ACCOMMODATIVE MICROFLUCTUATIONS, CRYSTALLINE LENS TENSION, CILIARY BODY THICKNESS, AND REFRACTIVE ERROR IN CHILDREN

A Thesis
Presented in Partial Fulfillment of the Requirements
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ABSTRACT

Purpose: To investigate the relationship between microfluctuations in accommodation, resting tension on the crystalline lens, ciliary body thickness, and refractive error in children.

Methods: Subjects were 49 children, ages eight to 15 years. Subjects wore habitual correction over their left eye and an infrared filter over the right eye during accommodative measurements. Monocular accommodation was measured continuously for two, 30-second periods using a PowerRef I at a sampling rate of 25 Hz while subjects viewed a high-contrast target at 0.25 m. The high (1.0-2.3 Hz) and low (0-0.6 Hz) frequency components of the power spectrum from a Fast Fourier Transform of the accommodative response were used in analysis. Resting tension on the crystalline lens was assessed by measuring the amplitude of the oscillations of the crystalline lens following a rightward 20° saccadic eye movement. Ciliary body thickness was measured two millimeters posterior to the scleral spur from images obtained with a Zeiss Visante™
OCT. Cycloplegic spherical equivalent refractive error was obtained with the Grand Seiko autorefractor.

Results: The mean ± SD spherical equivalent refractive error was −1.00 D ± 2.25 (range −6.00 D to +3.44 D). Greater power in the high-frequency component of accommodative microfluctuations was associated with thinner ciliary bodies, lower ages, and more hyperopic refractive errors. No statistically significant relationship was found for the low-frequency component or root mean square of accommodative microfluctuations and refractive error.

Conclusions: High-frequency microfluctuations of accommodation appear to be suppressed with thicker ciliary bodies. These variations in accommodation need to be observed in a longitudinal study to better assess the functional significance of their relationship to ciliary body size and refractive error.
Dedicated to my family
for all of their support and encouragement
throughout the years.
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1.1 Accommodation

Accommodation is an important part of vision as the power and shape of the crystalline lens change so retinal image quality can be maintained at near as well as distance. It is part of the near triad of miosis, convergence, and accommodation that is regulated by the parasympathetic system and the Edinger-Westphal nucleus.\(^1\)

Accommodation was first localized to the crystalline lens by Thomas Young who was able to rule out several competing theories of how the eye was able to focus clearly at different distances such as through changes to corneal curvature or the axial length of the eye.\(^2\) Helmholtz later proposed a basic explanation of the accommodative process.\(^3\)

Helmholtz’s accommodative theory is still accepted today by the majority of scientists. According to the Helmholtz model of accommodation, the ciliary muscle is the only active element; the rest is biomechanical forces at work in the eye. When the ciliary muscle is in a relaxed state, the ciliary ring diameter is at a maximum and the tension on the anterior lens zonules is increased, pulling on the lens capsule and lens and altering the
inherent shape and dioptric power of the crystalline lens. When the ciliary muscle contracts during accommodation, the ciliary ring diameter and tension on the anterior lens zonules are reduced, allowing the lens capsule and lens to return to their inherent shape and dioptric power. When the ciliary muscle contracts it also pulls the ciliary ring forward and stretches the choroid and posterior zonules resulting in an anterior movement of the lens during accommodation.⁴

Fincham and Graves viewed the accommodative process in a patient with aniridia and confirmed the Helmholtz theory, observing a decrease in lens diameter with accommodation.⁵,⁶ He also found that without the lens capsule, the lens remained in a non-accommodated state. Furthermore, he found that in non-accommodating animals, the lens capsule is uniform but in accommodating animals the lens capsule is thinnest near the poles and thickest in the mid-periphery.⁵

Although the Helmholtz theory is widely accepted, other theories still exist. Coleman proposed that with ciliary muscle contraction the vitreous is pulled forward against the lens causing the anterior surface of the lens to steepen.⁷,⁸ Schachar’s theory states that ciliary muscle contraction causes increased tension on equatorial lens zonules while releasing tension on anterior and posterior zonules. This theoretically results in an increase in the equatorial diameter of the lens and steeper radii.⁹¹¹ However, studies have confirmed both that the eye is able to accommodate after a vitrectomy¹² and that the equatorial diameter of the crystalline lens decreases during accommodation.¹³
There are four main components of accommodation according to Heath.\textsuperscript{14} Reflex accommodation is the adjustment of the refractive state to maintain focused retinal images and is the largest and most important component of accommodation. The primary stimulus of reflex accommodation is blur. Vergence accommodation is induced by the neurological linking of the accommodative and fusional vergence system mediated through the CA/C ratio. Proximal accommodation is due to the perceived nearness of an object. Tonic accommodation represents baseline neural innervation in the absence of any other stimulus.\textsuperscript{14} It is important to be aware of what components of accommodation are affected by the experimental design.

1.2 Development of Accommodation

Infants are able to accommodate relatively accurately by an age of three months,\textsuperscript{15} although the infant accommodative response is more variable than adult responses.\textsuperscript{16, 17} Other aspects of accommodation, such as depth of focus\textsuperscript{18} and accommodative dynamics in response to steps of blur input,\textsuperscript{19} also mature rapidly during the first three months of life.

Accommodation in young children has not been carefully studied due to inherent difficulties with comprehension and attention.\textsuperscript{4} There are several studies on accommodation in school-age children. Amplitude of accommodation gradually decreases with age.\textsuperscript{20-22} Rouse and colleagues (1984), using MEM dynamic retinoscopy, found that accommodative lag slowly decreased from kindergarten to adult levels by
sixth grade. However, more recent studies using Nott retinoscopy found no difference in accommodative lag across different age groups (range: 4 to 15 years). It appears that by twelve years of age, accommodative measures are similar to normal adult values. However, this may reflect increased motivation and understanding of the tasks with maturity as much as it does physiological changes to the accommodative system.

1.3 Accommodative Microfluctuations in Research

Accommodative microfluctuations are small variations in the refractive power of the eye. They were discovered by Collins with an infrared optometer. The temporal characteristics of the oscillations were first described in detail using Fourier analysis almost fifty years ago by Campbell. A high-frequency component (1.3-2.2 Hz) and low-frequency component (<0.5 Hz) were identified by Campbell et al. and confirmed by other laboratories. The role of accommodative microfluctuations is controversial with theories ranging from simple system feedback instability and biological noise to periodic feedback to the eye to maintain steady-state accuracy. If the microfluctuations are biofeedback, they may be an intrinsic part of the accommodative system and actively changed to optimize accommodation, or they may be independent of the system but still utilized to enhance function. Accommodative microfluctuations have been found to be similar between eyes.

Further study has shown that the location of the high-frequency component peak varies between subjects within the range of high frequencies. It has been correlated
with the arterial pulse rate.\textsuperscript{33, 34} The peak frequency has been shown to decrease with application of a topical beta-adrenergic receptor antagonist, that also reduces arterial pulse,\textsuperscript{35} and it increased with elevated arterial pulse, such as during exercise.\textsuperscript{36} The high-frequency component seems to reflect noise from the arterial pulse in the accommodative plant because it is not lenticular in origin.\textsuperscript{36, 37} The power of the high-frequency component reaches a maximum near the center of the accommodative range or around \(-3.00\) to \(-5.00\) D, and then it decreases approaching the near point.\textsuperscript{38, 39}

The amplitude of the microfluctuations increases with accommodation until around \(4.00\) D,\textsuperscript{29, 40} particularly in the increasing power of the low-frequency component.\textsuperscript{41, 42} Increases in power of the low-frequency component have also been associated with larger ocular depth-of-focus from decreasing pupil size,\textsuperscript{43} pupils smaller than \(2\) mm,\textsuperscript{44} decreasing target luminance,\textsuperscript{45} and increasing blur,\textsuperscript{46} indicating that the low-frequency component may play a role in control of the accommodative response.\textsuperscript{36, 47} The low-frequency component microfluctuations are too slow to play a role in dynamic accommodation, but may contribute to the steady-state accommodative response.\textsuperscript{31} Using ultrasound techniques, a low frequency fluctuation in the anterior and posterior lens surface position was detected.\textsuperscript{37}

1.4 Accommodative Microfluctuations and Refractive Error

More recently, differences in the characteristics of microfluctuations have been observed between refractive error groups. These findings are summarized in Figure 1.
Seidel and coworkers (2003) first studied accommodative microfluctuations and refractive error in subjects in their early twenties who were classified as either emmetropes, early-onset myopes, or late-onset myopes. Both groups of myopes in the study had progressed around −0.50 over the past year. Seidel had subjects accommodate on a −4.00 D Maltese cross stimulus in a Badal system for 100 seconds. He found that subjects with late-onset myopia had a significant increase in root mean square accommodative microfluctuations and low-frequency component power when compared to subjects with emmetropia and early-onset myopia. No difference in the high-frequency component was observed between groups. Seidel hypothesized that late-onset myopes may require a larger low-frequency component of accommodation to guide the accommodative response across the increased depth of field. When Seidel repeated the same experimental setup but used free-space viewing instead of the Badal system, the differences between groups disappeared. Also, no differences were found between monocular or binocular viewing.

Harb and colleagues also studied accommodative microfluctuations and refractive error in subjects in their early twenties. Accommodative lag was not correlated with refractive error in the study subjects. The subjects were dilated with 2.5% phenylepherine to ensure the pupils were an adequate size for measurements to be taken with a PowerRefractor. Subjects read binocularly for 10 minutes on a computer screen while their accommodative data was recorded. Harb and colleagues found that myopes had more variable accommodation measured as the standard deviation as well as power in
both the low-frequency component and high-frequency component accommodative microfluctuations. The average accommodation between groups did not vary; accommodation was more variable about the average accommodation in myopes. The accommodative response also varied significantly between subjects in both groups.\textsuperscript{40}

Day and coworkers (2006) studied accommodative microfluctuations in subjects in their early twenties classified as emmetropes, early-onset myopes, and late-onset myopes. A Badal system with a Maltese cross target at several stimuli levels was used as the subjects accommodated for two minutes at each target level. Blinks were removed from that data and replaced with a straight line. Day found that subjects with late-onset myopia had more power in the high-frequency component unrelated to stimulus level and larger microfluctuations during distance viewing. The low-frequency component power did not increase as rapidly while viewing accommodative stimuli more distant than –3.00 D for subjects with late-onset myopia as it did for other refractive groups.\textsuperscript{41}

Langaas and coworkers (2008) first studied accommodative microfluctuations and refractive error in children. All of the children in the study were less than fifteen years old. The mean age of the emmetropic group was 13.51 and the mean age of the myopic group was 14.14. There was no difference in accommodative lag between emmetropes and myopes. Langaas used a PowerRefII as subjects accommodated on letter targets at varying distances for two seconds. He found more variability, measured as standard deviation, in the accommodative response of myopes than emmetropes.\textsuperscript{48}
1.5 Accommodative Microfluctuations and Age

Candy and coworkers have recently investigated accommodative microfluctuations in infants. They found that infants have a larger root mean square accommodative response than adults. The accommodative pattern was similar between infants and adults, increasing, particularly in the power of the low-frequency component accommodative microfluctuations, as the accommodative stimulus increased. Candy presented several possible explanations for the findings: increased body and head movement, larger depth of field, more hyperopic and astigmatic refractive error, more lens elasticity, and cortical immaturity in infants as compared to adult subjects.\textsuperscript{16}

Other studies have looked at accommodative microfluctuations and presbyopia. Studies have found that accommodative microfluctuations are decreased in presbyopes with the predominant theory that the decrease is due to loss of elasticity with aging.\textsuperscript{49, 39}

1.6 Ciliary Body Anatomy

The ciliary body is composed of stroma and the ciliary muscle. The ciliary muscle is a smooth muscle and composed of three orientations of fibers: longitudinal, radial, and equatorial.\textsuperscript{50} The muscle inserts anteriorally into the scleral spur and trabecular meshwork.\textsuperscript{50} Posteriorly, it attaches by elastic tendons to the choroidal stroma.\textsuperscript{50} The inner surface is bounded anteriorally by the pars plicata and posteriorally by the pars plana.\textsuperscript{50}
The ciliary body grows rapidly for the first two years of life and continues growing past the age of six. Ciliary body size has been related to refractive error in both children and adults.

1.7 Crystalline Lens Anatomy

The ciliary body is connected to the crystalline lens by a system of lens zonules. The posterior lens zonules extend from near the posterior attachment of the ciliary muscle towards the pars plicata. The anterior zonules originate in the valleys of the pars plicata and pass through the circumlental space between the ciliary muscle processes and equatorial region of the crystalline lens. The zonular plexus system of fine fibers anchors the ciliary epithelium to the lens zonules.

Surrounding the crystalline lens, the lens capsule is a thin acellular elastic basement membrane. It is composed of Type IV collagen and glycoaminoglycans. The capsule is thickest in the midperipheral region and thinnest at the lens poles.

On the anterior surface of the crystalline lens is a monolayer of epithelial cells. It is responsible for regulation of the osmolarity of the lens. The cells of the epithelium elongate and become lens fibers which make up the majority of the crystalline lens. The crystalline lens is composed of an inner nucleus and an outer cortex.
1.8 Characteristics of Myopic Eyes

Myopic eyes have a more prolate ocular shape resulting from a longer axial length than equatorial length. The eye elongates axially but not equatorially as it elongates in myopia.\textsuperscript{54} There is relative hyperopic peripheral refraction in myopic eyes as opposed to relative myopic periphery refraction in emmetropic and hyperopic eyes.\textsuperscript{54} The ciliary body size is also increased in myopic eyes.\textsuperscript{52,53}

Accommodation is affected in progressing myopes. The AC/A ratio is increased in myopes and an elevated AC/A ratio is a risk factor for developing myopia in the next year.\textsuperscript{55} An elevated AC/A ratio indicates that more convergence is required for each diopter of accommodation. After the onset of myopia, accommodation is less accurate. This is evidenced by the increased accommodative lag in progressing myopes.\textsuperscript{56,57} It is controversial whether accommodative lag increases before\textsuperscript{56} or after\textsuperscript{57} the onset of myopia.

Due to the unique characteristics of myopic eyes, Mutti and coworkers (2006) have proposed a hypothesis that equatorial restriction is involved in the onset of myopia. “Excessive ciliary and/or lenticular stretch in the enlarged myopic eye may produce increased equatorial tension, which may account for the structural and accommodative characteristics that are associated with myopia. Structurally ciliary tension may also explain the prolate ocular shape that is found in myopia.”\textsuperscript{57} Accommodative microfluctuations may be another aspect of accommodation that is affected by the increased equatorial tension in myopic eyes.
1.9 Role of Crystalline Lens Tension and Ciliary Body Thickness

Recently, there have been reports showing that the thickness of the ciliary body is related to refractive error in both children and adults.\textsuperscript{53, 58} Microfluctuations have a peak at the midrange of accommodation and decrease at the range limits suggesting biomechanical involvement.\textsuperscript{4} There have been relatively few studies regarding accommodative microfluctuations in school-age children and none of those studies have evaluated mechanical factors related to the crystalline lens or whether ciliary body anatomy affects microfluctuations. The aim of this study was to determine if there is a relationship between accommodative microfluctuations and tension on the crystalline lens or ciliary body size in children.
CHAPTER 2

METHODS

2.1 Subjects

Subjects were recruited via electronic mail sent to faculty, staff, and students at The Ohio State University College of Optometry, flyers given to the parents of eligible patients that visited the Ohio State Optometric Services Clinic, subjects who completed participation in the Adolescent and Child Health Initiative to Encourage Vision Empowerment (ACHIEVE) study, letters sent through the mail to parents of recent Ohio State Optometric Services Clinic patients who met inclusion criteria, and word of mouth.

Forty-nine subjects ages eight to 15 years (mean = 11.4 years, SD = 2.2 years) participated in the study. Exclusion criteria were a history of ocular surgery or amblyopia, corrected vision with habitual correction of worse than 20/40, and medications that interfere with eye movement or accommodation. Written informed consent was obtained from a parent or guardian of each subject, and written assent was
obtained from each subject. The study was approved by the Institutional Review Board of The Ohio State University.

All measurements were made on right eyes only. Cycloplegia was achieved by instilling one drop of 0.5% proparacaine followed by two drops of 1% tropicamide separated by five minutes. Cycloplegic measurements were made 25 minutes after the second drop of tropicamide. Cyclopegic, spherical equivalent refractive error in the right eye was obtained from the mean of five readings with the Grand Seiko WR-5100K (Grand Seiko Co., Hiroshima, Japan) autorefractor. The mean ± SD spherical equivalent refractive error was −1.00 D ± 2.19 (range −6.00 D to +3.44 D).

2.2 Accommodation Measurements

Throughout the experiment, subjects wore their habitual correction over their left eye and an infrared filter over their right eye that occluded vision while still allowing measurements to be taken. Monocular accommodative response for measurements of microfluctuations was measured using a PowerRefractor (MultiChannelSystems, Reutlingen, Germany) at a sampling rate of 25 Hz and a test distance of 1 m. The sampling rate of the PowerRefractor is greater than the Nyquist limit. The instrument has a range of −8.75 to +4.00 D which was adequate because all subjects were wearing habitual correction. For measurements to be taken a pupil size larger than 3.7 mm is required which was achieved by dim room illumination. Its use has been established for measuring refractive error in children, as well as for the measurement of
accommodative microfluctuations.\textsuperscript{16, 40} While positioned in a head and chin rest to minimize head movement, subjects viewed a high-contrast Maltese cross target (angular subtense: 1.4°) at a distance of 0.25 m (−4.00 D) continuously for two, 30-second periods while measurements were taken. A sample measurement is shown in Figure 2.

To improve the confidence in the analyses, five 10-second segments of data were used for a frequency resolution of 0.1 Hz.\textsuperscript{59} Matlab Version 7.1 (Mathworks, Natick, MA) was used to write a program (Appendix) that identified blinks by missing data points and changes greater than 10 D/s that are faster than physiologically possible\textsuperscript{40} and filtered erroneous data points to form a line using the average of the points before and after the erroneous data to connect the valid data points. For each segment, the fast Fourier transform (FFT) was calculated and then averaged for the five segments. The area under the curve of the mean power spectrum was integrated to find the component power in the high-frequency (1.0-2.3 Hz) and low-frequency (0.0-0.6 Hz) ranges according to previous classifications.\textsuperscript{28, 40} A sample power spectrum is shown in Figure 3.

Accommodative lag was also measured using the Grand Seiko autorefractor. Subjects viewed a 4.00-D stimulus, a single row of 20/100 letters, through a Badal lens system. A similar lag measurement has been used in other studies.\textsuperscript{53, 64} The mean of five measurements was used in analysis.
2.3 Crystalline Lens Tension Measurement

In a 1995 report, Deubel and Bridgeman described oscillations of the crystalline lens after saccadic eye movements. Their work documents the idea that the eye is not “inelastic” and that the crystalline lens moves under both viscous and elastic forces during high-speed, saccadic eye movements. The authors demonstrated that the Purkinje tracking system will be contaminated by these oscillations when studying the main sequence of saccade dynamics. They also demonstrated that the oscillations of the crystalline lens are dependent upon the “stiffness” of the accommodative system. Two conditions that should lead to less tension on the crystalline lens, increased accommodation and younger age, were both associated with larger, post-saccadic oscillations of the crystalline lens.

Purkinje images I and IV were created with a single pipe fiber optic light source with an infrared pass filter. Oscillations in the right eye, which was occluded with a Wratten 89 B infrared filter, were video-taped. The left eye was unoccluded to allow for fixation between the two saccadic targets. Digital video files of the saccadic eye movements were recorded with a digital video camera (pco.1200hs, The COOKE Corporation, Romulus, MI) at the rate of 1000 frames per second.

All saccadic eye movements were 20° in magnitude to maximize velocity of the saccade while preserving accuracy and therefore maximizing the oscillations of the crystalline lens. Measurements from five saccadic eye movements made in a rightward direction under cycloplegic conditions were included in analysis. Upon review of each
video file, if the subject was found not to have completed a smooth, full saccadic eye movement, that video file was discarded and another trial was completed and recorded. The saccadic targets at distance were “+” symbols at 4.0 meters.

All video files were imported into Matlab® for analysis. Files were “batch processed” in a Matlab® program. Briefly, the Matlab® program identified Purkinje images I and IV through a routine that isolated the two Purkinje images based on a texture analysis, intensity analysis, and finally a “roundness” and size analysis. Once Purkinje images I and IV were isolated in the individual frames of the video, the horizontal position of Purkinje images I and IV were identified through a center of mass function and recorded in separate arrays for each Purkinje image in the video file. Arrays were exported from Matlab® as Microsoft Excel® spreadsheets. The difference in the horizontal position between Purkinje images I and IV was calculated through subtraction of x-axis coordinates. Graphs of the difference in Purkinje images I and IV (Figure 4) were created for each video file. Specific features were extracted from each of the curves to serve as measurements of the crystalline lens oscillations. The amplitude of the crystalline lens oscillation at the end of the saccade was termed “A,” which was the difference between Max and Min in Figure 4. The value of A served as our primary measurement of crystalline lens oscillation.
2.4 Ciliary Body Measurement

Images of the nasal ciliary body of the right eye were obtained with the Zeiss Visante™ Anterior Segment Optical Coherence Tomographer (OCT). Measurements were made under cycloplegia as previously described. A thickness measurement at 2.0 mm posterior to the scleral spur (Figure 5) was used in analysis (CBT2), as the thickness at this location has been previously shown to be negatively correlated with refractive error.

2.5 Statistical Analyses

There were three dependent variables of interest: high-frequency component, low-frequency component, and root mean square of accommodative microfluctuations. The distributions of the high- and low-frequency components were skewed toward higher powers. To make them less skewed for the purpose of modeling them as outcomes in a regression, the variables underwent a logarithmic transformation and were tested with a Shapiro-Wilk test for normality.

The independent variables of interest were ciliary body thickness at 2.0 mm posterior to the scleral spur (CBT2), the amplitude of the crystalline lens tension measurements following saccadic eye movements (A), and refractive error. Saccadic velocity, mean accommodation measured with the PowerRefractor, pupil size, gender, and age were independent variables used as controls. Using multiple regression, each dependent variable (high-frequency component, low-frequency component, and root
mean square) was regressed separately on CBT2, A, refractive error, saccadic velocity, mean accommodation, pupil size, gender, and age.
3.1 General Sample Characteristics

The general characteristics of the study sample are listed in Figure 6. There was a strong, negative correlation between refractive error and age ($r = -0.55$, $p < 0.0001$), i.e., older subjects tended to be myopic while younger subjects tended to be emmetropic or hyperopic. Refractive error was also negatively correlated with ciliary body thickness ($r = -0.31$, $p = 0.03$). There were also correlations between age and accommodative microfluctuations in the log of the high-frequency component ($r = -0.38$, $p = 0.01$), log of the low frequency component ($r = -0.29$, $p = 0.05$), and log root mean square ($r = -0.36$, $p = 0.01$). Mean accommodation measured with the PowerRefractor was correlated with refractive error ($r = 0.33$, $p = 0.02$). Amplitude of crystalline lens oscillations, saccadic velocity, pupil size, and gender did not have statistically significant correlations with any outcome or predictor variables.
The relationship between accommodative lag and refractive error in this sample is shown in Figure 7. Note that the myopes in this sample do not have the characteristically higher accommodative lag when compared to emmetropes.

3.2 Multiple Regression Models of Accommodative Microfluctuations and Ciliary Body Thickness or Crystalline Lens Tension

In the multiple regression model, there was a negative, statistically-significant relationship between the log of the high-frequency component and ciliary body thickness (Figure 8). There was also a negative statistically-significant relationship between the log of the high-frequency component and age. This indicates that older subjects and subjects with thicker ciliary bodies have smaller microfluctuations of accommodation in the high-frequency component. Only the age control variable was statistically significant in the log low-frequency component or log root mean square model (Figures 9 and 10).

3.3 Models of Accommodative Microfluctuations and Refractive Error

There was a positive, statistically-significant relationship between the log of the high-frequency component and refractive error (Figure 11). This indicates that the more hyperopic refractive errors have larger microfluctuations of accommodation in the high-frequency component. Mean measurement of refraction during accommodation was also significant in this model. Refractive error was also significant in the log low-frequency
component model (Figure 12). Both refractive error and mean measurement of refraction
during accommodation were significant in the log root mean square model (Figure 13).
CHAPTER 4

DISCUSSION

In this study, greater ciliary body thickness was associated with reduced power in the high-frequency component of accommodative microfluctuations. The power of the high-frequency component accommodative microfluctuations decreases by 86% for every 50 µm increase in ciliary body thickness. The high-frequency component has previously been associated with the arterial pulse. These results suggest that thicker ciliary bodies may dampen the effects of pulse on accommodation. Greater ciliary body thickness has also been associated with a decreased amount of accommodative lag in adults and increased amounts of myopia in children. Although the role of an increased ciliary body thickness in juvenile-onset myopia is still unclear, it appears that the increased thickness of the ciliary body may actually improve the stability of the high-frequency portion of the accommodative response.

The low-frequency component of accommodative microfluctuations has previously been found to be lenticular in origin. Because no relationship was found
between the low-frequency component and tension on the crystalline lens, it can be hypothesized that the low-frequency component of accommodative microfluctuations is not affected by variation in tension on the lens capsule and/or zonules and must instead originate from some inherent property of the lens itself or feedback control noise in neural input to accommodation.\textsuperscript{38}

More hyperopic refractive error was associated with higher powers of high-frequency accommodative microfluctuations in this study. This differs from what others have previously reported.\textsuperscript{40-42, 48} Because increasing myopic refractive error was associated with increasing age in this sample, the myopic subjects were likely to have completed their myopia progression. In addition, some of the younger subjects with refractive error more in the emmetropic range may have just begun the process of developing myopia. This is illustrated in Figure 7 which shows a normal accommodative lag in most of the myopic subjects, with a few low myopes/emmetropes having higher accommodative lag. Accommodative lag has been shown to increase at myopia onset,\textsuperscript{57} but the differences in accommodative lag between myopes and emmetropes disappear once myopia progression has ended.\textsuperscript{68, 69} Mutti and coworkers have found that an elevated response AC/A ratio in non-myopic children was associated with a greater risk of myopia development during the next year.\textsuperscript{55} Perhaps, accommodative microfluctuations are another feature of accommodation that is transiently affected by myopia progression. If relationship between accommodative microfluctuations and myopia onset were evaluated in a longitudinal study, it would be interesting to determine if larger accommodative
microfluctuations would be predictive for myopia development in a manner similar to the AC/A ratio.

Others have found that accommodative microfluctuations were increased in subjects with late-onset myopia who were assumedly still progressing.\textsuperscript{41, 42} Langaas and co-workers studied subjects with early onset myopia and although no difference in accommodative lag was found between emmetropic and myopic subjects, myopic subjects were found to have more variable accommodation.\textsuperscript{48} The subjects in the study by Langaas and co-workers were older than subjects in this study and their individual refractive error phenotype was likely already expressed. Because accommodative problems may be more likely during active myopia progression, studying accommodative abnormalities is challenging in a cross-sectional study where children may be at different, unknown stages of myopia development. Thus, accommodative microfluctuations need to be assessed in a longitudinal study to determine how much age, ciliary body thickness, and refractive error progression affect the microfluctuations.

Another source of variance between studies that should be addressed is the differences in methods for measuring accommodative microfluctuations. Harb and coworkers (2006) measured accommodative microfluctuations after prolonged periods of reading when the subject may have been fatigued.\textsuperscript{40} It may be important to differentiate anatomical and fatigue-related accommodative microfluctuations. The present study did not evaluate accommodative stability under fatigue.
Finally, there may be one other difference between this study and previous studies that might account for why the results of this study differed from previous studies. The microfluctuation measurements in this study were not normally distributed, which is an assumption for the statistical analyses that were used. Other studies in the literature do not report the use of normalized data in their analyses or even if a transformation of the data was necessary. This issue should also be considered in future studies.

The results of this study provide some insight into how microfluctuations of accommodation change with age. The mean power values for both the high-frequency component, low-frequency component and root mean square in the present study are smaller than those found by Candy in infants, but larger than those found by Day in young adults. The accommodative ability of children in this study was not yet adult-like, but more advanced than the infant state. Although it has been suggested that microfluctuations of accommodation may decrease as the neurological system matures, there may be an additional explanation. The results of the present study suggest that a thicker ciliary body provides a more stable accommodative response. Thus, some of the decrease in accommodative microfluctuations may be also due to the increase in the size of the ciliary body as children become older.

Accommodative microfluctuations represent an interesting component of accommodation which is known to be affected by the development of myopia. A longitudinal study is needed to determine if accommodative microfluctuations increase
before, during, or after myopia progression and the role of the ciliary body thickness in the process
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seidel 2003</td>
<td>Emmetropes, early-onset myopes, late-onset myopes &lt;br&gt;Mean age 21.4 +/- 3.9 years &lt;br&gt;Myopes had all progressed -0.50 over the past year</td>
<td>Badal system with modified Canon  &lt;br&gt;Ten seconds of accommodation to 4D Maltese Cross target</td>
<td>Late-onset myopes had greater RMS &lt;br&gt;Incease in low frequency component in late-onset myopes &lt;br&gt;High-frequency component similar between all groups</td>
</tr>
<tr>
<td>Seidel 2005</td>
<td>Emmetropes, early-onset myopes, late-onset myopes</td>
<td>Free-space viewing  &lt;br&gt;Ten seconds of accommodation to 4D Maltese Cross target</td>
<td>No significant differences between groups &lt;br&gt;No significant difference between monocular and binocular viewing</td>
</tr>
<tr>
<td>Harb 2006</td>
<td>Myopes and emmetropes &lt;br&gt;Age 22-28 &lt;br&gt;Lag not correlated with refractive error</td>
<td>Free-space viewing with PowerRef  &lt;br&gt;Read for 10 minutes at 1.5D, 2.5D, and 3.5D targets</td>
<td>Accommodative variability related to refractive error and stimulus level &lt;br&gt;Myopes more variable standards deviation, low-frequency component, and high-frequency component</td>
</tr>
</tbody>
</table>

Figure 1. Microfluctuations and refractive error in research.
<table>
<thead>
<tr>
<th>Study Year</th>
<th>Group Description</th>
<th>Experimental Method</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2006</td>
<td>Emmetropes, early-onset myopes, late-onset myopes</td>
<td>Badal system with modified Shin-Nippon</td>
<td>Emmetropes and early-onset myopes increased in low-frequency component with accommodation</td>
</tr>
<tr>
<td></td>
<td>Mean age 23.6 +/- 4.2 years</td>
<td>Accommodated at 0D, 1D, 2D, 3D, and 4D target for two minutes</td>
<td>Late-onset myopes had more variable accommodation at distance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High-frequency component larger in late-onset myopes</td>
</tr>
<tr>
<td>Langaas 2008</td>
<td>Emmetropes mean age 13.51 years, Myopes mean age 14.14 years, All subjects less than 15</td>
<td>Free-space viewing with PowerRefII</td>
<td>Myopes more variability measured as standard deviation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Accommodated at 0.25D, 2D, and 4D targets for two seconds</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 continued
Figure 2. Sample accommodative measurement.
Figure 3. Sample power spectrum.
Figure 4. Lens tension measurement.
Figure 5. Ciliary body thickness measurement.
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.43</td>
<td>2.23</td>
</tr>
<tr>
<td>Cycloplegic Refractive Error (D)</td>
<td>−1.00</td>
<td>2.19</td>
</tr>
<tr>
<td>Ciliary Body Thickness 2mm posterior to the scleral spur (CBT2) (µm)</td>
<td>604.06</td>
<td>104.28</td>
</tr>
<tr>
<td>Crystalline Lens Oscillations (degrees)</td>
<td>2.07</td>
<td>0.67</td>
</tr>
<tr>
<td>Saccadic Velocity (degrees/second)</td>
<td>0.37</td>
<td>0.06</td>
</tr>
<tr>
<td>Pupil Size (mm)</td>
<td>5.87</td>
<td>0.77</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>−3.80</td>
<td>1.96</td>
</tr>
<tr>
<td>Low-Frequency Component (D²/Hz)</td>
<td>0.0422</td>
<td>0.0608</td>
</tr>
<tr>
<td>High-Frequency Component (D²/Hz)</td>
<td>0.0049</td>
<td>0.0047</td>
</tr>
<tr>
<td>Root Mean Square (D)</td>
<td>0.49</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Figure 6. General characteristics of study sample.
Figure 7. Refractive error vs. accommodative lag.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>p-value</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>−5.76</td>
</tr>
<tr>
<td>Age (relative to 8 years)</td>
<td>0.004</td>
<td>−0.19</td>
</tr>
<tr>
<td>Gender (Female = 1)</td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>Saccadic velocity</td>
<td>0.93</td>
<td>0.29</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>0.27</td>
<td>−0.07</td>
</tr>
<tr>
<td>Pupil Size</td>
<td>0.44</td>
<td>−0.13</td>
</tr>
<tr>
<td>Ciliary body thickness</td>
<td>0.03</td>
<td>−0.003</td>
</tr>
<tr>
<td>Amplitude of lens oscillations</td>
<td>0.35</td>
<td>−0.30</td>
</tr>
</tbody>
</table>

Figure 8. Multiple linear regression model for the log of the high-frequency component accommodative microfluctuations as a function of ciliary body thickness and other independent variables in children.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>p-value</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>−3.85</td>
</tr>
<tr>
<td>Age (relative to 8 years)</td>
<td>0.03</td>
<td>−0.20</td>
</tr>
<tr>
<td>Gender (Female = 1)</td>
<td>0.81</td>
<td>0.09</td>
</tr>
<tr>
<td>Saccadic velocity</td>
<td>0.77</td>
<td>−1.35</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>0.96</td>
<td>−0.005</td>
</tr>
<tr>
<td>Pupil Size</td>
<td>0.16</td>
<td>−0.35</td>
</tr>
<tr>
<td>Ciliary body thickness</td>
<td>0.13</td>
<td>−0.003</td>
</tr>
<tr>
<td>Amplitude of lens oscillations</td>
<td>0.54</td>
<td>−0.28</td>
</tr>
</tbody>
</table>

Figure 9. Multiple linear regression model for the log of the low-frequency component accommodative microfluctuations as a function of ciliary body thickness and other independent variables in children.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>p-value</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>−0.79</td>
</tr>
<tr>
<td>Age (relative to 8 years)</td>
<td>0.008</td>
<td>−0.10</td>
</tr>
<tr>
<td>Gender (Female = 1)</td>
<td>0.96</td>
<td>−0.008</td>
</tr>
<tr>
<td>Saccadic velocity</td>
<td>0.95</td>
<td>−0.10</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>0.34</td>
<td>−0.04</td>
</tr>
<tr>
<td>Pupil Size</td>
<td>0.16</td>
<td>−0.14</td>
</tr>
<tr>
<td>Ciliary body thickness</td>
<td>0.20</td>
<td>−0.001</td>
</tr>
<tr>
<td>Amplitude of lens oscillations</td>
<td>0.33</td>
<td>−0.18</td>
</tr>
</tbody>
</table>

Figure 10. Multiple linear regression model for the log root mean square accommodative microfluctuations as a function of ciliary body thickness and other independent variables in children.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>p-value</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>−5.85</td>
</tr>
<tr>
<td>Age (relative to 8 years)</td>
<td>0.50</td>
<td>−0.04</td>
</tr>
<tr>
<td>Gender (Female = 1)</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>0.006</td>
<td>−0.18</td>
</tr>
<tr>
<td>Pupil Size</td>
<td>0.18</td>
<td>−0.20</td>
</tr>
<tr>
<td>Refractive Error</td>
<td>0.0005</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Figure 11. Multiple linear regression model for the log of the high-frequency component accommodative microfluctuations as a function of refractive error and control variables in children.
### Predictor | p-value | Parameter Estimate
--- | --- | ---
Intercept | | −3.88
Age (relative to 8 years) | 0.61 | −0.05
Gender (Female = 1) | 0.81 | 0.09
Mean accommodation-PowerRefractor (D) | 0.26 | −0.11
Pupil Size | 0.06 | −0.44
Refractive Error | 0.02 | 0.25

Figure 12. Multiple linear regression model for the log of the low-frequency component accommodative microfluctuations as a function of refractive error and control variables in children.
<table>
<thead>
<tr>
<th>Predictor</th>
<th>p-value</th>
<th>Parameter Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>−0.82</td>
</tr>
<tr>
<td>Age (relative to 8 years)</td>
<td>0.43</td>
<td>−0.03</td>
</tr>
<tr>
<td>Gender (Female = 1)</td>
<td>0.91</td>
<td>−0.01</td>
</tr>
<tr>
<td>Mean accommodation-PowerRefractor (D)</td>
<td>0.04</td>
<td>−0.08</td>
</tr>
<tr>
<td>Pupil Size</td>
<td>0.06</td>
<td>−0.17</td>
</tr>
<tr>
<td>Refractive Error</td>
<td>0.008</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Figure 13. Multiple linear regression model for the log root mean square accommodative microfluctuations as a function of refractive error and control variables in children.
LIST OF REFERENCES


APPENDIX

Matlab program used for analyses of accommodative microfluctuations
function [editdata, block1, R, block2] = prog(data)

data1 = cat(2, data(:, 1), data(:, 2));
data2 = cat(2, data(:, 3), data(:, 4));
data3 = cat(2, data(:, 5), data(:, 6));
data4 = cat(2, data(:, 7), data(:, 8));
data5 = cat(2, data(:, 9), data(:, 10));

% DATA1
%%% get rid of NAN
place = isnan(data1(:, 2));
c = 0;
while place(1) == 1
    data1(1, :) = [];
    c = c + 1;
    place = isnan(data1(:, 2));
end
if c > 0
    data1(:, 1) = data1(:, 1) - (0.04 * c);
end
N = length(data1(:, 2));
while place(N) == 1
    data1(N, :) = [];
    N = length(data1(:, 2));
    place = isnan(data1(:, 2));
end

tempdata(:, 1) = data1(:, 1);
tempdata(:, 2) = data1(:, 2);
tempdata(any(isnan(tempdata), 2), :) = [];

%%% descriptives
mu = mean(tempdata(:, 2));
sigma = std(tempdata(:, 2));
n = length(tempdata);
%%% get rid of blinks
%%% calculates difference between points
k = 1;
while k < n
    difference(k, 1) = tempdata(k + 1, 1) - tempdata(k, 1);
    difference(k, 2) = tempdata(k + 1, 2) - tempdata(k, 2);
    change(k, 1) = [difference(k, 2) / difference(k, 1)];
    if abs(change(k, 1)) > 10
        minusmean(k, 1) = tempdata(k, 2) - mu;
        minusmean(k + 1, 1) = tempdata(k + 1, 2) - mu;
        k = k + 1;
    end
end
if abs(minusmean(k,1)) > abs(minusmean(k+1,1))
    tempdata(k,:)=[];
    k=k-1;
else
    tempdata(k+1,:)=[];
    k=k-1;
end
end

k=k+1;
n=length(tempdata);
end

N=length(data1);
m=1;

while m<=N
    if j>n
        correct(m,1)=[1];
        m=m+1;
    elseif data1(m,1)==tempdata(j,1)
        correct(m,1)=[0];
        m=m+1;
        j=j+1;
    elseif data1(m,1)~=tempdata(j,1)
        correct(m,1)=[1];
        m=m+1;
    end
end

while correct(1)==1
    data1(1,:)=[];
    data1(:,1)=data1(:,1)-.04;
    correct(1)=[];
end

N=length(data1);
v=1;
stop=0;
while stop==0
    if correct(v,1)==1
        a=v+1;
        if a==N
            data1(v,:)=[];
            stop=1;
        elseif correct(a,1)==1
            a=a+1;
        end
    end
end
while correct(a,1)==1
  if a==N
    data1 (v:1:a,:)=[];
    stop=1;
    else
      a=a+1;
    end
  end
  data1(v,2)=data1(v-1,2)+((data1(a,2)-data1(v-1,2))/(a-(v-1)));
  v=v+1;
  else data1(v,2)=data1(v-1,2)+((data1(a,2)-data1(v-1,2))/(a-(v-1)));
  v=v+1;
  end
  else
    v=v+1;
  end
  if v==N
    stop=1;
  end
end

if data1(1,1)~=0.4
  v=data1(1,1)-0.4;
  data1(:,1)=data1(:,1)-v;
end

figure, plot (data1(:,1), data1(:,2))

%%%descriptives
mu1=mean(data1(:,2));
sigma1=std(data1(:,2));
n1=length(data1);
diff1=data1(:,2)-mu1;
rms1=norm(diff1)/sqrt(n1);

%clear
place=[];
N=[];
tempdata=[];
mu=[];
sigma=[];
n=[];
difference=[];
%DATA2
%%%get rid of NAN
place=isnan(data2(:,2));
c=0;
while place(1)==1
    data2(1,:)=[];
    c=c+1;
    place=isnan(data2(:,2));
end
if c>0
    data2(:,1)=data2(:,1)-(.04*c);
end
N=length(data2(:,2));
while place(N)==1
    data2 (N,:)=[];
    N=length(data2(:,2));
    place=isnan(data2(:,2));
end

tempdata(:,1)=data2(:,1);
tempdata(:,2)=data2(:,2);
tempdata(any(isnan(tempdata),2),:) = [];

%%%descriptives
mu=mean(tempdata(:,2));
sigma=std(tempdata(:,2));
n=length(tempdata);
%%%get rid of blinks
%%%calculates difference between points
k=1;
while k<n
    difference(k,1)=tempdata(k+1,1)-tempdata(k,1);
    difference(k,2)=tempdata(k+1,2)-tempdata(k,2);
    change(k,1)=[difference(k,2)/difference(k,1)];
    if abs(change(k,1))>10
        minusmean(k,1)=tempdata(k,2)-mu;
        minusmean(k+1,1)=tempdata(k+1,2)-mu;
        if abs(minusmean(k,1))> abs(minusmean(k+1,1))
            tempdata(k,:)=[];
            k=k-1;
    end
end
else
    tempdata(k+1,:)=[];
    k=k-1;
end
end

k=k+1;
n=length(tempdata);
end

N=length(data2);
m=1;
j=1;
while m<=N
    if j>n
        correct(m,1)=[1];
        m=m+1;
    elseif data2(m,1)==tempdata(j,1)
        correct(m,1)=[0];
        m=m+1;
        j=j+1;
    elseif data2(m,1)~=tempdata(j,1)
        correct(m,1)=[1];
        m=m+1;
    end
end

while correct(1)==1
    data2(1,:)=[];
data2(:,1)=data2(:,1)-.04;
correct(1)=[1];
end

N=length(data2);
v=1;
stop=0;
while stop==0
    if correct (v,1)==1
        a=v+1;
        if a==N
            data2(v,:)=[];
            stop=1;
        elseif correct(a,1)==1
            while correct(a,1)==1
                if a==N
                    data2 (v:1:a,:)=[];
                end
            end
        end
    end
end
stop=1;
else
a=a+1;
end
end
data2(v,2)=data2(v-1,2)+((data2(a,2)-data2(v-1,2))/(a-(v-1)));
v=v+1;
else data2(v,2)=data2(v-1,2)+((data2(a,2)-data2(v-1,2))/(a-(v-1)));
v=v+1;
end
else
v=v+1;
end
if v==N
  stop=1;
end
end
if data2(1,1)~=0.4
  v=data2(1,1)-0.4;
  data2(:,1)=data2(:,1)-v;
end
figure, plot (data2(:,1), data2(:,2))

%%% descriptives
mu2=mean(data2(:,2));
sigma2=std(data2(:,2));
n2=length(data2);
diff2=data2(:,2)-mu2;
rms2=norm(diff2)/sqrt(n2);

%clear
place=[];
N=[];
tempdata=[];
mu=[];
sigma=[];
n=[];
difference=[];
change=[];
minusmean=[];
correct=[];
a=[];

%DATA3
%%%get rid of NAN
place=isnan(data3(:,2));
c=0;
while place(1)==1
    data3(1,:)=[];
    c=c+1;
    place=isnan(data3(:,2));
end
if c>0
    data3(:,1)=data3(:,1)-(0.04*c);
end
N=length(data3(:,2));
while place(N)==1
    data3 (N,:)=[];
    N=length(data3(:,2));
    place=isnan(data3(:,2));
end

tempdata(:,1)=data3(:,1);
tempdata(:,2)=data3(:,2);
tempdata(any(isnan(tempdata),2),:)=[];

%%%descriptives
mu=mean(tempdata(:,2));
sigma=std(tempdata(:,2));
n=length(tempdata);
%%%get rid of blinks
%%%calculates difference between points
k=1;
while k<n
    difference(k,1)=tempdata(k+1,1)-tempdata(k,1);
    difference(k,2)=tempdata(k+1,2)-tempdata(k,2);
    change(k,1)=[difference(k,2)/difference(k,1)];
    if abs(change(k,1))>10
        minusmean(k,1)=tempdata(k,2)-mu;
        minusmean(k+1,1)=tempdata(k+1,2)-mu;
        if abs(minusmean(k,1))>abs(minusmean(k+1,1))
            tempdata(k,:)=[];
            k=k-1;
        else
            tempdata(k+1,:)=[];
            k=k-1;
        end
    end
end
k=k+1;
n=length(tempdata);
end

N=length(data3);
m=1;
j=1;
while m<=N
    if j>n
        correct(m,1)=[1];
m=m+1;
    elseif data3(m,1)==tempdata(j,1)
        correct(m,1)=[0];
m=m+1;
j=j+1;
    elseif data3(m,1)~=tempdata(j,1)
        correct(m,1)=[1];
m=m+1;
    end
end

while correct(1)==1
    data3(1,:)=[];
data3(:,1)=data3(:,1)-.04;
correct(1)=[];
end
N=length(data3);
v=1;
stop=0;
while stop==0
    if correct (v,1)==1
        a=v+1;
        if a==N
            data3(v,:)=[];
            stop=1;
        elseif correct(a,1)==1
            while correct(a,1)==1
                if a==N
                    data3 (v:1:a,:)=[];
                    stop=1;
                else
                    a=a+1;
                end
            end
        end
    end
end
data3(v,2)=data3(v-1,2)+((data3(a,2)-data3(v-1,2))/(a-(v-1))); v=v+1;
else data3(v,2)=data3(v-1,2)+((data3(a,2)-data3(v-1,2))/(a-(v-1))); v=v+1;
end
if v==N stop=1;
end

if data3(1,1)~=0.4
  v=data3(1,1)-0.4;
  data3(:,1)=data3(:,1)-v;
end

figure, plot (data3(:,1), data3(:,2))

%%%descriptives
mu3=mean(data3(:,2));
sigma3=std(data3(:,2));
n3=length(data3);
diff3=data3(:,2)-mu3;
rms3=norm(diff3)/sqrt(n3);

%clear
place=[];
N=[];
tempdata=[];
mu=[];
sigma=[];
n=[];
difference=[];
change=[];
minusmean=[];
correct=[];
a=[];

%DATA4
%%%get rid of NAN
place=isnan(data4(:,2));
c=0;
while place(1)==1
    data4(1,:)=[];
    c=c+1;
    place=isnan(data4(:,2));
end
if c>0
    data4(:,1)=data4(:,1)-(0.04*c);
end
N=length(data4(:,2));
while place(N)==1
    data4(N,:)=[];
    N=length(data4(:,2));
    place=isnan(data4(:,2));
end

tempdata(:,1)=data4(:,1);
tempdata(:,2)=data4(:,2);
tempdata(any(isnan(tempdata),2),:) = [];

%%%descriptives
mu=mean(tempdata(:,2));
sigma=std(tempdata(:,2));
n=length(tempdata);
%%%get rid of blinks
%%%calculates difference between points
k=1;
while k<n
    difference(k,1)=tempdata(k+1,1)-tempdata(k,1);
    difference(k,2)=tempdata(k+1,2)-tempdata(k,2);
    change(k,1)=[difference(k,2)/difference(k,1)];
    if abs(change(k,1))>10
        minusmean(k,1)=tempdata(k,2)-mu;
        minusmean(k+1,1)=tempdata(k+1,2)-mu;
        if abs(minusmean(k,1))> abs(minusmean(k+1,1))
            tempdata(k,:)=[];
            k=k-1;
        else
            tempdata(k+1,:)=[];
            k=k-1;
        end
    end
end
k=k+1;
n=length(tempdata);
end

N=length(data4);
m=1;
j=1;
while m<=N
   if j>n
      correct(m,1)=1;
m=m+1;
   elseif data4(m,1)==tempdata(j,1)
      correct(m,1)=0;
m=m+1;
j=j+1;
   elseif data4(m,1)~=tempdata(j,1)
      correct(m,1)=1;
m=m+1;
   end
end

while correct(1)==1
   data4(1,:)=[];
data4(:,1)=data4(:,1)-.04;
correct(1)=[];
end
N=length(data4);
v=1;
stop=0;
while stop==0
   if correct(v,1)==1
      a=v+1;
      if a==N
         data4(v,:)=[];
         stop=1;
      elseif correct(a,1)==1
         while correct(a,1)==1
            if a==N
               data4(v:1:a,:)=[];
               stop=1;
            else
               a=a+1;
            end
         end
      end
   end
   data4(v,2)=data4(v-1,2)+((data4(a,2)-data4(v-1,2))/(a-(v-1)));
v=v+1;
else data4(v,2)=data4(v-1,2)+((data4(a,2)-data4(v-1,2))/(a-(v-1)));
v=v+1;
end
else
v=v+1;
end

if v==N
stop=1;
end

end

if data4(1,1)~=0.4
v=data4(1,1)-0.4;
data4(:,1)=data4(:,1)-v;
end

figure, plot (data4(:,1), data4(:,2))

%%% descriptive
mu4=mean(data4(:,2));
sigma4=std(data4(:,2));
n4=length(data4);
diff4=data4(:,2)-mu4;
rms4=norm(diff4)/sqrt(n4);

% clear
place=[];
N=[];
tempdata=[];
mu=[];
sigma=[];
n=[];
difference=[];
change=[];
minusmean=[];
correct=[];
a=[];

% DATA5
%%% get rid of NaN
place=isnan(data5(:,2));
c=0;
while place(1)==1
    data5(1,:)=[ ];
    c=c+1;
    place=isnan(data5(:,2));
end
if c>0
    data5(:,1)=data5(:,1)-(0.04*c);
end
N=length(data5(:,2));
while place(N)==1
    data5(N,:)=[];
    N=length(data5(:,2));
    place=isnan(data5(:,2));
end

tempdata(:,1)=data5(:,1);
tempdata(:,2)=data5(:,2);
tempdata(any(isnan(tempdata),2),:)=[];

%%%descriptives
mu=mean(tempdata(:,2));
sigma=std(tempdata(:,2));
n=length(tempdata);
%%%get rid of blinks
%%%calculates difference between points
k=1;
while k<n
    difference(k,1)=tempdata(k+1,1)-tempdata(k,1);
    difference(k,2)=tempdata(k+1,2)-tempdata(k,2);
    change(k,1)=[difference(k,2)/difference(k,1)];
    if abs(change(k,1))>10
        minusmean(k,1)=tempdata(k,2)-mu;
        minusmean(k+1,1)=tempdata(k+1,2)-mu;
        if abs(minusmean(k,1))> abs(minusmean(k+1,1))
            tempdata(k,:)=[];
            k=k-1;
        else
            tempdata(k+1,:)=[];
            k=k-1;
        end
    end
    k=k+1;
end
n=length(tempdata);
N=length(data5);
m=1;
j=1;
while m<=N
    if j>n
        correct(m,1)=[1];
m=m+1;
    elseif data5(m,1)==tempdata(j,1)
        correct(m,1)=0;
m=m+1;
j=j+1;
    elseif data5(m,1)~=tempdata(j,1)
        correct(m,1)=1;
m=m+1;
    end
end

while correct(1)==1
    data5(1,:)=[];
data5(:,1)=data5(:,1)-.04;
correct(1)=[];
end
N=length(data5);
v=1;
stop=0;
while stop==0
    if correct(v,1)==1
        a=v+1;
        if a==N
            data5(v,:)=[];
            stop=1;
        elseif correct(a,1)==1
            while correct(a,1)==1
                if a==N
                    data5(v:1:a,:)=[];
                    stop=1;
                else
                    a=a+1;
                end
            end
            data5(v,2)=data5(v-1,2)+((data5(a,2)-data5(v-1,2))/(a-(v-1)));
v=v+1;
        else
            data5(v,2)=data5(v-1,2)+((data5(a,2)-data5(v-1,2))/(a-(v-1)));
v=v+1;
        end
    else
        data5(v,2)=data5(v-1,2)+((data5(a,2)-data5(v-1,2))/(a-(v-1)));
v=v+1;
    end
end
end
else
    v=v+1;
end

if v==N
    stop=1;
end

if data5(1,1) ~= 0.4
    v=data5(1,1)-0.4;
data5(:,1)=data5(:,1)-v;
end

figure, plot (data5(:,1), data5(:,2))

%%%descriptives
mu5=mean(data5(:,2));
sigma5=std(data5(:,2));
n5=length(data5);%clear
diff5=data5(:,2)-mu5;
rms5=norm(diff5)/sqrt(n5);

%FFT
x=n1;
if n2<x
    x=n2;
end
if n3<x
  x=n3;
end
if n4<x
  x=n4;
end
if n5<x
  x=n5;
end

R1=fft(data1(1:x,2));
R1(1)=[];

R2=fft(data2(1:x,2));
R2(1)=[];

R3=fft(data3(1:x,2));
R3(1)=[];

R4=fft(data4(1:x,2));
R4(1)=[];

R5=fft(data5(1:x,2));
R5(1)=[];

r=R1+R2+R3+R4+R5;
R=r/5;
nyquist=12.5;
N=length (R);
l=round(N/2);
P=((abs(R(1:l))/N).^2)*2;
F=(1:l)/l*nyquist;
figure, plot (F,P)
axis([0,3,0,.012])

F=F';
b=1;
while F(b)<.6
  b=b+1;
end

LFC=trapz(P(1:b));
c=b;
while F(c)<.1
c=c+1;
end
MFC=trapz(P(b:c));
k=c;
while F(k)<2.3
   k=k+1;
end
HFC=trapz(P(c:k));

%OVERALL
editdata=[data1(1:x,:),data2(1:x,:),data3(1:x,:),data4(1:x,:),data5(1:x,:)];
mu=(mu1+mu2+mu3+mu4+mu5)/5;
sigma=(sigma1+sigma2+sigma3+sigma4+sigma5)/5;
rms=(rms1+rms2+rms3+rms4+rms5)/5;
block1=[F, P, ];
block2=[F(1), mu, sigma, rms, LFC, MFC, HFC];
end