ASTIGMATISM AND OCULAR STRUCTURAL CORRELATES IN CHICKS

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Astigmatism and Ocular Structural Correlates in Chicks

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CERTIFICATE OF ORIGINALITY

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

Signature:_____

Name: Chin Hung Geoffrey Chu

Date: 16th March, 2015

DEDICATION

This document is dedicated to all my family members, especially my loving parents, grandparents and brothers.

ABSTRACT

Purpose: To study: 1) the effects of hemiretinal form deprivation on eye growth; 2) the effects of experimentally induced astigmatism on corneal accommodation; and 3) the effects of optically imposed astigmatism on eye growth.

Methods: White Leghorn chicks were used. The treatment started from 5 days of age and usually lasted for 1 to 3 weeks. Refractive status was measured by using a Hartinger refractometer. Eye shape profiles and corneal topography were measured by using an eye shape imaging system and a videokeratography, respectively. Right eye served as the treated eye while left eye served as untreated control; a separate group of birds received no treatment served as the control group.

In *Exp.1* (Chapter 2), hemiretinal form deprivation was used to cover the visual fields corresponding to four retinal regions: superior (SRD), inferior (IRD), temporal (TRD) and nasal (NRD). Refractive changes over three weeks were recorded and the eye shape profiles along four meridians were captured at the end of the experiment. In *Exp.2* (Chapter 3), the characteristics of corneal accommodation in normal chicks and chicks with experimentally induced astigmatism were studied. The videokeratography provided a continuous recording of the changes in the corneal profile over time, allowing further characterization of corneal accommodation. In *Exp.3*

(Chapter 4), crossed-cylinder lenses were used for optically imposing astigmatism. The effects of orientation (45, 90, 135 and 180) and magnitude (+4.00DS/-8.00DC and +2.00DS/-4.00DC) on corneal topography and eye shape profiles were studied.

Results: *Exp.1.* Differences in refractive status and eye shape profiles were found when different retinal regions were form-deprived. SRD group exhibited the highest magnitudes of spherical-equivalent (M) among the four treatment groups. Astigmatism was also induced, but only subtle differences were found across the treatment groups. *Exp.2.* Bi-directional changes in corneal accommodation were found in normal and astigmatic chicks. The magnitudes of positive accommodation were associated with those of the induced astigmatism. *Exp.3.* Both the orientation and magnitude of optically imposed astigmatism influenced the characteristics of induced astigmatism. Chicks treated with WTR astigmatism (minus cylinder axis 90) developed the highest magnitude of induced astigmatism, whereas those treated with ATR astigmatism (axis 180) developed the lowest magnitude of astigmatism. Both corneal and internal astigmatism contributed about 50% of the refractive astigmatism.

Conclusions: These studies extended our current knowledge about the role of visual error signals on the genesis of astigmatism. In particular, the changes in ocular biometric parameters from the anterior (*i.e.*, corneal

curvature) to the posterior segment (*i.e.*, eye shape profile) should be considered during astigmatic eye growth.

PUBLICATIONS ARISING FROM THE THESIS

Chu, C. H., Deng, L., and Kee, C. S. (2012). Effects of hemiretinal form deprivation on central refractive development and posterior eye shape in chicks. Vision Research, 55, 24-31.

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Chu, C. H., and Kee, C. S. (2015). Effects of Optically imposed Astigmatism on Early Eye Growth in Chicks. PLoS One, 10 (2): e0117729. doi:10.1371/journal.pone.0117729

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LIST OF ABBREVIATIONS

1st	First
2nd	Second
3rd	Third
ADH	Accumulated Differences in Area Along the Horizontal Meridian
ADV	Accumulated Differences in Area Along the Vertical Meridian
AL	Axial Length
AL/ED	Axial Length/Equatorial Diameter Ratio
am	Ante Meridiem
ANOVA	Analysis of Variance
ATR	Against-The-Rule Astigmatism
CA	Corneal Astigmatism
CCD	Charge-Coupled Device
CLOC	Circle of Least Confusion
C-J0	Corneal Cosine Jackson Crossed-Cylinder Component
C-J45	Corneal Sine Jackson Crossed-Cylinder Component
CR	Corneal Radius
CMZ	Circumferential Marginal Zone
ED	Equatorial Diameter
e.g.	For example
Exp	Experiment
FK	Flattest Corneal Curvature
FRD	Full Retinal Form Deprivation
H	High magnitude
h	Height
IA	Internal Astigmatism
i.e.	That Is
IOD	Inter-ocular Difference
IRD	Inferior Retinal Form Deprivation
K	Corneal Curvature
l	Length
L	Low Magnitude
LE	Left Eye
LED	Light Emitting Diode
M	Spherical-Equivalent / Mean Ocular Refraction
max.	Maximum
MHM	Most Hyperopic Meridian
MK	Mean Corneal Curvature
MMM	Most Myopic Meridian
MPA	Maximum Positive Accommodation
n	n-number
n	Refractive index

NA	Negative Accommodation
NPA	Maximum Negative Accommodation
NRD	Nasal Retinal Form Deprivation
ns	No Significant Difference
p	P Value
P0	Day of Hatch
P2	2 Days Post-Hatching
P5	5 Days Post-Hatching
P12	12 Days Post-Hatching
PA	Positive Accommodation
pm	Post Meridiem
PMMA	Polymethyl Methacrylate
R	Radius of Curvature
r	Pearson's correlation
r_s	Spearman's rho
r	Radius of Corneal Curvature
r_A	Radius of Anterior Corneal Curvature
r_P	Radius of Posterior Corneal Curvature
RA	Refractive Astigmatism
RE	Right Eye
R-J0	Refractive Cosine Jackson Crossed-Cylinder Componen
R-J45	Refractive Sine Jackson Crossed-Cylinder Component
SE	Standard Error
SK	Steepest Corneal Curvature
SRD	Superior Retinal Form Deprivation
TRD	Temporal Retinal Form Deprivation
VKS	Videokeratography System
vs.	Versus
w	Width
WTR	With-The-Rule Astigmatism
XCyl	Crossed-Cylinder Lens

LIST OF SYMBOLS AND UNITS OF MEASURE

&	And
Δ	Change
°	Degree
°C	Degree Celsius
D	Diopter
DC	Diopter Cylindrical
DS	Diopter Spherical
=	Equal to
”	Inch
>	Larger Than
≥	Larger Than or Equal to
lux	Lux
mm	Mini-meter
%	Percentage
±	Plus And Minus
α	Significance level
<	Smaller Than
≤	Smaller than or Equal to
-	To

KEYWORDS

Astigmatism

Corneal accommodation

Corneal curvature

Crossed-cylinder lens

Emmetropization

Eye shape

Hemiretinal deprivation

Positive accommodation

Negative accommodation

Videokeratography

CHAPTER 1 INTRODUCTION

The aim of this study was to investigate the relationship between astigmatism and ocular structural correlates. In particular, changes in the shape of cornea and sclera were measured in chicken eyes with astigmatism induced by visual manipulations.

Chapter 1 introduced the background of this study and defined the common terminology used in this thesis. Subtopics included the characteristics of astigmatism in humans and animals, the factors related to the genesis of astigmatism, and the potential role of astigmatism in eye growth.

Chapter 2 presented the study on the effects of hemiretinal form deprivation on refractive error development in chicks, particularly astigmatism, and posterior eye shape in chicks. An imaging system for measuring the changes in eyeshape profile was introduced. This study was published in *Vision Research* 55 (2012): 24-31.

Chapter 3 presented the study on the effects of the presence of experimentally induced astigmatism on corneal accommodation in chicks. This study also determined whether corneal accommodation in chicks was detectable under natural viewing conditions: that is with no artificial stimulation, anesthesia, nor the use of lid retractors. A videokeratographer

was developed for measuring corneal parameters. This study was published in *Vision Research* 98 (2014): 26-34.

Chapter 4 presented a study implementing the two abovementioned instruments to confirm whether chick is capable of compensation of imposed astigmatism and on the effects of the orientation and magnitude of imposed astigmatism on the shape of cornea and sclera in chicks. The videokeratography and eye shape profile imaging systems tested in the previous two chapters were used in this study. The content of this chapter has been accepted for publication in *PLOS One* (2014).

Chapter 5 gave general conclusions of this thesis and provided future directions in this area.

Definition of Astigmatism

Discovery of astigmatism

Astigmatism is an optical defect that degrades the retinal image quality along different meridians to different extents. This defect was first noted by Sir Isaac Newton who described the meridional variation in optical power and the formation of line foci in the mid 17th century (Cox, 2010; Newton, 1652). In 1793, Thomas Young discovered ocular astigmatism, a difference in degree of refraction along different meridians when measuring refraction and accommodation of his eyes (Donders and Moore, 1864; T. Young,

1801). He further noted that his vision was improved by inclination of the spectacle glass. In fact, de la Hire, in 1694, remarked that a circular object would appear as an oval image when the crystalline lens was tilted (Levene, 1977). To determine the origin of ocular astigmatism, Young neutralized the effective power of his cornea by immersing in water. In this study, he described the asymmetries in cornea, pupil, lens, and the distances of the fovea and optic nerve from the visual axis; he concluded that the astigmatism was caused by the asymmetries of ocular components (Atchison and Charman, 2010; T. Young, 1801). In 1824, Sir George Airy, whose refractive error was considerably higher than Young's, found the visual distortion produced by lens tilting unacceptable, and invented cylindrical lens which can improve the degraded visual quality due to his ocular astigmatism subsequently (Levene, 1966).

Components of astigmatism

The cornea and crystalline lens are the two main refractive components of the eye, astigmatism is largely corneal in origin (V. Dobson, Miller and Harvey, 1999; Keller, Collins, Carney et al., 1996; Kershner and Brick, 1984; Lyle, Grosvenor and Dean, 1972; Maples, Atchley and Hughes, 1996) and most of the corneal power is due to the anterior corneal surface. In the Gullstrand schematic eye, the refractive power of the anterior surface (radius of curvature, r_A=7.7mm) is +48.83D and the posterior surface

(r_P=6.8mm) is only -5.88D (Atchison and Smith, 2000; Gullstrand, Von Kries and Nagel, 1924), *i.e.*, approximately 12% of the anterior corneal power (Lam and Douthwaite, 2000). Only about -0.305D posterior corneal astigmatism was found in human adults (18 to 65 years old) (Dubbelman, Sicam, V. A. D. P. and Van der Heijde, G. L., 2006). A compensatory effect of the posterior corneal surface (usually against-the-rule, *i.e.,* when the minus-cylindrical axis is oriented within 90±30 degrees) to the anterior corneal astigmatism (usually with-the-rule, *i.e.,* when the minus-cylindrical axis is oriented within 180±30 degrees) (Dunne, Royston and Barnes, 1991; Ho, Liou, Tsai et al., 2010; Kelly, Mihashi and Howland, 2004) has been suggested, but the posterior corneal astigmatism was only weakly correlated with the internal astigmatism (Piñero, Ruiz-Fortes, Pérez-Cambrodí et al., 2014).

Internal astigmatism (IA) (Alpins, 1993; Alpins, 2001; S. S. Duke-Elder and Abrams, 1970; Lyle, Grosvenor and Dean et al., 1972), also known as ocular residual astigmatism, is defined as the remaining astigmatism after subtracting the corneal astigmatism from refractive astigmatism. Based on the Javal's rule (Auger, 1988; Banks and Held, 2012; Grosvenor, Quintero and Perrigin, 1988; Javal, 1890; Keller, Collins, Carney et al., 1996), the internal astigmatism in human is about 0.50D, against-the-rule (Sarver, 1969) (however, also see (Piñero, Ruiz-Fortes, Pérez-Cambrodí et al., 2014)). The internal astigmatism is supposed to be caused by the lenticular

toricity and the decentration and/or tilt of the optical axis of the crystalline lens with respect to that of the cornea (Tscherning, 1920). These kinds of aberration due to imperfections in the shape of the cornea or the lens are considered as on-axis astigmatism. Previous studies found that the crystalline lens is usually tilted from 3° to 7° about the vertical axis with the temporal side located 0° to 3° more forward (Tscherning, 1920; Zeeman, 1908). In human infants, the lenticular astigmatism is usually about 0.50D against-the-rule (Hofstetter and Baldwin, 1957) and mainly due to the toricity of the posterior lens surface (Carter, 1963; Mutti, Mitchell, Jones et al., 2004; Neumueller, 1953). Some studies (P. Artal, Guirao, Berrio et al., 2001; Kelly, Mihashi and Howland et al., 2004) found that the corneal and internal aberrations partially compensate for each other. While several studies (V. Dobson, Miller and Harvey et al., 1999; Grosvenor, Quintero and Perrigin et al., 1988; Grosvenor and Ratnakaram, 1990; Mutti, Mitchell, Jones et al., 2004; Shankar and Bobier, 2004) reported that lenticular astigmatism did not compensate for anterior corneal astigmatism, other studies showed contradictory results (P. Artal, Guirao, Berrio et al., 2001; P. Artal, Berrio, Guirao et al., 2002; Kelly, Mihashi and Howland et al., 2004; Mas, Espinosa, Domenech et al., 2009; Park, Oh and Chuck, 2013; Tabernero, Benito, Alcón et al., 2007). Whether lenticular astigmatism is compensatory for corneal astigmatism in chicks remains unclear, a study in chicks found a thicker crystalline lens in eyes treated with plano/-9.00DC

lenses, but no significant difference was found in those treated with plano/+10.00DC lenses (Irving, Callender and Sivak, 1995).

Definition and classification of astigmatism

"Astigmatism" ("a-stigma" in Greek) describes a "non-point" image formed by an optical system from a point object, can exist in two distinct forms, on-axis and off-axis astigmatism. The former is due to the asymmetric optical system about the optical axis, for instance, asymmetric curvatures at different meridians and/or misalignment of the optical components. The latter is the aberration caused by object away from the axis of a perfectly symmetrical optical system (see following section for details). In clinical practice, a "regular" astigmatism" refers to the astigmatism produced by the difference of two principal refractive meridians (with the maximum and minimum powers) perpendicular to each other (Figure 1-1). It follows that an "irregular" astigmatism refers to the one produced by two principal powered meridians that are not perpendicular to each other. In the rest of this thesis, "astigmatism" refers to a regular astigmatism. Previous studies have found that ametropia is associated with refractive (D. I. Flitcroft, 2013; Gonzalez Blanco, Sanz, Juan et al., 2008) and/or axial abnormalities of the eye (D. I. Flitcroft, 2013; Gonzalez Blanco, Sanz, Juan et al., 2008; Mutti, Hayes, Mitchell et al., 2007). For instance, myopic eyes usually had a long eyeball (Curtin, 1977; Mauget-FaŸsse, Cornut, Quaranta El-Maftouhi et al.,

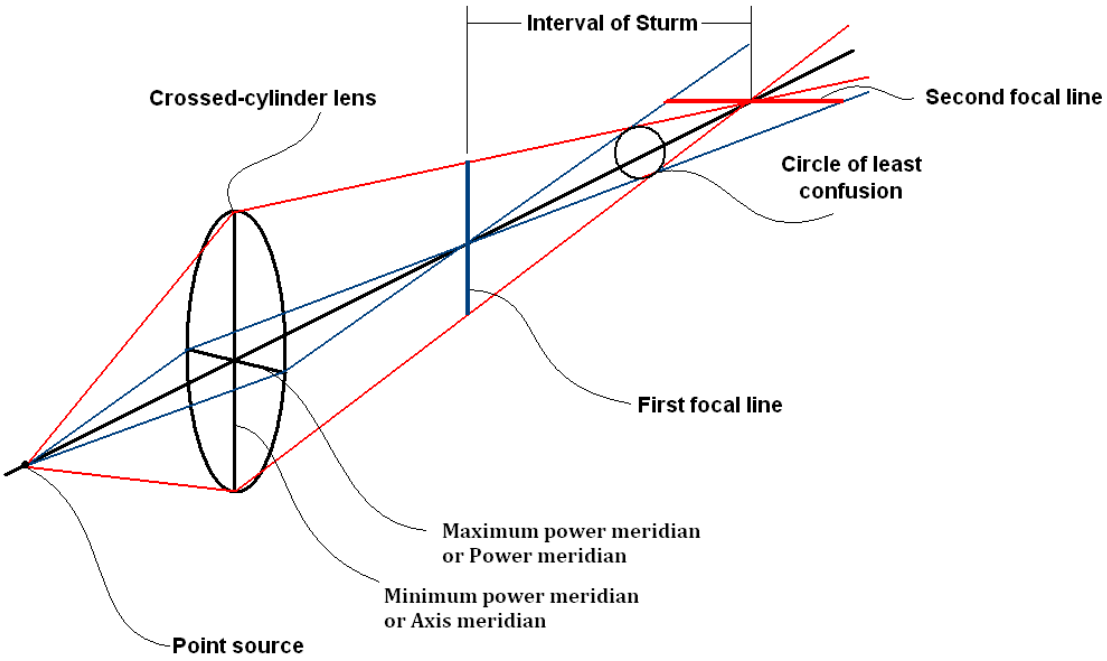

Figure 1-1 Astigmatism is the difference in refractive powers between two principal powered meridians. The dioptric distance between the line foci associated with the first (due to the tangential plane) and second (due to the sagittal plane) focal line is the magnitude of astigmatism. The “circle of least confusion” is located in the middle of the two line foci.

2006), a steep refracting surfaces (Carney, Mainstone and Henderson, 1997), or both (Grosvenor and Scott, 1994; Llorente, Barbero, Cano et al., 2004); and hyperopic eyes appeared to show the opposite pattern (Curtin, 1977; Grosvenor and Scott, 1994; Llorente, Barbero, Cano et al., 2004; Mauget-FaŸsse, Cornut, Quaranta El-Maftouhi et al., 2006). Despite the fact that astigmatism is a very common ametropia (V. Dobson, Miller and Harvey et al., 1999; V. Dobson, Harvey and Miller, 2007; Hoffmann and Hütz, 2010; Miller, Dobson, Harvey et al., 2001), most of the previous works have focused mainly on spherical equivalent. Compared to myopic and hyperopic errors, astigmatic errors are more complicated because astigmatism varies not only in magnitude but also in orientation. Each principal powered meridian produces a line focus, and the relative locations of the two line foci with respect to the retinal plane result in five different astigmatic conditions ─ simple or compound myopic astigmatism, simple or compound hyperopic astigmatism, and mixed astigmatism (Figure 1-2). In this thesis, WTR and ATR astigmatisms were defined as negative-cylinder axes oriented within 180±30 and 90±30 respectively; axes outside these ranges were defined oblique astigmatism. For statistical analyses, astigmatism was also decomposed into two vector components J0 and J45 (see Figure 1-3 for details).

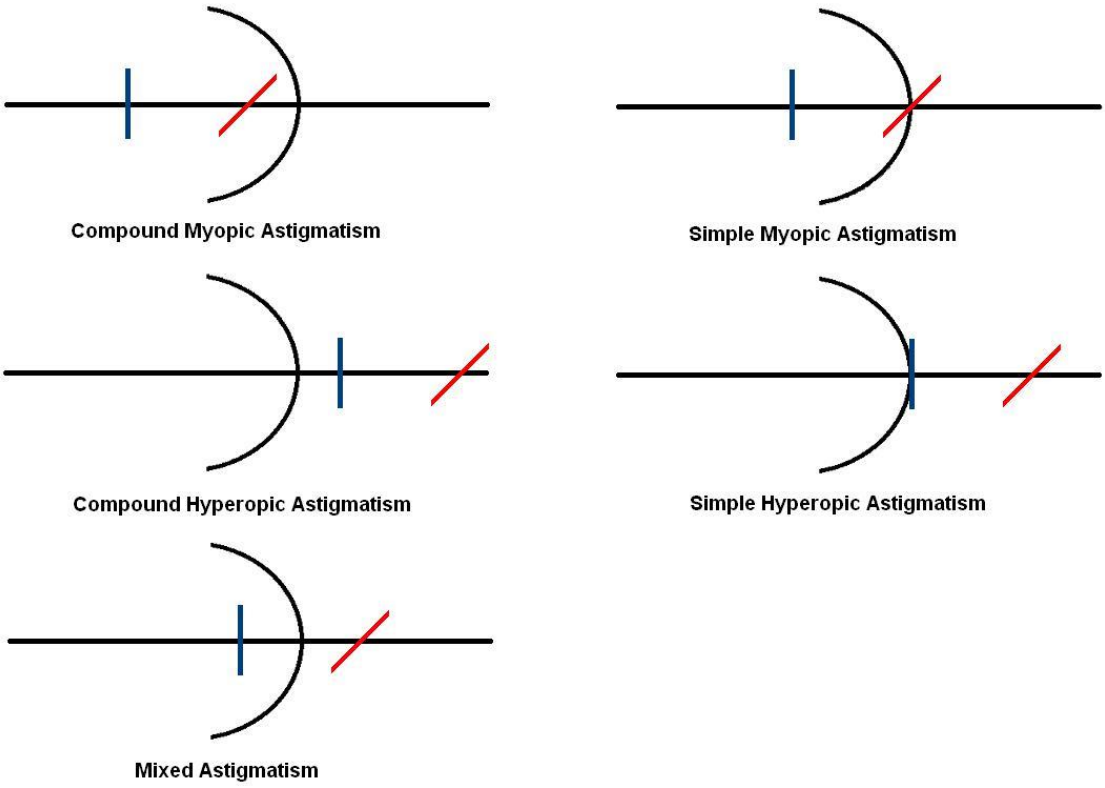

Figure 1-2 The five different types of astigmatism are characterized by the relative locations of the two line foci with respect to the retinal surface (assuming that the object is a cross). Maximum power meridian (red) and minimum power meridian (blue).

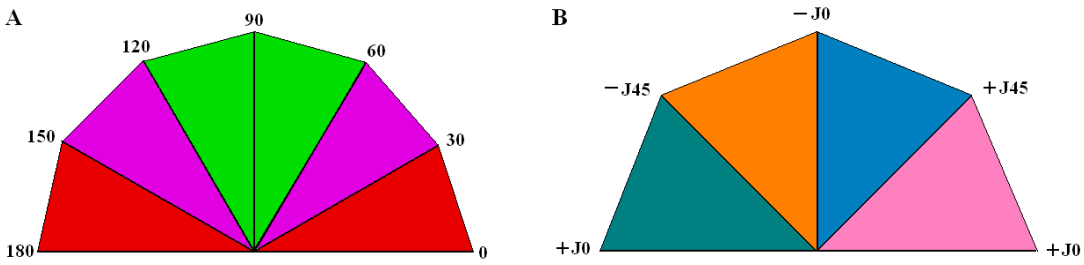

Figure 1-3 A) According to the axis orientation, astigmatism can be categorized into with-the-rule (180±30, area in red), against-the-rule astigmatism (90±30, area in green), or oblique astigmatism (area in purple). B) Based on the principle of Fourier analysis, astigmatism can also be decomposed into two vector components J0 and J45. In general, astigmatism can be described by one of the categories: +J0 and +J45 (region in pink), +J45 and –J0 (region in blue), -J0 and –J45 (region in orange), and –J45 and +J0 (region in green). Therefore, +J0 and –J0 can sometimes be alternatives of WTR and ATR astigmatisms, while the J45 indicates the oblique astigmatism.

Eyeshape and refractive status

An eye is frequently described as an “eyeball”. A normal human eye is thought to be a regular round shape (Donders and Moore, 1864). However, empirical studies showed that the length (*l*), height (*h*), and width (*w*) of a human eye were different (Deller, O'Connor and Sorsby, 1947; Sorsby and O'Connor, 1945), and the proportion of these dimensions was related to refractive errors (Atchison, Jones, Schmid et al., 2004; Sorsby, 1953; Spooner, 1957). For instance, it has been reported that the changes in *l:h:w* ratio associated with the changes in refraction were about 2:1:1 (Deller, O'Connor and Sorsby et al., 1947) to 3:2:1 (Atchison, Jones, Schmid et al., 2004), but eyeshape was not significantly different between the emmetropes and hyperopes in these studies (see also (Atchison, Pritchard, Schmid et al., 2005; Verkicharla, Mathur, Mallen et al., 2012)). Fledelius and Goldschmidt (Fledelius and Goldschmidt, 2010) pointed out that the axial length is only slightly larger than the equatorial diameter, and close to spherical even in high myopic eyes (≥6.00D). In fact, most emmetropic retinal shapes are oblate rather than spherical (Atchison, Pritchard, Schmid et al., 2005). In recent years, a study reported that the retinal shape was less oblate in myopes compared to that in emmetropes (Verkicharla, Mathur, Mallen et al., 2012). In support of Van Alphen’s claim: “corneal power and axial length are by far the most important factors in determining the refraction, …, any compensation by decreased lens power or increased chamber depth becomes immaterial (Van Alphen, G. W. H. M., 1961)”,

Spooner (Spooner, 1957) found that the axial length was longer than the transverse diameter (horizontal) and the central corneal radius of curvature was smaller in the myopic eyes, whereas the transverse diameter was longer than axial length and the central corneal radius of curvature was larger in the hyperopic eyes. In accordance with Spooner's results, recent studies (Carney, Mainstone and Henderson et al., 1997) also found that the cornea of myopic eyes flattened less rapid in the periphery than those of emmetropic eyes. In other words, steeper corneal curvatures were found in myopes. According to Stenström's data, the correlation between axial length and corneal radius of curvature was +0.31 (Van Alphen, G. W. H. M., 1961). With respect to the ratio of axial length to corneal radius of curvature (AL/*r*), a high correlation was reported between AL/*r* and spherical equivalent (Llorente, Barbero, Cano et al., 2004), and a previous study has postulated that a high AL/*r* as a risk factor for juvenile-onset myopia (Grosvenor and Goss, 1998). All these human studies indicate that an "eyeball" is not simply a "ball" and different types of ametropia are associated with altered ocular dimensions and radius of curvatures. In contrast to the human eyes, the eyeshape in birds can be classified into three types (globular, flat, and tubular), and the eyeshape of chicks belongs to the flat type (Walls, 1942). Despite the fact that numerous studies using animal models have demonstrated a close relationship between ocular axial dimensions, corneal curvature and myopia development, only a few studies focused on the morphogenesis of astigmatism. Astigmatism has been

linked to the changes in anterior (Weale, 1988) and posterior eyeshapes in human (Buckingham, 1993; Weale, 1988), whether there is a causal relationship remains unclear.

In visual optics, the paraxial region is the region around the optical axis containing light rays of slope angle up to 2°, equivalent to 0.4mm in diameter of the macular region (Emsley, 1952). Foveal refraction is confined within the paraxial region, while peripheral refraction is the measurement outside this region. Relative peripheral refraction, which is defined as a relative change in refraction with respect to paraxial refraction, has been used to predict the ocular shape since the 30's of last century (Ferree, Rand and Hardy, 1931; Hoogerheide, Rempt and Hoogenboom, 1971; Millodot and Lamont, 1974; Rempt, Hoogerheide and Hoogenboom, 1971). Although myopic eyes were frequently associated with a change in peripheral refraction (Atchison, Pritchard and Schmid, 2006; Logan et al., 2004; Millodot, 1981; Mutti, Sholtz, Friedman et al., 2000; Seidemann and Schaeffel, 2002), Rosén et al. (Rosen, Lundstrom, Unsbo et al., 2012) has questioned the role of peripheral refraction in the development of myopia based on the results of earlier studies.

Furthermore, the off-axis (also called peripheral or oblique) astigmatism, or third order aberration, the major type of monochromatic aberrations, increases with eccentricity away from the fovea (Gustafsson, Terenius,

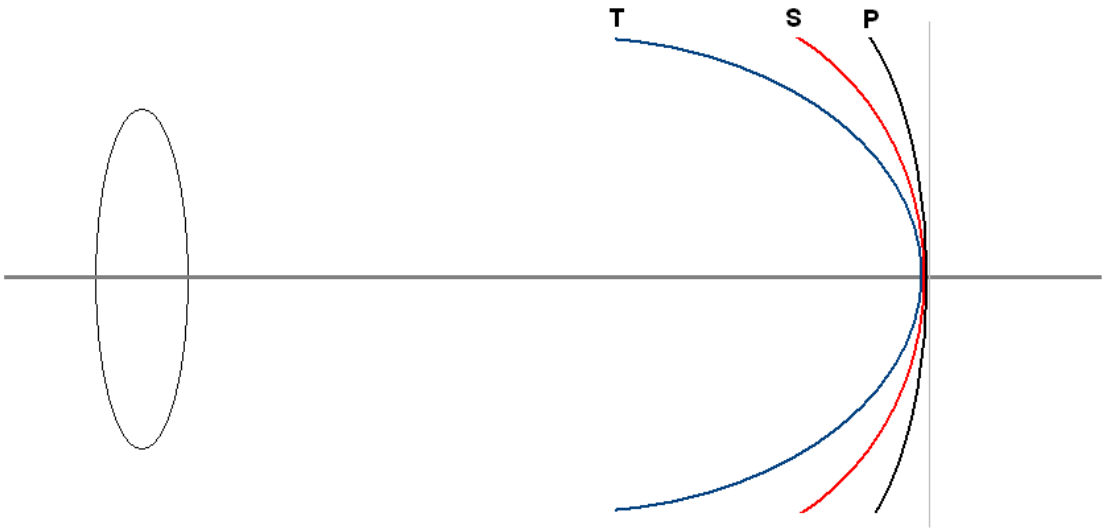

Figure 1-4 Illustrates the image surfaces formed by an astigmatic lens where P is the Petzval surface; T the tangential surface; and S the sagittal surface. According to the third order aberration theory, the longitudinal separation between T and P is three times the separation between S and P (Rabbetts, 2007). Therefore, when the image is in focus at the center, the tangential and sagittal are out of focus at the periphery, with the tangential (or circumferential) details blurred to a greater extent than sagittal (or radial) details.

Buchheister et al., 2001; Millodot, 1981) due to mismatch between two image shells (tangential and sagittal[1], see Figure 1-4) and retinal shell. The differences in image contrast in radial and circumferential contours provide defocusing signals to the retina which have been considered as a cue (Wallman, 1993) or a hindrance (Fulton, Hansen and Petersen, 1982) to the refractive eye growth. As noted earlier, refractive errors developed as a result of the failure of a coordinated eye growth (Spooner, 1975) especially during the critical period (Daw, 1995). Many studies proposed that myopia is axial in nature (Atchison, Jones, Schmid et al., 2004; Spooner, 1957). Myopic eyes usually have longer eyeball than emmetropic eye; high myopes sometimes have abnormal eye shapes, e.g., staphyloma (Curtin, 1977; Curtin, Iwamoto and Renaldo, 1979). Weale (Weale, 1988) tried to explain the relationship between oblated eyeshape and corneal ellipticity (physiological astigmatism); and he found the deformation of cornea was explicable in terms of tensile forces in the globe. Although close association between high astigmatism and toricity of the sclera was found (Buckingham, 1993; Weale, 1988), the causal relationship has not been definite. Flitcroft wrote: “for any given paraxial focal length and standard clinical refraction, the profile of peripheral field curvature is sensitive to the asphericity of the corneal and lenticular surfaces” (D. I. Flitcroft, 2012). In his review article, a ray tracing program was used to generate “how changes in both eye shape and ocular surface asphericity can alter the off-

[1] Field curvature is another type of monochromatic aberration that closely related to the off-axis astigmatism. An image formed by a curved lens is focused on a curved surface, the Petzval surface (Rabbetts, 2007).

axis spherical equivalent refraction without changing on-axis refraction (D. I. Flitcroft, 2012)". According to his demonstration, prolate corneal and scleral profiles both create higher peripheral hyperopia, whereas oblate corneal and scleral profiles create relatively more peripheral myopia. Most importantly, Flitcroft underscored that it is impossible to speculate eye shape solely from off-axis refraction data, and vise versa.

Characteristics of Astigmatism in humans and animals

The characteristics of astigmatism found in both human and animals are species-dependent. In humans, with-the-rule astigmatism is predominantly found in school-aged children (V. Dobson, Miller and Harvey et al., 1999; S. P. Fan, Rao, Cheung et al., 2004; Mohindra and Nagaraj, 1977; Mohindra, Held, Gwiazda et al., 1978; Shih, Hsiao, Tung et al., 2004), while the axis of astigmatism shifts to against-the-rule in the elderly (Asano, Nomura, Iwano et al., 2005; Gudmundsdottir, Jonasson, Jonsson et al., 2000; Sawada, Tomidokoro, Araie et al., 2008). The prevalence of astigmatism can be astonishingly high, up to 63% to 78% (Howland, Atkinson, Braddick et al., 1978; Howland and Sayles, 1984; Thorn, Held and Fang, 1987), in certain ethnic groups. In hatchling chicks, about 8.00D ATR astigmatism is found (Schmid and Wildsoet, 1997). In monkeys, about 90% of the infants had <1.00D astigmatism, astigmatism greater than 1.00D was predominantly ATR (Kee, Hung, Qiao-Grider et al., 2002). It is so interestingly to observe that the infantile astigmatism decreases with age, with mirror symmetric

axis in both eyes in monkeys (Kee, Hung, Qiao et al., 2005)), and in humans (Guggenheim, Zayats, Prashar et al., 2008; Mckendrick and Brennan, 1997). The association between astigmatism and spherical ametropia was also frequently reported in human (Fulton, Hansen and Petersen et al., 1982; Green, 1871; Gwiazda, Grice, Held et al., 2000; Heidary, Ying, Maguire et al., 2005), monkeys (Huang, Hung and Smith, 1997; Kee, Hung, Qiao-Grider et al., 2003), chicks (Irving, Sivak and Callender, 1992; Kee and Deng, 2008). Monkeys treated with plus-cylindrical lens developed higher degrees of oblique astigmatism and strong association with hyperopia. The association was mainly due to the reduced vitreous chamber depth (Huang, Hung and Smith et al., 1997). On the other hand, the presence of oblique astigmatism has been associated with a higher risk of developing amblyopia (Abrahamsson and Sjöstrand, 2003; Charman and Voisin, 1993; V. M. Dobson, Tyszko, Miller et al., 1996; V. Dobson, Miller, Harvey et al., 2003; Miller, Dobson, Harvey et al., 2000).

Whether an eye could compensate for optically imposed astigmatism remains controversial. In the past 40 years, tremendous effort has been devoted to study factors associated with myopia and hyperopia development using well-established protocols such as form-deprivation myopia, lens-induced myopia and lens-induced hyperopia. In contrast to these spherical ametropia animal models, the development of an animal model for astigmatism has not been successful. In 1991, a pioneer study

used a plano/-9.00DC cylindrical lens with axis oriented either vertically (n=3) or horizontally (n=2) over the left eyes to induce astigmatism in chicks (Irving, Callender and Sivak, 1991). As no corneal changes were detected, they suggested that the refractive changes, in particular astigmatism, were due to alteration of the vitreous chamber but not accommodation. However, the validity of their suggestions was confined by the small sample size. Induction of astigmatisms with a plano/-9.00DC cylindrical lens at different orientations (45, 90, 135, and 180 degrees) was attempted in a separate experiment (Irving, Sivak and Callender et al., 1992). The highest and lowest magnitudes of induced astigmatism were respectively found at 45 (about 6.00DC; n=4) and 135 (about 2.00DC; n=4) degrees. In the follow-up study (Irving, Callender and Sivak et al., 1995) with larger sample size [plano/-9.00DC (n=23), plano/+10.00DC (n=16) at 45, 90, 135, and 180 degrees], the highest and lowest induced astigmatisms were found when the plano/+10.00DC lens was oriented at, respectively, 45 (3.75±2.50D) and 135 (1.00±1.50D) axis orientations. In contrast, the highest and lowest induced astigmatism were found when the plano/-9.00DC lens was oriented at 45 (5.75±1.50D), and 135 and 180 degrees (2.25±1.00D and 2.25±1.50D) respectively. About 50% of this induced astigmatism was contributed by the cornea. On the contrary, one study learnt that it was unlikely to compensate for optically imposed astigmatism by morphological changes in cornea or lens in chicks (Thomas and Schaeffel, 2000). Also, lid sutured monkeys frequently exhibited astigmatism without clear evidence of

changes in corneal or lenticular curvatures (Raviola and Wiesel, 1985). In fact, the crystalline lens in chicks has been suggested to be isolated from the refractive development (Hayes, Fitzke, Hodos et al., 1986; Nathan, Crewther, Crewther et al., 1984; Pickett-Seltner, Weerheim, Sivak et al., 1987; Sivak, Ryall, Weerheim et al., 1989; Troilo, Gottlieb and Wallman, 1987). Anyhow, the results of compensatory astigmatism for optically imposed astigmatism could not be replicated in the following studies (Laskowski and Howland, 1996; Phillips and Collins, 2000; Schmid and Wildsoet, 1997; Thibos, Cheng, Phillips et al., 2001; Thomas and Schaeffel, 2000), presumably due to the differences in age, breed of birds, as well as methodology (*e.g.*, lens aperture, lens design) between these studies (Irving, Sivak and Callender et al., 1992; Schmid and Wildsoet, 1997). The internal astigmatism contributed to the induced astigmatism was practically unconfirmed since Irving et al. (Irving, Callender and Sivak et al., 1995) found no differences in anterior chamber depth and lens thickness between the treated and untreated fellow eyes. Smith and colleagues (Kee, Hung, Qiao-Grider et al., 2003; Smith, Huang and Hung, 1998) tried to repeat the experiment in young monkeys treated with crossed-cylindrical lens (+1.50DS/-3.00DC), and found that the induced astigmatism was mostly corneal in nature. However, the astigmatic responses were insensitive to orientation cues, and the axis of the induced astigmatism was typically oblique regardless of the imposed axis orientation (Kee, Hung, Qiao-Grider et al., 2003; Smith, Huang et al., 1998). In line with the results of human

research (V. Dobson, Howland, Moss et al., 1983), Smith et al. (Smith, Huang et al., 1998) suggested that the cylinder-rearing monkeys appeared to adjust their accommodation for the most myopic meridian (MMM). Broadly speaking, most of the cylinder-rearing monkeys showed hyperopic shifts at the end of the treatment, but some of the monkeys were myopic (Huang, Hung and Smith et al., 1997). It is also worthy noting that yoking effects of visual manipulations on the fellow eye's astigmatism were reported in both monkeys (Kee, Hung, Qiao-Grider et al., 2003) and chicks (Kee and Deng, 2008).

Factors Related to the Genesis of Astigmatism

Astigmatism has been associated with several factors including age, refractive errors, ethnicity, extraocular muscle tension, eyelid tension, systemic diseases, and genetic profile. Human eye is 75% of the adult size at birth, reaches about 96% by the age of 3 years, and continues to grow gradually between 3-14 years of age (Larsen, 1971; Song, Kim, Lee et al., 2007 Sep; Sorsby and Sheridan, 1960). At birth, the axis of astigmatism varies considerably (Mohindra, Held, Gwiazda et al., 1978) and its magnitude usually decreases with age (Abrahamsson, Fabian and Sjöstrand, 1988; V. Dobson, Fulton and Sebris, 1984; Ehrlich, Atkinson, Braddick et al., 1995; Howland, Atkinson, Braddick et al., 1978; Mohindra, Held, Gwiazda et al., 1978). Permanent neuronal changes in visual cortex will lead to refractive (Boniuk, 1973; Read, Vincent and Collins, 2014) or

meridional amblyopia (Cobb and MacDonald, 1978; Daugman, 1983; V. M. Dobson, Tyszko, Miller et al., 1996; Freeman, Mitchell and Millodot, 1972; Mitchell, Freeman, Millodot et al., 1973) in case significant amount of astigmatism in children is left untreated. On the other hand, a significant positive association between the magnitudes of astigmatism and myopia has been extensively reported (Ehrlich, Atkinson, Braddick et al., 1995; S. P. Fan, Rao, Cheung et al., 2004; Farbrother, Welsby and Guggenheim, 2004; Fulton, Hansen and Petersen et al., 1982; Gwiazda, Grice, Held et al., 2000; Kaye and Patterson, 1997). In this respect, Gwiazda et al. (Gwiazda, Grice, Held et al., 2000) found that the infants who had significant against-the-rule astigmatism were more likely to develop myopia in school age, but others failed to find such an association (Goss and Shewey, 1990; Parssinen, 1991).

Several lines of studies link the ocular mechanical forces to astigmatism. First, Howland and Sayles (Howland and Sayles, 1985) proposed that an unequal extraocular muscle tension may deform the adjacent scleral shape and lead to astigmatism. This hypothesis is supported by the linkage between the changes in cylindrical axis (with-the-rule to against-the-rule astigmatism) with the reduced power of medial rectus in aging population (Marin-Amat, 1956), the significant corneal flattening during convergence (Löpping and Weale, 1965), and the significant change in corneal astigmatism after strabismus surgery (Denis, Bardot, Volot et al., 1995;

Preslan, Cioffi and Min, 1992). This hypothesis is also supported by the linkage between albinism, retinitis pigmentosa, idiopathic nystigmus and with-the-rule astigmatism (Nathan, Kiely, Crewther et al., 1986). Not only the magnitude of astigmatism is affected, paresis of the superior oblique muscle is the most frequent cause of axial deviation (Metz, 1984; Rutstein and Eskridge, 1990; Von Noorden, Murray and Wong, 1986). On average, about 5° exclyclodeviation was detected in patients with superior oblique paresis. Second, Grosvenor (Grosvenor, 1978) proposed that corneal astigmatism is a result of the interaction between the tightness of eyelids and the rigidity of cornea. S'ev Shilo (Shilo, 1977) speculated that the interaction of a firm eyelid with the pliable anterior eyecoat may lead to an astigmatic cornea. Furthermore, lid retraction could alter the corneal toricity (Lieberman and Grierson, 2000; Wilson, Bell and Chotai, 1982), and both the axis and magnitude of astigmatism are associated with the morphology of eyelids (Grey and Yap, 1986; Read, Collins and Carney, 2007a; Wilson, Bell and Chotai et al., 1982). Lastly, some congenital ocular diseases with moderate astigmatism are caused by increased lid tension, for example, ptosis (Cadera, Orton and Hakim, 1992), chalazia (Nisted and Hofstetter, 1974), blepharophimosis (Beckingsale, Sullivan, Wong et al., 2003), eyelid haemangiomas (Dubois, Milot, Ingrid et al., 2006; Robb, 1977), dacryoceles, dermoid tumors (Bogan, Simon, Krohel et al., 1987), epiblepharon (Preechawai, Amrith, Wong et al., 2007). However, one study found no significant correlation between the modulus of elasticity of eyelid and

corneal toricity if age of the participants was controlled (Vihlen and Wilson, 1983).

Systemic diseases have also been associated with astigmatism. For instance, astigmatism was reported in patients with herpes simplex keratitis (Beigi, Algawi, Foley-Nolan et al., 1994), Graves' ophthalmopathy (Mombaerts, Vandelanotte and Koornneef, 2006), Craniosynostotic syndrome (Khan, Nischal, Dean et al., 2003), and Down syndrome (Kim, Hwang, Kim et al., 2002; Little, Woodhouse and Saunders, 2009). Furthermore, tilted optic disc has been associated with high corneal (Bozkurt, Irkec, Gedik et al., 2002; Jonas, Kling and Gründler, 1997; Vongphanit, Mitchell and Wang, 2002) and lenticular astigmatism (Gündüz, Evereklioglu, Er et al., 2002). These observations indicate that factors either directly or indirectly affect the cornea, eyelids, ocular muscles, or the facial features may promote the genesis of astigmatism. To some extent, the phenotypes are determined by genes. In other words, the etiology of refractive errors is multifactorial (T. L. Young, Metlapally and Shay, 2007). The genetic influence on astigmatism has been studied by comparing the refractive errors in monozygotic and dizygotic twins (Hammond, Snieder, Gilbert et al., 2001; J. M. Teikari and O'Donnell, 1989; J. Teikari, O'Donnell, Kaprio et al., 1989; Valluri, Minkovitz, Budak et al., 1999). In contrast to the strong genetic influences on myopia (Goss and Jackson, 1996; Wu and Edwards, 1999; Yap, Wu, Liu et al., 1993; Zadnik, Satariano, Mutti et al.,

1994) and hyperopia, the genetic influences on astigmatism are relatively low (Clementi, Angi, Forabosco et al., 1998; Dirani, Islam, Shekar et al., 2008; Hammond, Snieder, Gilbert et al., 2001; J. M. Teikari and O'Donnell, 1989; J. Teikari, O'Donnell, Kaprio et al., 1989; Valluri, Minkovitz, Budak et al., 1999). A recent meta-analysis study found a candidate gene for astigmatism, VAX2, which plays an important role in the dorsal-ventral development of the eye. However, their results were not at genome-wide level of significance (Lopes, Hysi, Verhoeven et al., 2013). Although no definite gene has been identified to be responsible for refractive astigmatism, evidence for single-model-locus inheritance of corneal astigmatism was defined (Clementi, Angi, Forabosco et al., 1998). The platelet-derived growth factor receptor alpha (PDGFRA) gene was identified as associated with the corneal curvature in both Asians and Europeans (Q. Fan, Zhou, Khor et al., 2011; Han, Chen, Fan et al., 2011), while the FKBP12-rapamycin complex-associated protein 1 (FRAP1) variants influenced corneal curvature in Asians only (Han, Chen, Fan et al., 2011). Basically, refractive errors can be classified into two main categories: physiological and pathological. Pathological astigmatism, for example, can be a consequence of a dominant effect of corneal deformation, like keratoconus (Ihalainen, 1986) and cornea plana (Tahvanainen, Forsius, Karila et al., 1995). In such cases, several loci for both diseases have been identified. In contrast, physiological astigmatism is polygenic and no responsible gene has been identified. Nature and nurture of astigmatism

have been disputable over decades. Subtle genetic differences between strains may lead to different response to the same visual manipulation. As illustrated in a classical experiment, topically applied atropine and optic nerve section have been reported to inhibit form deprivation in pigtail monkeys, but not in rhesus monkeys (Raviola and Wiesel, 1985). To a certain extent ethnicity, extraocular muscle tension, eyelid tension, inherited diseases as well as ocular dimensions, especially corneal curvatures, are all determined by genes. In essence, the gene-gene or gene-environment interactions make the problem more complicated.

Role of Astigmatism in Eye Growth

Ever since the first report on lid-sutured induced myopia in higher primates (Wiesel and Raviola, 1977), visual experience has consistently been shown to play an important role in refractive development in many other species (fish (Shen, Vijayan and Sivak, 2005), amphibians (Schaeffel, Hagel, Eikermann et al., 1994), birds (Hodos and Kuenzel, 1984; Wallman, Turkel and Trachtman, 1978), and primates (Norton and McBrien, 1992; Smith, Maguire and Watson, 1980; Smith and Hung, 1995; Troilo and Judge, 1993)). Further studies showed that the spatial and temporal properties of visual signals can influence the ocular compensatory responses in the presence of visual manipulations (Wallman and Winawer, 2004). In response to partial-retinal form deprivation (Hayes, Fitzke, Hodos et al., 1986; Wallman, Gottlieb, Rajaram et al., 1987) or hemifield lenses (Diether

and Schaeffel, 1997), only the form-deprived or defocused area of the eye was affected, suggesting that the eye growth was controlled by a "local" retinal mechanism (Troilo and Wallman, 1991). In addition, optic nerve and ciliary nerve sectioning (Schmid and Wildsoet, 1996; Shih, Fitzgerald and Reiner, 1994; Troilo, Gottlieb and Wallman et al., 1987) provided further evidence to support the hypothesis that the local retinal mechanism was neither dependent on the central nervous system nor under the control of accommodation. There are hints that visual manipulations not only affected the posterior globe but also the anterior structure of the eye. The alteration of corneal shape has been reported in ametropic monkeys (Kee, Hung, Qiao-Grider et al., 2003; Smith, Huang and Hung, 1998) and chicks (Hodos, 1990; Irving, Sivak and Callender et al., 1992). In addition, Schmid and Wildsoet (1997) (Schmid and Wildsoet, 1997) also found that constant light rearing chicks developed smaller cornea with reduced astigmatism, whereas form-deprived chicks developed larger cornea with higher astigmatism when compared with the normal untreated eyes. It has been suggested that astigmatism could guide emmetropization (Howland, 1982), because most human infants exhibit a significant amount of astigmatism and the magnitude of astigmatism reduces during normal maturation (Atkinson, Braddick and French, 1980; Ehrlich, Atkinson, Braddick et al., 1995). However, the presence of astigmatism early in life is also associated with myopia later in life (Ehrlich, Atkinson, Braddick et al., 1995; Fulton, Hansen and Petersen et al., 1982). This is possibly due to the image

degradation caused by the uncorrected astigmatism that triggers the onset of form-deprivation myopia (Smith, Huang et al., 1998).

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The Three Hypotheses in this thesis

Due to the paucity of research on astigmatism and its structural correlates, this thesis aimed to answer questions including:

- Does hemiretinal form deprivation lead to astigmatism?
- Does hemiretinal form deprivation at different retinal regions produce similar magnitude of changes in eyeshape?
- Can the presence of astigmatism affect corneal accommodation?
- Can chick eye compensate for optically imposed astigmatism?
- How do the orientation and magnitude of optically imposed astigmatism influence refractive development and eyeshape?

The three key hypotheses tested in this thesis were:

Hypothesis 1: hemiretinal form deprivation leads to region-specific ocular changes and astigmatism of specific characteristics (Chapter 2);

Hypothesis 2: the corneal accommodation in chicks is not related by the presence of astigmatism (Chapter 3);

Hypothesis 3: the eye growth regulating mechanism is sensitive to the orientation and magnitude of optically imposed astigmatism (Chapter 4).

Guided by the refined methodology and results in the first two experiments, the changes in corneal and scleral shapes in response to optically imposed astigmatism were studied in the third experiment. A more in-depth introduction and discussion of each study can be found in the following three chapters.

CHAPTER 2 EFFECTS OF HEMIRETINAL FORM DEPRIVATION ON CENTRAL REFRACTIVE DEVELOPMENT AND POSTERIOR EYE SHAPE IN CHICKS.

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Introduction

Access to normal visual experience is essential for normal refractive development during early eye growth. Ever since lid-sutured macaque monkeys were first reported to develop abnormally long eyeball and axial myopia (Wiesel and Raviola, 1977), the profound effects of early visual form deprivation on inducing axial elongation and refractive error development were further confirmed in other animal species tested (guinea pig (Howlett and McFadden, 2006); fish (Shen, Vijayan and Sivak et al., 2005); tree shrew (Sherman, Norton and Casagrande, 1977); marmoset (Troilo and Judge, 1993); chicks (Wallman, Turkel and Trachtman et al., 1978)). More astonishingly, when nasal or temporal retina was obstructed by translucent occluder in chicks, only the corresponding part of the posterior globe protruded and became myopic (Wallman, Gottlieb, Rajaram et al., 1987), regardless of whether the optic nerve was intact or not (Troilo, Gottlieb and Wallman et al., 1987). Importantly, this “local mechanism” has also been reported in infant rhesus monkeys recently; specifically, covering the temporal retina increased vitreous chamber depth and relative myopia only at the temporal side of the eyeball (Smith, Huang, Hung et al., 2009).

Because the central region of the retina provides the finest visual acuity, it is important to learn how visual experience across the visual field affects the central refractive development. In humans, it was once reported that people who had relative hyperopic errors in horizontal principal power meridians at the peripheral field were more prone to myopia development (Hoogerheide, Rempt and Hoogenboom et al., 1971; Rempt, Hoogerheide and Hoogenboom et al., 1971). Although a recent study (Rosen, Lundstrom, Unsbo et al., 2012) questioned the interpretation of the results in these studies, increasing evidence from animal models suggests that optical error signals imposed on peripheral retina may act as a cue for regulating eye growth. In infant rhesus monkeys, covering the animal's peripheral retina by a diffuser with unobstructed central vision induced axial myopia; strikingly, the recovery from this induced myopia with unrestricted vision was virtually unaffected in the absence of an intact fovea (Smith, Kee, Ramamirtham et al., 2005). In chicks, it has been shown that diffusers covering different extents of peripheral retina have significant impacts on the magnitude of axial ametropia (Irving, Callender and Sivak et al., 1995; R. A. Stone, Pendrak, Sugimoto et al., 2006). Taken together, both the presence of local mechanism and the regulating role of peripheral vision on central refractive development indicate a potential relationship between peripheral eye shape and axial ametropia. Although the classifications of ametropic groups according to estimated eye shape is not yet conclusive, it has been suggested that the incorporation of biometric parameters

associated with 3-dimensional eye shape could be useful in studying refractive error development (R. A. Stone and Flitcroft, 2004).

Despite growing evidence of the interaction between eye shape and central refractive development, very little is known about the relationship between eye shape and manifest astigmatism. Given the facts that astigmatism is frequently associated with ametropic eyes (humans (Alward, Bender, Demske et al., 1985; Guggenheim and Farbrother, 2004; Kaye and Patterson, 1997; Parssinen, 1991), monkeys (Kee, Hung, Qiao et al., 2005), chicks (Kee and Deng, 2008)) and that alterations in ametropic axial growth are primarily related to structural and molecular changes that occur at the posterior segment (Rada, Matthews and Brenza, 1994; Siegwart and Norton, 1999), it is reasonable to speculate that astigmatism is related to abnormal posterior eye growth. This hypothesis is in line with the suggestion that axial eye growth may alter anterior ocular structures through stretching (Mutti, Zadnik, Fusaro et al., 1998; Van Alphen, G. W. H. M., 1986), and the correlation found between the changes in axial length and corneal power during early infancy (Mutti, Mitchell, Jones et al., 2005). This study aimed to determine the effects of hemiretinal form deprivation on central refractive development and eye shape using chicks as an animal model.

Materials and Methods

Animal Subjects

White Leghorn chickens (*Gallus gallus domesticus*, n=87) were used. They were reared in a temperature controlled (about 22ºC) animal facility on a 12-hour light / 12-hour dark lighting cycle, with food and water provided *ad libitum*. The average light illuminance was approximately 100 lux at chick's eye level. Care and use of the animals were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocol was reviewed and approved by the Animal Subjects Ethics Sub-committee of The Hong Kong Polytechnic University.

Visual Manipulations

All diffusers used in this study were heat-molded using 0.5mm-thick white polystyrene plastics. A full retinal diffuser, which had a dome shape with an internal aperture diameter of 13mm and a sagittal height of 4mm, was cut into two equal halves to make the hemiretinal diffuser. These hemiretinal diffusers were used to cover superior, inferior, temporal or nasal retina by using the chick's pupillary center as a reference point (see Figure 2-1A for an illustration). Each hemiretinal diffuser, which was first glued to the hook side of a Velcro ring, was attached to the loop side of a Velcro ring that was glued to feathers around the animal's right orbit. All the left eyes were untreated and used as control.

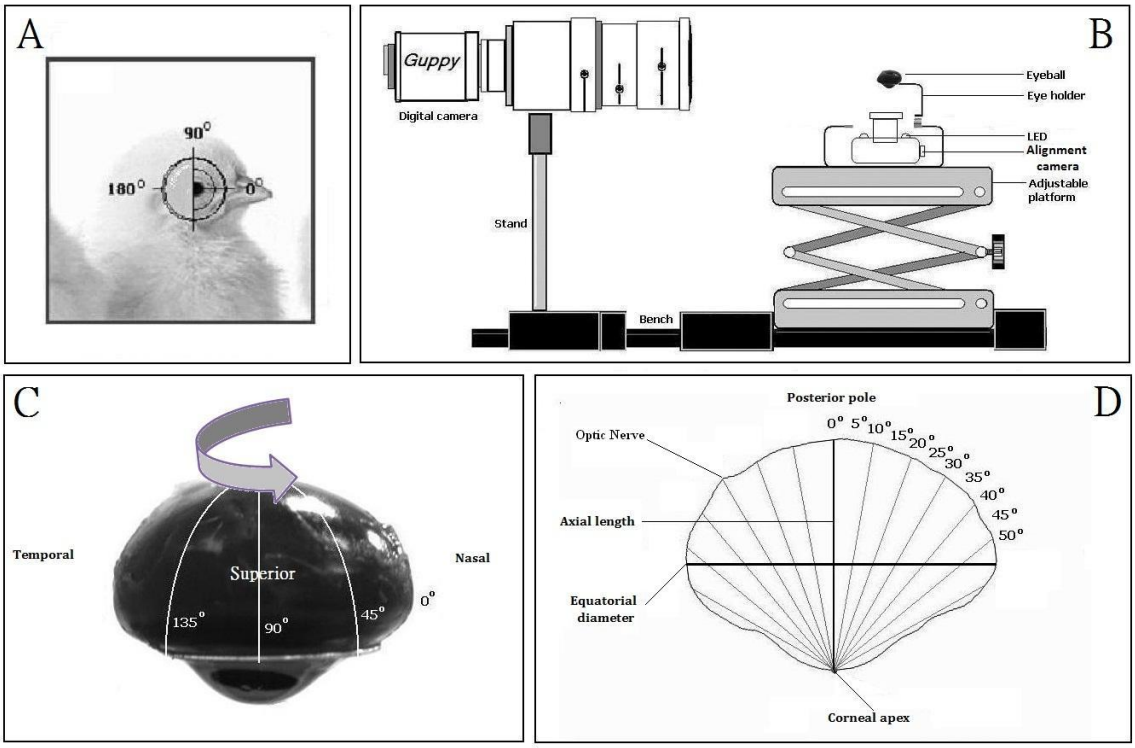

Figure 2-1 A) Hemiretinal diffusers were used to deprive half of the visual field by aligning the diffuser's edge with pupil centre. In this example, the nasal retinal is form deprived. B) Schematic diagram of the set-up of imaging system, the pupillary axis of the eyeball was aligned with the optical axis of an alignment camera guided by eight LEDs built around the camera's aperture; the eye shape profile was captured by the Guppy CCD camera. C) The profiles of the enucleated eyeball at four meridians were captured consecutively by revolving the eyeball around the pupillary axis. D). The edge of each eyeball's profile was first extracted by a custom MatLab algorithm and posterior ocular parameters were derived for eccentricities up to 60° in 5° intervals on each side.

After baseline refractions were carried out at 5 day of age, the animals were randomly assigned to receive one of the four visual manipulations: superior ("SRD", n=17), inferior ("IRD", n=14), temporal ("TRD", n=23) or nasal retinal form deprivation ("NRD", n=23). The hemiretinal diffusers were daily removed daily for cleaning purposes throughout the treatment period.

Refractometry

Refractive errors were measured on day 5 post-hatching and weekly after that for 3 weeks by a Hartinger refractometer (Jena Coincidence Refractometer, Model 110, Carl Zeiss Meditec, Jena, Germany) modified for small pupils (Wallman and Adams, 1987). During refractions, birds were anaesthetized with Isoflurane inhalation (1.0% to 1.5% in Oxygen for rapid induction and low percentage of possible complications (Furtado and Andrade, 2013)). Although isoflurane administration can lead to dopaminergic alteration in human (Votaw, Byas-Smith, Voll et al., 2004) and cycloplegia in normal chick eyes (Wallman and Adams, 1987), no significant effect on astigmatism measurements in chick has been reported (Schmid and Wildsoet, 1997). After the chick was anesthetized, the palpebral fissure was aligned horizontally, and the lower eyelid was pulled down gently using a lid retractor without causing any distortion of the refractometer mire. Refractive errors appear to vary according to the size, strength and position of the lid retractor in conjunction with eyelid tension. Furthermore, because

the cornea does not behave in accordance with Gauss's law of elastic dome (Sokol, Tammaro, Haji et al., 2005), the coupling ratio is not equal to one (*i.e.,* the change in steep K is not equal to the change in flat K). Thus, the effect of lid retractor may affect not only the astigmatic components, but also the spherical components. Therefore the design and application of lid retractor should be treated with caution. Nonetheless, in practice, previous studies (Kee and Deng, 2008; Schmid and Wildsoet, 1997) have shown that the presence of lid retractor produced minimal effect on both spherical-equivalent (0.20D to 0.70D) and astigmatism measurements. For each datum, three sets of measurements were taken for each eye and the average was calculated using an algorithm based on power vectors analysis (Thibos, Wheeler and Horner, 1997). Unless otherwise stated, the data were presented as inter-ocular differences (treated eye–untreated eye; no yoking of eye growth was assumed) in spherical equivalent M, R-J0 and R-J45 astigmatic vector components (Thibos, Wheeler and Horner et al., 1997), (Spherical equivalent (M) = S + C/2; R-J0 = -1/2•C•cos(2α); R-J45 = -1/2•C•sin(2α); where S is the magnitude of the spherical power, C the magnitude of the cylindrical power and α the axis of the minus cylinder) (Thibos, Wheeler and Horner et al., 1997). All the measurements were taken at about the same time of the day (10:00am±1hour) to minimize the effects of potential diurnal variations on refractive error measurements (Johnson, Lytle, Troilo et al., 2004).

Eye Shape Profile Imaging

Eyeball images were acquired along four different meridians to extract posterior eye shape profile. After the last refractions were performed on day 26 post-hatching (*i.e.*, 3 weeks of treatment), subsets of chicks from each treatment group (SRD, n=9; IRD, n=8; TRD, n=10; NRD, n=11) were sacrificed by carbon dioxide asphyxiation. Each eye was first land-marked with a fine-tip marker on its sclera at 12 o'clock (superior) position, enucleated, cleared of extraocular tissues and muscles, and photographed. The setup of imaging device is illustrated in Figure 2-1B: the enucleated eyeball was rested on an eye holder with its anterior part facing down and its pupil center aligned with the optical axis of an alignment camera (USB camera, Polar Techno-color Ltd., HK). The alignment was judged by using the corneal reflexes of eight LEDs around the camera. Once the alignment was fixed, images of the eyeball's profiles along each of the four meridians were captured consecutively using a CCD camera (Camera: Guppy F-036B, Lens: C-mount lens, 50mm, Tamrom 23FM50SP, high resolution, f/2.3; Allied Vision Technologies, Staltroda, Germany) by revolving the eyeball around the pupillary axis through the eye holder (Figure 2-1C). The acquired image was later processed using a custom MatLab algorithm (MatLab; The MathWorks, Natick, MA) to determine the eye shape parameters. In particular the posterior eye shape profile was represented by extracting ocular lengths measured from central to peripheral eccentricity

up to 60°, in 5° intervals, using the corneal apex as a reference (Figure 2-1D). Furthermore, to determine the effects of hemiretinal form deprivation on posterior eye shape, the ratio of axial length (AL) to equatorial diameter (ED) along a particular meridian was calculated for each bird. The AL was derived from the vertical dimension enclosed by the corneal apex and a point on the posterior scleral surface, whereas the ED was derived from the widest horizontal dimension in each image (Figure 2-1D). For SRD and IRD birds, AL/ED data were calculated from the images acquired along the superior-inferior (vertical) plane only; for TRD and NRD birds, the AL/ED values came from the dimensions measured using the images along the temporal-nasal plane (horizontal) only. For comparison purposes, eye shape profile was recorded from a separate group of birds reared with similar protocol except that the right eyes were treated with monocular full retinal form deprivation (FRD, n=10).

Data analysis

Statistical analyses were carried out using SAS Enterprise Guide 4.1 (SAS Institute Inc., Cary, NC). Because our primary aim was to determine the effects of hemiretinal form deprivation on central refractive component and eye shape parameters, the data of FRD treated birds were therefore not included in the statistical tests. Repeated measure analyses (via proc mixed (Thiébaut, Jacqmin-Gadda, Chêne et al., 2002)) were applied to test

the effects of treatment, treatment duration and their interaction on treated eyes. If the interaction was statistically significant, the treatment effect was subsequently tested by one-way ANOVA and the effect of treatment duration was tested by one-way repeated measures ANOVA. In addition, if ANOVA revealed a significant difference, Tukey's post hoc test was conducted to identify which pairs of treatment were significantly different and Dunnett's post hoc test was conducted to test on which day the change from baseline (day 5) was significant. Pairwise Spearman's correlation coefficients between AL/ED ratio and refractive components were computed and tested for significance. Significance level was set at $\alpha=0.05$.

Results

Effects on inter-ocular difference in spherical equivalent, R-J0 and R-J45

There were significant interactions between treatment and treatment duration in spherical equivalent (M), R-J0 and R-J45 astigmatic components (all interactions, $p \leq 0.05$). Overall, both treatment and treatment duration had significant effects (all $p<0.03$) on these three refractive components.

Treatment Effect (by treatment week)

Spherical equivalent (M)

At baseline, no significant differences were found among the four treatment groups in spherical equivalent (ANOVA, $p=0.17$). After one week,

significant treatment effects were found in spherical equivalent (ANOVA, $p<0.0001$). As illustrated in Figure 2-2, IRD group had significantly less myopic/more hyperopic spherical equivalent compared to the other three groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.01$). In contrast, SRD group had more myopic spherical equivalent than the other three treatment groups (Tukey's adjustment for pairwise comparison, all $p\leq0.01$). After two weeks of treatment, significant treatment effects in spherical equivalent still persisted (ANOVA, $p<0.0001$), with the SRD group exhibited more myopic spherical equivalent compared to the other three groups of birds (Tukey's adjustment for pairwise comparison, all $p\leq0.0001$). At the end of the three-week treatment period, the treatment effects were still statistically significant (ANOVA, all $p<0.0001$): the SRD group had significantly more myopic spherical equivalent than the other three groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.01$), whereas the IRD group had consistently low amount of hyperopia (+1.24±0.36D), and which was significantly different from SRD and NRD groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.005$) but not to the TRD group (Tukey's adjustment for pairwise comparisons, $p=0.09$).

R-J0 and R- J45

At baseline, no significant differences in R-J0 and R-J45 components were

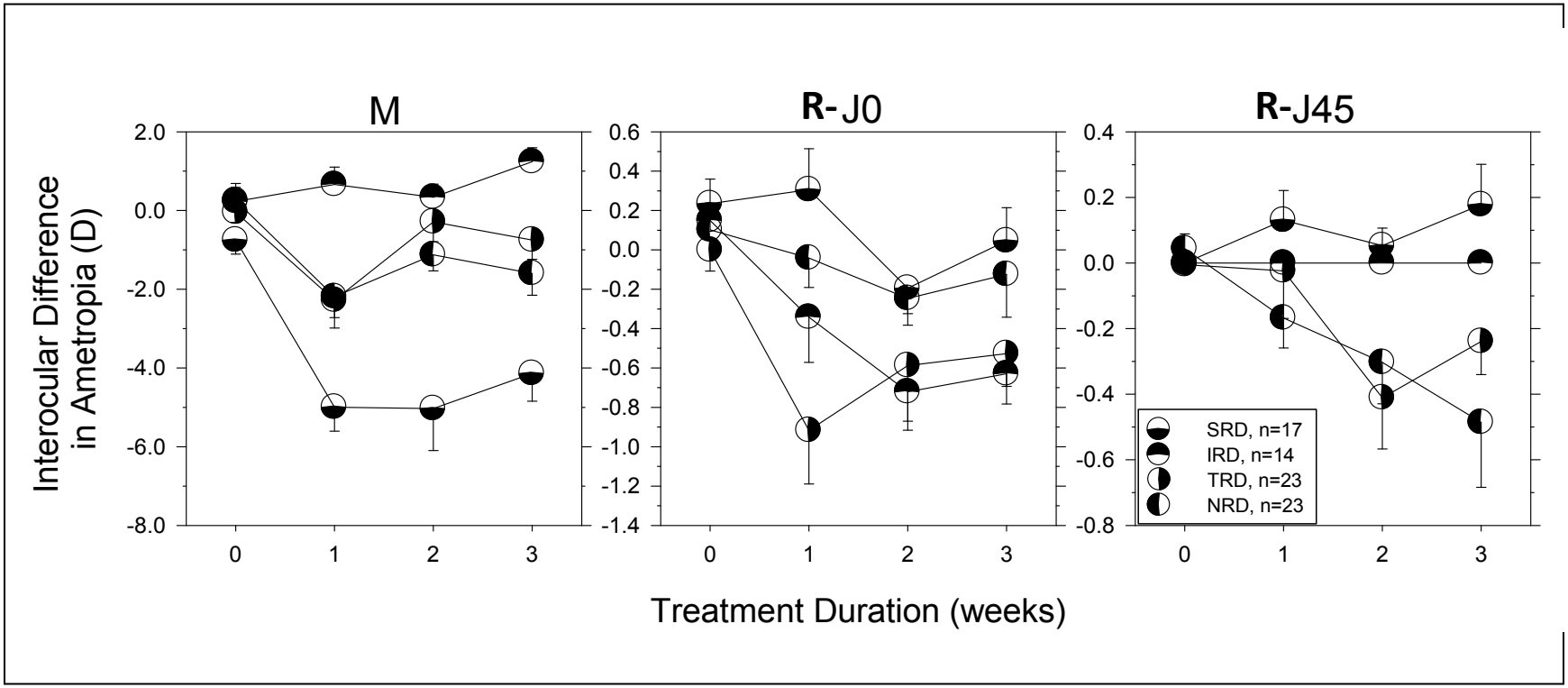

Figure 2-2 Longitudinal changes (mean±SE) of the inter-ocular differences in spherical-equivalent refractive error, M, and astigmatic components, R-J0 and R-J45 over the 3-week treatment period.

found among the four hemiretinal treated groups (ANOVA, all p>0.39). After one week, significant treatment effects were found on R-J0 (ANOVA, p=0.001) but not on R-J45 (ANOVA, p=0.29). In particular, the TRD group had more minus R-J0 component compared to both NRD and SRD groups (Tukey's $p \leq 0.02$) but not to the IRD group (Tukey's p=0.32). On week two, significant treatment effects were found on R-J45 (ANOVA, both p=0.02) but not on R-J0 (ANOVA, p=0.25). The TRD group had R-J45 component significantly more minus compared to those of SRD group (Tukey's p=0.04). At the end of the 3-week treatment period, significant treatment effects were found on both R-J0 and R-J45 (ANOVA, both $p \leq 0.03$), the TRD exhibited more minus R-J0 compared to SRD with borderline significance (Tukey's, p=0.066) and the NRD exhibited more minus R-J45 compared to the SRD group (Tukey's $p \leq 0.01$).

Treatment Duration Effect (by treatment type)

Spherical equivalent (M)

Treatment duration had significant effect on spherical equivalent for the SRD, NRD, and TRD groups (all $p \leq 0.04$) but not for IRD group (p=0.08). For both SRD and NRD groups, the relative changes from baseline in spherical equivalent at all three time points (*i.e.*, 1st, 2nd and 3rd weeks) were significant (Dunnett's post hoc tests, all $p \leq 0.04$) except on the 1st week of NRD group (Dunnett's post hoc test, p=0.065). For TRD group, the

changes from baseline in spherical equivalent was significant only on the 1st week (p=0.02).

R-J0 and R- J45

Treatment duration had significant effects on R-J0 component for all (all p≤0.03) except NRD groups (p=0.24), and on R-J45 component for the NRD group only (p=0.012). With respect to baseline, significant more minus R-J0 was found on 2nd week for SRD group (Dunnett's post hoc test, p=0.02), on 1st and 3rd weeks for TRD group (Dunnett's post hoc test, p<0.01), and on 2nd and 3rd weeks for IRD group (Dunnett's post hoc test, p<0.007). For the NRD group, significant more minus R-J45 was found on 3rd week only (Dunnett's post hoc test, p=0.03).

Posterior Eye shape Parameters

Hemiretinal form deprivations produced an enlarged eyeball in general with local expansion corresponding to the deprived region. Figure 2-3 illustrates the posterior eye shape profile (mean length+SE) as a function of eccentricity with reference to the corneal apex for the four hemiretinal treatment groups (half-filled symbols), a full retinal form-deprived group (filled symbols), and all the fellow untreated eyes as a control group (open symbols) after the 3 weeks observation period. Eye shape profiles along

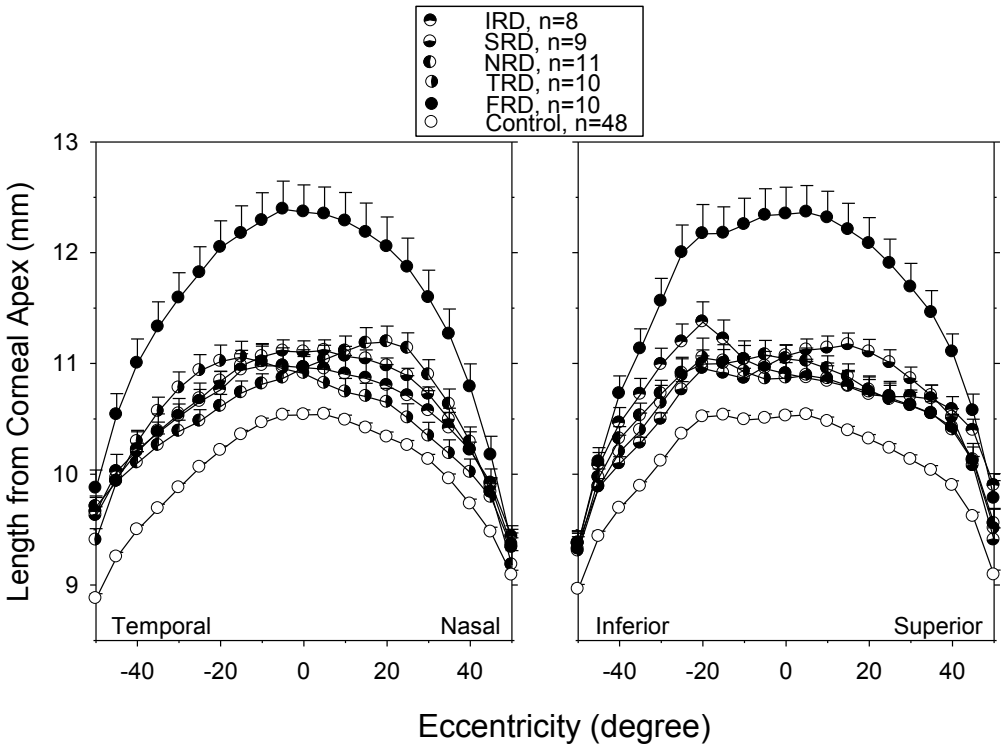

Figure 2-3 Posterior ocular dimension (mean+SE) as a function of eccentricities with respect to corneal apex for hemiretinal treated (semi-filled symbols) and fellow untreated eyes (open symbol). Data along the horizontal (left) and vertical meridians (right) were presented with their anatomical positions indicated on the x-axes. Data from full retinal form-deprived (filled symbols) birds were presented for reference purposes. Note that the standard errors for control eyes were small (max.=0.048) and were thus hidden by the symbols.

the horizontal (left) and vertical (right) meridians were presented with the corresponding anatomical locations indicated on x-axes. Compared to the fellow untreated eyes, all hemiretinal form deprivations resulted in an overall enlargement of eyeball with a protruded area corresponding to the form-deprived region. It should be noted that this enlarged posterior segment applied to both covered and uncovered regions, which could partly be due to a reduction in light level with the proximity of hemiretinal diffuser. Furthermore, the differences between hemiretinal and full retinal form deprivations were more pronounced near the posterior pole but appeared to diminish near 50° eccentricities. To illustrate the ocular expansion due to hemiretinal and full retinal form deprivations at all meridians, Figure 2-4 plots the percentage increase in eye dimension (treated eye /fellow untreated eye) for five eccentricities (*i.e.*, 10°, 20°, 30°, 40° and 50°) from central. For each eccentricity, percentage increase at the eight locations (two locations on each meridian) was calculated individually and averaged for each treatment group. In the polar plot, each datum represents an average increase in percentage (radius) at a particular retinal location (see Figure legend) for a treatment group. To visualize the local effects more easily, the data for the same treatment group are color coded as shown in Figure legend. Compared to full retinal form deprivation (black lines), which produced symmetric eye expansion for virtually all measured meridians in the posterior pole from 10° to 40° eccentricities (see statistics in the following paragraph), the hemiretinal treatment groups resulted in

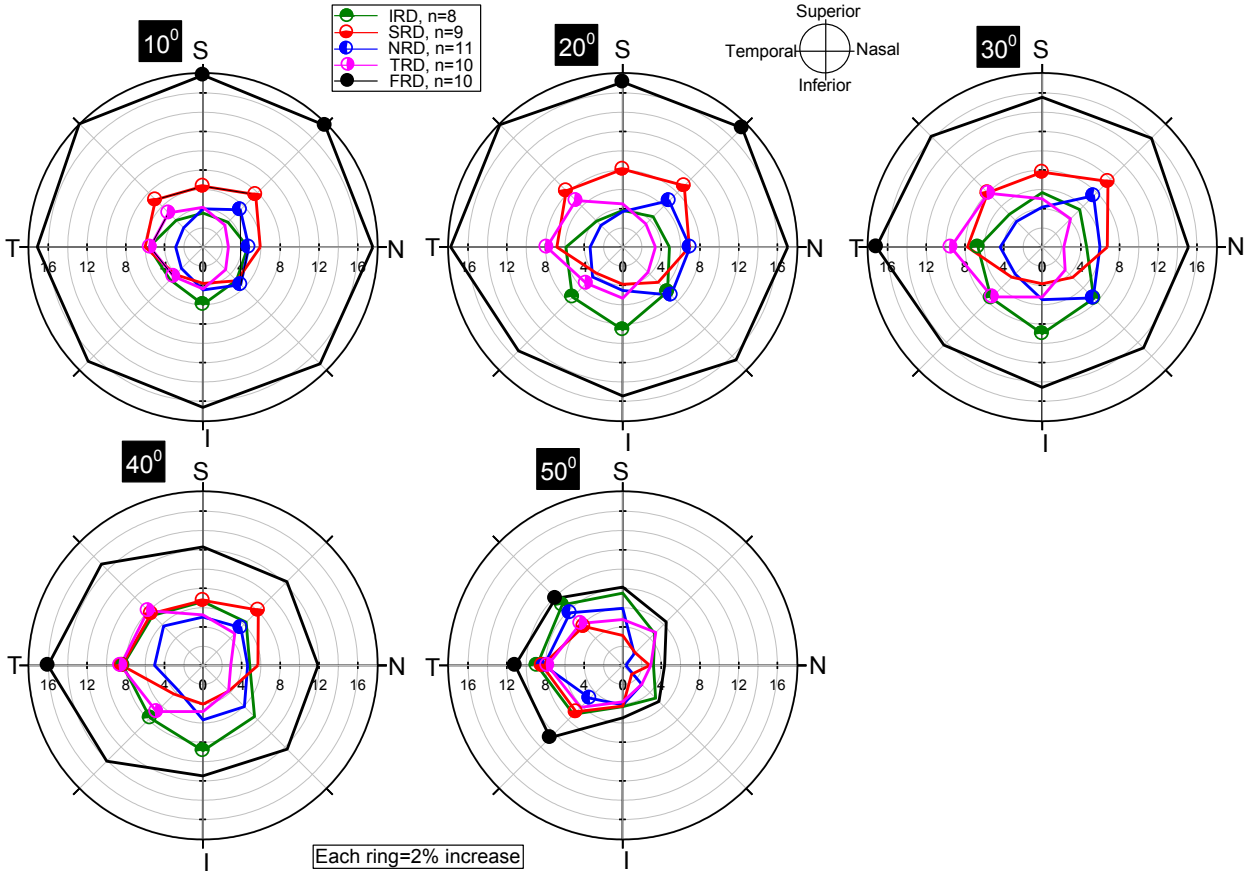

Figure 2-4 Percentage increase in ocular dimensions for treated eyes with respect to fellow untreated eyes (treated eyes / fellow untreated eyes) at five eccentricities. Data for the five treatment groups are color coded as shown in the legend. Each ring represents a 2% increase in the treated eye relative to the fellow eye. A symbol on one side of each meridian represents a statistical significant asymmetric expansion.

asymmetric posterior expansions typically near the central (axial) regions but these asymmetric local effects appear to diminish gradually towards 40° eccentricity. For instance, by comparing the SRD (red lines) and IRD (green lines), one would notice much bigger expansions at, respectively, superior and inferior regions from 10° to 40° eccentricities; however, these treatment effects disappeared at 50° eccentricity. At 50° eccentricity, which was nearby the eye's equator (see Figure 2-1D), although both full retinal and hemiretinal form deprivations still produced relatively bigger eye sizes compared to their fellow untreated eyes, all treatment groups exhibited larger expansion only on the temporal side of the eyeball. To determine if individual treatments had produced asymmetric eye growth on individual meridians, for each of the five eccentricities, the differences in eye expansion between the two opposite locations (*i.e.*, temporal–nasal; superior–inferior; superonasal-inferotemporal; or superotemporal-inferonasal) were calculated for each bird and the group's data were tested to see if the values were statistically significant from zero. As marked in Figure 2-4, any location where a symbol was inserted represented an "asymmetric expansion" along a particular meridian, *e.g.*, asymmetric eye growths were consistently found in SRD group at superior position (superior–inferior>0) from 10° to 40° eccentricities. Further analyses showed that the treatment effects of hemiretinal form deprivation on asymmetric expansion at all four meridians were statistically significant for all (one-way ANOVAs, all $p<0.01$) except the 50° eccentricity (one-way

ANOVAs, all p>0.19).

Figure 2-5 A and B shows the effects of hemiretinal form deprivation on spherical equivalent and AL/ED ratio for the fellow untreated (open) and treated eyes (filled) at the end of the 3-week treatment period. No significant differences in spherical equivalent or AL/ED ratio were found in the fellow untreated eyes across the four treatment groups (ANOVA, all p>0.18). A noteworthy finding was that the fellow eyes also experienced myopic shifts as the deprived eyes, suggesting the possibility of "yoking" of the eyes in responding to unilateral hemiretinal deprivation (R. A. Stone, Liu, Sugimoto et al., 2003; Wildsoet and Wallman, 1995). In contrast, significant treatment effects were found on spherical equivalent and AL/ED in the treated eyes (ANOVA, $p \leq 0.006$). Similar to the results shown in Figure 2-2, this SRD subset also had significantly more myopic spherical equivalent than the other three subsets of treated birds (Tukey's adjustment for pairwise comparisons, all $p \leq 0.01$). More importantly, not only did SRD group show significantly higher AL/ED ratio compared to IRD and NRD groups (both $p \leq 0.03$ after Tukey's adjustment), the IRD group also had significantly smaller AL/ED ratio compared to TRD group (p=0.01 after Tukey's adjustment). In addition, correlation analyses of the 38 treated eyes indicated that spherical equivalent (Spearman's r_S =–0.55, p<0.001), but not R-J0 (Spearman's r_S =0.17, p=0.30) and R-J45 (Spearman's r_S =–0.10, p=0.56), was significantly correlated with AL/ED ratio.

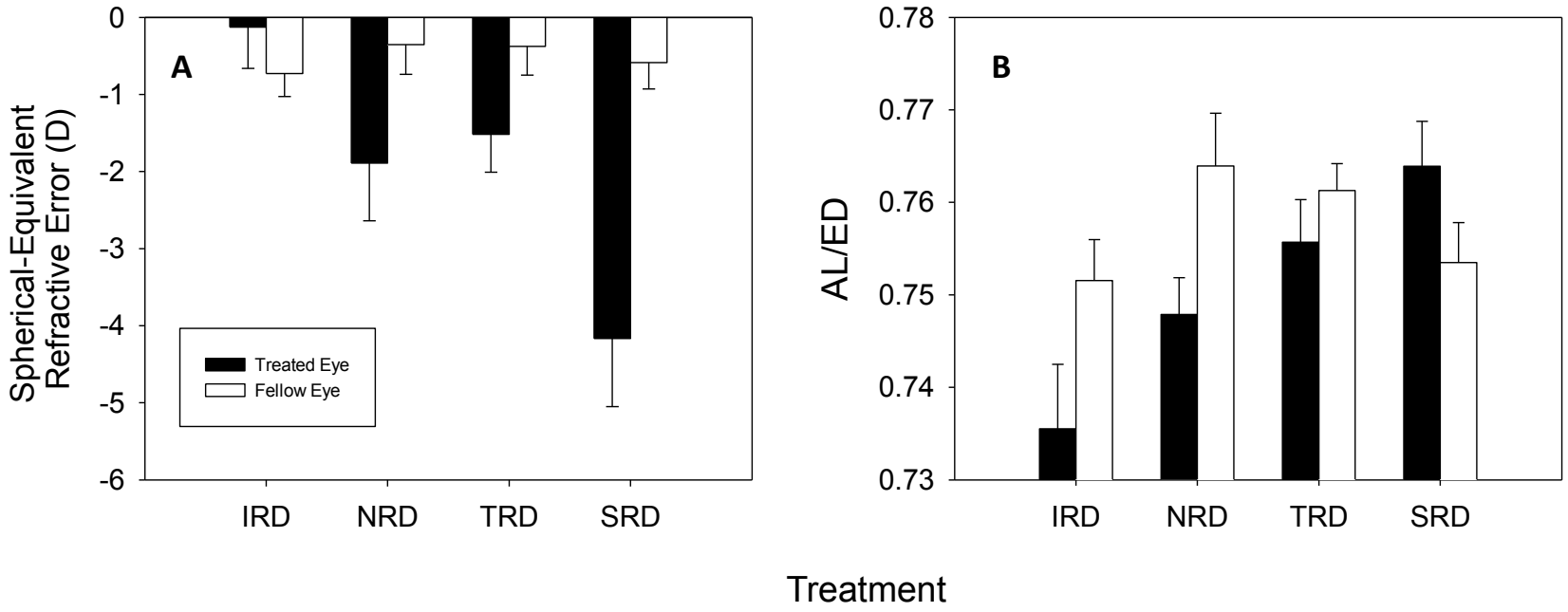

Figure 2-5 Spherical-equivalent refractive error (M) and AL/ED ratio for treated (filled bar) and fellow untreated eyes (open bar) at the end of 3-week treatment period (mean+SE).

Discussion

Our key findings were: 1) the effects of hemiretinal form deprivation on central ametropia and eye shape may vary depending on which hemiretinal was deprived; 2) the induced astigmatism showed subtle differences in magnitudes and properties across the four hemiretinal treatment groups; 3) the spherical-equivalent refractive error in these hemiretinal form-deprived chicks was correlated with AL/ED ratio.

Our results provide further evidence that hemiretinal form deprivation could also alter central ametropia in chicks. The magnitude of this induced myopia, however, was much smaller when compared to previous studies which partially form-deprived retina with diffuser placed 10° beyond the pupillary center or diffuser with a trapezium opening (Troilo, Gottlieb and Wallman et al., 1987; Wallman, Gottlieb, Rajaram et al., 1987), suggesting a more sensitive/plastic region within the 10° central retina. We believe that this lower magnitude of central ametropia came about because the translucent occluder we used to bisect the pupil might have exposed the treated eyes to more than half of the visual field due to eye movements and/or viewing behavior. As shown in Figure 2-3, the corresponding treatment-induced changes in eye shape and dimension were more obvious at 20° to 30° eccentricities, whereas those changes within the 10° eccentricity were smaller in magnitude. Given the facts that chicks could

exert 10° to 20° lateral eye movements (Schippert and Schaeffel, 2006), and that only brief periods of unrestricted vision could significantly attenuate the effects of form-deprivation or defocus-induced myopia (Kee, Hung, Qiao-Grider et al., 2007; Napper, Brennan, Barrington et al., 1997; Shaikh, Siegwart. Jr. J. T. and Norton, 1999; Smith, Hung, Kee et al., 2002; Winawer and Wallman, 2002), it is possible to cover more than half of the retina, like those device used by Wallman and coworkers (Wallman, Gottlieb, Rajaram et al., 1987), the changes in central ametropia and ocular dimensions would have been larger. In this respect, previous studies using occluders (R. A. Stone, Pendrak, Sugimoto et al., 2006) or spherical lenses (Morgan and Ambadeniya, 2006; Schippert and Schaeffel, 2006) with central aperture (*i.e.*, unrestricted central visual field) have consistently shown that central ametropia may be induced only if the critical region around the central retina in chicks is covered (see also Irving et al. 1995 (Irving, Callender and Sivak et al., 1995)). Our results provide further evidence, even if the central retina in the treated chicks might have been partially exposed to unrestricted vision, that covering the four hemiretinal sectors can still produce different impacts on central ametropia (Figure 2-2). Among the four hemiretinal treated groups, IRD and SRD birds exhibited the biggest contrast in the magnitudes of central ametropia and AL/ED ratio. The differential effects of covering inferior and superior retina were also reported in previous studies using chicks (Langford, Linberg, Blaylock et al., 1998; Miles and Wallman, 1990; R. A. Stone, Pendrak, Sugimoto et al.,

2006) and guinea pigs (McFadden, 2002; Zeng and McFadden, 2010). Specifically, using diffusers with apertures oriented eccentrically for chicks to access inferior-nasal or superior-temporal retina (R. A. Stone, Pendrak, Sugimoto et al., 2006) have found that the magnitude of central myopia was much higher in birds with superior-temporal retina covered than those birds with inferior-nasal retina covered. Similarly, as reported in two abstracts, McFadden (*IOVS* 2002; 43: ARVO E-abstract 189) (McFadden, 2002) and Zeng and McFadden (*IOVS* 2010; 51: ARVO E-Abstract 1736) (Zeng and McFadden, 2010) also reported that guinea pigs became more myopic when superior retina was covered with a partial diffuser. It remains unclear whether this differential susceptibility to superior-inferior retinal deprivations is related to regional variations in retinal function and/or ocular structural plasticity. At cellular level, there is evidence that the embryonic developmental pattern is distinctly different between rod and cone photoreceptor subtypes in chicks, with rods developed earlier and distributed more abundantly in the inferior retina compared to cones (Bruhn and Cepko, 1996). Furthermore, the bullwhip and mini-bullwhip cells, retinal cell types which have lately been proposed to regulate eye size in chicks (Fischer, Ritchey, Scott et al., 2008), were also found to distribute asymmetrically in, respectively, ventral and dorsal circumferential marginal retinal regions (Fischer, Skorupa, Schonberg et al., 2006). Further studies are much in need to determine whether this regional variation in cell types can influence the mechanism regulating central refractive development and

eye shape. It would also be interesting to find out if the higher susceptibility to superior retinal form deprivation would lead to ocular pathologies commonly found at superior fundus (*e.g.*, retinal hole and tear) in humans (Kanski, 1989).

The magnitude of induced astigmatism also varied depending on the retinal region receiving form deprivation, albeit its pattern is different from those shown by spherical power components (Figure 2-2). First, although SRD group exhibited the highest magnitudes of spherical-equivalent among the four groups of birds, the same group developed the lowest magnitude of R-J0 compared to other groups. Second, the signs of R-J0 components were negative for all treatment groups but the signs of R-J45 components were somewhat varied across the treatment groups. Specifically, unlike TRD and NRD treatments, which both induced negative R-J45 components, the SRD treatment resulted in a positive R-J45 component. To our knowledge, other than the recent finding that covering the nasal visual field with either diffuser or -3.00D lens produced significantly higher magnitudes of manifest astigmatism in monkeys (Hung, Huang and Smith III, *IMC* 2010, Poster 44), this is the first study which shows that hemiretinal form deprivation could have significant impacts on manifest astigmatism and produced subtle differences in individual astigmatic components in chicks. It should be noted, however, that although subtle differences were found on R-J45 components, the magnitudes of R-J45 were smaller than R-J0 components. Thus, of

those treated eyes that exhibited more than 1.00D of manifest astigmatism, the proportions of against-the-rule (axes range=60 to 120), with-the-rule (axes range= 0 to 30 and 150 to 180), and oblique astigmatisms (observed axes= 35, 135 and 140) were indeed quite similar after 1 week (ATR=75.9%; WTR=22.2%; Oblique=1.85%; total n=54) and 3 weeks of treatment (ATR=82.7%; WTR=13.5%; Oblique=3.8%; total n=52). Strictly speaking, the IRD, TRD and NRD exhibited against-the-rule astigmatism, while only the SRD developed oblique astigmatism after three weeks of treatment. How this disproportionately higher prevalence of against-the-rule astigmatism came about remains uncertain despite significant differential treatment effects on posterior eye shape. Because asymmetric ocular expansions were consistently found at 50° temporal side of all treatment groups (Figure 2-4), it would be interesting to study the influence of eye shape profile near equator or anterior to equator on the characteristics of astigmatism induced. Equally importance is to relate the contribution of corneal astigmatism to this induced astigmatism, a project that we are planning to do using a custom-made corneal topography system for small animals' eyes.

Several human studies, using either imaging techniques (Cheng, Singh, Kwong et al., 1992; Deller, O'Connor and Sorsby et al., 1947) or peripheral refractions (Mutti, Sholtz, Friedman et al., 2000), have noted a tendency for myopes and hyperopes to exhibit, respectively, more prolate and oblate eye

shape (for a review see Stone and Flitcroft, 2004 (R. A. Stone and Flitcroft, 2004). However, a reanalysis of previous peripheral refractions data (collected only at 30° temporal retina (Mutti, Sholtz, Friedman et al., 2000)) indicates that classifying refractive groups according to the geometry of eye shape has its limitation; in essence, different kinds of eye shape could be found within each refractive group (R. A. Stone and Flitcroft, 2004). In this respect, our results showed that spherical equivalent was also moderately but significantly correlated with AL/ED ratio (Spearman's r_S=–0.55, p<0.001), indicating that myopia was associated with a more prolate/less oblate eye shape. It should be noted, however, that the AL/ED ratio was calculated based on the values acquired at the presumably most responsive meridians for individual treatment group. As reflected in Figure 2-4, subtle differences in eye shape at all meridians across the four treatment groups were also noted near 50° eccentricity. If AL/ED ratio was recalculated based on the averaged values of all four meridians, the correlation between spherical equivalent and AL/ED ratio actually became even stronger (Spearman's r_S=–0.65, p<0.001), supporting the idea that 3-dimensional eye shape may be a better indicator in relating with central refractive status.

Our results may have important implication on clinical practice. In particular, a comparison of various ophthalmic interventions for controlling myopia progression indicates that the executive bifocal is an effective lens type (Smith, Campbell and Irving, 2013). Because our results showed that

superior retina is most susceptible to form deprivation myopia, it is possible that at the eye's primary gaze the executive bifocal which provides universal addition power for the superior retina might have slowed the myopia progression by providing myopic-defocus related inhibiting signals. This region-specific mechanism needs further studies.

CHAPTER 3 BI-DIRECTIONAL CORNEAL ACCOMMODATION IN ALERT CHICKS WITH EXPERIMENTALLY INDUCED ASTIGMATISM

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Introduction

The extent to which the cornea, the major refractive component of the eye, plays a role in accommodation is still controversial. Although prior studies found 0.40D to 0.72D corneal accommodation in humans aged between 20 to 40 years old (Pierscionek, Popiolek-Masajada and Kasprzak, 2001; Yasuda, Yamaguchi and Ohkoshi, 2003; Yasuda and Yamaguchi, 2005), negative results have also been reported (Bannon, 1946; Buehren, Collins and Carney, 2003; He, Gwiazda, Thorn et al., 2003; Read, Buehren and Collins, 2007; Rosenfield and Gilmartin, 1987). These inconsistent results may be possibly due to methodology differences, difficulties in detecting subtle changes in corneal curvature, or an artifact of eye movement. In contrast to the findings in humans, there is stronger evidence for corneal accommodation in several avian species, including the chicken, which has been proposed as a good model for studying corneal accommodation, because of its prominent amplitude of corneal accommodation (Glasser, Troilo and Howland et al., 1994; Troilo and Wallman, 1985). Previous studies showed significant corneal steepening accompanied with lenticular accommodation (Glasser, Troilo and Howland et al., 1994; Murphy, Glasser and Howland, 1995; Ostrin, Liu, Choh et al., 2011; Schaeffel and Howland, 1987; Troilo and Wallman, 1987) and the total accommodation (*i.e.*,

lenticular plus corneal accommodations) can be over 25.00D (Glasser, Troilo and Howland et al., 1994; Schaeffel, Glasser and Howland, 1988). Indeed, corneal deformation has been estimated to contribute 40.0% to 50.0% (about 6.00D to 9.00D) of the ocular accommodation (Fazio, Grytz, Bruno et al., 2012; Glasser, Troilo and Howland et al., 1994; Schaeffel and Howland, 1987; Troilo and Wallman, 1987). Nevertheless, some studies could not detect any corneal accommodation in chicks (Beer, 1892; Sivak, Hildebrand, Lebert et al., 1986).

Corneal accommodation in chicks has been reported to occur due to the contraction of a longitudinal Crampton's muscle (Walls, 1942). This muscle is the anterior portion of the striated ciliary muscle which originates at the sclera with the scleral occiscles acting as a supporting base (Glasser, Troilo and Howland et al., 1994; Murphy, Glasser and Howland et al., 1995). A direct connection of the muscle to the corneal inner lamella creates a circumferential tension that alters corneal curvature upon muscle contraction. In empirical studies, changes in chick corneal curvature have been measured either by an infrared photokeratometer (García de la Cera, E., Rodríguez, de Castro et al., 2007; Schaeffel and Howland, 1987; Troilo and Judge, 1993) or by a modified keratometer (Irving, Sivak and Callender et al., 1992; Troilo and Wallman, 1987). Ocular accommodation was induced either pharmacologically by treatment with nicotine (Glasser, Troilo and Howland et al., 1994; Schmid and Wildsoet, 1997; Troilo and Wallman,

1987), or electrophysiologically by stimulation of the Edinger-Westphal nucleus (Glasser, Troilo and Howland et al., 1994; Troilo and Wallman, 1987). However, the extent to which experimental manipulations to stimulate corneal accommodation mimic the natural action of the system is still unclear.

Astigmatism is a refractive error frequently associated with myopia (or "nearsightedness") and hyperopia (or "farsightedness") in humans (Read, Collins and Carney, 2007b) and animal models (monkeys (Kee, Hung, Qiao et al., 2005); chicks (Kee and Deng, 2008)). It has been hypothesized that the presence of astigmatism may facilitate the accuracy of accommodative response by utilizing the contrast cues associated with the two principal refractive meridians (Howland, 1982); thus the significant astigmatism found in infants could potentially interfere with the eye's focusing strategy and signaling pathway during early eye growth. However, despite the high prevalence of astigmatism found across different nations (see a summary figure in Kee, 2013), the functional role of astigmatism during normal and abnormal refractive development remains unclear (Kee, 2013). The present investigation had two key aims. First, we sought to identify whether we could detect corneal accommodation in chicks under natural viewing conditions: that is no artificial stimulation, anesthesia, nor the use of lid retractors. Second we sought to test hypothesis that corneal

accommodation in chicks is influenced by the level of either refractive or corneal astigmatism.

Materials and Methods

Animal Subjects

Twenty-two White Leghorn chicks (*Gallus gallus domesticus*) were hatched and raised in a temperature- and light-controlled animal room at The Hong Kong Polytechnic University. The light/dark cycle was 12hr/12hr (7:00am to 7:00pm) and the light illumination was about 100lux at the chicks' eye level. Food and water were provided *ad libitum*. Care and use of the animals were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocol was reviewed and approved by the Animal Subjects Ethics Sub-committee of the university.

Manipulations

In this study, sixteen chicks treated by optical manipulations (see below) that developed >1.00D of corneal astigmatism were included; six age-matched untreated chicks served as controls. To induce astigmatism, the right eyes of the treated birds were covered, from day 5 to day 12 post-hatching, with a crossed-cylindrical lens (+4.00DS/–8.00DCx45, n=3; +4.00DS/–8.00DCx90, n=3; +2.00DS/-4.00DCx180, n=3), a slit aperture

(0.5mm widthx10mm height; horizontal slit, n=3; vertical slit, n=2), or a spherical spectacle lens (+15.00D, n=1; -15.00D, n=1). The fellow eyes were left untreated (we refer to these eyes as, “untreated fellow eyes”). The purpose of this study was to determine the effect of astigmatism on corneal accommodation, so the effects of manipulations on corneal accommodation were not studied in details. Each lens or slit aperture was first glued to a Velcro ring with Norland Optical Adhesive (Norland Products Inc., New Brunswick, NJ, USA) and later attached to the Velcro ring’s adhesive mate, which was glued (Pattex leather contact adhesive, Dusseldorf, Germany) to the feathers around the right eye. During the treatment period, the devices were cleaned every morning. All measurements were performed at 12 days of age.

Measurements

Refractive status was measured under anesthesia with a modified Hartinger refractometer as described in Experiment 1 (Materials and Methods). After refractometry, corneal parameters were measured in alert chicks using a custom-made videokeratography system (VKS) under dim illumination without using lid retractors. To avoid the potential effects of the diurnal effects (Campbell, Bunghardt, Kisilak et al., 2008; Johnson, Lytle, Troilo et al., 2004), the refractions and corneal curvature measurements were performed at 9:00am to 11:00am and 1:00pm to 5:00pm, respectively. The

components of refractive errors (*i.e.*, M, spherical equivalent; MMM, most myopic meridian; MHM, most hyperopic meridian; RA, refractive astigmatism; R-J0 and R-J45, the two astigmatic components of RA) and corneal curvature parameters (*i.e*., MK, mean corneal curvature; FK, flattest corneal curvature; SK, steepest corneal curvature; CA, corneal astigmatism; C-J0 and C-J45, the two astigmatic components of CA) were decomposed using power vector analysis (Thibos, Wheeler and Horner et al., 1997).

The videokeratography system (VKS)

A Placido-ring videokeratography system (VKS) was custom-built for chick eyes. The instrument comprised of a dome (80mm in radius) with an inner aperture of 12mm diameter to house a telecentric imaging system (CCD camera: Guppy AVT F-046, Edmund Optics, NJ, USA). The dome surface has 16 concentric bright rings around the inner aperture (see Figure 3-1A). Unlike our previous version (Xu, Kee, Zhou et al., 2009), the current system used a series of white LEDs (illumination LEDs), instead of a circular fluorescent light, to provide even illumination for the Placido-rings (see Figure 3-1A). A bird could usually maintain its posture for 1-2 minutes if the head was fixed on a headrest with its body laid on an adjustable platform to avoid eye, head, or body movement. In addition, a high speed camera was employed and all images were carefully screened before included for analysis. A good image should have all Placido-ring images concentric with

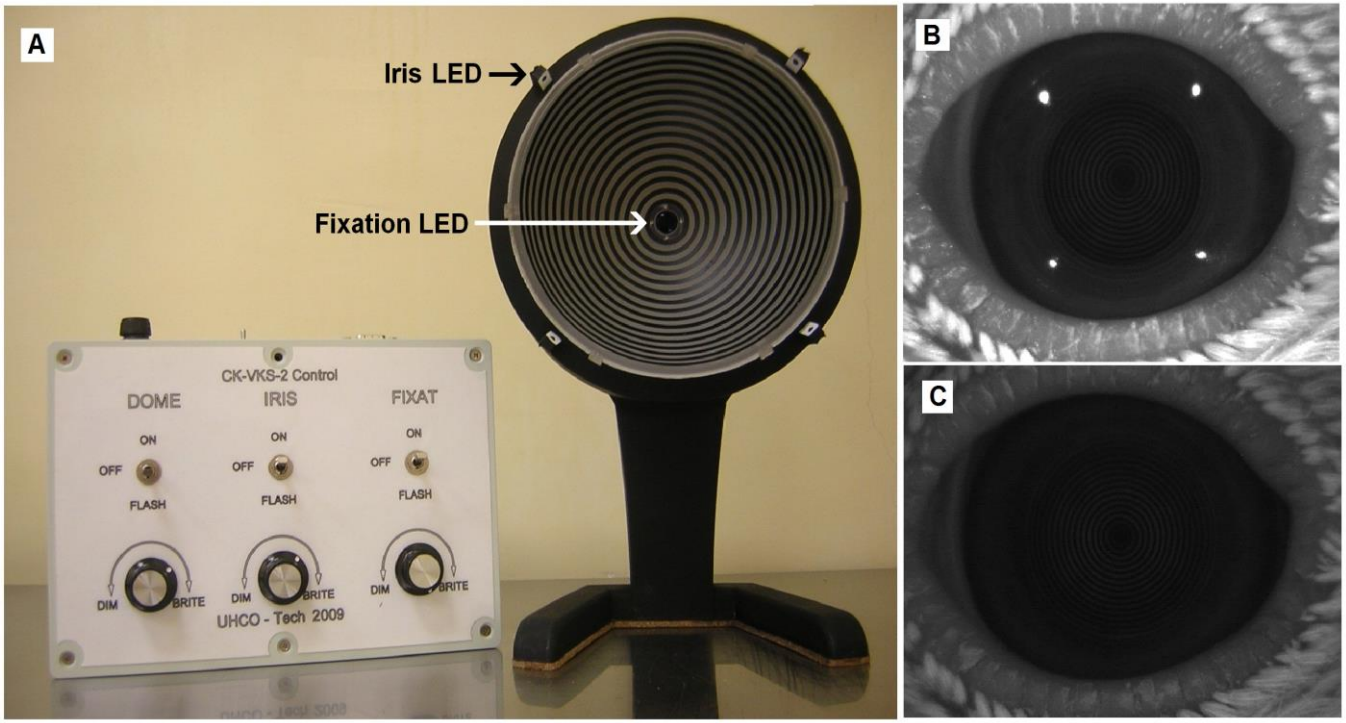

Figure 3-1 A.) The Placido-ring dome (right) and the control box (left) of the videokeratography system. B.) The alignment of the Placido-rings with the pupil center of chick was accomplished by switching on the four iris LEDs. C.) A series of images were acquired for analysis after the iris LEDs were switched off.

the pupil center and the seventh ring (from central aperture) in sharp focus. To align the center of Placido-rings with the subject's pupil center, four infrared LEDs were installed at the outer perimeter of the dome to illuminate the pupil (Figure 3-1A, "iris LED"). These LEDs can be switched off independently after a good focus and a good alignment were achieved (Figure 3-1 B and C). Another four red LEDs were installed near the inner aperture to serve as fixation targets to attract chick's attention (Figure 3-1A, "Fixation LED"). Once the image was aligned and focused at a working distance of 80mm, the iris LEDs were switched off and a series of images were captured in multiple-shot mode (frame rate=49.4 frame per second) using the software (AVT Fire Package version 3.0) provided by the CCD camera.

To derive the common corneal biometric parameters, images of good quality (sharply focused with good alignment) were selected and analyzed via a user interface written in MatLab software (see Appendix for details). All corneal parameters were calculated from the central 2.80mm diameter because the instrumental noise was the lowest (0.18D) when compared to other smaller diameters (see Appendix for details).

Corneal Accommodation

When the chick's attention was directed to the fixation LEDs, only the iris LEDs were switched off (*i.e.*, the fixation LEDs were still switched on) and a series of continuous frames were captured using the multiple-shot mode as described above (500 to 1500 frames, 10.1 and 30.3 seconds duration, respectively). The fixation LEDs, located at 80mm working distance (*i.e.*, 12.50D), were the only stimuli for positive accommodation; no stimulus was used to stimulate the negative accommodation. This procedure was performed on each eye consecutively for all birds. After excluding all distorted or disrupted images from the 500 to 1500 frames acquired from each bird, we were able to identify consecutive frames with obvious changes in Placido-ring images (*i.e.*, changes in the ring-to-ring width) while the center of the Placido-rings did not appear to shift in direction. These changes can be found in all eyes within 30 to 40 consecutive frames, thus all these images were analyzed for the changes in mean corneal curvature (MK) as a reflection of corneal accommodation. The series of frames acquired for each eye were measured for corneal parameters and analyzed for the following statistical parameters. For each eye, the mode of mean K was identified as the most frequently recorded mean K. The positive (PA) and negative (NA) corneal accommodations were defined as the differences in MKs of the mode from the higher and lower values, respectively. Although there was no fixation target for NA, we considered the status with flatter than normal (i.e., resting status) corneal curvature as a reflection of "negative" accommodation, the NA as observed in this study

may well be due to sympathetic limb of accommodation. In addition, the maximum positive (MPA) and maximum negative accommodation (MNA) were identified as the highest and the lowest values from each series of frames of each eye.

The temporal pattern of corneal accommodation between the treated and fellow control eyes were examined in two ways: long intervals, and short intervals. For 4 birds (control, n=1; treated, n=3), we studied the changes in mean K over approximately 300 frames per eye for 4 birds with varying degrees of refractive astigmatism (0.50D to 2.70D in their right/treated eyes, see Figure 3-2 for details). These four birds were chosen because interruptions due to poor image quality, eye movement, and/or lid closure were minimal over a long interval of consecutive frames. For another 18 birds, data from shorter intervals (30 to 40 frames) were analyzed.

Data analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Illinois, U.S.A.). One-way ANOVAs are used to determine if the refractive and corneal parameters are significantly different across the untreated fellow eyes of the treated birds, the right and the left eyes of control birds. One-way ANOVA was also used to determine if there were significant differences in individual parameters across the treated eyes of the

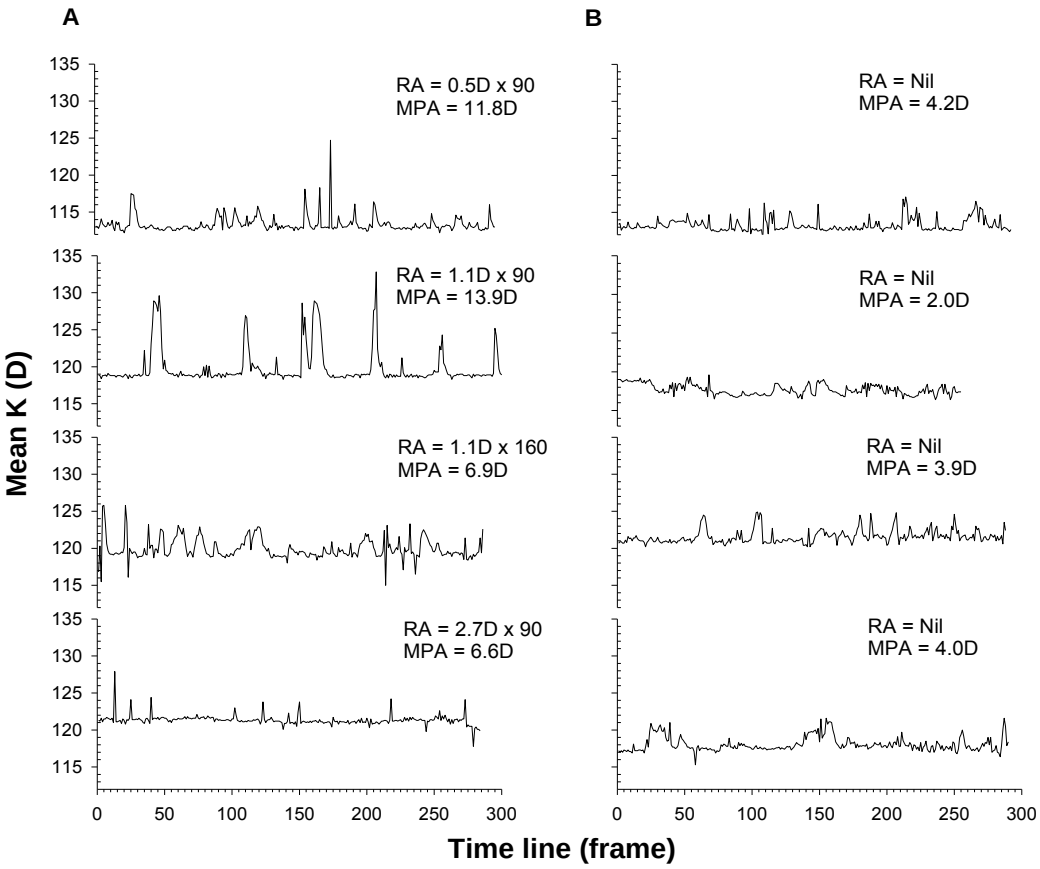

Figure 3-2 The changes in the magnitude of MK over 300 consecutive frames in the treated/right (A) and untreated fellow/left eyes (B) for a control bird (top panel) and three treated birds with different magnitudes of refractive astigmatism. In each plot, the magnitude and axis of refractive astigmatism (RA) and the maximum positive accommodation (MPA) are inserted.

treatment groups (*i.e*., crossed-cylindrical lenses, spherical lenses, and slit apertures). Paired *t*-test was used to determine the differences between the treated and untreated fellow eyes in the treated birds. Pearson's correlation analyses are used to determine if the magnitudes of corneal accommodation in the fellow eyes (*i.e*., right and left eyes) are correlated, as well as whether the magnitudes of corneal accommodation and astigmatism were correlated (*i.e*., right and left eyes of all birds). In all tests, significance level was set at 95% level of confidence. Unless otherwise indicated, data are presented as mean and standard error (mean±SE).

Results

Effects of visual manipulations on refractive errors and corneal curvature

Neither the refractive (spherical equivalent, most myopic meridian, most hyperopic meridian, refractive astigmatism, R-J0, and R-J45) nor the corneal parameters (mean K, FK, SK, corneal astigmatism, C-J0, and C-J45) were significantly different across the untreated fellow eyes of the treated birds, the right and left eyes of the control birds (one-way ANOVA, all $p \geq 0.16$). As summarized in Table 3-1, the treated eyes exhibited significantly higher most myopic meridian, refractive astigmatism, corneal astigmatism, and R-J0 when compared to their fellow untreated eyes (paired *t*-tests, all $p<0.05$); all other refractive (spherical equivalent, most hyperopic meridian, R-J45) and corneal parameters (mean K, FK, SK, C-J0, C-J45) were not significantly different between the treated and fellow

	Treated Group (n=16)		Control Group (n=6)	
	Treated Eye	Fellow Eye	Right Eye	Left Eye
M (D)	-1.95±1.55 (-12.20 to +13.21)	+0.47±0.19 (-0.38 to +1.54)	+0.67±0.32 (-0.38 to +1.71)	+0.93±0.36 (-0.38 to +2.06)
MMM (D)	-3.90±1.58 * (-13.57 to +12.68)	+0.33±0.19 (-0.90 to +1.19)	-0.43±0.32 (-0.38 to 1.54)	+0.76±0.40 (-0.90 to +1.86)
RA (D)	3.14±0.39 *** (1.05 to 6.58)	0.28±0.14 (0.00 to 1.74)	0.47±0.14 (0.00to 1.05)	0.35±0.18 (0.00 to 1.05)
R-J0 (D)	-0.94±0.35* (-3.29 to 1.38)	-0.14±0.07 (-0.87 to 0.00)	-0.23±0.07 (-0.52 to 0.00)	-0.17±0.09 (-0.52 to 0.00)
CA (D)	1.53±0.19 *** (0.47 to 3.09)	0.59±0.08 (0.21 to 1.25)	0.57±0.20 (0.19 to 1.48)	0.75±0.19 (0.34 to 1.66)

Table 3-1 Refractive errors measured after 1 week of treatment or at equivalent age (P12). Data are presented as mean±SE, the range is presented in parentheses. Statistic significance between treated and fellow eyes is marked with asterisk(s) * $P<0.05$, and *** $P<0.001$. M, spherical equivalent; MMM, most myopic meridian; RA, refractive astigmatism; R-J0, refractive J0; CA, corneal astigmatism.

untreated eyes in the treatment groups. The magnitudes of refractive and corneal astigmatism for all eyes as a group were significantly correlated ($r=0.69$, $p<0.001$). With respect to the refractive and corneal parameters in the treated eyes, only mean K, FK, and SK showed significant treatment effects (one-way ANOVAs, all $p<0.02$), with the eyes treated with spherical lenses (mean K: 116.70±2.60D, FK: 115.95±2.45D, and SK: 117.50±2.80D) showing significantly flatter corneal curvature (Tukey's pairwise tests, all $p<0.05$) than those treated with crossed-cylindrical lenses (mean K: 121.21±0.64D, FK: 120.52±0.63K, and SK: 122.03±0.64D) or slit apertures (mean K: 121.92±0.72D, FK: 121.480.84D, and SK: 122.62±0.76D). However, note that there were only two birds treated with spherical lenses, a flatter corneal curvature was found in the +15.00D treated eye (mean K:114.1, FK:113.5, and SK:114.7) than the -15.00D treated eye (mean K:119.3, FK:118.4, and SK:120.3); thus the flatter corneal curvature in this treatment group was mainly due to the +15.00D treated eye.

Corneal accommodation

Longer interval (n=4)

Figure 3-2 shows the temporal changes in mean K over 300 consecutive frames of the right (A) and left eyes (B) for a control bird (top row) and three treated birds (bottom three rows, the right eyes were the treated eyes). The sequence of birds was arranged from top to bottom according to the

magnitude of refractive astigmatism. As can be observed from this figure, the mean K was frequently maintained at a certain level for all eyes, but both the treated and fellow eyes clearly showed bi-directional changes in mean K from this level. In general, the changes in mean K usually took a longer duration for positive (PA, about 200msec) than negative accommodation (NA, about 100msec), and the magnitudes of positive corneal accommodation showed more variability between fellow eyes (control: RE=+1.26±0.20D vs. LE=+1.20±0.29D treated: RE=+2.24±0.44D vs. LE=+1.20±0.29D) when compared to the negative corneal accommodation of fellow eyes (control: RE=-0.33±0.15D vs. LE=-0.44±0.18D; treated: RE=-0.46±0.5D vs. LE=-0.39±0.11D). On the other hand, although the maximum positive corneal accommodation in the four treated/right eyes (Figure 3-2A) were all higher than those in the untreated/left eyes (Figure 3-2B), there were no correlations between the magnitudes of maximum positive corneal accommodation with refractive astigmatism or corneal astigmatism in these four birds. Figure 3-3 compares the frequency distributions of mean K between the fellow eyes of the four birds, the sequence of birds followed that of Figure 3-2. For all eight eyes, the modes of mean K occupied 45±4.6% (range: 32.0% to 65.0%) of the time, and the deviations from the mode of mean K (*i.e.*, excluding the mode) were within 1.00D in 25.2±3.3% (range: 12.0% to 36.0%) and 12.1±3.2% (range: 4.7% to 28.0%) of the time for positive and negative corneal accommodation, respectively.

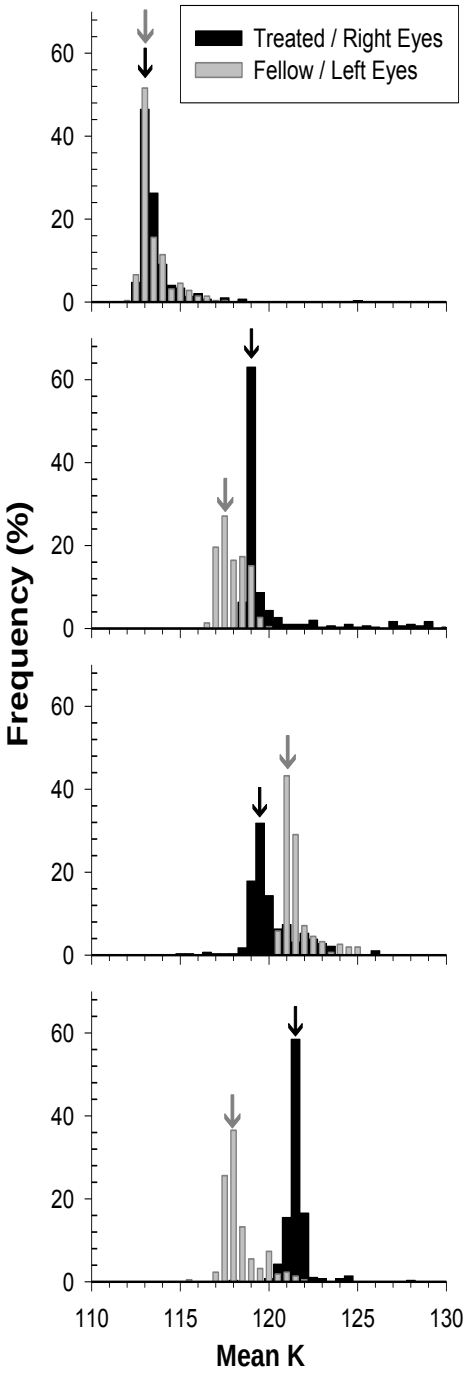

Figure 3-3 The frequency distributions of MK in the treated/right (dark bars) and untreated fellow/left eyes (gray bars) for the four birds in Figure 3-2. The modes of accommodation are marked off with arrow heads.

Figure 3-4 shows the frequency distributions of the changes in corneal astigmatic magnitude (A) and axis (B) for the four birds in the same sequence as Figures 3-2 and 3-3. These changes were calculated by subtracting the modes of each parameter from the corresponding values. On average, the changes in corneal astigmatism during these intervals were within ±1.00D for 99.1±0.4% of the time (ranges: control/untreated fellow eyes: 99.0% to 100.0%; treated eyes: 97.2% to 98.9%), indicating that under most circumstances the corneal astigmatism contributed to at most 0.50D of changes in mean K (since 1.00D cylindrical power=0.50D spherical-equivalent power). On the other hand, the astigmatic axis changed by less than ±20 degrees in 75.2±9.1% of the time, with more variation in the control/untreated eyes than treated eyes (ranges: control/untreated fellow eyes: 22.6% to 90.9%; treated eyes: 72.7% to 97.9%), probably due to the higher instrumental noise when measuring eye with low corneal astigmatism (see appendix and Figure 6-1). Although significant correlations were found between the changes in mean K and astigmatic axis within the three right/treated eyes (*i.e*., the top three right eyes in Figure 3-4B), the correlations were usually low and varied in sign (Pearson's r=-0.24, +0.24, -0.36, all $p<0.001$), indicating that corneal accommodation did not produce consistent pattern of change in the direction of astigmatic axis.

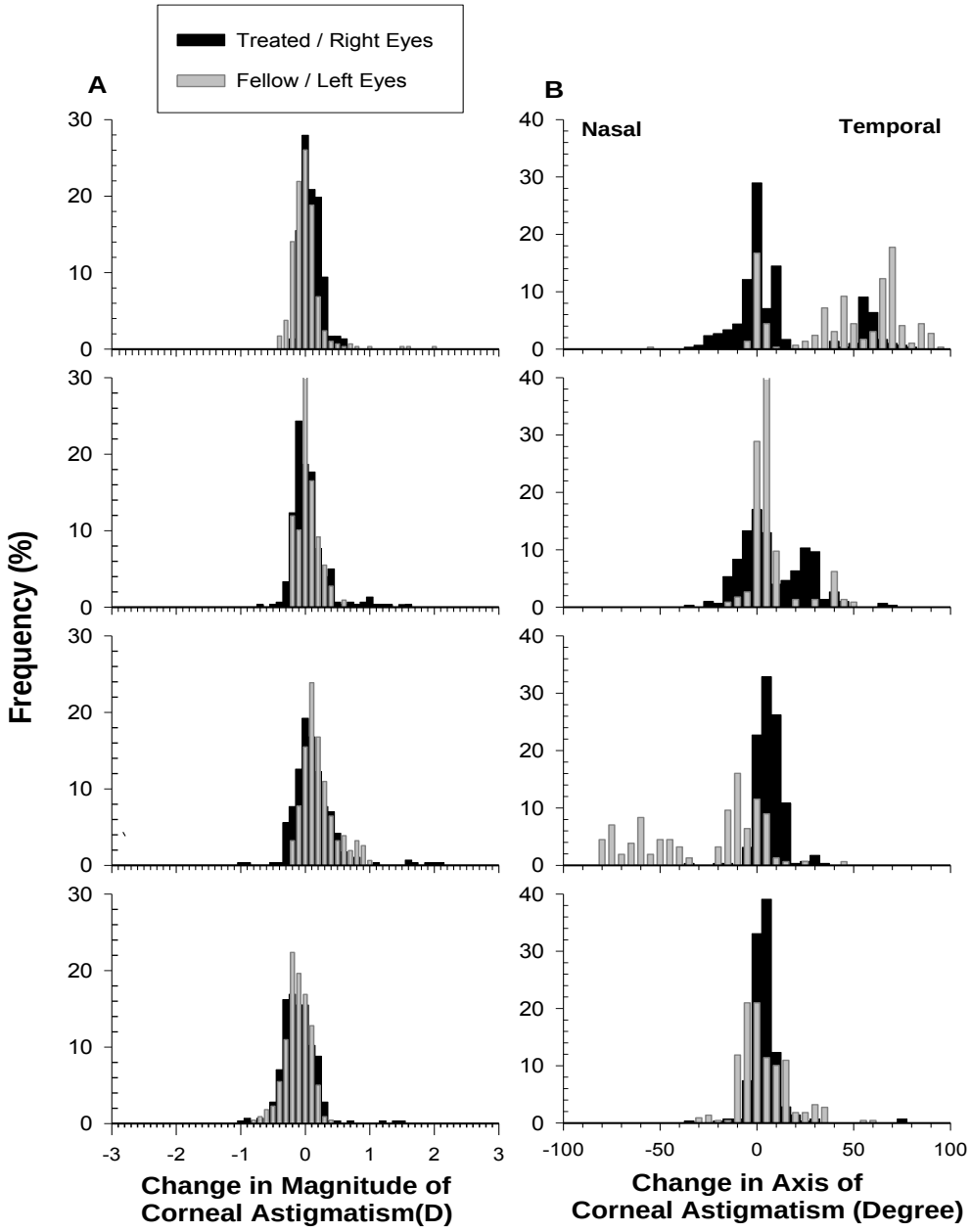

Figure 3-4 The frequency distributions of the changes in magnitude (A) and changes in axis (B) of corneal astigmatism for the four birds in Figure 3-2. The changes are determined by the differences from the mode.

Shorter interval (n=22)

Table 3-2 summarizes the magnitudes of corneal accommodative changes as well as the corresponding changes (relative to the corresponding modes) in astigmatic magnitude and axis. Except the negative corneal accommodation in the fellow eyes of the treated group ($r=0.64$, $p<0.01$), no significant correlations between the fellow eyes were found in all other parameters for the treated and control groups ($r=0.08$ to 0.69, all $p\geq0.10$). Similar to the refractive status (Table 3-1), no significant difference in any of the corneal parameters was found across the untreated fellow eyes of the treated birds and the right and left eyes of the control birds (one-way ANOVAs, all $p\geq0.11$). However, the positive corneal accommodation (+2.24D vs. 1.26D, paired *t*-test, $p<0.05$), standard deviation of positive corneal accommodation (0.39D vs. 0.23D, paired *t*-test, $p<0.01$), and maximum positive corneal accommodation (+7.53D vs. +4.38D, paired *t*-test, $p<0.01$) were all significantly higher in the treated eyes when compared to their untreated fellow eyes. In contrast, the negative corneal accommodation, standard deviation of negative corneal accommodation, and maximum negative corneal accommodation were not significant different between the treated and untreated fellow eyes (paired *t*-tests, all $p\geq0.29$). One-way ANOVAs showed that there was no treatment effect on any of the corneal accommodative changes (all $p\geq0.38$). Interestingly, when data from all eyes were pooled, both the positive corneal

	Treated Group (n=16)		Control Group (n=6)	
	Treated Eye	Fellow Eye	Right Eye	Left Eye
Positive Accommodation				
MPA (D)	+7.53±0.81 ** (3.00 to 15.70)	+4.38±0.53 (1.70 to 9.40)	+4.67±1.47 (1.80 to 11.80)	+4.15±1.16 (1.90 to 9.70)
Δ CA (D)	0.02±0.16 (-1.37 to 1.20)	-0.09±0.07 (-0.82 to 0.40)	-0.14±005 (-0.28 to -0.02)	-0.09±0.14 (-0.69 to 0.13)
Δ Axis of CA (°)	3.21±3.49 (-14.40 to 36.00)	-4.50±5.79 (-53.00 to 38.00)	-12.17±10.89 (-61.00 to 4.00)	-26.78±9.48 (-59.00 to -1.70)
Negative Accommodation				
MNA (D)	-0.92±0.23 (-3.90 to -0.20)	-0.73±0.12 (-2.30 to -0.30)	-0.73±0.19 (-1.30 to -0.40)	-0.87±0.27 (-1.70 to -0.30)
Δ CA (D)	0.09±0.17 (-0.75 to 2.12)	-0.14±0.08 (-1.02 to 0.33)	-0.09±0.17 (-0.75 to 0.27)	-0.15+0.18 (-0.36 to 0.31)
Δ Axis of CA (°)	0.03±2.64 (-15.00 to 26.00)	-3.02±4.58 (-41.00 to 15.00)	-1.83±12.78 (-47.00 to 27.00)	7.67±10.49 (-27.00 to 50.00)

Table 3-2 Corneal Accommodation measured after 1 week of treatment or at equivalent age (P12). Data are presented as mean±SE, the range is presented in parentheses. Statistical significance between treated and untreated fellow eyes is marked with asterisk(s) ** P<0.01. MPA, maximum positive accommodation; MNA, maximum negative accommodation; ΔCA, change in magnitude of corneal astigmatism; ΔAxis of CA, change in axis of corneal astigmatism.

accommodation and maximum positive corneal accommodation were significantly correlated with refractive (positive corneal accommodation vs. refractive astigmatism: r=0.34; maximum positive corneal accommodation vs. refractive astigmatism: r=0.34, both $p<0.05$), but not corneal astigmatism (positive corneal accommodation vs. corneal astigmatism: r=0.13; maximum positive corneal accommodation vs. corneal astigmatism: r=0.10, both $p\geq0.41$). Figure 3-5 illustrates the low but significant correlation between the maximum positive corneal accommodation and refractive astigmatism. On the other hand, positive corneal accommodation was significantly correlated with negative corneal accommodation (r=-0.67, $p<0.001$), but there was no correlation between maximum positive corneal accommodation vs. maximum negative corneal accommodation (r=-0.06, p=0.71), maximum positive corneal accommodation vs. spherical equivalent (maximum positive corneal accommodation vs. spherical equivalent: r=-0.22, p=0.16) or maximum negative corneal accommodation vs. spherical equivalent (r=0.08, p=0.59), nor between the maximum level of accommodation and the change in astigmatic axis (maximum positive corneal accommodation vs. ΔAxis: r=-0.08, p=0.60; maximum negative corneal accommodation vs. ΔAxis: r=-0.03, p=0.86; Table 3-2).

Discussion

The key findings in this study are: 1) both the control and treated eyes in

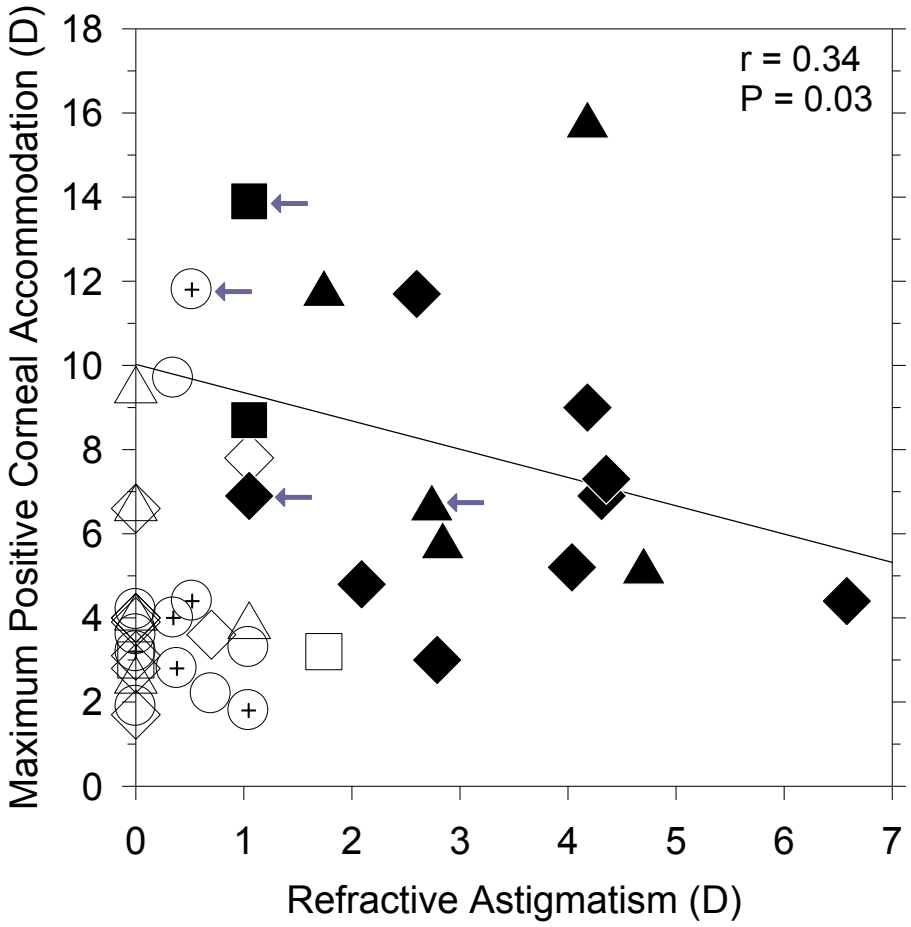

Figure 3-5 The maximum positive corneal accommodation is plotted as a function of refractive astigmatism for the treated (filled symbols) and untreated fellow / control eyes (open symbols). Low but significant correlation was found when all data were pooled. ◇, Crossed-cylinder lens; □, Spherical lens; △, Slit aperture; ⊕, Control right eye; ○, Control left eye. The four birds with data measured from longer intervals are labelled with arrow heads (←). The solid line is the simple regression line between RA and MPA of treated right eyes.

alert chicks demonstrated frequent increases (positive corneal accommodation) and decreases (negative corneal accommodation) in corneal curvature, with positive corneal accommodation showing much higher magnitudes than negative corneal accommodation; 2) the magnitudes of refractive astigmatism and maximum positive corneal accommodation were correlated.

Non-anaesthetized chicks were capable of altering their corneal curvature to become steeper or flatter, although the magnitudes of positive corneal accommodation and maximum positive corneal accommodation were much higher than negative corneal accommodation and maximum negative corneal accommodation (Figures 3-2 and 3-3; Table 3-2). Despite the differences in methodologies (see introduction section) and the age of animals (4 to 10 weeks vs. 12 days) in previous studies with ours, the maximum magnitudes of corneal accommodation reported in previous studies were very similar to what we found in the untreated/control chick eyes (current: 9.40D to 11.80D; Glasser, Troilo and Howland et al., 1994: 9.00D; Schaeffel and Howland, 1987: 9.00D to 10.00D; Troilo and Wallman, 1987:10.00D). On average, the maximum positive corneal accommodation in the untreated/control eyes ranged from 4.15D to 4.67D, only 4 out of these 28 eyes exhibited maximum positive corneal accommodation more than 6.00D (Figure 3-5). Assuming that the 80mm working distance had stimulated 12.50D of total accommodation, our results suggest that the

corneal accommodation contributes about 32.8% to 37.4% of the total ocular accommodation response.

One novel finding in this study was that astigmatic eyes appeared to show a higher maximum positive corneal accommodation. Compared to their untreated fellow eyes, the eyes exposed to various visual manipulations not only developed significant amounts of refractive and corneal astigmatism but also exhibited higher positive corneal accommodation and maximum positive corneal accommodation (Tables 3-1 and 3-2). Furthermore, when data from all eyes in this study were pooled, the magnitudes of refractive astigmatism and positive corneal accommodation or maximum positive corneal accommodation were weakly but significantly correlated (Figure 3-5). It should be noted that during the same intervals when the positive corneal accommodation responses were observed, the changes in corneal astigmatism rarely exceeded 1.00D and the astigmatic axis did not show consistent pattern of change (Figure 3-4 and Table 3-2). A previous study (Schmid and Wildsoet, 1997) using topical agents to stimulate (nicotine) or inhibit (vecuronium bromide) ocular accommodation in chicks also did not find significant changes in the magnitude of astigmatism (0.60D and 0.10D changes, respectively). Likewise, Schaeffel and Howland (1987) also found no significant changes in astigmatic magnitude when alert chicks were accommodating. Taken together, these results indicate that the positive accommodation in chicks is accompanied with very little, if any, changes in

ocular astigmatism, arguing against the presence of accommodative astigmatism in chicks. On the other hand, because astigmatism results in two line foci, it may interfere with the end point of the ocular accommodative system is possible (Howland, 1982). For instance, the presence of induced astigmatism could increase the variability of accommodative behavior is well documented (Stark, Strang and Atchison, 2003). Compared to untreated fellow eyes, the astigmatic treated eyes showed a higher frequency of time spent on positive corneal accommodation (long interval data) and an increased variability of positive corneal accommodation (standard deviation of positive corneal accommodation, Table 3-2), it is possible that these accommodative behaviors in the astigmatic eyes have resulted in higher magnitudes of positive corneal accommodation and maximum positive corneal accommodation. While it should be realized that the corneal accommodation was captured at separate time interval for each eye, the fact that the magnitudes of accommodative parameters were very similar across the untreated/control eyes. (Table 3-2) indicates that the corneal accommodations captured were representative. Thus, the higher magnitudes of positive corneal accommodation and maximum positive corneal accommodation in the treated eyes are more likely to be due to the presence of significant astigmatism and not simply by chance.

Compared to positive corneal accommodation and maximum positive corneal accommodation, negative corneal accommodation and maximum

negative corneal accommodation were much smaller in magnitudes (Figures 3-2 and 3-3, Table 3-2). To our knowledge, only one previous study, reported in abstract form (Troilo, Li and Howland, 1993), documented the features of maximum negative corneal accommodation in chicks; approximately 4.00D of negative accommodation in 2 to 3 week-old non-anesthetized chicks with no measure of corneal accommodation. Thus, our study provides, for the first time, clear evidence of negative corneal accommodation in alert chicks (Figures 3-2 and 3-3, Table 3-2). Although the magnitudes of maximum negative corneal accommodation in this study were only about a quarter of the negative accommodation in the previous study, both findings support the presence of bi-directional changes in accommodative function in chicks. Further study is needed with respect to the underlying mechanism and the functional significance of this negative accommodation.

There were two major limitations in this study: First, the inclusion of birds treated with a variety of different visual manipulations. Because the primary aim of this study was to determine the influence of astigmatism on corneal accommodation, any treated bird exhibiting >1.00D corneal astigmatism was included. The variations in number of chicks in different groups (e.g., n=1 in groups treated with spherical lens) made it impossible to examine the effects of manipulations differentially. Second, the fixation target for stimulating accommodation. Although the eye's fixation and alignment were

monitored carefully during the imaging process, the movements due to breathing could not be avoided; thus, together with the fact that no fixation stimulus was provided for negative accommodation, we cannot rule out the possibility that the negative corneal accommodation was an artifact of eye movement or a consequence of sympathetic limb of accommodation.

In conclusion, we detected bi-directional changes in corneal accommodation by measuring corneal changes in alert chicks. The presence of weak but significant correlation between refractive astigmatism and maximum positive corneal accommodation suggest that the presence of astigmatism might interfere with the image quality and in turn affect the accommodative mechanism.

CHAPTER 4 EFFECTS OF OPTICALLY IMPOSED ASTIGMATISM ON EARLY EYE GROWTH IN CHICKS

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Introduction

Astigmatism is a very common refractive error but its etiology remains elusive (Cox, 2010; Kee, 2013; Read, Collins and Carney, 2007b; T. L. Young, Metlapally and Shay et al., 2007). Uncorrected astigmatism not only degrades the contrast of retinal image at both distance and near, the presence of significant astigmatism with specific orientation has also been associated with amblyopia (Abrahamsson and Sjöstrand, 2003; V. Dobson, Miller, Clifford-Donaldson et al., 2008; Gwiazda, Mohindra, Brill et al., 1985; Mohindra, Held, Gwiazda et al., 1978) and myopia development (Ehrlich, Atkinson, Braddick et al., 1995; Fulton, Hansen and Petersen et al., 1982; Kaye and Patterson, 1997). The prevalence of astigmatism generally declines during childhood (Howland, Atkinson, Braddick et al., 1978; Mohindra, Held, Gwiazda et al., 1978). However, in American Indian, a population known to exhibit high prevalence of significant astigmatism (Harvey, Dobson and Miller, 2006; Harvey, Dobson, Miller et al., 2011; Miller, Dobson, Harvey et al., 2000), prescription of spectacle correction even during early school years did not appear to improve visual functions to normal level (V. Dobson, Miller, Harvey et al., 2003). These findings, together with asthenopia (Lansche, 1966), tilted optic disc (Bozkurt, Irkec, Gedik et al., 2002; Jonas, Kling and Gründler et al., 1997; Vongphanit,

Mitchell and Wang et al., 2002), and abnormal retinal electrophysiology frequently found in astigmats (D. I. Flitcroft, Adams, Robson et al., 2005), spur the needs for understanding the etiology of astigmatism with new approach. Although several factors including genes (Clementi, Angi, Forabosco et al., 1998; Hammond, Snieder, Gilbert et al., 2001), ethnicity (Abraham and Volovick, 1972; Cowen and Bobier, 2003; S. P. Fan, Rao, Cheung et al., 2004; Mandalos, Peios, Mavracanas et al., 2002; Mohindra, Held, Gwiazda et al., 1978; Montés-Micó, 2000; Shih, Hsiao, Tung et al., 2004), nutrition (Lyle, Grosvenor and Dean et al., 1972), age (Fledelius and Stubgaard, 1986; Saunders, 1981), and spherical refractive errors (*i.e.*, myopia and hyperopia) (V. Dobson, Harvey and Miller et al., 2007; Goss, 1999) have been associated with astigmatism in humans, the effect of environmental factor is still unclear.

Visual experience plays an important role in refractive development. In response to form deprivation and spherical defocuses, a wide variety of animal models developed refractive errors (Benavente-Perez, Nour and Troilo, 2012; Hung, Crawford and Smith, 1995; Marsh-Tootle and Norton, 1989; Norton, Siegwart and Amedo, 2006; Shen and Sivak, 2007; Shen and Sivak, 2007; Sivak, Barrie, Callender et al., 1990; Smith, Harwerth, Crawford et al., 1987; Troilo, Nickla and Wildsoet, 2000; Wallman, Turkel and Trachtman et al., 1978; Wallman and Winawer, 2004). By the way of illustration, both chicks and macaque monkeys developed ametropia

primarily axial in nature, with the former animal model responsive to a broader range of spherical defocus than the latter [－30.00D to +15.00D (Irving, Sivak and Callender et al., 1992; Kisilak, Hunter, Huang et al., 2008) vs. －3.00D to +6.00D (Smith, 1998)]. However, could the growing eye alter its ocular components to compensate for astigmatic errors? Different laboratories have investigated this question, but the results were contradictory. An initial study in chicks showed partial compensation for optically imposed astigmatism with significant effects of axis orientation, the highest magnitude of induced astigmatism was found when imposing oblique astigmatism, and about 50% of the induced astigmatism was corneal in origin (Irving, Sivak and Callender et al., 1992; Irving, Callender and Sivak et al., 1995). However, similar results were not replicated subsequently, in chicks (Laskowski and Howland, 1996; McLean and Wallman, 2003; Phillips and Collins, 2000; Schmid and Wildsoet, 1997; Thibos, Cheng, Phillips et al., 2001; Thomas and Schaeffel, 2000) or in monkeys (Kee, Hung, Qiao-Grider et al., 2004; Smith, Huang et al., 1998). On the other hand, although the presence of astigmatism produced a slight myopic or hyperopic shift in some studies (Huang, Hung and Smith et al., 1997; Irving, Sivak and Callender et al., 1992; Laskowski and Howland, 1996; Phillips and Collins, 2000; Schmid and Wildsoet, 1997; Thibos, Cheng, Phillips et al., 2001), it did not appear to affect the compensatory response to spherical defocus (McLean and Wallman, 2003).

The inconclusiveness of previous studies has questioned about the capability of the eye to compensate for astigmatic errors. The primary purpose of this study was to examine how the chick's eye responds to imposed astigmatism with crossed-cylindrical lenses of different axis orientations and magnitudes. The secondary purpose was to determine the correlations between refractive, corneal, and eyeshape parameters in astigmatic eyeball.

Materials and Methods

Animal Subjects

Eggs of White Leghorn chickens (*Gallus gallus domesticus*) were hatched in the university's central animal facilities. The chicks were reared in a temperature controlled (22ºC) animal facility on a 12-hour light/12-hour dark lighting cycle (from 7:00am to 7:00pm), with food and water provided *ad libitum*. The average light illuminance was approximately 100 lux at the chick's eye level. Care and use of the animals were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocol was approved by the Animal Subjects Ethics Sub-committee of The Hong Kong Polytechnic University.

Experiments

Visual Manipulations

At 5-day post-hatching (P5), the chicks were randomly assigned to the treatment or control group. To impose astigmatism, a crossed-cylindrical lens (PMMA, 7.6mm base curve, 10.8mm diameter, 10.8mm optical zone; Conforma, VA, USA) of specific magnitude and axis was held in front of the right eye by using a Velcro mount, and the fellow eyes were left untreated. The optical effect of crossed-cylindrical lens has been illustrated elsewhere (McLean and Wallman, 2003; Raasch, 1995; Thibos, Wheeler and Horner et al., 1997). The opposing powers at two orthogonal meridians create no spherical power, and also no astigmatic power at 45° away from the principal meridians. The minus-cylindrical axis was carefully oriented for individual treatment groups with the palpebral fissure as a horizontal reference line (Irving, Callender and Sivak et al., 1991). During the treatment period (P5 to P12), the lens was removed daily for cleansing; any scratched or cracked lens was replaced immediately. If the lens was found detached, the data of the chick was excluded from further analysis.

The two experiments in this study determined whether and how the orientation and magnitude of optically imposed astigmatism altered early eye growth. In experiment A, the effects of the astigmatic axis on eye growth were determined by randomly assigning the chicks to wear a high magnitude (H) crossed-cylindrical lens of power +4.00DS/－8.00DC with the minus-cylindrical axis oriented at one of four axis orientations (45, 90,

135, and 180; n=20 in each group). These groups were referred to as H45, H90, H135 and H180, respectively. These four orientations were chosen for imposing with-the-rule ("WTR", H90), against-the-rule ("ATR", H180), and oblique astigmatisms (H45 and H135) which are commonly found in humans. Eight age-matched chicks received no treatment served as controls. Since we found significant effects of axis orientation on various biometric parameters in experiment A, in experiment B we tested the effects of magnitude on eye growth by adding two groups of chicks with a lower magnitude (L) crossed-cylindrical power +2.00DS/－4.00DC (L90, n=20; L180, n=18).

Biometric measures

The details of refraction method have already been described in the methodology in Chapter 2. In brief, the refractive status was measured along the pupillary axis using a modified Hartinger refractometer (Jena Coincidence Refractometer, Model 110, Carl Zeiss Meditec, Jena, Germany) in anaesthetized chicks (isoflurane inhalation, 1.0% to 1.5% in oxygen). For each datum, three independent measurements were taken and averaged using power vector analysis (Thibos, Wheeler and Horner et al., 1997). The seven refractive parameters (Spherical components: spherical-equivalent, M; most hyperopic meridian, MHM; most myopic meridian, MMM; Cylindrical components: refractive astigmatism, RA; the two vector components (Thibos, Wheeler and Horner et al., 1997), R-J0 and R-J45,

and the axis) were analysed. To avoid potential effects of diurnal variations on refractive status (Campbell, Bunghardt, Kisilak et al., 2008; Johnson, Lytle, Troilo et al., 2004; Nickla, 2006), all measurements were taken at approximately the same time of the day (10:00am±1hr).

After measuring the refractive changes in a large number of treated chicks, a subset of birds (n=8) were randomly assigned to each group was used for corneal topography and eyeshape imaging. Corneal curvatures and astigmatism were measured using a custom-made videokeratography system in alert chicks (see (Chu, Zhou, Zheng et al., 2014) for details). The system captured Placido-ring images (*i.e.,* the first Purkinje image) in multiple-shot mode and analysed the central 2.80mm-diameter cornea[2] using a custom MatLab algorithm (MatLab; The MathWorks, Natick, MA). In order to rule out the potential effect of accommodation on corneal curvature, only images acquired at relaxed accommodative status were used, these images were identified from 500-1500 Placido-ring images from each bird as demonstrating the most-frequently observed mean corneal curvature (Chu, Zhou, Zheng et al., 2014). The average values of the corneal curvatures along the two principal meridians were calculated, assuming a corneal refractive index[3] of 1.369 (Avila and McFadden, 2010;

[2] Since central 50% of the cornea was nearly spherical (Avila and McFadden, 2010)), we assumed that the cornea was spherical within this area which also covered the entire pupil and instrument noise was the minimum (Chu, Zhou, Zheng et al., 2014).

[3] 1.362 was used in other studies (Glasser, Troilo and Howland, 1994; Sivak, Bobier and Levy, 1978; Troilo and Wallman, 1987), and an "effective index" of 1.332 was suggested (personal communication with Dr. Frank Schaeffel).

Mandelman and Sivak, 1983) from three good images per eye at different time points. Further, seven corneal parameters (steepest curvature, SK; flattest curvature, FK; mean curvature (average of FK and SK), MK; corneal astigmatism, CA; the two vector components, C-J0 and C-J45; and the axis, were derived for further analyses.

Immediately after the chicks were sacrificed by carbon dioxide asphyxiation at the end of the experiment, eyes were enucleated and eyeball profiles were captured along the horizontal and vertical meridians, by an eyeshape imaging system described previously (Chu, Deng and Kee, 2012). A MatLab algorithm was written to extract the following ocular dimensions by referring to the corneal apex: the axial length (AL), ocular lengths up to 50° in 5° intervals (see Figure 4-1 for illustration), and equatorial diameters (vertical equatorial diameter, ED90; horizontal equatorial diameter, ED180). To study the changes in posterior eyeshape in response to cylindrical lens treatment, the inter-ocular differences in ocular dimensions between the treated/right eyes and the fellow/left eyes (*i.e.,* treated/right eye – fellow/left eye) were calculated first from central 0° to 50° eccentricity, in 5° intervals, and summated for the horizontal (ADH, accumulated differences in area along the horizontal meridian) and vertical meridians (ADV, accumulated differences in area along the vertical meridian). We used “unit area” as a general term to represent the unit for these two quantities. The difference

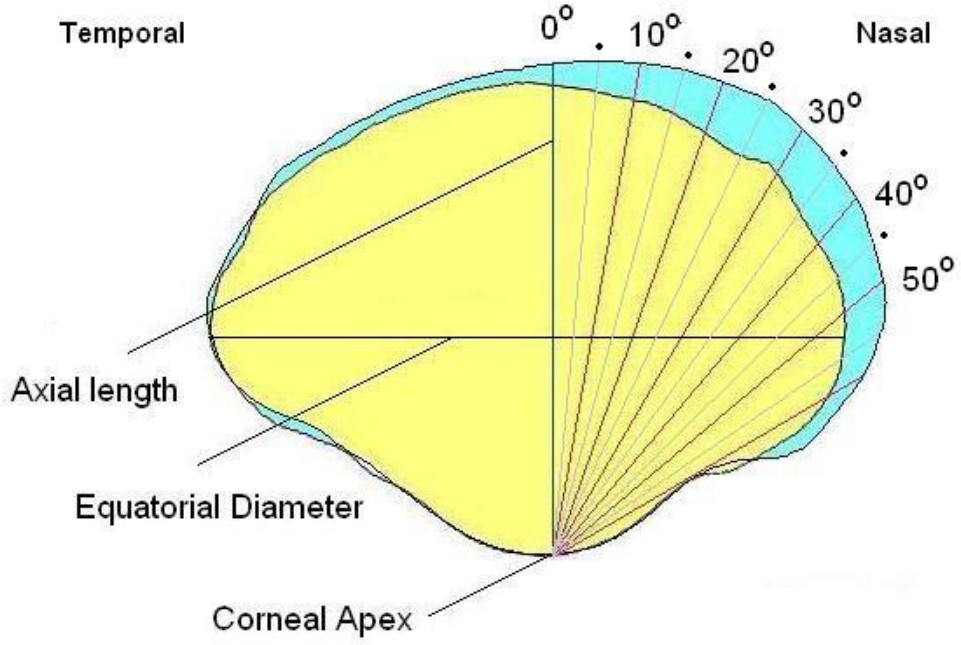


Figure 4-1 The eyeshape profiles of the horizontal meridian for the fellow eyes of a chick treated with +4.00DS/─8.00DCx90. The profile of the treated eye (blue area) is overlaid with that of the untreated fellow eye (yellow area). Axial length, equatorial diameter and ocular dimensions at different eccentricities (from 0° to 50° in 5º intervals), as identified by a MatLab program, are calculated with respect to the corneal apex. Identical image analysis protocol was applied to the vertical (superior and inferior regions) eyeshape profiles.

between ADH and ADV (ADH-ADV) was then calculated to show the meridional difference in ocular expansion of the posterior segment. In addition, the sum of ADH and ADV (ADH+ADV) was calculated to indicate the overall expansion of the posterior globe.

Internal astigmatism

As defined previously (S. S. Duke-Elder and Abrams, 1970; Lyle, Grosvenor and Dean et al., 1972), the internal astigmatism (IA) is the vectorial difference after subtracting the corneal astigmatism from refractive astigmatism (Alpins, 1993; Alpins, 2001; Thibos, Wheeler and Horner et al., 1997).

Data analysis

Statistical analyses were carried out using SPSS16 (SPSS, Inc, Chicago, Illinois, USA) and Oriana Version 4.01 (Kovach Computing Service). Statistical tests aimed primarily to determine the effects of crossed-cylindrical lenses on refractive, corneal and eyeshape parameters. Comparisons across groups were made by one-way ANOVAs. If the one-way ANOVA revealed significant effect, Tukey's pairwise post hoc comparisons were used to determine which groups were significantly different. Two-sample *t*-tests were used to determine the effects of the

astigmatic magnitude between H and L groups. Watson-Williams F-tests (Batschelet, 1981; Mahan, 1991) followed by pairwise comparisons were used to determine the treatment effects on axis orientations, the axis orientations per group were expressed as mean±angular deviation. Paired *t*-tests were used for the comparisons of parameters within eyes (*e.g.*, horizontal vs. vertical corneal curvatures) or between treated/right and fellow/left eyes. Pearson's correlation analyses were performed between refractive, corneal and eyeshape parameters. In all tests, significant level was set at the 95% level of confidence. Unless otherwise stated, all data were expressed in terms of inter-ocular differences (IOD) and mean±standard error (SE).

Results

Pre-treatment refractive status

At the onset of the two experiments, all of the refractive parameters (both spherical and cylindrical components) were not statistically different across the treatment and control groups (one-way ANOVA, all $p \geq 0.40$). The mean spherical equivalent (M) and refractive astigmatism (RA) in each group ranged from – 0.15D to +0.27D and from – 0.09D to +0.18D, respectively.

Post-treatment effects

Refractive status:

Effects of axis of astigmatism (Experiment A):

After 1 week of treatment (P12), there was no significant difference in the spherical equivalent or most hyperopic meridian across the treatment and control groups (one-way ANOVA, both $p \geq 0.11$). However, compared to the controls, the H45 and H90 groups developed significantly more negative most myopic meridian (H45= – 3.18±0.61D; H90= – 3.19±0.30D; and Controls= – 0.44±0.36D, one-way ANOVA with Tukey's post hoc tests, both $p < 0.05$). More importantly, refractive astigmatisms in the four treatment groups were all significantly higher than that in the controls (one-way ANOVA with Tukey's post hoc tests, all $p < 0.001$, see Figure 4-2B). As summarized in Table 4-1, the highest and the lowest magnitudes of induced refractive astigmatism were found in the H90 group (5.51±0.26D) and the H180 group (2.84±0.44D), respectively. As shown in Table 4-2, the four treatment groups also exhibited refractive astigmatisms of different axes (Watson-Williams F-test with pairwise comparisons, all $p < 0.005$, also see Figure 4-2A); the average axes for H45, H90, H135 and H180 were, respectively, 68±7, 84±10, 119±12, and 174±44 degrees. Further analyses of the astigmatic components showed that R-J0s were significantly different between the controls and all treatment groups (one-way ANOVA with Tukey's post hoc tests, all $p < 0.05$), whereas R-J45s were significantly different only between the controls and the H45 and H135 groups (one-way ANOVA with Tukey's post hoc tests, both $p < 0.001$).

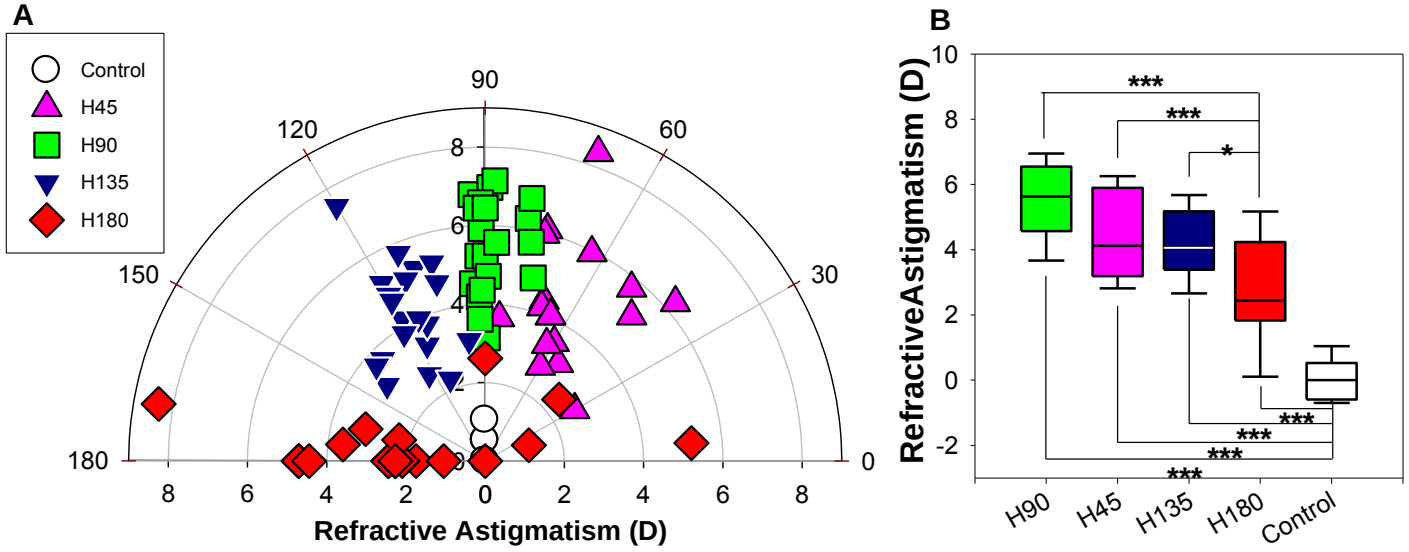


Figure 4-2 (A) Distributions of inter-ocular differences in refractive astigmatism (treated/right eye – fellow/left eye) after one week of cylindrical lens treatment (P5-P12) for the four treatment groups (+4.00DS/–8.00DC, n=20 in each group) with negative cylindrical axis oriented at one of the four directions (45, 90, 135, or 180), as well as the age-matched controls (n=8). The effects of the axis of cylindrical lens are represented by different coloured symbols as shown in the legend. For example, in chicks treated with H90, the +4.00DC and -4.00DC were oriented vertically and horizontally respectively; to compensate for this astigmatic error, the eyes should develop negative cylindrical axis at 90. As shown in A, the cylindrical lenses of different axes induced compensatory astigmatism in the four treatment groups. (B) The box plots of refractive astigmatism include the values of median (line inside the box), maximum (upper whisker), minimum (lower whisker), upper (upper border of box) and lower quartiles (lower border of box) for the controls and treatment groups at P12. The levels of significant differences in the magnitudes of refractive astigmatism across the treatment groups (lines above the boxes), or between treatment and controls (lines below the boxes), are indicated by asterisk: * $p \leq 0.05$, *** $p \leq 0.001$ (Tukey's post hoc tests).

	Crossed-cylinder Lens						Control
Lens Power	(H) +4.00DS/–8.00DC				(L) +2.00DS/–4.00DC		No Lens
Axis (°)	45 [a]	90 [a,b]	135 [a]	180 [a,b]	90 [b]	180 [b]	
n	20	20	20	20	20	18	8
M (D)	–0.94±0.64	–0.43±0.24	–0.39±0.69	0.06±0.35	0.29±0.17	0.74±0.29	–0.41±0.35
MMM (D)	–3.18±0.61*	–3.19±0.30*	–2.53±0.66	–1.36±0.44	–1.76±0.22[#]	0.14±0.27[##]	–0.44±0.36
MHM (D)	1.31±0.70	1.03±0.23	1.75±0.74	1.47±0.39	2.30±0.16	1.34±0.38	–0.42±0.34
RA (D)	4.48±0.34***	5.51±0.26***	4.29±0.27***	2.84±0.44***	4.10±0.16[#]	1.34±0.22	0.03±0.22
R-J0 (D)	–1.29±0.23**	–2.71±0.13***	–1.17±0.17*	1.22±0.25**	–2.02±0.07[##]	0.52±0.17	–0.01±0.11
R-J45 (D)	1.47±0.21***	0.23±0.47	–1.71±0.13***	–0.05±0.12	0.10±0.08	–0.03±0.02[#]	0.01±0.01

Table 4-1 Inter-ocular differences (treated/right eye–fellow/left eye) in refractive parameters (mean±SE) for controls and treatment groups. M=spherical-equivalent; MMM=most myopic meridian; MHM=most hyperopic meridian; RA=refractive astigmatism; R-J0 and R-J45, the two vector components of RA. In experiment A (indicated by "[a]"), the comparisons across the controls and treated groups was tested by one-way ANOVA followed by Tukey's test. In experiment B (indicated by "[b]"), the comparisons between high and low magnitudes of imposed astigmatism were tested by two-sample *t*-tests. The levels of significant difference are indicated by asterisk: * p≤0.05, ** p≤0.01, *** p≤0.001 in experiment A, and [#] p≤0.05, [##] p≤0.01, [###] p≤0.001 in experiment B.

Effects of magnitude of astigmatism (Experiment B):

In the two groups treated with 90° cylindrical axis, both the spherical equivalent and most myopic meridian (two-sample *t*-test, both p≤0.018), but not most hyperopic meridian (p=0.95), were significantly different between the H90 and L90 groups. Also, both the refractive astigmatism and R-J0 (two-sample *t*-test, both p<0.001), but not R-J45 (p=0.36), were significantly different between the H90 and L90. In the other two groups treated with 180° cylindrical axis, only most myopic meridian, refractive astigmatism, and R-J0 were significantly different (two-sample *t*-tests, all p≤0.025). One-way ANOVAs (all p≤0.009) followed by Tukey's post hoc tests (all p<0.001) showed that the H90, L90, and H180 groups developed significantly higher astigmatic components (refractive astigmatism and R-J0) than the controls. On the other hand, the axes of induced refractive astigmatism were not significantly different in both the H and L groups: H90 vs. L90=84±10 vs. 88±4; H180 vs. L180=174±41 vs. 172±58 (Watson-Williams F-test, both p≥0.39, see Table 4-2 and Figure 4-3A).

Corneal curvature:

Effects of axis of astigmatism:

Compared to controls (0.60±0.18D), all treatment groups except the H180

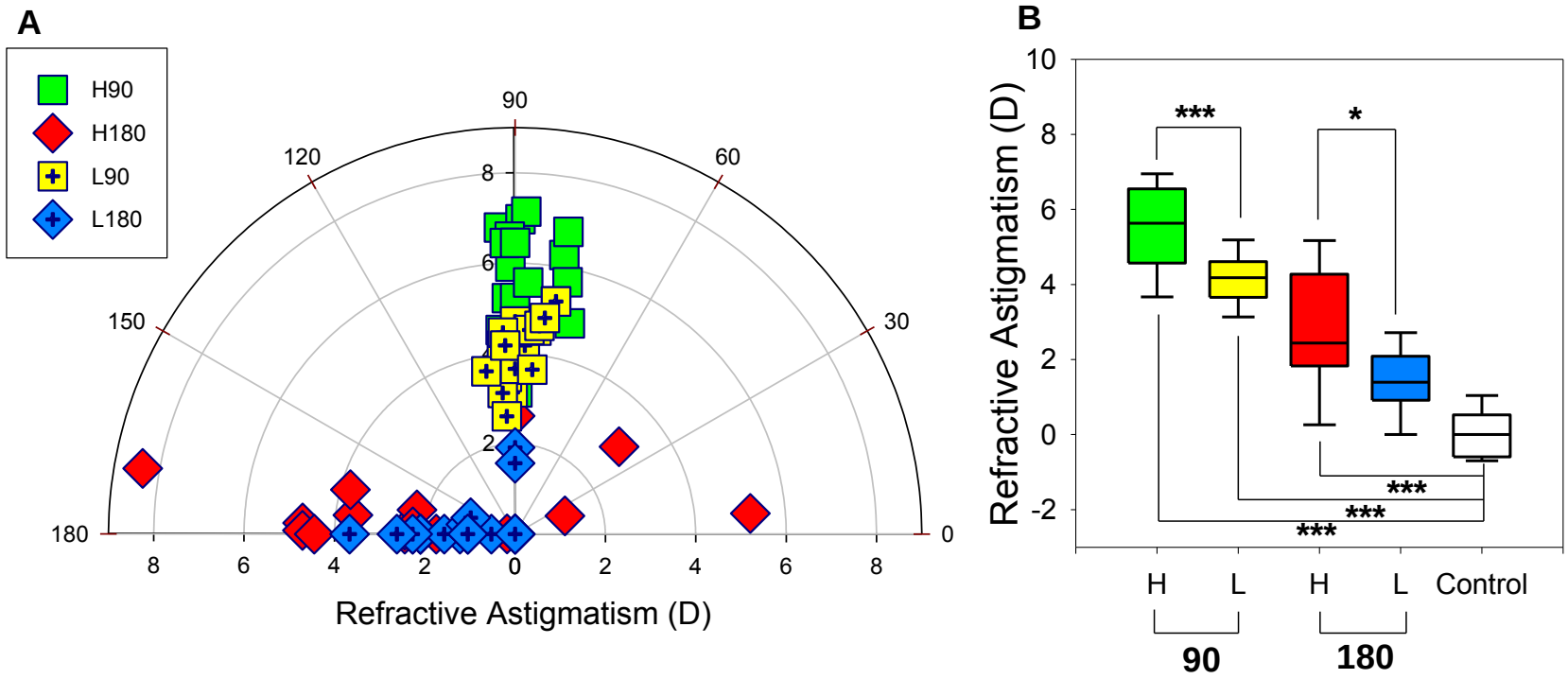


Figure 4-3 (A) Distributions of inter-ocular differences in refractive astigmatism for the four treatment groups with cylindrical lenses of two magnitudes ([H]:+4.00DS/–8.00DC and [L]:+2.00DS/–4.00DC) and two axis orientations (H90, H180, and L90; n=20 in each group; L180, n=18). See caption for Fig. 4-2 and text for details. (B) The box plots of refractive astigmatism include the values of median, maximum, minimum, upper and lower quartiles for each group (see Figure 2 for details). The levels of significant difference in the magnitudes of refractive astigmatism across the treatment groups (lines above the boxes, two-sample *t*-tests), or between treatment and control group (lines below the boxes, Tukey's post hoc tests) are indicated by asterisk: * p≤0.05, *** p≤0.001.

Lens Power		Crossed-cylinder Lens (H) +4.00DS/–8.00DC				Crossed-cylinder Lens (L) +2.00DS/–4.00DC		Control No lens
Axis	(°)	45 [a]	90 [a,b]	135 [a]	180 [a,b]	90 [b]	180 [b]	Axis (°)
M	(D)	-3.08±0.88	-0.78±0.40	-3.09±0.99*	0.43±0.54	0.13±0.38	0.22±0.48	-0.42±0.34
MMM	(D)	-5.03±0.78***	-3.22±0.54*	-4.95±1.01***	-0.74±0.61	-2.12±0.42	-0.06±0.40	-0.44±0.36
MHM	(D)	-1.13±1.01	1.66±0.35	-1.24±0.99	1.61±0.57	2.38±0.35	0.49±0.60	-0.42±0.35
RA	(D)	3.91±0.33***	4.87±0.43***	3.71±0.31***	2.35±0.51**	4.51±0.16#	0.55±0.34	0.03±0.22
CA	(D)	1.81±0.24**	2.27±0.22***	1.46±0.12	1.15±0.21	2.00±0.34#	0.74±0.14	0.60±0.18
IA	(D)	2.82±0.25***	3.05±0.37***	3.18±0.35***	1.50±0.29	3.48±0.18#	1.01±0.18	0.72±0.16
R-J0	(D)	–1.34±0.15***	–2.36±0.20***	–0.96±0.22	1.19±0.24**	–2.21±0.07#	0.52±0.12	–0.01±0.11
C-J0	(D)	–0.52±0.18*	–0.91±0.11***	0.04±0.16	0.51±0.12	–0.59±0.05	0.20±0.12	0.09±0.06
I-J0	(D)	–0.81±0.13*	–1.18±0.26**	–0.80±0.20	0.67±0.17*	–1.63±0.07##	0.41±0.07#	–0.10±0.11
R-J45	(D)	1.39±0.20***	0.42±0.21	–1.52±0.22***	–0.24±0.13	0.23±0.15#	–0.07±0.05#	0.01±0.01
C-J45	(D)	0.33±0.08*	–0.08±0.17	–0.51±0.11	–0.40±0.14	–0.11±0.18	–0.20±0.10	–0.16±0.07
I-J45	(D)	1.06±0.17*	0.55±0.24	–1.10±0.29***	0.16±0.07	0.34±0.20	0.13±0.11	0.17±0.07
RA	(°)	68±7	84±10	119±12*	174±44***	88±4	172±68	70±37
CA	(°)	79±11**	91+6*	125±20	152±22***	96±10	117±39	109±21
IA	(°)	61±10	82±21	17±15**	15±67*	82±9	92±109	1.4±49

Table 4-2 Inter-ocular differences (mean±SE) in spherical equivalent (M), most myopic meridian (MMM), most hyperopic meridian (MHM), refractive (RA), corneal (CA), and internal astigmatisms (IA) for a subset of birds from the treatment and control groups (n=8 in each group) with both refractometry and corneal topography measurements. Note that the astigmatic axes in the last three rows are calculated by circular statistics (mean±angular deviation) for the treated eyes only. In experiment A (indicated by "[a]"), the comparisons across the controls and treated groups were tested by one-way ANOVA followed by Tukey's test. In experiment B (indicated by "[b]"), the comparisons between high and low magnitudes of imposed astigmatism were tested by two-sample *t*-tests. The levels of significant difference are indicated by asterisk: * p≤0.05, ** p≤0.01, *** p≤0.001 in experiment A, and # p≤0.05, ## p≤0.01, ### p≤0.001 in experiment B. Comparisons for the astigmatic axis were performed by Watson-Williams F-tests followed by pairwise comparison tests.

group (p=0.095) developed significantly higher corneal astigmatisms (one-way ANOVA with Tukey's post hoc tests, all $p<0.05$). The highest and lowest magnitudes of corneal astigmatism among the treatment groups were found in the H90 (2.27±0.22D) and H180 groups (1.15±0.21D), respectively. The average axes of corneal astigmatism for the H45, H90, H135 and H180 groups were 79±11, 91±6, 125±20, and 152±21 degrees, respectively (see Table 4-2). The C-J0s of the H45 and H90 groups were significantly different from the H135, H180 groups, and the controls (One-way ANOVA with Tukey's post hoc tests, all $p\leq0.037$). However, C-J0s were neither significant different between the H45 and H90 groups nor among the H135, H180 and the control groups (all $p\geq0.116$). On the other hand, the effects of obliquely oriented cylindrical axes were found between the H45 and H135 groups: the C-J45s were significantly different between the H45 and H135 groups, and between the H45 and H180 groups (one-way ANOVA with Tukey's post hoc tests, both $p\leq0.001$).

Significant effects of cylindrical axis on corneal curvatures were found in the treated/right eyes (one-way ANOVA, all $p\leq0.006$) but not in the fellow/left eyes (one-way ANOVA, all $p\geq0.241$). Figure 4-4 compares the steepest (top symbols) and flattest corneal curvatures (bottom symbols) of the treated/right eyes across the control and treatment groups. As shown, both SK and FK were much steeper in H90 than the other groups. Significant differences in SK were found between multiple treatment groups (H90 vs.

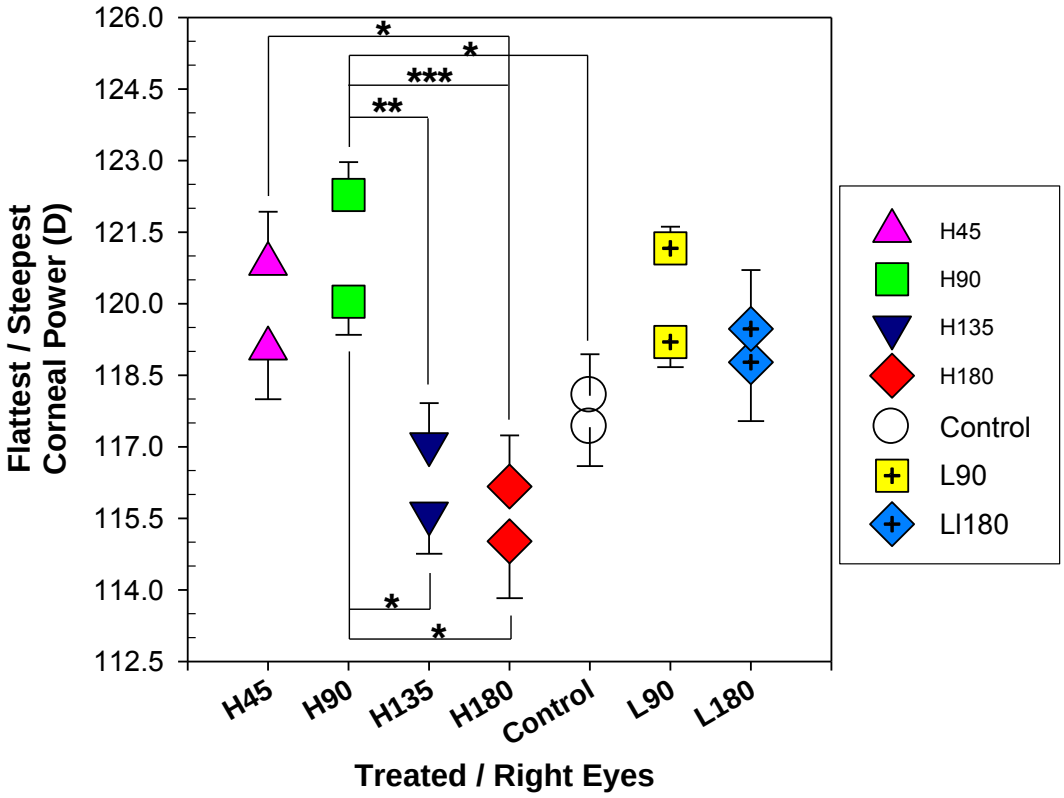


Figure 4-4 Comparisons of the steepest (top symbols) and the flattest corneal curvatures (bottom symbols) across the controls and treatment groups at P12 (treated/right eyes data only). The levels of statistical significant difference across the treatment groups (Tukey's post hoc tests), are indicated by asterisk: * p≤0.05, ** p≤0.01, ***p≤0.001. Although both SK and FK of L90 were not statistically different from others, they showed similar trends as those in H90.

controls, H90 vs. H135, H90 vs. H180, and H45 vs. H180; Tukey's post hoc tests, all p≤0.05), whereas significant differences in FK were found only between H90 and the other two treatment groups (H90 vs. H135; H90 vs. H180; Tukey's post hoc tests, both p≤0.05).

Effects of magnitude of astigmatism:

Significant difference in C-J0 was found only between the H90 and L90 groups (two-sample *t*-test, p=0.021). No significant differences in corneal astigmatism and C-J45 were found between the H and L groups of the same axis orientations (two-sample *t*-tests, all p≥0.055).

Eyeshape profile:

Axial length and Equatorial diameter

In general, the cylindrical-lens-wear produced an overall abnormal eyeshape. In the control group, no significant difference was found in AL (RE=9.11mm, LE=9.07mm), ED180 (RE=11.99mm, LE=11.92mm) or ED90 (RE=12.03mm, LE=12.08mm) between the left (LE) and right (RE) eyes (paired *t*-tests, all p≥0.191). In the treated groups, the ocular dimensions of the treated eyes were significantly longer and larger than those of their untreated fellow eyes (n=48, treated vs. fellow, AL: 9.29±0.04mm vs. 9.08±0.03mm; ED180: 12.26±0.04mm vs. 11.93±0.04mm; ED90: 12.22±0.05mm vs. 11.99±0.05mm, paired *t*-test, all p<0.001).

Effects of axis of astigmatism:

As shown in Table 4-3, the inter-ocular difference in AL of the H135 group (one-way ANOVA followed by Tukey's post hoc test, $p<0.05$), as well as the inter-ocular differences in ED90 of all treatment groups (one-way ANOVA followed by Tukey's post hoc tests, all $p<0.05$) were significantly larger than those of the controls. Furthermore, inter-ocular differences in ED180 of all except the H90 treatment groups were significantly larger than the controls.

In Figure 4-5, the differences in ocular dimensions (*i.e.*, treated/right eyes - fellow/left eyes, from 0° to 50° eccentricities in 5° intervals) towards the four peripheral regions along the vertical and horizontal meridians are compared. We found that only the nasal regions (50° eccentricities) were significant different between the controls and all treatment groups (one-way ANOVA with Tukey's post hoc tests, all $p\leq0.008$). To determine the effects of cylindrical lenses on posterior ocular asymmetry, we calculated the differences in area between the fellow eyes up to 50º eccentricity for the horizontal (ADH) and vertical meridians (ADV) were calculated. While the differences between the two meridians (ADH-ADV, Table 4-3) were analyzed, we found that the H180 showed significantly larger magnitudes than the H90 and control groups (one-way ANOVA followed by Tukey's post hoc tests, both $p\leq0.011$). When the total changes in ocular dimensions

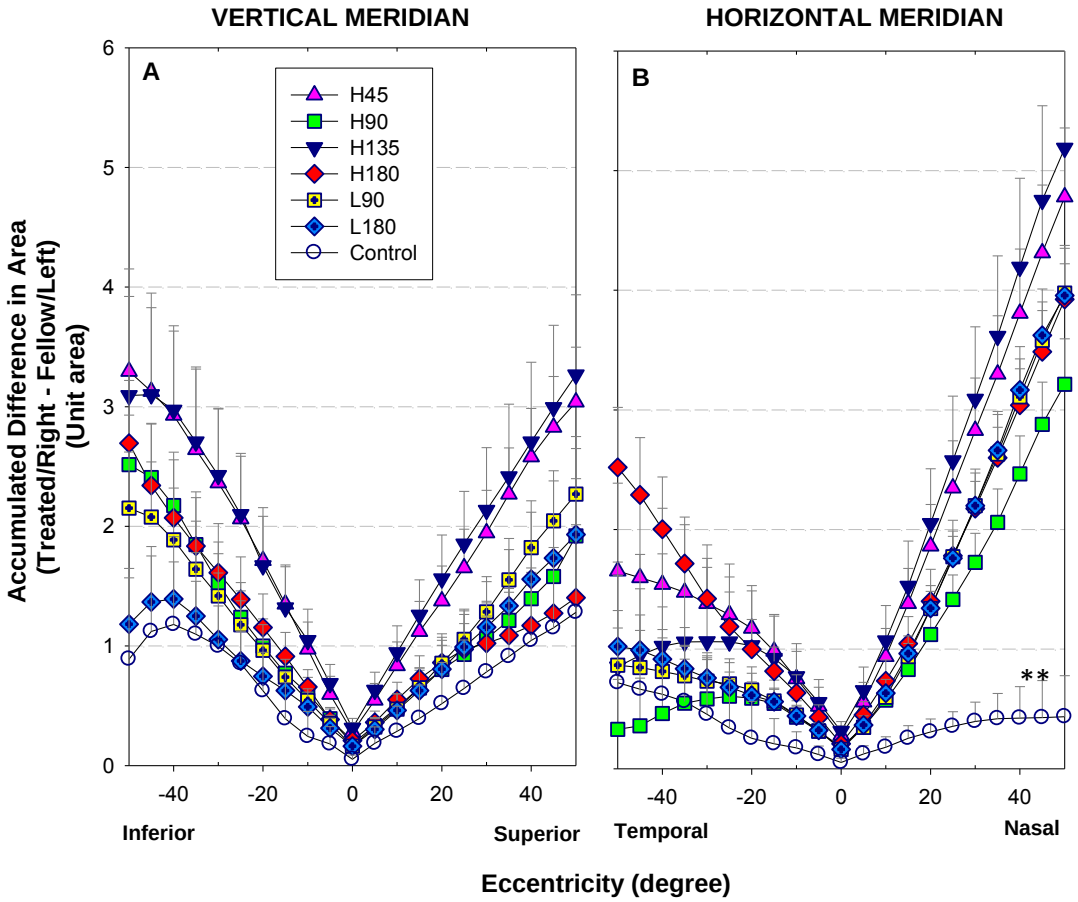


Figure 4-5 The regional differences in areas (treated/right eye–fellow/left eye, 5º intervals) were measured across different eccentricities along vertical (superior-inferior) (A) and horizontal (nasal-temporal) meridians (B). Note that the values at 0º showed the differences in axial length, not area. The levels of significant differences in area at eccentricity 50° between the treatment and control groups are indicated by asterisk: ** $p<0.01$ (Tukey's post hoc tests).

Lens Power	Crossed-cylinder Lens						Control
	(H) +4.00DS/–8.00DC				(L) +2.00DS/–4.00DC		No lens
Axis (°)	45 [a]	90 [a,b]	135 [a]	180 [a,b]	90 [b]	180 [b]	Axis (°)
Axial / Equatorial Dimensions							
AL (mm)	0.28±0.08	0.18±0.03	0.31±0.08*	0.21±0.05	0.16±.0.03	0.16±0.04	0.05±0.02
ED180 (mm)	0.37±.0.04**	0.25±0.05	0.34±0.06**	0.43±0.05***	0.30±0.07	0.29±0.06	0.08±0.06
ED90 (mm)	0.26±0.03**	0.30±0.06***	0.22±0.08*	0.31±0.03***	0.23±0.08	0.07±0.06	–0.06±0.08
Posterior Ocular Dimensions							
ADH-ADV (unit area)	0.10±0.70	–0.89±0.48	–0.32±0.47	2.35±0.61*	0.42±0.68	1.86±0.73	–1.02±1.01
ADH+ADV (unit area)	12.77±2.37*	7.98±1.36	12.40±3.12*	10.54±1.62	9.26±1.24	8.09±1.34	3.33±1.93
Regional Differences							
Nasal (unit area)	4.78±0.58***	3.21±0.38**	5.19±0.84***	3.93±0.45***	3.98±0.25	3.96±0.40	0.44±0.34
Temporal (unit area)	1.65±0.73	0.33±0.42	0.85±0.88	2.52±0.50	0.87±0.31	1.02±0.30	0.72±0.90
Superior (unit area)	3.04±0.46	1.92±0.48	3.27±0.67	1.40±0.55	2.27±0.48	1.93±0.72	1.28±0.73
Inferior (unit area)	3.30±0.86	2.52±0.41	3.09±0.83	2.69±0.52	2.51±0.47	1.18±0.47	0.89±0.68
FK (D)	–0.53±1.17	0.80±2.01	–0.85±0.63	–1.24±1.21	0.80±0.80	–0.18±0.97	–0.66±0.53
SK (D)	0.61±1.20	2.68±2.08*	–0.15±0.56	–0.65±1.05	2.03±0.87	–0.08±1.00	–0.81±0.50
MK (D)	0.04±1.17	1.74±2.04	–0.50±0.59	–0.94±1.13	1.42±0.83	–0.13±0.96	–0.74±0.51

Table 4-3 Inter-ocular differences in ocular dimensions (mean±SE) related to the eye shape profile for the controls and treatment groups (n=8 in each group). AL=axial length; ED180 & ED90, horizontal and vertical equatorial diameters, respectively; ADH & ADV, difference in area between the two eyes up to 50º eccentricity along the horizontal and vertical meridians, respectively. In experiment A (indicated by "[a]"), the comparisons across the controls and treated groups were tested by one-way ANOVA followed by Tukey's test. In experiment B (indicated by "[b]"), the comparisons between high and low magnitudes of imposed astigmatism were tested by two-sample *t*-tests. The levels of significant difference are indicated by asterisk: * p≤0.05, ** p≤0.01, *** p≤0.001 in experiment A, and [#] p≤0.05, [##] p≤0.01, [###] p≤0.001 in experiment B.

were compared (ADH+ADV, Table 4-3), we found that both H45 and H135 groups had significantly larger eye sizes than the controls (one-way ANOVA with Tukey's post hoc tests, both $p \leq 0.041$). In short, the H180 group showed meridional difference in posterior globe without an alteration in eye size; both H45 and H135 groups had significantly larger than normal eye sizes but no differences in ocular expansion between the two meridians; whereas H90 group did not show any difference from the controls in these two parameters.

Effects of magnitude of astigmatism:

Significant difference in the ED90 was found only between the H180 and L180 groups (two-sample *t*-test, $p=0.002$, Table 4-3), no significant difference in AL or ED180 was found between the H180 and L180 or between the H90 and L90 groups (two-sample *t*-tests, all $p \geq 0.082$).

When the differences in ocular dimensions (up to 50° eccentricity) at the four quadrants were analyzed, the H180 group was larger than the L180 group in both the temporal and inferior regions (two-sample *t*-tests, both $p<0.05$, Figure 4-5A and B); but, no significant differences were found in all four regions between the H90 and L90 groups (two-sample *t*-tests, all $p \geq 0.115$). As shown in Figure 4-6 A and B, there were also no significant differences in ADH-ADV, and ADH+ADV between H180 and L180 or

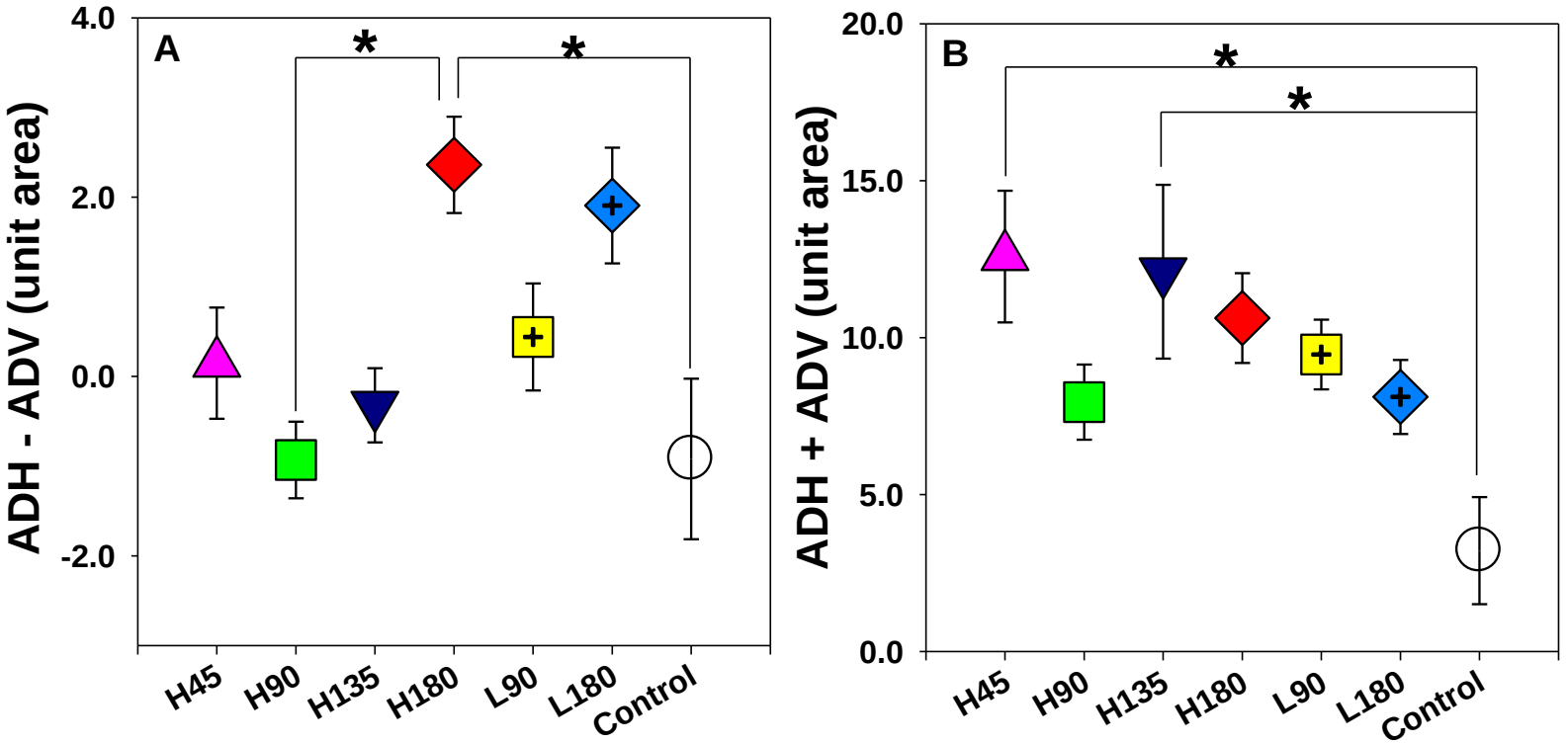


Figure 4-6 ADH and ADV indicate the area differences (treated/right eye–fellow/left eye) along vertical and horizontal meridians from 0° to 50° eccentricities. The difference (ADH–ADV) and the summation (ADH+ADV) of these parameters (mean±SE) are plotted in (A) and (B), respectively. The levels of statistical significant difference across the treatment groups (Tukey's post hoc tests), are indicated by asterisk: * $p<0.05$.

between H90 and L90 (two-sample *t*-tests, all p≥0.137). However, one-way ANOVA combined with Tukey's tests revealed that the (ADH-ADV) of the H180 group was larger than that of both the H90 and control groups (both p≤0.026).

Correlation analyses

Refractive, corneal, and internal astigmatisms

Data from the subset of birds with both refractions and corneal topography measurements were pooled for correlation analyses (n=112, both eyes from treated and control groups). Moderate to high correlations were found between the refractive and corneal astigmatic components (Pearson's correlation r=0.78, 0.84 and 0.61 for astigmatism, J0 and J45 components respectively, all p<0.001; Figure 4-7A-C), as well as between the refractive and internal astigmatic components (Pearson's r=0.94, 0.94 and 0.90 for astigmatism, J0 and J45 components respectively, all p<0.001; Figure 4-7D-F).

Spherical components and Eyeshape parameters

Table 4-4 shows significant correlations found between the refractive and eyeshape parameters. most hyperopic meridian, most myopic meridian, and spherical equivalent were significantly correlated (all p<0.001) with AL (Pearson's r= – 0.61, – 0.47 and – 0.57, respectively) and ADH+ADV (r = –

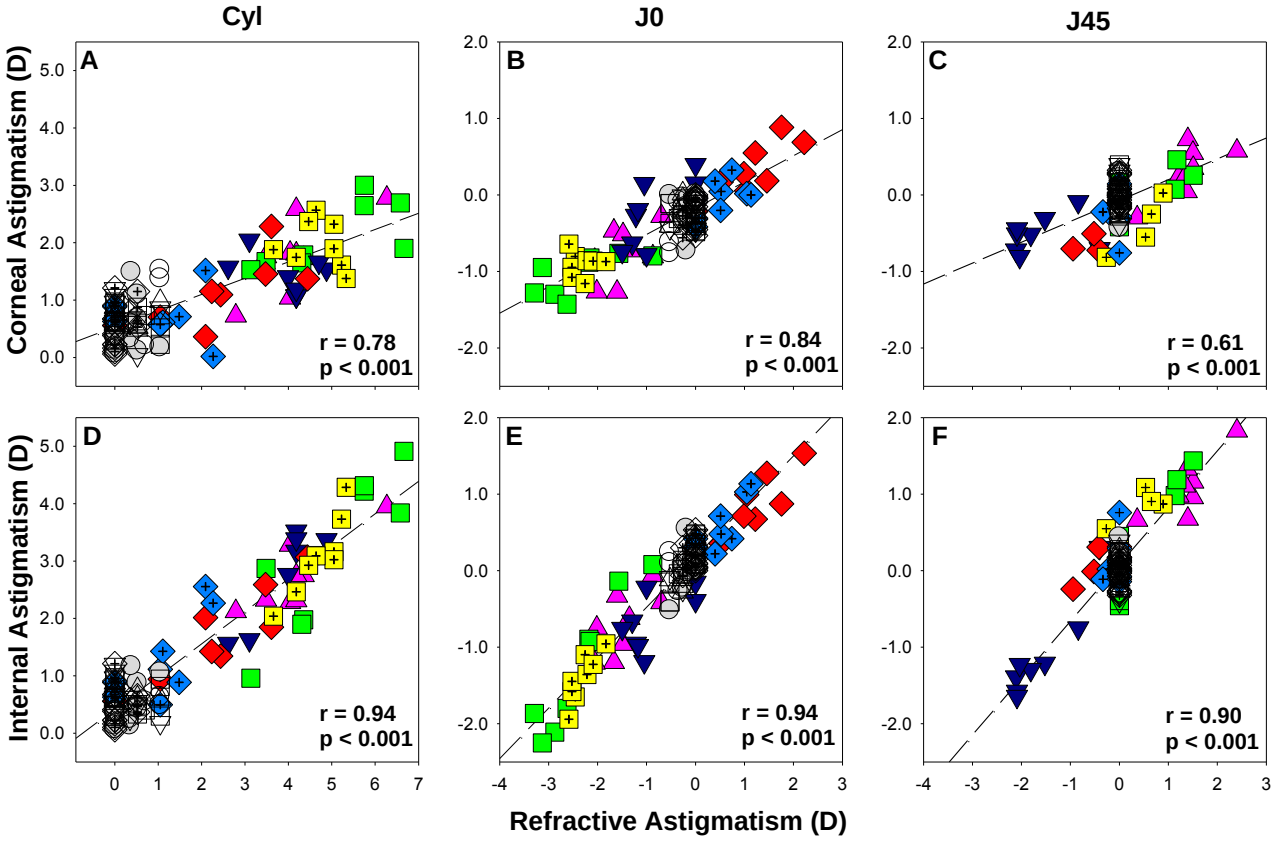


Figure 4-7 Data from both eyes of all treatment and control eyes (n=112) were included in the correlation analyses for refractive and corneal astigmatic components (top panel, A-C), and for refractive and internal astigmatic components (bottom panel, D-F).,Internal astigmatism is derived by calculation (filled symbols, treated/right eyes; open symbol, fellow/left eyes). ΔH45, □ H90, ▽ H135, ◇ H180, ⊞ L90, ◈ L180, O Control.

	AL (mm)	EDmean (mm)	ED180 (mm)	ED90 (mm)	ADH (unit area)	ADV (unit area)	ADH-ADV (unit area)	ADH+ADV (unit area)	S (unit area)	I (unit area)	N (unit area)	T (unit area)
MMM	–0.61***	–0.29*	-	-	–0.44***	–0.53***	-	–0.52***	–0.43***	–0.51***	–0.49***	-
MHM	–0.47***	-	-	-	–0.38***	–0.38***	-	–0.40***	–0.36**	–0.32*	–0.34**	–0.30*
M	–0.57***	-	-	-	–0.39***	–0.46***	-	–0.45***	–0.37***	–0.45***	–0.36**	–0.29*
RA	-	0.43***	0.28*	0.42***	-	0.33**	-	0.31*	0.27*	0.31*	0.45***	-
CA	-	0.31*	-	0.35**	-	-	-	-	-	-	-	-
IA	0.31*	0.33**	0.26*	0.32*	0.27*	0.36**	-	0.34**	0.35**	0.30*	0.45***	-
R-J0	-	-	-	-	-	-	0.36**	-	-	-	-	-
C-J0	-	-	-	-	-	-	0.35**	-	-	-	-	0.36**
I-J0	-	-	-	-	-	–0.30*	0.30*	-	–0.27*	–0.27*	-	-

Table 4-4 Pearson's correlation analyses between refractive, corneal parameters and eyeshape parameters (n=56). M=spherical-equivalent; MMM=most myopic meridian; MHM=most hyperopic meridian; RA=refractive astigmatism; R-J0 and R-J45, the two vector components of RA; AL=axial length; EDmean=average of ED180 and ED90; ED180 & ED90, horizontal and vertical equatorial diameters, respectively; ADH & ADV, difference in area between the two eyes up to 50º eccentricity along the horizontal and vertical meridians, respectively; T, N, I and S=difference in area between the two eyes up to 50º eccentricity at temporal, nasal, inferior and superior regions, respectively. The levels of significant difference between treatment and control groups are indicated by asterisk: * p≤0.05, ** p≤0.01, *** p≤0.001.

0.40 to – 0.52), but not with ED180, ED90, or ADH-ADV (all p≥0.22). All the three spherical components were significantly correlated (Pearson's correlations, all p≤0.02) with the differences in area at superior (r= – 0.36 to – 0.43), inferior (r= – 0.32 to – 0.51), and nasal regions (r= – 0.34 to – 0.49); but only most hyperopic meridian and spherical equivalent were significantly correlated (both p=0.03) with those at the temporal region (r= – 0.30 and – 0.29).

Astigmatic components and Eyeshape parameters

While refractive, corneal, and internal astigmatism were all significantly correlated with ED90 (Pearson's r=0.42, 0.35 and 0.32, respectively; all p≤0.02), only refractive and internal astigmatism were significantly correlated (all p<0.05) with ED180 (Pearson's r=0.28 and 0.26). In addition, both refractive and internal astigmatism were significantly correlated with the differences in area at the superior, inferior and nasal regions (Pearson's r=0.27 to 0.45, all p≤0.04), as well as ADH+ADV (Pearson's r=0.31 and 0.34, both p≤0.05). Interestingly, all the J0 components were significantly correlated with ADH-ADV (Pearson's correlations: R-J0: r=0.36; C-J0: r=0.35; I-J0: r=0.30; all p≤0.02). Furthermore, the internal astigmatism was significantly correlated with AL (Pearson's r=0.31, p=0.02), and I-J0 was significantly correlated with the differences in area at the superior and inferior regions (Pearson's correlations, both r= – 0.27, both p=0.04). On

the other hand, although C-J0 was also significantly correlated with the difference in area at the temporal side (Pearson's correlation, r=0.36, p=0.01), corneal astigmatism was not correlated with any other parameters (all p≥0.549).

Discussion

The main findings in this study were: 1) the chick eyes developed astigmatism after wearing crossed-cylindrical lens for a week; 2) the characteristics of the resultant astigmatism in the treated birds were influenced by the orientation and magnitude of imposed astigmatism; 3) the characteristics of the induced astigmatism were correlated with multiple eyeshape parameters.

Cylindrical lens wear also produced significant impacts on the corneal shape. The magnitudes of induced corneal astigmatism across the treatment groups varied in a similar fashion as those of the refractive astigmatism (Table 4-3). In the treated eyes, both the steepest and flattest corneal curvatures in the H90 group were significantly steeper than those of the H180 group (Figure 4-4). Furthermore, relative to their fellow untreated eyes, imposing WTR astigmatism (H90 and L90) resulted in steeper corneal curvatures whereas imposing ATR astigmatism (H180 and L180) produced flatter corneal curvatures along both principal meridians (Figure 4-4 and Table 4-3). As shown in Figure 4-4, imposing different astigmatic axes of

high magnitudes of astigmatism appeared to have a more dramatic effect on the steepest meridian: whereas significant differences in the flattest meridians were found only in three treatment groups, significant differences in the steepest meridians involved all treatment groups. It should be borne in mind that the resultant steepest meridians were oriented at different directions across the treatment groups (*e.g.*, horizontal meridian for H90 and vertical meridian for H180), the differential magnitudes and orientations of induced astigmatism across the treatment groups suggest that the induced ocular toricity may be related to the structural anisotropy occurred regionally and/or across different meridians. Thus, the results in young chicks showed that vision-dependent processes are capable of altering corneal shape and producing astigmatism. Several elucidations related to the ocular structures are worthy of consideration. Firstly, the corneal collagen fibrils are running in parallel to one another and oriented at orthogonal position to adjacent layers with the corneal base directly connected to the ciliary muscles (Coulombre, 1964; Trelstad and Coulombre, 1971). Secondly, the anterior segment is asymmetric at the horizontal plane, with the greatest temporal distance between equator and limbus (Murphy, Glasser and Howland et al., 1995). The intermediate ciliary muscle is suggested as the *depressor corneae*, with its greatest effect occurring temporally (Lord, Jr., Rexford, D., 1956). By contrast, the shortest ciliary muscle fibers, absence of intermediate ciliary muscle and poorly developed scleral venous sinus are found in the nasal quadrant. Thirdly, the

overlapping patterns of the scleral ossicles (Gallus gallus: 14 ossicles, type D (1,9;6,10) pattern (de Queiroz and Good, 1988)) allow the chick cornea to alter its toricity during accommodation (Glasser, Troilo and Howland et al., 1994; Walls, 1942). The ossicle numbers 1 (inferior) and number 9 (superior), “+” elements (on top of the others), are located nearly at the vertical meridian (i.e., the axis meridian); while the ossicle numbers 6 and 10, “-“ elements (under the others), provide a buffer for the movement of the axis meridian. In combination with our previous findings of corneal accommodations (Chu, Zhou, Zheng et al., 2014), we speculated that the cornea could respond to imposed astigmatism, for example, the H90 group by contraction of the ciliary muscles (*i.e.,* positive corneal accommodation), enhanced by the specific pattern of ossicles, to create an against-the-rule corneal profile (ossicles number 1and9 move forward, while number 6and10 move backward) for astigmatic compensation. Therefore, the cornea showed comparatively steeper curvatures in both principal meridians at the end of treatment, especially at the horizontal meridian due to ciliary asymmetry. As shown in Figure 4 and 8, no significant differences in the equatorial diameters and posterior globe between the H90, L90 groups and the controls were found. Perhaps, the scleral ossicles might have sufficient flexibility to counteract the positive accommodation. The reverse is the case; the cornea compensates for the imposed ATR astigmatism, the corneal curvatures are relatively flatter as a result of negative corneal accommodation. However, the magnitude of negative

corneal accommodation is smaller than that of positive corneal accommodation (Chu, Zhou, Zheng et al., 2014) that may be due to the limitation of the ossicles, and/or the cornea structure. Excessive negative accommodation might stretch the cornea and sclera which become flatter, along the horizontal meridian in particular. As a consequence, the magnitudes of induced astigmatism in both the H180 and L180 groups were significantly lower than the others. The corneal fibril arrangement, ciliary muscle asymmetry and the ossicle pattern provide flexibility for cornea to alter its profile, but also limitation for astigmatism compensation at the same time. Therefore full compensation was found in the L90 group, but not found in the H90 group even the magnitude of imposed astigmatism was doubled.

The refractive astigmatism found in this study was moderately correlated with the corneal astigmatism (r= 0.61 to 0.84; Figure 4-7A-C) and strongly correlated with the internal astigmatism (r= 0.90 to 0.94; Figure 4-7D-F). In terms of the magnitude of refractive astigmatism, corneal astigmatism contributed to about 40% (30% to 52%) of refractive astigmatism across the treatment group (Table 4-2). However, when the two astigmatic components (J0 and J45) were considered, the components of internal astigmatism contributed a larger proportion about 60% (51% to 70%) to the refractive astigmatism than those of corneal astigmatism in most of the treatment groups (Table 4-2). Our results showed that the internal

astigmatism not only contributed to the induced astigmatism but also correlated with multiple eyeshape parameters including the axial length, equatorial diameters, meridional (ADH and ADV) and regional changes in ocular dimensions (see Table 4-4). More importantly, similar to R-J0 and C-J0, the I-J0 was also correlated with the ADH-ADV. These results suggested that the differential changes at the posterior eye segment might have altered the normal balance of internal refractive components across different meridians and contributed to ocular toricity. Although no correlations have been established between refractive error and thickness of retina, choroid or sclera (Beresford, Crewther, Kiely et al., 2001), recent research found internal astigmatism was correlated with retinal topography (Oh, Oh, Yang et al., 2014). Also, several studies (Beresford, Crewther, Kiely et al., 2001; Nickla, Sharda and Troilo, 2005; Wildsoet and Wallman, 1995) found increase in choroidal thickness in defocus-induced hyperopic chick eyes, whereas only equatorial choroidal thickening was found in the birds treated with plus-cylindrical lens (plano/+10.00DS) (Irving, Callender and Sivak et al., 1995). Such regional choroidal expansion was also demonstrated in the partial form-deprived chick eyes followed by unrestricted vision (Wallman, Wildsoet, Xu et al., 1995). How the retinal topography change with imposed astigmatism is still open to question. It should be noted that the internal astigmatism in this study was derived from calculation; neither the curvature nor the dimension of internal ocular components was measured. Thus, it remains unclear what were the

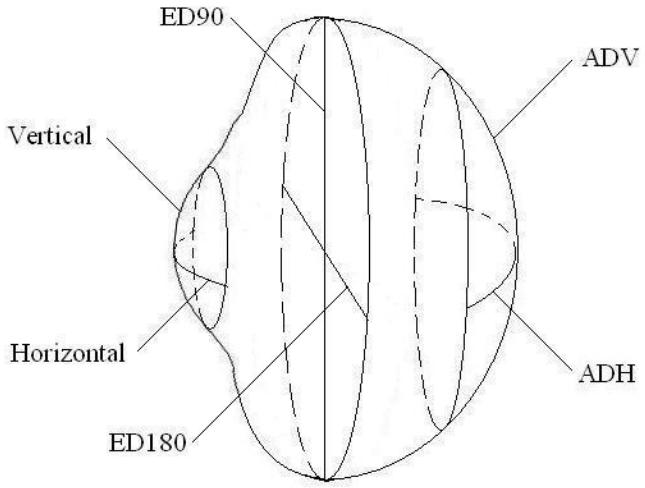
structural correlates for these internal toricities. In this respect, it has been suggested that the internal astigmatism may be due to the variation of posterior corneal astigmatism (Piñero, Ruiz-Fortes, Pérez-Cambrodí et al., 2014), refractive index in crystalline lens (Birkenfeld, de Castro, Ortiz et al., 2013) and the tilting and/or decentration of the crystalline lens with respect to visual axis (S. Duke-Elder, 1993). In addition, since the crystalline lens is located inside the eyeball (Gündüz, Evereklioglu, Er et al., 2002; Jonas, Kling and Gründler et al., 1997), so the nasal-temporal asymmetric ocular expansions as observed in this study (Figure 4-5) might have influenced the on-axis refractive status. Similar to the effects of tilting a spectacle lens with respect to the visual axis (Jalie, 2008; Sarver, 1963), one may speculate that the relatively larger nasal ocular expansions compared to the temporal (Figure 4-5) might have tilted the crystalline lens with its nasal margin located more anteriorly than the temporal margin, resulting in oblique astigmatism along the 90° axis. The reasons for the eyes treated with imposed astigmatism of different magnitudes and axes all resulted in the nasal ocular expansion remains unclear. One possibility is that the optic nerve head and pecten at the inferior-temporal posterior quadrant in chicks may have restricted potential eye growth at the temporal region (Nalbach, Wolf-Oberhollenzer and Remy, 1993). Another possibility is the higher ganglion cell density on the nasal retina where may be more sensitive to visual manipulation than that on the temporal side (Y. X. Chen and Naito, 2000; Y. Chen, Wang, Shibata et al., 2004). Future works using the latest

imaging technology may help to determine the origins of this internal astigmatism.

Unlike previous studies which showed a slight myopic shift (minus-cylindrical lenses, in chicks (Irving, Sivak and Callender et al., 1992; Laskowski and Howland, 1996; Phillips and Collins, 2000; Schmid and Wildsoet, 1997)) and hyperopic shift (plus-cylindrical lenses, in chicks (Irving, Callender and Sivak et al., 1995; Schmid and Wildsoet, 1997); or crossed-cylindrical lenses, in chicks (McLean and Wallman, 2003) and monkeys (Huang, Hung and Smith et al., 1997; Smith, Huang et al., 1998)), our chicks did not show a significant shift in spherical-equivalent refractive error (Table 4-1). Instead, in addition to the induced astigmatism, we found that the imposed astigmatism altered the eyeshape parameters and multiple eyeshape parameters were correlated with both spherical (M, MHM, and MMM) and astigmatic components (Table 4-4). However, the eyeshape parameters that were correlated with spherical components do not necessarily also correlated with astigmatic components. First, whereas all spherical components (*i.e.,* M, MHM, and MMM) were negatively correlated with axial length, only internal astigmatism was positively correlated with axial length. Second, whereas the spherical components only correlated with the ADH+ADV, the three J0 components only correlated with ADH-ADV. Third, whereas all spherical components were correlated with ADV and ADH, only RA and I-J0 were correlated with ADV or ADH. Fourth, whereas

MMM was negatively correlated with the average equatorial diameter, the refractive, corneal and internal astigmatisms were positively correlated with nearly all equatorial dimensions. Thus, the different eyeshape parametric changes associated with spherical and astigmatic components as observed in this study cautious the use of conventional measure such as ocular axial length when characterizing the impacts of changes on the posterior eye segment in the development of astigmatism.

Another interesting finding from this study is the differential effects of imposing WTR and ATR astigmatism on eyeshape parameters. As summarized in Figure 4-8, imposing WTR astigmatism (H90 and L90) produced significantly steeper horizontal corneal curvature than vertical curvature; imposing ATR astigmatism (H180 and L180) produced flatter horizontal corneal curvature (H180 and L180, only H180 reached statistical significance). In contrast, imposing ATR astigmatism produced significantly greater posterior ocular expansions in the horizontal than the vertical meridian, but this effect was not observed in the groups treated with WTR astigmatism. The horizontal equatorial diameter was also significantly larger in one of the ATR-treated groups (L180) but not in any of the two WTR-treated groups. Thus, optically imposed WTR and ATR astigmatisms appear to have stronger effects on, respectively, the anterior and posterior eye shapes. However, even with these contrasting effects on the different segments of eyeball, only the most ametropic meridian in H45 and H90 was



	Corneal Radius of Curvature (Horizontal vs. Vertical)	Equatorial Diameter (ED180 vs. ED90)	Post. Area Expansion (ADH vs. ADV)
H90	— ***	ns	ns
L90	— ***	ns	ns
H180	+ *	ns	+**
L180	ns	+*	+*

Figure 4-8 Comparisons of the effects of imposing WTR (H90 & L90) and ATR (H180 & L180) astigmatism on the horizontal and vertical meridians of corneal radius of curvature, equatorial diameter, and posterior ocular expansion. As illustrated in the schematic diagram, the comparisons were made for corneal curvatures of horizontal vs. vertical meridians; equatorial diameters of the horizontal (ED180) vs. vertical directions (ED90); and the difference in area up to 50° eccentricity of the horizontal (ADH) vs. vertical meridians (ADV). The table on the right summarizes the results of comparisons, the "+" and "–" signs indicate a significantly higher (horizontal>vertical) and lower (horizontal<vertical) values respectively, the "ns" represents no significant difference. The levels of significant difference, using paired *t*-test, are indicated by asterisk: * p≤0.05, **p≤0.01, *** p≤0.001.

significantly different from controls, the spherical equivalent refractive error was not significantly different between the controls and any of the treatment groups (Table 4-1). These results suggest that, at least within the range that we tested, the ocular parametric changes in response to astigmatic error cues may be quite specific regionally and probably independent from those observed under form deprivation and spherical defocus (Schaeffel, Troilo, Wallman et al., 1990; Wallman, Turkel and Trachtman et al., 1978). Possibly, these differential effects of specific astigmatic cues on the individual ocular dimensions had rendered the relatively lower correlations between the refractive changes and axial length (Table 4-4). As postulated in previous studies, the mechanisms controlling the growths of anterior chamber and vitreous chamber, (Barutchu, Crewther and Crewther, 2002; Wildsoet and Pettigrew, 1988), as well as those regulating the growths of equatorial diameter and axial length, (Fischer, Ritchey, Scott et al., 2008) may be independent from each other.

After one week of cylindrical lens treatment, virtually all treated eyes developed astigmatism and the amount of induced astigmatism varied dependent on the axis orientation and magnitude of astigmatism imposed by the lenses (Figures 4-2 and 3). The highest and lowest magnitudes of induced astigmatism were found, respectively, in the treatment groups that experienced a week of WTR (H90: 5.51±0.26D) and ATR astigmatisms (L180: 1.34±0.22D). Only the L90 group developed a magnitude

(4.10±0.16D) and an axis (88±4) that appeared to compensate fully for the 4.00DC imposed astigmatism (Table 4-1). In contrast to our findings, several eariler studies using higher magnitudes of optically imposed astigmatism (10.00DC to 16.00DC, usually plano-cylindrical lenses) did not show clear compensatory astigmatic changes in chicks (Schmid and Wildsoet, 1997; Laskowski and Howland, 1996; Phillips and Collins, 2000; Thibos, 2001). However, in the pioneer study (Irving, Callender and Sivak et al., 1995) that employed similar paradigm (P0 or P2 birds worn plano/+10.00DC or plano/-9.00DC for 7 days) as ours, partial compensations in refractive astigmatism were found when the plano/+10.00DC lenses were oriented at 135 (3.75±0.63D, the highest) or 45 axes (1.00±0.38D, the lowest). One possibility for this discrepancy across studies is that the visual signals and its effects imposed by the high-powered cylindrical lenses (*i.e.,* 10.00DC) might have approached the operating limits of the sensory mechanism and/or the structural correlates. It is necessary to konw that we used relatively lower magnitudes of cylindrical lenses (8.00DC and 4.00DC) and each principal powered meridian only imposed either 4.00D or 2.00D of defocus. Even with these lower powers of cylindrical lenses, the chicks only compensated partially in most of our treatment groups. Many biometric parameters were not significantly different between the H and L groups suggest that even the H lenses might have approached the limits of the operating mechanisms. Another possibility is the starting age in different experiments. Since

hatchling chicks typically exhibit significant amounts of natural astigmatism (Schmid and Wildsoet, 1997), wearing cylindrical lenses immediately after hatching might have confounded the visual error signals used for regulating refractive development. Furthermore, age-dependent anatomical changes were also noted in normal post-hatched chicks with respects to corneal flattening (Avila and McFadden, 2010; Irving, Sivak, Curry et al., 1996) and the orientation of collagen circumscribing the central cornea (Boote, Hayes, Young et al., 2009). Thus, the differences in experimental methodology and paradigm may be the possible reasons for the discrepancy reported in these studies. Regardless, the current study, which included a large number of animals and biometric measures from anterior to posterior ocular segment, demonstrates that chicks are capable of compensating for astigmatic error signals and the regulatory mechanism is sensitive to the axis orientation and magnitude of imposed astigmatism.

In conclusion, the current study extends our understanding of astigmatic eye growth in chick and provides new insights into the effects of optically imposed astigmatism on corneal and eyeshape parameters.

CHAPTER 5 GENERAL CONCLUSIONS

The key findings of this thesis were:

1. While expansion of the posterior eye globe was found in corresponding region of all treatment groups with hemiretinal form deprivations, different characteristics in refractive errors were found. The superior-retina form-deprived group (SRD) was the most myopic and the least astigmatic, whereas the inferior-retina form-deprived group (IRD) was the least myopic. In addition, the temporal-retina form-deprived group (TRD) was the most astigmatic. Our results suggest regional variation in the susceptibility to refractive error.

2. Although “astigmatic accommodation” is still a controversial topic; it has been hypothesized as a mechanism to compensate for the optical defects due to astigmatic error. In the second experiment, we showed that both positive and negative corneal accommodations in chicks were still functional in the presence of significant experimentally induced astigmatism. The magnitudes of maximum positive corneal accommodation were increased in those animals exhibiting higher refractive astigmatism.

3. It is believed that astigmatism is originated from the two main refractive components, the corneal and crystalline lens, but the role of posterior eyeshape in sphericalization is still uncertain. In the last experiment, our

results showed that chicks could compensate for optically imposed astigmatism and this capability may vary with the orientation and magnitude of visual error signals. In addition, significant correlations were found between the astigmatic components and parameters related to posterior eye shape.

In accordance with previous findings (Miles and Wallman, 1990; Zeng and McFadden, 2010; Zeng, Bowrey, Fang et al., 2013), our results showed that the highest amount of form deprivation myopia and the lowest amount of induced astigmatism were found in the superior-retina form-deprived group and also indicated that the superior retina (or lower field) was the most sensitive to form deprivation. Lower field myopia was widely reported in different animal species (Hodos and Erichsen, 1990; Murphy, Howland and Howland, 1995; Schaeffel, Hagel, Eikermann et al., 1994; Zeng, Bowrey, Fang et al., 2013), but the results of human researches were contradictory (For: Seidemann et al., 2002 (Seidemann and Schaeffel, 2002) and Berntsen et al. 2010 (Berntsen, Mutti and Zadnik, 2010) and Against: Atchison et al. 2006 (Atchison, Pritchard and Schmid et al., 2006) and Ehsaei et al. 2011 (Ehsaei, Mallen, Chisholm et al., 2011)). Nevertheless, the results highlight the importance of understanding the influence of the superior retina to the entire eye growth and refractive status. On the other hand, a nasal-temporal variation along the horizontal meridian was consistently found in both animal (monkey (Huang, Hung, Ramamirtham et

al., 2009; Hung, Ramamirtham, Huang et al., 2008), marmoset (Totonelly and Troilo, 2008), birds (Glasser, Murphy, Troilo et al., 1995; Lord, Jr., Rexford, D., 1956; Murphy, Glasser and Howland et al., 1995)) and human (Dunne, Misson, White et al., 1993; Logan et al., 2004; Logan, Singh and Gilmartin, 2005; Lotmar and Lotmar, 1974; Radhakrishnan, Allen, Calver et al., 2013; Rempt, Hoogerheide and Hoogenboom et al., 1971; Schultz D. N., 1977; Singh, Logan and Gilmartin, 2006) researches. It is worth noting that the animal researches suggested that local retinal mechanism (Wallman, Turkel and Trachtman et al., 1978) and the peripheral visual experience (Smith, Ramamirtham, Qiao-Grider et al., 2007) play an important role in controlling eye growth. According to the “blur hypothesis”, localized image degradation leads to regional ocular expansion. Stone et al. (2006) (R. A. Stone, Pendrak, Sugimoto et al., 2006) demonstrated that the effect of peripheral visual experience decreased with increasing size of the central aperture. They also found that chicks developed significant amount of myopia (about -16.0D) when deprived the superior-temporal retina, and became hyperopic (about +1.50D) when the interior-nasal retina was deprived. Taken together with our results (see Figure 2-2), we conjectured that unrestricted central retinal vision may not be crucial for inhibiting myopia development, on the contrary, the superior peripheral retinal area may take up the most important role in myopia control.

The association between partial form deprivation and astigmatism was

studied in the first experiment. As aforementioned, though the superior retina deprived group was the most myopic, it was the least astigmatic. Compared with other treatment groups, the orientation of the astigmatism was oblique instead of against-the-rule. In retrospect, previous works showed inconsistent results in correlations between astigmatism and spherical ametropia. Several human studies found that the magnitude of astigmatism was increased with the degree of myopia (Gwiazda, Grice, Held et al., 2000; Heidary, Ying, Maguire et al., 2005; Kaye and Patterson, 1997), but the others found it independent of the spherical equivalent (Ehrlich, Braddick, Atkinson et al., 1997). Another study showed that the "astigmatic axis is related to the level of spherical ametropia" (Farbrother et al., 2004). In monkeys, astigmatism frequently associated with reduced vitreous chamber growth rates and hyperopia (Huang, Hung and Smith et al., 1997). This observation in monkeys could only have been applicable in chicks when considering the SRD and IRD groups in the presence study. Nonetheless, similar properties in spherical and astigmatic components of both TRD and NRD, *e.g.,* M and R-J0, within the range of the two extremes (*i.e.,* SRD and IRD), are also worthy of consideration. These findings suggested that the temporal and nasal retina might not be the major determinant in ametropia development. At molecular level, VAX2 is an eye-specific homeobox gene, particularly involved in the establishment of a physiological asymmetry of the ocular dorsal-ventral axis (Barbieri, Broccoli, Bovolenta et al., 2002). The physiological asymmetry may provide an

explanation of the different responses found in the superior and inferior retina form-deprived groups. A locus of the VAX2 gene was associated with astigmatism in accordance with a recent study (Lopes, Hysi, Verhoeven et al., 2013). On this aspect, despite no definite answer, the dorsal-ventral retina would be worth our while to investigate its role in astigmatic eyegrowth.

Another finding of experiment 1 is the correlation between AL/ED ratio and spherical equivalent. Although the AL/ED ratio may not be as representative as the AL/r ratio when describing the spherical equivalent, the AL/ED ratio is more about the effect of posterior eyeshape on the spherical equivalent. Since the growth of axial length and equatorial diameter of an eye are independent (A. R. Stone, Lin, Iuvone et al., 1990), the disproportionate growth of these two parameters may lead to different eyeshape (*i.e.,* prolate: AL>ED; oblate: AL<ED or spherical: AL=ED; similar to the "oblateness" = 1-AL/ED described in other studies (Ishii, Iwata and Oshika, 2011)), as a result, different refractive statuses. A recent clinical study (Lim, Chong, Tan et al., 2013) found that the eye shape of new born children, at least in Asian, was prolate. Unlike human, the eyeball of chick is flat, so the eye shape is usually oblate, and the AL/ED ratio of emmetropic eye aged between 12 to 26 days is about 0.758 (or the oblateness=+0.242). When the eye becomes more myopic, the AL/ED ratio will increase and the eye shape will become less oblate (*i.e.,* the oblateness becomes less

positive). In fact, the growth of equatorial diameter is under the influence of two types of retinal neurons, bullwhip (in ventral circumferential marginal zone, CMZ) and mini-bullwhip (in dorsal CMZ) cells whose terminals release the glucagons peptide to suppress the equatorial eye growth. On the contrary, inhibition of the functions of these cells will lead to excessive equatorial growth (Fischer, Ritchey, Scott et al., 2008). Therefore, the AL/ED ratio could be an useful parameter for studying the relationship between refractive errors and posterior eyeshape.

Although the corneal accommodations in chick have been extensively studied, the effect of experimentally induced astigmatism on corneal accommodation was not included. In experiment 2 (Chapter 3), a weak but significant positive correlation was found between induced astigmatism and positive corneal accommodation. Like human, the accommodation is usually expressed in positive terms resulting from parasympathetic innervation of the ciliary muscle (Glasser, Troilo and Howland et al., 1994; Glasser, Murphy, Troilo et al., 1995; Murphy, Glasser and Howland et al., 1995) which is striated (West, Sivak and Doughty, 1991a) in chick instead of smooth. However, the mechanism of negative corneal accommodation whether a sympathetic innervation exist in chick or not is still unknown. Even though the destruction of the Edinger-Westphal nucleus (Schaeffel, Troilo, Wallman et al., 1990), section of optic or cilary nerve (Schmid and Wildsoet, 1996; Shih, Fitzgerald and Reiner et al., 1994; Troilo, Gottlieb and

Wallman et al., 1987) did not prevent experimentally induced ametropia; did not cause significant morphological differences in ciliary muscle and ciliary fold structure between control and myopic chicks were found (West, Sivak and Doughty, 1991b); did increase refractive error variability and hyperopic shift. Wildsoet and Scmid pointed out that “an intact optic nerve is required for accurate emmetropization (Wildsoet and Schmid, 2000).” Even though the mechanism of accommodation involved in astigmatic compensation still opens to question, accommodation may probably play a relatively important role in astigmatic compensation since higher precision might be required for compensating for both magnitude and axis orientation. Although the mechanism of negative accommodation is not known, taken together with the results from experiment 3, the characteristics of the ocular structures in chicks, such as corneal fibril arrangement, asymmetry of ciliary muscles, and the pattern of scleral ossicles provide evidence to support the hypothesis of “astigmatic accommodation” (Irving, Sivak and Callender et al., 1992; Irving, Callender and Sivak et al., 1995).

In the last experiment (Chapter 4), I attempted to address a controversial topic about astigmatic compensation in chicks. A replicated experimental design by imposing crossed-cylindrical lenses at four different orientations over the right eyes of the chicks for examining how is the astigmatic compensation related to eyeshape. In experiment A, our results demonstrated the capability of astigmatic compensation in chick eye was

orientation-sensitive, and dose effect of astigmatic compensation was shown in a supplementary experiment (B). Together, both magnitudes and axis orientations of induced astigmatism were significantly different between the H and L groups. Compensation for imposed ATR astigmatism was more difficult than for imposed WTR astigmatism. Several anatomical factors, such as the arrangement of corneal fibrils, asymmetry of ciliary muscles, and the pattern of scleral ossicles, may be crucial for characterizing the accommodation pattern. Positive accommodation not only produced a steeper corneal curvature but also ATR corneal profile, while negative accommodation flattened the cornea and produced WTR astigmatic profile.

Another new finding in this study is the relationship between astigmatism and posterior eyeshape. Although astigmatism is mainly attributed to the cornea, our results indicated that the experimentally induced astigmatism was also associated with the changes in posterior eyeshape profile. As calculated in experiment 3, internal astigmatism could contribute as much as 60% of the refractive astigmatism in chicks. The significant correlations between the internal astigmatism and the posterior eyeshape suggested that the astigmatic compensation might also take place internally by altering the toricity of ocular components. On the other hand, as presented in experiment 1, the highest magnitude of induced astigmatism was found in the TRD group (TRD vs. NRD=2.58±0.38D vs. 0.79±0.31D; Tukey's post hoc, p=0.008). Interestingly, in experiment 3, only the posterior ocular

expansions at the temporal region were correlated with the C-J0 (see Table 4-4). These results suggest that the mechanism regulating astigmatic eye growth may be more sensitive to a local mechanism restricted to the temporal region. Further studies are needed to determine the impacts of local mechanism on refractive development.

There are several limitations in these studies: First, the movement of chick eye, much less than that of human eye, is about 20°. To eliminate the effect of eye movement on hemiretinal form deprivation, covering 10° more from the midline was suggested (Troilo, Gottlieb and Wallman et al., 1987). Although lower field myopia was widely reported in many animal species, the habitual eye position of chick was not known. Also, the eye movements at different gazes have not clearly defined: for instance, the limitation of the vertical gaze may not be the same as horizontal gaze. So, the areas covered by the hemiretinal diffuser at different positions may not be equal. If down gaze was the habitual eye position, larger retinal area would be deprived and the hump of the graph would be closer to the center (see Figure 2-3), and as a result higher myopia would be developed. However Figure 2-3 shows the humps of the graphs of the four hemiretinal form-deprived groups at about 20° away from the central axis, and indicates that the eye movement and eye position did not play important roles. Clearly, the axial changes of the momentarily restricted central region of the hemiretinal form-deprived groups were due to peripheral changes because very brief

period of unrestricted vision are sufficient to prevent deprivation myopia (Napper, Brennan, Barrington et al., 1995). The current experimental design allowed examination of the effects of peripheral form deprivation on on-axis refraction. Second, different manipulations for inducing astigmatism in experiment 2 may mask the effects of manipulations themselves. The different effects on accommodation between plus lens and minus lens; between spherical and astigmatic lenses, as well as between optical lens and diffuser are not known. If the hypothesis of "astigmatic accommodation" is right, the presence of astigmatism might overwhelm the manipulation effects on corneal accommodation. Unless induced astigmatism was attribute to the ocular components other than the cornea and crystalline lens. Third, although the corneal videokeratographer was carefully designed, the movements of the animal cannot totally be avoided, such as eye, head movements as well as body movement due to breathing. Forth, it is unclear whether the partial astigmatic compensation in experiment 3 is due to the limitations of treatment duration and/or the ocular structures. According to our results from experiment 1, the maximums of form deprivation myopia were found after week 1, and the J0 components after week 2, however the J45 components of both TRD and NRD developed slowly over weeks. In combination with the results from Kisilak et al. (Kisilak, Hunter, Huang et al., 2008) on hyperopic defocus compensation, treatment period may be one of the factors for complete astigmatic compensation. By contrasting the results from experiment A and B, only the low-magnitude,

vertical-oriented group showed complete astigmatic compensation, but not the high-magnitude, vertical-oriented or the low-magnitude, horizontal-oriented groups. Fifth, the crystalline lens curvature was not measured and thus the contribution of lenticular astigmatism cannot be confirmed. Previous animal study (Suburo and Marcantoni, 1989) found that avian lens shape could be affected by the vitreal pressure and/or scleral support. Because the location of lens is correlated with the shape of globe (Gündüz, Evereklioglu, Er et al., 2002; Jonas, Kling and Gründler et al., 1997), we also cannot rule out the possibility that the significant correlation found between internal astigmatism and eyeshape was due to the alteration of lenticular toricity. Sixth, only the paraxial refractive errors were measured in these studies, it is unclear how the peripheral refraction is related to the changes in corneal and posterior eyeshape.

Based on what I have learned and in consideration of the limitations in these studies, I would like to offer three suggestions for further research in this area. First, I suggest determining the operating limit during astigmatic compensation. For spherical-lens compensation, it usually takes about two weeks for complete compensation within a range of spherical defocus, I would suggest to extent the treatment period to a longer duration to determine if maximum astigmatic compensation can be achieved. Second, I would like to suggest future research that will include A-scan ultrasonography or OCT. These diagnostic/imaging devices will provide

additional information on the ocular dimensions of different ocular components. Third, it would be important to determine whether and how the experimentally induced astigmatism recovers.

Appendix

Calibration of VKS

The radius of curvature was calibrated with five chromium steel balls (Grade 25, AISI 52100, USA) of known diameters that cover a range of corneal radii in young chicks (5/32" (3.97mm), 3/16" (4.76mm), 7/32" (5.56mm), 1/4" (6.35mm), and 9/32" (7.14mm)). A steel ball was fixed on a platform with its surface cleaned with alcohol before measurements. Five topographic images of the steel balls were taken for each ball with refocusing between measurements. Using a calibration curve (r^2=0.99) compiled from the results of all steel balls, the corneal radius of curvature (*r*, measured in mm) was converted into the corneal power (K, *i.e*., corneal curvature) using the formula K=(*n*-1)/*r*; where n=1.369 is the corneal refractive index of chicks (Avila and McFadden, 2010; Irving, Sivak, Curry et al., 1996; Mandelman and Sivak, 1983) (1.362 was used in other studies (Glasser, Troilo and Howland et al., 1994; Sivak, Bobier and Levy et al., 1978; Troilo and Wallman, 1987), and an "effective index" of 1.332 for better prediction was suggested (personal communication with Dr. Frank Schaeffel). Finally, 1.369 was selected for comparison purpose),. To be able to analyze astigmatic cornea, we further derived six biometric parameters: SK, the meridian with steepest curvature; FK, the meridian with the flattest meridian; MK, the average value of SK and FK; corneal astigmatism (CA), the dioptric difference between SK and FK; C-J0 and C-J45, the power vectors calculated from the corneal astigmatic magnitude and axis (Thibos, Wheeler and Horner et al., 1997).

Figure 6-1 plots the changes in meridional corneal power with respect to mean K of ten chicks who exhibited a range of corneal astigmatisms. As shown, the meridional corneal powers changed smoothly through the 180° meridians, with the SK and FK separated by 90°, indicating that the corneal astigmatism found in chicks was due to a regular change in meridional corneal shape (*i.e.*, regular astigmatism). Compared to birds with higher magnitudes of astigmatism, those with lower magnitudes exhibited slightly more variability in meridional corneal powers, probably due to the relatively higher instrumental noise when measuring lower magnitudes of change.

Images were analyzed using an algorithm written in MatLab software. Specifically, each image was first processed to enhance the rings' regions using a Gabor filtering with an adaptive thresholding strategy. After these processed rings were identified in a coarse-to-fine fashion and labeled digitally, the radial distance of each ring from the origin was detected using the Hough transform (Bryan, 2000; Duda and Hart, 1972). The radial distance was then smoothed using a median filter and converted to radii using the method proposed by Carvalho et al. (Carvalho, Romão, Tonissi et al., 2002). The radii within three pre-selected areas, 1.50mm, 2.10mm and 2.80mm diameters of the central cornea, were segmented into 360° semi-meridians, summed, and averaged for the conventional 180° meridians according to clinical notation.

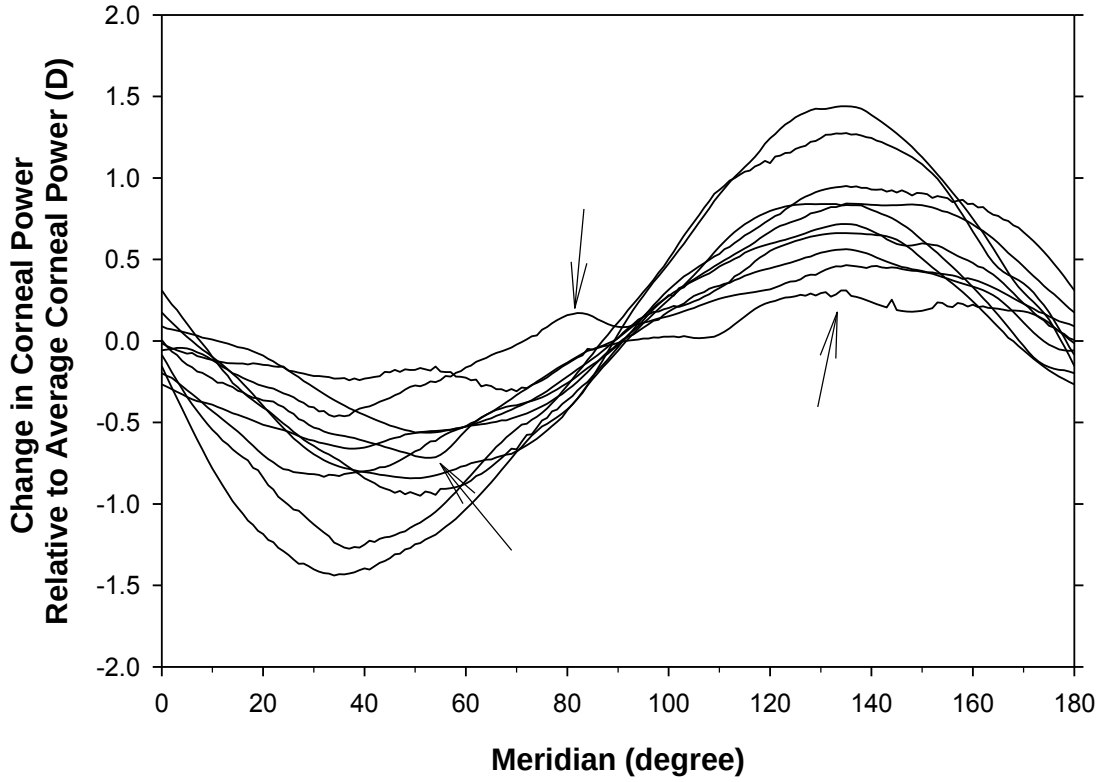


Figure 6-1 Changes in meridional corneal power relative to MK in ten birds with different magnitudes of corneal astigmatism. The steepest meridians for all birds were arbitrarily aligned at 135° to show the sigmoidal changes in corneal power across the 180° meridians. The arrows marked the higher variabilities in meridional corneal powers in birds with lower magnitudes of astigmatism.

The accuracy of the instrument for measuring the three central corneal areas (1.50mm, 2.10mm, and 2.80mm diameter) were determined by calculating the difference of the measured values from the real values of three steel balls (2.78mm, 3.18mm, 3.57mm). Five images, separated by re-alignment and re-focusing, were acquired consecutively from each ball. The data of the five images were averaged using power vector analysis (Thibos, Wheeler and Horner et al., 1997) and subtracted from the real values.

Reliability and Repeatability

Steel balls

Repeated measures of the three steel balls showed that the accuracy of the instrument (measured value minus real value) was 0.18D for the largest tested areas (maximum differences: 1.50mm: 0.45D, 2.10mm: 0.32D, and 2.80mm: 0.18D) in all six corneal parameters. There were no significant differences across the three tested areas in mean K, FK, and C-J0 astigmatic components. Although significant differences across the three tested areas were found for corneal astigmatism, SK and C-J45 astigmatic components (one-way ANOVAs, all $p<0.001$), Tukey's tests (all $p<0.001$) showed that the maximum differences between two tested areas for astigmatism, SK and C-J45 were only, respectively, 0.33D (1.50mm vs. 2.80mm), –0.32D (1.50mm vs. 2.80mm), and –0.17D (1.50mm vs. 2.80mm).

Measurements of the astigmatic components showed a greater instrumental noise for smaller tested area (maximum differences from real value among the three steel balls: 1.50mm vs. 2.80mm: corneal astigmatism =–0.45 vs. –0.18; C-J0=–0.02 vs. –0.01; and C-J45=–0.22 vs. –0.09). Because of the higher accuracy and lower instrumental noise with wider tested area, only data of the 2.8mm diameter central cornea were used for the analyses in this study.

Alert chicks eyes

Six sets of images (50 to 100 images per set) were collected from each of the treated eye for 12 birds from a separate experiment. These birds were treated monocularly with crossed-cylindrical lens and developed different degrees of corneal astigmatism (see corneal astigmatism results in Figure 6-1). Each set of images was separated by a re-alignment which often took less than 2 minutes. From each set of data, one image with good quality was manually selected, *i.e.*, there were six images from each of the twelve eyes. To see if different numbers of images would affect the outcome measures, the mean values of 5 and 3 randomly selected images from each bird were compared. Because no significant differences were found between the means of 5 versus 3 images for all six corneal parameters (*i.e.*, SK, FK, mean K, corneal astigmatism, C-J0 and C-J45; all $p \geq 0.78$), the

repeatability of the instrument was tested by comparing the means from the first and second sets of 3 images.

The Bland-Altman plots in Figure 6-2 illustrate the repeatability of the six corneal parameters for these 12 treated birds. As reflected from the distributions of the six parameters, the crossed-cylindrical lens treatment produced a wide range of corneal curvature and astigmatic components. Despite this significant treatment effect, the mean differences and 95% limits of agreement (in parentheses) for the six parameters were small: mean K, –0.02D (+0.25, –0.25); SK, –0.03D (+0.26, –0.26); FK, –0.01D (+0.25, –0.25); corneal astigmatism, 0.02D (+0.21, –0.21); C-J0, 0.00D (+0.24, –0.24); and C-J45, 0.01D (+0.29, –0.29). In addition, there were no systematic changes across the dioptric ranges measured in all six parameters, and 83% of the repeated measurements differed by less than 0.25D.

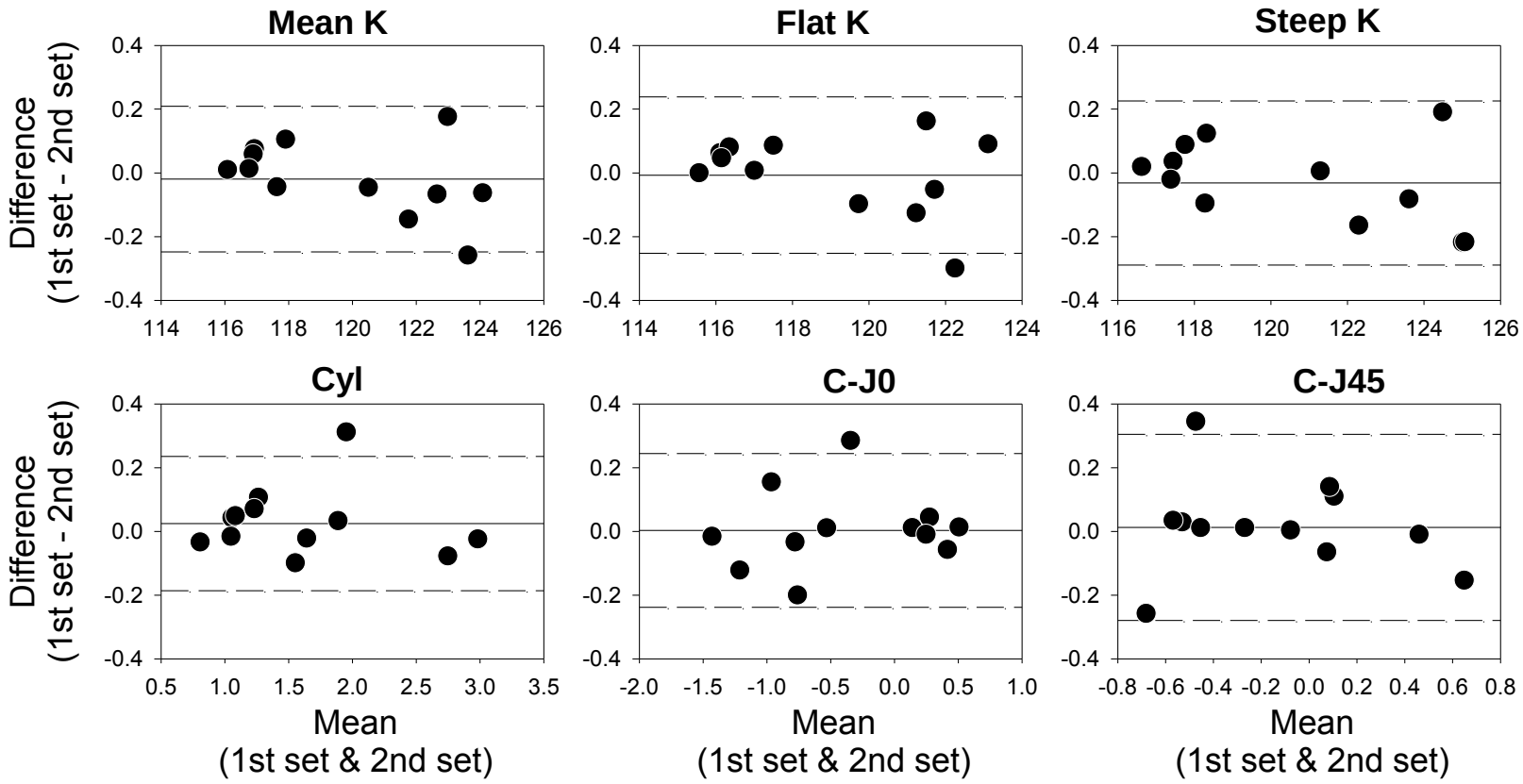


Figure 6-2 Short-term repeatability of MK, FK, SK, CA, C-J0 and C-J45 for videokeraography in chicks. The averages and differences of the two sets of consecutive readings are plotted on the abscissa and ordinate, respectively. *Solid line*: mean difference; *dashed lines*: 95% limits of agreement.

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