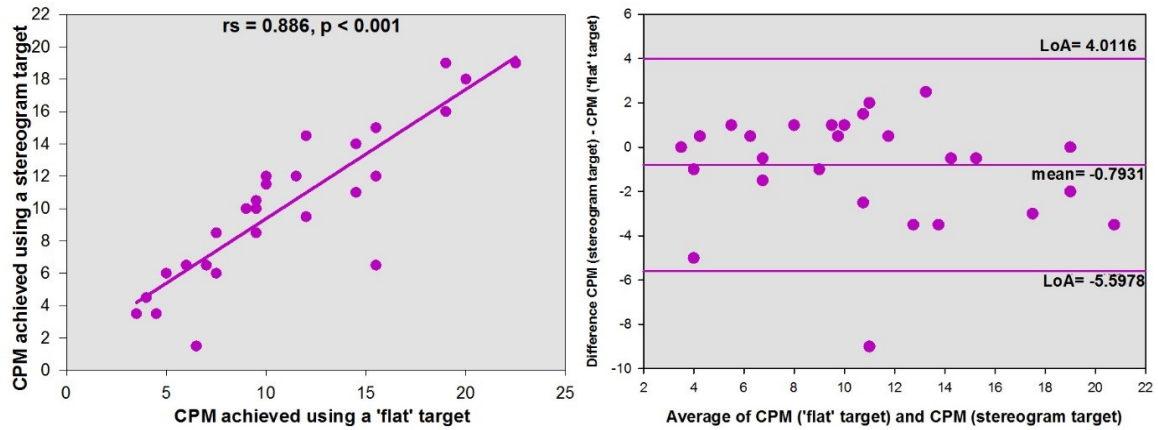

Figure 3.13 The correlation coefficient and Bland and Altman agreement between binocular PcAF using different targets

The Bland and Altman plots (Figures 3.12 and 3.13) show good agreement between the tests when different targets were used, however, there were large limits of agreement between the use of a 'flat' target and a stereogram target of 6.3 CPM for TAF, and 9.6CPM for PcAF.

3.4 Discussion

AF is a valid and repeatable clinical test, which is useful to investigate symptomatic accommodative dysfunction (Zellers et al., 1984). Due to its subjective nature, the technique is prone to inaccuracies, particularly relating to the response time of the subject and practitioner performing the test. Furthermore the technique has been shown to induce significant visual stress, and therefore errors relating to ocular suppression, which are likely to affect binocular measures of AF. In an attempt to improve subjective measures of AF, the present investigation demonstrates the validity of a novel patient-controlled accommodative facility device. The PcAF allowed for separate identification of the ToA and ToD during the AF cycle allowing comparability with the equivalent metrics derived from the accommodative dynamic profile. Given the potential flaws in the previous studies that have examined the effects of using a stereogram target on binocular AF, this study also re-examined this influence, with a group of healthy pre-presbyopes. Binocular AF was assessed using both a stereogram target and a non-stereogram target and results were compared. The stereogram target reduced the CPM achieved during TAF by 1.3 CPM; in contrast the stereogram target had no effect on the CPM during PcAF.

3.4.1 Validation of the PcAF

Both monocularly and binocularly measures of the CPM observed via TAF and PcAF were within the range of normative values reported by Zellers et al. (1984). The TAF test relies on a subjective end criterion; relying on the subject to appreciate and then relay when a target appears clear. Hence a proportion of the time for each cycle includes an inherent time delay that is related to the subject and practitioner reaction times; the reaction time of the subject in appreciating the target as 'clear', then indicating this to the practitioner (usually verbally), consequently leading to the practitioner flipping the lenses.

The PcAF provided the means to allow the subject to control the lens flip thus removing the need for the verbal signal and the practitioner actioning the lens change. This would have also reduced the cognitive demand of the task (Kedzia et al., 1999). When compared with the traditional AF test, PcAF results showed a faster ToAC to TAF and a higher number of CPM. In comparison objective measures of accommodative dynamics ToAC, demonstrated a faster ToAC than PcAF, suggesting that a significant proportion of the PcAF ToAC is related to the subject's reaction time.

Current measures of ToA and ToD assessed via PcAF, (1.99s and 2.71s, respectively) and accommodative dynamics (1.96s and 1.59s, respectively) are within a similar range of subjective response times found by Radhakrishnan et al. (2007) (2.39s and 2.04s respectively) and Allen et al. (2010) (1.89s and 2.64s, respectively). The strong correlations and Bland and Altman plots, demonstrate good agreement between the

ToA, ToD and ToAC found using the PcAF and AD. However, proportional bias was present with slower values of ToD; there was a greater reduction in ToD with PcAF, than in ToD with AD. This could have been due to a ceiling effect inherent with measurements of accommodative dynamics; any measurement of time derived from the accommodative dynamic profile will be limited to the total time that each target is presented. In this study, this time was 5 seconds, however from Figure 3.7 it can be seen that the measurement threshold remained at approximately 2.5 seconds.

The ToD and ToAC were found to be significantly slower when assessed with PcAF compared to AD. It is likely that the inherent time delay from the subject's response and subjectivity during PcAF explains this discrepancy.

PcAF data showed good intra- and inter-observer reliability. Although repeatability was reduced binocularly, with both the TAF and PcAF, possibly due to the increased complexity of the task and stress that the binocular test puts on the visual system. Moreover good concordance was observed between PcAF, TAF, and AD metrics suggesting that PcAF is a valid method for the assessment of AF.

3.4.2 The effect of a stereopsis target on binocular AF testing

The mean binocular TAF using a stereogram target was 9.8CPM, which is within the normal values suggested by Zellers et al. (1984) of 8CPM ±5, and in line with previous studies (Burge, 1979, Griffin et al., 1977, Wick et al., 2002b).

In the present investigation, the stereogram target significantly reduced the CPM achieved during binocular TAF by 1.3CPM, which is less than the reduction observed in previous studies. Griffin et al. (1977) reported a reduction in binocular CPM achieved with a stereogram target of 3.5CPM in 'normal' subjects and 5CPM in 'suspect' subjects. Their criteria for 'suspect' was defined as subjects who struggled to keep bifoveal fixation during testing, or achieved a 'low' score (of less than 9 CPM). The specific inclusion/exclusion criteria for the study was not stated. Therefore, subjects with other binocular vision dysfunction may have been included, which would have potentially exaggerated the effects of using a stereogram target during binocular AF in the 'normal' group. In a similar study, Burge (1979) conducted an investigation which excluded subjects with strabismus, or reduced amplitude of accommodation. The study found a significant reduction of 3.5CPM when using a stereogram target. However, there was a 1.5CPM difference noted between the right eye and left eye; the investigators fail to report whether this difference was statistically significant. The contrasting findings between the present investigation and that of Burge's study may have also been due to the age ranges included: the study assessed a large age range of 6 to 30 years. Children tend to have reduced AF compared to adults (Hoffman and Rouse, 1980, Hennessey et al., 1984a, Wick and Hall, 1987, Scheiman et al., 1988, Wick et al., 2002b) and therefore using this large age range could have exaggerated the reduction in CPM achieved with a stereogram. Zellers et al. (1984) found that in adults, a monocular difference of greater than 4CPM between the eyes would be suspicious. Therefore, the widely cited

3.5CPM reduction in binocular AF with a stereogram target found by Griffin, Graham and Clausen (1977) and Burge (1979) although statistically significant may not be clinically significant. Similarly in this study although 1.3CPM may be statistically significant, it may not be clinically significant. The large limits of agreement found in the Bland and Altman plots comparing the use of the stereogram and 'flat' targets, for the TAF and PcAF, and the agreement between the binocular AF tests between the 'flat' and stereogram target with the PcAF, would support this supposition. Further research is required to explore the clinical significance of the reduction in CPM achieved when using a stereogram across different age groups, and between 'normal' and 'suspect' groups.

3.4.3 Considerations of this study

There are some limitations of the PcAF, firstly the taken to flip the lenses (300ms) increases the ToA, ToD, SoAC, decreasing the total number of CPM achieved. The shortest time between flip measured was 0.7s, of which 300ms would represent 43% of the total time. Secondly, the time intervals were recorded to the nearest 0.1s, which would have limited the accuracy of the accommodative times recorded.

During this study, the target that was viewed by the subject did not vary during TAF, PcAF or AD. Randomizing the target letters between lens presentations may have prevented the subject from anticipating the target and thus may have improved the reliability of the test. The choice of a consistent target was made so that the AF systems used in this study closely emulated the TAF used in clinical practice. During TAF Levine

et al., (1985) and Zellers et al. (1984) found a slight in-test training effect; and a better flip rate in the second eye. To mitigate the learning effect, the order in which the monocular and binocular tests, including whether TAF or PcAF were tested first, was randomised. Intra-observer repeatability in this study was tested using a subject who would have been classed as having normal AF using the criteria set by Zellers et al. (1984). The good intra-observer repeatability of the AF metrics found during this study would support findings by Levine et al. (1985) that subjects with normative AF did not significantly improve AF CPM on repeated testing.

3.4.4 Conclusions and future work

In summary the key findings of this study were:

- PcAF was a valid and repeatable method of assessing AF.
- Using a stereogram target reduced the CPM achieved in a sample of young adults with 'normal' binocular function.

Future work will seek to modify the PcAF, by improving the accuracy of times recorded. Further investigations will include examining the effects of in-test fatigue and subject age on the ToA and ToD (McKenzie et al., 1987, Rouse et al., 1989, Rouse et al., 1992). Further exploration using a more systematic approach in quantifying groups of symptomatic and non-symptomatic accommodative dysfunction, and normal and

'suspect' binocular function, is needed to further understand the effects of using a stereogram during binocular AF. It would also be useful to examine the effects of using a stereogram during binocular AF across different ages.

Chapter 4: Optimising the calculation methods for analysing depth of focus from defocus curves

4.1 Introduction

Given that the overarching aim of this thesis is to examine the suitability of the methods for assessing accommodative function in pre-presbyopes, presbyopes and pseudophakes, the previous two chapters conducted investigations of methods for measuring accommodation on pre-presbyopes and presbyopes. Therefore, in this chapter, a pseudophakic group has been included in the study population, in an investigation into the measurement of depth-of-focus. Thus, providing the opportunity to compare the methods for assessing depth-of-focus across age groups.

Depth of focus (DoF) is the dioptric range over which an object can still be resolved (Wang and Ciuffreda, 2004). In contrast, range-of-focus is defined as the physical distance range over which an object can be resolved (Pieh et al., 2002, Schmidinger et al., 2006, Alfonso et al., 2009, Maxwell et al., 2009). DoF can be measured by determining the VA through optical lenses of varying powers, this is the most common method for constructing and assessing defocus curves in the phakic eye (Rosenfield et al., 1996, Wold et al., 2003, Ostrin and Glasser, 2004a, Gupta et al., 2007, Gupta et al.,

2008, Antona et al., 2009, Leon et al., 2012, Momeni-Moghaddam et al., 2014). In the accommodating eye, VA can be maintained with negative lens defocus but decreases with positive lens defocus. The magnitude of the dioptric range over which the acuity is maintained is dependent upon both the amplitude of accommodation (AoA) and the individual's tolerance to optical blur (known as true DoF). In defocus curve assessment the terms AoA and DoF are often used interchangeably, however these are separate co-dependant metrics. The AoA is often derived from a defocus curve as the highest powered negative lens where VA is maintained to a set cut-off criterion. The DoF is quantified by two different metrics: the range-of-focus and the area-of-focus. The range-of-focus is defined as the dioptric range over which VA can be maintained above the cut-off criterion (Gupta et al., 2008), as shown in Figure 4.1.

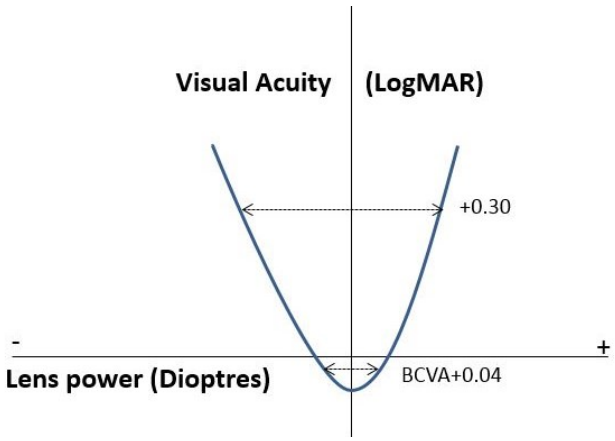

Figure 4.1: An example of a defocus curve of a presbyopic patient, showing the range-of-focus metric derived with an absolute cut-off of +0.30logMAR and a relative cut-off of BCVA+0.04logMAR

The area-of-focus calculates the area of vision seen above a cut-off criterion and quantifies both the range and depth-of-focus (Buckhurst et al., 2012) (Figure 4.2).

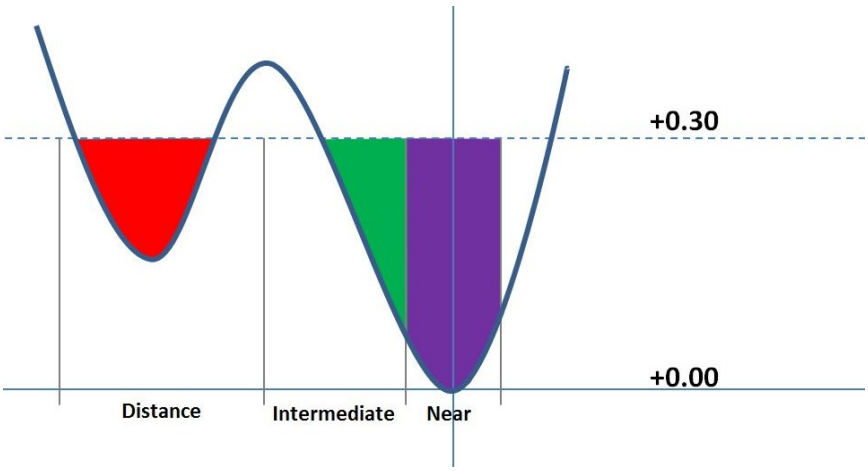

Figure 4.2: The method used by Buckhurst et al. (2012) to analyse defocus curves of a subject fitted with a MIOL

The set cut-off on a defocus curve, at which the AoA or DoF is determined, influences the dioptric value attained from the evaluation. This cut-off can be relative or absolute (Figure 4.1); if relative then the chosen VA level is dependent on the BCVA whereas an absolute criterion is independent of the subject's BCVA.

One limitation of measuring the DoF using minus lenses, is that the VA measured via negative defocus is generally an under-estimation of the actual VA which can be achieved at the equivalent distance. This underestimation of VA is due to the lack of physiological miosis associated with the near vision triad response and the minification of the target by the optical lens power (Pieh et al., 2002), although this latter source of error can be compensated for mathematically (Gupta et al., 2008).

Accurate and repeatable methods in constructing and analysing defocus curves, are necessary to assess the DoF clinically and in a research setting. Currently there is much variation in the methods used, as summarised in Table 4.1, including:

- The distance at which the test is performed
- Method of analysis
- The step size between optical defocus levels
- Randomisation or non-randomisation of target
- Randomisation or non-randomisation of lens power

4.1.1 Target distance

Defocus curves have been constructed with subjects viewing targets at both distance and near. There has been some debate over whether this variation in test distance would have any significant clinical implications. Ostrin and Glasser (2004a) suggested that using a distance target as a stimulus to accommodation, leads to a general under-estimation of AoA in younger subjects due to the absence of proximal cues. In contrast, Momeni-Moghaddam et al. (2014) demonstrated that a distance (6m) target, gave significantly lower AoA compared to a near (40cm) target; the magnitude of difference was amplitude dependant. The investigators concluded that the difference in the AoA response was likely to be due to the pupil miosis and proximal cues from the near vision target, both of which would be absent when using a distance target. Therefore, the study concluded that although the AoA found via defocus curves using distance and near targets correlate significantly, the two measures are not clinically interchangeable.

4.1.2 Method of analysis

The cut-off criteria used to derive the AoA or DoF can be either absolute (Ostrin and Glasser, 2004a, Momeni-Moghaddam et al., 2014) or relative to the BCVA (Rosenfield and Cohen, 1996, Lovie-Kitchin and Brown, 2000, Wold et al., 2003, Gupta et al., 2008, Antona et al., 2009, Buckhurst et al., 2012, Leon et al., 2012, Wolffsohn et al., 2013). Figure 4.1 shows a defocus curve of a typical presbyope, with the DoF calculated at both a relative criteria of BCVA+0.04logMAR and an absolute criteria of +0.30logMAR. Gupta et al. (2008) recommended using a relative criterion of BCVA +0.04logMAR, to derive AoA, which is based on the variability of VA measurements (Raasch et al., 1998, Lovie-Kitchin and Brown, 2000). When deriving the DoF via the area-of-focus Wolffsohn et al. (2013) compared the use of an absolute criteria of +0.30logMAR (the legal driving standard within the UK (DVLA, 2015)) to a relative cut-off criterion of BCVA +0.10logMAR in a study with IOL implants. The higher threshold of +0.10logMAR was used instead of BCVA+0.04logMAR to allow for the reduced near visual acuity with IOLs. It was concluded that the relative criteria of BCVA +0.10logMAR was more prone to erroneous errors when compared to the absolute criteria of +0.30logMAR (Wolffsohn et al., 2013).

4.1.3 Step size between lens presentations

Increased test duration whilst establishing AoA can lead to patient fatigue affecting results. In response to this Wolffsohn et al. (2013) investigated the effect of using step changes of 0.50DS, 1.00DS and 1.50DS in groups of patients with different multifocal IOL

designs. The study found that a larger step size of 1.00DS and 1.50DS adversely affected the results when a relative cut-off criteria was used: the shorter range-of-focus derived using a relative cut-off is more sensitive to variations in the defocus curve (Wolffsohn et al., 2013).

4.1.4 Randomisation of lens power and visual target

Presenting lens powers in a predictable order, or using static letters as a target during the construction of defocus curves could lead to a learning effect, and over-estimating the final AoA or DoF calculated. Gupta et al. (2007) and Gupta et al. (2008) investigated the effects of randomising these two factors; both studies concluded that the AoA measured was not significantly affected by randomising the order of the lens powers presented, or changing the letters used as a target. However, these studies still encouraged the use of randomisation of both the lens power and the target presented (Gupta et al., 2007, Gupta et al., 2008) to minimise any learning effects (Altman and Bland, 1999).

Study	Target	Cut-off Criteria	Other Comments
Distance targets			
Gupta et al. (2007)	LogMAR chart at 6 metres	Not stated	
Gupta et al. (2008)	LogMAR chart at 6 metres	First negative lens which blurred to BCVA+0.04LogMAR	+0.036LogMAR is the variation found in repeated measures of VA (Raasch et al., 1998, Lovie-Kitchin and Brown, 2000)
Ostrin and Glasser (2004a)	Letter chart at 6 metres	First negative lens which blurred 6/6	0.25DS step changes
Wold et al. (2003)	Letter chart at 6 metres	Most negative lens where the BCVA was still achievable.	
Momeni-Moghaddam et al. (2014)	Snellen chart at 6 metres	Most negative lens at which 6/9 could be read	0.25DS step changes
Near Targets			
Antona et al. (2009)	Corresponding VA of 0.9logMAR at 40cm	The first negative lens which induced the first point of sustained blur, +2.50D	0.25DS step changes
Leon et al. (2012)	Letters 0.7mm high, at 33 cm	The first negative lens which induced the first point of sustained blur +3.00D	
Rosenfield and Cohen (1996)	One line above the BCVA on near chart at 40cm	The first negative lens which induced the first point of sustained blur	Does not mention compensation for near distance was used
Momeni-Moghaddam et al. (2014)	Equivalent 6/9 line on Reduced Snellen chart at 40cm	Last minus lens at which 6/9 could be read on the reduced Snellen +2.50D	0.25DS step changes

Table 4.1: The different defocus-curve methodology used to obtain AoA or range-of-focus from defocus curves in phakic subjects

4.1.5 Defocus curves compared to other methods of measuring accommodation

Objective techniques for measuring accommodation are known to be more repeatable and reliable than alternative subjective methods such as defocus curves (Glasser, 2006). Subjective methods often over-estimate accommodation levels due to blur cues and a subjective end point (Wold et al., 2003, Ostrin and Glasser, 2004a, Leon et al., 2012). It has been proposed that objective methods are better suited to examine the efficacy of presbyopia treatment strategies (Ostrin and Glasser, 2004a, Wold et al., 2003). However, in comparison with the crystalline lens, an IOL causes Purkinje images III and IV to be laterally displaced and brighter resulting in aberrant auto-refractor and power-refractor measurements. Therefore, a combination approach of using objective and subjective methods to assess pseudophakic accommodation is advocated (Langenbucher et al., 2003, Sheppard et al., 2010). Defocus curves are often used and are the preferred subjective method for assessing the effectiveness of IOLs for restoring accommodation (Gupta et al., 2007). As discussed in Section 1.6.2 in comparison to the Push-Up test, defocus curves have been shown to measure lower AoA. This has been attributed to a number of factors including: the minification effects from the increase in negative lens power, and a lack of both pupil miosis and proximal cues during defocus curve measurements, and the variation in target size during the push-up test (Rosenfield and Cohen, 1996, Ostrin and Glasser, 2004a, Gupta et al., 2008).

4.1.6 Assessment of defocus curves in the pseudophakic eye

The pseudophakic eye is unable to accommodate and hence a reduction in VA is expected when introducing both positive and negative defocus lenses. Multifocal IOLs (MIOLs), accommodating IOLs (AIOLs) and extended depth-of-focus IOLs (EDoF IOLs) have all been developed with the aim of restoring functional near vision in pseudophakic eyes. During DoF assessment a pseudophakic eye implanted with one of these lenses should demonstrate an improved tolerance to negative defocus lenses

4.1.6.1 Multifocal intra-ocular lens (IOL) designs

Multifocal intraocular lenses (MIOLs) work on the principle of simultaneous vision, achieved by diffractive or refractive designs, providing a double or triple peaked defocus curve (Rosenfield et al., 1996, Schmidinger et al., 2006, Alfonso et al., 2009, Antona et al., 2009, Maxwell et al., 2009, Wolffsohn et al., 2013, Buckhurst et al., 2012, Leon et al., 2012, Momeni-Moghaddam et al., 2014). However, it is difficult to describe the profile of MIOLs using either absolute or relative cut-off criterions, due to the multi-peaked defocus curve. Buckhurst et al., (2012) developed an area metric to describe the areas of vision at distance, intermediate and near following MIOL implantation, as shown in Figure 4.2 (Buckhurst et al., 2012). By examining areas of the defocus curves in these three portions, the describing metric assessed both range-of-focus and VA.

4.1.6.2 Accommodative IOL lens designs

The first generation of commercially available accommodative IOLs were based upon the optic shift principle and were designed to translate anteriorly with accommodation. However, studies have failed to demonstrate that these accommodative IOLs provide a significant magnitude of accommodative change. Cleary et al. (2010) utilised a relative cut-off criteria of +0.04LogMAR and showed similar AoA values for both monofocal (0.64 ± 0.37) and accommodative IOL (0.93 ± 0.35) groups (Cleary et al., 2010).

The second generation of accommodative IOLs were based on a dual optic telescopic system. These IOLs have a high-powered positive anterior lens and negative posterior lens. In the unaccommodated state, the capsule holds the lenses close to each other. With accommodative effort the capsule compresses the haptics, forcing a separation of the two lenses. The anterior lens of a dual optic IOL has a higher power than the optic of the single lens system; this means that these lenses are able to produce higher accommodative power (McLeod et al., 2007). Ossma et al (2007) assessed defocus curves on subjects implanted with the Synchrony dual optic lens. To demonstrate the accommodative power of the lens, defocus curves were examined using a relative cut-off criteria, and compared with a matching monofocal group. The Synchrony group maintained an increased range-of-focus in comparison with the monofocal group and the authors concluded that this provided evidence of the accommodative power of the IOL (Ossma et al., 2007). On further examination of these findings, it is evident that the gradient of the negative and positive slopes were similar for both IOLs. If accommodation was present then a shallower gradient of curvature should be present

with negative defocus, in comparison to positive defocus. The results suggest that the increased range-of-focus observed was due to increased DoF, rather than increased accommodation. As such, these conclusions need to be viewed with caution and highlights the need for a valid method of analysing defocus curves for pseudophakic assessment.

Since 2014 an emerging category of IOL known as extended-depth-of-focus IOL (EDoF IOL) have been made commercially available. The classification that defines a lens as EDoF has yet to be fully specified and as such there appears to be some overlap between EDoF IOLs and MIOL designs: The Symfony EDoF IOL incorporates a low addition diffractive surface producing a second defocus curve peak (Gatinel and Loicq, 2016). Neither the range-of-focus nor the area-of-defocus metrics have been validated for assessing the defocus curve profile of EDoF IOLs. The range-of-focus metrics to describe the profile of EDoF IOLs may not be suitable as they do not differentiate between the positive and negative defocus gradients. The area metric proposed by Buckhurst et al., (2012) was developed to assess the specific visual profiles of multifocal IOLs and may not be suitable for EDoF lenses.

To accurately assess and compare the efficacy of EDoF IOLs, there is a clinical need to standardise the defocus curve analysis.

4.1.7 Study aims

The aim of this study was to assess the validity of defocus curve metrics in phakic and pseudophakic eyes. In phakic eyes the validity of a range of defocus metrics against both

amplitude of accommodation (AoA) and accommodative dynamics (AD) was assessed. AoA is considered the gold standard clinical test of accommodation despite the influence of depth-of-focus. In addition accommodative dynamics is a valuable tool for validation as it assesses accommodation independently of depth-of-focus.

The investigation also assessed the validity of the range of defocus metrics against reading speed in pseudophakic eyes with EDoF IOL correction. Reading speed was chosen, as in comparison to near acuity, reading speed provides a real life measure of usable near vision. AoA and accommodate dynamics were not relevant with pseudophakic eyes given that these subjects do not accommodate.

The secondary aims of this study were to examine the relationship between the push-up test, accommodative dynamics and the defocus curve metrics.

4.2 Methods

4.2.1 Phakic Subjects

Forty two healthy adults (18 males and 24 females) with a mean age of 31, SD ±8 years (range 19 – 49) were recruited for this study. The inclusion criteria for the study included:

- Aged between 18 and 50 years old.
- Correctable VA in soft contact lenses at least 0.0 LogMAR or better.

The exclusion criteria for the study was:

- Current or previous ocular pathology, injury or surgery
- Current or previous binocular vision disorder
- Diabetes mellitus

4.2.2 Pseudophakic Subjects

A further 30 subjects (7 males, 18 females) with a mean age of 67, SD ± 6 years (range 55 - 74) were recruited from BMI Southend Hospital (Southend on Sea UK). The inclusion criteria for this study included:

- Previously had bilateral cataract surgery or bilateral clear lens extraction within the last 3-6 months.
- A postoperative best corrected distance VA of at least 0.1 LogMAR.
- Aged between 40 and 70 years old.

The exclusion criteria for this study included:

- Current or previous ocular pathology, injury or surgery (other than cataracts or lens extraction.
- Capsular opacification, LASIK and YAG capsulotomy.
- Corneal astigmatism of greater than 1.50DC.

The 30 subjects recruited had been implanted within the previous 3 to 6 months, with one of two types of lenses:

- Group one; 15 subjects who had been implanted bilaterally with the Tecnis 1-piece acrylic monfocal IOL (Abbott Medical Optics Inc. Santa Ana, California).
- Group two; 15 subjects who had been implanted bilaterally with the Symfony EDoF IOL (Abbott Medical Optics Inc. Santa Ana, California).

Ethical approval was obtained from the Plymouth University Ethics committee and the study was performed in accordance to the tenets of the Declaration of Helsinki. Informed written consent was obtained from each subject prior to commencement of both parts of this study.

4.2.3 Phakic Evaluation

All 42 subjects were examined by a single observer (NS). Intra-observer repeatability was assessed on a subset of 24 subjects: these subjects attended a second visit separated by at least 24 hours and were examined by the same observer (NS). Inter-observer repeatability was assessed on a subset of 20 subjects: these subjects attended an additional visit and were examined by a second observer (PB). The two observers were blind to all data measurements collected previously.

4.2.3.1 Refraction

A full objective and subjective monocular refraction was performed on each eye, to ensure that the refractive error was within the inclusion criteria. The mean spherical refractive error of the study population was RE: -0.90DS ±2.10, LE: -0.88DS ±2.00. Any

refractive error greater than -0.50DS, +0.75DS and -0.75DC was corrected using soft contact lenses. At this point, any subjects who could not achieve a VA of 0.0 LogMAR were excluded.

4.2.3.2 Defocus curves (minus-to-blur)

Data for the defocus curves for each subject was collected with the use of trial lenses and with the subject viewing a logMAR test chart using *Thomson test chart 2000* (Thomson Software Solutions, Hatfield, Herts, UK.). The procedure was performed with the fellow eye occluded. Lenses between the range of +2.00DS to –15.00DS were presented monocularly in 1.00DS steps. The VA was recorded for each presentation. In between each presentation, the letters on the vision chart were randomised to minimise any learning effects. The process was then repeated on the second eye.

4.2.3.3 Push-Up Test

AoA was measured using the RAF rule monocularly and binocularly. The target (a single word on the N5 line) was initially presented 50cm away from the subject (2.00D demand of accommodation). Subjects were asked to identify if the word appeared clear. If clear, the target was slowly moved towards the subject, until the first point of sustained blur was reported. This was recorded as the break point. The target was then retracted until the target was reported as clear, this was recorded as the recovery. The procedure was

repeated until three readings were recorded, and mean break and recovery were calculated. The total mean of the break and recovery was recorded as the AoA.

4.2.3.4 Time for Accommodative Change (ToAC)

The ToAC was derived from the monocular accommodative dynamic profile, which was measured using the Grand Seiko Auto REF WAM-5500 with a motorised Badal adaption system, as described in Section 2.2.3.

4.2.4 Pseudophakic evaluation

All subjects were examined 3-6 months post-operatively. During this visit the following tests were performed.

4.2.4.1 Refraction

A combination of retinoscopy, autorefraction and a full subjective refraction at 6 m were used to determine the refractive power. The mean spherical refractive error was RE: +0.30DS ±0.75, LE: +0.42DS ±0.75. Any refractive error was corrected by placing trial lenses at the back of the trial frame whilst assessing the defocus curve.

4.2.4.2 Binocular defocus curves

The logMAR test chart at 6m was used to measure the binocular VA with each defocus lens. Lenses were presented binocularly, within the range of +1.50 to –5.00D in 0.50D steps. The letters on the test chart were randomised between measures.

4.2.4.3 Assessment of Critical Print Size in the pseudo-phakic population

To assess the near visual function, the *Radner Reading Chart* was used to measure the reading speed. This reading test consists of paragraphs of different sized text. The test has high repeatability due to the standardized structure and construction of the sentences used (Stifter et al., 2004, Radner and Diendorfer, 2014). The structure is composed of three lines, fourteen words, and between eighty-two and eighty-four letters. The construction of the text is such that the nouns, verbs and syllables are consistently positioned across each sentence (Radner et al., 1998, Radner and Diendorfer, 2014). *The Radner Reading Chart* can quantify three reading metrics: the reading acuity (RA) which is determined as the smallest sized print that is resolved, the maximum reading speed (MRS) which is the fastest reading speed (when print size is not a limiting factor), and the critical print size (CPS) defined as the smallest size print size which allows maximum reading speed to be maintained (Radner et al., 1998, Radner and Diendorfer, 2014). Each participant had their MRS measured for each of the different sized text, from which the CPS was determined with visual inspection of the results.

4.2.5 Statistical Analysis

4.2.5.1 Evaluation of the defocus curves

4.2.5.1.1 Correction of Effective Power and Magnification of the Defocus Curves

Any spectacle lens placed in front of the eye has a magnifying effect on the retinal image. The magnification is dependent on the lens shape, refractive index and the distance between eye and lens (Gupta et al., 2008) (Equation 4.1). As discussed in section 4.1, minus lenses have a minification effect on the target, which can lead to an under-estimation of the VA. Positive lenses have a magnification effect on the target, which can lead to an over-estimation of the VA. To compensate for these optical effects a correction was applied to every VA measured in the defocus curve (Equation 4.1).

$$SM = \frac{1}{\left(1 - \frac{t}{n} F1\right) * (1 - dFs)}$$

Equation 4.1

Where SM = Spectacle Magnification, t = Lens thickness, n = Refractive Index, F1 = Front surface power, d = Back Vertex Distance, and Fs = Lens power.

The back vertex distance of a lens also influences its effective power at the ocular plane. Thus a correction was used to determine the effect of defocus at the ocular plane (Equation 4.2).

$$Od = \frac{Fs}{(1 - dFs)}$$

Equation 4.2

Where Od = Ocular defocus, Fs = Lens power and d = Back Vertex Distance

4.2.5.1.2 Curve Fitting

Matlab software was used to plot defocus curves and calculate DoF (see Appendix 3). The defocus curves were constructed plotting lens power in dioptres (x-axis) against LogMAR VA (y-axis). A 9th order polynomial was fitted to each data set to create the defocus curves (Wolffsohn et al., 2013) (equation 4.3). The curve fitting process was limited to 100 iterations for each curve.

$$y=a+bx+cx2+dx3+ex4+fx5+gx6+hx7+ix8+jx9$$

Equation 4.3

For each of these defocus curves the following metrics were calculated:

1. The area-of-focus with 0.30 logMAR as the cut-off (0.30area)
2. The area-of-focus with 0.04 log MAR as the cut-off (0.04area)
3. The range-of-focus (in dioptres) between the two points on the curve where 0.30 intercepts x-axis (0.30dist)
4. The range-of-focus (in dioptres) between the two points on the curve where 0.04 intercepts x-axis (0.04dist)

A relative cut-off criteria of BCVA+0.04logMAR was used as proposed by Gupta et al. (2008). The absolute criteria of +0.30logMAR was used, as it is equivalent to the legal driving standard in the UK (DVLA, 2015).

4.2.5.1.3 Calculating range-of-focus

The absolute criteria of +0.30LogMAR and relative criteria of BCVA+0.04 LogMAR were used to calculate the range-of-focus. The Newton-Raphson method is used to find the roots of a function (Ypma, 1995), by adjusting the polynomial function by 0.3 (or BCVA +0.04), x can be calculated when y = 0.3 (Equation 4.4). In the present study, the Newton-Raphson method was used to calculate x when y = 0.3, and x when y= best VA +0.04.

$$x_1 = x_0 - \frac{f(x_0)}{f^{'}(x_0)} = x_0 - \frac{(a-0.3) + bx_0 + cx_0^2 + dx_0^3 + ex_0^4 + Fx_0^5 + gx_0^6 + hx_0^7 + ix_0^8 + jx_0^9}{b + 2cx_0 + 3dx_0^2 + 4ex_0^3 + 5Fx_0^4 + 6gx_0^5 + 7hx_0^6 + 8ix_0^7 + 9jx_0^8}$$

Equation 4.4

To increase the accuracy of the estimation of x when y=0.3, the resultant x1 from Equation 4.5 is used as x_n and utilised in Equation 3.5 until the % error is reduced to 0 (Equation 4.6).

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)} = x_0 - \frac{(a-0.3) + bx_n + cx_n^2 + dx_n^3 + ex_n^4 + Fx_n^5 + gx_n^6 + hx_n^7 + ix_n^8 + jx_n^9}{b + 2cx_n + 3dx_n^2 + 4ex_n^3 + 5Fx_n^4 + 6gx_n^5 + 7hx_n^6 + 8ix_n^7 + 9jx_n^8}$$

Equation 4.5

$$\%error = \frac{(x_{n+1} - x_n)}{\left(\frac{x_{n+1} + x_n}{2}\right)}$$

Equation 4.6

The Newton-Raphson method was used to determine each intersection of the curve at 0.3 LogMAR. The range-of-focus (0.30dist) was calculated as the dioptric distance over

which VA was better than 0.3 LogMAR. The range-of-focus (0.04dist) was calculated as the dioptric distance over which VA was better than the BCVA+0.04LogMAR.

4.2.5.1.4 Calculating area-of-focus

The polynomial equations for each curve were integrated such that the area of defocus (LogMAR*m-1) could be derived (Equation 4.7).

$$\int_{a_1}^{a_0} ax + bx^2 + cx^3 + dx^4 + ex^5 + Fx^6 + gx^7 + hx^8 + ix^9 + jx^{10}$$

Equation 4.7

The upper area limit was defined as either +0.30LogMAR or BCVA +0.04 and bound the area of defocus. Separate areas were calculated for the positive and negative area of defocus. The negative area of defocus was subtracted from the positive defocus area to create an accommodative area metric (Equation 4.8 and Figure 4.3). The resulting area-of-focus metric describes the range and area of VA seen, beyond what would be expected by an absolute presbyope. In an absolute presbyope this area-of-focus would expected to be zero.

Area-of-focus = negative area-of-focus – positive area-of-focus

Equation 4.8

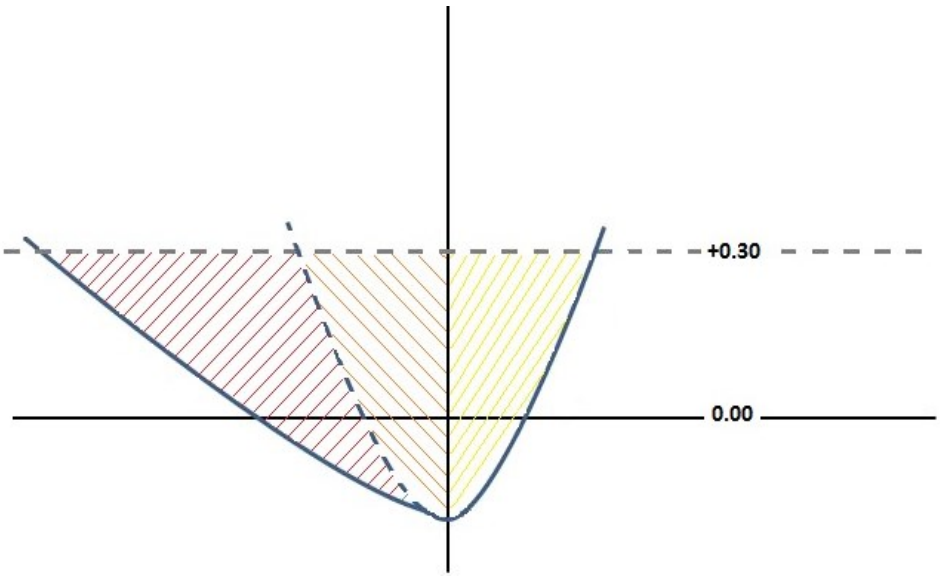

Figure 4.3: The area-of-focus metric (shaded in red) was calculated by finding the area-seen on the positive side of the graph and on the negative side of the graph. The positive area-seen (shaded in yellow) was then subtracted from the negative area-seen.

4.2.5.2 Evaluation of accommodative dynamics curves

The speed of accommodative change (ToAC) was derived from the accommodative dynamics via the same methods as outlined in Section 2.2.5.

4.2.5.3 Assumption of Normality

Visual inspection of descriptive statistics, histograms, box-plots and Sharpiro-Wilks tests were conducted to determine whether the accommodative metrics followed a normal distribution. All of the DoF and accommodative metrics measured in the phakic population were found to be normally distributed with the exception of ToAC, and 0.04area. All of the DoF and near visual function metrics measured in the pseudophakic population followed a normal disruption.

4.2.5.4 Comparison of Eyes

A two-way repeated measures ANOVA was used to investigate if there was a significant difference between the right and left eye for each accommodative metric measured. The ANOVA was constructed with the metrics assessed as within-subject factors and eye (right or left) as between-subject factors.

4.2.5.5 Repeatability

To test intra-observer and inter-observer repeatability ICC was calculated: two-way mixed single measures (absolute agreement);

$$ICC\ (absolute, 2) = \frac{subject\ variability}{(subject\ variability + measurement\ error) \div 2}$$

Bland and Altman plots were also constructed: intra-observer repeatability was tested by comparing the defocus curves assessed at two separate visit by the first examiner (NS). Inter-observer repeatability was tested by the defocus curves measured during the first visit, by the first observer (NS), and the third visit by the second observer(PB).

4.2.5.6 Correlations between the DoF metrics and other accommodative metrics

Pearson's correlation coefficient was used to assess the association between the DoF metrics 0.30dist, 0.30area and 0.04dist, with both AoA and age. Similarly Spearman's

Rho test was used to assess the relationship between both AoA and age with the 0.04 area metric.

Spearman's Rho correlation coefficient was used to investigate the association between the DoF metrics and ToAC.

Pearson's correlation coefficient was utilised to investigate the relationship between the binocular CPS from the *Radner reading chart* and the DoF metrics derived in the pseudophakic population. Additionally, for the DoF metrics that showed a significant correlation, a forward stepwise regression analysis was completed to investigate which DoF metric best predicted the CPS.

4.2.5.7 Metric sensitivity to different designs of IOLs

Two-way repeated measures ANOVAs were examined to determine which metrics derived from the binocular defocus curves of the pseudophakic population, could detect the difference between a monofocal and EDoF IOL. The ANOVA was constructed with the metrics assessed as within-subject factors and lens design as between-subject factors (monofocal and Symfony EDoL).

4.3 Results

4.3.1 Phakic population

Repeated measures ANOVA for all measures of accommodation showed no significant difference between right and left eyes ($F_{2,6}$ =0.194, p=0.661). Hence only the RE data were considered for further analysis; the means or medians and spread of data are shown in Figure 4.4.

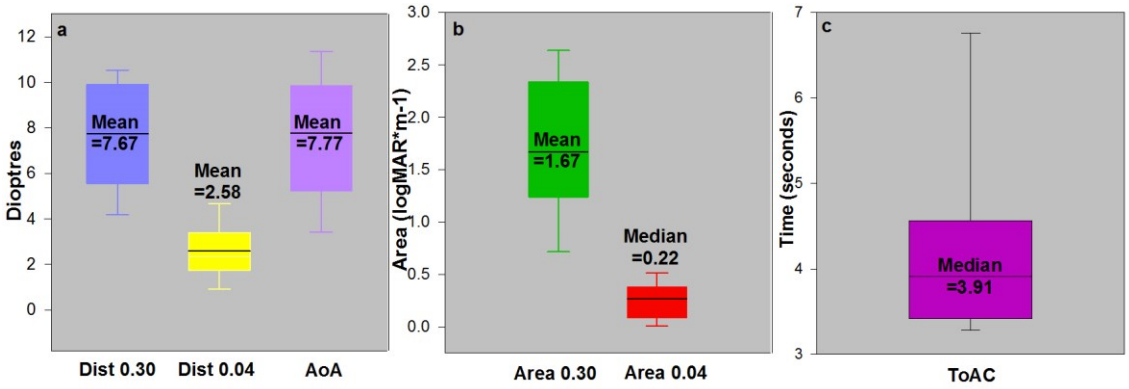

Figure 4.4: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of the data for the accommodative metrics measured: a. dist0.30, dist0.04 and AoA, b. area 0.30 and area0.04, and c. ToAC

4.3.1.1 Correlations between the DoF metrics and the Push-Up test

Figure 4.5 displays the correlation coefficients between the AoA found via the push-up test and each defocus curve calculation method. The 0.30dist metric showed the strongest positive correlation with AoA. No significant correlation was found between AoA and the 0.04area metric.

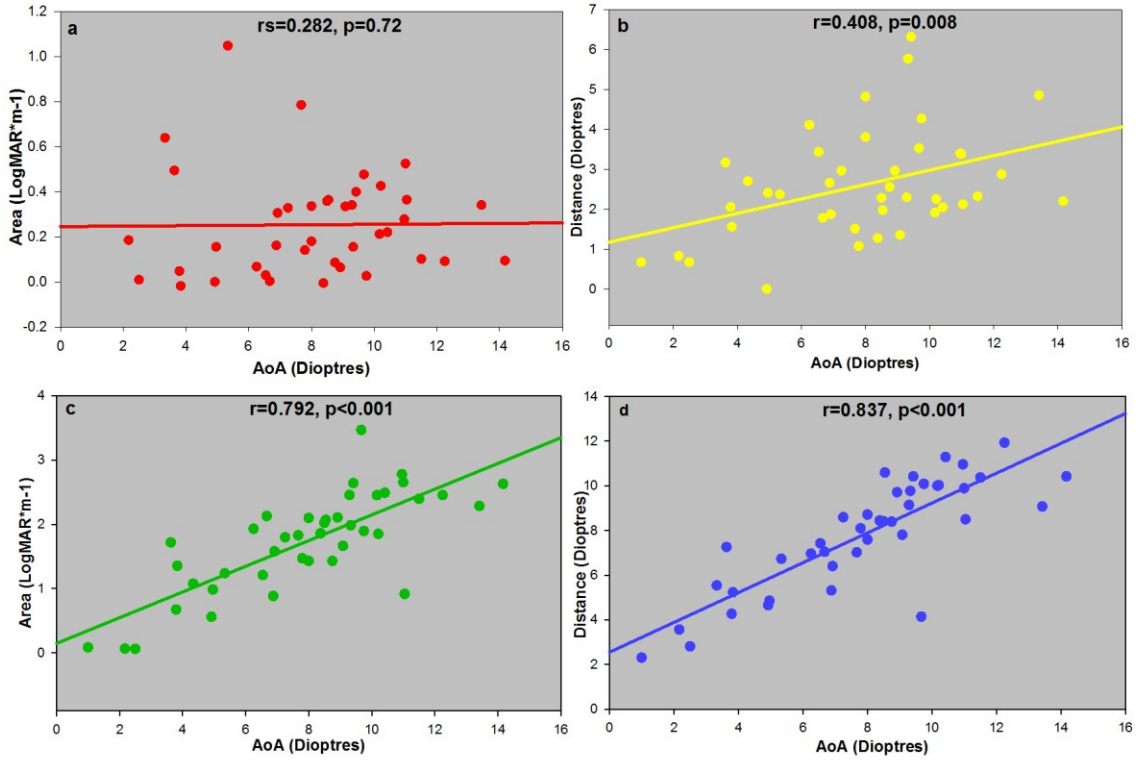

Figure 4.5: The correlation coefficient between AoA (via the Push-Up method) and: a 0.04area, b. 0.04dist, c. 0.30area, d. 0.30dist.

4.3.1.2 Correlations between the DoF metrics and ToAC

Figure 4.6 shows the Spearman's Rho correlations between the defocus curve metrics and ToAC. It can be seen that the correlations were significant when the cut-off criterion was set at +0.30 logMAR; the highest level of correlation was seen with the 0.30area metric.

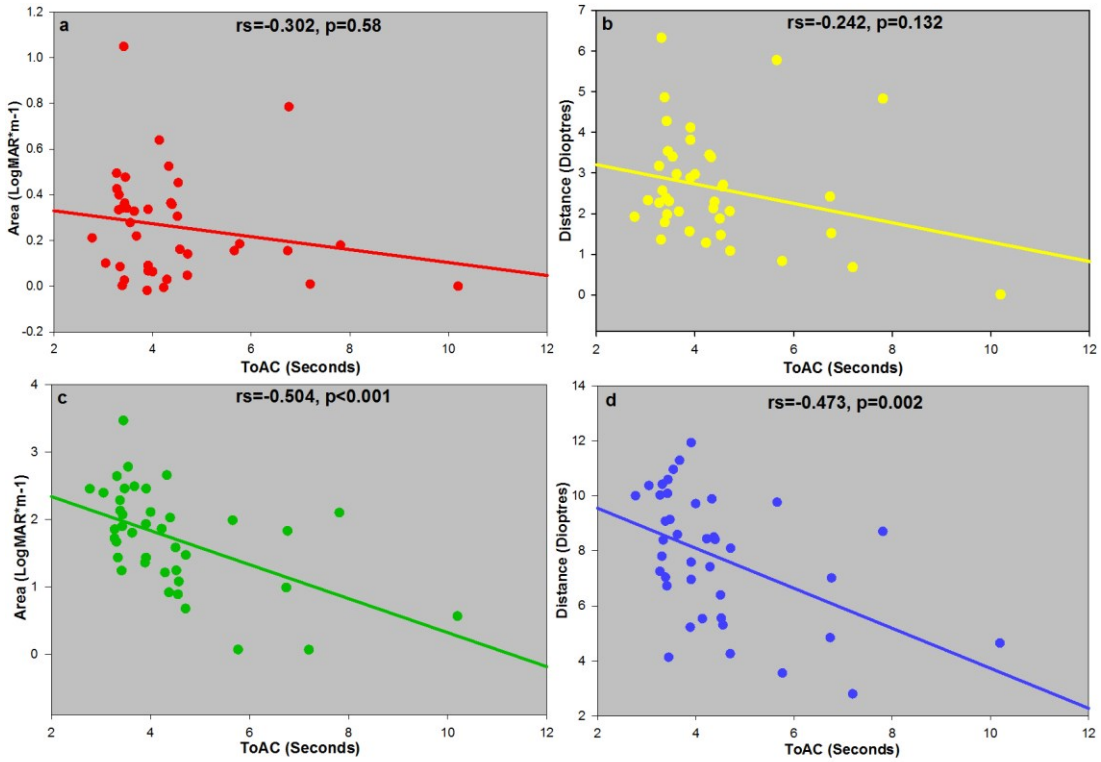

Figure 4.6 Spearman's Rho correlation coefficient between the ToAC and each metric for calculating the depth of focus: a. 0.04area, b. 0.04dist, c. 0.30area, d. 0.30dist

4.3.1.3 Correlations between DoF metrics and age

Except the 0.04dist metric, all other measures of accommodation showed a significant inverse correlation with age. The association with age was greatest when the metric cut-off was set at +0.30 logMAR rather than +0.04 logMAR (Figure 4.7).

4.3.1.4 Repeatability

Intraclass correlation coefficents (ICCs) were examined to assess intra-observer and inter-observer repeatability, these are displayed in Table 4.2.

Of the defocus curve measurements both intra-observer and inter-observer repeatability was highest with the +0.30 logMAR cut-off in comparison to the BCVA+0.04 logMAR cut-off. Figure 4.8 and 4.9 displays the Bland and Altman plots for both the inter- and intra-observer repeatability for all accommodation metrics evaluated.

		Intra-observer repeatability	Inter-observer repeatability
ToAC		0.491	0.575
AoA		0.969	0.947
DoF	**0.30_{dist}**	0.975	0.914
	0.30_{area}	0.935	0.870
	0.04_{dist}	-0.118	-0.051
	0.04_{area}	0.693	0.474

Table 4.2: The ICC intra-observer repeatability and inter-observer repeatability

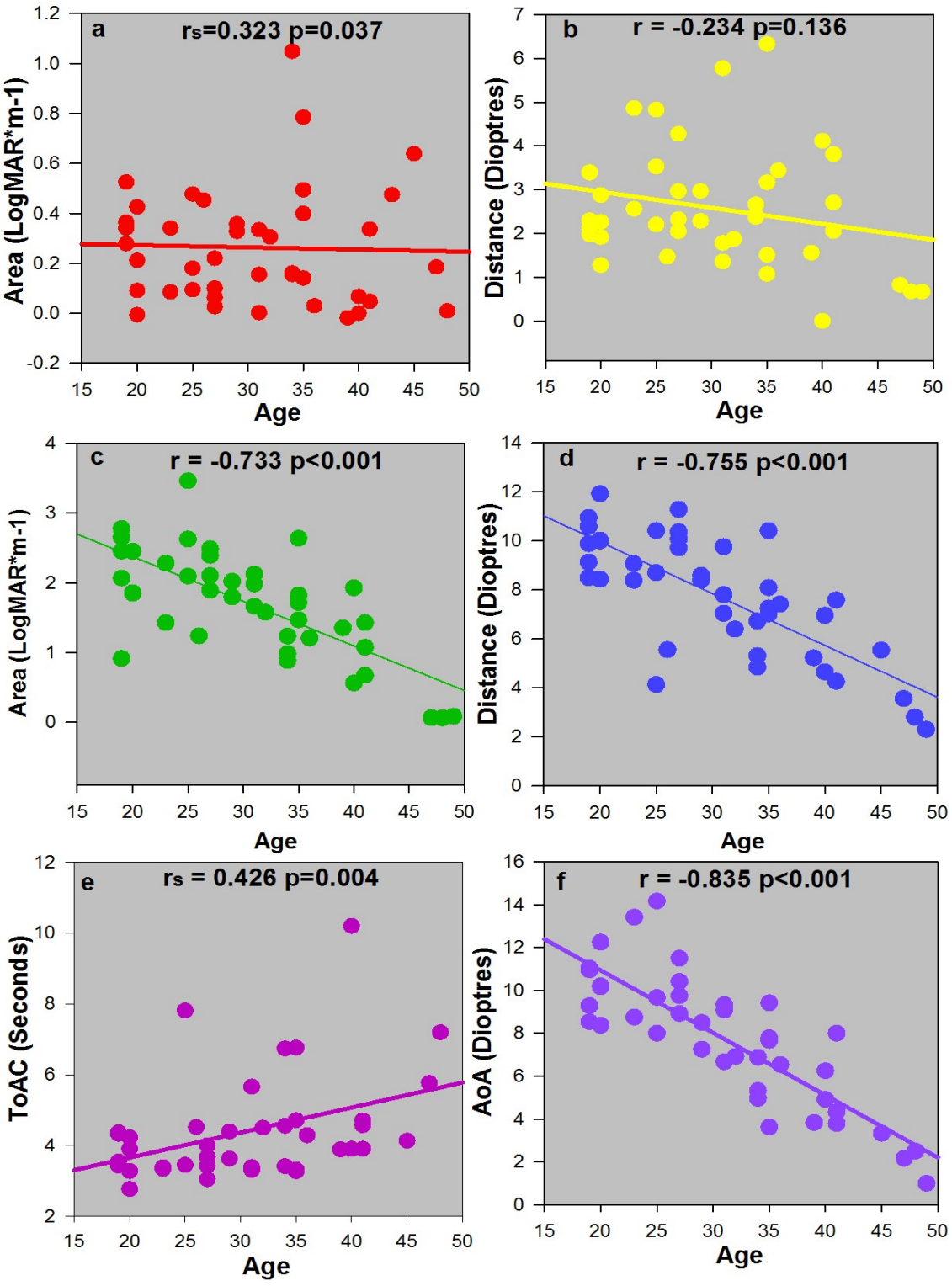

Figure 4.7 Pearson's correlation coefficient between the age and each accommodation metric: a. 0.04area, b. 0.04dist, c. 0.30area, d. 0.30dist, e. AoA, and f. ToAC.

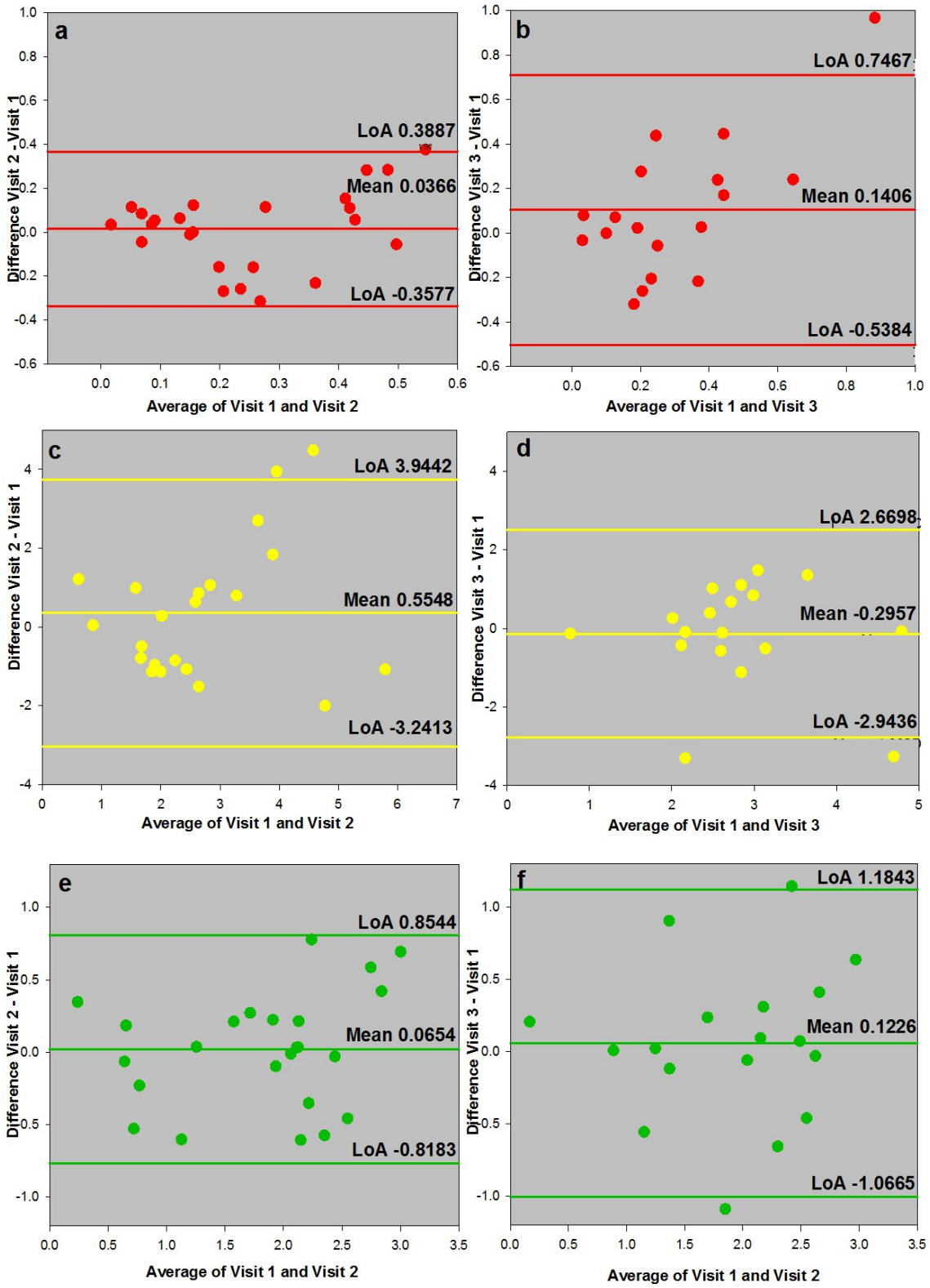

Figure 4.8: Bland and Altman plots of a. intra-observer repeatability 0.04area, b. inter-observer repeatability 0.04area, c. intra-observer repeatability 0.04dist, d. inter-observer repeatability 0.04dist, e. intra-observer repeatability 0.30area, f. inter-observer repeatability 0.30 area.

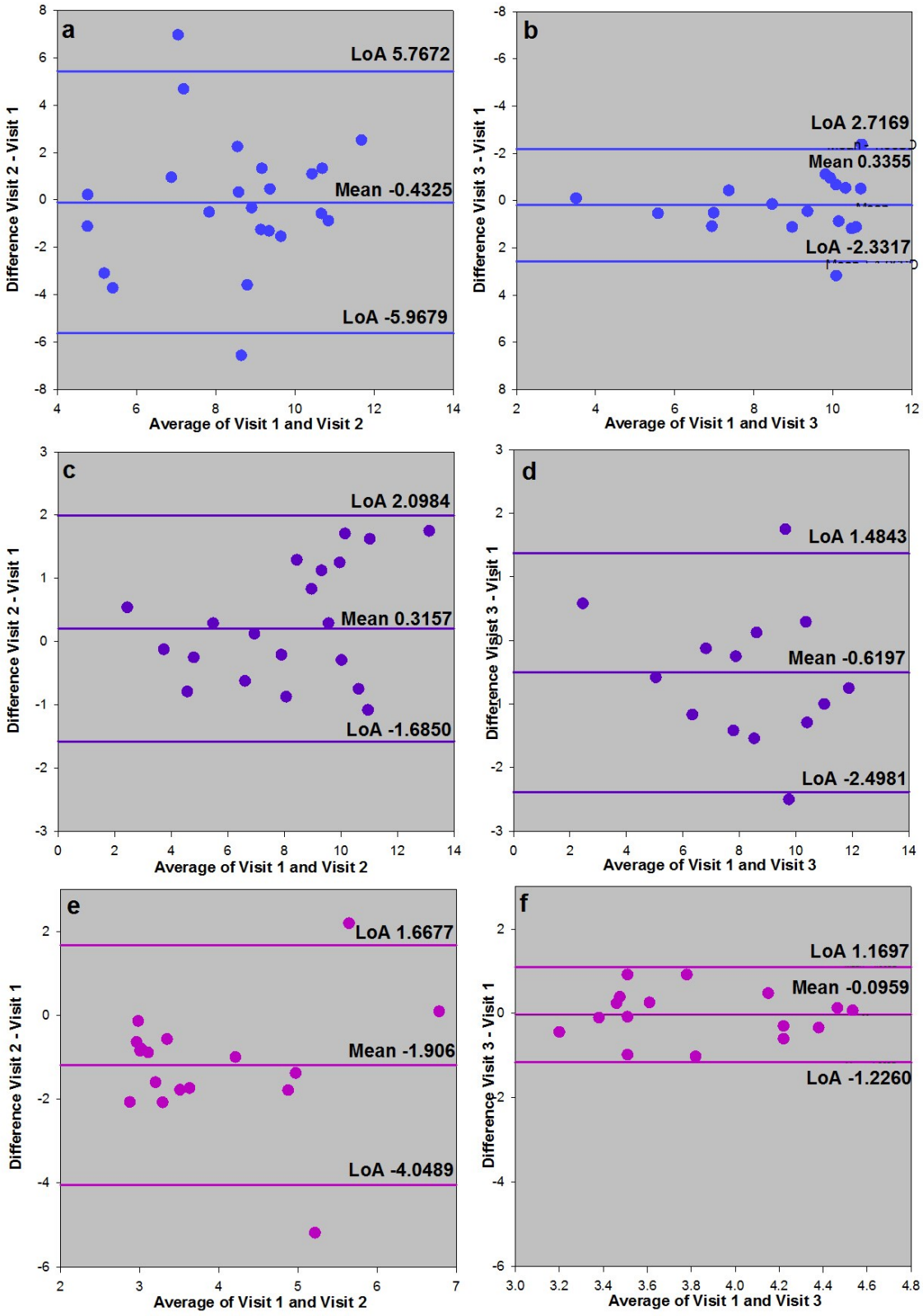

Figure 4.9: Bland and Altman plots of a. intra-observer repeatability 0.30dist, b. inter-observer repeatability 0.30 dist, c. intra-observer repeatability AoA, d. inter-observer repeatability AoA, e. intra-observer repeatability ToAC, f. inter-observer repeatability ToAC.

4.3.2 Pseudophakic populations

Figure 4.10 show the means and spread of data for all of the metrics examined.

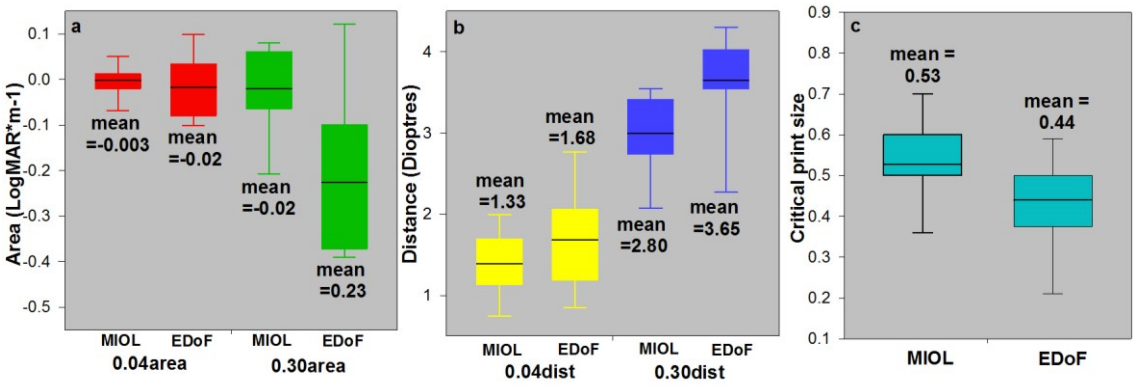

Figure 4.10: Box plots displaying the median, 10th, 25th, 75th and 90th percentiles of data for monofocal IOL and EDoF IOL groups: a. the area-of-focus metrics, b. the range-of-focus metrics, c, the Radner CPS

4.3.2.1 <u>The ability of the metric to differentiate between IOL-designs</u>

A significant difference was found between the lens design ($F_{2,6}$=10.249, p<0.004). Further post-hoc analysis with independent 2-tailed t-tests, (Table 4.3) demonstrated that the cut-off of BCVA+0.04logMAR did not identify a significant difference between the two lens designs with either the area-of-focus or range-of-focus metric. The cut-off of 0.30logMAR showed significant differences between the lens designs, with the Symphony EDoF IOL having a significantly larger range-of-focus and area-of-focus than the monofocal IOL.

DoF Metric	t	p
0.30dist	3.007	0.006
0.30area	3.842	0.001
0.04dist	1.4828	0.198
0.04area	0.610	0.553

Table 4.3: The independent t-tests comparing the monofocal and Symfony EDoF lens designs

4.3.2.2 Correlations between the DoF metrics and the Radner CPS

The mean CPS in the monofocal group was 0.53 ±0.12, whilst for the Symfony EDoF group it was 0.44 ±0.11. The Pearson's correlation between the DoF metrics derived from the defocus curves and the Radner CPS in the pseudophakic population are displayed in Figure 4.11. Only the 0.30dist metric demonstrated a significant inverse correlation (r =-0.487, p =0.014).

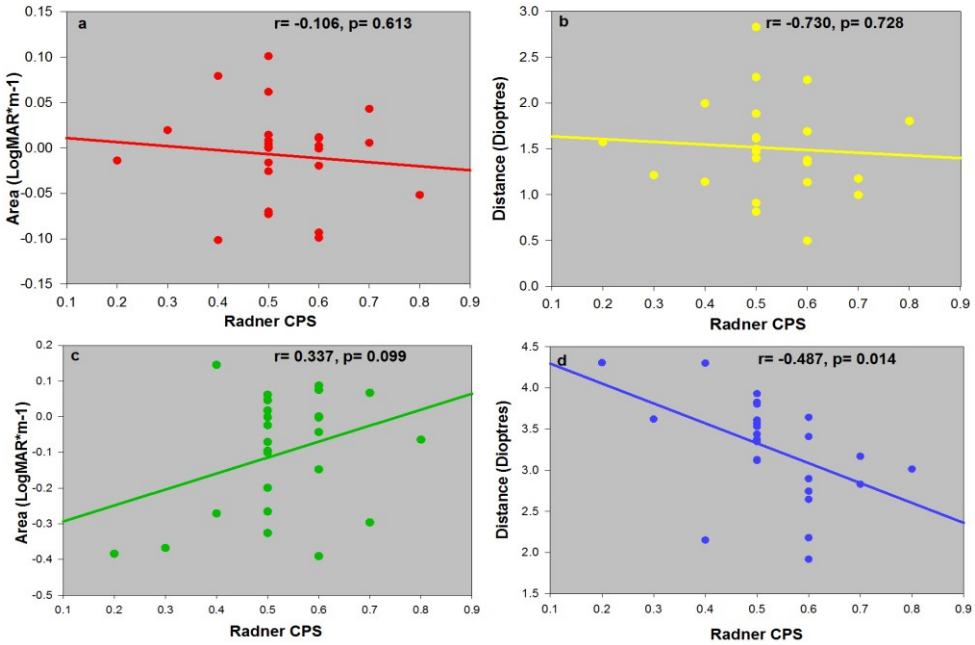

Figure 4.11 Pearson's Correlations between the Radner CPS and each metric for calculating the defocus curve: a. 0.04area, b. 0.04dist, c. 0.30area, d. 0.30dist

4.4 Discussion

The aims of this study were to assess a range of defocus curve metrics in both phakic and pseudophakic eyes. In phakic eyes repeatability was assessed as well as the agreement with the results of AoA and accommodative dynamics. In eyes implanted with an EDoF IOL the agreement between defocus curve metrics and the results of reading speed were determined.

4.4.1 Repeatability of defocus curve metrics

The defocus curve metrics calculated with an absolute criterion of +0.30logMAR (0.30area and 0.30dist) demonstrated a high level of intra- and inter-observer repeatability when compared with the relative BCVA +0.04logMAR cut-off (0.04area and 0.04dist). This can be explained by considering the likelihood of a false positive result. The narrower threshold for the relative cut off areas (0.04area and 0.04dist) leave these measurements very sensitive to small variations in visual acuity measurements resulting in poor repeatability. Gupta et al (2008) who proposed the 0.04dist metric did not assess its repeatability.

4.4.2 Correlation of defocus curve metrics with other measures of accommodation

In comparison to the metrics calculated with the cut-off of BCVA+0.04log MAR, AoA and ToAC, showed higher levels of correlations with the metrics calculated with an absolute

criterion of +0.30logMAR (0.30area and 0.30dist). This finding is in contrast to a study by Gupta et al. (2008), which concluded that the AoA derived from defocus curves using a BCVA+0.04logMAR cut-off demonstrated a stronger correlation and more clinically acceptable limits of agreements than an AoA derived using +0.30logMAR. The discrepancy may be explained by the demographical differences between the study populations and methodological differences. It can be surmised that the poor repeatability of the relative cut off metrics (0.04_{area} and 0.04_{dist}) adversely affects a pre-presbyopic population rendering the results unreliable.

Similar results were found in the pseudophakic population, the relative cut-off metrics (0.04area and 0.04dist) failed to significantly correlate with reading speed despite it having a strong correlation with the absolute metrics (0.30area and 0.30dist).

Determining whether the range-of-focus or area-of-focus metric is the most valid and repeatable within the phakic population is more ambiguous. With the +0.30logMAR cut-off, both the range-of-focus and area-of-focus metric demonstrated comparable inter- and intra-observer repeatability, and comparable correlations with age, push-up test AoA and the ToAC. Therefore both metrics, when derived using an absolute criteria of +0.30logMAR, are valid and repeatable whilst describing the DoF in a phakic population. It would be anticipated that a participant fitted with an EDoF lens would achieve a greater DoF than a participant fitted with a monofocal lens. Therefore, it would be expected that the EDoF group have a significantly greater range-of-focus and area-of-focus than the monofocal group at both cut-off criterion. However, this study found that these metrics only differentiated between a monofocal IOL and EDoF IOL if an

absolute cut-off criteria was utilised. This would support the findings of Buckhurst et al. (2012), who established that the area-of-focus and range-of-focus metrics, with the absolute criteria of 0.30logMAR could determine between a monofocal IOL and MIOL designs. However, Buckhurst et al. (2012) noted that only the area-of-focus metric could differentiate between the different types of refractive and diffractive designs of the MIOL used. This would suggest that a metric quantifying the area of vision seen with an absolute cut-off criterion would provide a more valid overall assessment of the visual out-come after implantation with EDoF IOLs and MIOLs, than a range-of-focus metric. Conversely, when this study further examined the correlations between the Radner CPS scores and each accommodative metrics a significant correlation was demonstrated with only the 0.30dist metric.

It would be anticipated that due to the increased depth-of-focus offered by EDoF IOLs, subjects in this group would score better on a near visual function test. Buckhurst et al. (2012) assessed near vision function using a subjective rating system to quantify patient satisfaction with near vision, after IOL implantation. They found that the subjective near vision rating correlated highest with near area-of-focus ($r_s = 0.484$, $P < 0.001$), than with the range-of-focus metric ($r_s = 0.408$, $P = 0.001$). These findings contrast with the findings in this study, that Radner CPS correlated only with 0.30dist metric (r =-0.487, p =0.014). These observations may be due to the difference in the calculation methods used in quantifying and calculating the area-of-focus metric. This finding would suggest that the 0.30dist metric was the most sensitive in detecting the greater DoF expected in subjects with the EDoF lens.

One of the limitations of this study was the use of step changes of 1.00DS, despite previous studies recommending step changes of no greater than 0.50DS if a relative criteria is used (Wolffsohn et al., 2013). However, Wolffsohn et al. (2013) found that if an absolute cut-off criteria was used, a larger step size of 1.00DS did not affect the range-of-focus metric. The present study included young adults of 19 years and older, where some of the younger subjects had a Push-Up test AoA of up to 14.16D; therefore it was appropriate that the depth-of-focus needed to be measured over a large range (2.00DS to -15.00DS). If step changes of 0.50DS were used this would involve a total of 35 lens presentations, compared to the total of 18 lens presentations with 1.00DS steps. The investigators deemed that this greater number of lens presentations would have a greater risk of results being affected by fatigue bias. Furthermore, the study which recommended step changes of 0.50DS examined a pseudophakic population with multifocal IOLs (Wolffsohn et al., 2013), whereas all of the subjects examined using 1.00D step changes, in this study were phakic subjects. Therefore further studies to understand the effect of the size of step changes on defocus curves measured in young phakic populations would lead to a greater understanding of the balance needed between step changes and fatigue bias.

4.4.3 Conclusion

In summary the key findings of this study were:

- The repeatability of the defocus curve metrics was higher when an absolute cut-off criteria of 0.30logMAR was used.
- The dioptric range over which a subject can see 0.30logMAR consistently demonstrated the best repeatability, and highest correlations with other measures of accommodation in phakic eyes, and near vision function in eyes implanted with the EDoF IOL.

Chapter 5: Investigating the *in vivo* ciliary muscle shape change during accommodation

5.1 Introduction

It is apparent from previous chapters that whilst there are many accommodative metrics that can be measured, there are common problems around the subjectivity of response. Being able to measure the physical changes in the ciliary body during the accommodative response *in vivo* is an alternative approach that could be useful in future experiments. An understanding of the normal structure and function of the human ciliary muscle is desired for numerous reasons.

The compromises in visual function associated with the traditional methods of correcting presbyopia have driven the development of novel methods of restoring accommodation, including accommodating IOLs (Glasser, 2008). An improved understanding of the interactions between an accommodating IOL and the ciliary muscle could better inform IOL design and placement within the eye (Richdale et al., 2013, Richdale et al., 2016).

As discussed in Section 1.8, the ciliary muscle may also have a functional role in the development of accommodative dysfunction and myopia (Oliveira et al., 2005, Bailey et al., 2008, Schultz et al., 2009, Sheppard and Davies, 2010). Studies have shown that ciliary muscle thickness varies with refractive error in children and young adults; the

ciliary muscle in myopic eyes have been found to be longer (Sheppard and Davies, 2010) and thicker in the posterior region (Oliveira et al., 2005, Bailey et al., 2008, Schultz et al., 2009, Buckhurst et al., 2013, Kuchem et al., 2013, Pucker et al., 2013). The hyperopic defocus model states that increased axial length and relative hyperopic peripheral refractive error can be a predictor of myopic development (Mutti et al., 2007, Charman and Radhakrishnan, 2010). After the onset of refractive error, myopes have demonstrated increased accommodative lag (Gwiazda et al., 2005, Mutti et al., 2006), higher accommodative convergence/stimulus to accommodation ratios (Mutti et al., 2000, Gwiazda et al., 2005, Mutti et al., 2017), and increased accommodative fluctuations (Schultz et al., 2009). The exact role of ciliary muscle morphology in myopigenis is unclear, but studies have suggested that there is likely to be a significant relationship between the two (Bailey et al., 2008, Sheppard and Davies, 2010, Buckhurst et al., 2013, Pucker et al., 2013).

5.1.1 Imaging the *in vivo* ciliary muscle

Historically, studying the ciliary muscle *in vivo* has been challenging due to the position of the iris in human eyes (Strenk et al., 2006). Prior to the availability of anterior eye imaging devices, much of the research on the ciliary muscle had been performed *in vitro*; on patients with aniridia, or using animal models (particularly Rhesus monkeys). With improved medical imaging technology, MRI scanners and ultrasound bio-microscopy have been used to investigate ciliary muscles thickness with ageing and accommodation

(Strenk et al., 1999, Strenk et al., 2006, Strenk et al., 2010, Kasthurirangan et al., 2011, Sheppard et al., 2011). More recently with the introduction of the anterior segment time-domain optical coherence tomography (TD-ASOCT) there is now a non-invasive means to capture highly detailed images of the crystalline lens and the ciliary muscle *in vivo*.

Optical coherence tomography (OCT) produces highly detailed 3D images using interference technology. A low-coherence infra-red light-source is split into a sample beam, and a reference beam. As the light source scans across an ocular structure, the sample beam reflections from the ocular tissues coincide with the reference beam reflections from a mirror, resulting in interference (Wolffsohn, 2008). In TD-ASOCT, a reference mirror is moved within an interferometer, to measure the resulting coherence (the difference in frequency and phase) of the interference. This movement continuously varies the distance between the reference mirror and beam splitter, such that this distance matches the time delay of the sample beam. The variation in the optical length of the reference beam determines the axial depth of the ocular tissues; this is utilised to construct A-scans. Multiple A-scans are aligned to create 2D images. It is the mechanical movement of the reference mirror that limits the scan time, resolution and clarity of images, of TD-ASOCT (Wolffsohn, 2008, Drexler et al., 2014).

Swept source OCT (SS-OCT) overcomes the limitations posed by the movement of the reference mirror, by keeping it stationary, and instead employing a tunable narrow-width laser source to sweep through the light spectrum (Drexler et al., 2014). The spectral pattern of the resulting interference between the reflections from the sample

beam and reference beam are assessed; this allows the reflections from each layer to be measured concurrently. In SS-OCTs the scan speed is determined by the wavelength of the swept source laser (Drexler et al., 2014). Compared to TDOCT, SSOCTs offers greater axial resolutions, and faster scan times (Drexler, 2004).

The Zeiss Visante anterior segment TD-ASOCT (Carl Zeiss Meditec Inc., Dublin, CA, USA) was the first commercially available AS-OCT used to assess the *in vivo* human ciliary muscle. TD-ASOCT imaging has been used to study the effects of ageing and accommodation, on biometric measurements of the crystalline lens (anterior lens surface, anterior lens curvature, lens thickness, and lens equivalent refractive index), and the ciliary muscle (ciliary muscle length, thickness, total cross-sectional area, and differences between the nasal and temporal portions), in both children and adults (Bailey et al., 2008, Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lewis et al., 2012, Lossing et al., 2012, Richdale et al., 2012, Buckhurst et al., 2013, Pucker et al., 2013, Richdale et al., 2013, Richdale et al., 2016).

5.1.2 Ciliary muscle changes with age

Much of the evidence derived from ASOCT studies support the findings of *in vitro* and animal model studies but some disparities are evident. In their AS-OCT study, Sheppard and Davies (2011) support the *in vitro* observations of Pardue and Sivak (2000) reporting a decrease in ciliary muscle length with age. In contrast during a study on Rhesus monkeys, Tamm et al. (1992b), found no change in overall ciliary muscle length with

increasing age. In Rhesus monkeys the ciliary muscle appears to move posteriorly with age, as apposed to the anterior movement observed during *in vitro* and *in vivo* human studies, (Strenk et al., 1999, Pardue and Sivak, 2000, Strenk et al., 2004a, Strenk et al., 2010, Sheppard and Davies, 2011).

Furthermore, *in vivo* studies that have reported on the change in ciliary muscle thickness with age, in humans, have reported conflicted results; Richdale et al. (2013) found no significant changes in thickness, at any point along the ciliary muscle with age. However, Sheppard and Davies (2011) found that (in emmetropes) the posterior temporal ciliary muscle thickness decreased, whilst maximum anterior ciliary muscle thickness increased, both nasally and temporally. This study also found an overall inward shift of ciliary muscle mass, and a decrease in anterior length (the distance between the scleral spur and point of vertical maximum thickness).

5.1.3 Ciliary muscle changes with accommodation

There is general consensus among AS-OCT studies that during accommodation the overall length of the ciliary muscle reduces, whilst the anterior ciliary muscle thickness increases, and the posterior ciliary muscle becomes thinner (Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016). Investigators have also confirmed the forward and inwards movement of the ciliary muscle mass with accommodation, which releases zonular tension (Sheppard and Davies, 2010, Lossing et al., 2012, Richdale et al., 2013).

In Rhesus monkeys there is a reduction in the contractility of the ciliary muscle with increasing age, which significantly contributes to presbyopia (Lutjen-Drecoll et al., 1988). Conversely TD-ASOCT studies are in general agreement that the change in ciliary muscle contractility, in humans, does not change with age (Sheppard and Davies, 2011, Richdale et al., 2013, Shao et al., 2015, Richdale et al., 2016). Furthermore, the overall length and anterior length of the ciliary muscle continues to decrease with accommodation, independently of age (Sheppard and Davies, 2011, Shao et al., 2015). In order to further assess the ciliary muscle response to accommodation, several investigators have examined the ciliary muscle thickness change per dioptre of accommodation (Sheppard and Davies, 2010, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016); the methodology and results from these studies are displayed in Table 5.1.

5.1.4 Considerations of AS-OCT methodologies when examining the ciliary muscle

Various attempts have been made to standardise the methodologies utilised when investigating the ciliary muscle with AS-OCT, (Sheppard and Davies, 2010, Kao et al., 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Laughton et al., 2015) but disparities still exist (see Table 5.1).

5.1.4.1 Refractive index

As discussed in section 1.8 images produced by AS-OCT include some optical distortions. These optical distortions result from the refraction of light at each surface edge as the OCT scanning light passes through different mediums; this results in loss of detail of the structures imaged (Ortiz et al., 2010). As discussed in section 1.8, to compensate for these optical distortions, a refractive index (RI) appropriate to each ocular structure needs to be applied to each image. The RI of the ciliary muscle and sclera are estimated to be 1.382 and 1.48, respectively (Tearney et al., 1995, Dirckx et al., 2005). However, there are some disparities between the exact RI and way the RI is applied to the image data during image analysis; the vast majority of investigators have applied a RI of 1.48 for the sclera and 1.38 or 1.382 for the ciliary muscle (Bailey et al., 2008, Sheppard and Davies, 2010, Kao et al., 2011, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Laughton et al., 2015).

Study	N	Age (years)	Refractive Error	Ciliary Muscle	Pharmacological agents, Accommodative demands	RI*	Mean thickness (µm)
Bailey et al. (2008)	53	8-15	'All types' Mean= -1.13D ±2.26	RE nasal	Proparacaine Tropicamide 1% x 2 drops	1.000	CMT1 899 CMT2 601 CMT3 326
Sheppard and Davies (2010)	50	19-34	Between -10 and +6	RE nasal & temporal	0D, 4D, 8D Measured before OCT (using WAM-5500)	1.382	Temporal: CM25 550, CM50 347, CM75 174, CMT2 405 Nasal: CM25 535, CM50 297, CM75 152, CMT2 347
Sheppard and Davies (2011)	79	19-70	Between 9.50D and +2.33D	RE nasal and temporal	0D, 4D, 8D Measured before OCT (using WAM-5500)	1.382	Nasal CMT2 327
Lossing et al. (2012)	25	23-28	Any if BCVA >6/6 in contact lenses	RE Temporal	1D, 4D Measured simultaneously (using Power Refractor)	1.38	CMTmax 825 CMT1 796 CMT2 598 CMT3 367
Buckhurst et al. (2013)	62	18-40	-10.06 to +4.38	RE & LE nasal & temporal	NA	1.388	Temporal: CMT1 529, CMT2 335, CMT3 189 Nasal: CMT1 556, CMT2 319, CMT3 170
Kuchem et al. 2013	29	18-40	-2.56 to +3.29	RE & LE	NA	1.38	CMTmax 855 CMT1 826 CMT2 598 CMT3 348

Table 5.1: Previous studies, which have used AS-OCT to image the ciliary muscle. *RI = Refractive Index. All studies utilised the Zeiss Visante AS-OCT, apart from **, which did not state the type of AS-OCT used. CMT1: ciliary muscle thickness at 1mm from the scleral spur, CMT2: ciliary muscle thickness at 2mm from the scleral spur, CMT3: ciliary muscle thickness at 3mm from the scleral spur, CM25: ciliary muscle thickness at 25% from the scleral spur, CM50: ciliary muscle thickness at 50% from the scleral spur, CM75: ciliary muscle thickness at 75% from the scleral spur

Study	N	Age (years)	Refractive Error	Ciliary Muscle	Pharmacological agents, Accommodative demands	RI*	Mean thickness (µm)	
Pucker et al. 2013	269	6-14	-4D to +7.75	RE nasal	NA	1.38	CMTmax CMT1 CMT2 CMT3	809 778 527 280
Richdale et al. (2013)	26	30-50	-0.50D to +0.50	RE temporal	Phenylephrine 0, 2, 4, 6D	1.41 1.38	CMTmax CMT1 CMT2 CMT3	870 800 490 270
Shao et al. 2015**	33	20-39	-7.75 to plano	LE	0D & 6D			
Richdale et al. 2016	91	30-50	-10.90D to +1.75D	RE Temporal	Phenylephrine 0, 2, 4, 6D Measured simultaneously (using Power Refractor)	1.41 1.38	CMT1 CMT2 CMT3	790 510 300

Table 5.1 (continued) Previous studies, which have used AS-OCT to image the ciliary muscle. *RI = Refractive Index. All studies utilised the Zeiss Visante AS-OCT, apart from **, which did not state the type of AS-OCT used. CMT1: ciliary muscle thickness at 1mm from the scleral spur, CMT2: ciliary muscle thickness at 2mm from the scleral spur, CMT3: ciliary muscle thickness at 3mm from the scleral spur, CM25: ciliary muscle thickness at 25% from the scleral spur, CM50: ciliary muscle thickness at 50% from the scleral spur, CM75: ciliary muscle thickness at 75% from the scleral spur

5.1.4.2 Reference points

Much of the literature on AS-OCT and the ciliary muscle is based upon manual examination of the OCT images. As such when assessing changes in ciliary muscle parameters with accommodation a challenge is posed when anatomically corresponding points need to be identified to allow accurate comparisons between the relaxed and accommodative status (Bailey, 2011). In earlier *in vivo* studies, Bailey et al. (2008) and Oliveira et al. (2005) measured the ciliary muscle thickness at designated distances posterior to the scleral spur. Sheppard and Davies (2010) stated that this does not consider the fact that ciliary muscle thickness and length varies with refractive error (Oliveira et al., 2005), and therefore comparing pre-determined points along a ciliary muscle between emmetropic, myopic, and hyperopic eyes may fail to examine the equivalent anatomical points. Therefore, Sheppard and Davies (2010) proposed that the full length of the ciliary muscle should be assessed and thickness measurements are taken at 25%, 50% and 75% of the length relative to the scleral spur. In principal this technique should improve comparability between refractive groups, however there has been some debate as to how accurately the full length of the ciliary muscle can be assessed with the current resolution offered by TD-ASOCT (Bailey, 2011).

5.1.4.3 Accommodative lag

In studies examining ciliary muscle change with accommodation, several investigators have noted a significant accommodative lag to a near stimulus (Charman, 2008, Lossing

et al., 2012, Richdale et al., 2013). Therefore, to accurately assess the ciliary muscle shape change per dioptre of accommodation, the accommodative response of the eye should be measured, rather than assumed to be accurate to the accommodative demand of the target. Hence, accommodation should ideally be assessed simultaneously whilst imaging the ciliary muscle (Lossing et al., 2012).

To this end some investigators have implemented the use of power refractors to monitor accommodative response whilst the images are captured with the Visante AS-OCT (Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016). Due to technical restrictions relating to the size and design of the AS-OCT, many studies have been unable to do this and have therefore either assumed an accommodative response equal to the accommodative demand of the target, or measured the accommodative response to the a target either prior to, or following imaging (Sheppard and Davies, 2010).

5.1.4.4 Software for ciliary muscle image analysis

Previous studies have utilised the Visante AS-OCT's built-in software to analyse images to extract biometric measurements of the ciliary muscle (Bailey et al., 2008, Sheppard and Davies, 2010, Sheppard and Davies, 2011). However, there are numerous limitations in employing this software to assess the ciliary muscle. These include:

- Automatically applying RIs to an image of the ciliary muscle, which are more appropriate for the anterior cornea (*n*= 1.000), the central cornea (*n*= 1.338) and

the posterior cornea (*n*= 1.343). Whilst the exact RI of the sclera and ciliary muscle are not compensated for.

- Using straight-line callipers to measure the ciliary muscle length or distances from the scleral spur does not account for scleral curvature. Individual variation in scleral curvature would lead to increased variability in the thickness of the ciliary body particularly across the posterior portion (Kao et al., 2011).
- The Visante software cannot be used to measure cross-sectional area. This biometric measurement could provide an improved understanding of the physiology and morphology of the ciliary muscle; especially where studies on ciliary muscle thickness have shown conflicting findings (Bailey et al., 2008, Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lewis et al., 2012, Lossing et al., 2012, Richdale et al., 2012, Buckhurst et al., 2013, Pucker et al., 2013, Richdale et al., 2013, Richdale et al., 2016) .

To combat these limitations investigators have developed semi-automated software programmes to allow image analysis on images exported from the Vistante OCT (Kao et al., 2011, Laughton et al., 2015). These programmes have been designed such that once a landmark, such as the scleral spur, has been selected manually by an observer, the appropriate RI for the sclera (approx. *n*= 1.41) and the ciliary muscle (approx. *n*= 1.38) are applied to the images and edges of the ciliary muscles detected. Various lengths, thicknesses and cross-sectional areas can then be measured taking into account the specific curvature of the sclera. Although full automation of this software could remove

any observer variation in selecting specified landmarks, this would be difficult to achieve due to the challenges in ensuring uniformity during image acquisition (Laughton et al., 2015). A significant limitation of the Kao et al. (2011) software (which has been utilised in studies by Lossing et al., (2012), Richdale et al., (2012), Richdale et al., (2013) and Richdale et al., (2016)), is that the edge detection algorithm combined the ciliary muscle with the pigmented ciliary epithelium, which overestimates the biometric measurements.

5.1.4.5 Tomey SS-ASOCT

The newer Tomey CASIA SS-1000 AS-OCT (Tomey, Nagoya, Japan) is an SS-ASOCT and has the ability to produce scans of the ciliary muscle with a higher resolution than the Zeiss Visante TD-ASOCT. A comparison between the two AS-OCTs, and their equivalent modes, most suited to imaging the ciliary muscle, is summarised in Table 5.2.

When imaging the ciliary muscle with the Visante OCT, the position of the image plane is approximated by viewing a real-time image of the subject's eye on the video monitor. As such the system does not provide the facility to identify specific points on the anterior eye for scanning. Therefore, ensuring uniformity of the specific location of the cross-section of the ciliary body used for analysis during repeated measurements, is difficult. One unique feature of the Tomey CASIA is that the area of the conjunctiva, over which the scan is acquired, is visible during both image acquisition and before image analysis. This scan area can be utilised to select a specific plane from which the image to be

analysed, can be selected. With this function it is possible to identify a physiological marker on a subject's eye as a reference point (RP) which is selected for image analysis. This facility should increase the repeatability and accuracy of repeated ciliary muscle measurements.

	Visante	**Casia**
Light Source	Superluminescent LED	Swept-source laser
Wavelength	1310nm	1310 nm
Mode	High-resolution corneal scan	Angle 'HD'
Axial resolution	18µm	10µm
Transverse resolution	60µm	30 µm
Scan resolution (per line sampling)	512 a-scans	2048 a-scans
Scan area	10mm x 3mm	8mm x 8mm
Scanning time	0.25 seconds	0.2 seconds

Table 5.2: The specifications of the Visante TD-ASOCT and Tomey CASIA SS-ASOCT in the equivalent modes, most suited to imaging the ciliary muscle

Another feature of the Tomey CASIA software is the tools for cross-sectional area analysis. This feature allows analysis of both the entire cross-sectional area of the ciliary muscle, and segment cross-sectional areas. This would allow a more in-depth analysis of the morphology of the ciliary muscle during accommodation and provide improved

understanding of the changes in ciliary muscle physiology with refractive error and ageing.

5.1.5 Aims of this study

The overarching purpose of this study was to evaluate the use of an anterior segment swept source spectral OCT (Tomey CASIA 1000 SS-ASOCT) for the assessment of the ciliary muscle during accommodation. The three main aims were:

1. To investigate whether the Tomey CASIA AS-OCT can be used to examine the ciliary muscle changes that occur with accommodation.
2. To investigate if the technique can be improved (determined by assessing inter- and intra-observer repeatability) by using a reference point to select a specific location of ciliary muscle for cross section scan analysis.
3. To investigate whether ciliary muscle area can be used to assess the ciliary muscle morphology during accommodation, and whether this metric is valid and repeatable.

5.2 Methods

5.2.1 Subjects

Thirty healthy adults (11 males and 19 females) of mean age of 30, ± SD 9 years (range 19 – 48) were recruited for this study. The inclusion criteria was adults aged between 18 and 50 years old with a VA correctable in soft contact lenses to at least 0.0logMAR or better. The exclusion criteria for this study included any current or previous ocular pathology, injury, surgery or binocular vision abnormality, and diabetes mellitus.

Ethical approval was obtained from the Plymouth University Ethics committee and the study was performed in accordance to the tenets of the Declaration of Helsinki. Informed written consent was obtained from each subject prior to commencement of both parts of this study.

5.2.2 Refraction

Objective and subjective monocular refraction was performed on both eyes. One eye was randomly selected for further analysis. The mean spherical refractive error was -0.76DS ±2.44. Any refractive error, outside of the range of -0.50DS to +0.75DS and above 0.75DC, was corrected using soft contact lenses.

5.2.3 Accommodative Response

Due to the physical dimensions of the Tomey CASIA AS-OCT, simultaneous measurements of accommodation during image acquisition were not possible. Therefore, the accommodative response was measured using a WAM-5500 open field auto-refractor (Grand Seiko Co. Ltd., Hiroshima, Japan), prior to image acquisition. The contralateral eye was occluded and subjects were asked to focus on a Maltese cross target. A full aperture, convex, +5D badal lens of 2 inch diameter was used to create a 4D accommodative demand. Subjects were instructed to 'focus on the cross and to make it as clear as possible'. Although presbyopic subjects may struggle to clearly focus on, or maintain a clear focus on a 4D target, it is known that a blurred target will stimulate

ciliary muscle contraction (Strenk et al., 1999, Strenk et al., 2006, Strenk et al., 2010, Sheppard and Davies, 2011). Three measurements were taken, and then the process was repeated with the patient viewing a target at 6 metres, to simulate 0D accommodative demand. The dimensions of the WAM restricted the power of the badal lens to 5D, therefore it was not possible to achieve 8D accommodative demand. The difference between the distance best vision sphere and near best vision sphere was calculated to determine accommodative change. The process was repeated three times and the mean accommodative change was recorded.

5.2.4 Image Acquisition

The equipment was set up as shown in Figure 5.1; to acquire scans of the temporal and nasal ciliary muscle, whilst the subject viewed the external target with the ipsilateral eye.

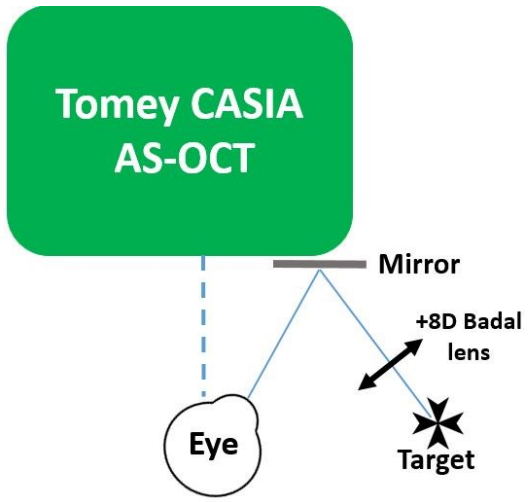

Figure 5.1: The set-up of the equipment for capturing images of the accommodative apparatus

The subject viewed the Maltese cross in a mirror attached to the Tomey CASIA AS-OCT, the location of the Maltese cross was set to achieve an eccentric gaze of approximately 40 degrees whilst the head was in the primary position on the chin rest. The real time on screen video feed from the Tomey CASIA AS-OCT was use to ensure the correct eccentric gaze angle. Two-dimensional images were obtained in the 'Angle HD mode'. In this mode the Tomey CASIA AS-OCT captures up to 2048 A-scans covering an area of 8mm x 8mm in 0.2 seconds (Tomey, 2017).

Scans were acquired for both the temporal and nasal ciliary muscle whilst the subject viewed a Maltese cross at distance and at accommodative demands of 4D and 8D; the 8D demand allowed near-maximal induced accommodative change without using a pharmaceutical agent (Sheppard and Davies, 2010). The order of the presentation of accommodative demands was randomised for each subject in order to avoid bias.

5.2.5 Image Analysis

5.2.5.1 Identification of a conjunctival reference point

The Tomey CASIA AS-OCT, displays a real time video image of the conjunctiva during image acquisition. Following this a still image is displayed that shows the location of each A-scan line. This feature allows the user to select an A-Scan for analysis that corresponds with a specific RP on the conjunctiva (for example a conjunctival blood vessel), as illustrated in Figure 5.2. In this study, a RP on the conjunctiva was used to ensure that all images analysed, for a single subject would be conducted in the same location.

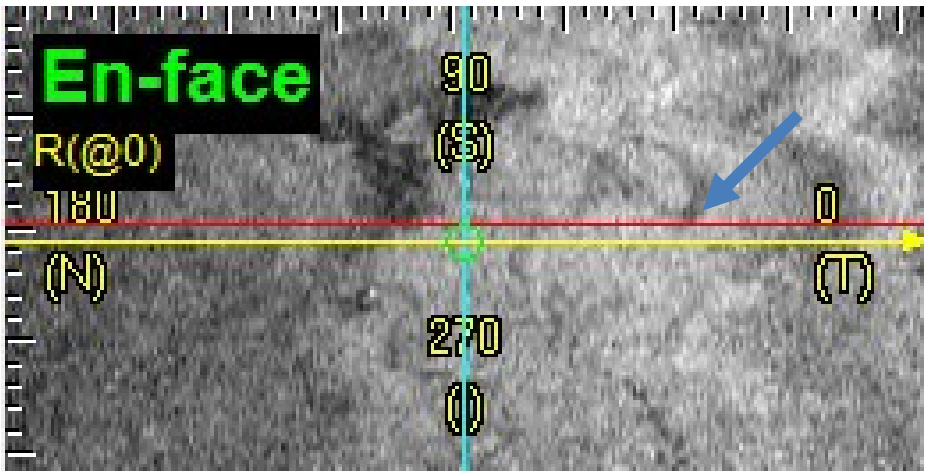

Figure 5.2: The display of the 8mm x 4mm area of conjunctiva over which the Tomey AS-OCT captures the image of the ciliary muscle. *In this image the physiological RP chosen was the inferior loop of conjunctival blood vessels, as shown by the blue arrow. The red line once aligned with the RP illustrates the plane of the image analysed. This ensured that subsequent images were analysed along the same plane of the ciliary muscle.*

5.2.5.2 Measurements of ciliary muscle thickness and area

Each image was analysed by one examiner using the in-built image analysis software. The '2D Analysis' tool provided by the Tomey software was utilised to measure ciliary muscle thickness and area. In built callipers were used to measure the thickest vertical point of the ciliary muscle (CMT_{Max}). The measurement being taken from the anterior ciliary muscle-scleral boundary to the posterior ciliary pigment epithelial surface. In addition to the thickest point, sequential anterior-to-posterior measurements were taken at 1mm (CMT_1), 2mm (CMT_2) and 3mm (CMT_3) increments from the scleral spur (see Figure 5.3).

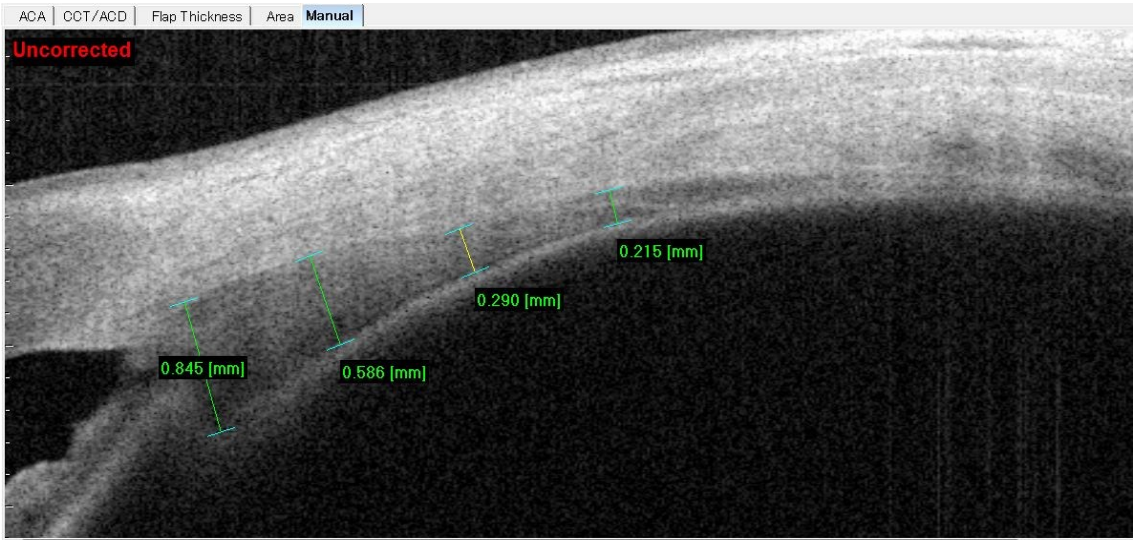

Figure 5.3: Measurements of the ciliary muscle being taken using callipers of CMT_{max}, CMT_1, CMT_2, and CMT_3.

Four area measurements were assessed. To define the zones, for ciliary muscle area analysis, the callipers used for thickness measurements were positioned at the 1mm, 2mm, and 3mm locations as described above. The defined areas were between; the scleral spur to 1mm (CMA_1), 1 to 2mm from the scleral spur (CMA_2), 2 to 3mm from the scleral spur (CMA_3), and the scleral spur to 3mm (CMA_{total}) as shown in Figure 5.4. For the purpose of this analysis the 'Area' tools facility on the Tomey CASIA AS-OCT software was selected and used to outline the boundaries of the ciliary muscle for each area. Once the borders of an area are defined the software automatically calculates the area.

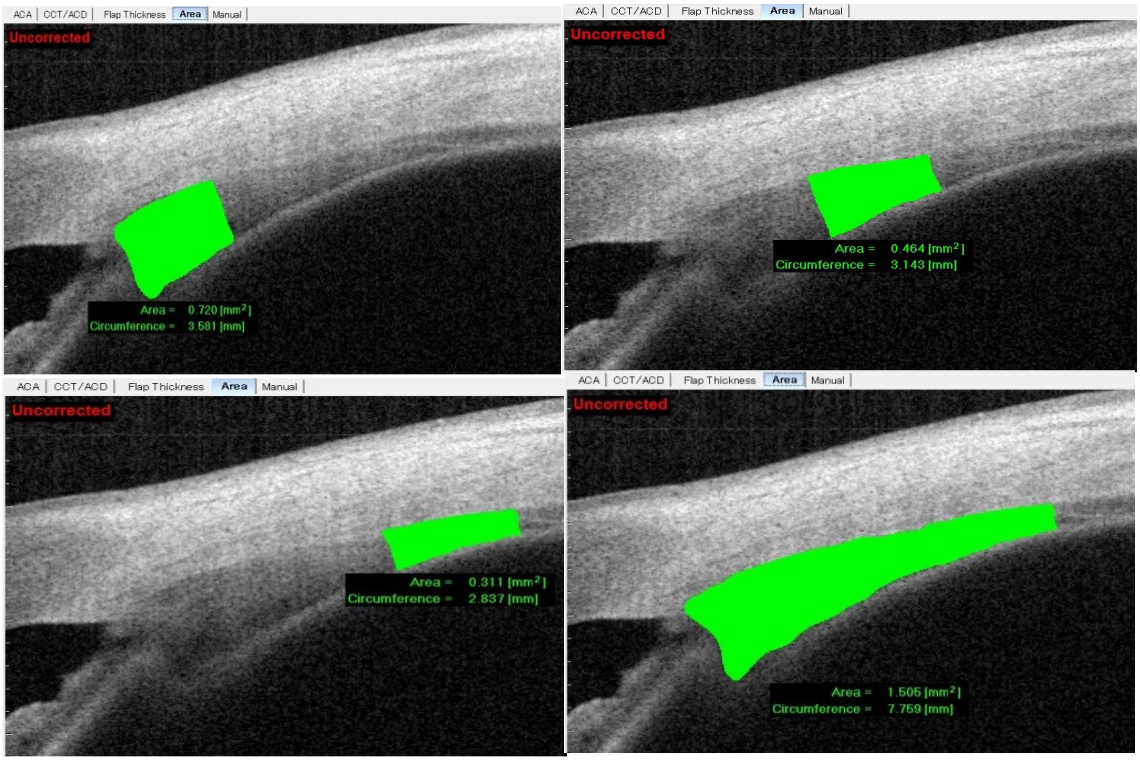

Figure 5.4: Calculations of CMA_1 (top-left), CMA_2 (top-right), CMA_3 (lower-left), and CMA_{total} (lower-right).

To attain accurate measures of ciliary muscle thickness, the vast majority of investigators have applied a RI of 1.48 for the sclera and 1.38 or 1.382 for the ciliary muscle (Sheppard and Davies, 2010, Kao et al., 2011, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Laughton et al., 2015). For this study a RI of 1.38 was applied for all ciliary muscle measurements to minimise the effects of optical distortion produced by variation in refractive indices of the tissues.

5.2.6 Repeatability of the thickness and area measurements

To determine intra-observer repeatability a single subject was assessed on ten separate visits by one examiner. The examiner captured nasal and temporal ciliary muscle images for each accommodative demands of 0D, 4D, and 8D. Implementing the above protocol

for ciliary muscle parameters, one examiner (NS) evaluated the images in a random order and was blind to the knowledge of which accommodation level was being assessed.

To determine inter-observer repeatability a subset of 20 subjects was assessed by a second examiner (MN). The second examiner captured nasal and temporal ciliary muscle images independently using the protocol described above. The two examiners analysed the ciliary muscle images following agreed identification of a nasal and temporal RP. Each examiner were masked to the results of the other.

5.2.7 The use of a reference point to increase accuracy and repeatability of measurements with the Tomey AS-OCT

The utility of a conjunctival RP during image analysis was examined in this study. Specifically the study sought to examine if the intra-observer repeatability improved when using a RP. Ciliary muscle images were captured and analysed using three distinct protocols:

1. Images were captured on one participant at ten separate visits. The use of a RP was avoided for image acquisition and analysis.
2. Images were captured on one participant at ten separate visits. A RP was used for image acquisition and analysis.
3. Images were captured on a single visit and analysed at 9 positions at regular intervals (every 0.44mm) across the scan area (Figure 5.5). This was conducted

to examine the effect of vertical scan location across superior and inferior ciliary muscle.

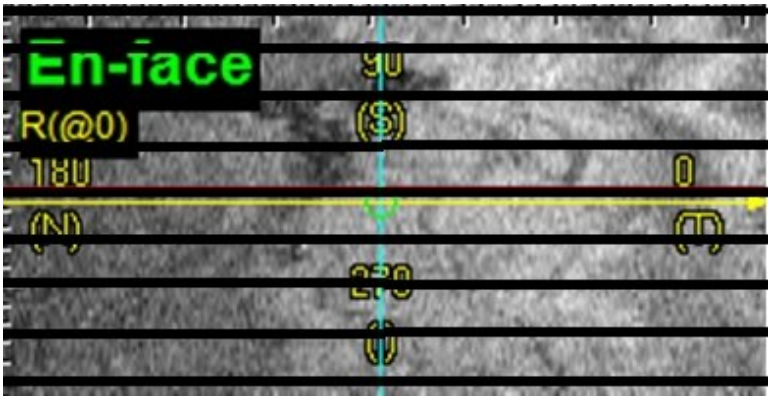

Figure 5.5: The scan area displayed by the Tomey CASIA AS-OCT during image acquisition and analysis, with black lines depicting the nine positions at which scans were analysed

The temporal muscle was used in line with previous studies (Sheppard and Davies, 2010). Identifying the scleral spur is critical as a point of reference for image analysis, and studies have found the scleral spur to be more difficult to identify in the temporal ciliary muscle compared to the nasal ciliary muscle (Sakata et al., 2008). Therefore, measures of the temporal ciliary muscle are likely to show greater variability.

5.2.8 Statistical analysis

5.2.8.1 Assumption of Normality

Following visual inspection of descriptive statistics, histograms, box-plots and Sharpiro-Wilks tests, it was determined that all of the ciliary muscle thickness and area metrics followed a normal distribution.

5.2.8.2 Change in ciliary muscle thickness and area with accommodation

A three-way mixed repeated measures ANOVA was performed to test the difference in ciliary muscle metrics between the two eyes with eye (right/left) as the between-subject variable and accommodative level and ciliary muscle metric as the within subject variables. The same three-way mixed repeated measures ANOVA was performed to determine if there was a significant difference between the temporal and nasal ciliary muscle: muscle location (nasal or temporal) was the between subject variable and accommodative level and ciliary muscle metric were the within-subject variables. Where a significant difference was found then multiple one-way ANOVAs were used with accommodation as the between subject variable and ciliary muscle metric as the within subject variable. The Bonferroni *post hoc* was used to identify the pair-wise differences between accommodative levels.

5.2.8.3 Per dioptre changes in ciliary muscle thickness and area with accommodation

The change in ciliary muscle thickness and area between 0 and 4D was calculated. The area and thickness change per dioptre of accommodation was derived by dividing each value by the individual subject's accommodative response to a 4D target.

5.2.8.4 Repeatability

Inter-observer repeatability was investigated using ICC for each nasal and temporal ciliary muscle metric at different accommodative levels in SPSS, using the following formula.

$$ICC\ (absolute, 2) = \frac{subject\ variability}{(subject\ variability + measurement\ error) \div 2}$$

Intra–observer repeatability was assessed using co-efficent of variation (CoV% = (standard deviation/mean) * 100). Bland and Altman plots were constructed to visualise the agreement of inter-observer measurements (see Appendix 4).

5.2.8.5 The use of a reference point with the Tomey CASIA OCT

The ten measurements acquired for each of the three separate protocols were used to calculate the coefficient of variation (CoV) to examine if the intra-observer repeatability improved when using a reference point.

5.3 Results

5.3.1 Change in ciliary muscle thickness and area with accommodation

Figure 5.6 shows the box plots for each of the ciliary muscle thickness and area measurements. Table 5.3 and 5.4 displays the means and ANOVA results for the ciliary

muscle area and thickness measurements (respectively) at 0D, 4D and 8D of accommodative demand.

No significant difference was found between the nasal and temporal CMT_{max}, CMT_1, CMT_2, or CMT_3, with a 0D accommodative demand (F_1= 2.315 p= 0.319). In comparison, nasal CMA_2 was found to be was significantly greater than temporal CMA_2 (p= 0.006).

Changes in the accommodative demand had a significant effect on both the nasal ($F_{2,3}$= 19.316, p< 0.001) and temporal ($F_{2,3}$= 15.889, p< 0.001) ciliary muscle thickness measurement. Post-hoc analysis revealed that with increasing accommodative demand CMT_{max} and CMT_1 increased whilst CMT_2 and CMT_3 decreased; this trend was evident for both nasal and temporal CMT (Table 5.4).

Accommodative demand also influenced the nasal ($F_{2,3}$= 29.516, p< 0.001) and temporal ($F_{2,3}$= 10.188, p< 0.001) ciliary muscle area. Post-hoc analysis revealed that the nasal and temporal CMA_{total} and CMA_1 increased with accommodative demand between 0D and 4D although between 4D and 8D only the nasal CMA_{total} and CMA_1 demonstrated a significant increase. In contrast CMA_2 and CMA_3 remained unchanged with increasing accommodative effort.

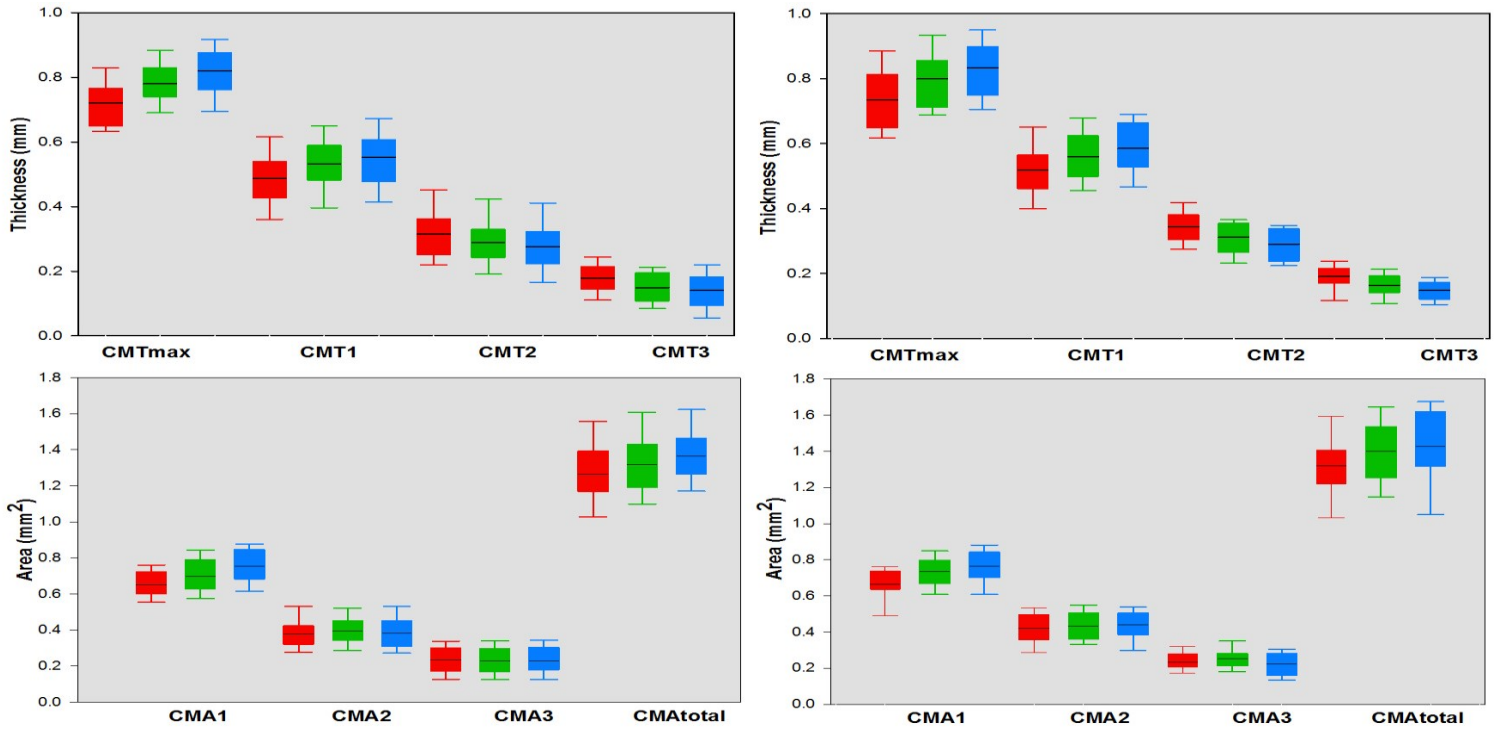

Figure 5.6: Boxplots displaying the means, 10th, 25th, 75th and 90th percentiles of data for: a. nasal ciliary muscle thickness (top left), b. temporal ciliary muscle thickness (top right), c. nasal ciliary muscle area (lower left) and d. temporal ciliary muscle area (lower right). Whilst the subject was viewing a target of 0D (a distance target), 4D of accommodative demand, 8D of accommodative demand.

Area	Nasal			Temporal		
	0D	4D	8D	0D	4D	8D
Total	1.266 ± 0.191	1.318 ± 0.200	1.364 ± 0.203	1.319 ± 0.189	1.400 ± 0.170	1.425 ± 0.210
Area		0D/4D p<0.001	4D/8D p<0.001		0D/4D p=0.002	4D / 8D p=0.670
		0D /8D p=0.004			0D/8D p=0.002	
CMA1	0.651 ± 0.084	0.697 ± 0.105	0.754 ± 0.106	0.664 ± 0.097	0.735 ± 0.093	0.764 ± 0.103
		0D/4D p=0.003	4D/8D p<0.001		0D/4D p=0.002	4D/8D p=0.364
		0D/8D p<0.001			0D/8D p<0.001	
CMA2	0.380 ± 0.084	0.394 ± 0.080	0.383 ± 0.094	0.421 ± 0.097	0.435 ± 0.099	0.439 ± 0.086
		0D/4D p=0.067	0D/4D p=0.586		0D/4D p=1.000	4D/8D p=1.000
		0D/8D p=1.000			0D/8D p=0.563	
CMA3	0.235 ± 0.078	0.227 ± 0.081	0.226 ± 0.084	0.234 ± 0.072	0.254 ± 0.081	0.222 ± 0.073
		0D/4D p=1.000	4D/8D p=1.000		0D/4D p=1.000	4D/8D p=1.000
		0D/8D p=1.000			0D/8D p=1.000	

Table 5.3: The means (mm), standard deviations and ANOVA results for the ciliary muscle area measurements at 0D, 4D and 8D of accommodative demand

Thickness	Nasal			Temporal		
	0D	4D	8D	0D	4D	8D
CMT_{max}	0.721 ± 0.089	0.779 ± 0.087	0.819 ± 0.086	0.735 ± 0.104	0.799 ± 0.100	0.833 ± 0.101
	0D/4D p<0.001	4D/8D p<0.001		0D/4D p<0.001	4D / 8D p<0.001	
	0D /8D p<0.001			0D/8D p<0.002		
CMT_1	0.489 ± 0.097	0.531 ± 0.102	0.552 ± 0.101	0.519 ± 0.084	0.559 ± 0.117	0.586 ± 0.083
	0D/4D p<0.001	4D/8D p=0.003		0D/4D p<0.001	4D/8D p<0.001	
	0D/8D p<0.001			0D/8D p<0.001		
CMT_2	0.318 ± 0.079	0.292 ± 0.072	0.278 ± 0.076	0.344 ± 0.064	0.312 ± 0.063	0.291 ± 0.066
	0D/4D p<0.001	4D/8D p=0.001		0D/4D p<0.001	4D/8D p<0.001	
	0D/8D p<0.001			0D/8D p<0.001		
CMT_3	0.177 ± 0.054	0.151 ± 0.056	0.141 ± 0.060	0.191 ± 0.045	0.164 ± 0.042	0.148 ± 0.039
	0D/4D p<0.001	4D/8D p=0.003		0D/4D P<0.001	4D/8D P<0.001	
	4D/8D p<0.001			0D/8D p<0.001		

Table 5.4: The means (mm), standard deviations and ANOVA results for the ciliary muscle thickness measurements at 0D, 4D and 8D of accommodative demand

5.3.2 Changes in ciliary muscle thickness and area per dioptre of accommodation

The mean accommodative response to the 4D target was 2.52D±0.62D. There was no significant difference between the nasal and temporal ciliary muscle thickness, and area changes.

Ciliary Muscle Thickness (mm)			Ciliary Muscle Area (mm^2)		
	Nasal	**Temporal**		**Nasal**	**Temporal**
CMT_{max}	+0.028 ±0.029	+0.026 ±0.016	**CMA_{total}**	+0.026 ±0.036	+0.036 ±0.049
CMT_1	+0.019 ±0.016	+0.018 ±0.013	**CMA_1**	+0.023 ±0.038	+0.029 ±0.041
CMT_2	-0.012 ±0.015	-0.014 ±0.011	**CMA_2**	+0.006 ±0.016	+0.007 ±0.030
CMT_3	-0.007 ±0.006	-0.026 ±0.012	**CMA_3**	-0.002 ±0.025	+0.008 ±0.027

Table 5.5: The mean (with standard deviation) of the per dioptre change in ciliary muscle thickness and area for a 4D stimulus.

5.3.3 Repeatability

Inter-observer repeatability was found to show good agreement, with all ICC values ≥0.714. Overall ICC was greater for ciliary muscle thickness compared to ciliary muscle areas (Table 5.6).

For intra-observer repeatability, the CoV values were between 0.28% and 3.79% (Table 5.7). With increasing distance from the scleral spur, the repeatability for both the thickness and area measurements of the temporal ciliary muscle reduced. This trend

can be observed for all accommodative demands. Area measurements demonstrated poorer repeatability than the thickness measurements.

	Temporal			Nasal		
	0D	**4D**	**8D**	**0D**	**4D**	**8D**
CMTmax	0.911	0.770	0.959	0.952	0.984	0.900
CMT1	0.957	0.968	0.807	0.942	0.788	0.800
CMT2	0.933	0.804	0.735	0.714	0.873	0.957
CMT3	0.837	0.862	0.833	0.939	0.948	0.923
CMATotal	0.896	0.857	0.794	0.926	0.887	0.972
CMA1	0.854	0.830	0.803	0.717	0.921	0.965
CMA2	0.751	0.717	0.847	0.972	0.891	0.978
CMA3	0.874	0.728	0.883	0.759	0.914	0.959

Table 5.6: The ICC values for inter-observer repeatability, for all of the measurements taken from both the nasal and temporal ciliary muscle, whilst viewing accommodative demands of 0D, 4D and 8D.

	Temporal			Nasal		
	0D	**4D**	**8D**	**0D**	**4D**	**8D**
CMTmax	0.44%	0.28%	0.58%	1.27%	1.24%	0.63%
CMT1	1.18%	1.18%	0.46%	1.00%	0.83%	1.07%
CMT2	1.50%	1.58%	1.02%	1.28%	1.28%	2.52%
CMT3	1.78%	1.78%	3.59%	3.48%	2.98%	3.79%
CMATotal	0.76%	0.39%	0.58%	0.59%	0.63%	1.07%
CMA1	0.51%	0.41%	0.50%	0.43%	0.61%	0.59%
CMA2	1.29%	1.17%	1.67%	1.68%	1.42%	2.21%
CMA3	2.53%	1.62%	1.67%	1.22%	2.23%	3.33%

Table 5.7: The CoV% intra-observer for all of the measurements of both the nasal and temporal ciliary muscle, whilst viewing accommodative demands of 0D, 4D and 8D.

5.3.4 Repeatability when using a conjunctival reference point

As can be seen in Table 5.8, improved levels of intra-observer repeatability were observed for both area and thickness measurements when a RP was used for image analysis (protocol 2). The lowest levels of repeatability were found when area or thickness were measured at the 9 positions across the scan area (protocol 3).

In general with increasing distance from the scleral spur, the repeatability for both the thickness and area measurements reduced across all protocols.

	Protocol 2	**Protocol 1**	**Protocol 3**
	Images were captured on one participant at ten separate visits. A reference point was used for image analysis.	Images captured on one participant at ten separate visits. The use of a reference point was avoided for image analysis.	Images were captured on a single visit and analysed at 9 positions at regular intervals across the scan area.
CMTmax	1.27%	4.53%	3.05%
CMT1	1.00%	8.07%	6.45%
CMT2	1.28%	7.07%	11.90%
CMT3	3.48%	17.94%	25.07%
CMATotal	0.59%	2.53%	5.87%
CMA1	0.43%	5.64%	3.42%
CMA2	1.68%	8.74%	7.95%
CMA3	1.22%	7.37%	16.40%

Table 5.8: The CoV values for the temporal ciliary muscle whilst viewing a distance target, using a RP, no RP and across one scan.

5.4 Discussion

The primary purpose of this study was to evaluate the novel features of the Tomey CASIA AS-OCT, and to assess the influence of its features when examining the ciliary muscle *in vivo.* Images were captured whilst the subject simultaneously viewed accommodative demands of 0, 4 and 8D. The proprietary software within the Tomey CASIA AS-OCT, allows measurement of the ciliary muscle thickness and cross-sectional area. The study utilised this software to examine the change in cross-sectional area of three distinct zones of the temporal and nasal ciliary muscle, relative to the scleral spur, during accommodation.

5.4.1 Overall changes in the area of the ciliary muscle with accommodation

The increase in CMA_{total} and CMA_1 with accommodative demand corresponds with the observed increase in anterior CMT (CMT_{max} and CMT_1). These findings further support the supposition that during accommodation the ciliary muscle mass shifts inwards and anteriorly (Croft et al., 2001, Charman, 2008, Sheppard and Davies, 2010, Richdale et al., 2012, Lossing et al., 2012, Richdale et al., 2013, Richdale et al., 2016).

Since CMT_1 and CMT_2, showed a respective increase and decrease with accommodation, CMA_2 remained constant. Unexpectedly, CMA_3 showed no significant change with accommodation despite reduced measures of CMT_2 and CMT_3. Potential changes in the

curvature of the ciliary muscle-sclera and the ciliary muscle-anterior chamber interfaces may partly explain the constant nature of CMA_3. Indeed the repeatability of all ciliary muscle metrics examined was poorer posteriorly and this may explain the ambiguity of the CMA_3 results.

Other sources of error may originate from the technique used to measure CMA. During image analysis with the Tomey CASIA software the circumference of the ciliary muscle cross-sectional area is highlighted and once the outline is complete, the software automatically calculates the area. One possible source of error in this method is that on automatic fitting of the area the corner regions became more bulbous. Such artificial changes are likely to have overestimated the area measurement and potentially explain the contrasting finding between the lack of change observed in cross-sectional area, as thickness measurements decreased during accommodation.

The overall repeatability of ciliary muscle area was lower than the ciliary muscle thickness measurement. In view of the ambiguous results and reduced repeatability it may be surmised that utilising the area function offered by the Tomey CASIA AS-OCT in its present form has limited clinical and research value.

Future work around designing a software programme which is compatible with the Tomey AS-OCT images to outline and calculate the sectioned cross-sectional areas, could improve the accuracy of area measurements.

5.4.2 The use of a reference point in scan acquisition and image analysis

Much of the literature on CMT and accommodation is based upon studies that have manually examined the thickness changes with accommodative demand. A major criticism of the manual method of assessing CM images is the accuracy with which the examiners can identify the same anatomical points on different AS-OCT images. For example in studies that have used the scleral spur as the reference point for subsequent CMT measurements, accurate identification of the scleral spur between images is vital.

The present study highlights the difference in variability of the ciliary muscle metrics when image analysis was performed with and without reference to a conjunctival RP. Repeatability was found to be significantly lower when a RP was not used.

The improvement in CoV values in every ciliary muscle measurement when a RP was used, demonstrates how this feature of the Tomey CASIA AS-OCT software improves both the repeatability and accuracy of measurements.

The Visante OCT has been widely used for assessing the *in vivo* ciliary muscle but it does not have the facility to identify a RP. However, there is a clear rational from the results of this study to recommend that a RP should be utilised in any future studies which include repeated scanning of the ciliary body, to maximise accuracy and repeatability.

5.4.3 Ciliary muscle thickness

The present values of mean ciliary muscle thickness observed with the Tomey CASIA AS-OCT are in accordance with values reported from previous studies (Sheppard and Davies, 2010, Lossing et al., 2012, Richdale et al., 2012, Buckhurst et al., 2013, Richdale et al., 2013, Richdale et al., 2016). However, as Table 5.1 shows there is some discrepancy in measures of ciliary muscle thickness in the literature.

Discrepancies between mean CMT values reported in the literature and the results of the present study are likely to be due to variations in the methodology used to analyse the images. In this study a refractive index of 1.38 was applied across the data set to correct for the optical distortion, however, there is no standardisation in the methods of how the refractive index correction is applied. Bailey et al., 2011 suggests that the refractive index should only be applied parallel to the image axial scan depth (the co-ordinates of the y-axis) (Bailey, 2011). The inbuilt Vistante software does not allow for such refractive index manipulation of the images and thus, custom designed image-analysis software have been created for these purposes (Kao et al., 2011, Laughton et al., 2015). The semi-automated programme designed by Laughton et al (2015) which fits polynomial curves to the air/sclera boundary and the inner and outer ciliary muscle boundary applies tiered refractive index correction to scleral (n = 1.41) and ciliary muscle tissue (n=1.38). A significant limitation of this study was that the proprietary software within the Tomey CASIA OCT does not allow this tiered application of refractive indices.

The straight-line callipers used in this study do not take into account the curvature of the sclera which is likely to vary between individuals (Bailey, 2011; Kao et al., 2011). The ciliary muscle is known to be longer in myopic eyes, and therefore the scleral curvature is likely to differ between refractive error groups (Bailey et al., 2008, Schultz et al., 2009). This study used subjects with a range of refractive errors and therefore it is likely that the scleral curvature varied between subjects. The CMT values found in this study were generally lower than those found previously (see Table 5.1); the use of straight line callipers may partly account for these results (Bailey, 2011, Kao et al., 2011).

Other factors that are likely to have contributed to the disparities between study results include variations in edge-detection algorithms. Some of which appear to combine both the pigmented ciliary epithelium along with the ciliary muscle, and therefore over-estimate ciliary muscle thickness (Kao et al., 2011).

5.4.4 Overall changes in the thickness of the ciliary muscle with accommodation

With increasing accommodative demand the present study found CMT_{max} and CMT_1 to increase where as CMT_2 and CMT_3 reduced; this trend was found for both the temporal and nasal ciliary muscle. These findings are in general agreement with previous studies examining CMT and accommodation (Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016).

Our findings of a posterior ciliary muscle thinning are not corroborated by all previous studies; Sheppard and Davies (2010) failed to detect a change in the posterior ciliary muscle thickness, which is likely to be due to the different methods used to quantify the distances from the scleral spur at which thickness measurements were taken.

In their study examining ciliary muscle morphology in children during accommodation, Lewis et al. (2012) hypothesised that the CMT_2 position may act as a 'fulcrum' point along the muscle as it showed no significant change with accommodation. This is contrary to the findings of this present study and other studies of the ciliary muscle in adults, where a significant reduction in thickness was observed with increasing accommodative demand (Sheppard and Davies, 2010, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016). Therefore, the observations of (Lewis et al., 2012) may be attributable to differences in the age group examined.

In agreement to previous studies, the CMT increase was greater for the 0D to 4D demand when compared to the 4D to 8D demand (Sheppard and Davies, 2010). In view of the age range included in this study the variation in accommodative lag between subjects may partly explain the lack of proportional increase in thickness expected for the 4-8D demand. Although ciliary muscle contraction can still occur to the same degree, regardless of whether the subject can see a target clearly (Strenk et al., 1999, Strenk et al., 2006, Strenk et al., 2010, Sheppard and Davies, 2011).

5.4.5 Differences between nasal and temporal muscles

In support of the present findings, Sheppard and Davies (2010), (2011), and Buckhurst et al. (2013) found no significant difference between the nasal and temporal anterior ciliary muscle thickness.

In contrast to Sheppard and Davies (2010), who found that the posterior temporal ciliary muscle was significantly thicker at CM2, CM50 and CM75, the present study showed symmetry between the nasal and temporal portions of the posterior ciliary muscle thickness. Furthermore, Sheppard and Davies (2010) found a greater reduction in thickness in the anterior length of the temporal ciliary muscle, with accommodation, indicating that the temporal muscle is more contractile than the nasal muscle.

Further work to investigate possible asymmetry between the nasal and temporal ciliary muscles is required. Ideally, this would involve imaging the nasal and temporal portions simultaneously; however, this would require acquiring scans in a mode giving lower resolution images of the ciliary muscle, which may affect the accuracy of results.

5.4.6 Repeatability

Interestingly it was noted that despite the good to excellent inter- and intra-observer repeatability values demonstrated for each ciliary muscle metric measured, the measurement variability was greater for the posterior ciliary muscle, compared to the anterior ciliary muscle. Furthermore, when no RP was utilised for scan acquisition and image analysis, the increase in CoV was markedly greater in the posterior region of the

ciliary muscle. This would suggest that there is greater intra-subject variability in ciliary muscle thickness posteriorly.

The contrast in results between the thickness and area measurements posteriorly would suggest that using distinct cross-sectional areas to quantifying changes in the ciliary muscle, during accommodation, is not a valid metric.

5.4.7 Per dioptre changes in thickness of the ciliary body with accommodation

Due to the dimensions of the Tomey CASIA AS-OCT it was not possible to measure accommodation whilst simultaneously acquiring ciliary muscle images as recommended by Lossing et al., (2012). Despite this limitation the change in ciliary muscle thickness per dioptre of accommodation was in alignment with previous studies (Table 5.9).

5.4.8 Conclusion

In summary the key findings of this study were:

- The repeatability of the ciliary muscle area, using the Tomey CASIA AS-OCT software, was lower in comparison to ciliary muscle thickness.
- The use of a conjunctival reference point improved the repeatability of ciliary muscle measurements and should be used in all future studies examining the ciliary body when possible.

- In line with previous studies, the ciliary muscle thickens anteriorly and thins posteriorly, with accommodation (Sheppard and Davies, 2010, Sheppard and Davies, 2011, Lossing et al., 2012, Richdale et al., 2012, Richdale et al., 2013, Richdale et al., 2016).
- The increase in thickness and area of the anterior ciliary muscle with accommodation observed in this study, further supports Helmholtz's theory of accommodation (Croft et al., 2001, Charman, 2008, Sheppard and Davies, 2010, Richdale et al., 2012, Lossing et al., 2012, Richdale et al., 2013, Richdale et al., 2016).

	Ciliary Muscle Thickness change per dioptre of accommodation to a 4D target (mm)						
	This Study		Previous studies				
	Nasal	Temporal	Richdale et al., (2016) Temporal	Richdale et al., (2013) Temporal	Lossing et al., (2012) Temporal	Richdale et al., (2012) Temporal	Sheppard and Davies (2010) Temporal
CMT_{max}	+0.028 (±0.029)	+0.026 (±0.016)		+0.026	+0.018	+0.025	
CMT_1	+0.019 (±0.016)	+0.018 (±0.013)	No significant change	+0.013	+0.012	+0.015	CM25 +0.071
CMT_2	-0.012 (±0.015)	-0.014 (±0.011)	Significant decrease	-0.011	No significant change	-0.011	-0.021
CMT_3	-0.007 (±0.006)	-0.026 (±0.012)	Significant decrease	-0.015	-0.012	-0.017	CM75 No significant change

Table 5.9: The mean (and standard deviation) of per dioptre changes in ciliary muscle thickness with accommodation. CMT1: ciliary muscle thickness at 1mm from the scleral spur, CMT2: ciliary muscle thickness at 2mm from the scleral spur, CMT3: ciliary muscle thickness at 3mm from the scleral spur, CM25: ciliary muscle thickness at 25% from the scleral spur, CM50: ciliary muscle thickness at 50% from the scleral spur, CM75: ciliary muscle thickness at 75% from the scleral spur

Chapter 6: Crystalline lens ageing study (CLAS): does smoking status and other lifestyle factors affect accommodation?

6.1 Introduction

As discussed in section 1.9, ageing is an extremely complex process, due to the interaction between different ageing theories and the influence of physiological and environmental factors (Troen, 2003, Jin, 2010, Bao et al., 2014, Goldsmith, 2015, Libertini, 2015, Goldsmith, 2016). One of the theories known to play a central role in the ageing of the crystalline lens is the free-radical theory, which states that if levels of ROS exceeds the levels of anti-oxidant defence molecules, oxidative stress occurs leading to damage to the surrounding biological structures.

Aerobic respiration, which occurs abundantly in the crystalline lens, produces endogenous ROS. Lifestyle can affect both the endogenous ROS, and exogenous ROS, that a human can be exposed to. For example, tobacco smoke and UV exposure are exogenous sources of ROS, whilst having a large amount of central adiposity can increase exposure to endogenous ROS (Savini et al., 2013).

Cigarette smoke contains numerous noxious substances such as carbon monoxide, formaldehyde, nitric oxides, and peroxides. Each inhalation from a cigarette contains approximately 1015 ROS or free radicals. Not only does this increase exposure to exogenous ROS, but reduces the antioxidant defence mechanisms, further increasing oxidative stress levels (Alberg, 2002, Northrop-Clewes and Thurnham, 2007, Nita and Grzybowski, 2017). Smokers are also known to have reduced levels of many of the anti-oxidants defence molecules. Whether this is solely due to a reduced dietary intake is unclear (Schleicher et al., 2009), however studies have shown that after correcting for fruit and vegetable intake, smokers still had a reduced concentration of plasma vitamin C than non-smokers (Lykkesfeldt et al., 2000). This suggests that vitamin C has a higher metabolic turnover in smokers (Kallner et al., 1981).

Central adiposity is the accumulation of both subcutaneous fat and visceral fat around the abdomen. As discussed in section 1.10.1, excessive visceral fat is associated with modifying glucose and lipid metabolism, and increased chronic inflammation; resulting in greater levels of oxidative stress (Fernández-Sánchez et al., 2011, Lumeng and Saltiel, 2011, Savini et al., 2013). Furthermore, excessive central adiposity is associated with reduced levels of anti-oxidants (Canoy et al., 2005, Andersen et al., 2006, Kaidar-Person et al., 2008); potentially further increasing the oxidative stress levels and chronic inflammation. Systemic chronic inflammation in humans increases the risk of metabolic syndrome disorders (Dandona et al., 2005, Agarwal et al., 2016, Kim et al., 2016) and is

associated with increased rates of biological ageing (Tzanetakou et al., 2012, Babizhayev et al., 2014).

Interestingly, as part of the multi-centred European Prospective Investigation of Cancer Study (EPIC-study), Khaw et al. (2008) created a lifestyle model with subjects scoring points for healthy lifestyle factors including: smoking status, physical activity levels, plasma vitamin C levels (indicating fruit and vegetable intake) and alcohol intake. The study found that if four high-risk lifestyle factors were followed, there was a four times greater chance of mortality, equivalent to 14 years in age, compared to if four low-risk lifestyle factors were followed. This suggested that biological ageing in humans is heavily influenced by lifestyle.

Presbyopia and opacification of the crystalline lens are age-related changes. Pathai (2013) suggested that the mechanisms underlying systemic ageing are also responsible for ageing within the crystalline lens, with the opacification of the crystalline lens being proposed as a biomarker for ageing. Supporting this theory numerous studies have demonstrated that cataracts are linked to a significantly greater mortality rate (West et al., 2000, Hennis et al., 2001, Nucci et al., 2004, Truscott, 2005, Truscott and Zhu, 2010). Truscott and Zhu (2010) suggested that the increasing protein concentration in the crystalline lens, and the changes to life-long proteins are responsible for both the loss of accommodative function and increasing opacification of the crystalline lens. Therefore, the ageing mechanisms, which can affect protein production or protein maintenance, are likely to influence the rate of ageing within the crystalline lens. The free radical

theory causing oxidative stress and chronic inflammation is known to accelerate the age-related protein changes in the crystalline lens associated with cataract formation (Sohal, 2002, Harper et al., 2004, Truscott, 2005, Graw, 2009, Babizhayev et al., 2011, Michael and Bron, 2011). A higher risk of cataract formation has been associated with conditions related to increased sources of endogenous ROS including: metabolic syndrome conditions, (Paunksnis et al., 2007, Lindblad et al., 2008, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013), diabetes (Janghorbani et al 2000, Lindblad et al 2008, Hegde and Varma 2005, Hashim and arina 2012), and obesity/raised BMI (Glynn et al., 1995, Schaumberg et al., 2000, Lim et al., 2009, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013).

A higher risk of cataract formation has also been associated with increasing levels of exogenous ROS e.g. smoking (Cumming and Mitchell, 1997, Klein et al., 1999, Klein et al., 2003, Raju et al., 2006, Xu et al., 2006, Navarro Esteban et al., 2007, Tan et al., 2008c, Wu et al., 2010, Ye et al., 2012). Furthermore, the anti-oxidant defence mechanism in the crystalline lens is known to decline with increasing age (Zhang and Augusteyn, 1994, Spector, 1995), leaving lens nucleus cells and lens proteins more susceptible to damage from oxidative stress (Giblin, 2000). This progressive loss of the oxidation defence mechanisms in the lens accompanies a reduction in chaperone activity (Harding, 1970). This has led to many researchers exploring the potential links between cataract formation and nutrient intake. As discussed in section 1.10.6 these studies have found conflicting results (Leske et al., 1991, Sperduto et al., 1993, Seddon et al., 1994, Leske et

al., 1998, Lyle et al., 1999, Cumming et al., 2000, Mares-Perlman et al., 2000, AREDS I, 2001, Jacques et al., 2001, Kuzniarz et al., 2001, Christen et al., 2003, Olmedilla et al., 2003, Christen et al., 2004, Kvansakul et al., 2006, Christen et al., 2008, Dherani et al., 2008, Maraini et al., 2008, Tan et al., 2008a, Christen et al., 2010, Chew et al., 2013, Kang et al., 2016). However, during a review of the available literature Weikel et al. (2014) recommended that maintaining a vitamin C intake of 135 mg per day (exceeding the daily recommend intake of 40mg), and a protein intake of 100 to 150g (greater than the daily recommended intake of 55g), over a long term period may be beneficial in preventing nuclear sclerotic cataracts. Overall it has been concluded that further data from observational studies and interventional studies is required before any firm conclusions can be made (Chew, 2013, Weikel et al., 2014).

With presbyopia having similar underlying physiological ageing processes as cataracts (Truscott and Zhu, 2010), it is known that some of the risk factors that are associated with cataracts, such as age and diabetes can also affect the accommodative ability of an individual (Skarbez et al., 2010). There is a gap in the literature as to whether other modifiable risk factors which influence the physiological levels of oxidative stress associated with cataract can also affect accommodative function, and therefore the rate of development of presbyopia. The purpose of this study is to investigate and identify links between accommodative function and lifestyle factors such as smoking, levels of physical activity, body shape, alcohol intake, and nutrient intake.

6.1.1 Aims and Objectives

The main aim of this cross-sectional study was to investigate whether the 'EPIC' lifestyle scoring system, found to predict mortality risk (Khaw et al., 2008), or a modified version 'CLAS', was associated with accommodative function, mainly AoA, AF, and ToAC. Further aims of this study were to identify:

- Whether accommodative function is associated with smoking status.
- If the total daily energy intake, daily protein intake, or the intake of anti-oxidants such as: vitamin C, vitamin E, are associated with accommodative function.
- If body shape is associated with accommodative function.
- If alcohol intake is associated with accommodative function.
- Whether levels of physical activity are associated with accommodative function.

6.2 Methods

6.2.1 Subjects

Seventy healthy adults (21 males and 49 females) with a mean age of 33, SD ±3 years (range 30 - 40) were recruited for this study across four sites:

1. Patients of Plymouth University's Centre of Eye Care Excellence.
2. Staff and students at University of Plymouth.

3. Patients who had attended Livewell SouthWest Stop Smoking service.
4. Advertisements in local Plymouth press.

The inclusion criteria for this study included healthy individuals between the ages of 30 and 40 years old, and a BCVA in soft contact lenses of better than 0.0logMAR. The exclusion criteria included current or previous ocular pathology or trauma, currently taking medications known to affect accommodation, diabetes mellitus and pregnancy.

Ethical approval was obtained from The National Institute for Social Care and Health Research Academic Health Science's Research Ethics Committee (Powys Teaching Health Board) and the study was performed in accordance to the tenets of the Declaration of Helsinki. Informed written consent was obtained from all subjects, following an explanation of the procedures involved, and prior to any data being collected.

6.2.2 Refraction

Objective and subjective monocular refraction was performed on each eye, the mean spherical equivalent refractive error found was -0.65DS ±1.55. Any refractive error greater than -0.50DS, +0.75DS or 0.75DC was corrected using soft contact lenses. At this point, any subject who could not achieve a BCVA of 0.0 LogMAR or better, was excluded.

6.2.3 Accommodation measurements

The right or left eye was randomly selected for all accommodation measurements. During the following procedures the contralateral eye was occluded.

6.2.3.1 Push-Up test

Monocularly, the subject was instructed to focus on a near vision target on the RAF rule (a word of size N5), presented at 40cm from the subject's eye. The subject was asked 'to report when the word first appears blurry'. The target was moved slowly towards the subject, at the first point of reported blur, the target was stopped and the subject asked if the target became clear. If so, the target was moved further towards the subject until the first point of sustained blur was reached. This was recorded as the break point. The target was then moved slowly away from the subject and the subject was asked 'to report when the target first becomes clear'. This was recorded as the recovery point. This was performed three times so that a mean could be calculated. AoA was calculated by taking an average of the mean break point and the mean recovery point.

6.2.3.2 Accommodative facility

Accommodative facility was performed monocularly. The subject was presented with a near vision target at a viewing distance of 40cm, and instructed to look at a four letter word on the N5 line. The -2.00D lens was presented first; the subject was asked to report

when the target first became clear, at which point the lens was flipped to the +2.00D lens until the subject reported the target as 'clear'. This was classed as one rotation. After an initial 'practice', with at least two rotations, or until the subject understood the test, a timer was started and the number of full rotations (presentation of -2.00 and +2.00) achieved within one minute was recorded.

6.2.3.3 Time for accommodative change from the accommodative dynamic profile

Accommodative dynamics were measured using Grand Seiko Auto-refractor WAM-5500 with a motorised Badal adaption as described in section 2.2.3. From the accommodative dynamic profile the time for accommodation (ToA), time for disaccommodation (ToD) and time for accommodative change (ToAC) were derived as described in section 2.2.5.

6.2.4 Questionnaires

All subjects completed three questionnaires (see Appendix 5). The first captured data on age, gender, ethnicity, social status, occupation, hobbies, and alcohol and smoking history. The second was a Food Frequency Questionnaire (FFQ) to capture information about the participant's diet over the past year (Bingham et al., 2001). FFQs capture information about nutritional dietary intake over a set period time, normally within the last 12 months, by asking the subject to estimate how often they have consumed

different types of foods and beverages. Despite the limitations of these methods, including relying on subjects to honestly report intake and gross estimations of portions size, FFQs have been validated in numerous populations (Fallaize et al., 2014, Tayyem et al., 2014), and have good agreement with other methods of investigating nutritional status, such as 7-day diet diaries and biomarkers (Brunner et al., 2001). FFQs are less time consuming or invasive than 7-day food diaries and biomarker testing. The specific FFQ used in this study has been validated for use in the UK, and used in a large-scale lifestyle study: the European Prospective Investigation of Cancer study (EPIC-study) (Bingham et al., 1994). Thirdly, subjects completed an International Physical Activity Questionnaire (IPAQ) which has been validated to capture information on physical activity in the proceeding 7 days (Craig et al., 2003).

6.2.5 Body Parameters

6.2.5.1 Body-Mass Index

Height was measured in meters, using a Marsden Leicester Height Measurer (Marsden Group, Rotheram, UK). With their shoes removed, the subjects were asked to stand with their feet flat on the floor and close together, and to stand as tall as possible. Two measurements were taken. If these were within 1cm of agreement an average was taken, and if the repeated measurements had a difference of 1cm or greater the two measurements were repeated.

Weight was measured using scales, in kilograms, again with shoes and heavy clothing removed. From this the BMI was calculated using the following formula:

$$BMI = \frac{weight\ (kg)}{height\ (m)^2}$$

Equation 1

The BMI of the subject was then used to classify them into one of the following groups (WHO, 2000):

- Underweight: If BMI <18.5
- Normal weight: If BMI was between 18.5 and 24.9
- Overweight: If BMI was between 25 and 29.5
- Obese if BMI was ≥30

6.2.5.2 Waist-hip ratios

The waist and hip circumferences were measured in line with the World Health Organisation (WHO) recommendations (WHO, 2008) using a stretch-resistant tape measure. Subjects were asked to stand up straight, with their feet together and to take a few normal breaths. The waist measurement was taken at the end of expiration; the waist portion was identfied as the midpoint between the lower side of the last palpable rib and the top of the iliac crest. The hip measurement was identified as the widest portion around the buttocks. This was repeated twice, if the repeated measurements

were within 1 cm of agreement then an average was calculated, and if the repeated measurements had a difference of 1cm or greater then the two measurements were repeated. From this the waist-hip ratio was calculated using the following formula:

$$Waist - hip\ ratio = \frac{waist\ circumference\ (cm)}{hip\ circumference\ (cm)}$$

Equation 2

Subjects were then categorised into two groups using the WHO's waist-hip ratio criteria for a 'healthy' waist-hip ratio (<0.9 for men or <0.85 for women) or an 'at risk' (of obesity-related healthy conditions), waist-hip ratio (≥0.9 for men or ≥0.85 for women) (WHO, 2008).

6.2.6 Statistical Analysis

6.2.6.1 Assumptions of normality

After visual inspection of descriptive statistics, histograms, box-plots and Sharpiro-Wilks tests all of the accommodation metrics were found to have a normal distribution, with the exception of AF, which was found to be non-normally distributed.

6.2.6.2 Compensated amplitude of accommodation (c.AoA)

A scatter plot was constructed with the AoA (y-axis) versus age (x-axis) and a linear regression line was fitted using Sigmaplot *(Version 13.0, Systat Software Inc. San Jose, California, USA*) using equation 3.

$$y = y0 + ax$$

Equation 3

From this *a* was derived, and compensated AoA (c.AoA) values were calculated to align each measurement to the average age of all subjects (34 years), effectively removing age factor from the AoA measurements, using equation 4, where y is the measured AoA and *x* is the subject's age.

$$C.AoA = \left(\left[\frac{100}{(a \times x) \times (a \times 34)}\right] \div 100\right) \times y$$

Equation 4

6.2.6.3 Associations between smoking status and accommodative function

All subjects were first classified into two groups: *ever-smoked* or *never-smoked*, with the *ever-smoked* group including all subjects who were current, or past-smokers. The c.AoA and ToAC were compared between the two groups using two-tailed independent t-tests, AF was compared between the two groups using a Mann-Whitney U test.

Subjects were also sorted into three groups: *current smokers* (for any subject who had smoked at least one cigarette in the last year), *past-smokers*, and *non-smokers*. A One-Way ANOVA was used to compare the c.AoA and ToAC between the three groups. A Kruskal-Wallis test was used to compare AF between the three groups.

6.2.6.4 Associations between diet and accommodative function

Analysis of the FFQ was completed using FETA software (Mulligan et al., 2014), the average daily intake of portions of fruit and vegetables, vitamin C, vitamin E, protein and total energy were derived. The correlations between vitamin C, vitamin E, protein and total energy, and each of the above accommodative parameters was investigated using Pearson's correlation coefficient or Spearman's Rho correlation test. Subjects were then sorted into two groups as to whether they had reported to consume less than five portions of fruit and vegetables per day, or five or more portions of fruit and vegetables per day. Five portions of fruit and vegetables per day was chosen in line with public health guidelines at the time of the study (Moseley, 2013).

Independent two-tailed t-tests were used to compare the c.AoA and ToAC between the groups. A Mann-Whitney U test was used to compare AF between the two groups.

6.2.6.5 Associations between alcohol consumption and accommodative function

Information about weekly alcohol consumption was derived from the first lifestyle questionnaire (Appendix 5). The correlation between weekly units of alcohol consumed and accommodative function was investigated using Pearson's and Spearman's Rho correlation coefficent tests. Subjects were then sorted into two groups: '*moderate drinkers'* defined as drinking between 1 and 14 units per week, and *'non-moderate drinkers*' defined as drinking less than one unit of alcohol per week or greater than 14 units of alcohol per week (Khaw et al., 2008). Two-tailed independent t-tests and Mann-Whitney U tests were used to compare the accommodative parameters between the '*moderate*' and '*other'* drinkers.

6.2.6.6 Associations between exercise and accommodative function

Analysis of the IPAQ was completed manually using Excel 2016 Software (Microsoft, Redmond, USA), to calculate each subject's MET-minute scores. The MET-minutes score is a quantification of the energy required to do a set physical activity, for an average 60 kilogram person, multiplied by the duration of activity (in minutes) (Ainsworth et al., 2000). To investigate any relationship between MET-minute scores and accommodative function, the accommodative parameters were correlated with the MET-minute score using Pearson's or Spearman's Rho Signed rank test. Furthermore, each subject was classified into one of three groups: '*inactive*', '*minimally active'* or '*HEPA active*' (Health enhancing physical activity), as defined by the IPAQ criteria (IPAQ, 2004), briefly this is:

- *HEPA active*, either:
    - High-intensity activity on a least 3 days per week, contributing at least 1500 MET-minutes to the weekly MET score, or
    - Seven or more days of vigorous or moderate intensity activity, or
    - walking contributing to at least 3000 MET-minutes to the weekly MET score.
- *Minimally active*, either:
    - Three or more days of vigorous activity lasting at least 20 minutes per day.
    - Five or more days of walking or moderate intensity activity, lasting at least 30 minutes per day.
    - Five or more days of any combination of: vigorous or moderate activity or walking which contributes a minimum of 600 MET-minutes to the weekly MET score.
- *Inactive*: Any subject who does not meet the criteria for the other two categories.

One-Way ANOVAs were used to compare the c.AoA and ToAC between the three groups. A Kruskal-Wallis test was used to compare AF between the three groups.

6.2.6.7 Associations between body shape and accommodative functions

The correlations between BMI and each of the accommodative parameters was investigated using Pearson's and Spearman's Rho correlation coefficent tests.

All of the subjects in this study had a BMI greater than 18.5, therefore the accommodative parameters were firstly compared between two groups: '*normal weight*' (BMI <25) and '*overweight'* (BMI ≥25) using independent two-tailed t-tests and Mann-Whitney U tests. Secondly, the over-weight group was further split into '*overweight*' (BMI between 25 and 29.5) and '*obese'* (BMI ≥30). The accommodative parameters were compared between the *normal weight, overweight,* and *obese* groups using one-way ANOVAs and a Kruskal-Wallis test.

The correlations between waist-hip ratio and each of the accommodative parameters was investigated using Pearson's and Spearman's Rho correlation coefficient tests. Two-tailed independent t-tests and Mann- Whitney U tests were used to compare the accommodative parameters between the '*healthy*' waist-hip ratio and the '*at risk'* waist-hip ratio groups.

6.2.6.8 Associations between the EPIC-study lifestyle model and accommodative function

Subjects were each scored for each positive lifestyle factor that they demonstrated, in line with the model used by Khaw et al. (2008), summarised in Table 6.1. This gave each

subject a lifestyle score between zero (being the least healthy) and four (being the most healthy). Accommodative parameters were compared between the groups using One-Way ANOVAs and a Kruskal-Wallis test. Furthermore, linear regression was used to see whether lifestyle score could predict a subject's accommodative function.

Lifestyle Factor	Score	
	Zero	One
Smoking status	Current smoker	Non-smoker or past smoker
Fruit and Vegetable intake	<5 portions per day	≥5 portions per day
Physical Activity	Inactive or minimally active	HEPA-active
Alcohol intake	<1 unit per week or >14 units per week	1 to 14 units per week

Table 6.1: The scoring system for the EPIC-study lifestyle model

6.2.6.9 Associations between the CLAS lifestyle model and accommodative function

An alternative lifestyle model termed the Crystalline Lens Ageing Study (CLAS) model was proposed, utilising the same lifestyle factors as the EPIC model, substituting physical activity levels for waist-hips ratio. This measurement of central adiposity was included because the association between obesity and cataracts is well documented (Glynn et al., 1995, Schaumberg et al., 2000, Paunksnis et al., 2007, Lim et al., 2009, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013), whereas no study has found an association between physical activity and cataracts. As discussed previously, as central adiposity increases chronic inflammation and oxidative stress, it is possible that this accelerates the age-related changes in the crystalline lens, leading to a loss of accommodative

function. Table 6.2 provides a summary of how the subjects were scored using the CLAS model. Again, this gave each subject a lifestyle score between zero (being the least healthy) and four (being the healthiest). Accommodative parameters were compared between the groups using One-Way ANOVAs and a Kruskal-Wallis test. Furthermore, linear regression was used to assess whether lifestyle score could predict a subject's accommodative function.

Lifestyle Factor	Score	
	Zero	One
Smoking status	Current smoker	Non-smoker or past smoker
Fruit and Vegetable intake	<5 portions per day	≥5 portions per day
Waist-hips ratio	At-risk*	Healthy*
Alcohol intake	<1 unit per week or >14 units per week	1 to 14 units per week

Table 6.2: The scoring system for the CLAS lifestyle model.
**** as classified by the WHO (2008)***

6.3 Results

6.3.1 The association between smoking and accommodative function

The subjects were first classified by their smoking history into two groups *ever-smoked* (n=32) or *never-smoked* (n=38). The mean or median and spread of data of the accommodative metrics for these two groups are shown in Figure 6.1. Smoking history demonstrated no association with accommodative function; there was no significant

difference in ToAC (t= -1.285, p= 0.203), c.AoA (t= 0.495, p= 0.622) or AF (U= 606.5, z= -0.018, p= 0.986) between the *ever-smoked* and *never-smoked* groups.

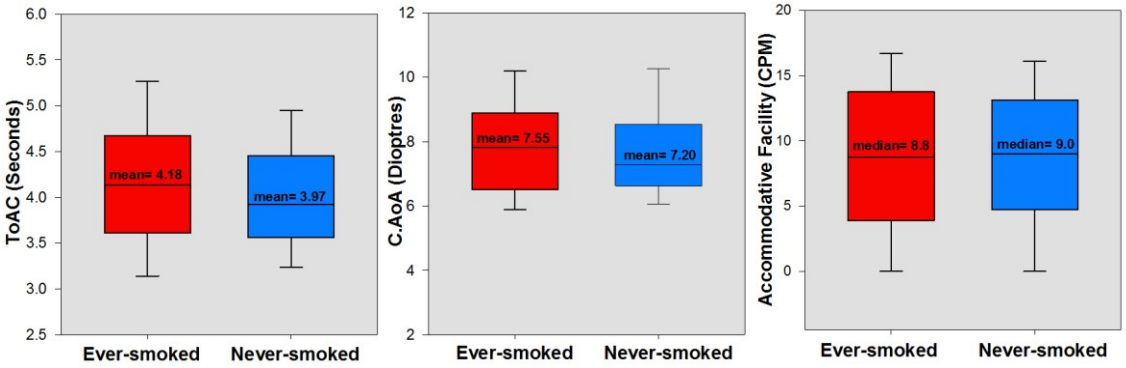

Figure 6.1 Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each of the accommodative parameters for the 'Ever-smoked' and 'Never-smoked' groups

Further investigation involved examining the association between current smoking status and accommodative function. The subjects were split into three groups: *current-smokers* (n=13), *past-smokers* (n=19), and *never-smoked* (n=38), the mean, medians and spread of data for these groups of each accommodative metric are shown in Figure 6.2.

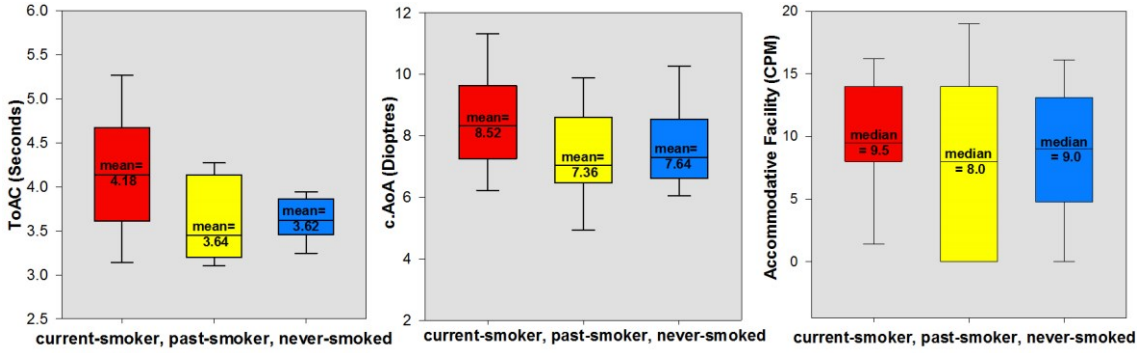

Figure 6.2 Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each of the accommodative parameters for the 'current-smokers', 'past-smokers' and 'never-smoked' groups

There was a significant difference in the ToAC between current-smokers, past-smokers and never-smoked groups, $F_{(2,68)} = 6.975$, $p < 0.002$. Post-hoc pairwise comparisons with

Bonferroni correction revealed that the current-smokers had significantly slower ToAC than the past-smokers (p= 0.016) and the never-smoked (p< 0.008). There was no significant difference in the C.AoA ($F_{(2,68)}$= 2.156, p= 0.124) or AF ($H_{(2,68)}$ = 1.177, p= 0.555) between the *current-smokers*, *past-smokers* and *non-smokers* groups.

6.3.2 The associations between diet and accommodative function

There was no correlation between any of the accommodative parameters and daily total energy intake, vitamin C intake, vitamin E intake or protein intake (Table 6.3). There was no significant difference in the accommodative abilities between subjects who consumed five or more portions of fruit and vegetables per day (n=27), and those who consumed less than five portions per day (n=43) (Figure 6.3). ToAC: t= 0.237, p= 0.814, c.AoA: t= 0.975, p= 0.975, AF: U= 404.00 Z= -1.880, p= 0.60.

Total Daily Intake	ToAC	C.AoA	AF
Energy	r= 0.113, p= 0.363	r= -0.120, p= 0.324	r_s= -0.146, p= 0.234
Vitamin C	r= -0.45, p= 0.721	r= 0.027, p= 0.828	r_s= 0.227, p= 0.062
Vitamin E	r= 0.30, p=0.808	r= -0.046, p=0.705	r_s= -0.073, p= 0.555
Protein	r= 0.18, p=0.888	r= -0.015, p=0.902	r_s= -0.040, p= 0.749

Table 6.3: The correlation coefficients of the accommodative parameters measured and daily intake of: total energy, vitamin C, vitamin E and protein

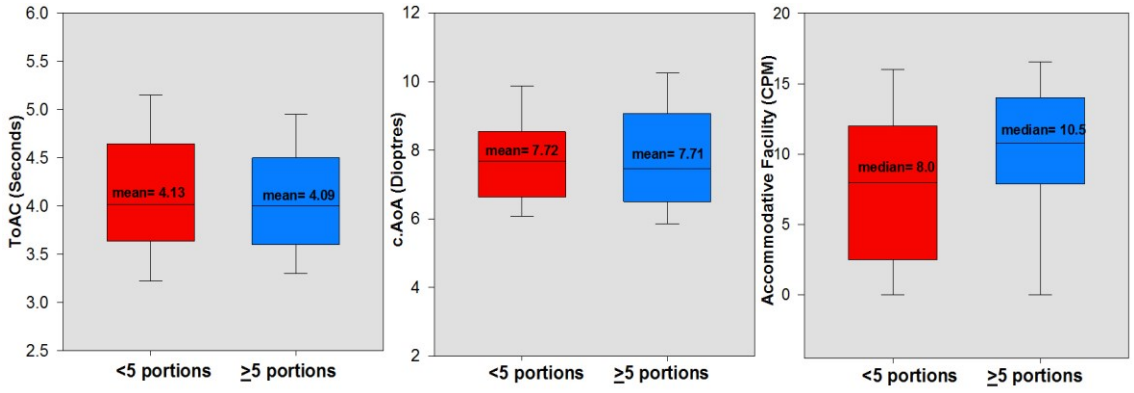

Figure 6.3: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each accommodative parameter, for subjects who had an average daily consumption of <5 portions of fruit and vegetables, and ≥5 portions of fruit and vegetables per day

6.3.3 The associations between alcohol consumption on accommodative function

There was no correlation between weekly units of alcohol consumed and accommodative function (C.AoA: r= -0.052, p= 0.670, ToAC: r= -0.176, p= 0.152, AF: r_s= 0.055, p= 0.651). Alcohol consumption demonstrated no association with c.AoA or ToAC; there was no significant difference in these c.AoA or ToAC between the moderate drinkers (n=49), and non-moderate drinkers (n=21) (Figure 6.4, t= 1.440, p=0.154 and t= -1.277, p=0.206, respectively). However, moderate drinkers achieved a significantly greater number of CPM during AF (median= 10CPM) compared to subjects who were either non-drinkers or heavy-drinkers (median= 8CPM) (Figure 6.4, U= 350.000, Z= -2.014, p< 0.044).

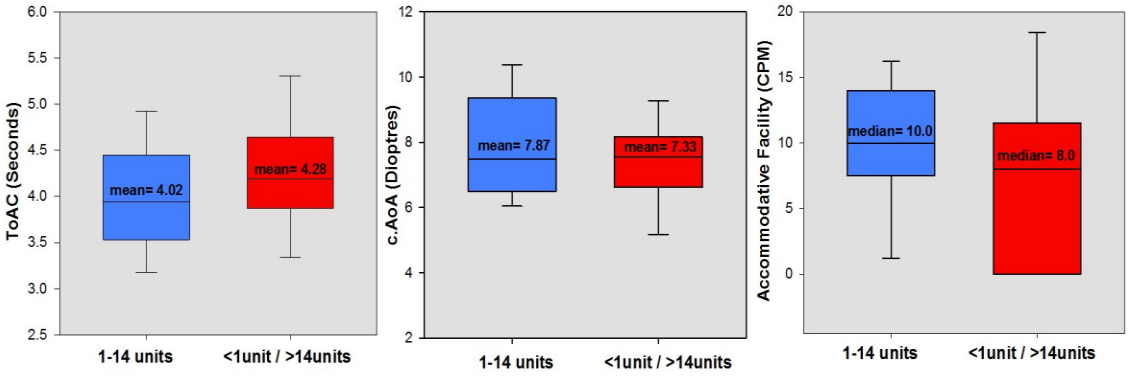

Figure 6.4: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each accommodative parameters for the moderate drinkers and non-moderate drinkers groups

6.3.4 The associations between body shape and accommodative function

There was no correlation between BMI and any of the accommodative parameters measured (C.AoA: r= -0.008, p= 0.723, ToAC: r= -0.43, p= 0.723, AF: r_s= -0.73, p= 0.548). There was no significant difference in any of the accommodative parameters between the *normal-weight* (n=46) and *over-weight* (n=25) groups (Figure 6.5: c.AoA: t= -0.231, p= 0.818, ToAC: t=-0.60, p= 0.952, AF: U= -0.73, p= 0.548), nor the *normal-weight*, *over-weight*, or *obese* groups (Figure 6.6: c.AoA: $F_{(2,68)}$= 2.291, p= 0.109, ToAC: $F_{(2,68)}$= 0.75, p= 0.476 $H_{(2)}$= 4.209, p= 0.122).

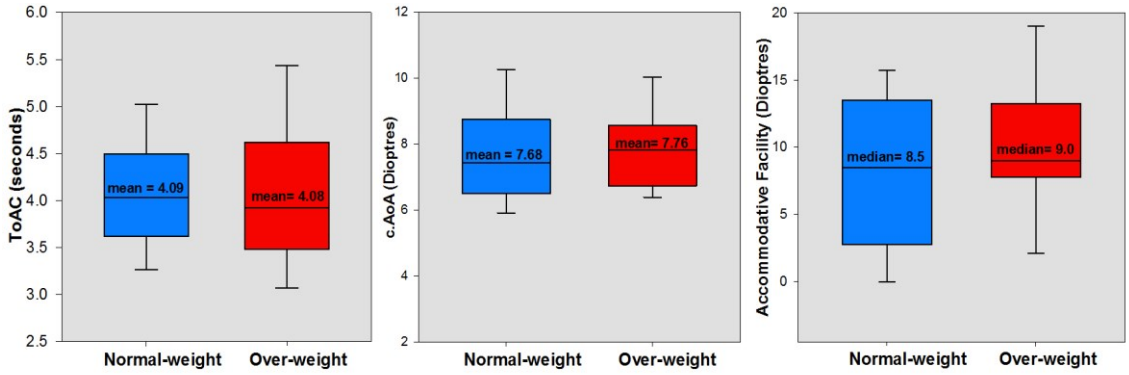

Figure 6.5: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each accommodative parameters for the 'normal-weight' and 'over-weight' groups as defined by BMI

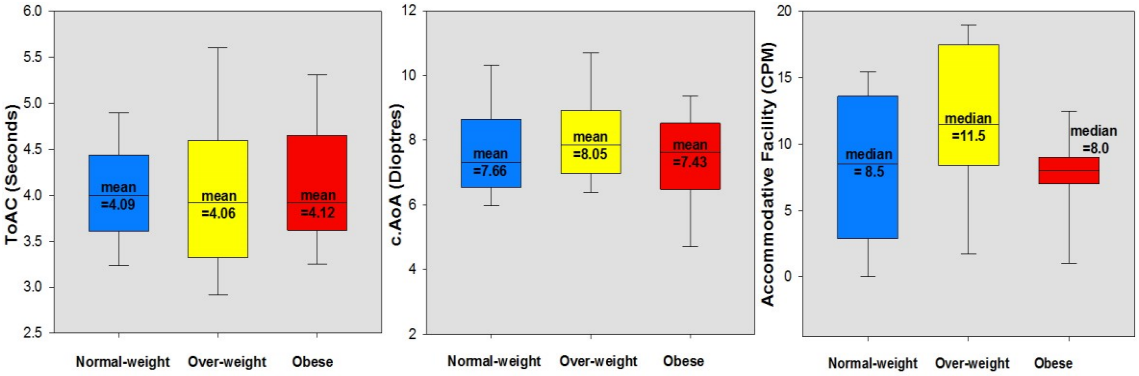

Figure 6.6: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each accommodative parameters for the 'normal-weight', 'over-weight' and 'obese' groups as defined by BMI

Waist-Hips ratio did not correlate with accommodative parameters (C.AoA: r= -0141, p= 0.242, ToAC: r= 0.207, p= 0.088, AF: r_s= -0.034, p= 0.778). There was no significant difference between the C.AoA or AF between the *normal* and *at-risk* waist-hips groups (t= 0.154, p= 0.878; *U*=550.500, Z=-0.363, p= 0.717, respectively). However the ToAC was found to be significantly quicker (t= -1.997, p= 0.05) in subjects with a *normal* waist-hips ratio (mean= 4.00s) than in subjects with an *at-risk* hips-ratio (mean= 4.37s), Figure 6.7.

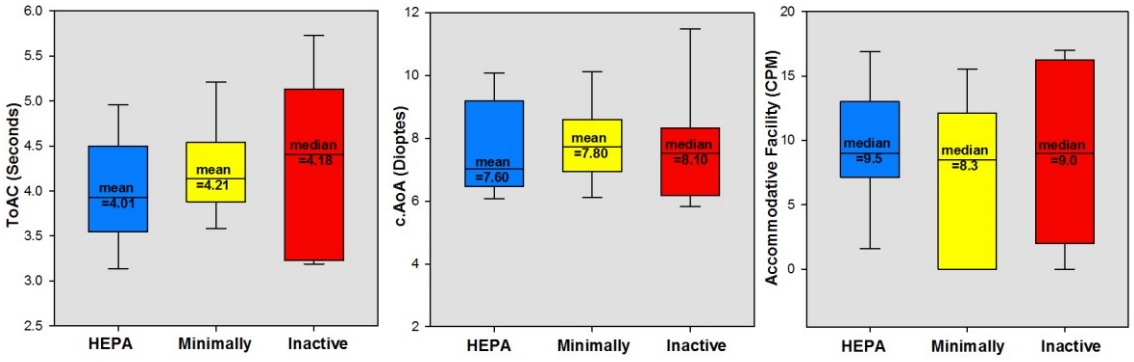

Figure 6.7: Box plots displaying the median, 10th, 25th, 75th and 90th percentiles of data for each accommodative parameters for the 'normal' and 'at-risk' groups as defined by waist-hip ratio

6.3.5 The associations between exercise and accommodative function

There was no correlation between the subjects' MET score and any of the accommodative function measurements (c.AoA: r= 0.71, p= 0.558, ToAC: r= -0.41, p= 0.736, AF: r_s= 0.156, p=0.197). Furthermore, there was no significant difference between the accommodative function measurements and the 'inactive' (n=9), 'minimally-active' (n=20) or 'HEPA-active' (n=41) levels of physical activity (Figure 6.8 ToAC: (F(2)= 0.772, p=0.477, C.AoA: $F_{(2)}$ = 0.301, p= 0.743, AF: $H_{(2)}$ = 0.621, p= 0.312 . Post-hoc pairwise comparison tests using Bonferroni correction, demonstrated that there was no significant difference between each of the groups for all accommodative metrics.

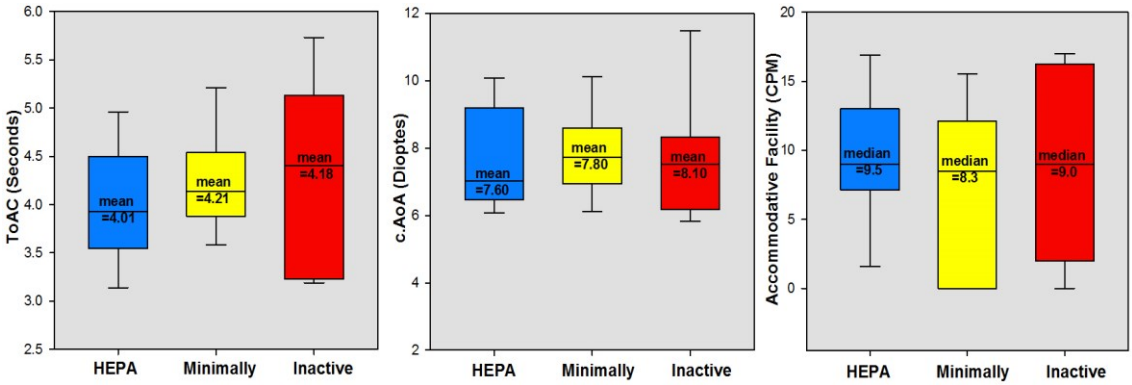

Figure 6.8: Box plots displaying the mean or median, 10th, 25th, 75th and 90th percentiles of data for each of the accommodative parameters for the 'high', 'moderate' and 'low' exercise groups

6.3.6 The effects of lifestyle on accommodative function using the EPIC model

Once subjects were scored according to the EPIC lifestyle model, there was an uneven number of subjects in each group, with a general skew to a higher lifestyle score: (score 1: n=7, score 2: n=21, score 3: n=30, score 4: n=12. Box plots displaying the means or medians of the accommodative parameters across the lifestyle groups are shown in Figure 6.9.

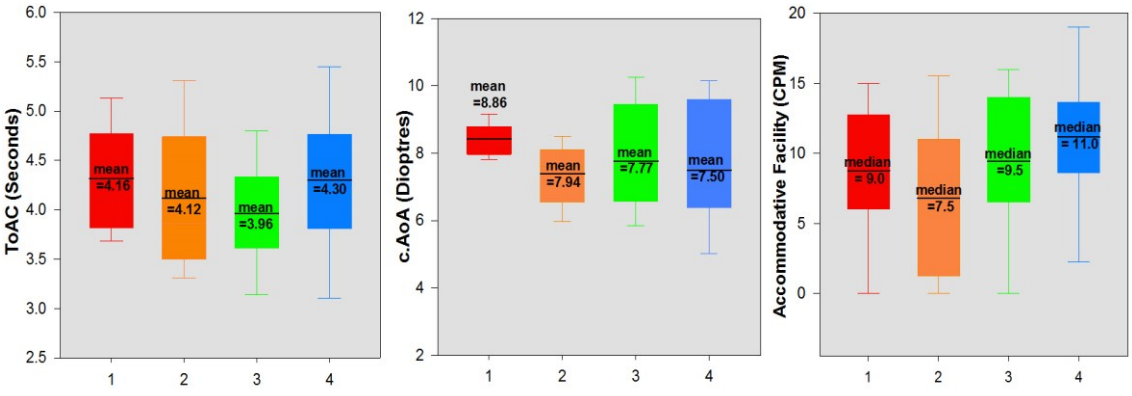

Figure 6.9: Box plots displaying the median, 10th, 25th, 75th and 90th percentiles of data for each of the accommodative parameters, for each of the EPIC lifestyle scoring groups.
Score 1, Score 2, Score 3, Score 4

A One-way ANOVA with EPIC lifestyle model score as the between subjects groups, and post-hoc pairwise comparisons with Bonferroni correction, revealed no significant difference between the lifestyle groups for ToAC ($F_{(3,68)}$ = 0.821, p= 0.487) or c.AoA ($F_{(3,68)}$ =1.557, p= 0.208). A Kruskal-Wallis Test with EPIC lifestyle model score as the between subjects groups, with post-hoc Mann-Whitney U-Tests with Bonferroni correction revealed no significant difference between the lifestyle groups for AF ($H_{(3,68)}$ =6.031, p =0.110).

Single linear regression revealed that the lifestyle score could not predict the accommodative function: ToAC (t= 0.239, p= 0.812), C.AoA (t= -1.002, p= 0.320), and AF (t= 1.484 p= 0.143).

6.3.7 The effects of lifestyle on accommodative function using the CLAS model

Once subjects were scored according to the CLAS model one subject had a score of zero, to allow further statistical analysis this subject was included in the group scoring 1. With the CLAS model there was a general skew of subjects falling into lower lifestyle groupings, again these groupings had uneven sizes: 1: n=12, score 2: n=26, score 3: n=25, score 4: n=6. Box plots displaying the means or medians of the accommodative parameters across the lifestyle groups are shown in Figure 6.10.

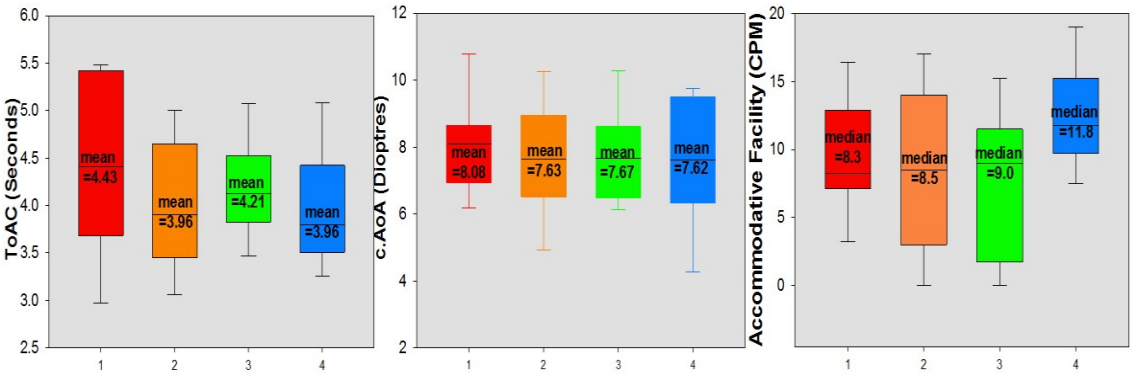

Figure 6.10: Box plots displaying the median, 10th, 25th, 75th and 90th percentiles of data for each of the accommodative parameters, for each of the CLAS lifestyle scoring groups.
Score 1, Score 2, Score 3, Score 4

One-way ANOVAs with CLAS lifestyle model score as the between subjects groups, and post-hoc pairwise comparisons with Bonferroni correction, revealed no significant difference between the lifestyle groups for ToAC ($F_{(3,69)}$ = 1.269, p= 1.269) or ($F_{(3,69)}$ = 0.238, p= 0.869). A Kruskal-Wallis Test with the CLAS lifestyle model score as the between subjects groups, and post-hoc Mann-Whitney U-Tests with Bonferroni correction revealed no significant difference between the lifestyle groups for AF ($H_{(3,69)}$ =2.387, p=0.496).

Single linear regression revealed that the lifestyle score could not predict the accommodative function: ToAC (t= 1.801, p=0.076), C.AoA (t= -0.587, p=0.559), and AF (t= 0.341, p=0.734).

6.3.8 Post-hoc Power calculations

Post-hoc power calculation was conducted using *G*Power;* using the c.AoA as the between-group comparison in the CLAS lifestyle model, with an α of 0.05 and the study population size (n=70) a power of 0.09 was achieved. In order to achieve a statistical power of 0.80 a study population of 1052 would be required (Faul et al., 2007, Faul et al., 2009).

6.4 Discussion

The primary aim of this cross-sectional study was to investigate whether the 'EPIC' lifestyle score or 'CLAS' lifestyle score, was associated with accommodative function, mainly AoA, AF, and ToAC. In doing so the associations between smoking status, diet and alcohol, body shape, and physical activity levels, and accommodative function, were examined. Current smokers or subjects with greater central adiposity were found to take longer to complete an accommodative change, furthermore, moderate drinkers were found to achieve more CPM during AF.

6.4.1 Smoking status

Smoking is known to increase the overall level of ocular inflammation, which increases the levels of oxidative stress and reduces the levels of endogenous anti-oxidants, in the crystalline lens (Galor and Lee, 2011). This has been associated with an overall

increased risk of cataracts in smokers, (Cumming and Mitchell, 1997, Klein et al., 1999, Klein et al., 2003, Raju et al., 2006, Xu et al., 2006, Navarro Esteban et al., 2007, Tan et al., 2008c, Wu et al., 2010, Ye et al., 2012), and at a younger age compared to non-smokers (Tan et al., 2008c, Wu et al., 2010). Furthermore, a dose-response curve has been demonstrated; with risk of cataract formation increasing alongside the packs of cigarettes smoked, and years smoked (Klein et al., 1999, Klein et al., 2003, Wu et al., 2010) (Klein et al., 2003). In this study, current smokers took longer to complete an accommodative change cycle than past-smokers or non-smokers. This association could suggest that the increased levels of oxidative stress, in *current smokers* could affect how quickly the accommodative response occurs. The similarity between the mean ToAC in the *past-smokers* and *never-smoked* groups would suggest that after smoking cessation, there is some recovery of the oxidative stress levels and anti-oxidant defence mechanism. This would be in agreement with other studies, which have found a reduced risk of cataract extraction dependant on when smoking is ceased (Kang et al., 2016). Therefore, by including *current-smokers* and *past-smokers* together in the *ever-smoked* group, any significant difference between the *never-smoked* and *ever-smoked* groups could have been masked, which could be why smoking status was not found to affect ToAC in the EPIC lifestyle model. There was no significant difference in c.AoA or AF between any of the groups describing smoking status, this could have been due to the relative small number of current-smokers recruited in this study. To further explore the effects of smoking status on accommodative ability, future work is required and should include a greater number of *current smokers* and *past-smokers*, and examine the

number of cigarettes smoked, and the years passed since smoking cessation in the 'past-smokers' group. There were a much lower proportion of *current-smokers* than *non-smokers* and *past-smokers* included in this study, smokers consisted of 18.3% of the sample population, which is representative of the proportion of smokers in the UK (approximately 17.2%) (ONS, 2017b).

This study did not capture data on e-cigarette use.

6.4.2 Dietary intake

Despite both vitamin C and vitamin E constituting key components of the anti-oxidant defence mechanism in the crystalline lens, and numerous studies associating a higher level of dietary anti-oxidant intake with reduced cataract formation (Jacques, 1999, Cumming et al., 2000, Jacques et al., 2001, Kuzniarz et al., 2001, Dherani et al., 2008, Tan et al., 2008a, Kang et al., 2016), this study found no association between anti-oxidant intake and accommodative function. This could have been due to the relatively low proportion of subjects in this study consuming fewer than five portions of fruit and vegetables per day. The Family Food Report, which reports the food purchased each year within the UK, stated that in 2015 an average of 3.9 portions of fruit and vegetables per person, per day were bought (DEFRA, 2017). The average daily portions of fruit and vegetables reported to be consumed in this study was 7.3, and therefore the dietary habits of the study population may not best represent the dietary habits of the target

population. Also it is generally known that subjects tend to over-report fruit and vegetable intake on Food Frequency Questionnaires (Bingham et al., 2001, Brunner et al., 2001). The specific FFQ used in this study has been validated to accurately reflect plasma vitamin C concentrations (Bingham et al., 2001), this is despite vitamin C being very labile; its content can be affected by season, shelf life, storage time, and cooking practices. However, the FFQ asked about dietary intake over the previous 12 months, which may not be representative of the dietary intake over a life-time, other limitations that could have affected these results include errors in memory and natural seasonal variations in eating habits.

Interventional studies have shown that nutritional supplements have little impact on reducing the risk of cataracts in subjects with a nutritionally adequate diet (AREDS I, 2001, Christen et al., 2003, Christen et al., 2004, Christen et al., 2008, Christen et al., 2010, Chew et al., 2013). This suggests that there could be a ceiling threshold of the antioxidant defence mechanism in the crystalline lens, above which increasing the dietary intake of antioxidants has no further effect on reducing oxidative stress. There may have been too few subjects in this study with an anti-oxidant intake below this threshold to have an adverse effect on the crystalline lens, and the accommodative mechanism. To further investigate if there is an association between anti-oxidant intake and accommodative function, a larger study sample would be necessary, including more subjects with a lower anti-oxidant intake. Furthermore, investigation is needed to establish whether the reported intake of fruit and vegetable portions or blood plasma

levels of vitamin C best represent the anti-oxidant defence mechanisms within the crystalline lens.

6.4.3 Alcohol

Moderate drinkers were found on average to achieve more CPM during AF. This result could support the findings of the longitudinal portion of the Blue Mountains Eye Study, which found that both heavy drinkers (> 2 standard drinks per day) and non-drinkers had a significantly increased likelihood of cataract extraction surgery, in comparison to moderate drinkers (1-2 standard drinks per day) (Kanthan et al., 2010). These findings would further support the proposed 'U' or 'J' shaped dose-response curve between alcohol intake and age-changes in the crystalline lens, which has been further evidence in a recent meta-analysis of alcohol intake and risk of cataracts by Gong et al. (2015). This J-shaped curve has been observed for alcohol intake and age-related cardiovascular disease, suggesting that moderate alcohol intake has a cardioprotective effect (Rehm et al., 2010). Although, non-drinkers, those that have stopped drinking and occasional periods of heavy drinking are known to skew the data on this relationship (Roerecke and Rehm, 2014). The similarity in the relationships between cataract development, and cardiovascular disease with alcohol intake could be due to the two disorders sharing many risk factors including: smoking, obesity, diabetes, hyperlipidaemia and hypertension (Nemet et al., 2010). Therefore, some caution must be taken as to whether the J-shaped curve association between alcohol intake and age-related changes

in the crystalline lens has any clinical significance; more work to understand the underlying physiological mechanisms of this relationship is needed.

Guides were provided whilst subjects completed the questionnaires, as to how many units each common measure of alcohol contains, however, subjects are known to under-report the number of units of alcohol consumed. This could have skewed the data in this study, so that the association of heavy drinking on AF could be greater than was reported.

6.4.4 Body shape

Subjects with a waist-hip ratio classified as 'at-risk', took longer on average to change their focus than subjects with a 'normal' waist-hip ratio, however this finding was not apparent when comparing subjects as classified by their BMI as being over-weight or a 'normal' weight. This could have been due to the limitations of using BMI measurements alone to identify obesity; BMI does not discriminate between muscle and fat tissue, and therefore very different body compositions can have similar BMIs, increasing the risk of an alpha error (Kok et al., 2004). Conversely, waist-hip ratios include an assessment of visceral fat and has been confirmed as a predictor of chronic disease (Balkau et al., 2006, WHO, 2008, Kwakernaak et al., 2013, Kim et al., 2016).

Numerous studies have found an association between lens changes leading to cataract formation and obesity (Glynn et al., 1995, Schaumberg et al., 2000, Paunksnis et al., 2007,

Lim et al., 2009, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013). This association has been found by quantifying both BMI, and central adiposity (Glynn et al., 1995, Schaumberg et al., 2000, Paunksnis et al., 2007, Lindblad et al., 2008). The exact mechanism behind the relationship between obesity and cataracts is unknown, however the increase in oxidative stress levels in obesity is well-documented (Agarwal et al., 2016), and therefore could increase the levels of ocular oxidative stress in the crystalline lens. 35.2% of the study sample population were classified as either over-weight or obese, which is lower than the reported UK average of 64.8% (Public Health England, 2017), again suggesting the study population did not best represent the target population.

6.4.5 Physical Activity

Exercise offers numerous benefits for health, and protection against many chronic age-related diseases (Martinson et al., 2001, Hurley and Reuter, 2011, Booth et al., 2012, Kokkinos, 2012). There is little known about the long-term effects of exercise on ocular diseases and physiology (Gale et al., 2009). Short term effects of exercise have been associated with increases in ocular perfusion pressure (Gale et al., 2009, Risner et al., 2009, Yip et al., 2011, Schmidl et al., 2012), and both an increase and decrease in intraocular pressure dependant on the type of exercise performed (Gale et al., 2009, Risner et al., 2009). This study found no association between exercise levels and accommodative function. The results in this study could have been affected by the relatively low portion of subjects categorised as physically inactive (12%), this is in

contrast to findings from a nationwide survey by Sport England (2016), which found the portion of inactive adults to be closer to 29% (with a similar, but not identical criteria for classifying active and inactive). It is known that subjects tend to over-report their physical activity levels, which would have further compounded this finding (Sallis, 2010). Furthermore, the IPAQ asks subjects about physical activity undertaken in the last seven days only and it is not particularly sensitive to change in physical activity levels (Bauman et al., 2009). Therefore the MET-minute scores and activity level found in this study may not have been representative of a subject's physical activity level over a longer period of time, or accurately reflect their levels of physical activities over their lifetime.

6.4.6 Lifestyle Models and accommodative function

Despite some of the individual lifestyle factors showing associations with ToAC and AF, once lifestyle was classified by either the EPIC or novel CLAS model, no association with accommodative function was found. There are numerous possible reasons for this, firstly it can be seen that there were relatively fewer subjects in the lower and upper groups with each lifestyle model due to recruitment constraints. This would have increased the chance of a Type II error in all mean comparisons tests. As previously discussed the lifestyle demographics of the study population did not always represent the lifestyle demographics of the target population (of 30 to 40 year olds within the UK). The study population had a greater proportion of subjects with a healthy weight, did more physically activity and consumed more portions of fruit and vegetables, than the

target population. This could have been due to the fact that in this study 86% of participants were either graduates or currently undergraduates. This would further suggest that the study population did not best represent the target population (ONS, 2013). Socio-economic status has also been found to be a risk factor for cataracts both in isolation and in conjunction with other modifiable risk factors (Cackett et al., 2008). Generally it has been found that a lower level of education (Mohan et al., 1989, Italian-American Cataract Study Group, 1991), a lower income, or a lower-entry job level are associated with a higher risk of cataracts (Reidy et al., 1998, Foster et al., 2003, Klein et al., 2003, Krishnaiah et al., 2005, Athanasiov et al., 2008). Overall, this study population and may have a lower risk of premature lens ageing, than the target population. This would limit how applicable the results of this study are. As demonstrated by the power stats calculations, a greater number of subjects and more even number of subjects across all of the groups should be included in further research.

Secondly, both of the models have given equal importance to each of the lifestyle factors. Within this study population, no association was found between accommodative function and fruit and vegetable intake, nor physical activity levels, in isolation, and therefore in combination with the other lifestyle factors, could have masked the effects of waist-hip ratio and smoking status on accommodative function. It is quite possible that one of the lifestyle factors would have a greater effect on age-related changes in the crystalline lens than some of the other factors. This has possibly been demonstrated in this study, as both smoking status and waist-hip ratio were both associated with

slower accommodative times. As well as in previous literature which has confirmed that a positive current smoking status and obesity is likely to increase oxidative stress in the crystalline lens, leading to age-related changes such as cataract formation (Glynn et al., 1995, Schaumberg et al., 2000, Klein et al., 2003, Xu et al., 2006, Paunksnis et al., 2007, Tan et al., 2008c, Lim et al., 2009, Wu et al., 2010, Sabanayagam et al., 2011, Ghaem Maralani et al., 2013, Ye et al., 2012). However, there is no evidence of an association between physical activity and cataract formation.

There are numerous interactions between lifestyle behaviours; for instance, smokers are generally known to have a poorer diet, drink more alcohol, do less physical activity and be overweight (Chiolero et al., 2008). Therefore, examining these factors in isolation could give misleading associations as has been previously discussed in the example of alcohol demonstrating a J-shaped dose-response curve with cataracts and cardio-vascular disease (Nemet et al., 2010).

Thirdly, lifestyle is dynamic: dietary habits, smoking status, alcohol consumption, body shape and levels of physical activity are likely to change over time (Hurley and Reuter, 2011, ONS, 2017b, ONS, 2017a). The FFQ used in this study asks subjects to consider their intake over the last year, and the IPAQ questionnaire asks subjects to consider their physical activity in the previous seven days. Questions about alcohol intake asked for estimations of current alcohol intake and information about smoking included current smoking status, and if a past smoker, when they last smoked. Therefore, it is unlikely that this study would not have been sensitive to changes in lifestyle. The methodological

constraints of this cross-sectional study has limited the information available to account for these changes. Ideally, a longitudinal study with long–term observations, utilising more objective measures instead of self-reporting, would provide more robust data.

Finally, these models considered only five single lifestyle factors; there are numerous lifestyle factors which could have an effect on the ageing processes of the crystalline lens including UV exposure (Robman and Taylor, 2005, Roberts, 2011, Na et al., 2014), which have not be considered. Furthermore, numerous non-modifiable lifestyle factors such as gender, ethnicity, and socio-economic status have been associated with the rate of presbyopia and cataract development. As discussed in section 1.7.4, the literature examining the relationships between these non-modifiable lifestyle factors and accommodative function have often had conflicting findings and these could be due to different methodologies used to examine the relationships. For example, Hunter and Shipp (1997) discussed how previous studies found that socio-economic status, such as level of education and income, inversely correlates with onset of presbyopia; however, their study did not confirm this link when comparing reading additions prescribed to patients to the average incomes of their zip codes. However, these conflicting findings could also suggest that when one factor is being examined in isolation, other modifiable and non-modifiable lifestyle factors need to be considered.

6.4.7 Conclusion

To our knowledge, this is the first study to examine the association between accommodative function and lifestyle. The key findings of this study are:

- Smokers and subjects who had an *at-risk* waist-hip ratios took more time for accommodative changes.
- No differences in the accommodative function were found between subjects classified as having a 'healthy' or 'un-healthy' lifestyle.
- The size and lifestyle demographics of the study population, limits the applicability of these results to a wider population.
- More work is need in this area to validate these findings, and then to explore possible mechanisms.

Chapter 7: Conclusions

7.1 Summary

A detailed understanding of the structure and physiology of the accommodative apparatus, and how these are affected by age and lifestyle, are vital for the development of a presbyopic treatment. The different parameters of the accommodative function can be assessed using various subjective and objective methods. However, there is much variation in these methods leading to ambiguity, which hinders the comparisons of study findings and the application of 'normative' values. Furthermore, standardisation between the tests would allow the relationships between the objective and subjective tests to be effectively examined. Therefore, the overarching aim of this thesis was to scrutinise the methods used to assess accommodative function and to evaluate the effect of lifestyle on accommodation.

7.2 Chapter 2: Validation of novel metrics from the accommodative dynamic profile

The dynamic profile of accommodation is often studied in a research setting to quantify parameters of accommodation by deriving different accommodative metrics. Numerous metrics have been utilised to describe the active response of the accommodative change, and yet there is often a lack of standardisation in how these

are derived, and little understanding of how these metrics relate to the subjective measurement of accommodative facility. Therefore, this study aimed to compare novel metrics quantifying how quickly the accommodative change occurred, to metrics currently used, and relate these to the accommodative facility test. In doing so, an improved understanding of how to standardise these metrics was obtained; the novel method of calculating latency of accommodation was more repeatable than methods used in previous studies, although this new latency metric did not prove to be interchangeable with the previous metric. The new metrics of time for accommodation, time for disaccommodation, and time for accommodative change demonstrated good repeatability and the strongest correlations with accommodative facility, and age. Therefore, when describing how quickly the accommodative change occurs from the accommodative dynamic profile, time metrics may provide more clinically relevant information relating to accommodative facility in comparison to accommodative latency metrics.

7.3 Chapter 3: Validation of a Patient-controlled Accommodative Facility Instrument.

There are some inherent limitations in the traditional method used to assess AF, which can affect the accuracy and repeatability of the results. These limitations include the speed at which the practitioner and patient can recognise and respond to a completed

accommodative change, and the application of the commonly quoted normative values. Numerous attempts to quantify the normative values for AF have been attempted, however the methodology used to investigate the effect of monitoring binocular fusion during the AF is inconsistent, therefore conclusions are limited. The study described in chapter 3 was conducted in two parts; the first aimed at validating a novel instrument to minimise the error caused by reaction times, the second part was aimed at investigating the effect of using a stereogram target during the AF test.

The Patient-controlled Accommodative Facility (PcAF) instrument was developed and validated, with a modified technique for assessing AF. The instrument allowed the patient to initiate the lens flip, removing the practitioner reaction time and, improving the accuracy of AF measurements. The instrument also automatically logged the time interval for each lens flip separately, allowing measurements of the subjective time for accommodation, disaccommmodation, and the accommodative change. Therefore improving the comparisons between the objective accommodative time metrics derived from the accommodative dynamic profile and AF. The PcAF instrument was found to provide a valid and repeatable assessment of AF, and provides the opportunity to further investigate the effect of in-test fatigue on time for accommodation and time for disaccommodation.

The PcAF and traditional AF test were then used to perform binocular AF tests with both a stereogram target, and a 'flat' target which did not monitor suppression. Using a stereogram target reduced the cycles per minute achieved during AF testing in young adults with normal binocular function, however further work is required to understand

the clinical relevance of this finding. Further studies are also required to examine the effects of monitoring suppression during AF in adults between the ages of 30 and 40 years, and in individuals with binocular abnormalities.

7.4 Chapter 4: Optimising the calculation methods for analysing depth of focus from defocus curves

Defocus curves are commonly used to derive information about depth-of-focus, and accommodation in phakic eyes. They are also used to assess the effectiveness of multifocal and extended depth-of-focus IOL designs, in restoring accommodative function. Yet further standardisation between the methods used to construct defocus curves and derive the metrics is required to enable closer comparisons between studies. The aim of chapter 4 was to compare the validity of four defocus curve metrics in a phakic and pseudophakic population. These metrics were compared with the amplitude of accommodation and the time for accommodative change in the phakic population, and with the Radner Critical Print Size in the pseudophakic population.

The range-of-focus metric with an absolute cut-off of +0.30logMAR was found to be the most valid and repeatable metric, when compared to an area-of-focus metric, and a relative cut-off criteria of best corrected visual acuity +0.04logMAR. This would suggest that future studies analysing the effectiveness of an accommodative IOL or extended

depth of focus IOL using defocus curves, should assess depth-of-focus by calculating the range-of-focus at a cut-off of +0.30 logMAR.

7.5 Chapter 5: Investigating the in vivo ciliary muscle shape change during accommodation

Before the introduction of AS-OCTs, studying the human ciliary muscle *in vivo* was limited by the position of the iris. With the introduction of the high-resolution and non-invasive instrument AS-OCT, researchers were presented with the opportunity to vastly expand the depth of knowledge of the structure and physiology of the human ciliary muscle *in vivo*. Subsequent research has focused on the changes in structure and function of the ciliary muscle during accommodation, with age, and its possible role in myopigensis. This research has been conducted using time-domain AS-OCT. The more recent development of swept source AS-OCT allows for faster scan times and superior image resolution. The swept source ASOCT TOMEY CASIA 1000 also has novel software features such as the real-time viewing of the conjunctival area over-which the scan is acquired, and tools to analyse portioned cross-sectional area of the ciliary muscle. Therefore, the purpose the study described in chapter 5 was to examine the use of the Tomey CASIA and its unique features in analysing changes in the ciliary muscle with accommodation.

Utilising the Tomey CASIA software a reference point was identified, along-which a specific location of the ciliary muscle was used for cross section scan analysis. This approach improved the repeatability of ciliary muscle measurements, and allowed for more accurate assessment of ciliary muscle morphology changes. It was concluded that the use of a physiological reference point on a patients conjunctiva, should always be employed in future studies examining repeated scans of the ciliary muscle, where possible.

Traditionally thickness measurements across the ciliary muscle have been used to assess the change in shape of the ciliary muscle during accommodation. The study explored the use of the Tomey CASIA's cross-sectional area measurement tool and examined the repeatability and validity of such a measure. The repeatability of the portioned cross-sectional area metric was found to be lower in comparison to the ciliary muscle thickness metric. Furthermore, thickness measurements demonstrated that the anterior portions of the ciliary muscle thickened with accommodation whilst the posterior thickness reduced. However, the area metric only identified a change of the anterior portion of the ciliary muscle. It was concluded that the area metrics were less reliable than thickness measurements in describing muscle morphology.

7.6 Crystalline lens ageing study (CLAS): does smoking status and other lifestyle factors affect accommodation?

The rate of biological ageing in humans is known to be influenced by many physiological and environmental factors. Age-related changes in the crystalline lens include the loss of accommodative ability and the loss of transparency. Truscott and Zhu (2010) have described how the underlying biological ageing mechanisms leading to the loss of accommodative function and reduced lens transparency, are similar. Therefore, it is possible that the development of both conditions could be influenced by the same physiological and environmental factors. Much of the current literature has concentrated on identifying lifestyle factors that can increase the risk of cataract development (Athanasiov et al., 2008, Lim et al., 2009, Lindblad et al., 2008, Robman and Taylor, 2005, Nita and Grzybowski, 2017). As yet, no studies have examined whether the lifestyle factors that have been identified as possible risk factors for cataracts, also affect the rate of development of presbyopia. This study explored the association between smoking status, body shape, anti-oxidant intake, alcohol consumption, and physical activity on the accommodative parameters of amplitude of accommodation, accommodative facility and time for accommodative change, in healthy adults between the ages of 30 and 40 years. Although being a current smoker and having greater central adiposity was associated with a slower time for

accommodative change, once lifestyle models were used, no association between lifestyle and accommodative function was found. However, application of this data may be restricted due to limitations of the study population including a non-representative study population. Further research is needed in this area on a larger study population to validate these findings.

7.7 Limitations and additional considerations of current work

There were a few known considerations or potential sources of error through-out these studies that were not controlled for; these include an uneven number of females and males, and refractive error groups in the study populations. Other potential sources of error could have arisen from the fatigue effects of repeated measures of accommodation, and reduced subject co-operation.

7.7.1 Effect of gender

As discussed in section 1.7.4 studies that have investigated if there is a physiological difference in the accommodative function of males and females have had inconsistent findings. (Kragha, 1986, Koretz et al., 1989, Millodot and Millodot, 1989, Pointer, 1995, Hunter and Shipp, 1997, Carnevali and Southaphanh, 2005, Burke et al., 2006, Nirmalan et al., 2006, Patel et al., 2006). In a review of this literature Hickenbotham et al. (2012)

concluded that there were no physiological difference in accommodative abilities between the genders, and that the contrasting findings of these studies could be explained by other physiological or environmental factors.

7.7.2 Inclusion of a range of refractive error groups

In all of the study populations there was generally a higher number of emmetropes and myopes, than hyperopes. Myopes generally have increased accommodative lags, compared to emmetropes and hyperopes (Gwiazda et al., 2005, Mutti et al., 2006, Millodot, 2015). However, lag was not utilised in any of the studies to describe the accommodative function. Studies which have investigated the relationship of refractive error and AoA have had varied results; McBrien and Millodot (1986) reported higher AoA in myopes, however, other studies reported lower AoA in myopes (Fong, 1997, Allen and O'Leary, 2006), whereas some studies have found no significant differences (Fisher et al., 1987, Anderson et al., 2008). Studies which have investigated the effect of refractive error on near AF, found no significant differences between refractive error groups (O'Leary and Allen, 2001, Allen and O'Leary, 2006, Pandian et al., 2006, Radhakrishnan et al., 2007). Therefore, the range of refractive errors used in these studies should not have had a large impact upon the results.

7.7.3 Fatigue affects and patient co-operation

For accurate assessment of accommodation, subjects must be co-operative through-out. Repeated measures of accommodation can cause fatigue effects, or loss of

concentration by subjects leading to under-estimations of the true accommodative function. To minimise the effects of this in all of the studies, rest breaks were given between tests, and the order of different accommodative tests were randomised.

7.7.4 Chapter 6: Crystalline lens ageing study (CLAS): does smoking status and other lifestyle factors affect accommodation?

The main limitations of the CLAS study limit the application of the findings to the target population are discussed in section 6.4.6. During analysis, it became apparent that the target population reported generally healthier lifestyles (i.e. consuming more portions of fruit and vegetables, and being more physical activity) than the target population. The methodology employed in this study would not have accounted for any previous changes in the lifestyle factors assessed, which could have affected the associations found with accommodative function. A longitudinal follow-up to this study would be useful to identify the potential impact that changes in lifestyle would have had.

The main limitation of this study was the reduced power (0.09) due to the small sample size (70). As stated in section 6.3.8 to obtain a power of 0.80 when using an ANOVA to compare the difference in c.AoA between the CLAS lifestyle groups, a sample size of n= 1052 was required, which would have been difficult to achieve in the time constraints of this study.

Due to the relatively small sample size, comparisons between groups with uneven numbers were carried out. This would have further affected the reliability of the results found. Due to the nature of study recruitment and drop-out this was difficult to control for. Ideally, this study would be repeated with a larger study population to confirm the findings.

7.8 Clinical implications and future work

7.8.1 Chapter 2: Validation of novel metrics from the accommodative dynamic profile

The novel metrics of time for accommodation investigated in this study were valid descriptors of the accommodative function, which more closely related to accommodative facility than the traditional metric of accommodative latency. Furthermore, the method proposed to define and derive accommodative latency proved to be more repeatable than the method used in previous studies, due to utilising curve fitting as opposed to visual inspection of the data. Therefore, the novel accommodative time metrics and latency metrics proposed in this study could be used in future research where descriptors of the speed of the accommodative change are desired.

Future work could involve deriving the peak velocity and time constants via curve fitting, and investigating their relationship to accommodative facility.

7.8.2 Chapter 3: Validation of a Patient-controlled Accommodative Facility Instrument

With minor improvements to the novel PcAF instrument, future work would begin by investigating the change in subjective time for accommodation and disaccommodation with age. Another study to investigate the effect of in-test fatigue on accommodative times would be clinically useful to increase the depth of understanding on the implications of the method of accommodative facility, commonly used in clinical practice.

Results from this study suggest that further exploration with a more systematic approach in quantifying groups of symptomatic and non-symptomatic accommodative dysfunction, and normal and 'suspect' binocular function, is needed to understand the effects of using a stereogram during binocular AF. It would also be useful to examine the effects of using a stereogram during binocular AF across different ages.

7.8.3 Chapter 4: Optimising the calculation methods for analysing depth of focus from defocus curves

The findings from this study suggest that the dioptric range over which a subject can see +0.30LogMAR should be utilised to describe the depth-of-focus from a defocus curve, when analysing the success of an accommodative IOL or extended depth-of-focus IOL in restoring accommodation.

7.8.4 Chapter 5: Investigating the in vivo ciliary muscle shape change during accommodation

Results from this study demonstrate that during investigations with repeated scans of the ciliary muscle, the use of a reference point on the conjunctiva to align with, to select the scan for analysis improves repeatability, and therefore should be used (where possible) in future studies.

The findings from this study also suggested that portioned cross-sectional area was a less repeatable metric that muscle thickness when investigating the shape change of the muscle, during accommodation.

The Tomey CASIA 1000 AS-OCT has a video function that could be utilised in future studies to analyse the change in shape of the ciliary muscle at different time intervals after the introduction of an accommodative target.

7.8.5 Chapter 6: Crystalline lens ageing study (CLAS): does smoking status and other lifestyle factors affect accommodation?

To our knowledge, this is the first study to examine the association between accommodative function and lifestyle. Although some associations were found between accommodative function and smoking status, and central adiposity, further work is required before applying these results to a wider population.

A cross-sectional study with a larger population size would be required. Some amendments to the methodology of how the lifestyle factors were assessed would improve the validity of the data collected. For instance, assessing plasma levels of vitamin C and vitamin E, and developing and validating a new physical activity questionnaire, which captures data for a time-period greater than 7-days. Ideally proceeding this, a longitudinal study would be conducted to confirm any findings, and identify the effect of any changes in lifestyle on accommodative function.

Accumulation of AGEs as a by-product of cross-linking of proteins, affects both skin and crystalline lens auto-fluorescence. AGE readers which measure skin auto-fluorescence and confocal biomicroscopes measuring lens auto-fluorescence have been validated to quantify the risk of cardiovascular disease, diabetes, and mortality (de Vos et al., 2014, Fokkens and Smit, 2016, Stirban, 2014, Burd et al., 2012). Future work could assess whether AGE measures of the crystalline lens or skin are associated with accommodative function or predict cataract development.

Appendices

A1 The Matlab code used to derive metrics from the accommodative dynamic profile

```
c% curvefitting_v1

clear all
filename = input ('Please input file name :   ', 's');
rawdata=xlsread(filename)

% for the first time taken to full accommdoative
 x = rawdata(:,1);
 y = rawdata(:,2);
[param]=sigm_fit(x,y)
[a] = param(1,1)
[b] = param(1,2)
[c] = param(1,3)
[d] = param(1,4)
 if param(1,2)>param(1,1)
    [y91] = param(1,2)*.99
else
     [y91] = param(1,1)*.99
end
[x91] = (-(log10(((b-a)/(y91-a))-1))/d)+c
[starttime] = x(1,1)
[measurementnumbers] = x91 - x(1,1)
[time] = measurementnumbers * 0.125

% for first accommodative latency
[y01] = (param(1,2)-param(1,1))/100 + param(1,1)
if param(1,2)>param(1,1)
    [y01] = (param(1,2)-param(1,1))/100 + param(1,1)
else
    [y01] = (param(1,1)-param(1,2))/100 + param(1,2)
end
 [x01] = (-(log10(((b-a)/(y01-a))-1))/d)+c
[measurementnumbers01] = x01 - x(1,1)
[lag] = measurementnumbers01 * 0.125


% for the second time taken to full accommdoative
 x2 = rawdata(:,5);
 y2 = rawdata(:,6);
```

```
[param2]=sigm_fit(x2,y2)
[a2] = param2(1,1)
[b2] = param2(1,2)
[c2] = param2(1,3)
[d2] = param2(1,4)
 if param2(1,2)>param2(1,1)
    [y92] = param2(1,2)*.99
else
    [y92] = param2(1,1)*.99
end
 [x92] = (-(log10(((b2-a2)/(y92-a2))-1))/d2)+c2
 [starttime2] = x2(1,1)
 [measurementnumbers2] = x92 - x2(1,1)

[time2] = measurementnumbers2 * 0.125

 % for second accommodative lag
 [y02] = (param2(1,2)-param2(1,1))/100 + param2(1,1)
 if param2(1,2)>param2(1,1)
    [y02] = (param2(1,2)-param2(1,1))/100 + param2(1,1)
else
    [y02] = (param2(1,1)-param2(1,2))/100 + param2(1,2)
end

[x02] = (-(log10(((b2-a2)/(y02-a2))-1))/d2)+c2
[measurementnumbers02] = x02 - x2(1,1)
[lag2] = measurementnumbers02 * 0.125

% for the third time taken to full accommdoative
 x3 = rawdata(:,9);
 y3 = rawdata(:,10);
 [param3]=sigm_fit(x3,y3)
 [a3] = param3(1,1)
 [b3] = param3(1,2)
 [c3] = param3(1,3)
 [d3] = param3(1,4)
 if param3(1,2)>param3(1,1)
    [y93] = param3(1,2)*.99
else
    [y93] = param3(1,1)*.99
end
 [x93] = (-(log10(((b3-a3)/(y93-a3))-1))/d3)+c3
 [starttime3] = x3(1,1)
 [measurementnumbers3] = x93 - x3(1,1)
 [time3] = measurementnumbers3 * 0.125

% for third accommodative lag
 [y03] = (param3(1,2)-param3(1,1))/100 + param3(1,1)
 if param3(1,2)>param3(1,1)
    [y03] = (param3(1,2)-param3(1,1))/100 + param3(1,1)
else
    [y03] = (param3(1,1)-param3(1,2))/100 + param3(1,2)
end
 [x03] = (-(log10(((b3-a3)/(y03-a3))-1))/d3)+c3
```

```
[measurementnumbers03] = x03 - x3(1,1)
[lag3] = measurementnumbers03 * 0.125


% for the fourth time taken to full accommdoative
 x4 = rawdata(:,13);
 y4 = rawdata(:,14);
 [param4]=sigm_fit(x4,y4)
 [a4] = param4(1,1)
 [b4] = param4(1,2)
 [c4] = param4(1,3)
 [d4] = param4(1,4)

if param4(1,2)>param4(1,1)
    [y94] = param4(1,2)*.99
else
    [y94] = param4(1,1)*.99
end
 [x94] = (-(log10(((b4-a4)/(y94-a4))-1))/d4)+c4

[starttime4] = x4(1,1)
[measurementnumbers4] = x94 - x4(1,1)
[time4] = measurementnumbers4 * 0.125

% for fourth accommodative lag
 [y04] = (param4(1,2)-param4(1,1))/100 + param4(1,1)
 if param4(1,2)>param4(1,1)
    [y04] = (param4(1,2)-param4(1,1))/100 + param4(1,1)
else
    [y04] = (param4(1,1)-param4(1,2))/100 + param4(1,2)
end
 [x04] = (-(log10(((b4-a4)/(y04-a4))-1))/d4)+c4
 [measurementnumbers04] = x04 - x4(1,1)
[lag4] = measurementnumbers04 * 0.125

 %for time taken to full disaccommodation
 x5 = rawdata(:,3);
 y5 = rawdata(:,4);
 [param5]=sigm_fit(x5,y5)
 [a5] = param5(1,1)
 [b5] = param5(1,2)
 [c5] = param5(1,3)
 [d5] = param5(1,4)
 if param5(1,2)>param5(1,1)
    [y95] = (param5(1,2)-param5(1,1))/100 + param5(1,1)
else
    [y95] = (param5(1,1)-param5(1,2))/100 + param5(1,2)
end
 [x95] = (-(log10(((b5-a5)/(y95-a5))-1))/d5)+c5
 [starttime5] = x5(1,1)
 [measurementnumbers5] = x95 - x5(1,1)
 [disaccommdation] = measurementnumbers5 * 0.125

%for disaccommodative lag
```

```
 x6 = rawdata(:,3);
 y6 = rawdata(:,4);
 [param6]=sigm_fit(x6,y6)
 [a6] = param6(1,1)
 [b6] = param6(1,2)
 [c6] = param6(1,3)
 [d6] = param6(1,4)

if param6(1,2)>param6(1,1)
    [y96] = param6(1,2)*.99
else
    [y96] = param6(1,1)*.99
end
 [x96] = (-(log10(((b6-a6)/(y96-a6))-1))/d6)+c6
 [starttime6] = x6(1,1)
 [measurementnumbers6] = x96 - x6(1,1)
 [disaccommdationlag] = measurementnumbers6 * 0.125

%for time taken to full disaccommodation
 x7 = rawdata(:,7);
 y7 = rawdata(:,8);
 [param7]=sigm_fit(x7,y7)
 [a7] = param7(1,1)
 [b7] = param7(1,2)
 [c7] = param7(1,3)

[d7] = param7(1,4)
 if param7(1,2)>param7(1,1)
    [y97] = (param7(1,2)-param7(1,1))/100 + param7(1,1)
else
    [y97] = (param7(1,1)-param7(1,2))/100 + param7(1,2)
end
 [x97] = (-(log10(((b7-a7)/(y97-a7))-1))/d7)+c7
 [starttime7] = x7(1,1)
 [measurementnumbers7] = x97 - x7(1,1)
 [disaccommdation2] = measurementnumbers7 * 0.125

%for disaccommodative lag
 x8 = rawdata(:,7);
 y8 = rawdata(:,8);
 [param8]=sigm_fit(x8,y8)
 [a8] = param8(1,1)
 [b8] = param8(1,2)
 [c8] = param8(1,3)
 [d8] = param8(1,4)
 if param8(1,2)>param8(1,1)
    [y98] = param8(1,2)*.99
else
    [y98] = param8(1,1)*.99
end
 [x98] = (-(log10(((b8-a8)/(y98-a8))-1))/d8)+c8
 [starttime8] = x8(1,1)
 [measurementnumbers8] = x98 - x8(1,1)
 [disaccommdationlag2] = measurementnumbers8 * 0.125
```

```
%for time taken to full disaccommodation
 x9 = rawdata(:,11);
 y9 = rawdata(:,12);
 [param9]=sigm_fit(x9,y9)
 [a9] = param9(1,1)
 [b9] = param9(1,2)
 [c9] = param9(1,3)
 [d9] = param9(1,4)
 if param9(1,2)>param7(1,1)
    [y99] = (param9(1,2)-param9(1,1))/100 + param9(1,1)
else
    [y99] = (param9(1,1)-param9(1,2))/100 + param9(1,2)
end
 [x99] = (-(log10(((b9-a9)/(y99-a9))-1))/d9)+c9
 [starttime9] = x9(1,1)
 [measurementnumbers9] = x99 - x9(1,1)
 [disaccommdation3] = measurementnumbers9 * 0.125

%for disaccommodative lag
 x10 = rawdata(:,11);
 y10 = rawdata(:,12);
 [param10]=sigm_fit(x10,y10)
 [a10] = param10(1,1)
 [b10] = param10(1,2)
 [c10] = param10(1,3)
 [d10] = param10(1,4)
 if param10(1,2)>param10(1,1)
    [y910] = param10(1,2)*.99
else
    [y910] = param10(1,1)*.99
end
 [x910] = (-(log10(((b10-a10)/(y910-a10))-1))/d10)+c10
 [starttime10] = x10(1,1)
 [measurementnumbers10] = x910 - x10(1,1)
 [disaccommdationlag3] = measurementnumbers10 * 0.125

 %for time taken to full disaccommodation
 x11 = rawdata(:,15);
 y11 = rawdata(:,16);
 [param11]=sigm_fit(x11,y11)
 [a11] = param11(1,1)
 [b11] = param11(1,2)
 [c11] = param11(1,3)
 [d11] = param11(1,4)

if param11(1,2)>param11(1,1)
    [y911] = (param11(1,2)-param11(1,1))/100 + param11(1,1)
else
    [y911] = (param11(1,1)-param11(1,2))/100 + param11(1,2)
end
 [x911] = (-(log10(((b11-a11)/(y911-a11))-1))/d11)+c11
 [starttime11] = x11(1,1)
```

```
 [measurementnumbers11] = x911 - x11(1,1)
 [disaccommdation4] = measurementnumbers11 * 0.125

%for disaccommodative lag
 x12 = rawdata(:,15);
 y12 = rawdata(:,16);
 [param12]=sigm_fit(x12,y12)
 [a12] = param12(1,1)
 [b12] = param12(1,2)
 [c12] = param12(1,3)
 [d12] = param12(1,4)
 if param12(1,2)>param12(1,1)
    [y912] = param12(1,2)*.99
else
    [y912] = param12(1,1)*.99
end
 [x912] = (-(log10(((b12-a12)/(y912-a12))-1))/d12)+c12
 [starttime12] = x12(1,1)
 [measurementnumbers12] = x912 - x12(1,1)
 [disaccommdationlag4] = measurementnumbers12 * 0.125


beep
subject = input('Please enter subject no :   ', 's');
activities = input('Please enter the testing activities :   ', 's');
trial = input('Please enter the trail number :   ', 's');
%group = input('Please enter Group no, Group 1= Normal, Group 2: Painful
Gp
%:   ');

% Export to excel file

angname='Study2allraw.xls';
fod = fopen(angname, 'a');
fprintf(fod, '%s\t',subject);
fprintf(fod, '%s\t',activities);
fprintf(fod, '%s\t',trial); % should analyse 3 trails in one matlab file
% fprintf(fod, '%7.4f\t',int_J_AP_L1);
% fprintf(fod, '%7.4f\t',J_ML_L1);
fprintf(fod, '%7.4f\t',time);
fprintf(fod, '%7.4f\t',time2);
fprintf(fod, '%7.4f\t',time3);
fprintf(fod, '%7.4f\t',time4);
fprintf(fod, '%7.4f\t',lag);
fprintf(fod, '%7.4f\t',lag2);
fprintf(fod, '%7.4f\t',lag3);
fprintf(fod, '%7.4f\t',lag4);
fprintf(fod, '%7.4f\t',disaccommdation);
fprintf(fod, '%7.4f\t',disaccommdation2);
fprintf(fod, '%7.4f\t',disaccommdation3);
fprintf(fod, '%7.4f\t',disaccommdation4);
fprintf(fod, '%7.4f\t',disaccommdationlag);
fprintf(fod, '%7.4f\t',disaccommdationlag2);
fprintf(fod, '%7.4f\t',disaccommdationlag3);
```

```
fprintf(fod, '%7.4f\t',disaccommdationlag4);
fprintf(fod, '%7.4f\t',a);
fprintf(fod, '%7.4f\t',a2);
fprintf(fod, '%7.4f\t',a3);
fprintf(fod, '%7.4f\t',a4);
fprintf(fod, '%7.4f\t',b);
fprintf(fod, '%7.4f\t',b2);
fprintf(fod, '%7.4f\t',b3);
fprintf(fod, '%7.4f\t',b4);
fprintf(fod, '%7.4f\t',c);
fprintf(fod, '%7.4f\t',c2);
fprintf(fod, '%7.4f\t',c3);
fprintf(fod, '%7.4f\t',c4);
fprintf(fod, '%7.4f\t',d);
fprintf(fod, '%7.4f\t',d2);
fprintf(fod, '%7.4f\t',d3);
fprintf(fod, '%7.4f\t',d4);
fprintf(fod, '%7.4f\t',a5);
fprintf(fod, '%7.4f\t',a7);
fprintf(fod, '%7.4f\t',a9);
fprintf(fod, '%7.4f\t',a11);
fprintf(fod, '%7.4f\t',b5);
fprintf(fod, '%7.4f\t',b7);
fprintf(fod, '%7.4f\t',b9);
fprintf(fod, '%7.4f\t',b11);
fprintf(fod, '%7.4f\t',c5);
fprintf(fod, '%7.4f\t',c7);
fprintf(fod, '%7.4f\t',c9);
fprintf(fod, '%7.4f\t',c11);
fprintf(fod, '%7.4f\t',d5);
fprintf(fod, '%7.4f\t',d7);
fprintf(fod, '%7.4f\t',d9);
fprintf(fod, '%7.4f\t',d11);
fprintf(fod, '%s\n',subject);
fclose(fod);
```

A2 Wilcoxon's Signed Rank test comparing each accommodative metric between the right and left eye

ToA: Z= -0.596, p= 0.551,

ToD: Z= -0.53, p= 0.958,

oToAC: Z=-0.158, p= 0.874,

LoA: Z= -1.109, p= 0.267,

LoD: Z= -1.727, p= 0.84,

pLoA: Z= -0.161, p= 0.872,

pLoD: Z= -1.041, p= 0.298,

AF: Z= -1.313, p= 0.189,

sToAC: Z= -0.965, p= 0.334

A3 The Matlab codes used to calculate the depth-of-focus metrics

0.04 area area-of-focus metric

```
% defocus_curve_fitting_V1
clear all;
hold off;
clc
filename=input ('Please input filename : ', 's')
rawdata= xlsread(filename)
%plotting graph
x = rawdata(:,1);
y = rawdata(:,2);
plot(x,y);
xlabel ('Lens Power, Dioptres');
ylabel ('Visual Acuity, LogMAR');
p = polyfit(x,y,8)
p1 = p
p1(1,9)= p1(1,9)-0.3
p2 = roots(p1)
syms x
n=1
fun = p(1,1)*x(n).^8+p(1,2)*x(n).^7 + p(1,3)*x(n).^6 + p(1,4)*x(n).^5 +
p(1,5)*x(n).^4 + p(1,6)*x(n).^3 + p(1,7)*x(n).^2 + p(1,8)*x(n) + p(1,9)
prompt1 = 'data1 = '
prompt2 = 'data2 = '
data1 = input(prompt1)
data2 = input(prompt2)
integration1 = int (fun,0,data2)
area1 = 0.3 * data2 - integration1
area_1d = double(area1)
integration2 = int (fun,data1,0)
area2 = 0.3 * abs(data1) - integration2
area_2d = double(area2)
prompt3 = 'subject = '
prompt4 = 'visit = '
prompt5 = 'eye = '
subject = input(prompt3)
visit = input(prompt4)
eye = input(prompt5)

% make a matrix
exportdata = [subject,visit,eye,area_1d,area_2d]

% Export to excel file
fod                                                                    =
fopen('/Users/nicolaszostek/Documents/Matlab_output/Curvefitting_v1_03
0_area_multiandmono.xls', 'a'); %file destination
fprintf(fod, '%7.4f\t',subject);
```

```
fprintf(fod, '%7.4f\t',visit);
fprintf(fod, '%7.4f\t',eye);
fprintf(fod, '%7.4f\t',area_1d);
fprintf(fod, '%7.4f\n',area_2d);
```

0.04dist range-of-focus metric

```
% defocus_curve_fitting_V2
clear all;
hold off;
clc
filename=input ('Please input filename : ', 's')
rawdata= xlsread(filename)
%Identify BCVA
data45 = -rawdata(3,2)-0.04
%plotting graph
x = rawdata(:,1);
y = rawdata(:,2);
plot(x,y);
xlabel ('Lens Power, Dioptres');
ylabel ('Visual Acuity, LogMAR');
p = polyfit(x,y,8)
p1 = p
p1(1,9)= p1(1,9)+(data45)
p2 = roots(p1)
syms x
n=1
fun = p(1,1)*x(n).^8+p(1,2)*x(n).^7 + p(1,3)*x(n).^6 + p(1,4)*x(n).^5 +
p(1,5)*x(n).^4 + p(1,6)*x(n).^3 + p(1,7)*x(n).^2 + p(1,8)*x(n) + p(1,9)
prompt2 = 'data1 = '
prompt3 = 'data2 = '
data1 = input(prompt2)
data2 = input(prompt3)
distance = (data1)-(data2)
prompt4 = 'subject = '
prompt5 = 'visit = '
prompt6 = 'eye = '
subject = input(prompt4)
visit = input(prompt5)
eye = input(prompt6)

% make a matrix
exportdata = [subject,visit,eye,distance]

% Export to excel file
fod                                                                    =
fopen('/Users/nicolaszostek/Documents/Matlab_output/Curvefitting_004_d
ist_comp_monoandmulti.xls', 'a'); %file destination
fprintf(fod, '%7.4f\t',subject);
fprintf(fod, '%7.4f\t',visit);
fprintf(fod, '%7.4f\t',eye);
fprintf(fod, '%7.4f\t',distance);
```

0.30area area-of-focus metric

```
% defocus_curve_fitting_V1
clear all;
hold off;
clc
filename=input ('Please input filename : ', 's')
rawdata= xlsread(filename)
%plotting graph
x = rawdata(:,1);
y = rawdata(:,2);
plot(x,y);
xlabel ('Lens Power, Dioptres');
ylabel ('Visual Acuity, LogMAR');
p = polyfit(x,y,8)
p1 = p
p1(1,9)= p1(1,9)-0.3
p2 = roots(p1)
syms x
n=1
fun = p(1,1)*x(n).^8+p(1,2)*x(n).^7 + p(1,3)*x(n).^6 + p(1,4)*x(n).^5 +
p(1,5)*x(n).^4 + p(1,6)*x(n).^3 + p(1,7)*x(n).^2 + p(1,8)*x(n) + p(1,9)
prompt1 = 'data1 = '
prompt2 = 'data2 = '
data1 = input(prompt1)
data2 = input(prompt2)
integration1 = int (fun,0,data2)
area1 = 0.3 * data2 - integration1
area_1d = double(area1)
integration2 = int (fun,data1,0)
area2 = 0.3 * abs(data1) - integration2
area_2d = double(area2)
prompt3 = 'subject = '
prompt4 = 'visit = '
prompt5 = 'eye = '
subject = input(prompt3)
visit = input(prompt4)
eye = input(prompt5)

% make a matrix
exportdata = [subject,visit,eye,area_1d,area_2d]

% Export to excel file
fod                                                                    =
fopen('/Users/nicolaszostek/Documents/Matlab_output/Curvefitting_v1_03
0_area_multiandmono.xls', 'a'); %file destination
fprintf(fod, '%7.4f\t',subject);
fprintf(fod, '%7.4f\t',visit);
fprintf(fod, '%7.4f\t',eye);
fprintf(fod, '%7.4f\t',area_1d);
fprintf(fod, '%7.4f\n',area_2d);
```

0.30dist range-of-focus metric

```
% defocus_curve_fitting_V3_distance_0.3
clear all;
hold off;
clc
filename=input ('Please input filename : ', 's')
rawdata= xlsread(filename)
%plotting graph
x = rawdata(:,1);
y = rawdata(:,2);
plot(x,y);
xlabel ('Lens Power, Dioptres');
ylabel ('Visual Acuity, LogMAR');
p = polyfit(x,y,8)
p1 = p
p1(1,9)= p1(1,9)-0.3
p2 = roots(p1)
syms x
n=1
fun = p(1,1)*x(n).^8+p(1,2)*x(n).^7 + p(1,3)*x(n).^6 + p(1,4)*x(n).^5 +
p(1,5)*x(n).^4 + p(1,6)*x(n).^3 + p(1,7)*x(n).^2 + p(1,8)*x(n) + p(1,9)
prompt1 = 'data1 = '
prompt2 = 'data2 = '
data1 = input(prompt1)
data2 = input(prompt2)
distance = (data1)-(data2)
prompt3 = 'subject = '
prompt4 = 'visit = '
prompt5 = 'eye = '
subject = input(prompt3)
visit = input(prompt4)
eye = input (prompt5)

% make a matrix
exportdata = [subject,visit,eye,distance]

% Export to excel file
fod                                                                    =
fopen('/Users/nicolaszostek/Documents/Matlab_output/Curvefitting_v1_03
0_distance_multiandmono.xls', 'a'); %file destination
fprintf(fod, '%7.4f\t',subject);
fprintf(fod, '%7.4f\t',visit);
fprintf(fod, '%7.4f\t',eye);
fprintf(fod, '%7.4f\t',distance);
```

A4

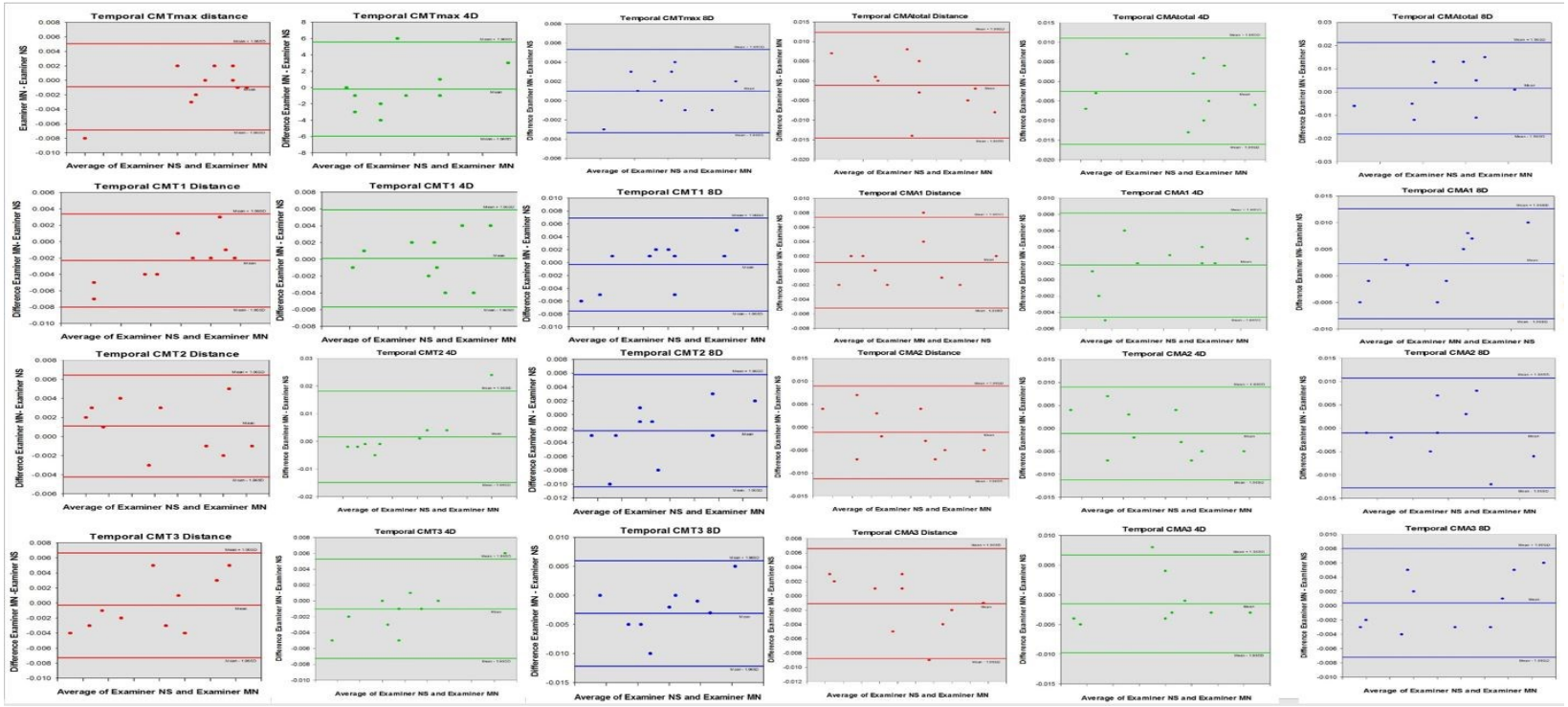

Figure A4.1 Bland and Altman plots demonstrating the inter-observer repeatability for each position along the temporal ciliary muscle, at 0D, 4D and 8D of accommodation

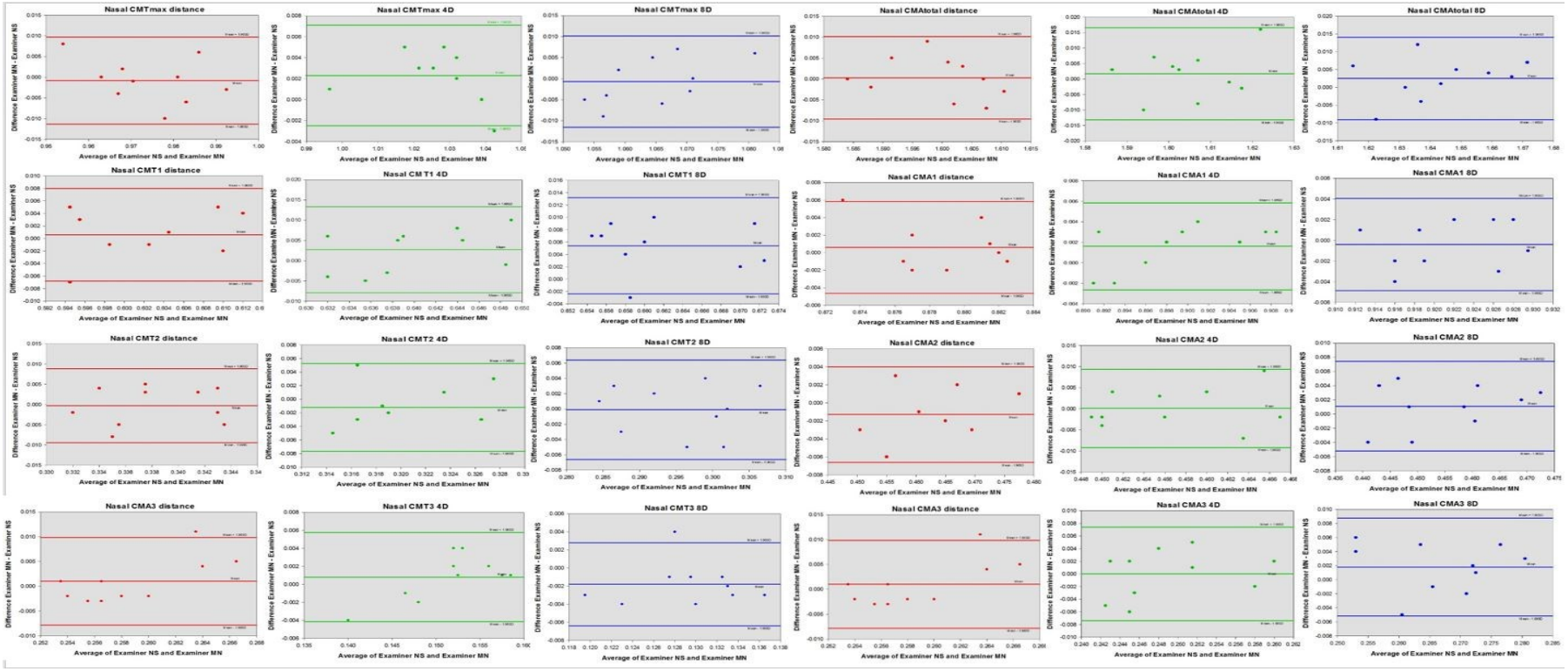

Figure A4.2 Bland and Altman plots demonstrating the inter-observer repeatability for each position along the nasal ciliary muscle, at 0D, 4D and 8D of accommodation

A5 Chapter 6 questionnaires

EYE & VISION RESEARCH WITH PLYMOUTH UNIVERSITY

QUESTIONNAIRE 1: TO BE COMPLETED BY THE PATIENT

PERSONNAL INFORMATION AND LIFESTYLE QUESTIONNAIRE

All information contained within this questionnaire is kept strictly confidential /Please complete all of the questions in this questionnaire as accurately as possible.

Gender: ☐ Male ☐ Female	DOB:

SECTION 1: ETHNICITY AND COUNTRIES LIVED IN		
1A. HOW WOULD YOU DESCRIBE YOUR ETHNICITY?		
☐ White British	☐ Black Other (please specify)	☐ Pakistani
☐ White Irish	☐ Mixed: White and Black African	☐ Bangladeshi
☐ White Other (Please specify).....	☐ Mixed: White and Black Caribbean	☐ Chinese
☐ Black British	☐ Mixed: White and Asian	☐ Other Asian (Please specify).....
☐ Black African	☐ Mixed Other (Please specify).....	☐ Other ethnic group (Please specify)
☐ Black Caribbean	☐ Indian	☐ Do not wish to specify)
1B. HAVE YOU LIVED IN THE UK ALL OF YOUR LIFE? Yes ☐ No ☐ (If no, please give details below of the other countries you have lived in with relevant dates)		

SECTION 2: EDUCATION AND INCOME		
2A. WHAT IS THE HIGHEST LEVEL OF EDUCATION WHICH YOU HAVE CURRENTLY ACHIEVED?		
☐ PhD	☐ Other higher education (eg diploma)	☐ GCSE (A-C)
☐ Masters/other post-graduate	☐ A levels/Highers	☐ GCSE (D-G)
☐ Degree	☐ ONC/National BTEC	☐ None
☐ Other (Please specify).....		
2B. WHAT IS YOUR PERSONNEL INCOME (BEFORE TAX AND OTHER DEDUCTIONS) FOR THIS YEAR?		
☐ Up to £ 9 999.99	☐ £15 000 - £19 999.99	☐ £ 30-£39 999.99
☐ £10 000 - £14 999.99	☐ £20 000 - £29 999.99	☐ £40 000+

SECTION 3: SMOKING AND ALCOHOL INTAKE			
3A. DO YOU CURRENTLY SMOKE?		☐ Yes (Got to 3D)	☐ No
3B. HAVE YOU SMOKED IN THE PAST?		☐ Yes	☐ No (Go to 3F)
3C. HOW MANY YEARS HAS IT BEEN SINCE YOU LAST SMOKED?		……years	
3D. FOR HOW MANY YEARS HAVE YOU/DID YOU SMOKE?		……years	
3E. WHAT DO YOU/DID YOU SMOKE? (Please tick all that apply and state the average number you have smoked in the last week. If you have stopped smoking please state the average number you smoked per day in the year before you quit)	☐ Cigarettes Number per day ……	☐ Cigars Number per day ……	☐ Pipe Number per day ……
3F. DO YOU DRINK ALCOHOL?		☐ Yes	☐ No
3G. HOW MANY UNITS DO YOU DRINK ON AVERAFE IN ONE WEEK? (Please use the guidelines below, please ask if you are unsure how to calculate this)		……units	
125ml glass wine = 1.5 units	250ml glass of wine = 3 units	Pint of low-strength beer/larger/cider = 2 units	
175ml glass wine = 2.1 units	Single shot of spirits = 1 unit	Pint of high-strength beer/lager/cider = 3 units	

SECTION 4: OCCUPATION AND HOBBIES		
4A. ARE YOU CURRENTLY EMPLOYED?	☐ YES (please state occupation) ……	☐ No
4B. ON AVERAGE HOW MANY HOURS PER DAY DO YOU SPEND DOING NEAR WORK? (EG. ON COMPUTERS/TABLETS/READING/SEWING)		……Hrs
4C. PLEASE STATE BELOW ANY ACTIVITIES/HOBBIES YOU DO IN A NORMAL WEEK (Please state: the time spent per week & whether this takes place indoors or outdoors).		
ACTIVITY/HOBBY	**NUMBER OF HOURS PER WEEK**	**INDOORS/OUTDOORS**
4D. ON AVERAGE HOW MANY HOURS PER DAY DO YOU SPEND OUTDOORS?		……Hrs

SECTION 5: MEDICAL AND OCULAR HEALTH			
5A: DO YOU HAVE DIABETES?		☐ Yes	☐ No
5B: HAVE YOU EVER BEEN DIAGNOSED OR TREATED FOR ANY OF THE FOLLOWING?			
☐ Strabismus/lazy eye	☐ Glaucoma	☐ Dry Eyes	☐ Retinal haemorrhage
☐ Corneal Ulcer	☐ Cataract	☐ Retinal detachment	☐ Eye Injury
☐ Uveitis/Iritis/keratitis	☐ Macular degeneration (AMD)	☐ Other (Please state)	

END OF QUESTIONNAIRE

FOOD FREQUENCY QUESTIONNAIRE

This questionnaire asks for some background information about you, especially about what you eat.

Please answer every question. If you are uncertain about how to answer a question then do the best you can, but please do not leave a question blank.

1. **YOUR DIET LAST YEAR**

For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (✓) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year.**

EXAMPLES:

For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✓	

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
POTATOES, RICE AND PASTA (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

For very seasonal fruits such as strawberries and raspberries you should estimate your average use when the fruits are in season, so if you ate strawberries or raspberries about once a week when they were in season you should put a tick in the column headed "once a week"

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
FRUIT (1 fruit or medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Strawberries, raspberries, kiwi fruit			✓						

Please estimate your average food use as best you can, and please answer every question - do not leave ANY lines blank. PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
MEAT AND FISH (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew or casserole									
Beefburgers									
Pork: roast, chops, stew or slices									
Lamb: roast, chops or stew									
Chicken or other poultry eg. turkey									
Bacon									
Ham									
Corned beef, Spam, luncheon meats									
Sausages									
Savoury pies, eg. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls									
Liver, liver paté, liver sausage									
Fried fish in batter, as in fish and chips									
Fish fingers, fish cakes									
Other white fish, fresh or frozen, eg. cod, haddock, plaice, sole, halibut									
Oily fish, fresh or canned, eg. mackerel, kippers, tuna, salmon, sardines, herring									
Shellfish, eg. crab, prawns, mussels									
Fish roe, taramasalata									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls									
Brown bread and rolls									
Wholemeal bread and rolls									
Cream crackers, cheese biscuits									
Crispbread, eg. Ryvita									
CEREALS (one bowl)									
Porridge, Readybrek									
Breakfast cereal such as cornflakes, muesli etc.									
POTATOES, RICE AND PASTA (medium serving)									
Boiled, mashed, instant or jacket potatoes									
Chips									
Roast potatoes									
Potato salad									
White rice									
Brown rice									
White or green pasta, eg. spaghetti, macaroni, noodles									
Wholemeal pasta									
Lasagne, moussaka									
Pizza									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DAIRY PRODUCTS AND FATS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Single or sour cream (tablespoon)									
Double or clotted cream (tablespoon)									
Low fat yogurt, fromage frais (125g carton)									
Full fat or Greek yogurt (125g carton)									
Dairy desserts (125g carton)									
Cheese, eg. Cheddar, Brie, Edam (medium serving)									
Cottage cheese, low fat soft cheese (medium serving)									
Eggs as boiled, fried, scrambled, etc. (one)									
Quiche (medium serving)									
Low calorie, low fat salad cream(tablespoon)									
Salad cream, mayonnaise (tablespoon)									
French dressing (tablespoon)									
Other salad dressing (tablespoon)									
The following on bread or vegetables									
Butter (teaspoon)									
Block margarine, eg. Stork, Krona (teaspoon)									
Polyunsaturated margarine (tub), eg. Flora, sunflower (teaspoon)									
Other soft margarine,dairy spreads (tub), eg. Blue Band, Clover (teaspoon)									
Low fat spread (tub), eg. Outline, Gold (teaspoon)									
Very low fat spread (tub) (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
SWEETS AND SNACKS (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Sweet biscuits, chocolate , eg. digestive (one)									
Sweet biscuits, plain, eg. Nice, ginger (one)									
Cakes eg. fruit, sponge, home baked									
Cakes eg. fruit, sponge, ready made									
Buns, pastries eg. scones, flapjacks, home baked									
Buns, pastries eg. croissants, doughnuts, ready made									
Fruit pies, tarts, crumbles, home baked									
Fruit pies, tarts, crumbles, ready made									
Sponge puddings, home baked									
Sponge puddings, ready made									
Milk puddings, eg. rice, custard, trifle									
Ice cream, choc ices									
Chocolates, single or squares									
Chocolate snack bars eg. Mars, Crunchie									
Sweets, toffees, mints									
Sugar added to tea, coffee, cereal (teaspoon)									
Crisps or other packet snacks, eg. Wotsits									
Peanuts or other nuts									
SOUPS, SAUCES, AND SPREADS									
Vegetable soups (bowl)									
Meat soups (bowl)									
Sauces, eg. white sauce, cheese sauce, gravy (tablespoon)									
Tomato ketchup (tablespoon)									
Pickles, chutney (tablespoon)									
Marmite, Bovril (teaspoon)									
Jam, marmalade, honey (teaspoon)									
Peanut butter (teaspoon)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)									
Coffee, instant or ground (cup)									
Coffee, decaffeinated (cup)									
Coffee whitener, eg. Coffee-mate (teaspoon)									
Cocoa, hot chocolate (cup)									
Horlicks, Ovaltine (cup)									
Wine (glass)									
Beer, lager or cider (half pint)									
Port, sherry, vermouth, liqueurs (glass)									
Spirits, eg. gin, brandy, whisky, vodka (single)									
Low calorie or diet fizzy soft drinks (glass)									
Fizzy soft drinks, eg. Coca cola, lemonade (glass)									
Pure fruit juice (100%) eg. orange, apple juice (glass)									
Fruit squash or cordial (glass)									
FRUIT **For seasonal fruits marked *, please estimate your average use when the fruit is in season**									
Apples (1 fruit)									
Pears (1 fruit)									
Oranges, satsumas, mandarins (1 fruit)									
Grapefruit (half)									
Bananas (1 fruit)									
Grapes (medium serving)									
Melon (1 slice)									
* Peaches, plums, apricots (1 fruit)									
* Strawberries, raspberries, kiwi fruit (medium serving)									
Tinned fruit (medium serving)									
Dried fruit, eg. raisins, prunes (medium serving)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
VEGETABLES Fresh, frozen or tinned (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Carrots									
Spinach									
Broccoli, spring greens, kale									
Brussels sprouts									
Cabbage									
Peas									
Green beans, broad beans, runner beans									
Marrow, courgettes									
Cauliflower									
Parsnips, turnips, swedes									
Leeks									
Onions									
Garlic									
Mushrooms									
Sweet peppers									
Beansprouts									
Green salad, lettuce, cucumber, celery									
Watercress									
Tomatoes									
Sweetcorn									
Beetroot									
Coleslaw									
Avocado									
Baked beans									
Dried lentils, beans, peas									
Tofu , soya meat, TVP, Vegeburger									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

YOUR DIET LAST YEAR, continued

2. Are there any **OTHER** foods which you ate more than once a week? Yes ☐ No ☐

If yes, please list below

Food	Usual serving size	Number of times eaten each week

3. What type of milk did you most often use?

Select one only

Full cream, silver ☐ Semi-skimmed, red/white ☐
Skimmed/blue ☐ Channel Islands, gold ☐
Dried milk ☐ Soya ☐
Other, *specify* ☐ None ☐

4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?

None ☐ Three quarters of a pint ☐
Quarter of a pint ☐ One pint ☐
Half a pint ☐ More than one pint ☐

5. Did you usually eat breakfast cereal (excluding porridge and Ready Brek mentioned earlier)? Yes ☐ No ☐

If yes, which brand and type of breakfast cereal, including muesli, did you usually eat?
List the one or two types most often used

Brand *e.g. Kellogg's*	Type *e.g. cornflakes*

6. What kind of fat did you most often use for frying, roasting, grilling etc?

Select one only

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

If you used vegetable oil, please give type eg. corn, sunflower ☐

7. What kind of fat did you most often use for baking cakes etc?

Select one only

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

If you used margarine, please give name or type eg. Flora, Stork ☐

8. How often did you eat food that was fried at home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

9. How often did you eat fried food away from home?

Daily ☐ 1-3 times a week ☐ 4-6 times a week ☐
Less than once a week ☐ Never ☐

10. What did you do with the visible fat on your meat?

Ate most of the fat ☐ Ate as little as possible ☐
Ate some of the fat ☐ Did not eat meat ☐

11. How often did you eat grilled or roast meat? ☐☐ times a week

12. How well cooked did you usually have grilled or roast meat?

Well done /dark brown ☐ Lightly cooked/rare ☐
Medium ☐ Did not eat meat ☐

13. How often did you add salt to food while cooking?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

14. How often did you add salt to any food at the table?

Always ☐ Rarely ☐
Usually ☐ Never ☐
Sometimes ☐

15. Did you regularly use a salt substitute (eg LoSalt)? Yes ☐ No ☐

If yes, which brand? ☐

16. During the course of last year, on average, how many times a week did you eat the following foods?

Food type	**Times/week**	**Portion size**
Vegetables (not including potatoes)	☐☐	medium serving
Salads	☐☐	medium serving
Fruit and fruit products (not including fruit juice)	☐☐	medium serving or 1 fruit
Fish and fish products	☐☐	medium serving
Meat, meat products and meat dishes (including bacon, ham and chicken)	☐☐	medium serving

17. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year? Yes ☐ No ☐ Don't know ☐

If yes, please complete the table below. If you have taken more than 5 types of supplement please put the most frequently consumed brands first.

Vitamin supplements	**Average frequency** Tick **one** box per line to show how often on average you consumed supplements									
Name and brand Please list full name, **brand** and strength	**Dose** Please state number of pills, capsules or teaspoons consumed	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Thank you for your help

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

END OF QUESTIONNAIRE.

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

References

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