VALIDATION OF OPTICAL COHERENCE TOMOGRAPHY-BASED CRYSTALLINE LENS THICKNESS MEASUREMENTS IN CHILDREN

MASTER'S THESIS

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> By Bret M. Lehman, B.S. * * * *

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Master's Examination Committee:

Approved by

Karla Zadnik, OD, PhD, Advisor

Melissa D. Bailey, OD, PhD

Mark A. Bullimore, MCOptom, PhD

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ABSTRACT

The purpose of this study was to evaluate the validity and repeatability of crystalline lens thickness measurements obtained by anterior segment optical coherence tomography (OCT). Optical coherence tomography utilizes infrared light and partial-coherence interferometry to produce a two-dimensional scan of the internal ocular structures *in vivo*. Changes in crystalline lens thickness are important to monitor for refractive error development, cataract progression, and anterior chamber alterations. Our goal was to develop an effective method of measuring the thickness of the crystalline lens with the Visante anterior segment OCT, to assess OCT repeatability, and to evaluate OCT validity as compared to A-scan ultrasonography.

Forty-seven normal children (mean age \pm SD = 11.06 \pm 2.30 years) had their crystalline lens thickness measured with the Visante anterior segment OCT (Carl Zeiss Meditec, Dublin, CA) and with conventional A-scan ultrasonography (Humphrey 820). The subjects' right corneas were anesthetized, and their right eyes were cyclopleged. Five A-scan ultrasonography measurements and three Visante OCT measurements were made per eye. Thirty-eight subjects had measurements at a second visit where three additional Visante OCT measurements were made.

The mean of the differences between the Visante OCT and A-scan ultrasonography was -0.045 mm (p = 0.017), indicating that the average measurement of crystalline lens thickness was thinner with the Visante OCT. The 95% limits of agreement were from -0.29 to 0.20 mm. When validity was assessed using only Visante OCT images that contained the corneal reflex, the mean of the differences was 0.019 mm (p = 0.11) with 95% limits of agreement from -0.091 to 0.13 mm. For the repeatability of the Visante OCT, the mean of the differences between visit one and visit two was -0.008mm (p = 0.25) with 95% limits of agreement from -0.088 to 0.072 mm. Repeatability improved when reassessed using only images that contained the corneal reflex; the mean of the differences for these images was -0.0001 mm (p = 0.97) with 95% limits of agreement from -0.030 to 0.030 mm. In comparison to A-scan ultrasonography, a statistically significant difference was observed only when images that did not contain the corneal reflex were included in the analysis. When assessing inter-visit repeatability, a statistically significant difference was not found in either case, but the limits of agreement were narrower when repeatability was assessed using only the images containing a corneal reflex.

The Visante OCT is a non-contact instrument that is simple to use, and it provides valid crystalline lens thickness measurements with excellent repeatability. Validity and repeatability were optimized when the Visante OCT images contain the corneal reflex.

Dedicated to my wife for her endless support and to my father for sending me in the right direction

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VITA

October 17, 1982	Born – Lexington, Ohio
May 2005	B.S. Zoology; Minor Neuroscience, Miami University
September 2005 – Present	Doctorate Student of Optometry The Ohio State University College of Optometry
September 2006 – Present	Graduate Student of Vision Science The Ohio State University College of Optometry

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CHAPTER 1

INTRODUCTION

Accurate measurements of crystalline lens thickness are important in studies of myopia progression (Zadnik et al., 1995; Mutti et al., 1998), refractive error (Garner et al., 1995), ocular accommodation (Richdale et al., 2008), presbyopia (Strenk et al., 2005), and cataract maturation (Jivrajka et al., 2008). During the process of emmetropization, for example, the eye adjusts for changes in axial length by altering the refractive power of the cornea and crystalline lens (Zadnik et al., 1992).

A variety of methods are available to measure the axial components of the eye. Ascan ultrasonography has been used for over 50 years and is still the gold standard for both clinical practice and research to measure the thickness of the crystalline lens. The drawback of this method is that the ultrasound probe physically touches the cornea and requires topical anesthesia prior to measurement, making this method challenging to use on children. In addition, precise positioning of the A-scan probe is difficult because there is no distinct landmark that can be used to align the probe with the cornea. A-scan ultrasonography has been shown to be insensitive to lens thickness changes less than or equal to the equivalent of 0.75 D (Kurtz et al., 2004); therefore, small changes in lens thickness due to accommodation, eye growth, or index of refraction changes may go undetected when measured using A-scan ultrasonography.

The Visante anterior segment optical coherence tomographer (OCT) (Carl Zeiss Meditec, Dublin, CA) is a new instrument designed to image the anterior segment of the eye. It utilizes low-coherence interferometry to create a detailed image of human tissue. Crystalline lens thickness measurements can be made from the images captured by this instrument. The Visante OCT is easy to use and does not require corneal contact, making it a more advantageous option for studying children. The Visante OCT has previously been shown to be a reliable tool to measure the anterior chamber angle (Li et al., 2007), (Dada et al., 2007), anterior chamber volume (Wang et al., 2007), and central corneal thickness (Li et al., 2007; Pinero et al., 2008). A recent study also used a model eye to assess the accuracy and validity of Visante OCT measurements and reported formulas that reduced errors when measuring a model eye (Dunne et al., 2007).

Recently, Zeng et al. (2009) demonstrated that Visante OCT crystalline lens measurements were significantly greater than A-scan ultrasonography-measured crystalline lens thickness measurements in adults (Zeng et al., 2009). Intraobserver and interobserver agreement was found to be better with the Visante OCT compared to Ascan. This study examined both elderly (> 49 years old) and young (18- 40 years old) subjects, but measurements were no performed in children. The difference in lens thickness measurement observed although statistically significant was not thought to be clinically meaningful. Unlike this project, the former study assessed lens thickness measurements in an uncyclopleged state and did not assess validity and repeatability in children.

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The current project assessed instrument agreement between the Visante OCT and conventional A-scan ultrasonography in the measurement of crystalline lens thickness. The between-visit repeatability of measurements made with the Visante OCT was also assessed. A better understanding of this method and the possible sources of error are necessary before OCT can be implemented into longitudinal evaluations of crystalline lens growth. The goals for this project were as follows:

- To determine instrument agreement between the Visante OCT and the A-scan ultrasonography for the measurement of crystalline lens thickness;
- To determine the between-visit repeatability of Visante OCT crystalline lens thickness measurements;
- To investigate the effect of capturing images that contain the corneal reflex on Visante OCT precision;
- To determine whether crystalline lens index of refraction is related to differences in lens measurements between the Visante OCT and A-scan ultrasonography; and
- To determine if the Visante OCT is a viable tool to measure crystalline lens thickness and is an alternative to A-scan ultrasonography.

CHAPTER 2

HISTORICAL REVIEW

The human crystalline lens is an integral part of alterations of refractive error that occur throughout life. Coordinated growth patterns of ocular components (i.e., cornea, crystalline lens, and axial length) determine a child's refractive error early in life through a process known as emmetropization (Grosvenor T 1994). Over the course of four years from ages 6 to10 years, the crystalline lens has been shown to thin nearly 0.20 mm to compensate for an average increase in axial length of 1 mm (Zadnik et al., 1995). As the crystalline lens continues to mature and more lens fibers are added within the capsular bag, the overall lens thickness increases linearly by 0.013 to 0.029 mm/year (Richdale et al., 2008). This continual addition of fibers and cells eventually create lens thickness changes that result in presbyopia and the development of cataracts (Shui and Beebe 2008). This increasing lens thickness plays an important role in ocular accommodation and the change in refractive error of the eye as it continues to age.

2.1 Ocular Biometry: A-scan Ultrasonography, Scheimpflug Photography, and MRI

2.1.1 A-scan Ultrasonography

Considering the impact of lens thickness on refractive error development, accurate imaging and measurement of the crystalline lens are essential to understanding its role in these processes. Measurements of lens thickness have historically been performed using A-scan ultrasonography, slit lamp Scheimpflug photography, and, more recently, magnetic resonance imaging (MRI). A-scan ultrasonography is considered the reference standard for *in vivo* measurement of lens thickness; however, this instrument has limitations.

A-scan ultrasonography works on the principle that the velocity of sound waves vary as they travel through different media. By determining the time necessary for sound waves generated by the ultrasound probe to travel through the crystalline lens, its thickness can be calculated. The faster the sound wave travels, for example, the shorter the crystalline lens. The drawback is that the A-scan assumes the same lenticular sound velocity of approximately 1641 m/s for each person (Koretz et al., 1989). Media changes within the crystalline lens such as refractive index or cataracts are known to alter the velocity at which sound travels through the lens. Previous studies have calculated that for every 50 meters/second error in assumed velocity of ultrasound, the thickness of the crystalline lens is over or underestimated by 0.1 mm (Jansson and Kock 1962).

In addition, the A-scan probe must physically touch the eye to make a measurement. This requires topical anesthesia and patient cooperation. The probe has the potential to indent the cornea and alter axial measurements. Lastly, there is no physical landmark that can be used to ensure that measurements are repeatedly being made at the same point through the ocular components. This can lead to intra- and inter-examiner error. To minimize this error, multiple measurements are often recorded and averaged. A method of measuring the thickness of the crystalline lens that does not require anesthetic, does not require corneal contact, and is more repeatable would be advantageous.

2.1.2 Scheimpflug Photography

Scheimpflug photography, a technique that has been around since the early 1900s and developed commercially for ocular biometry in the 1980s, is considered an alternative to A-scan ultrasonography (Sasaki et al., 1990). It is based on optical principles of camera movements and lens tilt to focus an image of a three-dimensional object (i.e., human crystalline lens) into a single plane of focus. Thus, an object with significant depth of field can be imaged with sharp clarity and optical accuracy. This technique has been shown to be repeatable (Edwards et al., 1988), in agreement with Ascan ultrasonography if a correction factor is added to the Scheimpflug measurement (Tong et al., 2003), and in agreement with MRI-based measurements (Koretz et al., 2004).

Scheimpflug photography has its own limitations. The refraction of light by the cornea and the anterior surface of the crystalline lens in Scheimpflug photography creates an optical distortion that generates an inaccurate measurement of the thickness of the crystalline lens (Dubbelman et al., 2001). It is feasible to correct this distortion; however, the index of refraction of the crystalline lens must be calculated (Dubbelman et al., 2001).

Despite the accuracies of Scheimpflug photography, its reliance upon complex algorithms to correct image distortions indicates the need for a non-invasive, physiologically accurate, repeatable instrument to measure the thickness of the crystalline lens.

2.1.3 Magnetic Resonance Imaging

MRI can also be used to measure the thickness of the crystalline lens. Excellent statistical agreement is shown between MRI and Scheimpflug photography (Koretz et al., 2004). MRI has been used to study and document the ocular physiological properties of the crystalline lens in presbyopes (Strenk et al., 2005), the anterior chamber (Tanitame et al., 2008), the cornea (Chang et al., 2007), and the crystalline lens itself (Kasthurirangan et al., 2008). High-resolution MRI images still have much lower resolution than optical coherence tomography. In addition, the expense and impracticality of this instrument make it challenging to use repeatedly in large-scale, longitudinal studies of the crystalline lens. These limitations once again establish the need for a high-resolution, lower-cost instrument more apparent.

2.2 Ocular Biometry: Optical Coherence Tomography

2.2.1 Principles of Optical Coherence Tomography

Optical coherence tomography (OCT) is an interferometric technique, utilizing the interference pattern of two infrared light waves, to produce a high-resolution, threedimensional image of biological tissue. This concept was applied to ocular structures in the early 1990s and the first *in vivo* measurements of the retina were achieved in 1993 (Fercher et al., 1993). The advantage of OCT, compared to other ocular biometry instruments, is that it penetrates deeper into ocular tissues while maintaining high resolution. Low-coherence interferometry, as applied by OCT, uses two super luminescent diodes (LEDs) that emit a broad range of frequencies of light at extremely short impulses. Two light waves travel to the tissue and either reflect or scatter. The scattered light is filtered out, while the coherent (non-scattered, reflected) light waves are collected and interference patterns are monitored. The image of the tissue is obtained because the areas that reflect large amounts of light create heavy interference patterns identifying the structure of the tissue. OCT requires no special preparation of the tissue being imaged, is non-invasive, creates an image of a live sample, uses no harmful radiation, and obtains resolution on the order of 10 µm.

Optical coherence tomography was first applied for ophthalmic purposes to the posterior segment of the eye including the fovea, retina, and optic nerve. The introduction of the Visante anterior segment OCT expands this technology to the management of corneal refractive surgery, keratoconus, glaucoma, and more. In this project, the Visante OCT was used to measure the thickness of the crystalline lens. The instrument was not originally intended to measure the properties of the crystalline lens and its surrounding structures including the ciliary body, choroid, and sclera. The purpose of this study was to determine if crystalline lens measurements made by the OCT are repeatable and in agreement with A-scan ultrasonography, a currently accepted method of obtaining measurements of crystalline lens thickness.

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2.2.2 Current Trends of Validity and Repeatability with the Visante OCT

Validity and repeatability of the Visante OCT has been assessed in the measurement of multiple anterior segment structures. Li et al. (2007) observed both intrasession repeatability and inter-session reproducibility of average anterior chamber angle size in light and dark. They found that not only was the Visante OCT capable of producing an objective and quantitative measurement, but it did so with good repeatability and reproducibility (Li et al., 2007a). Lavanya et al. (2007) compared anterior chamber depth measurements made by the Visante OCT to measurements made by the IOL-Master and the scanning peripheral anterior chamber depth analyzer (SPAC). It was found that the OCT gave systematically deeper anterior chamber measurements than SPAC and IOL-Master, but the differences found were small and unlikely to be clinically important (Lavanya et al., 2007). Dada et al. (2007) recently compared OCT to ultrasound in the measurement of central corneal thickness, anterior chamber depth, and peripheral iridocorneal angles. They found no significant difference between any of the anterior chamber parameters (Dada et al., 2007). Dunne et al. (2007) determined the accuracy of OCT on model eyes. They found that the Visante OCT was very accurate, and they determined that errors caused by distortion could be adjusted by applying a correction formula (Dunne et al., 2007). Richdale et al. (2008) demonstrated that the Visante OCT was capable of measuring crystalline lens thickness changes as a function of age and accommodation. Their results correlated well with similar studies using ultrasound, MRI, and Scheimpflug photography (Richdale et al., 2008).

As the versatility of the Visante OCT continues to expand, it is necessary to assess the repeatability and reproducibility of the instrument in all axial ocular

components. Recently, Zeng et al. (2009) compared human crystalline lens thickness measurements made by OCT to A-scan ultrasonography on both a phakic sample with lens opacity (> 49 years old) and on young adult subjects (18 to 40 years old) with clear lenses and active accommodation (Zeng et al., 2009). Measurements in their study were performed without cycloplegia. They compared one measurement of lens thickness made by the A-scan ultrasonography to one measurement of lens thickness made by OCT. The difference (OCT minus A-scan ultrasonography) for the elderly population was $0.135 \pm$ 0.150 mm (mean \pm SD) (p < 0.001) and for the young adult population was 0.101 \pm 0.111 mm (mean \pm SD) (p < 0.001). In addition, they found narrower 95% limits of agreement with OCT compared to A-scan ultrasonography for both inter-observer and intra-observer measurements. They attributed the statistically significantly thicker measurements made by the OCT to differences in operating principles (i.e., A-scan ultrasonography uses sound waves and Visante OCT uses infrared light waves) and accommodation. They did not assess the effect of sound wave velocity and crystalline lens refractive index on lens thickness measurement

2.4 The Changing Crystalline Lens

The index of refraction of the crystalline lens is established during embryonic development and changes throughout life (Atchison et al., 2008). Epithelial cells with their apical end facing inward toward the nucleus produce a basement membrane that surrounds the lens, known as the lens capsule. Within the lens, the central nucleus or embryonic nucleus is formed by the primary lens fibers. Secondary lens fibers arising

from the pre-equatorial, anterior epithelial cells continue to divide throughout life. These fibers form the juvenile nucleus and eventually give rise to the adult nucleus or cortex. With age, secondary lens fibers continue to be mitotically active; therefore, the density of the cortex changes continuously throughout life.

The changes in the ocular parameters in the emmetropic eve with age were recently well documented by Atchison et al. (2008) in a cross-sectional study with subjects 20 to 70 years old. Lens thickness, measured with the Oculus Pentacam, increased 0.024 mm/year, and lens equivalent index of refraction, measured with phakometry, decreased 0.0003/year (Atchison et al., 2008). Uhlhorn et al. (2008) recently measured the refractive index of the crystalline lens with OCT technology from 40 intact lenses from postmortem donors, aged 6 to 82 years. In this study, lenses were removed from the donor globe and the lens was inserted into a chamber to maintain adequate hydration. The mean \pm SD equivalent refractive index was 1.408 \pm 0.005, and a significant decrease was observed with age. The peak refractive index was suggested to be closer to 1.420 (Uhlhorn et al., 2008). Jones et al. (2007) recently conducted a crosssectional study (n=44, age 18-59 years) using MRI and found a significant increase in lens thickness with age but no age dependence on the central lens index of refraction (Jones et al., 2007). These results indicate that refractive index changes do occur in the crystalline lens with age; however, these changes are minimal compared to other ocular parameters such as lens thickness, axial length, and anterior segment depth, which are most likely responsible for changes in refractive error (Atchison et al., 2008). In addition, the changes in refractive index are more likely cortical rather than nuclear (Uhlhorn et al., 2008), (Jones et al., 2007).

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Extensive research has been conducted on the role of the ocular components in the process of emmetropization. Mutti et al. (2005) conducted a study on infants from three to nine months of age. It was shown that the refractive error decreased significantly over the six month time period. Axial growth appeared to be most influential; however, the crystalline lens flattened a substantial amount to contribute to the overall dioptric change in refractive error (Mutti et al., 2005). In addition Zadnik et al. (2004) reported two year longitudinal data from a study of ocular component changes in emmetropic children from ages six to 14 years. They found a coordinated flattening and thinning of the crystalline lens as the eye elongated axially with age. This synchronized growth pattern maintains emmetropia for children and emphasizes the essential role of the crystalline lens in ocular refractive development (Zadnik et al., 2004).

There is an ongoing debate regarding the etiology of myopia and the role of coordinated growth patterns of ocular components. Current thought is that the eye elongates both in the axial and equatorial dimensions. The equatorial expansion of the eye flattens the crystalline lens, reducing its dioptric power to maintain emmetropia as the eye continues to lengthen. This thinning and flattening of the crystalline lens was shown to abruptly stop around the age of ten years in a longitudinal study by Mutti et al. (1998). If the eye continues to grow in the axial dimension without a corresponding flattening and thinning of the crystalline lens, myopia is likely to result (Mutti et al., 1998).

Accurate assessment of crystalline lens thickness in ocular biometry is critical to uncover the role the crystalline lens in ocular refractive changes. The crystalline lens contributes to one-third of the overall dioptric of the eye, and small changes in its curvature, thickness, or refractive index have a large optical impact on refractive status.

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CHAPTER 3

METHODS

3.1 Recruitment and Sample Size Determination

Subjects were recruited by advertisements posted at The Ohio State University College of Optometry and letters sent to patients within the Optometry Services. A total of 53 children ages 8 to 15 years were recruited from The Ohio State University College of Optometry patient base, faculty, and students' families. The primary goal of the study was to determine the relationship between myopia development and crystalline lens oscillations after saccadic eye movements. This thesis, describing crystalline lens thickness measurements, was a secondary goal of the original study. Forty-seven children enrolled in the study completed both A-scan ultrasonography and Visante OCT measurements at the initial visit. The data obtained from these subjects was used for the current analysis.

The sample size calculation for the validity study was based on an alpha of 0.05, beta of 0.10, population variance of 0.10 mm (Zadnik et al., 1992), and an effect size of 0.06 mm (Zadnik et al., 1992). Based on these criteria, 30 subjects were needed. For repeatability, the sample size calculation was based on an alpha of 0.05, beta of 0.10,

population variance of 0.10 mm (Mohamed et al., 2007), and effect size of 0.10 mm (Mohamed et al., 2007). Based on these criteria, 11 subjects were needed for this analysis.

3.2 Eligibility Criteria

The Ohio State University's Biomedical Sciences Institutional Review Board, in accordance with the tenets of the Declaration of Helsinki, approved the study protocol. Subjects were educated on the purpose and the procedures of the study, and parental consent and child assent were obtained before enrollment into the study. No subjects were excluded based on race, gender, refractive error, or other ocular axial parameters. Subjects with crystalline lens opacities, binocular vision anomalies, or ocular disease/pathology in the anterior or posterior segment were excluded from the study.

3.3 Study Design

The study was designed to use the Visante OCT to develop a method to accurately measure crystalline lens thickness, to determine if lens measurements are valid compared to A-scan ultrasonography, and to assess repeatability of lens thickness measurements with the Visante OCT. Data for this study were obtained at two visits. At the first visit, the crystalline lens thickness was measured with A-scan ultrasonography and the Visante OCT. A subset of 38 children had Visante OCT crystalline lens measurements made at a second study visit that was approximately two weeks after the initial study visit. No visits were separated by more than three months. Data obtained from the first visit were used to

assess validity. Data obtained with the Visante OCT at visit one and visit two were used to assess repeatability.

3.3.1 Cycloplegia and Accommodation

Ocular accommodation changes the thickness of the crystalline lens. In studies involving pre-presbyopic patients, it has been reported that lens thickness increased from 42 to 72 µm per diopter of accommodation (Richdale et al., 2008). Visante OCT measurements induce minimal to no accommodation when the internal fixation target is set to the distance refraction (Zeng et al., 2009); however, A-scan ultrasonography requires subjects to fixate on the blinking light of the probe, which unavoidably introduces ocular accommodation (Lavanya et al., 2007). To minimize the effect of accommodation on the measurement of lens thickness, all subjects were cyclopleged prior to measurements. Adequate cycloplegia was obtained with two drops of 1% tropicamide separated by five minutes (Egashira et al., 1993), (Mutti et al., 1994). All measurements were recorded after a minimum of 30 minutes from the instillation of the first drop of 1.0 % tropicamide.

3.3.2 A-scan Ultrasonography Measurement

Crystalline lens thickness was measured by handheld A-scan ultrasonography (Model 820, Allergan-Humphrey, San Leandro, CA) using a focused transducer with a frequency of 10 MHz in semi-automatic mode and a sound velocity of 1641 m/s. Measurements were always performed on the right eye. After 30 minutes, each subject's right cornea was topically anesthetized with one drop of 0.5% proparacaine. One experienced examiner (MDB) obtained five consecutive axial scans with the A-scan ultrasonography. The patients were asked to fixate the blinking light at the center of the probe, and alignment precision was attempted by gently placing the probe on the cornea at the center of the pupil. Ultrasound traces with small peaks from the cornea, anterior and posterior poles of the crystalline lens, or retina were deleted, and an effort was made to select traces with good component definition and minimal corneal indentation. The mean of the five recordings of lens thickness was used to compare to the measurements made by the Visante OCT (described below).

3.3.3 Visante OCT Measurement

The crystalline lens of the right eye was imaged with anterior segment optical coherence tomography (Zeiss Visante OCT Model 1000, Carl Zeiss Meditec, Dublin, CA). Three images were obtained immediately after the A-scan measurement by one experienced examiner (MDB). Version 1.0 of the Visante OCT software was used in this study. The Visante OCT is a non-contact, non-invasive, high-resolution device that uses infrared light to image the anterior segment of the eye and measure intra-ocular distances. The system uses a Michelson interferometer illuminated by partial or low-coherence light from a 1310 nm LED. A beam splitter creates two separate light rays, one targeting the sample and the other targeting the reference mirror. A signal is detected only when the reflections are nearly matched in time-of-flight. Similar to the scanning technology of the ultrasound B-scan, the Visante OCT acquires multiple scans to create a two-dimensional

image. Low-resolution images are produced from 256 scans in 125 milliseconds and a high-resolution image from 512 scans in 250 milliseconds (Meditech 2006).

The scanning of the crystalline lens is a non-contact procedure in which the subject focuses on an internal fixation target. The instrument clears an internal target based on the patient's refractive error as entered by the examiner. Visante OCT measurements were centered on the pupil, and the fixation angle was adjusted so that the eye did not appear tilted. Subjects were cyclopleged, as explained above, and images were captured a minimum of 30 minutes after instillation of the first drop of 1% Tropicamide. The crystalline lens was imaged on the "anterior segment single" scanning mode for low-resolution images and along the horizontal meridian (nasal-temporal angles at 0-180 degrees) in primary gaze.

Images gathered early in the study did not always contain the "corneal reflex," a white line passing centrally through the OCT image. The Visante OCT manual states this line is an indication that the eye is optimally aligned with the instrument (Meditec 2006). Dada et al. suggest that optimal alignment occurs when the fixation angle (the angle between the instrument's optical axis and the eye's line of sight) is at 0 degrees (Dada et al., 2007). Because the measurement of the crystalline lens is not an advertised use of the Visante OCT, this study sought to determine if the presence of the corneal reflex improved the repeatability of the crystalline lens measurement. Statistical analyses were performed for the entire dataset and again excluding any images that did not contain the corneal reflex. Figure 3.1 is an image of the crystalline lens with the corneal reflex captured by the Visante OCT. Note that after minimal examiner training, it is very easy to capture an image of the crystalline lens with a visible corneal reflex.

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Figure 3.1: Image of the crystalline lens captured by the Visante OCT in anterior segment single scanning mode Version 1.0. The central, vertical white line is the "corneal reflex" according to the manufacturer.

To determine whether all three Visante OCT lens images were necessary to obtain the level of repeatability that we report, we examined the improvement in intersession repeatability as the number of images with the corneal reflex beam present increased. The standard deviation of the differences in crystalline lens thickness between visit one and visit two was plotted as a function of the number of Visante OCT images taken.

A single observer (BML) using the Visante OCT internal caliper system measured crystalline lens thickness. In all cases, the thickest portion of the crystalline lens was measured. The mean of three measurements of crystalline lens thickness at visit one with the Visante OCT was compared to the mean of five measurements made by the A-scan ultrasonography to assess validity. The same protocol was followed at the second visit to capture three additional images of the crystalline lens. Repeatability was assessed by comparing the mean of the three measurements made by the Visante OCT at visit one to the mean of three measurements made at visit two. Figure 3.2 describes how validity and repeatability were assessed in a diagram representing the flow of the experiment.



Validity Figure 4.1: a. versus d. Figure 4.2: b. versus d. Figure 4.3: b. versus c.

Visit Two: e. Visante AS-OCT: all images (n=38) 3 LT – average f. Visante AS-OCT: corneal reflex (n=24) 3 LT – average **Repeatability** Figure 4.5: a. versus e. Figure 4.6: b. versus f.

Figure 3.2: Study design overview, sample size, and figure analysis. Lens thickness (LT) was measured on 47 subjects at visit one with both the Visante OCT and the A-scan ultrasonography. Lens thickness was measured on 38 subjects at visit two with the Visante OCT. These measurements were divided into subgroups and were used to assess validity and repeatability of the Visante OCT. The validity and repeatability boxes correspond to the figures in the text below.

3.3.4 Establishing the Crystalline Lens Index of Refraction

The Visante OCT software available for this study was originally designed to measure the ocular components in the anterior chamber and the cornea. In order to obtain an image of the entire crystalline lens, the focus of the instrument must be moved posterior to the cornea. If no adjustments are made, Version 1.0 of the software attempts to locate the cornea by designating both anterior and posterior corneal surfaces arbitrarily on the anterior portion of the crystalline lens. The software assigns a refractive index of 1.000 (air) for all structures anterior to the anterior corneal boundary, 1.388 (cornea) for all structures within the corneal boundaries, and 1.343 (aqueous) for all structures posterior to the posterior corneal boundary. To eliminate this problem, the refractive index of the entire image was set at 1.388 (cornea) because this is the refractive index available in the Visante software that is the closest to the physiological index of the crystalline lens, based on the index of refraction estimated by the Gullstrand #1 exact schematic eye (Rabbetts 1998). This model eye estimates the index of refraction of the lens as 1.406 and the cortex of the lens as 1.386. To assign an index of refraction of 1.388 to the entire image, the "edit tab" was located, and "surfaces" was clicked on. The entire anterior corneal boundary was placed at the top of the screen, and the entire posterior corneal boundary was placed at the bottom of the screen. Lens thickness measurements were made only after these changes were completed.

3.3.5 Physiological Index of Refraction of the Crystalline Lens

In the present study, we assess the effect of index of refraction of the crystalline lens on the measurement of lens thickness. Although the index of refraction was shown to have minimal changes with age (see above), the variation from person to person may cause significant variation of thickness measurements because the Visante OCT only applies one refractive index to the entire image.

The importance of calculating the physiologic index of refraction was previously stated. Video phakometry, as described by Mutti et al. (1992), was used to calculate the physiological index of refraction of the lens (Mutti et al., 1992). Phakometry is a noninvasive technique used to image the Purkinje images generated by the front surface of the cornea (Purkinje image 1), the front surface of the crystalline lens (Purkinje image 3), and the back surface of the crystalline lens (Purkinje image 4). Because the radius of curvature of the front surface of the cornea can easily be determined via keratometry, the height of Purkinje image 1 can be calculated. Therefore, the radii of curvature of both the anterior and posterior lens surfaces can be estimated by comparing the height of Purkinje image 3 to Purkinje image 1 and Purkinje image 4 to Purkinje image 1, respectively. Now, the eye can be simplified into an equivalent mirror closed system. The axial components of the eye (anterior chamber depth, lens thickness, and vitreous chamber depth) are measured via ultrasound. The index of refraction of the cornea, anterior chamber, and vitreous is assumed to be relatively constant from person to person. Finally, given the refractive error of the individual eye, the index of refraction of the lens can be determined because it is the only remaining variable. The index of refraction of the crystalline lens was calculated in this manner for 41 subjects.

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Index of refraction of the crystalline lens is known to vary across subjects and the change with age (Mutti et al., 1995). Knowing that the crystalline lens index of refraction is different for each of our subjects, it was possible to determine whether differences in measured lens thickness between the A-scan ultrasonography and Visante OCT are related to differences in crystalline lens refractive index (i.e., whether differences in lens thickness between instruments is related to lens refractive index). In other words, the physiological index of refraction could create a bias toward more or less accurate measurements of lens thickness. To determine if a relationship existed in the data, the crystalline lens index of refraction was calculated using video phakometry as explained above for 41 subjects. A linear regression was performed to examine the relationship between physiological refractive index and the difference between Visante OCT and Ascan ultrasonography lens thickness measurements. The index of refraction of each of our subjects was plotted against the mean of the difference in the measurement of crystalline lens thickness between A-scan ultrasonography and Visante OCT at visit one. Forty-one subjects were used for this analysis. Regression analysis was performed to determine if the slope was significantly different from zero.

3.3.6 Visante OCT Software Version 2.0

The Visante software has been updated since the data for this study were collected. In the current Visante software version, Version 2.0, the "anterior segment single" mode and built-in software caliper system cannot be used to measure the thickness of the crystalline lens. In Version 2.0, an image of the crystalline lens, i.e., an

image in which the cornea is not visible, can only be obtained using a new "raw image mode" that applies a refractive index of 1.00 (air) to the entire image. The software calipers cannot be used when an image is collected using the raw image mode.

To verify that accurate measurements can still be made in Version 2.0, 21 images captured with version 1.0 were converted to an index of 1.00 (air) by expanding the corneal boundaries as explained above. All images contained the corneal reflex and were exported as .jpg image files. The images were opened in image processing software (Matrox Inspector 4.0; Matrox Electronic Systems Ltd.; Quebec, Canada). The thickness of the crystalline lens was measured in pixels and converted to millimeters using the conversion 1 mm = 51 pixels. This conversion was determined by exporting an image with refractive index set to air (1.00) with a 1-mm line drawn by the Visante software. The number of pixels in the crystalline lens was measured in the image processing software. The total number of pixels was divided by 51 pixels to determine crystalline lens thickness in millimeters. Dividing this number by 1.388, we converted lens thickness measurement in air to and index of refraction of 1.388. Version 1.0 crystalline lens thickness measurements were compared to the measurements calculated under version 2.0 using a paired t-test.

The result of applying a uniform refractive index to the entire image is a nonphysiologic appearance within the aqueous and vitreous; however, with the method described above, no measurements are made outside of the lens. All measurements that reported are between two points along the longest axial dimension of the lens. Because OCT technology relies on optical path length when determining thicknesses, other structures in the image do not influence the optical path length through the crystalline

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lens, which is supported by our results. Figure 3.3 shows an image of the crystalline lens when the index of refraction is converted to air (1.00) and the scale bars used to determine the number of pixels in 1.0 mm.



Figure 3.3: Image of the crystalline lens from the Visante OCT version 2.0 converted to an index of refraction of air (1.00). Measurement bars of 1.0 mm as shown in the bottom left of the image were used to determine the number of pixels in 1.0 mm.

3.4 Statistical Analysis

Statistical analyses were performed using SPSS, version 15.0 (SPSS Inc, Chicago,

Illinois). Validity was determined by comparing the mean of the five A-scan

measurements of each subject to the mean of the three Visante OCT measurements of the

same subject at visit one. These means were compared using a two-tailed paired t-test,

and the difference between the means was compared to zero. Inter-session repeatability of the Visante OCT was assessed by comparing the mean of three measurements of crystalline lens thickness at the first visit to the mean of three measurements of crystalline lens thickness at the second visit. Repeatability was assessed using difference versus mean plots as described by Bland and Altman (Bland and Altman 1986). Bias of the method can be characterized by comparing the mean difference between the instruments to zero. The degree of repeatability is expressed by the 95% limits of agreement (mean \pm [1.96 x standard deviation]).

CHAPTER 4

RESULTS

4.1 Study Population Demographics and Sample Size

47 subjects completed the first visit, and 38 subjects returned for a second visit. The mean age \pm standard deviation (SD) of the subjects was 11.1 ± 2.3 years (range 8 to 15 years). The mean spherical equivalent refraction, as measured by cycloplegic autorefraction, was -1.21 ± 2.33 D (mean \pm SD). Of the 47 subjects, 22 were myopes and 25 were non-myopes. Myopic refractive error, for this project, is defined as more than -0.75 D of myopia from cycloplegic autorefraction in both meridians.

Of the 47 subjects that completed the first visit, 27 subjects had the corneal reflex in at least one of the Visante OCT images of the crystalline lens. Of these 27 subjects, there were four subjects who had the corneal reflex in two of the three images and one subject who had the corneal reflex in only one image. Lens thickness measurements from these five subjects were not included in the corneal reflex analysis for validity. Of the 38 subjects who returned for a second visit, 29 subjects had the corneal reflex in two or more of the three images taken. Of these 29 subjects, 24 subjects from visit one and visit two had the corneal reflex in at least two images of their crystalline lens. Only three of these 24 subjects did not have the corneal reflex in all three images. To increase sample size, these three subjects were used in our analysis of repeatability.

4.2 Validity of Visante OCT Lens Thickness Measurements

Descriptive statistics for both validity and repeatability for all images and for images containing the corneal reflex are displayed in Table 4.1.

	A-scan		Visante	ОСТ		
	Mean	±SD	Mean	±SD	Mean	±SD
Validity	_					
All images (n=47)	3.47	0.19	3.42	0.18		
Corneal reflex (n=22)	3.40	0.20	3.42	0.19		
Repeatability	_		Visit one		Vis	it two
All images (n=38)	_		3.47	0.18	3.47	0.18
Corneal reflex (n=25)			3.44	0.18	3.44	0.18

Table 4.1: Means and standard deviations in millimeters for A-scan ultrasonography and Visante OCT

4.2.1 Validity Including Images that Do Not Have the Corneal Reflex

Validity was analyzed in three different ways. First, comparing lens thickness measurements of all 47 subjects that completed the first visit, the mean crystalline lens thickness measurement by A-scan ultrasonography was significantly thicker than that measured with the Visante OCT ($t_{46} = 2.49$, p = 0.017). The mean \pm SD difference in crystalline lens thickness between the Visante OCT and A-scan ultrasonography was

 -0.045 ± 0.13 mm with 95% limits of agreement from -0.29 to +0.20 mm. Figure 4.1 is a difference versus mean plot for all subjects.



Figure 4.1: Validity difference versus mean plot (Visante OCT – A-scan; n = 47). The mean difference (solid line) was significantly different from zero (p = 0.017). The dashed lines outline the 95% limits of agreement.

4.2.2 Validity including only the Corneal Reflex Images

When validity was reassessed using only the Visante OCT images that contained the corneal reflex, the mean \pm SD of the differences was $+0.019 \pm 0.054$ mm with 95% limits of agreement from -0.09 to +0.13 mm. This was not a statistically significant difference (t₂₁ = -1.66, p = 0.11). Figure 4.2 is a difference versus mean plot using only the images that contained the corneal reflex.



Figure 4.2: Validity difference versus mean plot (Visante OCT – A-scan) for only the images where a corneal reflex was visible (n = 22). The mean difference (solid line) was not significantly different from zero (p = 0.11). The dashed lines outline the 95% limits of agreement.

4.2.3 Validity Version 1.0 Compared to Version 2.0

Validity was reassessed once again using version 2.0 software with the Visante OCT. We compared 21 individual lens thickness measurements made with version 1.0 to measurements from the same subjects made with version 2.0. No significant difference was observed (t_{20} = 1.642, p = 0.12). The mean ± SD of the differences was -0.014 ± 0.040 mm with 95% limits of agreement from -0.092 to +0.064 mm. Figure 4.3 is a difference versus mean plot of the validity of the measurements made in version 2.0 compared to version 1.0. This result demonstrates that valid measurements can still be made in Version 2.0 using the modified procedure described above in the methods section.



Figure 4.3: Validity difference versus mean plot (Visante OCT Version 1.0 - Visante OCT Version 2) using only images where a corneal reflex was visible (n = 22). The mean difference (solid line) was not significantly different from zero (p = 0.12). The dashed lines outline the 95% limits of agreement.

4.2.4 Physiologic Index of Refraction

Figure 4.4 displays the difference in lens thickness measurements versus the

phakometry-calculated crystalline lens index of refraction. A significant relationship was

not found (p = 0.360). The inter-subject variation in crystalline lens index of refraction

does not create a bias in lens thickness measurement.



Figure 4.4. Difference between Visante OCT and A-scan ultrasonography versus the phakometry-calculated index of refraction (n=41). A significant relationship did not exist (p = 0.360).

4.3 Repeatability of Visante OCT Measurements

4.3.1 Repeatability Including Images without the Corneal Reflex

The mean crystalline lens thickness measured at visit one was not significantly different from that measured at the second visit ($t_{37} = -1.16$, p = 0.25). The mean \pm SD of the differences in crystalline lens thickness between visits was -0.008 ± 0.041 mm with 95% limits of agreement from -0.088 to +0.072 mm. Figure 4.5 is a difference versus mean plot of the repeatability of all crystalline lens thickness measurements made with the Visante OCT.



Figure 4.5: Inter-visit repeatability difference vs. mean plot of the Visante OCT (visit one – visit two) (n = 38). The mean difference (solid line) was not significantly different from zero (p = 0.25). The dashed lines outline the 95% limits of agreement.

4.3.2 Repeatability Including only the Corneal Reflex Images

When repeatability was reassessed using only the images that contained the corneal reflex, the mean \pm SD of the differences between visits decreased to -0.00 ± 0.015 mm with 95% limits of agreement from -0.03 to +0.03 mm, which was not statistically significant (t₂₃ = -0.040, p = 0.97). Figure 4.6 is a difference versus mean plot of the between-visit repeatability of crystalline lens thickness measurements made with the Visante OCT when only images with a corneal reflex were considered.



Figure 4.6: Inter-visit repeatability difference vs. mean plot of the Visante OCT (visit one – visit two) for only the images where a corneal reflex was visible (n = 25). The mean difference (solid line) was not significantly different from zero (p = 0.97). The dashed lines outline the 95% limits of agreement.

4.4 Improved Repeatability with More Measurements

Figure 4.7 is a plot of the repeatability of Visante OCT lens measurements (the standard deviation of the differences between visits) as a function of the number of images. The best repeatability was obtained when all three images per session were included; therefore, to obtain the repeatability that we report, at least three lens images with the corneal reflex present must be obtained. Additional improvement in repeatability may be possible with additional images; however, the limits of agreement with three images (\pm 0.03 mm) are already superior to those found with ultrasound. The result of this analysis indicates that additional measurements improve the estimate of crystalline lens thickness. Although we do not report a plateau of images that maximize measurement accuracy, three images of the crystalline lens were sufficient to yield excellent repeatability.



Figure 4.7: Plot of repeatability (standard deviation of the differences between visits) as a function of the number of Visante OCT lens images obtained that contained the corneal reflex beam.

CHAPTER 5

DISCUSSION

Accurate ocular biometry measurements are important in studies examining the development of refractive error, crystalline lens growth, presbyopia, and cataract surgery. The repeatability of partial coherence interferometry measurement techniques has been shown to be better than A-scan ultrasonography for axial length measurements (Carkeet et al., 2004). In our study, we demonstrated both validity and excellent repeatability through a novel use of the Visante OCT to measure the thickness of the crystalline lens.

A-scan ultrasonography is currently used widely in pediatric research (Edwards et al., 2002; Gwiazda et al., 2003; Mutti et al., 2007) and the instrumentation is typically more readily available than other systems that can measure crystalline lens thickness, such as a Scheimpflug camera. Still, one might consider it to be disadvantageous in a study of children due to the use of local anesthetics and direct physical contact with the eye. In addition, the applanation technique may cause corneal indention and distort ocular component measurements (Lam et al., 2001). Moreover, there is no landmark to provide precise measurement of the same axial dimension. It is imperative to make precise axial measurements. It is estimated, based on geometric optics and the Gullstrand #1 model eye, that a change of approximately \pm 0.4 mm in axial length on either side of

been reported that repeatability for ocular components should be within \pm 0.1 mm (Storey and Rabie 1983; Michaels 1992). The repeatability of the A-scan ultrasonography for the measurement of crystalline lens thickness has been previously reported, and a summary is displayed in Table 5.1 (Jansson and Kock 1962; Storey and Rabie 1983; Rudnicka et al., 1992; Zadnik et al., 1992). Although the statistical analyses used across the studies in Table 5.1 are not consistent, all studies show repeatability greater than \pm 0.1 mm. In addition, Kurtz et al. showed that the A-scan is sensitive to lens thickness changes only if they exceed the measurements equivalent to 1.00 D using the conventional hand-held technique (Kurtz et al., 2004). For the reasons stated above, a more accurate measurement technique may be advantageous to effectively study lens thickness changes.

Research Group	Year	Repeatability*
Rudnicka (Rudnicka et al., 1992)	1992	± 0.120
Optimum Accuracy per manufacturer		
(Rudnicka et al., 1992)	1991	± 0.134
Jansson (Jansson and Kock 1962)	1962	± 0.135
Storey & Rabie (Storey and Rabie 1983)		± 0.160
Zadnik (Zadnik et al., 1992)	1992	± 0.200

*1.96 x standard deviation of mean differences

Table 5.1 Repeatability of A-scan ultrasonography

5.1 Index of Refraction

The Gullstrand #2 Simplified Schematic Eye establishes a refractive index for the

crystalline lens at 1.413. The Gullstrand #1 Exact Eye creates a heterogeneous lens with

an index of 1.386 for the cortex and 1.406 for the nucleus (Rabbetts 1998). *In vivo*, the crystalline lens has a gradient refractive index that increases from the cortex to the nucleus. Assigning one or even two refractive indices to the crystalline lens is not physiologically accurate. We are aware that the index assigned by the Visante OCT is equivalent neither to the lens refractive indices in the schematic eyes nor to the refractive indices of our subjects' crystalline lenses; however, the index of refraction assigned to the cornea (1.388) by the Visante OCT software is the closest option available to the estimated Gullstrand values. By extending the boundary limits of the cornea in the Visante OCT software, an index of refraction of 1.388 could be applied to the entire crystalline lens.

5.2 Validity of Visante OCT Crystalline Lens Thickness

In comparison to A-scan ultrasonography, the lens measurements made by the Visante OCT were not significantly different when using only the images with the corneal reflex; however, a statistically significant difference did exist when images of the crystalline lens that did not include the corneal reflex were included in the analysis. The mean difference was -0.045 mm, indicating that the A-scan produced slightly thicker measurements of the crystalline lens. Although this difference is significant, it is still far less than the best-reported repeatability of A-scan ultrasonography (Rudnicka et al., 1992) and may not be clinically meaningful. Again, based on the Gullstrand #1 Exact Eye, a change of 0.10 mm in axial length corresponds to a 0.25-D change in refractive error. Further, it has been estimated that the crystalline lens thickness increases by 0.06

mm per diopter of accommodative response (Zadnik et al., 1992). Still, we attempt to provide an explanation for this difference with three possible hypotheses.

5.2.1 Refractive Index

One hypothesis that might explain this result is that the refractive index assigned to the crystalline lens (1.388) using the Visante OCT software was greater than the refractive index underlying the assumptions made in A-scan ultrasonography, causing the Visante OCT lens thickness measurements to be slightly thinner. This is inferred from the following formula: Thickness (d) = Optical Path Length (OPL) \div index of refraction (n) or restated as OPL = n*d. In this project, the numbers reported are thickness (d) because the optical path length has already been divided by the index of refraction. For example, if the index of refraction were to increase then the thickness measurement must decrease. To assign a theoretical value, the average lens thickness as measured by the Visante OCT in a subset of patients from the repeatability analysis was 3.44 mm with the index of refraction set to 1.388. If this experiment were repeated with an assigned index of 1.488, the average thickness would be approximately 3.21 mm. Therefore, we can estimate for every 0.1 unit increase in index of refraction the thickness of the crystalline lens will decrease by approximately 0.23 mm.

The index of refraction applied to the crystalline lens in the Visante OCT underestimates the true index of refraction as calculated by video phakometry. Video phakometry assigns one index of refraction to the crystalline lens so that the mathematical calculation of refractive error for that individual eye is accurate. In reality,

as mentioned previously, the crystalline lens has a gradient refractive index with the highest index in the central nucleus and a lower index cortically. Even though video phakometry provides a good estimate of an average overall refractive index of the crystalline lens *in vivo*, the exact physiological index across the entire spectrum of the lens is not known. Because our results demonstrate that the index of refraction does not bias lens thickness measurement made be the Visante OCT, an underestimation of the actual index of refraction is irrelevant. Mathematical calculation through application of the optical path length formula confirms that a lower refractive index produces a longer thickness measurement and that a higher refractive index produces a shorter thickness measurement. This point is not necessary to belabor for the purposes of longitudinal and developmental studies in crystalline lens thickness with an instrument of high repeatability, like the Visante OCT. In addition, the Visante OCT produced valid measurements of crystalline lens thickness as compared to the A-scan ultrasonography; therefore, the underestimation of refractive index is not enough to bias lens thickness measurement in a manner that is significantly different than the current gold standard.

5.2.2 Off-axis measurement and Sound Velocity in A-scan Ultrasonography

Another possible reason for the small difference found could be that an off-axis portion of the crystalline lens was measured with one of the devices, resulting in a slight error in the thickness measurement. An off-center measurement produces a smaller measurement, and an oblique measurement produces a thicker or thinner result. This error can be minimized with the Visante OCT by capturing images with the corneal reflex and measuring the axial dimension of the thickest part of the lens closest to the reflex. In contrast, an off-center measurement is difficult to minimize with the A-scan ultrasonography because there is no way to ensure that the thickest portion of the lens is consistently measured. In addition, the A-scan assumes a specific velocity of sound as the sound waves pass through the ocular media. If the assumed velocity of sound in the crystalline lens is the same as the actual velocity, the measurement made by the A-scan will be physiologically inaccurate. This is of particular interest when studying children because the velocity of sound through a child's crystalline lens may differ slightly from the velocity of sound through an adult's crystalline lens.

5.2.3 A-scan Repeatability less than Mean Difference

Finally, the known repeatability of the A-scan biometry for the measurement of crystalline lens thickness ranges from ± 0.120 to ± 0.200 mm (Rudnicka et al., 1992; Zadnik et al., 1992), demonstrating that A-scan measurements are less precise than the Visante OCT in this study. This means that if this experiment was repeated, a different average thickness of the crystalline lens would possibly be found with A-scan measurements. The largest difference between the Visante OCT and the A-scan reported here (0.045 mm) may not be clinically meaningful because the difference is far less than the reported repeatability of A-scan ultrasonography.

5.3 Improved Validity with Corneal Reflex

The A-scan ultrasonography and the Visante OCT did not produce significantly different measurements of crystalline lens thickness when the results were analyzed using only Visante OCT images in which the corneal reflex was visible. The mean difference in crystalline lens thickness measurements between the instruments including all subjects was -0.045 mm compared to 0.019 mm when only the images with a corneal reflex were analyzed. The non-significant difference between the instruments indicates that the two devices' measurements were more similar when the corneal reflex was captured in the Visante OCT images. Because the mean value of the Visante OCT measurements did not change with or without the corneal reflex present, this may indicate that the small difference found using the larger sample was the result of the higher variability of A-scan measurements.

5.4 Physiological Index of Refraction

Lens index of refraction may differ slightly for each person. Figure 4.4 demonstrates that the small inter-subject differences in the physiological index of refraction have no effect on the measurement of crystalline lens thickness obtained with the two instruments. If the index of refraction were to create a bias in the measurement, the slope of the line would be significantly different from zero. This situation is potentially problematic because more error in lens thickness measurement would be associated with a higher or lower refractive index. Again, this association was not observed in our study reemphasizing that the Visante OCT is an accurate tool to measure crystalline lens thickness across a wide spectrum of varying lens indices of refraction.

5.5 Repeatability of Visante OCT Crystalline Lens Thickness

The Visante OCT has previously be reported to have excellent repeatability when measuring the cornea (Mohamed et al., 2007). Our results emphasize the repeatability of the Visante OCT but now, for the first time, in the human crystalline lens. The mean of the differences in Visante OCT crystalline lens thickness measurements between visits was not significant. The agreement between visits was much better in the subset of subjects who had a visible corneal reflex in all images (95% limits of agreement: ± 0.030 mm compared to ± 0.08 mm). To maximize repeatability, it is necessary to include the corneal reflex in crystalline lens images captured by the Visante OCT; however, even if the corneal reflex is not present in the image, the repeatability of the Visante OCT is still better than that of A-scan ultrasonography in this sample.

5.6 Visante OCT Software Version 2.0

Crystalline lens thickness measurements were made with Visante OCT version 2.0. Lens thickness measurements were mathematically converted to an index of refraction of 1.388. These measurements were compared, using a paired t-test, to lens thickness measurements made in version 1.0 with the index of refraction of 1.388 applied to the entire image. Because a significant difference was not observed comparing the two data sets, it is not necessary to further assess validity and repeatability of version 2.0. Therefore, although external image processing is required to obtain lens thickness measurements with version 2.0 of the Visante software, the newer software still provides a viable method of obtaining the thickness of the crystalline lens.

CHAPTER 6

CONCLUSIONS

Crystalline lens thickness measurements made with the Visante anterior segment OCT are valid compared to those made with A-scan ultrasonography. Measurements of crystalline lens thickness made with the Visante OCT are highly repeatable. The best repeatability was achieved with the OCT when the corneal reflex was visible in the crystalline lens image (± 0.030 mm). The best-reported repeatability of A-scan ultrasonography is ± 0.12 mm (Rudnicka et al., 1992), which is four times larger than what was found for the Visante OCT in this study. The Visante OCT is non-contact, easy to operate, and able to produce a detailed, two-dimensional, high-resolution image of the crystalline lens. Given the good agreement between with the A-scan ultrasonography and the excellent repeatability of the Visante OCT, strong consideration should be given to using OCT methods as an alternative for ocular biometry studies involving the crystalline lens.

LIST OF REFERENCES

- Atchison DA, Markwell EL, Kasthurirangan S, Pope JM, Smith G, and Swann PG.
 (2008) Age-related changes in optical and biometric characteristics of emmetropic eyes. J Vis. 8: 29 21-20.
- Bland JM and Altman DG. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1: 307-310.
- Carkeet A, Saw SM, Gazzard G, Tang W, and Tan DT. (2004) Repeatability of IOLMaster biometry in children. *Optom Vis Sci.* 81: 829-834.
- Chang Y, Wu HM, and Lin YF. (2007) The axial misalignment between ocular lens and cornea observed by MRI (I)--at fixed accommodative state. *Vision Res.* 47: 71-84.
- Dada T, Sihota R, Gadia R, Aggarwal A, Mandal S, and Gupta V. (2007) Comparison of anterior segment optical coherence tomography and ultrasound biomicroscopy for assessment of the anterior segment. J Cataract Refract Surg. 33: 837-840.
- Dubbelman M, van der Heijde GL, and Weeber HA. (2001) The thickness of the aging human lens obtained from corrected Scheimpflug images. *Optom Vis Sci.* 78: 411-416.
- Dunne MC, Davies LN, and Wolffsohn JS. (2007) Accuracy of cornea and lens biometry using anterior segment optical coherence tomography. *J Biomed Opt.* 12: 064023.
- Edwards MH, Li RW, Lam CS, Lew JK, and Yu BS. (2002) The Hong Kong progressive lens myopia control study: study design and main findings. *Invest Ophthalmol Vis Sci.* 43: 2852-2858.
- Edwards PA, Datiles MB, and Green SB. (1988) Reproducibility study on the Scheimpflug Cataract Video Camera. *Curr Eye Res.* 7: 955-960.
- Egashira SM, Kish LL, Twelker JD, Mutti DO, Zadnik K, and Adams AJ. (1993) Comparison of cyclopentolate versus tropicamide cycloplegia in children. *Optom Vis Sci.* 70: 1019-1026.

- Fercher AF, Hitzenberger CK, Drexler W, Kamp G, and Sattmann H. (1993) In vivo optical coherence tomography. *Am J Ophthalmol.* 116: 113-114.
- Garner LF, Yap MK, Kinnear RF, and Frith MJ. (1995) Ocular dimensions and refraction in Tibetan children. *Optom Vis Sci.* 72: 266-271.
- Grosvenor T SR. (1994) Role of the axial length/corneal radius ratio in determining the refractive state of the eye. *Optom Vis Sci.* 71: 573-579.
- Gwiazda J, Hyman L, Hussein M, Everett D, Norton TT, Kurtz D, Leske MC, Manny R, Marsh-Tootle W, and Scheiman M. (2003) A randomized clinical trial of progressive addition lenses versus single vision lenses on the progression of myopia in children. *Invest Ophthalmol Vis Sci.* 44: 1492-1500.
- Jansson F and Kock E. (1962) Determination of the velocity of ultrasound in the human lens and vitreous. *Acta Ophthalmol (Copenh)*. 40: 420-433.
- Jivrajka R, Shammas MC, Boenzi T, Swearingen M, and Shammas HJ. (2008) Variability of axial length, anterior chamber depth, and lens thickness in the cataractous eye. *J Cataract Refract Surg.* 34: 289-294.
- Jones CE, Atchison DA, and Pope JM. (2007) Changes in lens dimensions and refractive index with age and accommodation. *Optom Vis Sci.* 84: 990-995.
- Kasthurirangan S, Markwell EL, Atchison DA, and Pope JM. (2008) In vivo study of changes in refractive index distribution in the human crystalline lens with age and accommodation. *Invest Ophthalmol Vis Sci.* 49: 2531-2540.
- Koretz JE, Strenk SA, Strenk LM, and Semmlow JL. (2004) Scheimpflug and highresolution magnetic resonance imaging of the anterior segment: a comparative study. J Opt Soc Am A Opt Image Sci Vis. 21: 346-354.
- Koretz JF, Kaufman PL, Neider MW, and Goeckner PA. (1989) Accommodation and presbyopia in the human eye--aging of the anterior segment. *Vision Res.* 29: 1685-1692.
- Kurtz D, Manny R, and Hussein M. (2004) Variability of the ocular component measurements in children using A-scan ultrasonography. *Optom Vis Sci.* 81: 35-43.
- Lam AK, Chan R, and Pang PC. (2001) The repeatability and accuracy of axial length and anterior chamber depth measurements from the IOLMaster. *Ophthalmic Physiol Opt.* 21: 477-483.

- Lavanya R, Teo L, Friedman DS, Aung HT, Baskaran M, Gao H, Alfred T, Seah SK, Kashiwagi K, Foster PJ, and Aung T. (2007) Comparison of anterior chamber depth measurements using the IOLMaster, scanning peripheral anterior chamber depth analyser, and anterior segment optical coherence tomography. *Br J Ophthalmol.* 91: 1023-1026.
- Li H, Leung CK, Cheung CY, Wong L, Pang CP, Weinreb RN, and Lam DS. (2007a) Repeatability and Reproducibility of Anterior Chamber Angle Measurement with Anterior Segment Optical Coherence Tomography. *Br J Ophthalmol*.
- Li H, Leung CK, Wong L, Cheung CY, Pang CP, Weinreb RN, and Lam DS. (2007b) Comparative Study of Central Corneal Thickness Measurement with Slit-Lamp Optical Coherence Tomography and Visante Optical Coherence Tomography. *Ophthalmology*.

Meditec CZ (2006). Visante OCT User Manual: Model 1000, Carl Zeiss Meditec.

- Meditech CZ (2006). Visante OCT User Manual: Model 1000, Carl Zeiss Meditec.
- Michaels DD. (1992) Optics, refraction, and visual function. *Curr Opin Ophthalmol.* 3: 69-70.
- Mohamed S, Lee GK, Rao SK, Wong AL, Cheng AC, Li EY, Chi SC, and Lam DS. (2007) Repeatability and reproducibility of pachymetric mapping with Visante anterior segment-optical coherence tomography. *Invest Ophthalmol Vis Sci.* 48: 5499-5504.
- Mutti DO, Hayes JR, Mitchell GL, Jones LA, Moeschberger ML, Cotter SA, Kleinstein RN, Manny RE, Twelker JD, and Zadnik K. (2007) Refractive error, axial length, and relative peripheral refractive error before and after the onset of myopia. *Invest Ophthalmol Vis Sci.* 48: 2510-2519.
- Mutti DO, Mitchell GL, Jones LA, Friedman NE, Frane SL, Lin WK, Moeschberger ML, and Zadnik K. (2005) Axial growth and changes in lenticular and corneal power during emmetropization in infants. *Invest Ophthalmol Vis Sci.* 46: 3074-3080.
- Mutti DO, Zadnik K, and Adams AJ. (1992) A video technique for phakometry of the human crystalline lens. *Invest Ophthalmol Vis Sci.* 33: 1771-1782.
- Mutti DO, Zadnik K, and Adams AJ. (1995) The equivalent refractive index of the crystalline lens in childhood. *Vision Res.* 35: 1565-1573.
- Mutti DO, Zadnik K, Egashira S, Kish L, Twelker JD, and Adams AJ. (1994) The effect of cycloplegia on measurement of the ocular components. *Invest Ophthalmol Vis Sci.* 35: 515-527.

- Mutti DO, Zadnik K, Fusaro RE, Friedman NE, Sholtz RI, and Adams AJ. (1998) Optical and structural development of the crystalline lens in childhood. *Invest Ophthalmol Vis Sci.* 39: 120-133.
- Pinero DP, Plaza AB, and Alio JL. (2008) Anterior segment biometry with 2 imaging technologies: very-high-frequency ultrasound scanning versus optical coherence tomography. J Cataract Refract Surg. 34: 95-102.
- Rabbetts RB (1998). Bennett & Rabbetts' Clinical Visual Optics, Butterworth-Heinemann Ltd: p 208.
- Richdale K, Bullimore MA, and Zadnik K. (2008) Lens thickness with age and accommodation by optical coherence tomography. *Ophthalmic Physiol Opt.* 28: 441-447.
- Rudnicka AR, Steele CF, Crabb DP, and Edgar DF. (1992) Repeatability, reproducibility and intersession variability of the Allergan Humphrey ultrasonic biometer. *Acta Ophthalmol (Copenh)*. 70: 327-334.
- Sasaki K, Sakamoto Y, Shibata T, and Emori Y. (1990) The multi-purpose camera: a new anterior eye segment analysis system. *Ophthalmic Res.* 22 Suppl 1: 3-8.
- Shui YB and Beebe DC. (2008) Age-dependent control of lens growth by hypoxia. *Invest Ophthalmol Vis Sci.* 49: 1023-1029.
- Storey JK and Rabie EP. (1983) Ultrasound--a research tool in the study of accommodation. *Ophthalmic Physiol Opt.* 3: 315-320.
- Strenk SA, Strenk LM, and Koretz JF. (2005) The mechanism of presbyopia. *Prog Retin Eye Res.* 24: 379-393.
- Tanitame K, Sasaki K, Sone T, Uyama S, Sumida M, Ichiki T, and Ito K. (2008) Anterior chamber configuration in patients with glaucoma: MR gonioscopy evaluation with half-Fourier single-shot RARE sequence and microscopy coil. *Radiology*. 249: 294-300.
- Tong L, Wong EH, Chan YH, and Balakrishnan V. (2003) Agreement between Scheimpflug photography and A-scan ultrasonography in anterior segment ocular measurements in children. *Optom Vis Sci.* 80: 529-534.
- Uhlhorn SR, Borja D, Manns F, and Parel JM. (2008) Refractive index measurement of the isolated crystalline lens using optical coherence tomography. *Vision Res.* 48: 2732-2738.

- Wang N, Wang B, Zhai G, Lei K, Wang L, and Congdon N. (2007) A method of measuring anterior chamber volume using the anterior segment optical coherence tomographer and specialized software. *Am J Ophthalmol.* 143: 879-881.
- Zadnik K, Mutti DO, and Adams AJ. (1992) The repeatability of measurement of the ocular components. *Invest Ophthalmol Vis Sci.* 33: 2325-2333.
- Zadnik K, Mutti DO, Fusaro RE, and Adams AJ. (1995) Longitudinal evidence of crystalline lens thinning in children. *Invest Ophthalmol Vis Sci.* 36: 1581-1587.
- Zadnik K, Mutti DO, Mitchell GL, Jones LA, Burr D, and Moeschberger ML. (2004) Normal eye growth in emmetropic schoolchildren. *Optom Vis Sci.* 81: 819-828.
- Zeng Y, Liu Y, Liu X, Chen C, Xia Y, Lu M, and He M. (2009) Comparison of lens thickness measurements using the anterior segment optical coherence tomography and A-scan ultrasonography. *Invest Ophthalmol Vis Sci.* 50: 290-294.