

Chapter 3: Longitudinal changes of optical aberrations in normal and form-deprived myopic chick eyes

Resumen capítulo 3:

Cambios longitudinales de las aberraciones ópticas in ojos de pollo miopes y emétopes.

Realizamos medidas de refracción (con retinoscopía), longitud axial (con biometría por ultrasonidos) y aberraciones oculares (con un aberrómetro Hartmann-Shack de construcción propia) en 7 pollos “White-Leghorn” despiertos tratados con oclusión monolateral por difusores durante dos semanas. El tratamiento comenzó el primer día después del nacimiento y las medidas se realizaron en varios días hasta el día 13 de vida. Los ojos no ocluidos experimentaron un proceso normal de emetropización, es decir, los valores de hipermetropía disminuyeron a un ritmo de 0.2 ± 0.09 D/día y la longitud axial aumentó 0.05 ± 0.03 mm/día, mientras los ojos ocluidos desarrollaron miopía axial (1.50 ± 0.2 D/día y 0.12 ± 0.02 mm/día). Las diferencias entre ojos de los promedios de refracción y longitud axial para el día 13 fueron 17.43 D y 0.86 mm respectivamente. Las aberraciones de alto orden monocromáticas disminuyeron con la edad en ambos ojos. El promedio de la RMS (Raíz cuadrática media) para pupilas de 1.5 mm de diámetro disminuyó de 0.11 ± 0.03 micras en el día 0 hasta 0.06 ± 0.03 micras en el día 13 en los ojos ocluidos y de 0.12 ± 0.05 micras hasta 0.03 ± 0.01 micras en ojos sin ocluir. La calidad óptica en términos de MTF (Función de transferencia de modulación) también muestra una mejora con la edad. A partir del día 8 los ojos miopes tienden a mostrar valores significativamente mayores de aberraciones oculares y, por tanto, peor calidad óptica con corrección óptimo de desenfoque que los ojos emétopes. La degradación impuesta por las aberraciones es pequeña comparada con la impuesta por el desenfoque y el difusor. Esos resultados sugieren un mecanismo de disminución de las aberraciones durante el desarrollo que no está guiado por estímulos visuales. Los ojos miopes presentan una calidad óptica peor que los ojos control, sugiriendo que los cambios geométricos debidos al excesivo alargamiento del globo ocular también afectan a la calidad óptica de las distintas estructuras oculares.

This chapter is based on the article by García de la Cera et al. “Longitudinal changes of optical aberrations in normal and form-deprived myopic chick eyes”, *Vision Research* (2006) 46, 579-589.

The contribution of Elena García de la Cera to the study was to develop the methodology to measure ocular aberrations, to perform all calibrations, and data processing routines, the performance of the experimental measurements on chicks (ocular aberrations, retinoscopy and ultrasound biometry) and data analysis and interpretation.

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3.1. Abstract

We performed measurements of refraction (with retinoscopy), axial length (with ultrasound biometry) and ocular aberrations (with a custom-built Hartmann-Shack aberrometer) on seven awake White-Leghorn chicks occluded monolaterally with diffusers for two weeks. Treatment started on the first day after hatching (day 0) and measurements were conducted on several days between day 0 and 13. Non-occluded eyes experienced normal emmetropization (decreasing hyperopia at 0.2 ± 0.09 D/day and increasing axial length at 0.05 ± 0.03 mm/day), while occluded eyes developed axial myopia (1.50 ± 0.2 D/day and 0.12 ± 0.02 mm/day). Interocular differences in refraction and axial length by day 13 were on average 17.43 D and 0.86 mm, respectively. Monochromatic high order aberrations decreased with age in both eyes. Average RMS (for 1.5 mm pupil diameter) decreased from 0.11 ± 0.03 at day-0 to 0.06 ± 0.03 microns (day-13) in occluded eyes, and from 0.12 ± 0.05 to 0.03 ± 0.01 microns in non-occluded eyes. MTF-based optical quality metrics also show an improvement with age. However, while this improvement occurs in both eyes, after day 8 myopic eyes tend to show significantly higher amounts of aberrations (and consequently worse best-corrected optical quality) than normal eyes. The degradation imposed by aberrations is small compared to that imposed by defocus and the diffuser. These results suggest a decrease of aberrations during development which does not seem to be visually guided. Myopic eyes showed slightly worse optical quality than normal eyes, suggesting that the geometrical changes resulting from excessive ocular axial growth also affect the optical quality of the ocular components.

3.2. Introduction

There is compelling evidence, mostly from animal models, that the absence of a normal visual experience in the early stages of development compromises emmetropization, i.e. the normal ocular growth aiming at matching axial length of the eye to its optical power and achieving focused images on the retina (Wallman 1993; Wildsoet 1997; Smith 1998). It is well established that visual form deprivation, as well as other ways of altering the visual environment, produces axial elongation and myopia in a variety of species. The chick has been an extensively used animal model, myopia development has been achieved with lid closure (Yinon 1984), deprivation of form vision by placing opaque or translucent goggles in front of the eye (Hayes et al. 1986; Wallman and Adams 1987; Troilo and Wallman 1991), or restricting the contrast and spatial frequencies of the visual environment (Schmid and Wildsoet 1997). With the previous methods the eye growth control system runs open-loop with no possible feedback. Myopia has also been achieved by placing negative lenses in front of the animal's eye. In this case, the eye adjusts its growth to compensate for the imposed defocus (Schaeffel et al. 1988; Kee et al. 2001). It has also been observed that when normal vision is restored, even for short periods of time, the myopia tends to regress (Troilo and Wallman 1991). While many studies have been performed on chicks, the impact of visual experience on normal eye growth has also been demonstrated in primates (Weisel and Raviola 1977; Troilo et al. 2000). Also, pathology-related form deprivation in human infants (by eyelid closure, congenital cataracts or corneal opacities) has been associated to the development of myopia.

The investigation of possible relationships between optical aberrations and myopia seems suggestive, in particular since the causes of myopia are not well understood. Several studies have investigated potential correlation between high order aberrations and myopia (Liang et al. 1994; He et al. 1998; Moreno-Barriuso et al. 2001; Atchison et al. 1995; Paquin et al. 2002). However although some of these studies show a co-variability, a cause-effect relationship

cannot be inferred. Some results suggest that the constant degradation of the image quality produced by increased aberrations could disrupt the emmetropization process (Buehren et al. 2003). Also, results from clinical trials have shown that rigid contact lenses reduced the progression of myopia in children and adolescent subjects, compared to controls wearing soft contact lenses or spectacles (Perrigin et al. 1990; Khoo et al. 1999). Interestingly, aberration measurements on rigid gas permeable (RGP) contact lens wearers with and without the contact lens on have shown the capability of RGP contact lenses to correct for significant amounts of high order aberrations (Dorransoro et al. 2003). While those results are suggestive, there is no definite proof that aberrations could be a cause of myopia nor that cancelling aberrations could be a potential way of reducing excessive ocular growth. On the other hand, it has been argued that the presence of aberrations may provide cues to determine the sign of defocus, since interactions between high order aberrations and defocus (and as a consequence retinal image quality) change with the sign of defocus, and that these effects may be important in the emmetropization process (Wilson et al. 2002). Alternatively, the ocular enlargement of myopic eyes (and therefore different geometrical properties of the ocular components) could be the reason for the increased amount of aberrations found in myopic eyes. The question is whether the increased optical aberrations in myopic eyes is a cause or a consequence of myopia.

Unlike studies in animal models, to test cause-effect relationships in humans is complicated, due to the time cost of longitudinal studies and impossibility of intervening the ocular optical properties in infants. While chicks have been widely used as animal models of myopia, their optical quality has not been studied experimentally in much detail. In most studies, modelling and conclusions assume diffraction-limited optics. Coletta and co-workers (Coletta et al. 2003) reported optical quality (in terms of modulation transfer function) of normal and myopic chick eyes using a double-pass method. To our knowledge, two studies have attempted to measure monochromatic aberrations in younger chicks using Hartmann-Shack aberrometers (Liang et al. 1994; Liang and Williams 1997). The study from the University of Waterloo was published

(Kisilak et al. 2006) several months after our own study (García de la Cera et al. 2006).

In this chapter we present longitudinal measurements of refraction, axial length and monochromatic aberrations in occluded eyes and normal chick eyes during the first two weeks of development. The aims of the study presented in this chapter are to investigate:

- 1) longitudinal changes of aberrations during normal emmetropization;
- 2) the effect of myopia development on ocular aberrations;
- 3) possible effects of natural aberrations on myopia development;
- 4) the differences in optical quality in myopic and emmetropic eyes;
- 5) longitudinal changes of aberrations in myopic eyes.

Unlike myopia caused by lens treatment (where the lens+elongated eye tends to form an optically good system), form deprived eyes are subject to the continuous degradation produced by the diffuser. If we find that this treatment resulting in myopia also produces increased amounts of high order aberrations, we will favor the hypothesis that aberrations are a consequence, rather than a cause of myopia. In such a model, the enlargement of the eye (and subsequent modification of structural properties of the ocular components) would be the reason for the larger aberrations found in myopic eyes.

3.3. Methods

3.3.1. Subjects and experimental protocols.

Ten White-Leghorn chicks were used in this experiment. All experimental protocols were approved by the Institutional Review Boards and followed the tenets of Helsinki. Seven chicks were monocularly treated and measured periodically. Another three, two untreated and one treated, were measured only on the last day, as control subjects (to discard possible interferences from the repeated measurements). All chicks were labelled with color wires attached around their feet. Chicks were reared under fluorescent lighting (12h/12h

light/dark cycle conditions) in a cage inside a controlled heated room (24-28 °C). They were allowed to eat and drink ad libitum. Adequate measures were taken to minimize pain or discomfort.

The seven non-control chicks were initially measured in their first day after hatching. This day was named “day 0”. Days of age are therefore estimated adding one day to the measurement day. Immediately after the measurements, the right eye of each chick was occluded, and the non-occluded eye (left eye) was used as a reference. Occluders consisted of translucent diffusers which were manufactured with a sheet of plastic, moulded to obtain hemispherical translucent goggles (Frank Schaeffel, Personal communication). The occluders were attached with velcro rings glued to the feathers around the eye. They were only removed during measurements on days 0, 1, 4, 6, 8, 11 & 13. On these days we obtained measurements of refractive error, axial length and monochromatic aberrations in both eyes. An experimental session, including the three types of measurements, lasted typically five minutes per eye. All measurements were performed with the animals awake and under natural viewing conditions.

3.3.2. *Refraction and ultrasound biometry*

Refraction was measured using streak retinoscopy with trial lenses in the horizontal meridian. Chicks were awake and unanaesthetized. We did not use cycloplegia nor lid-retractors.

An adapted ultrasound biometer (Allergan Humphrey Mod. 826) was used for axial length measurements. The probe was adapted to the chick eye's dimensions using a 10-mm tube filled in with water and covered with paraffin film (Schaeffel and Howland 1991), as described in section 2.2.2. Measurements were conducted under topical anaesthesia, a drop of lidocaine 1%. Five data were obtained per condition.

3.3.3. Shack-Hartmann aberrometry

Aberrations were measured with a custom-built compact Hartmann-Shack (HS) wavefront sensor, which we built specifically to measure ocular aberrations in animal models (described in Section 2.1.1. of this thesis). The entire system is mounted on an x-y translational stage. The chick sits on an elevating platform mounted in front of the system, which was moved to ensure correct centration and focusing of animal's pupil. The animal usually stayed quiet during the measurement, allowing us to capture several images per eye (See Figure 3.1A). The number of spots captured per image was related to the pupil size. We estimated pupil diameter as the distance between the two most separated spots in a HS image. We found that pupil increased with age, from (treated/untreated eyes) on day 0 to 2.3/2.9 mm on day 13, on average. Figure 3.1 (B,C & D) shows three typical examples of HS images from chick eyes.

Zernike coefficients were obtained by modal fitting of the lateral deviations to derivatives Zernike polynomial expansion up to the 5th order. We obtained a maximum of 20 images per condition and selected the best five. Presence of artifact reflections, limited number of spots or low intensity were used as rejection criteria. Data were processed for the maximum pupil diameter

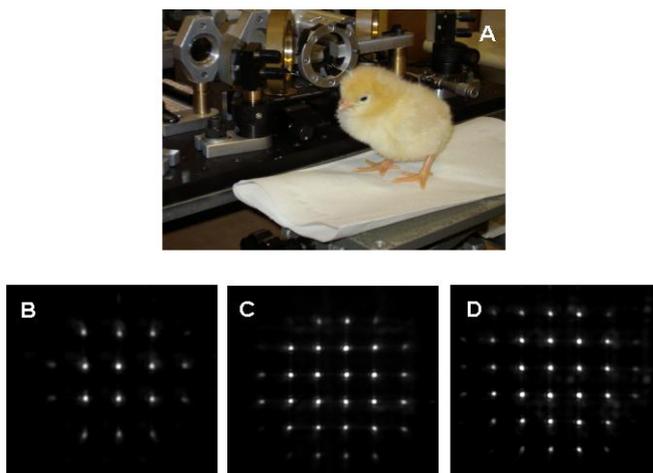


Figure 3.1 (A) Chick during an experiment session, photographed in front of the Hartmann Shack system. (B-D) Examples of Hartmann-Shack images on chick eyes (Chick #5): (A) day 0: before occlusion, (B) day 13: treated eye, and (C) day 13: untreated eye.

(ranging from 1.5 to 3.26 mm). However, for comparative purposes across eyes and days, the minimum pupil diameter of 1.5 mm was used. The optical quality of the eye was assessed in terms of individual Zernike terms or orders and root-mean-square wavefront error (RMS). Modulation Transfer Functions (MTF) and Point Spread Functions (PSFs) were also obtained from the wave aberrations. Strehl ratio was also used as an optical quality metric.

3.3.4. Statistical analysis

We used univariate ANOVA to test the changes with time and global differences between treated and non-treated eyes. Unpaired t-test was used to test differences between treated and untreated eyes on individual days.

3.4. Results

3.4.1. Ultrasound biometry and refraction

Figure 3.2 (A) shows axial length as a function of age in both eyes of the monolaterally treated chicks. Data from all chicks are shown, with squares representing non occluded eyes and the circles representing the corresponding occluded contralateral eye. While both eyes elongate during the first weeks of life ($p < 0.0001$; univariate ANOVA), the occluded eyes grow at a faster rate, and are significantly longer than the non occluded eyes ($p < 0.0001$; univariate ANOVA). The mean growth rate is 0.05 mm/day in non-occluded eyes and 0.12 mm/day in occluded eyes. Axial length increased from 7.2 ± 0.4 mm in non-occluded eyes and 7.1 ± 0.1 mm in occluded-eyes on average on day 0 (prior to treatment) to 7.9 ± 0.2 mm in non-occluded eyes and 8.8 ± 0.3 mm in occluded eyes on day 13. Control measurements on eyes that were left untreated or monolaterally occluded, but only measured on day 13 (to ensure that measurements did not interfere with normal emmetropization or the treatment) revealed similar results: 0.2 ± 0.3 mm interocular axial length difference in two chicks without any treatment, whereas 1.5 ± 0.1 mm interocular axial length

difference between the occluded and non occluded eyes of a non occluded chick.

Figure 3.2 (B) shows refraction as a function of age in both eyes of the monolaterally occluded chick eyes. Each color represents a chick (squares are non occluded eyes and circles are occluded eyes). According to refraction, all eyes were hyperopic on day 0 prior to treatment (OD: $+4.5 \pm 1.2$ D; OS: $+4.1 \pm 1.6$ D) but differences between eyes are statistically significant ($p < 0.0001$; univariate ANOVA) from day 1 ($p = 0.01$; unpaired t-test). Refraction tends gradually toward less hyperopic (non occluded eyes) or more myopic values (occluded eyes). Refraction changes at a rate of 0.21 D/day in the non occluded eye and 1.53 D/day in the occluded eye. By day 13, the non occluded eyes

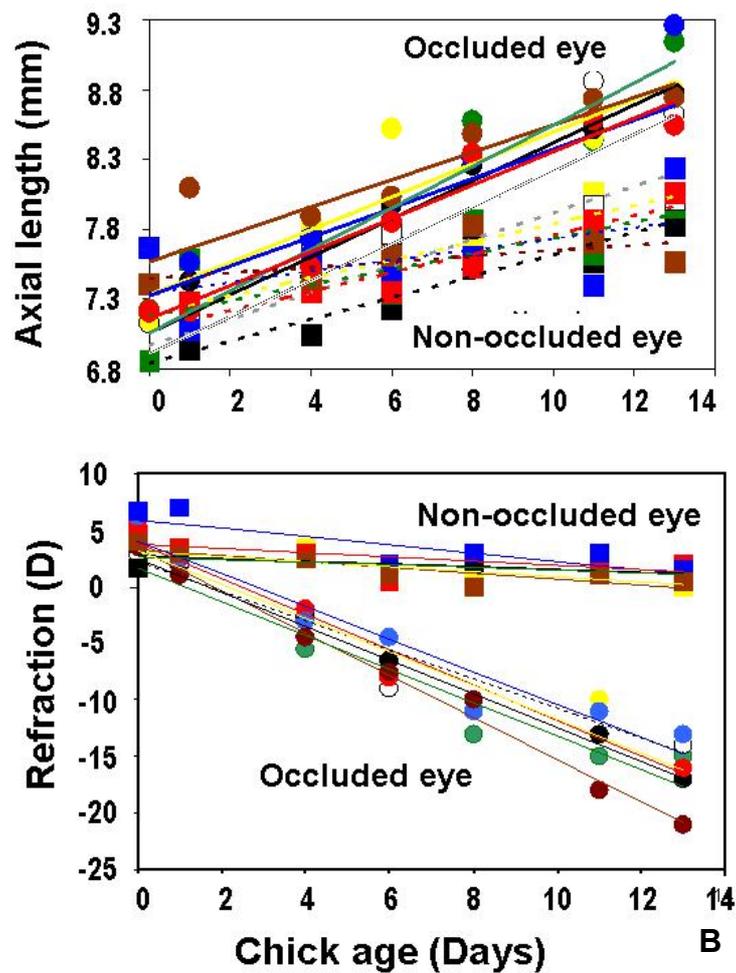


Figure 3.2 (A) Axial length as a function of age. (B) Refraction as a function of age. Each colour corresponds to a different chick. Squares symbols and dotted lines correspond to non-occluded eyes and circle symbols and solid lines correspond to occluded eyes.

show an average refraction of $+0.9 \pm 0.7$ D while occluded eyes show an average refraction of -16 ± 3 D. As we found for axial length, the non occluded chicks show the same trends in refraction as the chicks that were measured repeatedly throughout the study: Untreated non occluded chicks showed 0.5 D and 1.50 D difference between eyes respectively, while monilaterally occluded control chicks showed an interocular difference of 17 D.

3.4.2. Optical aberrations.

Figure 3.3 shows wave aberration patterns for days 0, 8 and 13 on chicks # 1 and # 7 corresponding to the eyes labelled in yellow and green respectively in Figure 3.2. Data are for 3rd and higher order aberrations and 1.5-mm pupil diameters. In both occluded and non occluded eyes, aberrations decrease with age and non-occluded eyes show lower amounts of aberrations than the occluded eyes. These trends are common in all eyes. Figure 3.4 shows longitudinal mean changes of 3rd and higher order RMS (A), 3rd order RMS only

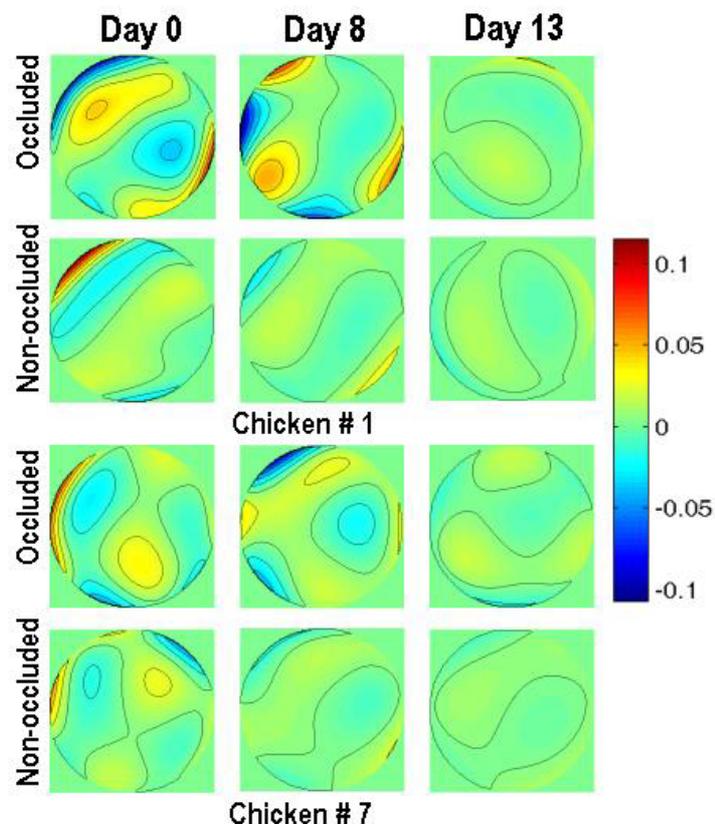


Figure 3.3 Wave aberration patterns for chick # 1 (represented in yellow in Figure 2) and # 7 (green) on days 0, 8 and 13. Data are for 3rd and higher order aberrations and 1.5mm.

and spherical aberration (B & C). For comparison, all RMSs have been computed for the same pupil diameter (1.5 mm). RMS decreases gradually and significantly with age ($p < 0.0001$; univariate ANOVA), and this happens in both non-occluded and occluded eyes, with differences being statistically significant between both groups ($p = 0.01$). Prior to occlusion (day 0), RMS is similar in both eyes ($p = 0.8$) but RMS is significantly higher in the occluded eyes on days 8 ($p = 0.005$; unpaired t-test), 11 ($p = 0.001$) and 13 ($p = 0.03$). From days 8 to 13, both eyes follow an approximately parallel decrease in RMS, with occluded eyes showing higher RMS values in all cases. We found larger intersubject variability in younger (days 0-4) than older chicks (days 6-13) with 0.05 microns vs. 0.02 microns average standard deviations across individuals, respectively. Measurements are also noisier in younger than older chicks: 0.2 and 0.08-microns standard deviations respectively for repeated measurements. Third and higher order RMS decreased from $0.12 \pm 0.05 / 0.11 \pm 0.03$ microns at day 0 to $0.03 \pm 0.01 / 0.06 \pm 0.03$ microns at day 13 for non occluded/occluded eyes. Third order RMS decreased from $0.09 \pm 0.04 / 0.08 \pm 0.02$ microns to $0.02 \pm 0.01 / 0.04 \pm 0.02$ microns.

Average changes of spherical aberration with age are shown in Figure 3.4 (in terms of RMS in C and 4th order spherical aberration Zernike coefficient in D). In the first 4 days the tendency is irregular in both non-occluded and occluded eyes, and tends to stabilize after day 6. Older non occluded eyes show spherical aberration very close to 0, while occluded eyes show slightly negative spherical aberration. On day 13, spherical aberration is practically 0 in both groups: -0.001 ± 0.006 and $+0.002 \pm 0.009$ microns in non occluded and occluded eyes respectively. Differences with age are not statistically significant ($p = 0.4$; univariate ANOVA), nor the differences between treated and non-treated eyes ($p = 0.1$).

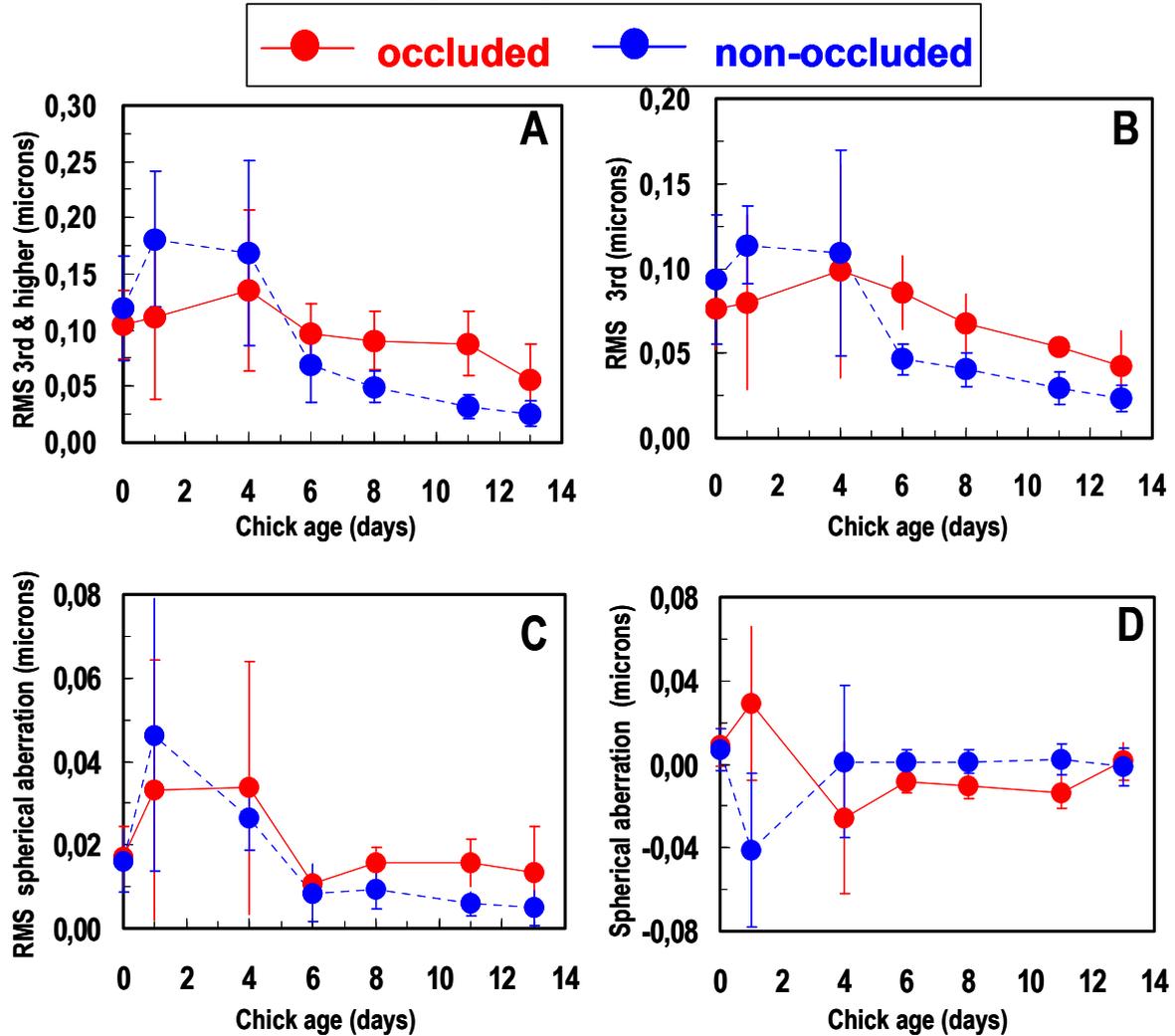


Figure 3.4. Mean 3rd and higher order RMS (A), 3rd order RMS (B), spherical aberration RMS (C), and 4th order spherical aberration Zernike coefficient (D) as a function of age, averaged across all chicks. Pupil diameter: 1.5mm. Blue circles and dotted lines correspond to non-occluded eyes and red circles and solid lines correspond to occluded eyes. Error bars stand for standard deviations.

3.4.3. Modulation transfer function.

Figure 3.5 shows mean MTFs (radial profile), for non occluded and occluded eyes, for several days throughout the experiment. The MTF for an ideal eye without aberrations is also shown for comparison. All data are for 1.5 mm pupil diameters. Prior to treatment, both eyes show similar optical quality in terms of Strehl ratio ($p=0.8$; unpaired t-test). Changes in Strehl ratio with age are significant ($p<0.0001$; univariate ANOVA) as well as the global differences between non occluded and occluded eye ($p<0.0001$; univariate ANOVA). On days 8 and 11 Strehl ratios are significantly better in the non occluded than in

the occluded eye ($p=0.006$ and $p=0.05$, respectively; unpaired t-test). As expected from the RMS values, optical quality improves from day 0 to day 13, gradually approaching the ideal MTF. Differences are reduced on day 13 ($p=0.06$), with the non occluded eyes being practically diffraction-limited at the end of the experiment. The MTF in non-occluded eyes is higher than in occluded eyes in all chicks except one on day 6, in all chicks on day 8 and 11

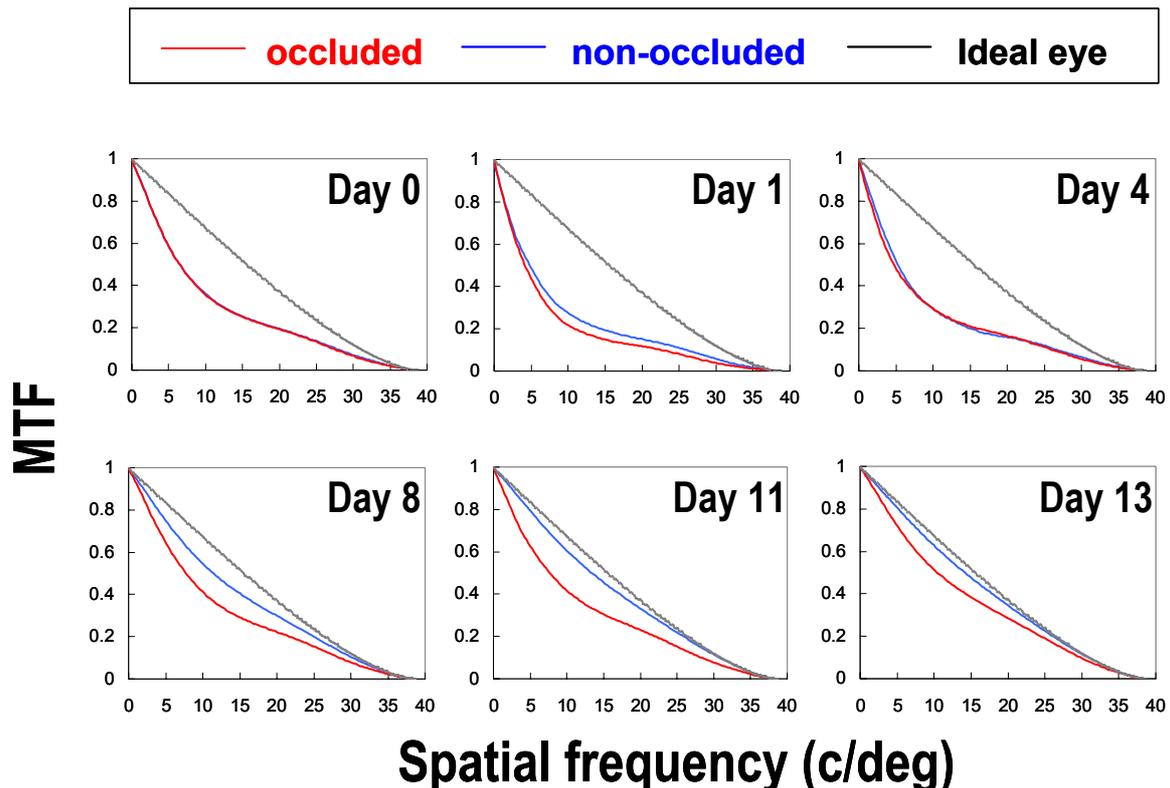


Figure 3.5 Mean MTFs (radial profile) averaged across all chicks, for non occluded (blue line) and occluded (red line) eyes on different days of the experiment (days 0, 1, 4, 8, 11, 13). Pupil diameter: 1.5 mm. The MTF of an ideal diffraction-limited eye of the same diameter is shown for comparison (black line).

and in all but two chicks on day 13. Figure 3.6 represents MTF ratios (non occluded/occluded eye) for all chicks for day 8. Values are greater than 1 for all spatial frequencies and subjects, indicating better optical quality in non-occluded eyes. MTF ratios (averaged across spatial frequencies) range between 1.08 for chick #5 and 2.02 for chick #1. Differences between the non-occluded and occluded eye tends to increase with spatial frequency and in some cases peak at mid spatial frequencies. Figure 3.7 shows modulation transfer as function of age for two different spatial frequencies, 1.5 c/deg and 7 c/deg, which seem to be relevant for the chick's visual system (Troilo and

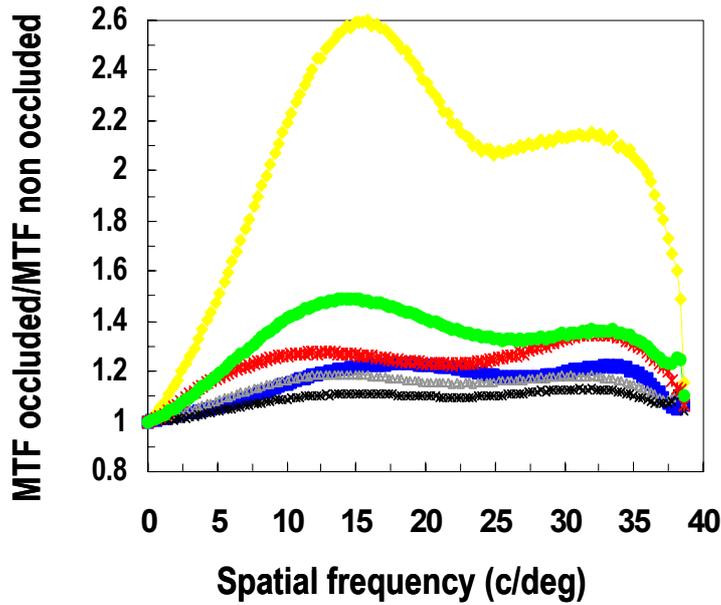


Figure 3.6 MTF ratios non-occluded/occluded eye for all chicks on day 8. MTF ratios (averaged across spatial frequencies). All data are for pupil diameter of 1.5 mm. Each color corresponds to a different chick.

Wallman 1991). After day 4, the occluded eye tends to show lower modulation than the non-occluded eye for 1.5 c/deg, but differences are in general not significant. However, for 7 c/deg differences are globally significant ($p=0.01$; univariate ANOVA). Figure 3.8 represents mean Strehl ratio (as a global image

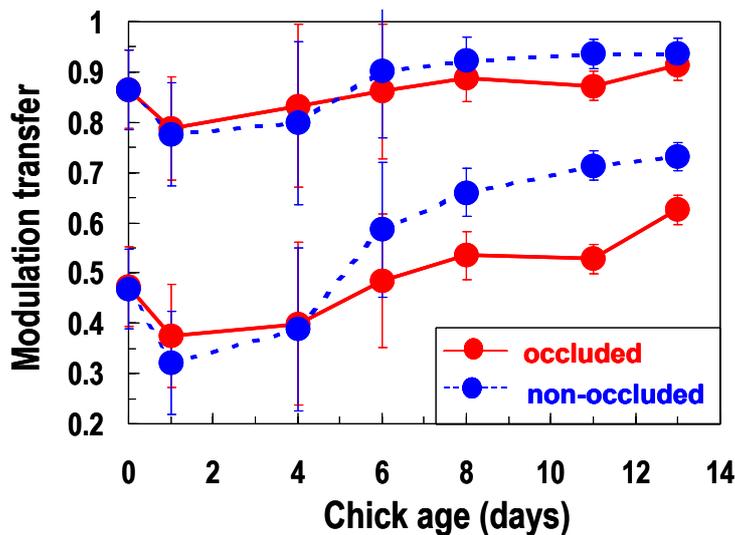


Figure 3.7 Mean modulation transfer as function of age (averaged across all chicks) for two different spatial frequencies, 1.5 c/deg and 7 c/deg. Blue circles correspond to non-occluded eyes and red symbols correspond to occluded eyes. Error bars stand for standard deviations.

quality metric) as a function of age, showing the consistent improvement of optical quality with age in both eyes, and significantly better optical quality in the non-occluded compared to the occluded eye ($p=0.02$ on day 13). Strehl ratio increases from 0.57 ± 0.10 on day 0 to 0.95 ± 0.05 on day 13 in non-occluded eyes, and from 0.56 ± 0.14 to 0.79 ± 0.15 for the occluded eyes. Interestingly, by the end of the experiment the optical quality is very close to diffraction limited (according to the Rayleigh criterion) for 1.5 mm pupils.

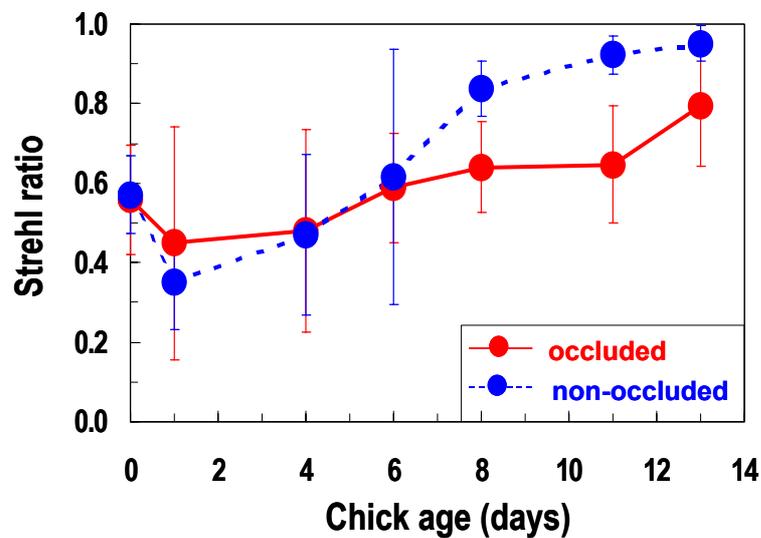


Figure 3.8 Mean Strehl ratio as a function of age. Blue circles and dotted lines correspond to non-occluded eyes and red circles and solid lines correspond to occluded eyes. Error bars stand for standard deviations

3.5. Discussion

3.5.1. Comparison with previous studies in experimental models

Our method of myopia induction in chicks by depriving forms has been widely used and studied. Normal eyes in our study developed as reported in the literature (starting moderately hyperopic, with a progressive tendency toward emmetropia). Our refraction and axial length changes in occluded eyes are consistent with results from previous studies in White Leghorn chicks, although for similar treatment periods our average myopia outcomes were slightly lower. A previous study (Guggenheim et al. 2002) found in a similar experiment with restricted vision in one eye and normal vision in the contralateral eye, interocular differences of 1.4 ± 0.4 mm in axial length and -26.4 ± 7 D in refraction, after 2 weeks of treatment. Our results on day 13 showed interocular differences of 0.9 ± 0.4 mm and -17 ± 3 D respectively. Another study (Schmid and Wildsoet 1997) using constant form deprivation with diffusers reported interocular differences of 0.49 ± 0.10 mm and 0.82 ± 0.20 mm in axial length, and -12 ± 3 D and -19 ± 6 D in refraction, on days 5 and 10 respectively. We obtained interocular differences of 0.5 ± 0.5 mm and 0.80 ± 0.3 mm in axial length and -10 ± 2 D and -14.8 ± 3 D in refraction, on days 6 and 11 respectively. While the outcomes are similar, we obtained slightly lower values of myopia induction. One reason for the differences between studies could have been the amount of diffusion produced by the occluder, since correlations between the amount of myopia induced and the density of the diffuser material have been demonstrated (Bartmann and Schaeffel 1994). Another potential factor contributing to lower myopia outcomes could have been the fact that we took out the occluders for brief time periods while we were taking the measurements, and given that additional measurements (Hartmann-Shack aberrations) required longer measurement times, chicks may have been exposed to longer periods of “normal viewing” than in previous studies. It has been shown (Schmid and Wildsoet 1997) that, if the treatment is interrupted with 20 minutes of “visual stimulation” each day, form-deprivation myopia is significantly reduced. However our control chick (monolaterally treated, but not

measured during intermediate days) developed an interocular refraction difference of 17 D, similar to the average refraction on day 13 that we found on the occluded eyes that participated in all measurements. Differences cannot be attributed to the fact that all chicks in our experiment were males since it has been shown (Guggenheim et al. 2002) that there is no sex-related difference in refraction data following form-deprivation, and if anything, slightly higher elongation (~ 0.2 mm) in males than females in three strains of chickens, included White Leghorn (Kisilak et al. 2002; Thibos et al. 2002; Campbell et al. 2003).

A previous study (Coletta et al. 2003) measured the modulation transfer function (MTF) using a double pass technique in older chickens' eyes, both normal and myopic after different treatments. Aberrometry allows individual assessment of individual Zernike terms, as well as estimates of point spread functions (PSF) and modulation transfer function for any pupil size and defocus, while the double-pass technique only allows measurement of MTF for the pupil size and focus correction of the measurement. Thibos and colleagues (Thibos et al. 2002) measured higher-order optical aberrations in normal chicks during the first week of life with a HS aberrometer. When normalized by pupil area, the equivalent defocus of all the Zernike modes decreased slightly with age, a tendency in agreement with our finding of the increasing optical quality with age (in our case for a constant pupil diameter). However, they concluded that the optical quality during the first week of life in the chick eye is significantly worse than in human adult eyes, while we found good optical quality in chicks (for 1.5 mm pupils), and close to diffraction-limit by day 13 in non treated eyes. Coletta et al. (Coletta et al. 2003) found relatively good optical quality in chick eyes, although worse than in human eyes. However, their data are for older chicks (from 3 to 6 weeks old) and larger pupils (4.50-mm mean pupil diameter) than in Thibos' or the study presented in this chapter. In any case, our results support Coletta et al.'s conclusions that optical quality is not limiting spatial resolution in chicks, since the MTF's cut-off frequencies are well above reported chicks spatial acuity: 1.5 c/deg from behavioral studies (Over and Moore 1981) or up to 8.6 c/deg from optokinetic nystagmus responses (Schmid and Wildsoet 1998).

Campbell and colleagues (Campbell et al. 2003) also found an improvement with age of the optical quality of young normal chicks, for 1.6-mm pupils. All reports show trends of decreased optical quality in myopic eyes, regardless the method of myopia induction. Coletta et al. (2003) found that myopic eyes had poorer optical quality than normal chicks. Unlike our study, where we induced myopia with diffusers, Campbell et al. induced myopia in chicks with -15 D lenses. They found that average optical quality (for 1.6-mm pupils) did not change between days 0 and 7, unlike control eyes that experienced a decrease in the amount of aberrations. For higher order aberrations alone, goggled eyes had significantly worse optical quality at day 7 than controls. While we also found significantly less aberrations in control eyes than in treated eyes, we found that higher order aberrations decrease in both normal and treated eyes. However, it should be noted that in our experiment, the most significant differences occur after day 8, and trends are observed when extending the experiment for at least five more days.

Similar tendencies were found recently in mammal models (Ramamirtham et al. 2004). These authors found that manipulation of visual experience with diffusers or spectacle lenses in young Rhesus monkeys resulted in greater amounts of ocular aberrations, with no significant differences in the magnitude or pattern of higher order aberrations between the control and treated groups before treatment and significant RMS differences ($0.09 \mu\text{m}$) by the end of the treatment period.

3.5.2. *An emmetropization of the optical aberrations?*

We found that aberrations tend to decrease during development in chicks. This was also found by Thibos et al. (Thibos et al. 2002) and Campbell et al. (Campbell et al. 2003) in normal chick eyes. While working with chicks allows longitudinal measurements, some cross-sectional measurements in the literature are suggestive that a similar tendency is found in humans. Human results reported by Brunette et al. (Brunette et al. 2003) showed that optical aberrations decrease during development. These authors measured optical aberrations in subjects ranging from 5.7 to 82.3 years and found that the

average optical quality in early childhood was significantly worse than in the advanced age, with aberrations decreasing during childhood and adolescence. It is well known that the optical aberrations of the crystalline lens (showing negative spherical aberration) partially compensate corneal aberrations (showing positive spherical aberration) in the normal young human eye, and that this compensation gets disrupted later in age (Calver et al. 1999; Guirao and Artal 1999; Mclellan et al. 2001). Brunette et al.'s cross-sectional data, as well as the mentioned longitudinal data in chicks, may suggest that the optimal performance found in young adults is reached after an optimization process that takes place during development. Other authors (Wang and Candy 2005), however found that the optical quality was as good in infants (5-7 weeks) as in young adults (younger than 40 years), with no significant difference in the levels of 3rd order monochromatic aberrations, and only a higher tendency in infants to show negative spherical aberration with adults eyes tending to show positive spherical aberration.

Aberration balance between optical components, and even more a potential improvement of the optical quality of the eye during development, may lead to consider an active process for the development of optical components. If an active visually guided process tunes the eye length to the power of the optical component, one may think of a similar system adjusting the optical and geometrical properties of the optical components to reduce high order aberrations and produce optimal image quality. Our results do not support such a system, or at least this process being visually guided. We found that the improvement of the optical quality with age occurs even in the eye occluded with diffusers, subject to dramatic image quality degradation. While a lens treatment may have provided a different approach to answering this question, excluding any visual feedback with the diffusers suggests that the tuning of optical aberrations of ocular components is likely the result of a pre-programmed process or just geometrical scaling but it does not seem to rely on visual experience to occur, at least to a great extent. These findings are in good agreement with a scaling model recently proposed by Howland (Howland 2005). This model, based on reported data of corneal curvature increase in White Leghorn chicks during the first week of life, shows that aberrations measured in

a growing eye at a constant pupil size decrease with time. A more elaborate model, including geometrical properties of the cornea and crystalline lens of the developing myopic and normal eye would be necessary to assess if scaling accounts for all decrease in aberrations and to explain the differences between both eyes. This question will be further addressed in Chapter 6, using more complex computer eye models.

3.5.3. *Optical aberrations and emmetropization*

We found higher amounts of optical aberrations in myopic eyes than in the normal control eyes after six days of treatment. While the differences are significant, the amount of blur produced by aberrations is minimal compared to the optical degradation produced by the diffuser or the developed refractive error. By day 13 even myopic eyes are close to diffraction-limited. These experiments shed light on possible relationships between aberrations and myopia development. There are several cross-sectional studies in humans reporting optical aberrations as a function of refractive error (Collins et al. 1995; Carkeet et al. 2002; Marcos et al. 2002; Paquin et al. 2002; Cheng et al. 2003; Llorente et al. 2004). Most studies found higher amounts of aberrations as myopia increased (Atchison et al. 1995; Coletta et al. 2003). Several studies only found a significant correlation for high myopes, and third order aberrations, but not spherical aberration (Marcos et al. 2002). One study (Carkeet et al. 2002) did not find correlations between refractive error and high order aberrations (for myopic Singaporean children, < 3 D), and another study (Cheng et al. 2003) on 200 normal human eyes failed to find correlations between high order aberrations and refractive errors (from +5.00 to -10.00). In the study presented in this chapter, in chicks interocular statistically significant differences in the amount of higher order aberrations only appear for amounts of myopia beyond -7.3 D. The fact that increased amounts of aberrations are found in higher myopes may lead to the hypothesis that aberrations may be a cause for myopia. Suggestive evidence of this hypothesis has been presented in the Introduction. Longitudinal measurements allow to shed light into the question whether higher aberrations are a cause or an effect of myopia development. Our experiment clearly favors the hypothesis that aberrations are a

consequence of the structural changes occurring in the excessively elongated eye: 1) We did not find that eyes with higher amounts of aberrations at birth emmetropized less efficiently; 2) The retinal image degradation imposed by diffusers induces myopia in the treated eyes. Unlike a potential treatment with lenses, where eye/lens system can project good optical quality images on the retina, a treatment with diffusers allows no visual feedback. Treated eyes turned out to be more aberrated most likely as a result of the treatment, but it is unlikely that the increased aberrations may have played any role at all in the development of myopia. 3) While aberrations are significantly higher in myopic eyes than in the normal eyes, the retinal image degradation induced is negligible compared to the degradation imposed by the diffuser and the induced defocus. While in the present experiment aberrations result from the myopia development, the next chapters test the hypothesis in the reversed direction (aberrations as a potential cause for myopia development).