Chapter 1. Introduction

The cornea and the lens of the eye project the images of the external world onto the retina. For adequate visual perception, this image should be focused on the photoreceptor layer (the cells that are responsible for light capture). One of the most extraordinary processes of ocular development in vertebrates is the coordination to adjust the axial length of the eye to the focal plane of the ocular optics (an active process called emmetropization). This tuning mechanism fails in a significant percentage of the population, who develop refractive errors (30% of the population in Europe and up to 85% in some Asian countries suffers from myopia) (Saw 2003; Kempen et al. 2004; Jorge et al. 2007). Despite its high prevalence, the etiology of myopia is not fully understood, although there is clear evidence that environmental factors play a major role (Saw 2003). Other high order aberrations, which are typically not measured in the optometry practice also contribute to retinal image degradation. In the young normal eye, the total aberrations of the eye are lower than those of the individual optical components, and whether this is a result of an active or a passive mechanism has been debated (Artal et al. 2001; Kelly et al. 2004; Tabernero et al. 2007; Marcos et al. 2008). In this thesis we investigated the potential role of ocular aberrations in myopia development, as well as the change of ocular aberrations during development, using experimental models of myopia.

1.1. Myopia

Myopia is a refractive error that allows detecting near objects clearly, but not distant objects. Images formed by a myopic eye from distant objects are perceived as blurred. This is due to the fact that parallel rays coming form infinite (distant objects) are focused in front of the retina, instead of on the retinal plane as occurs in the emmetropic eye when the accommodation is relaxed. The focus distance of the eye lenses is insufficient relative to eye dimensions, more specifically to axial length, which is the distance between the cornea and retinal photoreceptor layer. Figure 1.1 shows a scheme of a myopic eye, projecting distant objects in front of the retina and an emmetropic eye, perceiving distant objects in focus on the retina. Many studies around the world

suggest that myopia is associated mainly with a longer axial length due to enlargement of the vitreous chamber (Schaeffel et al. 1988; Hung et al. 1995; McBrien and Adams 1997; Gilmartin 2004) and secondly to greater corneal power (Grosvenor and Goss 1999).

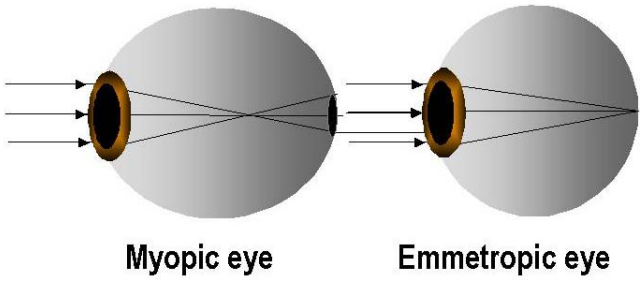

Figure 1.1 Image from a distant object is perceived as blurred and in an myopic eye, in contrast with emmetropic eye that focuses on the retinal plane.

1.1.1. Prevalence of myopia

Myopia in humans is a very common condition and has typically been associated to age and genetic factors (familiar antecedents, ethnic heritage…) as well as environmental factors (near work, social status, occupation…). Newborn eyes show commonly hyperopic errors: the cornea and lens are steeply curved, compared with their adult values, and the focal plane is short. During postnatal development, the focal plane moves away from the cornea (Chan and Edwards 1993; Pennie et al. 2001). This hyperopic shift toward emmetropia during development is inherent to the emmetropization process (Chan and Edwards 1993). During emmetropization two major changes occur in ocular growth: an increase of the vitreous chamber depth and a decrease of the cornea and lens power.

The fine tuning between power of ocular components of the eye and axial length is directed by a control mechanism to achieve an emmetropic eye. In emmetropic eyes the axial length matches the focal plane, allowing focusing of distant objects without accommodation. Often the emmetropization process is interfered by some reason, disrupting adequate ocular development, and resulting in hyperopic or, more frequently, myopic errors (e.g. (Zadnik and Mutti 1987; McBrien and Adams 1997)). An increased axial length, relative to the focal plane results in myopia. This relative increased length to eye power lenses

depends on several conditions and occurs in different situations. The prevalence of myopia changes across countries and types of society (Saw 2003). Myopia in the first stages of life is associated to immature eyes and usually reduces to emmetropia by one year of age (e.g (Fletcher 1955; Fledelius 1981; Varughese et al. 2005; Ziylan et al. 2006) although pre-term children have higher probability of developing myopia in later years (Larsson et al. 2003). At age five myopia is inexistent in some populations such as in rural china, 3% in Chile, 1-3% in North American and European societies, 4% in Japan and 3% in South Africa. But significantly higher prevalences are found in Taiwan or Singapore (12-28% in six and seven year old children), and the amounts of myopia as well as prevalence increase with age at a faster rate than in rural china, in suburban Chilean or north American and European primary school populations (Grosvenor 1987; Lin et al. 1999; Maul et al. 2000; Zhao et al. 2000; Morgan and Rose 2005; Saw et al. 2005).Also, it is common to find significant levels of myopia in school years in children who entered school as emmetropes (Quek et al. 2004; Morgan and Rose 2005). This general increasing tendency of the myopia prevalence continues to adult years, with prevalence of myopia in adults of 25.4% in USA, 26.6 % in West Europe , 30.01 % in Spain, 16.4% in Australia and again higher values in Asian populations: 80% in Singapore male school leavers or 82.2% in Chinese military conscripts (Grice et al. 1997; Montés-Micó and Ferrer-Blasco 2000; Kempen et al. 2004; Saw et al. 2005; Thorn et al. 2005; Anera et al. 2006). The prevalence rates of myopia are rising and is considered epidemic in some Asian populations (Saw 2003). Figure 1.2 shows myopia prevalence and progression in several Asian and Western populations. Other studies found different myopia prevalences associated to different conditions: Females and males (Krause et al. 1982; Kempen et al. 2004; Wickremasinghe et al. 2004), caucasian and African-american (Sperduto et al. 1983; Kleinstein et al. 2003; Hyman et al. 2005), years of school assistance (Rosner and Belkin 1987), high educational demands over several years (Lin et al. 1996; Kinge and Midelfart 1999; Kinge et al. 1999; Jorge et al. 2007), greater daily reading time (Angle and Wissmann 1980), higher near work demand (Goldschmith 1968; Curtin 1985; Ong and Ciuffreda 1995; McBrien and Adams 1997; Saw et al. 2002), place of residence (urban vs. rural) (Morgan and Rose 2005).

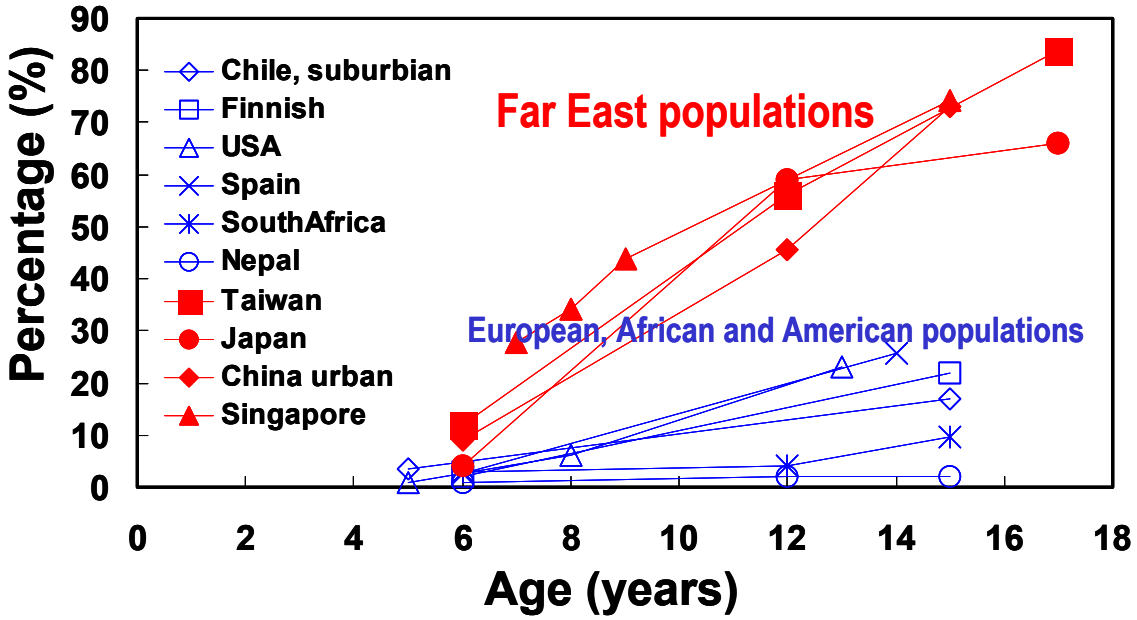

Figure 1.2 Prevalence (%) of myopia around the world as a function of age.

Myopia has been classified in several categories according to different etiological factors: simple myopia, characterized by normal visual acuity with optical correction and absence of other structural anomalies; night myopia at low illumination conditions due to the lack of accommodation stimulus; pseudomyopia produced by an apparent myopia due to unrelaxed accommodation and pathologic myopia, which is related to high myopia associated degenerative changes in the posterior segment of the eye and it is a pathologic disease more than a high refractive error (Ursekar 1983; Tokoro 1988). Grosvenor (Grosvenor 1987) proposed a system for classification of myopia on the basis of age-related prevalence and age of onset: congenital, youth-onset, early adult-onset, and late adult-onset.

1.1.2. Etiology of myopia

Myopia has been associated to different factors, both genetic and environmental (Pacella et al. 1999; Thorn et al. 1999; Saw 2003). It is often stated that whether myopia development is affected primarily by visual experience is a consequence of genetic background. Understanding the

physiological mechanism involved in myopia development is critical in for potential interventions, and consequently myopia prevention.

1.1.3. Genetic factors

Genetic factors are reported as a risk factor for myopia. The high prevalence of myopia found in specific ethnic groups suggests a role of the genes in this refractive error. On the other hand, the increase rate of myopia prevalence over the last generations in the same ethnic groups indicates that this effect can not be attributed to genes and environmental factors are also important (Lin et al. 1999; Saw 2003; Morgan and Rose 2005). Ocular growth involves several biological process (tissues, biochemical pathways…) since several genes are associated to eye growth (Feldkämper and Schaeffel 2003; McBrien and Gentle 2003; Schaeffel et al. 2003), indicating that there should be a biological basis for a contribution of genetic variation to refractive error (Morgan and Rose 2005). Several chromosomal localisations for inherited high myopia have been reported in genome-wide scans at least for high myopia, (Young et al. 1998; Young et al. 2001; Naiglin et al. 2002; Paluru et al. 2003; Gilmartin 2004; Young et al. 2005), but not for other more frequent types of myopia such as juvenile myopia (Mutti et al. 2002; Ibay et al. 2004).

Parental history studies show that myopia is more likely to occur when both parents are myopic and least when neither parent is myopic (Goss and Jackson 1996; Pacella et al. 1999; Mutti et al. 2002; Khandekar et al. 2005; Saw et al. 2005; Kurtz et al. 2007), although not all types of myopia have been related to parental history (Zadnik et al. 1994; Iribarren et al. 2005). However there are other factors present in these parental studies such as common environment and lifestyle similar in parents and children (Iribarren et al. 2002; Morgan and Rose 2005). Although heredity seems to be the most important factor in juvenile myopia when parents are myopes, other environmental factors such as increased near work, higher school achievement, and less time in sports activity are thought to play a role (Mutti et al. 2002). Therefore refractive error is affected with a set of variables and their interactions, with genetic and environmental being involved to various extents in the emmetropization process

(Saw 2003). An inappropriate visual stimulation may induce different ocular growth patterns and amounts of myopia depending on the individual's genetic predisposition. Twin studies show higher concordance for monozygotic twins than dizygotic (Chen et al. 1985; Hammond et al. 2001), and higher concordance in myopia values in twins with similar reading habits that discordant ones (Chen et al. 1985). The feedback (visual information which eye interprets for a correct eye growing signal in the emmetropization process) may be interfered by inherited metabolic conditions, developing myopia in predisposed subjects (Feldkämper and Schaeffel 2003).

1.1.4. Near work and related factors

The most relevant clinical environmental factor typically associated with myopia in humans is near work. Donders in 1864 (Donders 1864), associated near work requirements with refractive errors. Several studies show a greater prevalence of myopia and prevalence rates are highest among people who have occupations requiring near work (microscopists, visual display terminal workers…) (Tokoro 1988; Zylbermann et al. 1993; Simensen and Thorud 1994; McBrien and Adams 1997; Saw et al. 2002; Saw et al. 2007). In addition myopia is less common in populations where school is not compulsory (Young et al. 1969), as opposed to the high prevalence of myopia found in some Asian cities, such as Singapore, where school tasks are very demanding and require high levels of reading (Saw 2003). According to this hypothesis, the emmetropization process that results in myopia could be a consequence to improve focus in frequent readers (Mutti et al. 1996; Goss 2000), a sort of "near emmetropization". Higher progression rates are associated with earlier onset of myopia and with greater time spent on near work and less time spent outdoors, shorter reading distance, higher IOP, and esophoria at near. Although the question on how near work may trigger myopia and even the hypothesis is itself still under debate, ongoing clinical trials explore the effect of prescribing addition (positive refraction) for relaxing accommodative response with the aim of decreasing progression of myopia (Fulk et al. 2000; Gwiazda et al. 2003; Kurtz et al. 2007), However, not all studies have associated myopia progression with reading, short reading distance and close work or a reduction of myopia

progression by a decrease of the myopia by reading without glasses or positive refraction (Grosvenor et al. 1987; Hemminki and Parssinen 1987; Parssinen et al. 1989; Saw et al. 2002).

An old hypothesis is that intraocular pressure in the vitreous chamber is increased by sustained accommodation during near work requirements. Van Alphen in 1961 (Van Alphen 1961) proposed that the eye adjusts axial length to match the refractive power of the eye, the ciliary muscle and choroids control the intraocular pressure. When choroidal tension is insufficient to resist intraocular pressure, the ocular posterior segment would be stretched resulting in axial myopia. Higher values of intraocular pressure in myopic chicks than in hyperopic chicks have been reported, but this relationship between intraocular pressure and myopia is not clear (Schmid et al. 2003) and some studies (Goss and Caffey 1999) did not find statistical differences in IOP between myopic and emmetropic children. Other studies suggest that the ciliary muscle contraction during accommodation could influence scleral growth and eye shape directly, without involving intraocular pressure (Drexler et al. 1998). Greene (Greene 1980) proposed that stretching of the posterior sclera due to influence of extraocular muscles and pressure during ocular convergence could be the mechanism relating near work and myopia development, while others reject the idea of a mechanically stretching of sclera preceding myopia development (Ong and Ciuffreda 1995). Wildsoet & Wallman (Wallman et al. 1995) proposed a change choroidal thickness for compensating defocus in a chick model. In avian and mammalian models the sclera creep rate increased and decreased in order to modify axial length (Phillips et al. 2000).

Several studies have attempted to relate accommodative response differences with refractive state. It seems well established that myopes tend to have a smaller accommodative responses than non-myopes (Rosenfield 1998; Jiang 2000). The amount of accommodative convergence is elevated relative to accommodative response (AC/A ratios) in myopic children, therefore showing reduced accommodation, enhanced accommodative convergence and esophoria. Some have suggested increased tension in the crystalline lens increasing the effort to accommodate and enlarging the eye (Mutti et al. 2000).

The most extended hypothesis relating near work and myopia refers to the presence of a hyperopic blur associated to an accommodation lag (Gwiazda et al. 2005). Accomodative lag has been shown to be higher in myopes than hyperopes (Bullimore et al. 1992; He et al. 2005). Whether increased accommodation lag in myopes is a cause (due to the presence of hyperopic blur) or a consequence of myopia (due to decreased blur cues in myopia) is a question under debate. Some studies (Mutti et al. 2002) found that accommodative lag was not significantly different in children who became myopic compared with emmetropes previously to onset myopia. Higher accommodative lag was measured in children after the onset of their myopia, suggesting that increased accommodative lag could be a consequence rather than a cause of myopia. Whether accommodation is used as a signal to direct eye growth remains therefore unclear. In fact, intact accommodation is not necessary for proper emmetropization in experimental animal models, as it has be shown that some species are able to properly emmetropize and recover from induced refractive errors after lesion of the Edinger-Westphal nucleus or ciliary muscle (Wallman and Adams 1987; Troilo 1990).

1.1.5. Emmetropization process

The presence of an active mechanism during development that controls a fine tuning between the focal length of ocular components and axial length (known as emmetropization) is well established (Wallman 1993; Wildsoet 1997), although, as discussed above, it is not fully understood. It appears as if the eye was programmed to achieve optimal focus and a clear retinal image, with the process being visually guided. When retinal image quality gets degraded by some reason, the process gets disrupted, the eye continues to grow in the axial dimension (seeking best focus), therefore resulting in myopia. Several pathologies that affect the quality of the retinal image during development (congenital catarats, lid haemangioma, palpebral ptosis, vitreous haemorrhage...) are typically associated with significant amounts of myopia (Robb 1977; Hoyt et al. 1981; Rabin et al. 1981). The requirement of a clear

retinal image for a proper emmetropization has been extensively demonstrated using animal models.

Blur in the peripheral retina has also been considered to be an important factor in myopia development with potential interactions between the defocus at the fovea and peripheral retina (Wallman and Winawer 2004; Smith et al. 2005). Differences in ocular globe shape have been found across refractive errors in humans. Myopic eyes have been shown to exhibit larger axial length than equatorial diameter (Gilmartin 2006) and a hyperopic retinal periphery relative to the fovea (Drexler et al. 1998; Atchison et al. 2004; Singh et al. 2006). The eye has been proposed to grow axially as a result of interactive effects between foveal image and blurred peripheral retinal image (Ciuffreda et al. 2007). Also, recent experiments in monkeys (Smith et al. 2005) suggest that deprivation, imposed only in the periphery of the visual field, can induce foveal myopia, raising the hypothesis that peripheral refractive errors imposed by the spectacle lens correction could influence foveal refractive development also in humans (Smith et al. 2006). On the other hand, this effect has not been found in chicks, where defocus imposed on local retinal areas produce local changes in eye growth (Diether and Schaeffel 1997; Schippert and Schaeffel 2006).

1.1.6. Animal models of myopia

Animal models have allowed systematic investigations of the role of the visual environment in the regulation of the axial length and myopia. Visual form deprivation experiments in animals have shown that visual experience plays a major role in normal emmetropization and myopia development. By altering visual experience in new born animals, myopia can be artificially developed, and the role of different factors (type of treatment, duration, age of treatment, etc…) in the outcomes of refractive error can be systematically studied. Many different animals have been used as models for myopia: Chicks (Wallman et al. 1978; Yinon et al. 1980; Schaeffel and Howland 1988; Schaeffel and Howland 1991; Irving et al. 1992; Wildsoet and Wallman 1995; Schaeffel and Diether 1999), tree shrews (Sherman et al. 1977; Norton 1990), monkeys (Wiesel and Raviola 1977; Hung et al. 1995; Smith and Hung 2000), marmosets (Troilo and

Judge 1993; Whatham and Judge 2001; Troilo and Nickla 2002), cats (Ni and Smith 1989; Wilson et al. 2002), mice (Beuerman et al. 2003; Tejedor and de la Villa 2003; Schaeffel et al. 2004), fish (Kroger and Wagner 1996; Shen et al. 2005), etc.

1.1.6.1. Chick model

Chick (Gallus gallus domesticus) has been used more than any other model in myopia research. Although chicks are phylogenetically distant from humans it has proved to be an excellent model for myopia. The chicken eye is flat, i.e. with a shorter anterior posterior axis than other meridians. A scleral plate, a ring with ossicles, provides ocular support and shape. In addition, the chicken retina does not have blood vessels (Schuck et al. 2000) and is provided with a vascular projection from the retina into the vitreous chamber called pecten. The function of the pecten is not well known (Wolburg et al. 1999). Avian pecten arises from the optic nerve (Schuck et al. 2000) and it seems to play a role in the retina nutrition (Kiama et al. 1997; Wolburg et al. 1999), but other functions have also been suggested: intraocular PH regulation (Brach 1975), blood-retina barrier (Wolburg et al. 1999) (Schuck et al. 2000), regulation of intraocular pressure (Seaman and Himelfar.Tm 1963), and reduction of intraocular glare (Barlow and Ostwald 1972).

Additionally the chick retina does not show a fovea but an area centralis (Morris 1982) a high ganglion-cell-density area (Straznicky and Chehade 1987) and contains cone opsins red, green, blue, and violet, as well as the rod-specific opsin rhodopsin (Bruhn and Cepko 1996). Accomodation in chicks is achieved, apart from the lens, by the cornea. Ciliary muscle alters the corneal curvature for corneal accommodation and moving the ciliary body anteriorly as a part of the lenticular accommodative mechanism (Schaeffel and Howland 1987; Schaeffel et al. 1988; Glasser et al. 1995). The ciliary muscle also may serves in the regulation of aqueous dynamics within the eye (Murphy et al. 1995). Accommodation occurs independently in the two eyes. Figure 1.3 shows a section of an enucleated chick eye.

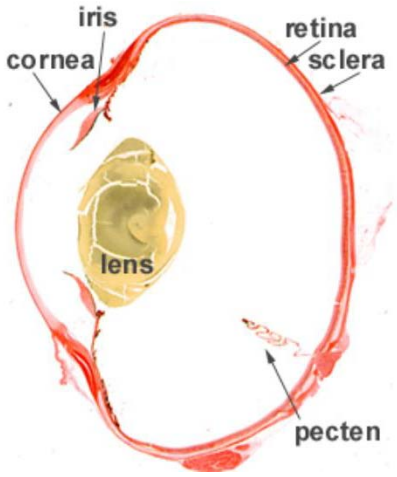

Figure 1.3 Sagital section of an enucleated chick eye. (Adapted from www.lsi.usp.br.txt)

Chicks are easily available animals, and their breading and feeding requirements are not demanding. They mature rapidly, so changes occur within days, making them very appropriate for lab experiments. One of the first techniques for depriving chicks from visual forms was eyelid closure (Yinon 1984), or using either occluders or plastic diffusers over the eye, avoiding alterations in the cornea and allowing light entrance in the eye but not forms (Wallman et al. 1978; Hodos and Kuenzel 1984; Schaeffel et al. 1988; Beresford et al. 2001; Guggenheim et al. 2002; Choh et al. 2006). Myopia induced by treatments involving eye lid closure, occluders or plastic goggles in young chickens, is called form-deprivation myopia. Myopia can also be achieved by defocusing retinal image with negative lenses. It has been shown that the chick eye emmetropizes to the new condition, by matching the axial length to the focal plane (which with a negative lens is placed posteriorly to the retina), i.e. producing a compensatory elongation of the eye (Schaeffel et al. 1988; Schaeffel and Howland 1991; Wildsoet and Wallman 1995; Park et al. 2003; Choh and Sivak 2005). The same effect has been found in the absence of accommodation, i.e. Edinger-Westphal nucleus ablation or ciliary nerve section (Schaeffel et al. 1990), and having removed a connection with the brain in optic nerve sectioned animals (Schmid and Wildsoet 1996; Diether and Wildsoet 2005). Evidence that the mechanism happens at the retina is further supported by observations in chicks that the axial elongation process is spatially local so that one portion of the eye may elongate while another portion remains normal. This has been observed in eyes exposed to translucent diffusers or to minus lenses that cover only a partial visual field or cylindrical lenses (Wallman and Adams 1987; Troilo and Wallman 1991; Irving et al. 1995; Diether and Schaeffel 1997), or in chicks raised in cages with low roof which results in a myopic shift in the inferior retina (Miles and Wallman 1990). Other way for altering visual experience in chick myopia models has been restricting image

contrast or spatial frequencies during post-natal development (Bartmann and Schaeffel 1994; Schmid and Wildsoet 1997).

Axial length and choroid thickness seem to be driven by circadian rhythms (Nickla et al. 1998; Nickla et al. 2001; Nickla 2006). Chicks can recover from myopia in intermittent form depriving treatments. When the visual restriction is briefly removed, ocular growth returns to normal rates (Wallman and Adams 1987; Troilo and Wallman 1991; Zhu et al. 2003), showing the existence of a regulatory mechanism driven by a visual cue.

1.1.6.2. Mouse model

There is a special interest in developing a myopia model in mice because the mouse genome has been completely sequenced and it can be manipulated. A mouse myopia model could reveal genetic and environmental factors for the same animal model. While a bright retinal reflection can be found in mice (Schmucker and Schaeffel 2004), their optical quality is believed to be low (Artal et al. 1998; Prusky et al. 2004). Several authors have investigated whether refraction can be induced with visual deprivation as in other animal models, but response to treatments are not so evident as in chick models. Tejedor et al. (Tejedor and de la Villa 2003) reported induced form deprivation myopia in mice, while Schaeffel et. al suggested technical difficulties in measuring changes in form deprived mice (Schaeffel and Burkhardt 2002). A difficulty of this model is to measure refractive error by retinoscopy. Mice eyes have pupil diameters smaller than 1.5 mm, and it is very difficult to observe retinal reflection shifts. It has been suggested that at least some mice strains, could not respond very efficiently to visual deprivation by ocular elongation (Schaeffel and Howland

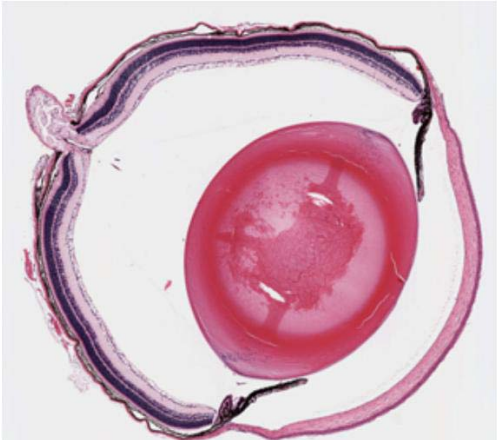

Figure 1.4 Section of an enucleated mouse eye (Adapted from www.uhnres.utoronto.ca)

2003; Schaeffel et al. 2004). Reports of refractive error obtained in form deprived mice eyes by photoretinoscopy indicate the development of low amounts of myopia and inhomogeneous distribution of the pupil brightness, indicating the presence of high order aberrations (Schaeffel et al. 2004). Figure 1.4 shows a section of an enucleated mouse eye.

1.1.7. Relating experimental myopia to human myopia

The normal refractive development in most animals appears to parallel human refractive development (Norton 1999): the distribution of refractive values, the progression toward emmetropia, the decrease of variability of refractive values (Norton and McBrien 1992;Pickett-Seltner et al. 1988; Prusky et al. 2004), the vitreous chamber elongation (Wallman et al. 1981), and the thinning of the choroid (McBrien 1998) are similarly found in myopic humans (Curtin 1985). Other changes are specific to each species: corneal curvature and increased variability in lens power in chicks (Troilo et al. 1995; Priolo et al. 2000), reduced lens thickness in tree shrews (McKanna and Casagrande 1978; Norton and Rada 1995). Infants with congenital cataracts, corneal opacities or retinopathy of prematurity typically develop myopia (Robb 1977; Hoyt et al. 1981; Gee and Tabbara 1988) suggesting that form deprivation early in life also results in myopia development in humans.

The hyperopic defocus in the accommodated eye during near tasks has been related to the hyperopic defocus imposed with negative lenses in animal models, which results in myopia development. However, findings in animal models showing that brief exposures to normal viewing counteract the effect of hyperopic defocus and prevent from myopia development questions that substantial near work is the major cause for myopia development, since that would only occur if near work was conducted continuously. Results from animal studies have consequences for possible myopia treatment in humans. For example, myopic defocus has been shown to have a protective role in myopia development (Flitcroft 1998), as it has been shown to protect against myopia in a chick model (Zhu et al. 2003) , and therefore it could be a possible treatment for myopia in humans.

In summary, animal models have revealed the existence of an active emmetropization mechanism controlled by visual experience. Experiments on animal models help to understand the role played by the visual environment in the development of myopia and to separate causal relationships from changes which are consequence of axial elongation and could explain some clinical observations in myopic humans. Studies are therefore needed to investigate which physiological properties of myopic eyes are a cause or a consequence of myopia, and to explore the causes of retinal degradation and their impact on myopia development, and the interactions between the different factors (genetic, environmental) involved in this process.

1.2. Ocular optical quality: Aberrations

Although in humans the most important refractive defects are spherical and cylindrical refractive errors, high order aberrations are also present, which also degrade retinal image quality. If the lenses of the eye (cornea and lens) were perfect, light from a point source at infinity would converge on the focal point at the retina. In the opposite sense, a point of light in the retina would emit a spherical wavefront, exiting the eye as a plane wave front. Phase deviations from these perfect wavefront, measured at the pupil plane, are known as wave aberrations.

Figure 1.5 represents a non-aberrated (top) and an aberrated (bottom) eye. In the non-aberrated eye images are only limited by diffraction. In aberrated eye, forms distorted images of an object; rays entering the eye through different pupil positions get deviated from the chief ray at the retinal plane, and a distorted wavefront travels toward the retina. Figure 1.6 also represents a non-aberrated and an aberrated eye. A point of light in the retina is emitted as a spherical wavefront in non-aberrated eye, but as an aberrated spherical wavefront in aberrated eye.

Wave aberrations are typically estimated from local ray aberrations, by either measuring the deviations of incoming beams from the principal ray

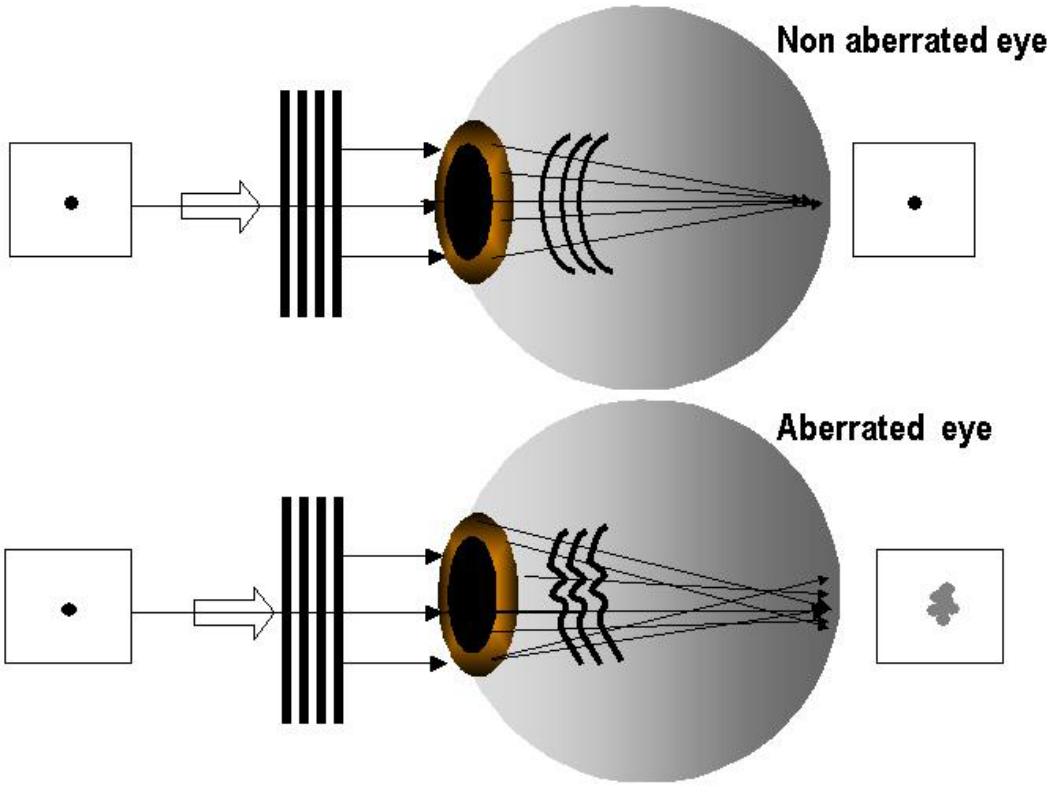

Figure 1.5 Image of a point source formed by a non-aberrated and an aberrated eye. For an incoming wavefront, rays entering the eye through different pupil positions converge on the same retinal location, and a spherical wavefront travels toward the retina.

(ingoing aberrometers such as spatially resolved refractometer or laser ray tracing, Figure 1.5)or sampling the wavefront as it exits the eye (such as Hartmann-Shack wavefront sensor, Figure 1.6). The magnitude of ray aberration for each pupil position is proportional to the local slope (derivatives) of the wavefront aberration, where □, □ are the horizontal and vertical coordinates of the ray aberration, Rp is the pupil radius, and W(x,y) is the wave aberration.

$$\alpha = \frac{1}{R_p}\frac{\partial W(x,y)}{\partial x}$$

$$\beta = \frac{1}{R_p}\frac{\partial W(x,y)}{\partial y} \qquad \text{Eq (1.1)}$$

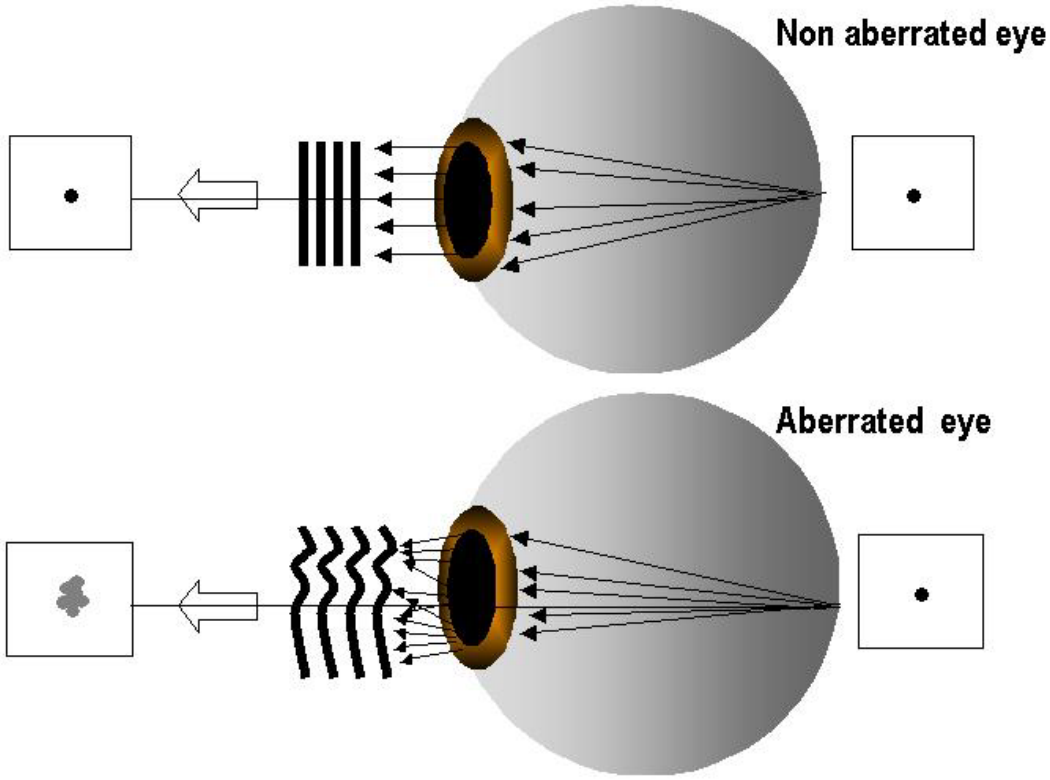

Figure 1.6 A light spot projected by the retina exiting the eye (non-aberrated, top, and aberrated eye, bottom).

1.2.1. Zernike polinomials

A Zernike polynomial expansion (ISO, "Standard 10110.A3 - The Zernike polynomials.") is the recommended basis for describing wave aberrations over circular pupils (Thibos et al. 2000), and will be the one used in this thesis. Among other advantages, Zernike polynomials are an orthogonal basis over the unit circle, and the lower orders represent typical refractive errors. Normally, Zernike coefficients are indexed using a double-index scheme: Z_0^0, Z_1^{-1}, Z_1^1… The subindex indicates the aberration type (order) and the upper index the individual aberrations in each order. Second order aberrations include defocus and astigmatism; third order aberrations include coma, and fourth order aberrations include spherical aberration. Figure 1.7 represents a set of Zernike polynomials up the 4th order.

$$W = \sum_{n}^{m} C_n^m Z_n^m$$

$Z_1^{-1}=x$
$Z_1^1=y$

$Z_2^{-2}=x^2-y^2$
$Z_2^0=2x^2+2y^2-1$
$Z_2^2=2yx$

$Z_3^{-3}=x^3-3y^2x$
$Z_3^{-1}=3x^{3+}3xy^2-2x$
$Z_3^1=3yx^2+3y^3-2y$
$Z_3^3=3yx^2-y^3$

$Z_4^0=1-6y^2-6x^2+6y^4+12x^2y^2-6x^4$

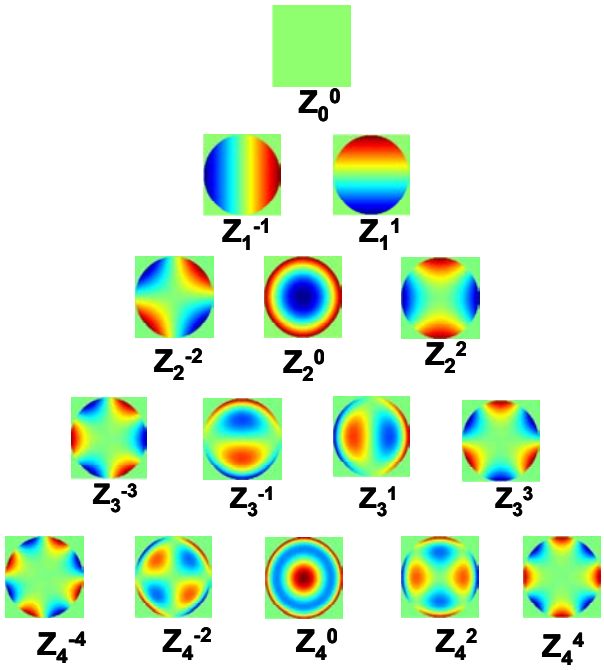

Figure 1.7 Zernike polynomial pyramid up to the 4th order

Aberrations (both magnitude and distribution) have been shown to vary widely across the population. Ocular aberrations in humans have been shown

to increase with age and change with accommodation (He et al. 1998; Mclellan et al. 2001; Artal et al. 2002; Fujikado et al. 2004; Wang and Koch 2004; Plainis et al. 2005; Chen et al. 2006). High order aberrations have been shown to increase with standard LASIK refractive surgery (Campbell et al. 1999; Moreno-Barriuso et al. 2001) and, as will be later shown, higher amounts of aberrations tend to be more associated with higher amounts of myopia.

1.2.2. Optical quality metrics

Several metrics estimated from the wave aberration have been proposed to describe the optical quality of the eye, with particular attention to those that correlate best with visual function (Thibos et al. 2000). For the purposes of this thesis, we will use metrics computed directly from the Zernike coefficients, as well as retinal image plane metrics.

1.2.2.1. RMS

The root-mean-squared (RMS) value of a particular term or mode is the RMS contribution of that term or the individual coefficients of that mode. The RMS expresses the deviation averaged over the entire wavefront. Making use of the orthogonality and normalization properties of the Zernike coefficients, the wavefront variance (RMS squared) can be simply derived from the squared coefficients.

1.2.2.2. Point Spread Function (PSF), MTF and OTF.

The point spread function is the image of a point object through the optical system. It is calculated as the squared magnitude of the inverse Fourier transform of the pupil function *P(x,y)*:

$$P(x,y) = A(x,y)e^{ikW(x,y)} \qquad \text{(Eq. 1.2)}$$

Where k is the wave number (2π/wavelength), *A(x,y)* is an apodization function (when the waveguide nature of cones is considered) and *W(x,y)* is the wave

aberration. *P(x,y)* is zero outside the pupil. The Optical Transfer Function (OTF) is the Fourier transform of the point-spread function (PSF) and the modulation transfer function (MTF) is the modulus of the OTF. The MTF represents the contrast loss as a function of spatial frequency, and accounts for the optical degradation imposed by both diffraction and high order aberrations.

1.2.2.3. Strehl ratio

Strehl ratio represents the maximum value of the PSF, normalized to the maximum of the diffraction-limited PSF, or equivalently, the volume under the OTF normalized by the diffraction-limited OTF.

1.2.3. Aberration measurement techniques

Aberrometers measure the ray aberration as a function of pupil position. This is proportional to the local slope (derivative) of the wave aberration, from which the wave aberration can be easily retrieved. Ocular aberrations are measured using different techniques (Spatially Resolved Refractometer –SRR-(He et al. 1998; Burns and Marcos 2000; Burns and Marcos 2001), Laser Ray Tracing –LRT-(Moreno-Barriuso et al. 2001), Hartmann-Shack –HS- (Liang et al. 1994; Thibos et al. 1999; Moreno-Barriuso et al. 2001), among others. Although all techniques measure ray aberrations, differences across instruments rely on either the psychophysical (requiring the participation of the subject) or objective (based on light reflected off the retina) nature of the technique; “ingoing” (aberrations measured as the test beam goes into the eye) or “outgoing” (as the wavefront emerges from the eye) direction of the measurement. For example, the SRR is a psychophysical and “ingoing” technique, as a test beam enters the eye through a series of entry pupil positions while a fixation cross is perceived through a centered pupil. The subject aligns the spot with the reference cross-target. The tilted angle is proportional to the derivative of the wave aberration at each entry pupil. The LRT is an objective “ingoing” technique based on the same principle, but now the deviation of the test ray from the principal ray is detected by a CCD camera placed on a plane conjugate to the retina. An x-y scanner synchronized to the

CCD camera allows to sample sequentially a series of pupil positions in a brief period of time. The Hartmann-Shack (HS) is an “outgoing” aberrometry technique based on the measurement of ray deviations at different pupil positions of a wave reflected by the retina from a light point source. A microlens array, placed on a pupil conjugate plane, focuses multiple spots (one per lenslet) onto a CCD camera. Each lenslet samples a small part of the wave-front corresponding to a certain pupil location. A regular pattern of spots would be obtained for an ideal non aberrated eye, while ocular aberrations produce an irregular pattern of spots. The deviation of each spot from the ideal position is proportional to the local derivative of the wave aberration. Figure 1.8 shows a schematic diagram of the Hartmann-Shack spot image capture in a non aberrated eye (regular pattern) and an aberrated eye (distorted pattern).

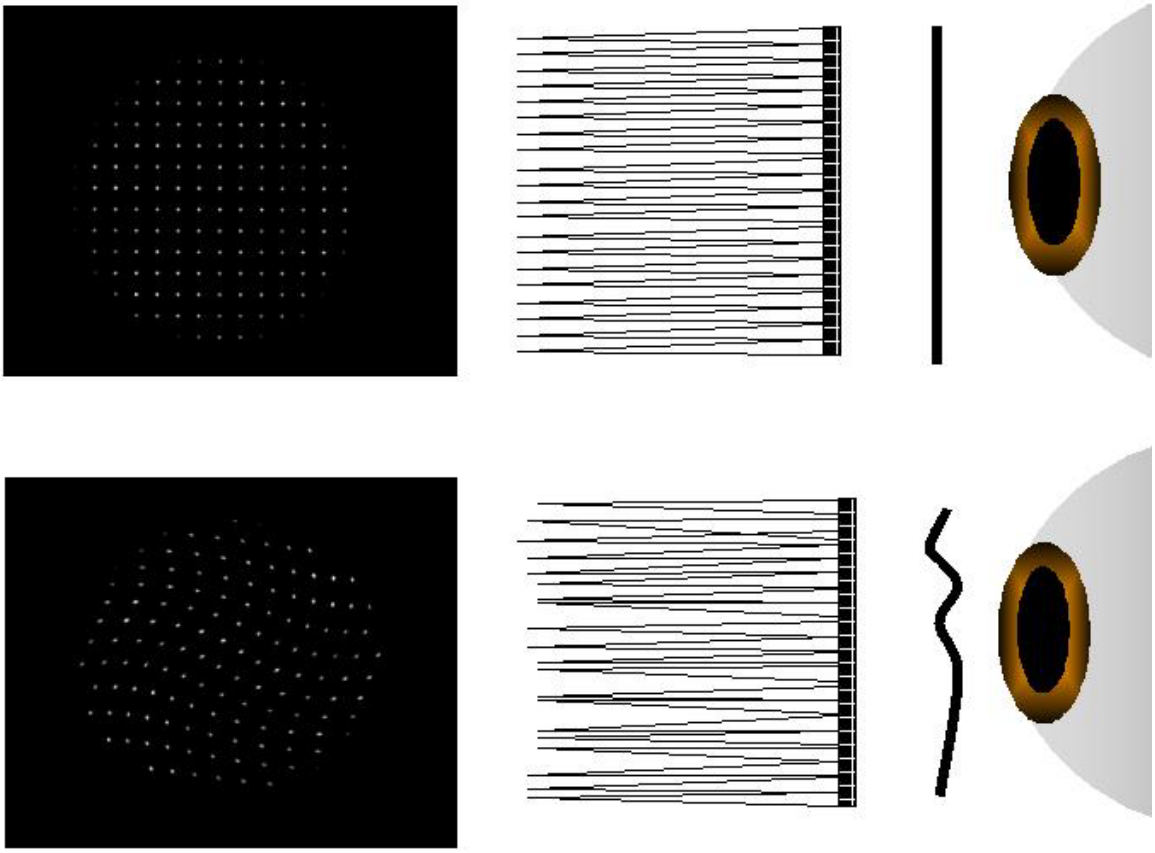

Figure 1.8 Harmtmann Shack pattern from non aberrated (upper) and aberrated eye (lower).

The advantage of this technique over the others described is that only one snapshot is necessary to obtain the wave aberration for the entire pupil. HS aberrometry is therefore an objective and fast technique. For normal human eyes it has been shown that the SRR, LRT and HS provide similar measurements of wave aberrations (Moreno-Barriuso et al. 2001; Marcos et al. 2002; Llorente et al. 2003).

In this thesis we developed a Hartmann-Shack aberrometer, which we considered to be the most adequate approach to measure aberrations in animal models, because it allows faster measurements and does not require a great collaboration from the subject.

1.2.4. Human ocular aberrations

The aberrations of the human eye have received increased attention in recent years. The availability of more efficient and reliable aberrometers, has allowed large population studies in normal subjects (Porter et al. 2001; Castejon-Mochon et al. 2002; Thibos et al. 2002), as well as the study of the relationship of ocular aberrations with different conditions (refractive error (Marcos et al. 2000; Marcos et al. 2001; Cheng et al. 2003; Llorente et al. 2004), aging (McLellan et al. 1999; Mclellan et al. 2001; Marcos et al. 2004), accommodation (He et al. 2000), keratoconus (Barbero et al. 2002), Hilos (Barbero 2003; Marcos et al. 2005; Marcos et al. 2007), refractive surgery (Moreno-Barriuso et al. 2000; Marcos 2001; Marcos et al. 2001; Marcos et al. 2001; Moreno-Barriuso et al. 2001; Llorente et al. 2004) etc. The ocular aberration pattern is the result of the contribution of corneal and crystalline lens aberrations and their interactions. The geometrical shapes (curvatures and asphericity) of the corneal and crystalline lens surface, and very likely the gradient index distribution of the crystalline lens, contribute to spherical aberration. Irregularities of the cornea, as well as the relative position of the optical elements (i.e. off-axis location of the fovea, pupil displacement) contribute to high order aberrations (Marcos et al. 2001).

Total ocular aberrations in the young normal eye are typically higher than corneal aberrations alone, indicating that internal optics (mainly crystalline lens) compensates part of the aberrations of the cornea. This interaction produces a balance of astigmatism and high order aberrations (Artal and Guirao 1998; Kelly et al. 2004). With age, the loss of this compensation due to changes primarily in the crystalline produces degradation of the ocular optics (Mclellan et al. 2001; Artal et al. 2002). This balance between corneal and internal aberrations has been debated to arise from either an active or a passive mechanism. An active and visually guided process would require a plasticity of the eye to reduce total aberrations and improve retinal image, i.e. an emetropization of high order aberrations by fine tuning of ocular surface geometry an positioning, somewhat similar to the fine tuning between optical power and axial length occurring in the emmetropization for refractive error. On the other hand, passive mechanism would result from purely geometrical factors (Artal et al. 2006; Marcos et al. 2008). In humans, some cross-sectional studies including infants and children report a decrease in aberrations with age, and suggest an emmetropization of high order aberrations. However, other studies of ocular aberrations in infants have been shown relatively low amounts of aberrations, suggesting that if there is a compensating process (visually guided or not) this is likely not very important (Wang and Candy 2005).

1.2.5. High order aberrations and myopia

Several studies have reported that (high) myopes have significantly higher amount of higher order aberrations than emmetropes, and shown significant correlations between spherical error and myopia (Collins et al. 1995; He et al. 2002; Marcos et al. 2002; Paquin et al. 2002). Spherical aberration is low in low myopes (<6 D) because there is a balance between corneal and internal (lens and others) aberrations. Marcos et al. (2002) showed several changes as a function of myopia: corneal spherical aberration increase toward more positive values and internal spherical aberration changed toward more negative values, keeping total spherical aberration constant. Cross-sectional as well as longitudinal studies show increased asphericity in higher myopic eyes

(Carney et al. 1997; Horner et al. 2000). Applegate (Applegate 1991) found coma and spherical aberrations in myopic eyes. Llorente et al. (Llorente et al. 2004) found significant differences in the optical and structural properties (corneal asphericity, corneal and ocular spherical aberration, apart from axial length) between hyperopes and myopes. While other studies did not found significant differences in myopes than emmetropic eyes (Cheng et al. 2003; He et al. 2005), there is a general agreement that high myopic eyes tend to be more aberrated.

1.2.6. Aberrations: Animal models.

As described before, defocus and astigmatism (second order aberrations) have been widely studied in a large number of species, particularly those used as experimental models for myopia. However, despite the fact that high order aberrations can degrade substantially the retinal image, and retinal image quality plays a role in emmetropization little is known on the optical quality and aberrations in animal models.

The first reports on chick eye is optical quality are controversial. A conference abstract reported that chicks showed worse optical quality and higher amounts of aberrations than humans (Thibos et al. 2002). However, this is in contrast with the good retinal image quality reported by Colleta et al. (Coletta et al. 2000; Coletta et al. 2003) using a double-pass technique. During this thesis, and posterior to the publication of our papers, measurements of ocular aberrations in chicks (Kisilak et al. 2006) were presented, confirming a an improvement of optical quality with age in chicks, and excellent optical quality at 2-weeks of age.

To our knowledge, no report of the aberrations and optical quality in the mouse eye has been presented before. The observations of inhomogeneous photoretinoscopic reflexes (Schmucker and Schaeffel 2004) and very recent reports of retinal imaging in mice using adaptive optics (Biss et al. 2007) confirm our results of poor optical quality in the mouse. In general, the rodent eye optical quality is believed to be highly degraded, according to double-pass

estimates (Artal et al. 1998) and ray tracing simulations using parameters from enucleated eyes (Hughes 1979) in rats. Huxlin et al. measured ocular aberrations in cats and found similar amounts than in humans (Huxlin et al. 2004). In primates, measurements of optical aberrations in marmosets showed that levels of higher-order aberrations of the marmoset eye are higher than in humans eyes but significantly lower than in anaesthetized animals, emphasizing the importance of measuring aberrations under awake conditions (Coletta et al. 2001; Coletta et al. 2003). Also in the marmoset, wavefront aberrations were shown to decrease with age and to be higher in myopic eyes (Coletta et al. 2004). Very recently, aberrations have been reported in the Rhesus Monkeys (Ramamirtham et al. 2004; Ramamirtham et al. 2006).

1.2.7. Why is it relevant to test relationships between aberrations and myopia?

There is evidence for potential relations between aberrations and myopia:

1. A degraded retinal image quality during ocular development produces myopia. As aberrations affect the visual experience degrading retinal images they could play a role in myopia development (Wilson et al. 2002).

2. There is a correlation between aberrations and myopia. Myopes seem to have higher aberrated eyes (Atchison et al. 1995; Marcos et al. 2002; Paquin et al. 2002).

3. An increase of corneal aberrations have been shown to occur for substantial time after extended reading, particularly in myopes and Asian subjects. This could represent a mechanism explaining the relationship between myopia development, near tasks and higher prevalence in Asian populations (Buehren et al. 2003).

4. There are clinical evidences of slower myopia progression in RGP (Rigid Gas Permeable) contact lens users, although there is controversy whether

this is purely a consequence of corneal shaping (Perrigin et al. 1990; Khoo et al. 1999). On the other hand, it has been shown that high order aberrations decrease when RGP contact lenses are worn (Dorronsoro et al. 2003).

5. The effect of form deprivation and lens treatments in myopia animal models will be limited by the tolerance to blur. Depth of focus is highly affected by ocular aberrations. However, the ocular aberrations in widely used animal models for myopia (such as the chick) or potential animal models for myopia (such as the mouse) have not been studied.

6. Longitudinal measurements of ocular aberrations during normal development and during development of myopia have never been studied. Those measurements will allow to test the hypothesis of active (visually guided) or passive (geometrical) mechanisms for the fine tuning of ocular components, particularly in animal models whose visual experience can be altered.

1.3. Hypothesis and goals.

The main goal of this thesis is to test relationships between ocular aberrations and myopia development in animal models (chicks and mice), which allow longitudinal measurements in relatively short periods of time and manipulation of visual experience. We will test the hypothesis that aberrations are a consequence of myopia, and that aberrations are a cause for myopia. We will also investigate the sources of optical aberrations in the normal and myopic eye of these models.

The specific goals of this thesis are:

1. Development of custom technology to measure optical aberrations and ocular biometry in animal model eyes. We developed a Hartmann-Shack wavefront sensor to measure total eye aberrations, a corneal keratometer to measure corneal radius of curvature, and adapted an

ultrasound biometer and streak retinoscopy to in vivo measurements in animal models (Chapter 2).

2. Measurement of optical quality in the normal chick eye, and changes of optical aberrations with development. We will address the question whether aberrations follow an emmetropization process, similar to refractive error (Chapter 3).

3. Measurement of optical aberrations and ocular biometry during myopia development in a form-deprived chick model. With these measurements we will assess if increased aberrations are associated with myopic eyes, i.e. if aberrations are a consequence of myopia (Chapter 3).

4. Development of a refractive surgery myopic chick model. We will investigate the potential of altering the emmetropization process by reshaping the cornea (nominally imposing a hyperopic defocus). We will study optical aberrations and ocular biometry in this model and will assess if increased aberrations can result in increased ocular elongation, i.e. if aberrations can be a cause of myopia (Chapter 4).

5. Measurement of optical aberrations in the wildtype mouse eye. We will assess relationships between the refractive error in the mouse and retinal image quality, as well as the impact of optical depth of focus on the possibilities to induce myopia (Chapter 5).

6. Development of computer eye models for the chick and mouse eye. We will explore the relationships between biometry and structure of the ocular components and the measured optical aberrations to understand the sources of aberrations in the developing (1-14 days) chick eye (normal and myope) and the 28-day old mouse eye (Chapter 6).