Variation of Ocular Parameters in Young Normal Eyes

THESIS

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Abstract

Refractive error is known to correspond to variation of ocular parameters. Myopia has been correlated to changes in retinal thickness, reduction in subjective visual sensitivity and changes in the intrinsically photosensitive retinal ganglion cell (ipRGC) mediated pupillary response, but no study has evaluated these parameters together in the same population of healthy, young subjects.

The purpose of this study was to conduct a comprehensive evaluation of ocular parameters in young, healthy subjects to establish a database of normal parameters for clinical comparison. Control data was directly compared to two subjects with color vision deficiency and two subjects with retinal dystrophy. Subjects with retinal dystrophy also underwent testing with adaptive optics scanning laser ophthalmoscope optical coherence tomography (AO-SLO-OCT).

Fifty-six eyes of 28 control subjects (mean age 25 ± 3 years, 16 female, 12 male) were divided into four groups based on spherical equivalent (SE). Total retinal thickness at the macula, ganglion cell interplexiform layer (GCIPL) thickness at the macula, and retinal nerve fiber layer (RNFL) thickness around the optic nerve head were measured with commercial OCT. Perimetry testing of the central 60 degrees of vision was performed. Of the 28 subjects that underwent OCT testing, 22 underwent pupillometry testing (mean age 25 ± 3. years, 12 female, 10 male) to determine if the
ipRGC pupil mediated response changes with SE, axial length (AL) and GCIPL thickness.

Mean macular thickness (P= 0.042) and GCIPL thickness (P= 0.001) decreased in more myopic groups. Before correcting for magnification error, the mean RNFL thinned with increasing myopia (P= 0.001). After correction for magnification, the relationship was insignificant (P= 0.443). All control subjects had normal visual sensitivity. GCIPL thickness was not associated with visual sensitivity (P= 0.051). Normalized pupil diameter 4.5 seconds after a 5-second blue flash, in the post-illumination pupillary response (PIPR), was not different between the least and most myopic subjects (P= 0.076), but was different between the shortest and longest eyes (P= 0.013). GCIPL thickness 2.5mm superior to the fovea (P= 0.019) and 2mm nasal to the fovea (P= 0.027) was thicker in less constricted pupils during the PIPR.

Commercial OCT revealed the subject with rod-cone dystrophy (RCD) had reduced macular thickness but normal cone density was found at the retinal locations imaged. AO-SLO-OCT suggests that a subject diagnosed with Best’s Vitelliform Macular Dystrophy showed reduced outer segment length compared to aged-matched controls, while the inner segment was less affected.

The findings of this study agree with some previous studies that have evaluated changes in ocular parameters with refractive error, but there are also conflicting results. Kang et al., after correcting for magnification, found that the mean RNFL thickened with increasing AL. Yuan et al. correlated reduced visual sensitivity to thicker RNFL. PIPR was correlated with axial length, but not with spherical equivalent. The increased GCIPL
thickness 2mm outside the fovea may be associated with difference in pupillary function.
The findings of this study can serve as a benchmark against which eyes with various retinal diseases can be compared.
Dedication

This document is dedicated to my family and friends.
Acknowledgments

I would like to express my most sincere thank you and appreciation to my advisor, Stacey Choi, who agreed to accept me as a student and supported my academic and scientific development during graduate school. I also must thank Nathan Doble for supporting my academic growth as well. Elaine Wells-Gray was absolutely essential to the development of this thesis as well as to enabling me to better understand the complexities of adaptive optics; for her time and guidance I am forever grateful and thankful. I also thank Andrew Hartwick and current and former members of his lab, Phil Yuhas and Patrick Shorter, for allowing me to include pupillometry in my study and mentoring me through its implementation. I thank Don Mutti and Jeffrey Walline for their time and for permitting me to use equipment from the BLINK study for my research.

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Chapter 1: Introduction

Extensive research has been completed to understand how ocular parameters change as a function of refractive error and likewise how ocular structure is related to ocular function. Previous studies have evaluated only a few parameters at one time and, therefore, the collective record of previously established correlations between ocular parameters as well as function have only been determined across different populations. The goal of this thesis is to establish a comprehensive and accurate metric of variation of retinal structure and function in the same young (18–40 years of age), healthy population with varying degrees of refractive error ranging from emmetropia to severe myopia. The introduction is, therefore, organized as an overview of the established work relating to retinal structure and function. Deficits in the comprehensiveness are highlighted in each topic, setting the stage for the remainder of this thesis to establish the first comprehensive overview of how ocular parameters change as a function of refractive error in the same young, healthy population. Two age-matched patients with retinal diseases and two age-matched subjects with mild color vision anomalies are included at the end of the thesis as a direct comparison to the normative data.

1.1 Retinal Structure

Retinal structures ranging from total retinal thickness at the macula, to ganglion cell-interplexiform layer thickness at the macula, to retinal nerve fiber layer (RNFL)
thickness around the optic nerve have been extensively examined to evaluate the effect of varying refractive error on these structures.

1.1.1 Macula

Five separate, recent investigations comparing commercial optical coherence tomography (OCT)-measured macular thickness to refractive error have reported contradicting results: four showed a statistically significant negative correlation between average macular thickness and myopic refractive error (Lam, Leung et al. 2007, Song, Lee et al. 2010, Zhang, He at al. 2011, Hwang and Kim, 2012) while one study showed no correlation (Luo, Gazzard et al. 2006). None of these five studies attempted to determine if changes due to refractive error are uniform across all retinal layers or if other layers, such as the outer retina or ganglion cell-interplexiform layer (GCIPL) are similarly affected by thickness changes. Additionally, none of these studies compared refractive error and macular thickness to subject and objective retinal function.

1.1.2 Retinal Nerve Fiber Layer Thickness

Individual patient data is usually compared to a built-in normative database in a commercial system to assess the extent of deviation from population averages. However, the effect of refractive error on the structure is not considered, causing potential false positive results. Thinning of the RNFL has been correlated with myopic refractive error and/or longer axial length (Budenz, Anderson et al. 2007, Kang, Hong et al. 2010). However, due to having some pseudophakic patients in their study, Budenz et al. substituted axial length for refractive error, leaving the interpretation of the refractive
relationship to be relatively superficially evaluated. Additionally, while RNFL thickness has been compared to visual sensitivity and pupil response, no investigation has evaluated both in the same population.

1.1.3. Ganglion Cell-Interplexiform Layer Thickness

A relatively comprehensive analysis has been conducted to determine what factors are correlated to thickness of the GCIPL in the normal population; thinning has been correlated to longer AL in five out of six regions around the fovea and thinner retinal nerve fiber layer thickness (Mwanza, Durbin et al. 2011). Thinning has also been correlated to more myopic refractive error and reduced visual field sensitivity (Araie, Saito et al. 2014). Studies evaluating the effect of refractive error or AL on GCIPL thickness have evaluated populations in the 4th decade or older; reference control data on younger subjects would therefore be valuable for comparison to ocular disease cases.

1.2 Retinal Function

Several studies have investigated changes in visual sensitivity in relation to refractive error using perimetry. Perimetry was used in this thesis to determine the subjective threshold of visual perception of white light in the central 48 to 60 degrees of vision. Another functional test used in this thesis was pupillary response. Pupillary response has been compared to refractive error in two published investigations as a means of understanding the dynamics of the intrinsically photosensitive retinal ganglion cells (ipRGC) and the effect of refractive error on ocular function (Adhikari, Pearson et al. 2015, Mulvihill 2016).
1.2.1 Visual Field

Correlation between the performance on visual field testing and ocular structure and refractive error has been well established for healthy subjects (Martin-Boglind 1991, Rudnicka and Edgar 1995, Aung, Foster et al. 2001, Czepita and Chmielewska 2004). Reduced sensitivity to light in the central 30 degrees of the visual field has been correlated to higher myopic refractive error on the Goldmann perimeter in 15 subjects with non-pathological myopia (Martin-Boglind 1991). Additionally, Czepita and Chmielewska found that average sensitivity, mean defect, and fluctuation values were reduced in young Polish myopes when tested with automated perimetry (Czepita and Chmielewska 2004). AL greater than 26mm and refractive error more myopic than -5.00D have both been shown to correlate with reduced visual field mean sensitivity and greater mean deviation on the automated Humphrey Visual Analyzer 30-2 (Rudnicka and Edgar 1995). In a study using the Humphrey Field Analyzer on normal myopes, spherical refractive error less myopic than -4.00D was not associated with decline in global field indices, nor was axial length, but subjects more myopic than -4.00D demonstrated a more negative mean deviation and their SE and AL was associated with reduced visual field indices; similar results were found if contact lenses were used instead of trial lenses (Aung, Foster et al. 2001). However, in a study with young adults with an average age of 28.3 with a mean SE of −4.79D in the right eye and −4.59D in the left eye, there was no association found between the refractive error and any of the visual field indices of the Octopus 1-2-3 (Interzeag, Schlieren, Switzerland) (Yuan, Feng et al. 2014). RNFL thickness has also been compared to Visual field performance. Thicker RNFL has been associated with a lower mean sensitivity and a higher mean deviation from normal,
even after adjusting for age, gender, optic disc area, ocular axial magnification scaling factor and refractive error (Yuan, Feng et al. 2014). The remaining studies on this topic have only involved comparing visual field performance to RNFL thickness in eyes with pathology. For instance, Ajtony et al. found that there was no association between RNFL average thickness and mean deviation (MD) and mean sensitivity (MS) in subjects with pre-parametric glaucoma as well as normal eyes as measured on the Stratus OCT and the 30-2 Humphrey 750 field analyzer SITA standard algorithm SITA(Ajtony, Balla et al. 2007). These studies in healthy controls did not concurrently evaluate the correlation of visual sensitivity to pupillometry, an objective measure of visual function.

1.2.2 Light Induced Pupillary Response

More recently, the relationship between pupillary response and refractive error has been investigated. A new class of photoreceptors was recently discovered known as intrinsically photosensitive retinal ganglion cells (ipRGC). These cells have their highest density 2mm from the primate fovea (Dacey, Liao et al. 2005). No study has compared the retinal thickness of this region to ipRGC function. These cells have been shown to contain melanopsin photopigment and are responsible for the pupillary light reflex (Lucas, Hattar et al. 2003) It has been shown that the higher the intensity of the stimulus light, the higher current in these cells, and the shorter the time to response peak current, which suggests that ipRGCs adapt to light (Do, Kang et al. 2009) . Specifically, these cells have been shown to photopotentiate with repeated light exposure; in other words, they fire more robustly with repeated exposure to light, resulting in a smaller pupil redilation over time (Mulvihill 2016). This photopotentiation effect has been shown to
correlate with refractive error: less myopic eyes have been shown to photopotentiate more than more myopic eyes when exposed to blue light compared to red light when exposed to repeated, alternating red and blue light (Mulvihill 2016). In essence, less myopic subjects’ pupils stay more constricted after repeated flashes of blue light when compared to the pupils of higher myopes. Additionally, these cells have been shown to have a peak sensitivity to blue light when compared to red light when attempting to account for a basal ipRGC footprint (Adhikari, Pearson et al. 2015). However, unlike Mulvihill, Adhikari et al. found that the amount of redilation measured 6 seconds after a single 10-second flash of blue light, also known as the post-illumination pupillary response (PIRP), was not correlated to refractive error (Adhikari, Pearson et al. 2015). Neither Mulvihill or Adhikari et al. determined whether their subjects were axial or refractive ametropes, as they did not measure axial length. If axial ametropia plays a role in ipRGC function, accounting for both refractive error and axial length may aid in determining why myopic eyes did not correlate to ipRGC function during the PIPR after one stimulus flash, but did when measuring the photopotentiation after multiple flashes.

1.3 Color Vision Deficiency

Two subjects with congenital red-green color vision deficiency (CVD) were evaluated in this study as a direct comparison to normal cases. When measured on OCT, red-green CVD has been known to alter foveal morphology, but to leave the remaining macular structure similar to that of normal subjects (Gupta, Laxmi et al. 2011). Additionally, subjects with the C203R mutation and associated red-green CVD have been known to have thinning of the outer nuclear layer and reduced cone density (Carroll,
Baraas et al. 2009). However, to the knowledge of this author, no study has compared the refractive error of congenital red green CVD subjects to commercial OCT retinal thickness.

1.4 Hyperopia

This thesis compares ocular parameters of subjects with emmetropic and myopic refractive error to one hyperopic subject as a direct comparison to normal emmetropes and myopes. The 1999 to 2004 National Health and Nutrition Examination Survey (NHANES) reported that the prevalence of hyperopia ≥3.00D is 1.2 % for males aged 20 to 39 (Vitale, Ellwein et al. 2008). While most studies evaluating the effect of refractive error on retinal thickness have utilized Asian, myopic populations, few have evaluated the effect of hyperopia in isolation. For instance, Luo et al’s evaluation of refractive effects on macular thickness had no hyperopic subjects more hyperopic than +1.10D spherical equivalent (Luo, Gazzard et al. 2006). Lam et al’s evaluation of the refractive error’s effect on macular thickness found that the most hyperopic subject was +3.25D SE but did have a mean refractive error in the myopic range of -6.13±4.52. When comparing macular thickness to refractive error, Wu et al. similarly had subjects hyperopic refractive error up to +3.30D but the mean refractive error was again myopic at -0.22±0.50D(Wu, Chen et al. 2008). Wu et al., Sato et al., Luo et al., Lam et al. and Hwang et al. grouped hyperopes with emmetropes or low myopes when evaluating macular thickness changes due to hyperopia and did not evaluate hyperopes as an independent group(Lam, Leung et al. 2007, Wu, Chen et al. 2008, Sato, Fukui et al. 2010, Hwang and Kim 2012). Zhang et al. did evaluate hyperopic refractive changes to macular thickness, but their subjects were
exclusively Chinese schoolchildren aged 8.63±1.64 years, a relatively specific population that may not be useful for comparison to developed adults. Kang et al’s evaluation of refractive error’s effect on RNFL thickness included subjects no more hyperopic than +1.10D (Kang, Hong et al. 2010). Further understanding of how hyperopic refractive error affects retinal thickness measurements in young adult populations is warranted.

1.5 Disease Cases

One subject with rod-cone dystrophy (RCD) and one subject with Best’s vitelliform macular dystrophy (BVMD) were evaluated in this study as a direct comparison to normal cases.

RCD is a hereditary retinal dystrophy. Hereditary retinal dystrophies are a heterogenous group of retinal dystrophies by which mutations in genes in the choroid, photoreceptor cells (PRC), and other retinal cells result in reduced vision function (Nentwich and Rudolph 2013). RCD is characterized by a gradual degeneration of rod photoreceptor cells (PRC) and later cone PRCs. The most common form of hereditary retinal disease is retinitis pigmentosa (RP), affecting one in 3000 people (Francis 2006). RP subjects have been evaluated using adaptive optics scanning laser ophthalmoscopy (SLO), suggesting that PRCs may drop out in regions (Roorda, Zhang et al. 2007). Reduced cone density in RCD, as measured by flood-illuminated AO fundus camera, has been correlated to reduced visual function (Choi, Doble et al. 2006). Using commercial OCT, macular thinning and RNFL thinning has been associated with RP (Garcia-Martin, Pinilla et al. 2012). Commercial OCT has also shown thinning in tissue adjacent to the foveola and can be used to identify epiretinal membranes and cystoid macular edema.
associated with the disease (Grigoropoulos 2009). AO-SLO imagery of RCD will be evaluated in this study.

Best’s Vitelliform Macular Dystrophy (BVDM) is an autosomal dominant or recessive inherited progressive macular dystrophy. BVDM is classically divided into four stages, which are primarily judged based on the clinical appearance of the macula: in stage one, the patient presents with an abnormal electrooculogram; in stage two, the retinal pigment epithelium (RPE) is disrupted; in stage 2 and 2a, a yolk-like vitelliform lesion exits in the macula which then becomes disrupted forming the “scrambled egg appearance” (stage 2a); in stage 3, the also known as the pseudohypopyon phase, the vitelline substance presents with a fluid and forms fluid level within the lesion; in stage 4a, the lesion is orange red with visible choroid; in stage 4b, the macula scars; in stage 4c, choroidal neovascularization occurs (MacDonald and Lee 1993). Stage V is the atrophic phase (Querques, Regenbogen et al. 2008). OCT has been shown to demonstrate a cross-sectional view of the macula in subjects with BVMD (Querques, Regenbogen et al. 2008). Commercial OCT has shown that stage 1 is correlated with changes in the outer retina, the intermediate stage is characterized by elevation and splitting of the RPE-outer retinal complex, and the atrophic stage consists of retinal thinning (MacDonald and Lee 1993). BVMD cone density and inner segment (IS) and outer segment (OS) measurements of PRC adjacent to active lesions have been previously measured using AO-SLO (Kay, Land et al. 2013). Both cone density measurements and inner and outer segment lengths in BVMD will be evaluated in this study using a combined AO-SLO-OCT system.
The complete body of ocular parameters in normal subjects is yet to be established in the same population. Therefore, in this thesis, we perform the first comprehensive investigation of retinal thickness, ocular biometry, pupillary function and visual sensitivity in the same cohort of 18 to 40-year-old healthy subjects. We then compare normal ocular parameter findings to ocular parameters of CVD cases, one hyperopic subject and two subjects with retinal disease.
Chapter 2: Materials and Methods

2.1 Subjects

Fifty-six eyes of 28 young adult control subjects (mean age 25 ± 3 years, 16 female and 12 male) were divided into four groups based on refractive error for commercial OCT analysis and comparison to disease cases. The four groups are (1) emmetropic eyes (+0.50D to -0.50D in spherical equivalent (SE)); (2) mild myopic eyes (SE of -0.75D to -2.00D); (3) moderate myopic eyes (SE of -2.25D to -4.75D) and (4) severe myopic eyes (SE of -5.00D to -8.75D). One additional normal subject with a hyperopic refractive error was not included into a group and was instead evaluated as a case study. 27 of the subjects were of Caucasian ethnicity and one was of Asian ethnicity.

Of the 29 normal subjects, 22 subjects (mean age 25 ± 3. years, 12 female and 10 male) participated in pupillometry testing. The 22 subjects were divided into two groups based on refractive error and axial length to determine if the ipRGC mediated pupil response differs between the lower and upper halves of refractive error of the control subjects of this study, as well as between the upper and lower halves of axial lengths. Right eye data was used. All subjects tested with pupillometry were ethnicly Caucasian.

Subjects were recruited primarily from The Ohio State University College of Medicine and College of Optometry with exception of three subjects who were not OSU students or staff. All subjects were required to (1) be between the ages of 18 and 40 years old, (2) have refractive error ranging from +4.00 D to -9.00 D SE, with a maximum
cylinder power of 2.00D, (3) have no previous history of eye diseases or surgeries, (4) have no color vision deficiency and (5) have visual acuity correctable to 20/20.

The Tenets of the Declaration of Helsinki were observed throughout the study, and the protocol was approved by the Institutional Review Board of the Ohio State University. Written informed consent was obtained after all procedures were fully explained to the subjects and prior to any experimental measurements. Both eyes of each subject were measured and evaluated for all tests except for the pupillometry testing, where only the right eye’s data was evaluated.

2.1.1 Color Vision Deficiency Subjects

CVD subjects met all the same criteria as control subjects with the exception that they have a known previous diagnosis of congenital dyschromatopsia. For their data to be included in the study, they were required to fail the Hardy Rand and Rittler (HRR) pseudoisochromatic plate (PIP) testing during the study. Both CVD subjects were red-green color deficient.

2.1.2 Retinal Dystrophy Subjects

Subjects with retinal dystrophy were chosen such that their ocular parameters, particularly refractive error, and systemic history reflected that of age-matched controls. No restrictions on best corrected visual acuity were placed on disease cases. One 38-year old male subject with RCD was recruited and one 20-year old female subject with BVMD was recruited.
2.2. Imaging Modalities

2.2.1 Fundus Photography

Digital fundus photography was conducted using a TRC-NW8 Non-Mydriatic Retinal Camera (Topcon Medical Systems Inc., Oakland, NJ, USA) to rule out gross pathology of the posterior pole, optic nerve and macula for each normal subject. Undilated flash-illuminated images centered on the optic nerve and macula were taken for each subject, permitting a comprehensive analysis of ocular structures. Should an image have resulted in poor quality, or lack of adequate reflection off the retina, subjects were given 1-2 minutes to enable redilation of their pupils, and an additional attempt at photographing the retina was made.

2.2.2. Commercial Optical Coherence Tomography

Commercial optical coherence tomography of the RNFL and macula was captured using the Zeiss Cirrus High Definition OCT Model 4000 (Carl Zeiss Meditec, Dublin, CA) and software version 6.0.2.81. This machine records retinal thickness from the RPE to the inner-limiting membrane (ILM) of the retina using a spectral domain OCT with a 840nm light source, capable of a resolution up to 5 micrometers and a scan rate of 27,000 scans per second.

2.2.3 Adaptive Optics (AO) - Scanning Laser Ophthalmoscope (SLO) - Optical Coherence Tomography (OCT)

Four normal subjects and disease subjects were imaged using an AO-SLO-OCT system. One RCD subject and one subject with Best’s disease was imaged. The details of
the combined AO-SLO-OCT system has been described elsewhere. (Wells-Gray EM 2015) but briefly, it simultaneously acquires cross-sectional OCT and enface SLO views of the retina at the cellular level. It measures ocular aberrations using a Hartman Shack wavefront sensor over a 7.15mm pupil and corrects them using a continuous-surface magnetic-membrane deformable mirror with 97 high-speed actuators. (DM; DM97-15, ALPAO, Montbonnot-Saint-Martin, France) prior to retinal imaging. Both SLO and OCT images are acquired at 60 Hz. A 680 ± 3nm light source (BroadLighter T- Series 680-HP, Superlum, Cork, Ireland) is used to acquire SLO images while OCT images are acquired using a superluminescent diode at 860 ± 70 nm (BroadLighter SLD T-860-HP, Superlum). All OCT results in this thesis were obtained in the line-scanning mode. The OCT B-scans comprise 600 A-scans, with a 40 kHz A-scan rate. The field of view for both modalities ranges from 0.7°×0.5° to 1.0°×1.5°. The power of the imaging lights is about 10 times below the American National Standards Institute’s maximum permissible exposure for ocular safety (Delori, Webb et al. 2007). Pupils were dilated using a drop of 1.0% Tropicamide and a drop of 2.5% Phenylephrine ophthalmic solutions prior to imaging. Refractive error was corrected with trial lenses and head motion was minimized using a combination of chin and forehead rest. Retinal locations were chosen by using an internal fixational target.

### 2.3 Ocular Parameters

#### 2.3.1 Ocular Biometry

Three axial length, keratometry, anterior chamber depth, and lens thickness was recorded for all eyes of control subjects and color vision deficient subjects using a
Lenstar LS900 Biometer (Haag-Streit, Koniz, Switzerland) by two trained technicians. Only the axial length data was used in the analysis.

2.3.2. Intraocular Pressure

Intraocular pressure (IOP) was attained by taking the average of three measurements of each eye on the Reichert 7 Auto-Tonometer (Amatech, Buffalo, NY), which is a non-contact tonometer (NCT). An average IOP between 10 and <22 was considered normal, and all control subjects fit within this criterion. IOP was used to rule out pathology and was not included in the analysis.

2.3.3. Refraction

All normal subjects required correction to 20/20 in each eye. Refractive error was attained through autorefraction or manual refraction by a trained investigator to ensure that each subject could read the 20/20 line—the endpoint of refraction. A randomized, wall-mounted LED Snellen eye chart was used during recognition acuity assessment of the minimum angle of resolution as well as during subjective refraction to determine if refractive requirements were met.

2.4. Retinal Function

2.4.1. Visual Field

Perimetry for control subjects was assessed using the Humphrey Visual Field (HVF) 750 series system (Zeiss, Humphrey Instruments, San Leandro, CA), and all control subjects were evaluated using the 30-2 Swedish Interactive Threshold Algorithm
(SITA) standard test. One RCD disease case underwent visual field testing but with the 24-2 SITA test. The 30-2 SITA standard test measures sensitivity of the central 60 degrees of visual field at 76 points that are separated by 6 degrees, while the 24-2 evaluates the central 48 degrees. The SITA test measures the subjective threshold for the perception of light at each location from high intensity to low intensity of light and vice versa.

2.4.2. Light-Induced Pupillary Response

Pupillometry was conducted using the RAPDx pupilometer (Konan Medical, Irvine, California) using custom software. The RAPDx recorded images of the pupil at ~40Hz. Blue light was used as a stimulus with a peak wavelength of 448nm and corneal irradiance of $2.70 \times 10^{12}$ photons/s/cm$^2$, as was red light of 608nm peak wavelength and irradiance of $2.58 \times 10^{12}$ photons/s/cm$^2$ (Shorter 2015).

2.4.3. Color Vision Assessment

All Color vision was assessed using HRR Pseudoisochromatic Plates. Any deficits in color vision perception resulted in disqualification as a control subject. Two subjects presented with color vision deficits, and all their testing data was recorded for comparison to control subjects with similar spherical equivalent.
2.5. Data Analysis

2.5.1. AO Images

2.5.1.1. Cone Density

A custom MATLAB algorithm (MathWorks, Natick, MA, USA) was used to register AO-SLO images to increase the signal to noise ratio prior to cone density measurements. To optimize the automated selection of cones in a custom cell-counting routine in MATLAB, regions of cones in each SLO image were selected (Figure 1). If vasculature was obscuring the view of cones, it was manually traced to subtract its surface area for the cone density calculation. The size of field of view is affected by the axial length (AL) of the eye, hence magnification factor was calculated for each eye based on the AL using the formula described in paper by Bennett et al. (Bennett, Rudnicka et al. 1994). In this equation (Equation 1), $q$ is the magnification factor and $x$ is axial length.

$$q = 0.01306 (x - 1.82)$$

Equation 1. Ocular magnification factor $q$ of the eye. Axial length in millimeters is $x$. Magnification factor $q$ is multiplied by the image size for an emmetrope to attain the true image size.
2.5.1.2. Inner and Outer Segment lengths of Cone Photoreceptors

Inner and outer segment length (ISL and OSL) of individual cone photoreceptors from registered AO-OCT images were measured using ImageJ (National Institute of Mental Health, Bethesda, Maryland, USA). ISL and OSL were measured at ten equally spaced locations on each B-scan. Measurements in pixels were converted to microns by multiplying the pixel values by 1.025.
Figure 2. Example of inner and outer segment measurement of cones. The second hyperreflective image below the IS-OS boundary was deemed to be the posterior border of the outer segment.

2.5.2. Commercial Optical Coherence Tomography

2.5.2.1. Macula

2.5.2.1.1 In-built Macular Cube

The macula was scanned using the Macular Cube 512x128 algorithm, which scans 512 consecutive linear a-scans within a series of 128 B-scans and creates a 6mm x 6mm 3-dimensional cube image of the retina around the fovea as seen in Appendix A. The data was then analyzed using the macular thickness analysis, which displays the average thickness of the retina in nine regions around the fovea: one central 1mm circle surrounded by two concentric rings, each broken into four equal quadrants located 3mm (inner macula) and 6mm (outer macula) around the fovea. The data for each region was then averaged by refractive error for comparison to disease cases. Of the 29 control
subjects that completed the Macular Thickness analysis, one had missing scan data in the inferior quadrant of the outer macula and therefore that data was unable to be included in analysis; the remainder of the scan was included.

2.5.2.1.2 *In-built GCIPL*

The GCIPL metrics were acquired using the same data and scans as the Macular Cube 512x128 algorithm. Using Zeiss’ ganglion cell analysis (GCA) algorithm, the software measures the thickness of the combination of the ganglion cell layer and the interplexiform layer of the inner retina in an elliptical annulus around the fovea. This annulus is broken into three equally sized superior sectors and three equally sized inferior sectors, as seen in Appendix B, which this study labelled based on relative location with respect to the fovea: inferior, superior, inferior nasal, superior nasal, inferior temporal and superior temporal. The vertical outer diameter of the annulus is 4mm and the inner vertical diameter is 1mm (Mwanza, Durbin et al. 2011). The horizontal inner and outer diameter is stretched by 20% (Mwanza, Durbin et al. 2011). While this data is used to monitor for glaucoma progression, it was used in this study to compare to pupil response and control biometry and refractive error.

2.5.2.1.3 *Caliper measurements of total retinal thickness and GCIPL*

The thickness of the total retina, as defined as the inner border of the inner-limiting membrane to the outer border of the RPE, as well as the GCIPL, was manually measured for all subjects that underwent pupillometry testing (Figure 3). The purpose was to attempt to correlate the thickness of the inner retina, the location of the highest
concentration ipRGCs, which is 2mm from the fovea, to ipRGC function. High Definition B-Scans were recorded across the fovea. Lateral magnification was accounted for using magnification factor $q$, from Bennett’s version of the Littman formula, to determine the location of 2.5mm and 2.0mm from the fovea for each eye. At that location, thickness was measured three times and the average was recorded. Thicknesses were manually measured using the caliper function included in the Cirrus OCT.

Figure 3. Manual measurement of the GCIPL and the total retinal thickness. To locate 2mm and 2.5mm from the fovea, magnification factor $q$ was multiplied by 2mm and 2.5mm respectively. The Cirrus OCT’s caliper function was then used to measure out the appropriate magnification-corrected distance from the fovea. At 2mm and 2.5mm, GCIPL thickness and total retinal thickness was manually measured three times using the caliper function and the average of the three measurements was recorded.
The difference between the GCIPL and the total retinal thickness was calculated to estimate the thickness of the outer retina in a 4.4mm circular ring around the fovea with an inner diameter of 1mm. This required estimation because the Macular thickness analysis data, which was used to measure total retinal thickness, is not an annular ellipse, but is instead a 6mm circle divided into three circular rings with 1mm, and 3mm, and 6mm outer diameters (Appendix A). Based on the 20% horizontal magnification, the elliptical annular ring of the GCA is 4mm tall vertically and 4.8mm wide horizontally with a 1mmx1.2mm central hole (Appendix B).

The GCA annual ellipse was estimated to be a circular ring with a 4.4mm diameter with a 1.0mm hole in the center (Figure 4). For each subject, the 6 GCA sectors were averaged to estimate the uniform average thickness for one 4.4mm circular ring. Additionally, all four quadrants of the macular thickness analysis data were averaged for both the both the 3mm (inner macula) and 6mm (outer macula) rings, creating inner and outer macula averages.

The area of scan overlap from the inner macula data from the macular thickness analysis and the 4.4mm ganglion cell circle (Figure 4) and the area of scan overlap from the outer macula from the macular thickness analysis and the 4.4mm ganglion cell circle were weighted to estimate outer retinal thickness in a 4.4mm circle. 36.30% of the total area of the 4.4mm GCIPL circular ring overlapped with the 3mm circular ring (inner macula) from the Macular Thickness Analysis scan and 63.70% of the area of the 4.4mm GCIPL circular ring overlapped with the 6mm circular ring (outer macula) from the
Macular Thickness Analysis data (Figure 4b). Therefore, 36.30% of the mean inner macula total thickness was added to 63.70% of the mean outer macular total thickness to estimate total retinal thickness for a 4mm circular ring with a 1mm inner diameter around the fovea.

![Figure 4. Superimposition of the macular thickness analysis scan over the ganglion cell analysis scan. The GCA scan is assumed to be a circle. (a) boundary summary (b) delineation of areas of overlap.](image)

2.5.2.2. Optic Nerve Head

2.5.2.2.1. Nerve Fiber Layer Thickness

The optic nerve was imaged using the Optic Disc Cube 200x200 scan, which scans 200 consecutive linear a-scans within a series of 200 B-scans, creating a three-dimensional cube image of the retina around optic nerve. The data was analyzed using Zeiss’ ONH and RNFL OU analysis algorithm which averages the thickness of the RNFL around the optic nerve head into 12 clock hour positions, 4 quadrants, and an overall average thickness value as seen in Appendix C.

The Optic Disc Cube 200x200 scan measures a 1.73mm circle around the ONH to measure the average RNFL thickness. Because of axial magnification, magnification of
this circle was accounted for using the Leung et al. modification (Leung, Cheng et al. 2007) of the Bennett et al. approach (Bennett, Rudnicka et al. 1994) in determining the true size of a fundus image. Using the Leung et al. application of this formula (Equation 2), which assumes an emmetropic axial length of 24.46mm (Leung, Cheng et al. 2007), and applies to the Cirrus and Stratus OCT (Kang, Hong et al. 2010), linear magnification of all RNFL OCT thickness measurements was found and axial thickness changes were subsequently accounted for.

\[ q = 0.01306 (x - 1.82) 3.382 \]

Equation 2. Modification of the Littman formula by Leung et al. Magnification factor \( q \) is multiplied by the image size to determine true image size on the retina. This equation only accounts for axial length, \( x \).

2.5.3. Visual sensitivity using static perimetry

The right eye perimetry was tested first, followed by the left eye. To maintain anonymity, each subject’s date of birth was rounded to the nearest quarter of a year and entered into the HVF prior to testing. Refractive error correction during testing was calculated using the HVF’s internal calculator. Cylindrical power was only corrected if it was greater than or equal to -1.00 D. The purpose of testing was to screen for any pathology or gross visual field defects on the mean deviation (MD) plot, which compares visual field sensitivity at each point to a database of controls, as well as on the pattern deviation plot, which displays concentrated visual field defects after correcting for elevations or depressions of the entire visual field relative to the control database. Any clinically significant concentrated field defects or general depression in the visual field,
as judged by an experienced examiner, would result in a disqualification. No control subjects were disqualified for visual field results. Additionally, the MD and pattern standard deviation (PSD) were recorded for comparison to ocular parameters, including refractive error. PSD data was not available for six out of 29 control subjects.

2.5.4. Pupillary Response using RAPDx

The RAPDx manufacturer gave permission for us to alter the characteristics of the RAPDx stimuli for this study. Pupillometry began with 5 minutes of dark adaptation. Four and a half of the minutes of adaptation consisted of facing the wall of a dark room, and the final 30 seconds of the adaptation consisted of looking into the RAPDx and adapting to the ambient light emitted by the two LCD screens in the pupillometer. After dark adaptation, subjects were exposed to the blue stimuli for five seconds followed by darkness for five seconds. After the five seconds of darkness, the subjects were exposed to five seconds red light, the control, followed by another 5 seconds of darkness. Pupil size was measured at 40hz. The timeline for testing is summarized on Figure 5.
Figure 5. Pupillometry stimulus timeline. 5 minutes of dark adaptation preceded the testing. Testing began with 5 seconds of blue light, followed by 5 seconds of darkness, then five seconds of red light, and ended with 5 seconds of darkness. Pupil diameter was recorded at 40Hz.

An Excel (Microsoft, Redmond, WA) macro as described by Shorter, was used to convert pupil diameter from pixels to millimeters, average the 40Hz data into 0.25s intervals, remove errors due to blinks, record the maximum and minimum pupil diameters of each eye to normalize pupil diameter from 0% to 100% total diameter, and generate graphs of each test. Data from each eye was manually evaluated for noise and artifacts. Noise and artifacts were judged based on the physiological limitations of the velocity of constriction and dilation: diameter changes exceeding 0.5mm/0.25sec were suspected as being erroneous and were deleted from the data set. Further, pupil diameter > 8.00mm or <1.00mm were assumed to be erroneous and were likewise deleted from the data.
2.5.5. Statistics

All statistical analysis was conducted using the Statistical Program for the Social Sciences (IBM Analytics, Chicago, IL) software version 24. Variables examined by bivariate analysis and tested for correlativity and statistical significance. P<0.05 was considered statistically significant. Ocular parameters of differing refractive groups were compared using a one-way ANOVA for significant differences between means. Post hoc analysis was conducted using the Tukey method. For commercial OCT average thicknesses, one-sample t-tests were used to determine if disease and CVD cases are statistically significant from aged-matched controls with similar refractive errors. For pupillometry, independent sample t-tests were used to compare population means between refractive and axial groups.
Chapter 3: Results

3.1. Refractive Error vs. Axial Length of Control Subjects

The mean SE of all normal subjects’ eyes was -2.42D ±2.15D. The average axial length of control eyes used for comparison between refractive groups was 24.34mm±1.05mm. Mean axial length between refractive groups was statistically significant (P= 0.001) in that increasing myopia is associated with axial elongation. Post hoc analysis indicates that average AL was statistically significant between all groups except for mild and moderate myopes (Table 1). The formula for magnification for the Cirrus OCT by Leung et al. assumes that the emmetropic eye is 24.46mm in axial length. Given that roughly 3.00D equates to 1.0mm of axial length, the average error by which the population of this study did not follow the model by Leung et al. for axial refractive error is -2.86D±2.26D, in that the population tended to be more myopic than their axial lengths would suggest.

3.2. Relationship between retinal Structure and refractive error

3.2.1. Macula

Table 1 summarizes all measurements taken from the macula on the Cirrus OCT, to include the total retinal thickness, the GCIPL thickness, outer retinal thickness, as well as inter-group comparisons of the mean measurements.


3.2.1.1. Total retinal thickness

In general, longer AL and myopic SE were associated with thinning of the macula. When comparing the average macular thickness between subjects divided by refractive error, a one-way ANOVA reports that the average mean between groups was statistically significant (P= 0.042), suggesting thinning with increasing myopia, as seen in Table 1. Additionally, significant differences between mean thicknesses of refractive control groups was found at the inferior and temporal quadrants of the inner macula (P= 0.010 and 0.001, respectively). The superior and nasal quadrants were not statistically significant between groups, nor were any quadrants of the outer macula. Macular volume was not significantly different between groups. Distribution of data and post hoc analysis are summarized on Table 1.

When all subjects are evaluated as one group, Pearson correlation indicates that increasing hyperopic spherical equivalent was significantly correlated with thickening of the average macular thickness (P= 0.018, r=0.315), as seen in Figure 6.

For the inner macula, increasing hyperopic spherical equivalent was associated with thickening of the inferior quadrant (P= 0.011, r=0.337), but the other quadrants were not correlated to spherical equivalent. For the outer macula, increasing hyperopic spherical equivalent was associated with thickening of the superior quadrant (P= 0.026, r=0.298), the inferior quadrant (P= 0.029, r=0.295), and the temporal quadrant (P= 0.001, r=0.494). All other quadrants of the outer macula were not associated with spherical equivalent.

The average macular thickness was not correlated to AL. For the inner macula, Pearson correlation indicated that longer axial length was significantly correlated with
retinal thinning in the superior quadrant ($P=0.040$, $r=-0.275$), inferior quadrant ($P=0.029$, $r=-0.293$), but not the nasal or temporal quadrants. For the outer macula, longer AL was correlated to the thinning of the inferior quadrant ($P=0.048$, $r=-0.268$), and the temporal quadrant ($P=0.004$, $r=-0.377$), but not the nasal or superior quadrants.

Figure 6. Mean macular thickness versus spherical equivalent. Pearson correlation was calculated for all normal subjects ($P=0.018$, $r=0.315$), suggesting thinning is significantly correlated to increasing myopic refractive error.
Table 1. Distribution of GCIPL and macular OCT data. All relationships calculated using a One-Way ANOVA with a Tukey post hoc analysis. Significance calculated at the \( p \leq 0.05 \) level. For total retinal thickness around the macula, mean macular thickness and the inferior and temporal quadrants of the outer macula were statistically different between at least two refractive groups, suggesting thinning with increasing myopia. The remaining regions were not statistically significant between groups. For the GCIPL, all regions were significantly different between at least two groups, also suggesting thinning with increasing myopia. Outer retinal thickness was thinner in moderate myopes than in emmetropes, but not significantly different between other groups.
3.2.1.2. Ganglion Cell-Interplexiform Layer Measurements

When comparing the average GCIPL thickness between refractive groups, a one-way ANOVA reports that the mean thickness between groups was statistically significant ($P=0.001$). Additionally, one-way ANOVA suggests significant differences between mean thicknesses for different refractive groups were found in all 6 regions of the annular ellipse (inferior $P=0.001$, superior $P=0.001$, inferior nasal $P=0.001$, superior nasal $P=0.001$, inferior temporal $P=0.005$, and superior temporal $P=0.001$), suggesting thinning is associated with greater myopic refractive error. For distribution of data and post hoc analysis see Table 1. Pearson correlation suggested thinning was associated with increasing myopia (Figure 7).

![Graph showing the relationship between GCIPL thickness and spherical equivalent.](image)

Figure 7. Mean GCIPL thickness versus spherical equivalent. Increasing myopia is associated with GCIPL thinning ($P = 0.001$, $r=0.430$).
3.2.1.3 Outer Retinal Thickness Measurements

When comparing the average outer retinal thickness between refractive groups, estimated as a 4.4mm circular disc with a 1mm inner diameter around the fovea, a one-way ANOVA reports that the mean thickness between refractive groups is statistically significant (P= 0.049). However, post hoc analysis indicated significance only existed between emmetropes and moderate myopes. A bivariate correlation indicated that there is no statistically significant correlation between outer retinal thickness and axial length. There is was significant positive correlation to the average macular thickness, the foveal thickness, all locations of the macular thickness analysis algorithm, the superior temporal region of the GCIPL scan (P= 0.43, r=0.272), but no correlation to any other regions of the GCIPL algorithm or quadrants of the RNFL thickness analysis.

3.2.2. Optic Nerve Head

Table 2 summarizes measurements taken around the optic nerve head before and after magnification.

3.2.2.1 RNFL Measurements

When comparing the average RNFL thickness between different refractive groups, a one-way ANOVA reported that the mean RNFL compared between groups was statistically significant before correcting for magnification error due to axial length (P= 0.001), as were all quadrants (p≤0.05) except the temporal quadrant (Table 2). After correction for magnification, no significant difference between average RNFL thickness was found between groups (P= 0.443), nor for any of the four quadrants (Table 2).
Pearson correlation indicated that longer axial length was associated with thinner RNFL thickness before lateral magnification (Equation 2) was accounted for ($P=0.001, r=-0.619$), but not after ($P=0.477, r=-0.097$). After magnification, with a Pearson correlation, none of the four quadrants’ mean thickness correlated to axial length ($P$ value ranging from 0.071 to 477). After magnification, RNFL thickness in all four quadrants was not correlated to SE either ($P$ value ranging from 0.511 to 0.934).

<table>
<thead>
<tr>
<th></th>
<th>Emmetropia</th>
<th>Mild Myopia</th>
<th>Moderate Myopia</th>
<th>Severe Myopia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RNFL Thickness Before Magnification</strong></td>
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<tr>
<td>Average Thickness (µm)</td>
<td>101.5±7.0*</td>
<td>95.1±7.3*</td>
<td>97.2±7.41†</td>
<td>86.71±3.35⁵⁶</td>
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<tr>
<td>Superior Quadrant (µm)</td>
<td>124.3±14.7⁵</td>
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<td>126.6±15.7</td>
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<td>Nasal Quadrant (µm)</td>
<td>85.2±6.8⁷</td>
<td>70.5±8.5⁸</td>
<td>67.0±9.6</td>
<td>64.29±12.7³⁴</td>
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<td>Inferior Quadrant (µm)</td>
<td>132.0±13.7⁴</td>
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<td>109.29±5.47⁵⁶</td>
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<tr>
<td>Temporal Quadrant (µm)</td>
<td>64.5±7.7</td>
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<td>66.5±11.7</td>
<td>65.00±4.55</td>
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<td><strong>RNFL Thickness After Magnification</strong></td>
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<tr>
<td>Average Thickness (µm)</td>
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<td>63.95±7.82</td>
<td>67.85±10.32</td>
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*Significant difference between Emmetropes and Mild Myopes
†Significant difference between Emmetropes and Moderate Myopes
‡ Significant difference between Emmetropes and Severe Myopes
§ Significant Difference Between Mild Myopes and Moderate Myopes
⁴Significant Difference Between Moderate Myopes and Severe Myopes
⁵Significant Difference Between Mild and Moderate Mypes
⁶Significant Difference Between Mild and Moderate Mypes

Table 2. Distribution of RNFL data. All relationships were calculated using a One-Way ANOVA with a Tukey post hoc analysis. Significance calculated at the $p \leq 0.05$ level. Mean RNFL thickness in all quadrants were significantly thicker in at least 2 groups.
3.3. Relationship between retinal function and refractive error

3.3.1. Visual Field

The relationship between refractive error and MD and PSD is summarized on Table 3. No statistical significance was found to exist between the MD or PSD between any of the four refractive groups. Pearson correlation indicated that average RNFL thickness was not significantly correlated to MD and PSD (P= 0.411, r = -0.112 and P= 0.659 and r= 0.068 respectively). Pearson correlation indicated that GCIPL thickness was not associated with MD or PSD (P= 0.051, r= -0.262 and P= 0.361, r = -0.140.)
respectively). Mean relative pupil constriction after red and blue stimuli was not correlated to PSD or MD.

<table>
<thead>
<tr>
<th></th>
<th>Emmetropia</th>
<th>Mild Myopia</th>
<th>Moderate Myopia</th>
<th>Severe Myopia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Mean Deviation</td>
<td>-0.083±0.78</td>
<td>-0.27±0.73</td>
<td>-0.21±1.35</td>
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<td>Average Pattern Standard Deviation (dB)</td>
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<td>1.66±0.21</td>
<td>1.76±0.51</td>
<td>1.45±0.24</td>
</tr>
</tbody>
</table>

*Significant difference between Emmetropes and Mild Myopes
*Significant difference between Emmetropes and Moderate Myopes
*Significant difference between Emmetropes and Severe Myopes
*Significant Difference Between Mild Myopes and Severe Myopes
*Significant Difference Between Moderate Myopes and Severe Myopes
*Significant Difference Between Mild and Moderate Myopes

Table 3. Distribution of Visual Field Data. All relationships calculated using a One-Way ANOVA with a Tukey post hoc analysis. Significance was calculated at the p≤0.05 level. No statistical significance was found mean MD or PSD when refractive groups were compared.

3.3.2. Pupillometry

The pupillometry results of short versus long axial length and more myopic versus less myopic groups are summarized on Table 4 and Figure 9.

3.3.2.1 Pupillary Response as a Function of Spherical Equivalent

When analyzing SE, using an independent samples t-test, normalized pupil constriction 4.5 seconds after a 5-second blue flash was not statistically different when comparing the average pupil size of the 11 least myopic subjects to the 11 most myopic subjects (P= 0.076). With red light, the control, there was no statistical significance
between refractive groups’ relative pupil constriction 4.5 seconds after the red flash (P= 0.918).

3.3.2.2 Pupillary Response as a Function of Axial Length

When analyzing difference between axial length groups 4.5 seconds after a 5-second blue flash, the average normalized pupil constriction of the 11 subjects with the shortest axial lengths was significantly different than the average normalized pupil constriction of subjects with the longest axial lengths (P= 0.013); the longer axial length eyes were less constricted than the shorter axial length eyes. With red light, the control, there was no statistical difference (P= 0.150).
Figure 9. Pupil Constriction as a function of refractive error and axial length. (a) Normalized pupil diameter 4.5 seconds after a red flash was not found to be statistically significant when comparing pupil diameters of more myopic to less myopic subjects. (c) After red light, pupil diameter of subjects with longer axial lengths did not have significantly different normalized pupil diameters when compared to shorter eyes. (d) With blue light, statistically significant differences in pupil diameter between the short and long axial length groups were found 4.5 seconds after the stimulus; shorter eyes were more constricted. (b) When comparing the more myopic to less myopic refractive groups, no significant difference in normalized pupil diameter existed after blue light.
Table 4. Pupil constriction after light stimuli as a function of refractive error and axial length. Independent samples t-test indicates that shorter eyes are significantly more constricted than longer eyes 4.5 seconds after blue light (P=0.013), but not after red light (P=0.150). More myopic eyes’ mean pupil constriction was not statistically different from less myopic eyes after blue light (0.076) or red light (P=0.918).

3.3.2.3. Manual measurement of the Ganglion Cell interplexiform layer 2mm and 2.5mm from the fovea on the Cirrus OCT

The GCIPL and the total retinal thickness were manually measured 2mm and 2.5mm around the fovea superiorly, inferiorly, nasally and temporally for all 22 subjects. The thickness data was compared between the 11 subjects with the shortest ALs and the 11 subjects with the longest ALs. Additionally, comparisons were made between the 11 least and 11 most myopic subjects. In general, more myopic eyes had thinner GCIPL layers and total retinal thicknesses. More myopic eyes had statistically thinner GCIPL and total retinal thickness at all locations except for the total retinal thickness 2.5mm nasal to the fovea and the GCIPL thickness superior to the fovea (Table 5). Longer eyes tended to have thinner GCIPL thickness and total retinal thicknesses. Longer eyes were found to have statistically significant thinning of total retinal thickness and GCIPL thickness at all locations except the temporal GCIPL thickness 2.5mm from the foveal and the total retinal thickness superior, nasal, and inferior to the fovea (Table 5).
Table 5. Manual measurement of the GCIPL and total retinal thickness as a function of refractive error and axial length. In most locations, more myopic eyes tended to have significantly thinner GCIPLs and total retinal thicknesses. In most locations, longer eyes tended to have thinner GCIPLs and total retinal thicknesses.

3.4. Correlation between retinal structure and function

3.4.1. Normalized Pupillary Constriction Compared to GCIPL thickness and Total Retinal Thickness

The manually measured GCIPL thickness and total retinal thicknesses of the 11 subjects with the least constricted pupils and 11 subjects with the most constricted pupils 4.5 seconds after both the red and blue stimuli were compared. When comparing the group of 11 subjects with the least constriction after red light compared to the group of 11 subjects with the most constriction after red light, no statistical significance was found.
between the manually measured total retinal thicknesses and GCIPL thicknesses at any of the 8 locations (Table 6).

When comparing the least constricted and most constricted pupils after blue light, no statistical significance was found between either groups’ total retinal thicknesses at any of the 8 locations measured (2.0mm and 2.5mm superior, inferior, nasal, and temporal to the fovea) nor 6 of the 8 GCIPL regions measured (Table 6). Statistical significance was found between mean GCIPL thickness measurements 2.5mm superior to the fovea (58.4±8.1µm vs 68.6±10.8µm for more constricted and less constricted pupils, respectively, P= 0.019), as well as the GCIPL thickness 2mm nasal to the fovea (80.0±7.6µm vs 88.9±9.9µm for more constricted and less constricted pupils respectively, P= 0.027).

The difference between the pupil constriction of the 11 subjects with the least constricted pupils and the 11 subjects with the most constricted pupils after red light was statistically significant (P= 0.001), but when the pupil constriction of these subjects were compared after their exposure to blue light, no statistical significance was found (P= 0.440). The difference between the more constricted and less constricted pupils 4.5s after blue light was statistically significant (P= 0.001), and these same subjects did not have statistically significant difference in their pupil constriction after red light (P= 0.446). No statistical significance was found between the mean AL or SE of the less constricted and more constricted pupil groups for any stimuli, red or blue (Table 6).
Table 6. Comparison of parameters of subjects with most and least normalized constriction after pupillometry stimuli. When comparing ocular parameters of the 11 subjects with the most constricted pupils compared to the 11 subjects with the least constricted after blue or red stimuli, no significant difference was found between the mean SE, mean AL, or mean magnification corrected RNFL. GCIPL was significantly thicker after blue stimuli in more constricted pupils 2mm nasal (P=0.027) to the fovea and 2.5mm superior to the fovea (P=0.019). No significant difference between mean thickness was found at any other measured location.

### 3.4.2 Normalized Pupil Constriction Compared to Average RNFL Thickness

An independent sample t-test indicates that no significant difference existed between the mean retinal macular thickness of the most constricted and least constricted pupils after the blue stimuli (P=0.052) and the red stimuli (P=0.907) (Table 6). No statistically significant Pearson correlation was found between magnification corrected RNFL thickness and pupil constriction 4.5 seconds after the red stimuli (P=0.785, r= -0.062), or the blue stimuli (P=0.051, r= 0.422). Without magnification correction, after red stimuli, no significant correlation was found between pupil constriction and RNFL thickness (P=0.934 r= 0.019), but statistical significant was found after the blue stimuli (P= 0.002, r =0.617).
3.5 Summary of range of normal variations in ocular parameters and function

Tables one through nine summarize the expected normal ocular parameters for various refractive groups of young, healthy subjects that range from emmetropia to severe myopia. The summary of the findings include:

- Mean macular thickness was significantly thinner in moderate myopes when compared to emmetropes; other groups did not differ significantly.
- Temporal quadrant outer macular thickness was thinner in severe myopes and moderate myopes when compared to emmetropes; other quadrants did not differ significantly.
- Myopic SE was correlated to thinning of average macular thickness as well as some quadrants of the inner and outer macula.
- All regions of the ganglion cell analysis, including the mean, had significant thickness differences between at least 2 of the 4 refractive groups; greater myopia was associated with GCIPL thinning.
- Mean outer retinal thickness was thicker in emmetropes compared to moderate myopes, but did not differ between other refractive groups.
- Mean RNFL thickness and all quadrants of the RNFL thickness analysis were significantly thinner in more myopic groups between at least two myopic groups.
except for the temporal quadrant which did not significantly differ between groups. After correcting for magnification, all quadrants did not differ significantly.

- MD or PSD does not change with refractive error. RNFL thickness and GCIPL thickness are not correlated to PSD or MD.
- Normalized pupil constriction was not correlated to MD or PSD
- After exposure to blue light, shorter eyes’ pupil were more constricted compared to longer eyes; pupil constriction did not differ between less myopic and more myopic subjects.
- Shorter AL and less myopic eyes tended to have thicker manually measured GCIPL thickness 2mm and 2.5mm around the fovea.
- Those that had the most constriction after blue stimulus had thicker manually-measured GCIPL in two of the 8 regions measured.
- Mean magnification-corrected RNFL was not correlated to ipRGC mediated PIPR but uncorrected RNFL thickness was positively correlated to the greater ipRGC mediated PIPR.

3.6 Color Vision Deficient Cases

Two red-green color vision deficient subjects were included in the study to compare against the normal population of subjects with equal SE. One-sample t-test data of the right eye was used.

Subject 20, a moderate myope, had significantly reduced average RNFL thickness, average GCIPL thickness, average macular thickness, and average outer retinal thickness compared to normal moderate myopes (P= 0.001, P= 0.002, P= 0.001, P=...
0.001, respectively). AL was significantly longer, and MD and PSD were significantly higher compared to normal moderate myopes (P= 0.001, P=0.001, P=0.001 respectively) (Table 7). Subject 13, a severe myope, had significantly shorter AL, thinner outer retinal thickness, reduced MD, and worsened PSD (P= 0.001, P= 0.001, P= 0.001, P= 0.001, respectively), but not significantly different macular thickness, GCIPL thickness, or average RNFL thickness (Table 7).

<table>
<thead>
<tr>
<th>Subject 20 (CVD)</th>
<th>Moderate Myopia</th>
<th>Subject 13 (CVD)</th>
<th>Severe Myopia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE (diopter)</td>
<td>-4.13</td>
<td>-3.85 ± 0.82</td>
<td>-7.00</td>
</tr>
<tr>
<td>AL (mm)</td>
<td>25.07*</td>
<td>24.38 ± 0.90*</td>
<td>24.03*</td>
</tr>
<tr>
<td>Number of Eyes</td>
<td>1</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Average Macular Thickness (µm)</td>
<td>271*</td>
<td>278.5 ± 13.8*</td>
<td>280</td>
</tr>
<tr>
<td>Average GCIPL Thickness (µm)</td>
<td>81*</td>
<td>83.4±4.0*</td>
<td>78</td>
</tr>
<tr>
<td>Average RNFL Thickness (µm)</td>
<td>85.2*</td>
<td>96.4±6.6*</td>
<td>89.8</td>
</tr>
<tr>
<td>Average Outer Retinal Thickness (µm)</td>
<td>190*</td>
<td>206.2±11.7*</td>
<td>202*</td>
</tr>
<tr>
<td>Average Mean Deviation (dB)</td>
<td>-3.03*</td>
<td>-0.21±1.35*</td>
<td>-2.43*</td>
</tr>
<tr>
<td>Average Pattern Standard Deviation (dB)</td>
<td>2.57*</td>
<td>1.76±0.51*</td>
<td>2.27*</td>
</tr>
</tbody>
</table>

Table 7 Color vision deficient subjects compared to control subjects of similar refractive error. With the exception of SE, all of subject 20’s ocular parameters were significantly different from age and refractive error matched controls. Subject 13 had a significantly shorter eye, thinner outer retinal thickness, reduced MD and reduced PSD relative to age and refractive error matched controls.

3.7 Hyperopic Subject

Subject 22 was a 23-year old Caucasian male. Subject 22 was not included into a refractive group for OCT evaluation because no other subjects were within 1.75D of his SE. His axial length was the shortest of all subjects (21.91mm right eye, 21.61mm left eye) and his refractive error was the most hyperopic (+2.25D for the right eye, +3.75D
for the left eye). Comparison of his right eye’s ocular parameters to Emmetropes is summarized on Table 8.

<table>
<thead>
<tr>
<th>Subject 22 (Hyperope)</th>
<th>Emmetropia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE Range (diopter)</td>
<td>+0.50 to -0.50</td>
</tr>
<tr>
<td>SE (diopter)</td>
<td>2.25*</td>
</tr>
<tr>
<td>AL (mm)</td>
<td>21.91*</td>
</tr>
<tr>
<td>Number of Eyes</td>
<td>1</td>
</tr>
<tr>
<td>Mean Macular Thickness (µm)</td>
<td>296</td>
</tr>
<tr>
<td>Mean GCIP Thickness (µm)</td>
<td>86</td>
</tr>
<tr>
<td>Mean RNFL Thickness (µm)</td>
<td>93.2*</td>
</tr>
<tr>
<td>Mean Outer Retinal Thickness (µm)</td>
<td>221.8*</td>
</tr>
<tr>
<td>Mean Mean Deviation (dB)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Mean Pattern Standard Deviation (dB)</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>23.37 ± 0.61*</td>
</tr>
<tr>
<td></td>
<td>290.3 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>85.6 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>96.6 ± 5.4*</td>
</tr>
<tr>
<td></td>
<td>215.5 ± 7.7*</td>
</tr>
<tr>
<td></td>
<td>-0.083 ± 0.78*</td>
</tr>
<tr>
<td></td>
<td>1.61 ± 0.39</td>
</tr>
</tbody>
</table>

*Significant difference between Subject 22 and Emmetropes
SE p=0.001, AL p=0.001, mean macular thickness p=0.096, mean GCIP thickness p=0.757, mean RNFL Thickness p=0.042, outer retinal thickness p=0.012, MD p=0.0677, PSD p=0.123

Table 8. Subject 22 (Hyperope) comparison to emmetropes. Subject 22 had thinner RNFL, shorter AL, thicker outer retina and greater visual sensitivity than emmetropes.

3.8 Disease Case Ocular Parameters Measured with Commercial Equipment

Ocular parameters of disease subjects are summarized on Table 9.
Table 9. Disease cases and ocular parameters measured with commercial equipment. Relative to emmetropes, subject 12 (BVMD) had significantly thinner macular tissue in all quadrants of the inner macula except the nasal quadrant. In the outer macula, the nasal and temporal quadrants were significantly thinner than other emmetropes. Mean macular thickness was not statistically different but foveal thickness was significantly thinner. Subject 16 had significantly reduced average macular thickness and foveal thickness. Subject 16’s (RCD) macular thickness was thinner in all quadrants of the inner and outer macula.

### 3.8.1 Best’s Vitelliform Macular Dystrophy

Subject 12 was a 20-year old mild African American female with a diagnosis of Best’s Vitelliform macular dystrophy of both eyes. Her best corrected visual acuity was unknown. She was known to have had injections of anti-VEGF medications, which are typically used to treat choroidal neovascularization. Her left eye was evaluated and compared to other emmetropic subjects. Visual field data was not available. Her ocular parameters compared to other emmetropes are summarized on Table 9. Her refractive error was -0.125D for her left eye, and her SE not was significantly different from other
emmetropes. Her mean macular thickness was not significantly different from other emmetropes, but the foveal thickness was significantly thinner (P= 0.006).

For the inner macula, the superior, inferior and temporal quadrants were significantly thinner than emmetropes (P= 0.001 for each quadrant), but the nasal quadrant was not. For the outer macula, the nasal quadrant (P= 0.018) was significantly thicker than emmetropic eyes and temporal quadrants (P= 0.003) was significantly thinner than emmetropes. Cross-sectional B-scan OCT images of her macula showed loss of the IS-OS boundary near the fovea, subfoveal deposits, and a superior RPE detachment (Appendix D).

The summary of AO imaging of subject 12 is summarized on Figure 10. Subject 12’s IS and OS lengths were measured with AO-OCT at 3° temporal to the fovea and were compared to an aged-matched healthy myopic control’s IS and OS thicknesses at 4° Temporal. Foveal IS and OS lengths were compared to control foveal data as well (Table 10).

AO-OCT suggests that the OS of subject 12 is thinner at the fovea and at 3° temporal to the fovea when compared to OS control data (Table 11). However, the IS length of subject 12 at the fovea and 3° temporal was found to be comparable to that of control data. This finding suggests that the OS thickness is more affected than the IS thickness.

Subject 12 showed greater variation of IS and OS length when compared to control data, as indicated by larger standard deviations and higher ratios of disease to control standard deviations (Table 11). The one exception to this trend is the OS 3° temporal to the fovea, which had a standard deviation roughly equal to control data.
Commercial OCT indicates that the fovea is more affected by BVMD than 3° temporal (Figure 10). Such a finding, paired with the normal variation of OS length 3° temporal suggests that 3° temporal is more normal than the fovea in subject 12.

AO-SLO data for subject 12 is summarized on Table 12. Measured cone density was divided by control cone density and cone reference density to determine the relative reduction in cone density. Foveal cone density of Subject 12 measured by AO-SLO suggests reduction in cone density relative to control, histological samples (Curcio, Sloan et al. 1990), and previous normal AO-SLO data (Wells-Gray E. M. 2016). Cone density of subject 12 3° nasal and temporal to the fovea are also relatively reduced to Curcio et al. and Wells-Gray et al. Of all locations measured, the greatest relative reduction in cone density was found at the fovea.

Relative to 3° temporal, the fovea in subject 12 had the greatest relative reduction in cone density and the greatest variation in IS and OS thickness, suggesting that PRC integrity was most affected at the fovea in subject 12.
Figure 10. Summary of AO-SLO-OCT imaging for subject 12 (BVMD). Cirrus OCT and fundus image for reference. (a) AO-OCT was recorded at the fovea and 3 degrees temporal. (b) AO-SLO was recorded at the fovea, 3 degrees nasal and 3 degrees temporal.

Table 10. AO-OCT inner segment and outer segment measurements. Ten measurements of the IS and OS were made in a 1° OCT image and averaged. Extrafoveal measurements in BVMD were made at 3° temporal while control measurements were made at 4° temporal.
Table 11. Comparison of mean IS and OS length in BVMD to control IS and OS length. Fractions less than one indicate reduced thickness relative to control data. Mean IS length was relatively similar between BVMD and the control subject at the fovea and 3° to 4° temporal. Mean OS Length was reduced in both locations in BVMD. Standard deviation was increased in BVMD at all locations except for the OS at 3°, where it was relatively similar.

<table>
<thead>
<tr>
<th></th>
<th>Fovea</th>
<th>3° to 4° Temporal</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVMD IS / Control IS</td>
<td>1.16</td>
<td>0.88</td>
</tr>
<tr>
<td>$\sigma_{BVMD IS} / \sigma_{Control IS}$</td>
<td>2.04</td>
<td>1.70</td>
</tr>
<tr>
<td>BVMD OS / Control OS</td>
<td>0.34</td>
<td>0.52</td>
</tr>
<tr>
<td>$\sigma_{BVMD OS} / \sigma_{Control OS}$</td>
<td>1.90</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Table 12. Cone density of subject 12 (BVMD) compared to control and reference data. Cone densities ($\rho_{cone}$) were calculated using custom MATLAB software. Fractions less than one indicate reduced cone density relative to control and reference data. Subject 12’s cone density was reduced at the fovea and 3° nasal and temporal to the fovea.

<table>
<thead>
<tr>
<th></th>
<th>Fovea</th>
<th>3° Temporal to the Fovea</th>
<th>3° Nasal to the Fovea</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho_{Cone Subject 12 (BVMD)}$</td>
<td>$8.21 \times 10^3$ cells/mm²</td>
<td>$7.08 \times 10^3$ cells/mm²</td>
<td>$1.06 \times 10^4$ cells/mm²</td>
</tr>
<tr>
<td>$\rho_{Cone Subject 8 (Control)}$</td>
<td>$7.08 \times 10^3$ cells/mm²</td>
<td>$6.78 \times 10^3$ cells/mm²</td>
<td>$9.41 \times 10^3$ cells/mm²</td>
</tr>
<tr>
<td>$\rho_{Cone Wells-Grey et al.}$</td>
<td>$0.12$</td>
<td>$0.04$</td>
<td>$0.41$</td>
</tr>
<tr>
<td>$\rho_{Cone Curcio et al.}$</td>
<td>$0.05$</td>
<td>$0.04$</td>
<td>$0.48$</td>
</tr>
</tbody>
</table>

3.8.2 Rod-Cone Dystrophy

Subject 16 was a 38-year old Asian-American male with RCD of both eyes. He was correctable to 20/40 in both eyes. He had a moderate myopic SE of the right eye (-3.75D) which was not statistically significant from the other 17 moderate myopes (Table 9). Statistically significant macular thinning relative to moderate myopes existed in all regions, as measured with commercial OCT. Mean deviation was significantly
reduced when compared to other moderate myopes (P= 0.001) but PSD was not significantly different (P= 0.069). His perimetry was conducted with the SITA 24-2, which measures the central 48 degrees of visual sensitivity.

Subject 16’s AO-SLO correspondence to perimetry and commercial SLO is summarized in Figure 11. AO-SLO cone density was measured at 8.49° superior temporal and 8.49° inferior nasal, corresponding to regions in his visual field with relative reduction in visual sensitivity, but also to the eccentric limits of the AO-SLO system. Cone density at these locations was compared to control cone density at 8° temporal and 8° nasal and found to not differ, as disease cone density divided by control was roughly equal to one (Table 13). Subject 16’s cone density was also compared to histological density (Curcio, Sloan et al. 1990) at 8.49° nasal and temporal as well as density measured using AO-SLO (Wells-Gray EM 2015)(Table 13); in both cases cone density in RCD did not differ from control data as RCD cone density divided by reference density was roughly equal to one. The regions selected on the visual field were adjacent to points of arguably clinically insignificant reduction in sensitivity from normal subjects. Given that his visual field was minimally affected, it is not surprising that cone density was not affected.
Figure 11. Subject 16 (RCD) AO-SLO correspondence to perimetry and Cirrus OCT fundus image. (a) Cirrus fundus image indicating locations where AO-SLO data was recorded. (b) Perimetry locations corresponding to a slight reduction in visual sensitivity 8.49° from fixation in the inferonasal field and superior temporal field. (c) AO-SLO image 8.49° superior temporal from the fovea with prominent blood vessel (d) AO-SLO image 8.49° inferior nasal from the fovea.

Table 13. AO-SLO for subject 16 (RCD) compared to control. Cone densities ($\rho_{cone}$) for Subject 16 (RCD) were measured at 8.49° superior temporal to the fovea and compared to a control’s density at 8° temporal. Cone density for subject 16 was also measured at 8.49° inferior nasal and compared to a control’s density at 8° nasal. Disease cone density at 8.49° superior temporal and 8.49° inferior nasal was compared to Curcio et al. and Wells-Gray et al. at 8.48° temporal and nasal, respectively. Dividing disease density by control and reference density indicates that disease cone density is not reduced at either location.
Chapter 4: Discussion

4.1. Retinal Structure

This is the first comprehensive study that combines changes in ocular parameters with pupil function, visual sensitivity, and retinal thickness as a function of refractive error in the same cohort of subjects. Previous studies correlating refractive error and retinal thickness in normal populations have typically only looked at one region of the retina per investigation, usually the macula or the optic nerve head. There have not been studies that have evaluated the effect of refractive error on both regions of the retina and concurrently evaluated pupillary response and visual sensitivity. While some of the findings agree with previous work, there are also discrepancies with previous findings; the following discussion evaluates the differences between studies that may have yielded data that conflicts with this study’s findings.

4.1.1 Macula

4.1.1.1 In-Built Macular Cube

We measured the total thickness, the GCIPL thickness and the outer macular thickness around the macula. Macular structural findings in this study, as tested by one-way ANOVA and Pearson correlation, suggest that total retinal thickness in the central 6mm of the macula thins with increasing myopia (Table 1). Four other studies with Asian subjects with ages ranging from 8.63±1.64 years to 55.6 ± 16.4 correlated thinning of the
mean macular thickness to higher myopic refractive error on either the Cirrus or Stratus 
OCT (Lam, Leung et al. 2007, Song, Lee et al. 2010, Zhang, He et al. 2011, Hwang and 
Kim 2012). However, in one previous study, when using the older stratus OCT on a 
sample of 104 Chinese school children aged 11.5±0.50 years with a mean SE of -1.38 
±1.57 D, refractive error was not associated with mean total macular thickness (Luo, 
Gazzard et al. 2006). Other than being one of a few studies that uses children as subjects, 
Luo, Gazzard et al. does not seem to have any inherent differences compared to studies 
that suggest thinning with increasing myopia. Age has been shown to influence mean 
macular thickness, suggesting thinning with increasing age (Song, Lee et al. 2010). 
However, Zhang et al. also used Asian children as subjects and found that mean total 
macula thickness is correlated with higher myopic refractive error. While most studies, 
including our own, suggest that mean total macular thickness decreases with increasing 
myopia, some studies disagree.

By one-way ANOVA testing, our study suggests that there are no significant 
differences between inner macular thicknesses of different refractive groups. However, 
Pearson correlation suggests that the inner macula thins in the inferior quadrant with 
increasing myopia. Previous studies have correlated increasing myopia with inner macula 
thinning (Huynh, Wang et al. 2006, Luo, Gazzard et al. 2006, Wu, Chen et al. 2008, Sato, 
Fukui et al. 2010, Zhang, He et al. 2011, Hwang and Kim 2012). However, another study 
with Chinese subjects found that there is no correlation (Lam, Leung et al. 2007). Except 
for Huynh, Wang et al., regardless of the outcome, these studies used predominantly 
Asian samples. Huynh, Wang et al., like our study, had a sample of both Asian and 
Caucasian subjects and found that the inner macula thins with increasing myopia.
Additionally, they found that after correcting for SE and AL, macular thickness was significantly thicker in Caucasian eyes when compared to East Asian eyes (Huynh, Wang et al. 2006). Our study had 28 Caucasian subjects and one Asian subject. Thickness variation is known to exist between ethnicities. Mean macular thickness is reportedly thicker in Caucasian eyes when compared to African American eyes (Kelty, Payne et al. 2008). Macular thickness measured on Caucasian eyes has been reported to be significantly thinner than East Asian and South Asian eyes (Tariq, Li et al. 2011). Given that Huynh, Wang et al. and our study both suggest thinning with Caucasian populations, but studies in strictly Asian populations conflict, it is possible that ethnicity might play a role in the relationship between refractive error and inner macular thickness; the inner macula in Caucasian eyes may tend to thin with increasing myopia while greater variation might exist in Asian populations.

By one-way ANOVA and Pearson correlation, our study suggests that the outer macular total retinal thickness thins with increasing myopia. Seven other studies using either the Zeiss Stratus or Cirrus OCT have found that at least one or all quadrants of the outer macula thin with increasing myopia (Huynh, Wang et al. 2006, Luo, Gazzard et al. 2006, Lam, Leung et al. 2007, Wu, Chen et al. 2008, Sato, Fukui et al. 2010, Song, Lee et al. 2010, Zhang, He et al. 2011, Hwang and Kim 2012). Again, except for Huynh et al., all studies used Asian populations. Our data would suggest that the outer macula does thin with increasing myopia in a predominantly Caucasian sample.
4.1.1.2 Ganglion Cell-Interplexiform Layer

Thinning of the automated and manually measured GCIPL layers were associated with axial elongation. The correlation between thinning and axial elongation has previously been established in populations with mean age of 34.4 to 48.5 years (Mwanza, Durbin et al. 2011, Araie, Saito et al. 2014, Takeyama, Kita et al. 2014). Our study also found that increasing myopic spherical equivalent is associated with thinning of the GCIPL thickness in normal controls. Takeyama et al. also previously determined this correlation in a Japanese population that had a more myopic mean refractive error of -3.47 ± 2.96D and a slightly older population of 34.88 ± 7.02 years. Given that previous studies found thinning of the GCIPL associated with myopic SE and longer AL in older, more myopic eyes, our study confirms that these trends persist in younger, less myopic subjects.

4.1.1.3 Outer Retinal Thickness

This study’s estimation of outer retinal thickness is the first attempt at using Zeiss’ algorithmic data to estimate the mean outer retinal thickness using the Cirrus OCT. This estimation assumes that the 4.8mm annular ellipse of the GCIPL algorithm is a 4.8mm circle (Figure 4). Based on how the area of the tissue was averaged, it assumes even distribution of GCIPL and macular thickness throughout the tissue. This method subtracts the GCIPL thickness from the total retinal thickness which yields the outer retinal thickness and the RNFL. The RNFL is not part of the outer retina, another factor that emphasizes that this method is an estimation of outer retinal thickness. Should the RNFL change dramatically in tissue adjacent to the fovea, and not remain relatively
constant, this may not be an accurate estimation of outer retinal thickness. This study found that the outer retinal thickness was only significantly different between emmetropes and moderate myopes. Changes in outer retinal thickness due to myopic refractive error has been evaluated in a study of 58 subjects on the Copernicus OCT (Zawiercie, Poland). In their study, the photoreceptor layer did not differ between the 29 emmetropes and the 29 myopes, but the difference in RPE thickness was significantly thinner for myopes(37.74 ± 4.30 µm in emmetropes versus 35.03 ± 4.21 µm in myopes)(Gella, Raman et al. 2011). Gella et al. likely used an included feature of the Copernicus that measures the IS+OS thickness, which would be the PRC thickness, as well as the feature that enables RPE thickness measurement. It is possible that Gella et al was more accurate and consistent than human selection for determining retinal layer borders, so it is possible that the RPE findings are likewise more accurate. However, both our study and Gella et al. did find some significant difference in outer retinal thickness between refractive error groups, both suggesting thinning with myopic SE. Future investigation of whether the estimated method using the Cirrus OCT or the automated method with the Copernicus OCT is more accurate. Investigations into refractive changes of the inner nuclear layer and outer nuclear layer are also merited. Additionally, future studies measuring the specific outer retinal layers on the Cirrus OCT are merited to determine the layers responsible for significant differences between refractive groups.
4.1.2 Optic Nerve Head

When evaluating the effect of SE on RNFL thickness, our study made two analyses: one without correction for lateral magnification and one correcting for lateral magnification.

Prior to accounting for magnification, Pearson correlation indicates that the RNFL thins with increasing myopic SE. One-way ANOVA also suggested mean RNFL thinning with increasing myopia, as severe myopes’ RNFL thickness were significantly thinner than each of the other refractive groups (Table 2). After correcting for magnification, SE did not correlate with mean RNFL thickness either with one-way ANOVA or Pearson correlation.

Before correcting for magnification, Kang et al. also found that the average RNFL thickness thinned with axial elongation and higher myopia (Kang, Hong et al. 2010). After correcting for lateral magnification, Kang et al. also found that mean RNFL thickness was not associated with spherical equivalent.

In our study, prior to correcting for lateral magnification, Pearson correlation indicated that the mean RNFL thins with increasing AL. After correcting for lateral magnification, no correlation was found when comparing mean RNFL thickness to AL. Thinning of the average RNFL without correcting for magnification has previously been correlated to longer axial length (Budenz, Anderson et al. 2007, Kang, Hong et al. 2010). Budenz et al. did not evaluate RNFL thickness after any magnification correction. However, Kang et al. did correct for magnification and found that RNFL thickens by 1.996 µm/mm with increasing AL (P < 0.001, R²= 0.074). The correlation is relatively weak with a low R² value, which does subtract from the significance of their correlation.
Our sample size was 56 eyes of 28 subjects and Kang et al. had 269 subjects; the threshold for finding significant correlations was likely lower in their sample, though it is also possible their larger sample size more closely matches the population mean. However, our population was predominantly Caucasian, while Kang et al. used an Asian population; differences in results may be due to ethnicity, as Budenz et al. also found that ethnicity does affect RNFL thickness (Budenz, Anderson et al. 2007). Specifically, Budenz et al. found that mean RNFL thickness with subjects of Caucasian ethnicity is significantly thinner than the RNFL thickness of subjects with Asian ethnicity (98.1μ ±10.9 versus 105.8μ ±9.2, respectively; P = 0.031).

4.2 Function

4.2.1 Visual Sensitivity

4.2.1.1 Visual Sensitivity and Refractive Error

No significant differences were found between the visual field mean deviations of different refractive groups. While a previous study did find a significant reduction in sensitivity of myopic subjects, the subjects affected had AL longer than 26mm and were more myopic than -5.00D (Rudnicka and Edgar 1995). Our study only had 7 eyes out of 56 with a spherical equivalent greater than or equal to -5.00D, and the average axial length was 24.34mm±1.05mm for all 56 eyes. Only four eyes in our study had axial lengths longer than 26mm. Therefore, it is likely our population was not a myopic or axially elongated enough sample to present the correlations as found by Rudnicka et al. Aung et al. did find that after -4.00D, a significant decline in visual sensitivity occurs with the 30-2 SITA testing on the HFA perimeter(Aung, Foster et al. 2001). Aung et al.
did use 146 subjects and the average refractive error was -6.35D± 3.72D with 33.6% of the subjects being -8.00D or more myopic. Again, it’s likely that the larger sample size and large volume of severe and possible degenerative myopes in their study promoted their findings into statistical significance; it is also likely again that the less myopic average refractive error our population was too low to yield significant findings relative to Aung et al. Our findings suggest that mild to moderate myopic populations do not have reduced central visual sensitivity.

4.2.1.2 Visual Sensitivity and RNFL Thickness

Our study found no significant correlation between RNFL thickness and visual field sensitivity. Yuan, Feng et al. did find statistical correlation between magnification-corrected RNFL thickness and visual sensitivity, as measured on the Cirrus OCT, in that lower sensitivity was associated with thicker RNFL (Yuan, Feng et al. 2014). The differences between Yuan, Feng et al. and our study is that they used an older population aged 28.3 ± 5.8 years with a range of 19-46, used a larger population of both eyes of 58 subjects, and used a different perimeter, the Octopus 1-2-3 automated perimeter (Interzeag, Schlieren, Switzerland). Additionally, Yuan et al. used more myopic eyes with an average refractive error ~4.79D ±1.66 for the right eye and ~4.59D±1.88 for the left eye. Kang et al. has shown that longer AL is associated with thickening of mean RNFL tissue, but thinning in some regions (Kang, Hong et al. 2010). Rudnicka et al. has correlated myopia greater than -5.00 with reduced visual sensitivity (Rudnicka and Edgar 1995); However, Rudnicka et al. had more myopic subjects than our study that aligns closer with Yuan et al., while Kang et al. reported a mean SE of-
2.521 +/- 2.297, close to our study. The population of Kang et al. was of Asian ethnicity, and mean RNFL thickness in subjects with Asian ethnicity has been shown to be thicker than that of subjects with Caucasian Ethnicity (Budenz, Anderson et al. 2007); it is possible that the thickening found in Yuan et al. and Kang et al. is associated with Asian ethnicity. Additionally, it is possible that the ethnic and myopic factors confounded in Yuan et al., resulting in a significant correlation between reduced visual sensitivity and increased RNFL thickness. Further, given that the age of the Yuan et al. population was higher than our study, a subclinical effect of age on visual sensitivity cannot be ruled out. A decrease in visual sensitivity of 0.101 dB/year from 20 to 40 years of age and 0.172 dB/year from age 40 to age 60, as measured on the HFA has been reported previously (Collin H 1988). Additionally, it is possible that the Octopus 1-2-3 perimeter may be more sensitive to loss of visual sensitivity. A 2015 study with 50 glaucoma patients with mean age of 49.8 years found that both the MD and PSD of the HFA and the Octopus 1-2-3 were comparable to one another (P= 0.001 and P= 0.002 for MD and PSD for the right eye and P= 0.001 for PSD and MD of the left eye) (Rangaswamy, Patel et al. 2010). However, the population in Rangaswamy, Patel et al. was younger and the subjects had optic neuropathy, so it still cannot be ruled out that the perimeter model is responsible for the lack of correlation found. Further investigation into the effect of refractive error on MD and PSD between perimeters on younger, healthy populations is therefore warranted.

4.2.1.3 Visual Sensitivity and GCIPL Thickness

Reduced MD has been correlated to thinning GCIPL in 195 normal subjects (Araie, Saito et al. 2014). While MD was almost statistically significant in our sample
(P= 0.051), it may not have attained significance due to the relatively smaller sample size of our study. GCIPL is reported to decrease with age (Mwanza, Durbin et al. 2011), as is visual sensitivity(Collin H 1988). The average age in Mwanza et al. was 46 and Collin et al. evaluated subjects in the third to seventh decade. Perhaps the younger population in our study did not perform as poorly on perimetry and therefore no correlation was found.

4.2.2 Intrinsically Photosensitive Retinal Ganglion Cells and Pupillary Response

4.2.2.1 Post-Illumination Pupillary Response Compared to Axial Length and Spherical Equivalent

The data of this study suggests that longer axial length is correlated with the melanopsin-controlled PIPR; after exposure to a 5 second flash of blue light, shorter eyes are more constricted than longer eyes. It has been established that in dark-adapted subjects aged 43.7± 14.4 years, 10 seconds of blue light elicits a stronger PIPR than 10 seconds of red light, when measured 6 seconds after exposure (P<0.001)(Adhikari, Pearson et al. 2015). The greater response to blue light is suggestive of ipRGCs mediating the PIRP, as the ipRGCs are more sensitive to blue than red (Adhikari, Pearson et al. 2015, Shorter 2015, Mulvihill 2016). The response to blue light in Adhikari et al. however, did not find a significant difference between the PIPR of groups divided by refractive error, nor did our study. Our findings suggest that axial and not refractive myopia mediates the PIPR. The mechanism of PIPR depending upon AL is unknown, but it is possible that the longer corridor for light to travel through results in less illumination of the retina and therefore reduced ipRGC hyperpolarization. Shorter AL has been shown to have reduced retinal illumination in model eyes (Quigley 2014). It may also be related
to the stretching of the retina with axial elongation that moves the ipRGC dendrites and cell bodies to the periphery of the retina where less illumination occurs. Greater eccentricity of the retina has been shown to have lower illumination (Kooijman and Witmer 1986). Our findings suggesting a significant difference in the PIPR between short and long eyes and not in myopic compared to less myopic eyes merits that further investigations should evaluate the structural influence of the eye on the PIPR.

4.2.2.2 Post-Illumination Pupillary Response Compared to RNFL Thickness

The Pearson correlation between magnification-corrected RNFL thickness and the PIPR after blue and red light, as found in our data, suggests that RNFL thickness is not correlated to ipRGC function. Independent samples t-test also indicates that RNFL thickness was not significantly thicker or thinner between groups with the most and least constricted pupils after either stimulus. However, after the blue stimuli, significant correlation was found between pupil constriction and RNFL thickness if magnification is not accounted for, in that thicker RNFL was associated with more constriction and greater PIPR. When accounting for both eyes of 18 control subjects and 56 glaucoma patients, Gracitelli et al. also found that a significant correlation between mean RNFL thickness on the Cirrus OCT and PIPR (P= 0.024, R² = 0.403). Gracitelli et al. measured pupil diameter 6 seconds after being exposed to 1 second of 470nm light (Gracitelli, Duque-Chica et al. 2014). Gracitelli et al. did not report correcting for magnification and it can be assumed they did not do so. Therefore, our results are consistent the Gracitelli et al.’s findings if magnification is not accounted for. Our study evaluated the pupil size 4.5 seconds after 5 seconds of a blue stimulus, which implies that the ipRGC PIPR is present.
earlier than 6 seconds. Gracitelli et al. did not evaluate SE or AL as our study did, but did require refractive error to be between ± 5.00D spherical equivalent and cylindrical power to be no greater than 3.00D. The lack of correlation for axial magnification corrected RNFL thickness to PIPR but correlation between uncorrected RNFL thickness and PIPR implies that AL may play a significant role in the ipRGC response or interpreting the ipRGC response. Our findings also imply that RNFL thickness, after magnification correction, is not correlated to ipRGC function.

4.2.2.3 Post-Illumination Pupillary Response Compared to Manually Measured GCIPL Thickness

The manually measured GCIPL thicknesses measured 2.0mm and 2.5mm around the fovea were only correlated to the blue PIPR in two out of the eight regions manually measured, which may be suggestive of a correlation to structure. The highest ipRGC density is 2.0mm from the fovea (Dacey, Liao et al. 2005). However, given that there are only ~3,000 ipRGCs per eye in the human retina, the likelihood that the physical thickness of the retinal would differ based on their structure or function is unclear. Contrarily, given that two of the eight measured regions do show some correlation to PIPR, and not to the red control stimulus, it is possible that the ipRGCs are responsible for this disparity. Further trials of this test should be run, possibly in 8 meridians instead of two or perhaps a series of circular b-scans 2mm and 2.5m around the fovea, which would optimize the sampling of tissue known to have the highest ipRGC density.
4.3 Color Vision Deficiency Subjects

The significant difference between commercial OCT findings of subject 20 compared to moderate myopes may be due to subject 20 being a more axial myope relative to the controls in this study; the longer axial length caused stretching of his retina, which reduced some of the reported thickness findings, as axial elongation has been correlated to macular thinning (Hwang and Kim 2012). The relative thinning is likely unrelated to his assumed X-linked congenital red-green color vision deficiency, as it has already been determined that other than foveal pit morphology, there is no structural difference between spectral domain-measured retinal thickness of congenital red-green deficient subjects, including anomalous trichromats, when compared to the normal population (Gupta, Laxmi et al. 2011). However, Gupta et al. did not analyze the effect of refractive error or axial length, but did list ±6.00D of refractive error as an exclusion criteria and reported that more research is necessary to support their findings. Without accounting for refractive error in the CVD and control groups, it’s possible that significant differences in mean refractive error between the CVD and control groups affected their structural results. It has been shown that subjects with C203R mutations with red green CVD have thinning of the outer nuclear layer (Carroll, Baraas et al. 2009). However, this mutation was not known to exist in subject 20. Few studies have evaluated retinal thickness in congenital color vision deficient subjects and more research is merited to distinguish if thinning is typical, such that those affected are not suspected of having other pathology.

Subject 13’s ocular parameters corresponded to his refractive group except for his relatively shorter axial length and his lower mean deviation. Given that there were only 7
normal severe myopes in this study, it’s possible that the average axial length for severe myopes found in this study is not representative of the true population average. However, it is more likely that Subject 13 is a refractive myope, as his AL was 24.93mm, which is shorter than the model based on Leung et al., which should equate to roughly -1.50D of myopia. However, Subject 13’s SE was -7.00D, suggesting significant refractive myopia. The reduction in MD to -2.43dB compared to the population average of +0.50±0.65dB MD may not be reliable given that subject 13 had 3 out of 17 fixation losses and 7% false positives, which could explain the relative reduction in visual sensitivity.

4.4 Hyperopic Subject

Subject 22 was a hyperopic 22-year old male that did not meet the refractive criteria of any other group. The NHANES reported 1.2% prevalence of hyperopia for males aged 20 to 39 (Vitale, Ellwein et al. 2008); therefore, it is likely that this study only recruited one hyperope because of the low prevalence in the population. He had the shortest axial length that was significantly shorter than the average emmetropic axial length. His outer retinal thickness was significantly thicker than the emmetropic mean. A subject with shorter axial length having a relatively thicker outer retina than the emmetropic population does agree with the findings of this study when mean outer retinal thicknesses are compared between refractive groups (Table 1). His mean RNFL thickness was significantly thinner than the emmetropic mean. The RNFL thinning relative to the emmetropic groups does not agree with this study, as this study found no significant difference between refractive groups’ mean RNFL thicknesses after correcting for magnification as well as no correlation between AL and RNFL thickness. Thinning with
reduced AL does, however, agree with Kang et al., which suggests thickening RNFL with increasing AL (Kang, Hong et al. 2010). Given the conflict of results, additional studies with higher young hyperopic study populations are warranted to completely understand the effect of hyperopic refractive error on retinal thickness.

4.5 Disease Cases

4.5.1 Subject 12: Best’s Vitelliform Macular Dystrophy

Commercial OCT reported thickening of the nasal quadrant of the outer macula of subject 12, which is consistent with a previous report of thickening of the region between the RPE and the IS-OS junction in subjects with Vitelliform Macular Dystrophy (Querques, Regenbogen et al. 2008). Vitelliform material is known to exist in stages 2 to stages 4 of BVDM, which may be responsible for the thickening and may suggest that subject 12’s left eye was in one of these stages (Parodi 2017). The atrophic and fibrotic stages, as found in vitelliform macular dystrophy, as measured on High Definition OCT, have also been associated with retinal thinning and loss of the IS-OS junction (Querques, Regenbogen et al. 2008). The IS-OS junction around the fovea was grossly absent in subject 12 without AO (Appendix D), suggesting it is possible that subject 12’s right eye may have instead been in the atrophic stage. The atrophic stage occurs after choroidal neovascularization; given her history of Anti-VEGF injections, the commercial OCT findings and clinical history therefore align.

Subject 12’s fovea having the largest relative reduction in cone density may be due to the presence of a central scotoma corresponding to the foveal lesion, resulting in poor fixation. Therefore, imaging may have occurred in regions outside of the foveal pit.
The greater relative thinning and greater variation in the OS of subject 12 is consistent with previous studies. In subjects with ocular disease and subsequent visual loss, it has been shown that greater OS variation and shorter OS length have been associated with reduced integrity of the PRC layer (Choi, Zawadzki et al. 2008). Our study supports this previous finding.

BVMD cone density of PRC adjacent to active lesions has been previously measured using AO-SLO on two patients with early BVMD and the BEST1 gene. In both subjects, the cone density nasal to the lesion was found to be normal compared to control data and contiguous with adjacent cones (Kay, Land et al. 2013). Our study examined the lesions themselves, as seen in Figure 10, which explains the reduction relative to Kay, Land et al.

4.5.2 Subject 16 (Rod-Cone Dystrophy)

Subject 16’s commercial macular OCT findings suggest significant macular thinning in most regions (Appendix E). RCD is characterized by degeneration of rod and cone photoreceptor cells. Diffuse thinning, as measured with the Macular Cube 512x128, when compared healthy controls, is consistent with a previous investigation with RP subjects that also used the Cirrus OCT (Garcia-Martin, Pinilla et al. 2012). Retinal thinning in RP has been found specifically in the outer nuclear layer in commercial OCT studies ($P = 0.029$) (Makiyama, Ooto et al. 2013). Although we observed significant general thinning of the macula, we cannot be certain whether the outer nuclear layer contributed to our finding.
While visual sensitivity was significantly reduced in the central 48 degrees of his visual field compared to other moderate myopes, the reduction is likely not clinically significant given the low mean deviation (-2.25). The visual field was reliable. It is worth noting that control subjects were tested with perimetry using the 30-2 SITA algorithm, the same testing algorithm as the 24-2 SITA used in the RCD case, but with more points at greater eccentricity. Therefore, if more eccentric retina was affected by RCD, it is possible that a 30-2 SITA test of subject 16 would have reported greater loss of visual sensitivity if more eccentric visual field were tested. RP, the most common rod-cone dystrophy, is characterized by gradual peripheral visual field loss, followed by ring scotoma, then tunnel vision (Hamel 2006). Subject 16’s peripheral visual sensitivity was essentially normal yet his OCT showed significant thinning relative to controls. This is in conflict to Garcia-Martin et al., which reported a MD of $-28.57\pm4.98$ in RP subjects with diffuse macular thinning, which is clinically significant.

While subject 16 had normal cone densities, previous AO retinal imaging findings have suggested that the PRCs are absent in some cases of RP (Choi, Doble et al. 2006, Roorda, Zhang et al. 2007). Clinical findings in RP are known to be heterogenous (Fahim, Daiger et al. 1993). The heterogenous nature of RCD and RP could therefore explain the disparity between our cone densities and that of previous investigations.

Additionally, our subject had reduced central visual function with 20/40 acuity; it is possible that he had poor fixation and therefore we did not measure the cone density of tissue with any reduced visual sensitivity. It is also possible that we happened to measure islands of tissue that were spared from degeneration.
Based on normal cone density measurements, the integrity of the cones appears to be normal. Although commercial OCT scans at the macula suggest diffuse thinning, the thinning at the macula is likely caused by a layer other than the PRC. While visual sensitivity was minimally affected, disease of the retina and optic nerve, such as glaucoma, have been shown to have structural changes that precede functional changes (Lisboa, Leite et al. 2012).

4.6 Limitations

One of the limitations of this study is that the sample size of this study was low and the average refractive error was less myopic compared to many other studies that evaluate refractive changes in ocular parameters. Trends such as reduced visual sensitivity and RNFL thinning that have previously been associated with myopia may have suggested statistical significance with a larger sample size because their sample sizes greater reflected true population average. However, the sample in this study, based on the formula by Bennett et al., was relatively more refractive than axially myopic. This might explain why the RNFL thickness was not significantly correlated to refractive error after accounting for axial magnification; perhaps other studies had more axial myopes and therefore the thinning effect of axial elongation was not as affected by the magnification correction in our study.

Another limitation is that lateral magnification due to axial length was not accounted for in volume scans such as the Macular Cube 512x128, a region with potentially high rates of change of tissue volume, slope and contour. A 6mm cube, as is used in the Macular Cube 512x128 may be significantly larger or smaller in shorter and
longer eyes, which may result in measuring more central or eccentric tissue, resulting in potentially erroneous thickness measurements if one is assuming a 6mm cube. The foveal slope, as measured by OCT, is 11.35±2.61 degrees before accounting for magnification and 12.16±2.86 after accounting for lateral magnification (Parthasarathy and Bhende 2015), however, which could create challenges in estimating the actual thickness of unmeasured adjacent tissue, given that contour changes relatively rapidly (Appendix A). Of nine studies that evaluated the effect of refractive error or axial length on macular thickness, only one, Ooto et al., accounted for lateral magnification (Ooto, Hangai et al. 2010). Of all nine studies evaluated, only Ooto et al. found no correlation between any region of the macula and axial length. Ooto et al. did not evaluate the effect of refractive error, however. Future OCTs should enable measurement and incorporation of axial length to account for lateral magnification due to axial elongation. Such a change would enable more accurate determination of refractive and axial changes in macular volume and thickness.

The lack of diversity of ethnicities of the subjects in this study is another limitation. While most the subjects in this study were Caucasian, most studies looking at retinal thickness compared to refractive error in healthy subjects used Asian populations, which may have different ocular parameters. For instance, Mwanza et al. reports that subjects of European descent have significantly thinner average GCIPL layers when compared to Hispanic and Asian subjects(Mwanza, Durbin et al. 2011).
4.7 Conclusion

This study is the first comprehensive study that evaluates the macular thickness, RNFL thickness, GCIPL thickness, visual sensitivity and PIPR in the same population. This study is also the first to evaluate the effect of axial length on the PIPR; while previous studies indicate that the PIPR was independent of refractive error, as did the current study, the structural component of axial length was shown to correlate to the PIPR: longer eyes have a weaker PIPR and do not stay as constricted after a 5 second flash of blue light. This is also the first study to correlate GCIPL thickness to SE in young, healthy subjects; both myopic SE and longer AL are associated with GCIPL thinning. The ocular parameters reported in this study of young, predominantly myopic adults can be used as a basis for comparison to disease case
References


Quigley, M., Powell, I., Walter, W. (2014). Optical modeling shows retinal illumination is increased in hyperopes and short axial length eyes. Is this the reason for increased AMD seen in these patients? Investigative Ophthalmology & Visual Science. **55**.


Appendix A. Example of a macular thickness analysis report from the Cirrus OCT.

<table>
<thead>
<tr>
<th>Macula Thickness : Macular Cube 512x128</th>
<th>OD</th>
<th>OS</th>
</tr>
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#### Macula OCT Analysis

- **OD**
  - Central Subfield Thickness (µm): 248
  - Cube Volume (mm³): 10.0
  - Cube Average Thickness (µm): 281

- **OS**
  - Central Subfield Thickness (µm): 267
  - Cube Volume (mm³): 16.0
  - Cube Average Thickness (µm): 297

*Legend:*
- Green indicates normal range.
- Red indicates abnormal range.
Appendix B. Example of a ganglion cell analysis report from the Cirrus OCT.
Appendix C. Example of a RNFL thickness analysis report from the Cirrus OCT.
Appendix D. Macular thickness analysis report for the left eye of Subject 12 (BVMD) as measured on the Cirrus OCT. The IS-OS boundary is absent near the fovea. Vitelliform deposits exist in the fovea and a serous retinal detachment is present superiorly.
Appendix E. Macular thickness analysis report for the right eye of Subject 16 (RCD) as measured on the Cirrus OCT.