# Age-Related Changes of the Human Eye

*Edited by*

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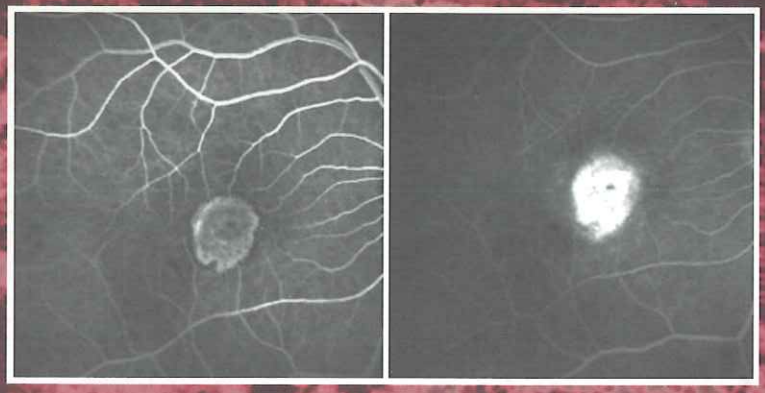
Luciano Cerulli



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*Cover illustration*: Figure 1, Chapter 2, by Janos Feher and Zsolt Olah, "Electron microscopy of aged orbicular muscle fibers." Figure 3a, Chapter 12, by Susanne Binder and Christiane I. Falkner-Radler, "Early face angiogram of a classic CNV."

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# Contents

# Chapter 5
# The Aging of the Human Lens

**Jorge L. Aliò, MD, PhD, Alfonso Anania, MD, PhD, and Paolo Sagnelli, MD**

**Abstract** Age-related lens changes include: a) the progressive increase in lens mass with age, b) changes in the point of insertion of the lens zonules, and c) a shortening of the radius of curvature of the anterior surface of the lens. With age, there is also decreased light transmission by the lens associated with increased light scatter, increased spectral absorption—particularly at the blue end of the spectrum—and increased lens fluorescence. Besides these physiological modifications, we must take into consideration the additional effects caused by exposure to external physical and chemical agents such as ultraviolet rays and drugs, which lead to considerable densitometric changes and consequently to modifications in optical lens quality. At present, new instruments allow the analysis, in clinical practice, of qualitative and quantitative alterations of the lens that occur with aging, confirming objectively the degradation of the optical quality of the crystalline lens.

**Keywords** crystalline, Lens, age related changes, human eye, cataract.

## Introduction

The crystalline lens of the eye is a principal component in the process of vision. To perform its role, the lens must be transparent and also have the capacity to rapidly alter its shape as it transitions between focusing on near and distant objects. Gross (light and scanning confocal microscopy) and ultrastructural (scanning, transmission, and freeze-etch electron microscopy) analysis of all vertebrate lenses reveals that lenses are composed of exceedingly long fiber-like cells that are of uniform cross-sectional shape (hexagonal) and size. These microscopic techniques also show that, in general, as these fibers are formed throughout life, they are overlain, in register, as age-related concentric growth shells. Thus, it has been proposed that the highly ordered arrangement of lens fibers contributes to lens transparency by transforming the individual fibers into a series of coaxial refractive surfaces.

Water and protein loss, modifications to membrane lipids, and protein modifications can result in the progressive increase in compaction folds. It follows that substantial senescent alterations in the structure of the embryonic and fetal nuclear fibers

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would lead to degradation of lens optical quality, especially since these fibers are located entirely within the region defined by the pupillary margin. While significant compaction of nuclear fibers occurs along the antero-posterior axis with aging, an even greater degree of compaction occurs in nuclear cataract formation. Therefore, there is convincing evidence that a senile cataract is an exaggerated final stage of age-related lens changes.

Clinical observations of aged lenses show increased light scatter even without overt visual impairment, and it has been demostrated that there is a degradation of the optical quality of the crystalline lens with aging that is associated with morphological changes such as thickness and density. The process of nuclear fiber compaction is probably multifactoral, as the lens is exposed to the cumulative effects of radiation, oxidation, and post-translational protein modifications. Additional changes include: a) the progressive increase in lens mass with age, b) changes in the point of insertion of the lens zonules, and c) a shortening of the radius of curvature of the anterior surface of the lens. With age, there is also decreased light transmission by the lens associated with increased light scatter, increased spectral absorption—particularly at the blue end of the spectrum—and increased lens fluorescence. Besides these physiological modifications, we must take into consideration the additional effects caused by exposure to external physical and chemical agents such as ultraviolet rays and drugs, which lead to considerable densitometric changes and consequently to modifications in optical lens quality. At present, new instruments allow the analysis, in clinical practice, of qualitative and quantitative alterations of the lens that occur with aging, confirming objectively the degradation of the optical quality of the cristalline lens.

## Lens Embryology

An in-depth study of lens embryology facilitates the understanding of fibers and suture development. [1-4] Lens formation is the result of a series of inductive processes.[5,6] The lens placode appears on the optic vesicle that protrudes from the forebrain, around the 25th day of gestation.[7] It is a thickening of the surface ectoderm[8]—a single layer of cuboidal cells—that invaginate into the neural ectoderm of the optic vesicle as the lens pit, becoming free from the surface by the 33rd day[9] (see Fig.5.1).

The cells at the anterior pole of the lens vesicle remain as epithelial cells—the cell number is controlled by apoptosis.[10] The posterior cells elongate as primary lens fibers that obliterate the lumen of the lens vesicle[11]—the retina largely determines this cytodifferentiation. The tiny developing lens is surrounded by a basement membrane that will become the lens capsule and is filled with nearly structureless primary lens fibers.[12] These cells expel their nuclei, mitochondria, Golgi bodies, and endoplasmic reticulum. This structure becomes a spherical, optically clear embryonic nucleus of 0.35 mm in diameter,[11] which stays unchanged throughout life[7] and is seen inside the Y sutures in the fully developed eye. In an embryo of

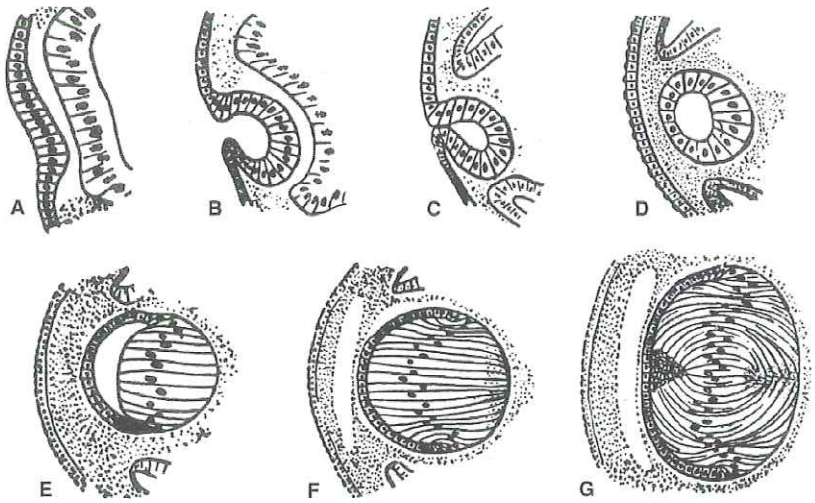


**Fig. 5.1** Scheme to show the development of the lens. (A) Lens thickening. (B) Lens pit. (C) Lens pit closing. (D) Lens vesicle. E) Elongation of cells of the posterior wall of the lens vesicle. (F) Obliteration of the cavity of the lens vesicle by cells of the posterior wall. (G) Formation of lens sutures by the meeting of fibers developed in the equatorial region (Mann I: The Development of the Human Eye. Grune Stratton, New York, 1950)

23 mm, equatorial *secondary lens fibers* derived from the anterior epithelium migrate forward under the anterior epithelium and backward directly beneath the capsule—meeting at the sutures that can be seen easily with slit-lamp microscopy as an upright anterior Y and an upside-down[7] posterior Y. The limbs of the Ys are often branched.

A large number of recent studies have focused on the involvement of polypeptide growth factors and cytokines in lens differentiation. These factors include fibroblast growth factors (FGFs), insulin and insulin-like growth factors (IGFs), transforming growth factors (TGFs),[13] platelet-derived growth factors (PDGFs), epidermal growth factors (EGFs) and several cytokines, including macrophage-migration inhibitory factor (MIF), and tumour necrosis factor-alpha (TNFα).[14] After birth, the equatorial fibers grow to form the cortex, meeting at more complex and less well-marked sutures—this growth continues until very shortly after death.

The tertiary vitreous condenses within the space between the ciliary body and the lens equator, forming the suspensory ligament of the lens at the fifth month of gestation.[7] The developing lens requires nutrition that is obtained through the tunica vasculosa lentis, which is a vascular network supplied posteriorly by the hyaloid artery (a branch of the primary dorsal ophthalmic artery) and anteriorly from an anastomosis with vessels in the pupillary membrane.[7] The *tunica vasculosa lentis* is first seen at about 35 days, and is most prominent at 65 days. It gradually regresses at about 85 days, and by term birth, only whispy remnants of the pupillary membrane are left, with a vestigial hyaloid artery (known as a Mittendorf's dot) attached to the axial posterior surface of the lens.[7,15]

## Morphology of the Human Lens

Roughly speaking, vertebrate lenses are asymmetrical, oblate spheroids of variable size and spheroidicity (see Fig. 5.2).

The lens, encaved in an elastic capsule, consists of: a) an anterior monolayer of epithelial cells, the pre-equatorial members of which exhibit mitotic activity throughout life; b) a superficial layer of elongating, differentiating, and maturing secondary fibers, and c) the main lens body that consists of fully matured primary (embryonal nucleus) and secondary fibers[16] (see Fig. 5.3). These fibres are characterized by a high protein content (35-40%), by the absence of nuclei, mitochondria, lysosomes, ribosomes and endoplasmatic reticulum, and are surrounded by increasingly less permeable membranes. All lens fibers are mutually anchored, securing minimal extracellular space, thus minimizing differences in the refractive index from fiber to fiber.

Many electron microscopic studies have been performed: initial investigations were undertaken by Wanko and Gavin[17] and Cohen[18] on mammals and humans, respectively. Many more followed describing fibers from a variety of mammals, including rat,[19,20] rabbit,[21] pig,[22] monkey,[23-26] and human.[24,27-32]

Recent technical advances in fixation methods for scanning electron microscopy (SEM)[25] and transmission electron microscopy (TEM)[33] have made it possible to analyze differentiated fibers in all regions of the lens. The cross-sectional area of fiber cells varied from region to region, with the smallest areas found in the compressed adult nuclear region and the largest found in the central embryonic

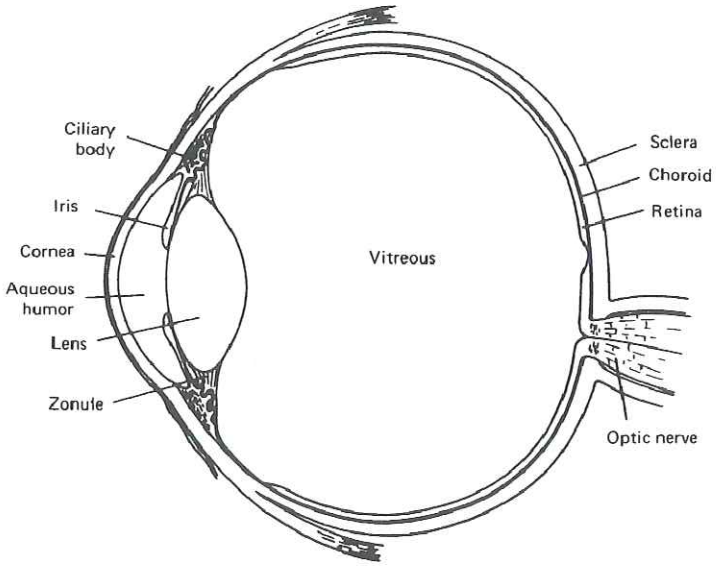


**Fig. 5.2** Diagrammatic section of the eye

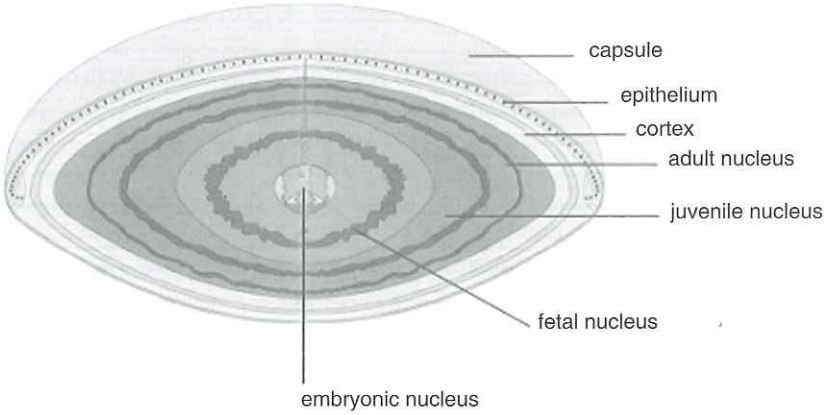


**Fig. 5.3** Diagram of an aged normal human lens, approximately to scale. The complex suture pattern is not shown. The epithelium and capsule are enlarged for clarity. (Modified from: Morphology of the Normal Human Lens. VL Taylor, KJ Al-Ghoul, CW Lane, VA Davis, JR Kuszak, and MJ Costello Investigative Ophthalmology & Visual Science, June 1996, Vol. 37, No. 7)

nucleus. Cellular organization was most ordered in the cortex, where radial cell columns were found. By contrast, cells were more irregularly packed in the center of the lens, where no apparent arrangement was observed. The cytoplasm of intact cells was smooth and homogeneous in all regions analyzed. The hardened nuclear core corresponds to the fetal and embryonic nuclei, the outer soft layer corresponds to the cortex, and the layer between, with intermediate hardness, corresponds to the adult and juvenile nuclei. The term *epinucleus* has been used to describe this intermediate layer of tissue corresponding to the adult plus juvenile nuclei.[34]

In the adult nucleus, the deep cortical fibers are compacted as they are internalized and become part of the adult fiber mass. Changes in shape occurred in the absence of significant membrane loss or turnover, which resulted in an increase of membranous undulations. The small variation in size of the adult nuclear fibers implies that cells seem to be affected equally by this compaction. Both mechanical compression (caused by the continual deposition of new fibers) and dehydration (regulated by the osmotic properties of the crystallins) are involved in this process of compaction.[35-37] Cells in the juvenile nucleus were, on average, twice the size of adult cells and approximately half the size of deep cortical cells. This suggests that juvenile cells are also compacted, but not to the degree that the adult cells are. Different developmental events or protein modifications may occur during the formation of juvenile cells, in contrast to adult cells, because they are formed before puberty. The crystallins of the juvenile cells may resist compaction because initially they may be more dehydrated than those of the adult cells.[16] Alternatively, some cells may grow in a larger form, or cells may fuse together during elongation.[38,39] Both these events would produce cells with larger areas.

Radial cell columns could be detected in adult and juvenile regions but were difficult to visualize because of the intricate folding of the membranes. Together,

the adult and juvenile nuclei comprise an annular ring that undergoes significant compaction with age and, importantly, is flanked by regions that are compacted relatively less with age. Biochemically, a correlation was observed between the protein modifications of lens crystallins and the lens regions.[40]

At the molecular level, packing of lens crystallins has been shown in the last decade to be random, with no evidence of crystalline regularity. Based on x-ray diffusion measurements *in vitro*, monomers (or small aggregates of crystallins) are thought to be associated closely. Therefore, at a critical distance, the light scattering of the concentrated protein solution decreases significantly, leading to transparency.[41] These long-term changes in radial cell thickness place constraints on cell shape, size, and packing. Possible cell-to-cell fusion also influences packing, especially near the poles and at the sutures. The simplest explanation for the observed changes in this annular ring is that the cytoskeleton is lost in the last stages of cortical differentiation and that the nuclear fibers are squeezed under pressure against a harder nuclear core (fetal and embryonic nuclei), resulting in gradual dehydration of crystallins and cellular compaction. Alternatively, self-association of the crystallins and the resultant decrease in osmotic pressure may induce dehydration of the cytoplasm.[37]

## *Lens Capsule*

### Anatomy of the Lens Capsule

The position of the lens in the optical system of the eye is assured by the attachment of the zonular fibers to the lens capsule, as well as the support provided by the vitreous and iris. The lens capsule and the zonular fibers constitute the link between the lens fiber substance and the ciliary muscle, and thus play an important role in transmitting the force of ciliary muscle contraction to change the shape of the lens fiber substance that is essential for accommodation. The geometrical pattern of the zonules is complex and varies significantly with age.

The zonular fibers attach to the lens in three separate groups: an anterior, an equatorial and a posterior group.[42] The attachment of the zonules to the lens is known to involve penetration of zonular fibers into the superficial lens capsule.[43,44] The anterior zonules attach to the outer surface of the lens capsule around the lens periphery in a rather broad zone, which increases with age from about 0.25–1.2 mm due to a relative inward displacement of the zonular insertion in the lens capsule.[45]

A zonular lamella has been described by many observers as a thin membrane surrounding the lens capsule.[46,47] Its existence, however, has been a matter of dispute and, according to Hogan,[43] the zonular lamella exists only in the equatorial region where the zonular fibers attach to the capsule. In an electron microscope study, Seland[44] confirmed the presence of a fibrillar surface layer in newborns, but showed that this fibrillar surface layer retracted from the anterior pole between the ages of 6 and 17. On the posterior capsule, a fibrillar surface layer does not seem to be a

constant feature, apart from the posterior zonular attachment.[43,44] The lens capsule encloses the lens fibers. The inner surface of the anterior lens capsule is in immediate contact with the lens epithelium, while the posterior lens capsule is in contact with the most superficial part of the posterior lens fibers.

## Ultrastructure of the Lens Capsule

The lens capsule is the thickest basement membrane in the body. When studied under light microscopy the lens capsule appears dense and homogeneous. Under the electron microscope, the lens capsule is found to be made up of parallel lamellae, more tightly packed toward the outer surface.[48] The lamellar structure of the lens capsule seems to disappear with age. In the posterior capsule, it disappears in childhood. In the anterior capsule it starts disappearing from the anterior pole in adulthood but persists in the equatorial and preequatorial regions corresponding to the metabolically most active part of the lens epithelium.[43,44,49]

Ultrastructurally, the support of the lens capsule is type IV collagen, which interacts with other glycoproteins and proteoglycans to form an extracellular matrix.[50-53] Type IV collagen is found only in basement membranes, and it is the only collagen that has been shown definitively to be present in basement membranes. Immunoelectron microscope studies of the lens capsule, however, also seem to show the presence of collagen types I and III.[54,55] Type IV collagen plays an important role in the formation of a resilient, three-dimensional molecular network.[56,57]

Compared to the fiber-forming collagens, the type IV collagen molecule is longer, more flexible, and contains frequent interruptions by non-collagenous sequences. The type IV collagen molecule possesses distinct end-region domains and exhibits several binding interactions that enable formation of a stable lattice-like network.

### Growth and Thickness of the Lens Capsule

The lens capsule continues to grow throughout most of life, growing in thickness anteriorly and increasing in surface area to adjust to the increasing volume of the lens. The anterior lens capsule is produced by the lens epithelium[58-60] and therefore reflects the activity of the epithelial cells, which undergo apparent morphological changes with aging. The epithelial cells become flattened—the number of organelles is reduced and become more difficult to distinguish because of an increasing density of the cytoplasmatic matrix.[49,61] The regional variation in thickness of the lens capsule changes markedly with age, which suggests a continuous modeling of the lens capsule with age. In contrast to the anterior lens capsule, which is synthesized by the lens epithelium and continues to grow and increase in thickness throughout most of life,[44,62,63] the human posterior lens capsule loses its epithelial cells in fetal life.[43]

It has been suggested that the posterior lens capsule is synthesized and secreted by nucleated cortical lens fibers, or synthesized by anterior epithelial cells and

secreted into the posterior aspects of the lens during the first part of life, after which the production of the posterior lens capsule is supposed to cease. Capsular thickness is not uniform but varies according to age and the location at which the measurements are taken.[44,62-64] Fisher and Pettet[62] examined unfixed lens capsules and found that thickness of the neonatal lens capsules varied from 3–5 μm at the posterior pole to approximately 11 μm at the equator. Thickness of the anterior pole and thickness beneath the insertion of the anterior zonules were found to increase with age, whereas thickness of the posterior lens capsule was found to be constant.

In pre-presbyopic eyes, the lens capsule was found to be thickest at the equator, whereas in old adults, the lens capsule was found to be thickest beneath the insertion of the anterior zonular fibres. Seland[44] examined fixed lens capsules and found that the neonatal lens capsule had a uniform thickness of about 4 μm and that thickness of the anterior as well as the posterior lens capsule increased gradually with age—most markedly in the peripheral region of the anterior lens capsule where thickness reached as much as 30 μm. In all age groups, the thinnest part of the lens capsule was found at the posterior pole.

The thickest part of the lens capsule in pre-presbyopic lens capsules was found to be in the mid-periphery of the anterior and posterior lens capsule. Thickness of the anterior peripheral zone was found to increase with age throughout the lifespan, whereas thickness of the posterior peripheral thickened zone was found to increase only in pre-presbyopic lens capsules and to decrease in the older age group.

## Mechanical Properties of the Posterior Lens Capsule

The anterior and posterior lens capsules differ in several aspects. The lamellar structure of the lens capsule disappears earlier with age in the posterior lens capsule than in the anterior lens capsule,[44] and differences have been described in the relative proportion of macromolecular components such as heparansulfate, proteoglycans, and fibronectin.[65,66]

This could indicate that the mechanical qualities of the anterior and posterior lens capsule are different. Mechanical strength of the posterior lens capsule (ultimate strain, ultimate stress, ultimate elastic modulus) was found to decrease markedly with age in a range similar to that of the anterior lens capsule.[48] The age-related loss of mechanical strength, however, seemed to begin earlier in the posterior lens capsule than in the anterior lens capsule. Ultimate load, which reflects the breaking strength of the lens capsule *in situ*, was significantly lower for the posterior lens capsule than for the anterior lens capsule.[48] This is in accordance with the fact that the posterior lens capsule is much thinner than the anterior lens capsule.

When looking at data pertaining to the accommodative function range (low strains), the mechanical quality of the posterior lens capsule was found to be similar to that of the anterior lens capsule in all age groups. This indicates that the mechanical properties of the lens capsule *in situ* vary proportionally with the regional variation in capsular thickness. The age-related loss of mechanical strength, however, seems to begin earlier in the posterior lens capsule than in the anterior lens capsule.

### Aging of the Lens Capsule

Studies of the human lens capsule indicate concurrently that aging of the human lens capsule is associated with a progressive loss of mechanical strength, which seems to parallel morphological changes in the lens capsule. Formed elements (inclusions) accumulate in the anterior lens capsule with age.[44,67] The laminated structure of the lens capsule disappears with age,[44,49] and the optical density of the lens capsule increases with age.[68,69] Peczon et al.[70] investigated age-related changes in the amino acid composition of the lens capsule and found a relative increase of noncollagenous amino acids and a decrease of collagenous amino acids (hydroxyproline) with age. Because collagen seems to be responsible for the mechanical strength of other soft connective tissues,[71] the age-related changes in the amino acid composition of the lens capsule also may have significance in the loss of mechanical strength.

The major structural component of the lens capsule is basement membrane type IV collagen, which is organized into a three-dimensional molecular network.[72] As discussed previously, the mechanical properties of the lens capsule correlate well with a network structure. The lens capsule is easily deformed at low deformations due to reorientation and alignment of the molecular network structure in the direction of deformation. As the elastic stiffness of the lens capsule at low deformations increases with age in pre-presbyopic eyes, and the extensibility correspondingly, decreases with age, this suggests geometrical changes in the molecular network structure with age.

One factor may be the increasing volume of the lens with age, which may cause stretching of the collagen network structure, thus limiting further deformation. Another factor may be an increased crosslinking of the molecular network structure with age, which also may limit deformation.[48] The collagen molecules in the lens capsule seem to be extremely long-lived. This provides great opportunity for post-translational modifications of the molecules, such as nonenzymatical glycosylation,[73,74] which can change the mechanical properties of the lens capsule through the formation of stable crosslinks.[75-77]

## *The Ocular Lens Epithelium*

### Ultrastructure of the Lens Epithelium

A single layer of cells—the lens epithelium—covers the anterior face of the lens that faces the cornea. The lens epithelium ends on the rims of the anterior surface. It contains cells in the central region that do not divide and are essentially *quiescent*, surrounded by a germinative-dividing zone of cells and followed (at the equatorial fringe) by the dividing cells that differentiate into fiber cells (see Fig. 5.4).

A remarkable feature of this epithelium is its capacity to divide and differentiate almost all through the life of an individual. This feature of sustained growth is very

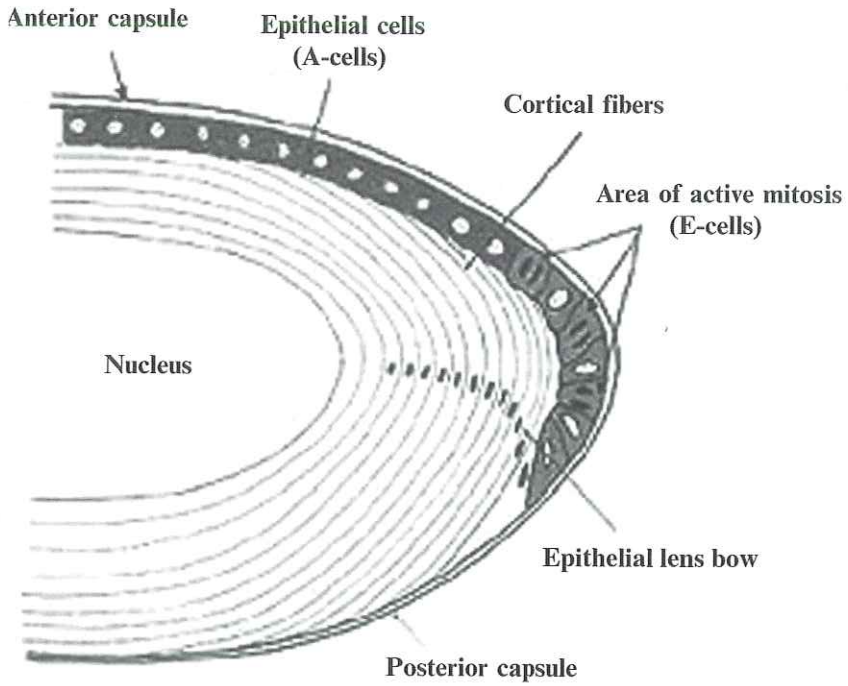


**Fig. 5.4** Simplified drawing of the ocular lens. Note that the epithelium has three regions of cells in nondividing phase (central epithelium), in dividing phase (germinative), and differentiating phase (equatorial). The anterior and posterior sutures are formed by the meeting of the elongating fiber cells that make the bulk of the lens mass. The surrounding capsule (shaded area around the lens) indicates that the basal surface of the epithelium and the fiber cells is on the outside, while the apical surface faces the inside of the lens. *apical interface*—an area of contact between the apical surfaces of the epithelial cells and the fiber cells is not indicated. It is the area just below the epithelial layer

much similar to its closest embryological sibling—the cells in the skin. The cells in the lens epithelium represent typical epithelial morphology: they are cuboidal, presenting a cobble-stone-like appearance in their native state and *in vitro*, if cultured without excessive passaging.

The diameter of human lens epithelial cells ranges from 9–17 μmm.[78] The cell size has been reported to increase with age,[79,80] which suggests a change in the cell density. Females have been reported to have higher cell density in the human lens epithelium than males.[81,82] Francois and Rabaey[83] observed lens epithelium under a phase-contrast microscope. They reported the presence of *pale polyhedral* and *dark, star-shaped* cell types. A recent *in vivo* study[84] using the noncontact specular microscopy recognized four morphological features of the live human lens epithelium. These were categorized as *linear furrows, columnar organization, puffy clouds*, and *black holes*.

The relationship between cell density and age is interesting, although controversial. An earlier report[82] that there is an age-related decrease in the cell density in the lens

epithelium has been recently confirmed.[84] This recent study calculated the loss of 675 cell/mm$^2$ in a 75-year life span (that amounts to a loss of 14% of the cells). This estimate is based on the unproven assumption that the rate of loss is linear with age, however it is not very different from that reported in the aging monkey lens central epithelium.[85] Others have found no such relationship.[86,87]

Karim et al.[87] reported a decrease in the mitotic index of lens epithelial cells under normal, as well as cataractous, conditions. Harocopos et al.[88] concluded that there was no relationship between cell density and the severity of cataracts, or between cell density and age. They did report, however, that the epithelium directly over the opaque area in cataractous lenses had higher cell density when compared to that overlaying the transparent regions. It is possible that a loss of a patch of cells overlying a cataractous fiber cell area may lead to the activation of cell division, and therefore higher cell density.

## The Epithelium as the Major Site of Transport, Metabolism and Detoxification

The overall metabolic status of the fiber cells in the absence of endoplasmic reticulum, mitochondria, and a nucleus, is comparatively very low.[89] There is no vascular system as we know it that would take nutrients to the fiber cells and remove metabolic/physiologic waste to replenish the intra- and intercellular milieu of the lens. Mere diffusion as a process to sustain the slow but substantial physiology of the ocular lens will be insufficient to accomplish this efficiently. A study of relative rates of transport across the anterior and posterior surfaces of the lens has led to the model of the *pump-leak* system.[90-92]

Lens epithelium is also a major site of detoxification and defense against oxidative insults[93,94] and is able to detoxify physiological concentrations of $H_2O_2$ enzymatically involving glutathione reductase, glutathione peroxidase, and the hexose monophosphate shunt.[94]

## Programmed Cell Death and the Lens Epithelium

The interest in the study of programmed cell death in the lens epithelium was generated recently by investigators probing the role of epithelium in cataractogenesis.[95] These studies are based on the hypothesis that the integrity of the lens epithelium is essential for the normal functioning of the lens, and that a decrease in the cell number of the epithelium may lead to changes in homoeostasis that may in turn lead to cataractogenesis.

The role that apoptosis plays in tissue development and morphogenesis is well established.[96] Apoptosis or cell death has been morphologically documented in the very early stages of the lens vesicle formation during development of the eye.[97] A role for apoptosis in regulating the size of the lens by controlling the number of cells that reach terminal differentiation into fiber cells remains a possibility.

Different metabolites[98] and drugs[99] have been reported to initiate apoptosis in lens epithelial cells in culture.[100] The research of Zigman,[101] Li and Spector,[95] Michael et al.[102] and Shui et al.[103] suggest that apoptosis may occur discretely in the lens epithelium and only in isolated cells that become susceptible. Such a thesis connotes two interesting corollaries:

- Any isolated apoptotic cell may be quickly eliminated by the surrounding cells as a protective response against the spread of cell death. Importantly, therefore, it points to the existence of a potent mechanism that strictly controls and inhibits cell death from spreading to neighboring cells.
- The presence or absence of apoptosis in the lens epithelium can be interpreted optimistically as a process that eliminates the dys functional cells to keep the rest of the epithelium healthy. A pathological state may precipitate when this ability to remove dysfunctional cells is compromised—for example, by aging or by exposure to harmful metabolites or environmental insults.

## *Lens Fiber Cells*

### Fiber Cell Organization and Development of Lens Sutures

The cells of the lens vesicle that were not induced to form primary fibers remain as a monolayer—the lens epithelium—that covers the anterior surface of the primary fiber mass. From this point on, further lens development and growth occurs throughout life in a manner similar to other stratified epithelia.

The lens epithelium constitutes the basal layer—however, whereas typically stratified epithelia have their progenitor cells distributed throughout the basal layer, the lens is unique in that its progenitor cells are sequestered as a distinct subpopulation within the lens epithelium known as the *germinative zone*, which comprises a narrow, peripheral, latitudinal band of the lens epithelium located just above the equator.[104] These cells undergo mitotic division, and selected daughter cells are induced to terminally differentiate and form *secondary fibers*.

As with primary fiber formation, the most apparent structural consequence of secondary fiber formation is the transformation of a cuboidal cell into a long fiber. While forming, however, primary fibers are fixed in position as they elongate essentially unidirectionally. Secondary fiber formation requires the forming fibers to rotate about their polar axis while simultaneously migrating posteriorly and elongating bidirectionally. Fiber rotation is complete when the long axis of a forming fiber is aligned parallel to the antero-posterior axis of the lens, and when the center of a forming fiber reaches the mid-point between the poles, which—by virtue of the fact that all vertebrate lenses are asymmetric, oblate spheroids—is posterior to the equator. As secondary fibers elongate, their anterior ends are insinuated between the lens epithelium and the primary fiber mass, while at the same time their posterior ends are insinuated between the primary fiber mass and the posterior lens capsule.

Maturation of secondary fibers is complete when they detach from the lens epithelium anteriorly, and the capsule posteriorly, to subsequently overlap with other newly mature fibers to form lens sutures.[105]

## The Contribution of Major Fiber Proteins to Sutural Development and Growth

In vertebrate lenses, MP26, gap junction (GJ) connexins, and MP19 are the major fiber intrinsic membrane proteins. The major intrinsic protein (MIP), or aquaporin0 (AQP0), is generally described as constituting more than 60 percent of the total fiber membrane protein.[106,107] The connexins (Cxs)[108,109] that form the lens GJ communicating channels, display a variety of expression patterns, channel regulation, and post-translational modifications during differentiation and aging of the lens cells.[110,111]

Lens epithelial GJs consist mainly of 1 connexin (Cx.1 or Cx43).[112] The Connexin50 is essential for normal postnatal lens cell proliferation.[113,114] Cortical fiber GJs consist of Cx.3 (or Cx46) and Cx.8 (or Cx50)—often coexisting in the same junctional plaques.[115,116] MP19 (also referred to as MP17, MP18 and MP20 in the literature) has also been described as the most abundant intrinsic membrane protein of lens fiber cells.[106] However, unlike MIP or the connexins, MP 19 bears no striking resemblance to any other reported protein family and, to date, has no defined structural or functional role. Both MIP and MP 19 co-localize with GJs in distinct regions of the lens.[117,118] Thus, it has been proposed that both MIP and MP 19 play some role in GJ formation, maintenance, or organization.

While it is well-documented and irrefutable that all vertebrate lenses contain the above described major fiber proteins, a review of the literature suggests that their density and distribution varies is species-specific—varying along fiber length and as a function of fiber depth and therefore of age. GJs primarily conjoin the mid-segment of fibers, or those segments of fibers not involved in sutures.[119,120] The function of these proteins is probably coordinated during fiber development.

## Lens Sutural Anatomy

Numerous studies have established that the vast majority of fibers are hexagonal in cross section,[121-126] with two broad faces oriented parallel to the lens surface and four narrow faces oriented at acute angles to the lens surface. During differentiation and maturation, the lens fiber membranes undergo typical changes. The lateral and apical surfaces of the hexagonal fibres change from smooth and studded with small ball-and-socket junctions in the superficial cortex, to covered with grooves-and-ridges in deeper cortical regions and the nucleus.

Freeze fracture studies[127] revealed that these surface changes are paralleled by changes in the internal organization of the fiber membranes. Epithelial and superficial fiber membranes are studded with a multitude of intramembrane particles (IMPs) and gap junctions (GJs). The IMPs represent intrinsic membrane proteins

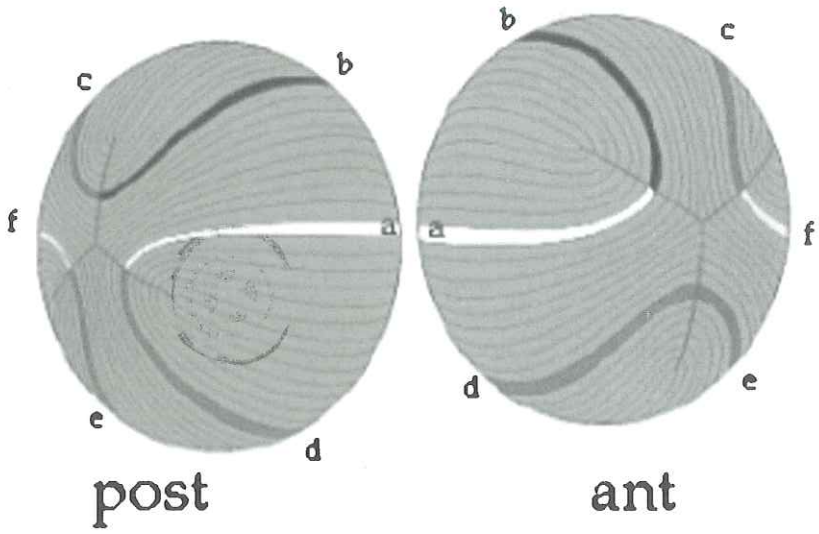


**Fig. 5.5** Formation of anterior Y and offset posterior Y sutures. In this view of the anterior and posterior surface, a number of curved fibers have been highlighted. The anterior ends of these fibers are paired (a-b-c-d-f) to form parts of anterior suture branches. By following these fibers along their length, it can be seen that as a result of opposite end curvature, the posterior ends of these fibers are paired with different fibers to form offset posterior suture branches (Modified from: Development of Lens Sutures. JR Kuszak, RK Zoltoski and CE Tiedemann Int. J. Dev. Biol. 48: 889–902, 2004)

that function as receptors, ion channels, transporters, and pores. GJs allow a direct cell-to-cell exchange of molecules up to 1500 Da.

Biochemical studies[128] showed that, upon maturation, the cholesterol-to-phospholipid ratio of lens membranes dramatically changes from 0.6-0.8 in superficial to over 5.0 in deep cortical and nuclear membranes. All this indicates that lens membranes, apart from those in the most superficial cortex, deviate from most cell membranes in the body. This is in line with electrophysiological studies showing that deep cortical membranes are non-leaky, have a high resistance and low capacitance, and have no or restricted cell-to-cell communication.[129,130]

In the human lens, fibers are partial or incomplete meridians—that is to say, upon completion of elongation, the vast majority of fibers do not have ends that extend to the poles (see Fig. 5.5).

During gestation primary fibers are neither uniform in shape nor size.[122,123,126,131,132] As such, the primary fiber cell mass, or embryonic lens nucleus, does not consist of growth shells overlain in the register to form ordered radial cell columns. The initial secondary fibers are similarly nonuniform in shape and size, and also lack an ordered arrangement. Only as lens development proceeds are the additional secondary fibers formed progressively more uniform in shape. The establishment of growth shells comprised of uniform fibers overlain in register as radial cell columns occurs

within 250–750 μM of the equatorial center of the lens. Coincidentally, this marks the beginning of suture formation.[133]

## Suture Formation after Birth

After birth, there are fundamental changes in the fiber differentiation program that result in the formation of progressively more complex iterations of star sutures during infancy, adolescence, and adulthood.

## Simple Star Suture Formation

Shortly after birth, a new (or secondary) anterior suture branch, and a pair of new (or secondary) posterior suture branches, begin to develop in relation to the extant suture branches within the infero-nasal quadrant. At the same time, the anterior ends of curved fibers that bracket the posterior suture branch are added to the extant primary anterior suture branches. Similarly, the posterior ends of the same curved fibers that bracket the anterior suture branch as a consequence of opposite end curvature, are added to the extant offset primary posterior suture branches. By the end of the infantile period, the anterior suture consists of three enlarged primary branches, and three new secondary suture branches—one completely formed, and two partially formed. The offset simple star posterior suture consists of three pairs of new secondary branches—a pair that are completely formed, two pairs that are only partially formed. All of the suture branches are arranged in a symmetrical, but nonidentical, simple star suture pattern.[133]

## Star and Complex Star Suture Formation

The essential parameters of *star* sutures formed during adolescence, and *complex star* sutures formed throughout adulthood, are demonstrated in Fig. 5.6.

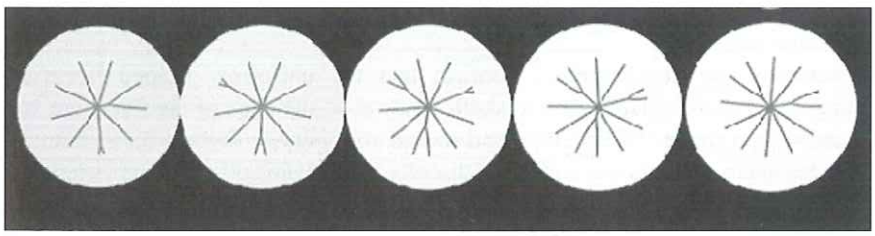

**Fig. 5.6** Key stage in the development of the complex star suture formed throughout adulthood. These sutures are progressively more complex through adolescence and adulthood

**Fig. 5.7** Intermediate-magnification SEM micrographic image of a portion of suture branches. The width and evenness of a suture branch is related to the degree of irregularity between fiber cell ends (Modified from: JR Kuszak, JG Sivak, JA Weerheim, Lens optical quality is a direct function of lens sutural architecture. Invest. Ophthalmol. Vis. Sci.S, vol. 32, 7:2119–2129, 1991)

These sutures are progressively more complex iterations (second and third rows, polar projections of anterior and posterior, respectively) of the simple star sutures formed through infancy. Throughout adolescence, the nine branched star sutures are formed as tertiary anterior suture branches, and tertiary pairs of posterior suture branches sequentially supplement the extant primary and secondary branches.[134] The different suture patterns formed during gestation, infancy, adolescence, and adulthood are the anatomical basis of the zones of discontinuity revealed by slit-lamp biomicroscopy.[134,135] Throughout life, anterior and posterior suture branch formation continues, and their distal ends extend to confluence at their respective poles.

Numerous structural studies confirm that the uniformly shaped fibers are arranged in highly ordered growth shells—however, the ends of the fibers are very nonuniform in shape.[133] Thus, their end-to-end arrangement to form suture branches produces naturally occurring regions of disorder aligned directly along the visual axis. In fact, by overlying suture branches in concentric growth shells, line and Y suture lenses produce disordered suture planes aligned directly along the visual axis (see Fig 5.7).

## The Physical Basis for Transparency of the Crystalline Lens

To perform its role in the process of vision, the lens must be transparent. The analysis of transmission in terms of the physical interactions between light and the structures of the lens is fundamental to understanding the changes in optical quality with aging.

The first author to study the physical concept of transparency in the lens was Trokel in 1962.[136] The physical interactions between light and the known structures of the lens (cortical fibers surrounded by cell membranes and protein fraction that comprises most of their cytoplasm) and the manner in which these molecular and microscopic structures affect the traversing light wave determines the transmission characteristics of the lens.

These characteristics depend upon the two processes of absorption and scattering. Absorption is the conversion of light from the incident beam to other forms of energy, such as heat or chemical energy. Scattering takes place when light passes over the elastically bound electrons in the atoms and molecules. The scattering interaction may be thought of as producing elastic vibrations that result in the emission of secondary light in all directions—thus, scattering also removes energy from the traversing beam. A distinction is made between light scattering by small and by large particles. Scattering by small particles occurs when the objects are smaller than the wave length of light, such as the soluble proteins of the lens. Large particles are larger than several wave lengths in size,[137] and are the structures that can be resolved by the light microscope.

Microscopic and submicroscopic structures cause the extinction of light, which determines the transmission characteristics of the lens. This extinction derives from the many processes summarized in Fig. 5.8 that show the absence of absorption and the major role of scattering in the extinction of visible light by the

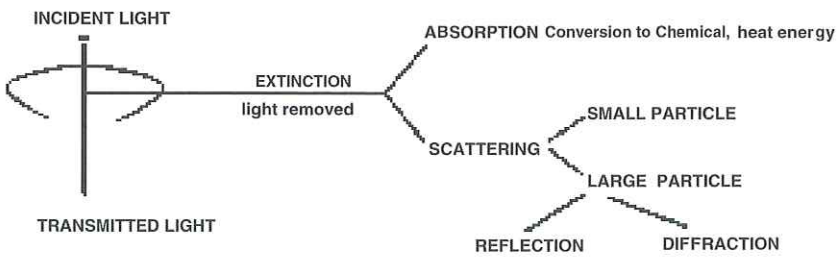


**Fig. 5.8** Summary of the processes that produce extinction of light in the crystalline lens. (Modified from: S Trokel The physical basis for transparency of the crystalline lens. Invest Ophthalmol 1:493, 1962)

lens. The physics of light scattering must be examined to understand the extinction characteristics of the lens.

## *Small Particle Scattering*

Completely regular crystalline matter will change only the velocity of traversing light without removing energy by scattering. It can be concluded that the high degree of light transmission of the intact lens fibers results from the spatial order of lens proteins in their normal state. The spatial order of protein molecules can be described by *p(r)*—the probability that two protein molecules are a distance r apart. The reduction in scattering due to local order has been derived by Zernike and Prins[138] in a general form:

$$\left[1-\frac{4\pi N}{V}\int_{o}^{\infty}(1-\rho(r))r^2\frac{\sin ksr}{ksr}dr\right]$$

This formula expresses the reduction of scattering of N particles in a volume (V), where $k = 2\pi/\lambda$, and $s = 2 \sin \theta/2$. The distribution function *p(r)* is normalized to unity when all r's are equally probable. This is the dilute solution in which this factor reduces to one, and no external interference of scattered light occurs.

Quantitative application of the Zernike-Prins factor to the lens proteins in the intact state is not now feasible because the exact dimensions and the spatial order of the proteins in the intact fiber are unknown. Qualitatively, the high concentration of the soluble proteins in the lens fiber must be accompanied by a degree of local order approaching a paracrystalline state. This results in the interference of scattered light and the transparency of the fibers.

## *Large Particle Scattering*

Although the nature of the physical interaction is the same, large particle scattering calls for mathematical treatment different from that of small particle scattering. Incident rays on an isotropic particle give rise to the phenomena of diffraction and reflection. The reflection is accompanied by refraction at the large particle surface. Diffraction and reflection can be considered special cases of scattering.

The phase contrast photomicrograph of an unstained section emphasizes those structures that cause large particle scattering (Figs. 5.9).

Thus, the lens transparency is made possible by a number of factors, including the regular arrangement of the lens fibers, the nonparticulate fiber cytoplasm, and the uniform distribution and paracrystalline state of proteins within the cells.[136-140] Kuszak et al.[141] have proposed that the arrangement of lens fibers depends strongly on the ability of newly formed cells to elongate in a pattern that meshes precisely

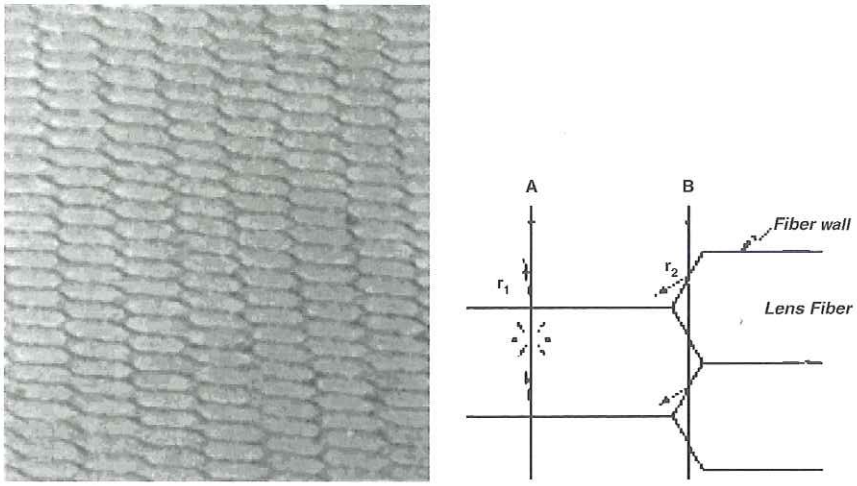


**Fig. 5.9** Comparison with a phase-contrast photomicrograph of a lens section made perpendicular to the fibers, and a diagram that represents the membrane pairs of the lens fiber walls as a single refractive discontinuity. $r_1$ is a ray reflected back to the light source, and $r_2$ is reflected at a 60-degree angle from the path of the transmitted beam. The cluster of short arrows represents scattering of light by the soluble proteins that comprise the fiber cytoplasm (Modified from: S Trokel S The physical basis for transparency of the crystalline lens. Invest Ophthalmol 1:493, 1962)

with the underlying cells. The lamellar conformation of lens proteins rather than helical structure may also contribute to transparency.[142] In addition, it has been proposed that a short-range, liquid crystal-like order of the crystallins is important for transparency of lens cytoplasm.[143]

In addition to the state of lens crystallins, the tight packing of the lens cells and the regulation of ion and water balance also play significant roles in maintaining the transparency of the normal lens. Consequently, the development of protein aggregates, cell membrane degeneration, the appearance of vacuoles, and the distortion of lens structure can all produce light scatter and the clinical observation of cataracts.[139]

## The Influence of Sutural Architecture on Lens Optical Quality

The morphology of lens sutures should be considered when evaluating the optical quality of crystalline lenses.A quantitative analysis of optical quality in line and Y suture lenses confirms that suture planes significantly degrade lens function (sharpness of focus).[144-146] Comparable studies in star suture lenses, however, show that the staggering of suture branches in concentric growth shells effectively minimizes the negative influence of suture planes on optical quality.[147]

Spherical aberration is a monochromatic anomaly that can be defined as the difference in focal length for light rays that pass through different points of a lens. Generally, light rays that pass through the periphery in vertebrate crystalline lenses can have longer focal lengths than those that pass through the center. This situation is known as negative or over corrected spherical aberration, and it is partially the result of lens structure. The net result of the additional growth shells of lens fiber cells throughout life is an ever-increasing lens mass.[144-147] Unlike other stratified epithelia, the strata of the lens (growth shells) are never sloughed off. Rather, they become more internalized as the lens grows.

The plasma membrane, the cytoplasm with specialized crystalline proteins, and the extracellular space between the lens fiber cells of the growth shells have different refractive indices. Thus, as the lens grows, a gradient of refractive index is established from the center of the lens to the periphery on the basis of variation in protein content. This gradient of refractive index, possibly in combination with the asphericity of lens shape, neutralizes positive spherical aberration.[144]

Numerous studies have shown that lens fiber cells are uniform in shape and are overlaid in precise alignment to produce radial cell columns between growth shells.[148-152] Thus, it has been proposed that the radial cell columns are a system of coaxial refractive surfaces that are partially responsible for lens transparency.[153,154] In contrast, scanning electron microscopy studies[147,152,155,156] show that the ends of lens fiber cells are variable in shape and are overlaid in imprecise alignment to produce irregular suture planes between growth shells. If the ordered alignment of uniform fiber cells into precise radial cell columns contributes to negative or corrected-spherical aberration, then the alignment of variably shaped lens fiber cell ends into imprecise suture planes could contribute to nonmonotonic spherical aberration. More importantly, the negative influence of sutures on optical quality increases with age.[157-160]

## Nuclear Fiber Compaction as a Function of Aging and Cataractogenesis

The substantial senescent alterations in the structure of the embryonic and fetal nuclear fibers can lead to degradation of lens optical quality, especially because these fibers are located entirely within the region defined by the pupillary margin. In fact, clinical observations of aged lenses show increased light scatter even without overt visual impairment.[161] Excessive senescent changes in the morphology of the nuclear lens fibers are likely to be most detrimental to lens optics, because these fibers are located directly along the visual axis. Therefore, age-related fiber compaction resulting in an increase in the membrane complexity along the light path may be a source of increased large particle scatter and ultimately, reduced lens optical quality with age.

Light scatter in the lens has been attributed to the interaction of the incident beam with both the cell membranes and the cytoplasmic proteins producing

respectively, large and small particle scatter.[162] It has been suggested that in normal lenses, the majority of light scatter originates from interactions with fiber membranes, which have a higher refractive index as compared to the cytoplasm.[163] The cytoplasm is virtually transparent due to the close association of the crystallin proteins that minimizes refractive index fluctuations.[164,165] Although numerous biochemical modifications have been noted in the cytoplasmic and membrane components of lenses with age-related nuclear cataracts, the sources of excessive light scatter have yet to be definitively identified. In nuclear cataracts, the signficantly increased fiber compaction may be one of the factors contributing to the excessive scatter in nuclear opacification.

The size and shape of the human lens changes dramatically during development and maturation. Assessments of human lens growth have established that the equatorial dimension of lenses increases at a greater rate than the polar (A-P axis) dimension.[166-168] While significant compaction of nuclear fibers occurs along the A-P axis with aging, an even greater degree of compaction occurs in nuclear cataract formation.[169] In most age-related nuclear cataracts, opacification begins in the lens center, and enlarges gradually. It has been noted clinically that cataracts often have reduced antero-posterior thickness in comparison to age-matched normal lenses.[170] However, the rate of compaction is not constant.

Morphometric analysis indicates that, in general, more compaction occurrs between young and middle-aged lenses than between middle-aged and aged lenses. Although initially surprising, this finding is temporally consistent with the onset of presbyopia near age 40. It is likely that condensation and compaction of nuclear fibers in early adulthood contribute to the lens hardening and loss of accommodative ability that characterize presbyopia.

The process of nuclear fiber compaction is probably multifactoral. The most obvious structural change is the formation of accordion-like folds, which account for much of the compaction along the A-P axis. These folds begin in early adulthood and increase in both frequency and amplitude with age. The early onset of structural changes may be due to controlled modifications in the cytoskeletal[171-174] and crystallin[175-178] proteins that accompany fiber cell maturation, and are probably necessary for long-term maintenance of fibers.

In the fourth through eighth decades, cumulative age-related changes—such as water and protein loss,[179,180] modifications to membrane lipids,[181,182] and protein modifications[183]—could result in the progressive increase in compaction folds. The further increase in nuclear fiber compaction in age-related nuclear cataracts is consistent with the extensive protein modifications,[184] dehydration,[185] and lipid peroxidation[186-188] known to occur in human cataracts. The major factor influencing compaction is most likely the loss of cytoplasmic water, which necessarily results in the loss of cell volume without reduction in cell surface area. The driving force for the loss of water may be the reported tendency of the crystallins to self-associate into larger aggregates with time, causing the nuclear cytoplasm to have a reduced osmolarity.[189,190] Such changes are essential for the high concentrations of proteins in nuclear cytoplasm to exist adjacent to cortical fiber cells with relatively high water content.

Further changes in the proteins and membrane lipids during cataract formation, specifically by oxidative damage,[191] may result in more extensive condensation of cytoplasmic proteins, as well as loss of protein and membrane fragments that lead to increased fluctuations in refractive index at cellular interfaces and increased light scattering.

## Biometric, Optical and Physical Changes in the Human Crystalline Lens with Aging

Optical and physical properties in the lens are closely related. The crystalline lens focal length and spherical aberration are profoundly influenced by the lens surface curvatures and gradient refractive index. The continued linear growth in mass and volume of the human lens after the age of five years and throughout the remainder of the normal life-span has been well documented.[192,193]

Glasser found the following results: a) the human lens grows throughout life and becomes heavier and larger in cross sectional area; b) there is a significant linear increase in lens weight with age; c) the lens equatorial diameters tend to increase up to age 70 and then decrease beyond this age; d) there is a significant linear increase in anterior lens surface radius of curvature up to age 65 and then a significant linear decrease after age 65; e) there is a tendency for an increased lens thickness with age; and f) the posterior lens surface curvature has a tendency to flatten with increasing age (see Fig. 5.10).

The thickness shows no significant age dependence, although it has a tendency to increase.[194] Moreover, the human lens shows an exponential increase in resistance to mechanical deformation with age from birth. Even though the predominant increase in hardness occurs after the age at which accommodation is completely lost, the increasing resistance demonstrates increased hardness of the human lens, which can account for the loss of accommodation. The age-dependent changes in the responses of lenses to mechanical deformation suggest that the human lens may loose elasticity and increase viscosity with age, and that this may account for the loss of accommodation with the development of presbyopia.[194]

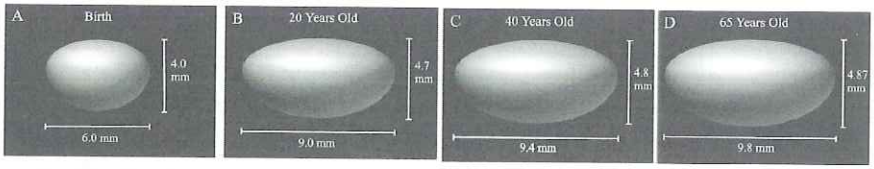


**Fig. 5.10** Changes in the lens equatorial and pole-pole dimensions with age. At the time of birth, (A) the lens is an asymmetric ellipse with an equatorial diameter approximately 1.5 times the anterior-posterior dimension. In the young adult (B) the equatorial diameter is close to twice the length of the anterior-posterior dimension, illustrating the unequal growth rate in the two lens axes. (C) and (D) also show that throughout adult life, the equatorial dimension of the lens increases faster than the polar dimension. (Modified from: Al-Ghoul KJ, Nordgren RK, Kuszak AJ et al Structural evidence of human nuclear fiber compaction as a function of ageing and cataractogenesis. Exp Eye Res 72:199, 2001)

## *The Change in Equivalent Refractive Index and the Lens Paradox*

Using Scheimpflug photography in a cross-sectional study of 100 subjects of various ages, Brown[195] demonstrated that the aging lens becomes more convex. He found a substantial decrease of the radius of the anterior lens surface from about 15 mm to approximately 8.5 mm between the age 20 and 80 years of age—the posterior lens radius declined from 8.5 to about 7 mm. This would imply an increase in lens power and a tendency toward myopia in the older eye, because other dimensions of the eye do not change significantly with age.[195] Between the ages of 30 and 65, however, a hypermetropic shift can be observed.[196] This paradoxical feature of the decrease of the radius of curvature of the crystalline lens with age without the eye becoming more myopic has been called the *lens paradox*.[197,198] To explain the lens paradox, there must be a compensating mechanism in the eye that prevents the eye from becoming myopic.

Because neither the axial length nor corneal curvature show considerable changes with age, a decrease of the refractive index of the lens has been suggested as such a compensating mechanism. Nevertheless, so far no empirical study has been able to show a decrease of the gradient refractive index with age. Pierscionek[199] found no significant age-dependent changes in the refractive gradient index measured in isolated lenses. Glasser and Campbell[200] also found no evidence in support of the lens paradox in isolated human lenses or in decapsulated human lenses. Dubbelman and Vander Heiide[201] confirm the existence of the lens paradox in the sense that they also found a decrease of the radius of curvature with age, but there are two major differences between their results and the results obtained by Brown.[195]

The first difference concerns the extent of the paradox. The average decrease of the anterior radius is 57 µm per year according to Dubbelman,[201] while Brown[195] found a value of about 100 µm per year, which is almost twice as large. The slight decrease of the radius of the posterior lens surface, approximately 17 µm per year, corresponds to the findings of Brown.[195]

The second difference concerns the absolute value of the anterior and posterior lens radius, which are both smaller than the values found by Brown. According to Dubbelman,[201] the difference for the anterior surface is more than 3 mm at the age of 18, which decreases to 0.9 mm at the age of 65. During aging, the posterior radius remains on average 2.3 mm smaller, but this can be explained by the fact that Brown did not correct for the refraction of the lens itself. However, Dubbelman's findings closely resemble the results of recent phakometric studies[202,203] the findings about the lens radii correspond with the radii of the Gullstrand nonaccommodated schematic eye, and also with the earlier measurements listed by Duke-Elder and Wybar.[204]

To explain the lens paradox, it was suggested that, with age, the increased sharpness of curvature was balanced with increased lens thickness.[197] However, Dubbelman's calculations demonstrated that thickening of the lens only cancels out 15 percent of the decrease of the equivalent refractive index needed to prevent the eye from becoming myopic with age.[201] According to the results of Brown,[195] Dubbelman[201] registered a small decrease of the refractive index with age. The origin of the decrease of the refractive index with age has remained unclear.

It is suggested that the water content of the lens should increase resulting in a decrease of the refractive power. However, most recent studies on this topic do not support each other. Siebinga et al.[205] measured an increase of water content in the nucleus with age, whiles Lahm et al.[206] measured the opposite. Clarke et al.[207] reported an increase of refractive index in the center of the lens, and another hypothesis is a change in the variation of the index gradient of the lens with age.

Using the results of Brown,[195] Smith et al.[208] proposed a model to describe this variation. They showed that slight changes in lens refractive-index profile would be sufficient to negate the more convex shape of the lens with age. Yet, if the lens radii of the present investigation were used, an even slighter change would suffice. In conclusion, then, recent studies confirm the existence of the lens paradox, although the decrease of the radius of the anterior lens surface, using Scheimpflug photography, is smaller than in earlier studies. There is a highly significant, but small decrease of the equivalent refractive index of the lens, which explains the lens paradox.

### *Crystalline Lens Position Modification with Age*

Using Purkinje images, Tscherning[209] first reported in 1898 that the human lens deviated 0.25 mm in the upper part and tilted six degrees in the infero-temporal direction. Yu Hu et al.[210] showed that the crystalline lens was not aligned perfectly along the visual axis, but its effect on refraction was limited.

Aging, associated with an increase in lens thickness[211,212] and a more anterior position,[213,214] and combined with a complex of anatomical predisposition (a short axial length, a shallow anterior chamber, and a small corneal diameter) and subsequent physiological factors, is conducive to anterior chamber angle closure and is considered to play a major role in the pathogenesis of Primary Angle-Closure glaucoma.[215,216]

The greater the contact between the anterior surface lens and the posterior surface of the iris, the greater the impediment to the anterior flow of the aqueous humour. In the lens position modification, the zonular apparatus plays an important role.

## Zonular Apparatus

### *Synthesis and Structural Organization of Zonular Fibers During Development and Aging*

Zonular fibers are a specific form of elastic extracellular matrix composed mainly of fibrillins. The major role of the zonule is to anchor the lens in the eyeball between the anterior and posterior chambers, holding the lens in the optical axis. A secondary role of the zonule is the transmission of accommodation forces from

the ciliary body to the lens. Clinical observations and a preliminary study have shown that zonules are more fragile during aging.[217]

### Zonular Fibers Electron Microscopy

Zonular fibers are composed of large bundles of microfibrils, each with a diameter of 12 nm. Microfibrils are more or less in contact with each other, depending on their location in the zonular apparatus. In the human eye, zonular microfibrils appear to become shorter and increasingly disorganized during aging.[217] This is supported by the *in situ* hybridization data, which clearly show an age-dependent decrease of fibrillin-1 mRNA expression as previously observed in the human aorta.[218]

During aging, a new fibrillar structure of fibrillin microfibrils appears with a 56-nm periodicity.[217] This new structure does not have the same periodic pattern as that of classical microfibrils. Banded elastin, however, has a periodicity of less than 50 nm and has never been described in the zonular bundles of microfibrils.[219] Other molecules could be involved in this structure, such as type VI collagen, which is a frequent partner of fibrillin-containing microfibrils in other organs (e.g., the nuchal ligaments). Hanssen[220] suggested that these modifications could only be the result of cross-linking between fibrillin microfibrils.

Crosslinks are often known to appear in long-lived proteins.[221] The formation of these putative crosslinked structures, which may be due to the transglutaminase activity demonstrated in zonular fibers,[222] may decrease the putative elastic properties of microfibrillar bundles. The low turnover of microfibrillar components may also act to increase this age related modification. In this regard, the appearance of these structures coincides with a physiological age-related modification of accommodation correlated with presbyopia. Gradual loss of elasticity, sclerosis of the lens, and concomitant atrophy of the ciliary muscles have all been proposed as the causes of this dysfunction.

### Anterior Shift of Zonular Insertion onto the Anterior Surface of Lens with Age

#### Anatomical Changes of the Zonular Insertion

Several studies have investigated the anatomy of zonular fibers in relation to lens structure.[223-227] In 1979, Farnsworth and Shyne[223] showed that:

- The distance between the zonular insertion and the lens equator increased with age
- The circumlental space (the distance from the equator to the ciliary body) decreased with age
- The distance between the zonular insertion and the ciliary body remained relatively constant Sakabe[228] supported the first two findings, but he found that the

distance between the anterior zonular insertion and the ciliary sulcus increased with age. A possible causal factor suggested is the change of lens contour. With lens growth, there is an increase in both frontal and sagittal diameters,[226-231] and a consequent decrease in radius of curvature of the anterior lens surface.[232] Sakabe[228] found that the diameter of the zonular-free zone decreased with age. Assuming that the attachment position of the zonules remains unchanged throughout life,[223] and that thickness of the anterior lens capsule increases (not decreases or is stretched or both) with age,[233] apparent decrease in the diameter of the zonular-free zone may be explained by increased convexity of the lens surface. This would result in an increase of anterior zonular insertion, because ciliary sulcus diameter does not change with age. The location of anterior zonular insertion appears to have clinical importance in the practice of cataract surgery, in which continuous curvilinear capsulorhexis is the technique of choice for the majority of surgeons. To create the capsulorhexis within the zonular-free zone, one should stay within the central 6.86-mm area of the anterior capsule, which is the area expected to be completely free from zonular fibers.[228] More importantly, this size decreases with age. If the capsulorhexis is not located in the center of the anterior capsule, the edge of the capsular opening can extend more easily to the position of anterior zonular insertion, resulting in a failure to accomplish continuous curvilinear capsulorhexis.

## Lens Metabolic Changes with Age and the Effects of External Agents

### *Introduction*

The lens is exposed to the cumulative effects of radiation, oxidation and postranslational modification. The alteration of proteins and other lens molecules impairs membrane functions and perturbs protein (particularly crystallin) organization, so that light transmission and image formation may be compromised. Damage is minimized by the presence of powerful scavenger and chaperone molecules. Progressive insolubilization of the crystallins of the lens nucleus in the first five decades of life, and the formation of higher molecular weight aggregates, may account for the decreased deformability of the lens nucleus which characterises presbyopia. Additional factors include the progressive increase in lens mass with age, changes in the point of insertion of the lens zonules, and a shortening of the radius of curvature of the anterior surface of the lens. With age, there is also a decrease in light transmission by the lens, associated with increased light scatter, increased spectral absorption (particularly at the blue end of the spectrum), and increased lens fluorescence.

A major factor responsible for the increased yellowing of the lens is the accumulation of a novel fluorogen—glutathione-3-hydroxy kynurenine glycoside—which makes a major contribution to the increasing fluorescence of the lens

nucleus that occurs with age. Because this compound may also crosslink with the lens crystallins, it may contribute to the formation of high-molecular-weight aggregates and the increases in light scattering that occur with age. Focal changes of microscopic size are observed in apparently transparent, aged lenses, and may be regarded as precursors of cortical cataract formation.[234,235]

## *Age-related Changes in Calcium, Sodium, Potassium and Lens Membrane Permeability*

The lens optical density increase with age, and the rate of increase, is much more apparent after the age of 40 years.[236] The lens also becomes increasingly colored (yellow) with age, and the intrinsic fluorescence also increases—all of these changes tend to degrade the optical properties of the lens.[237]

The smallest opacities are the so-called retrodots, which are present in normal, noncataractous lenses, and the frequency of their occurrence increases exponentially after 40 years of age.[238] They appear to be formed from multilayered membrane vesicles and have a surprisingly low protein content, but correspondingly high calcium concentration.[239,240] When there is a larger, but still localized breakdown in lens fiber structure, the formed opacities disturb the normal visual acuity, especially when they are located on or near the visual axis. Such lenses have near normal sodium and potassium concentrations but have elevated calcium levels.[241]

Calcium ions appear to have the ability both to disrupt the structure of the lens and also to protect the transparent, unaffected areas by sealing off the damaged fibers. The disrupting properties probably arise through activating the cysteine protease calpain, and several proteins of the structurally important lens cytoskeleton appear to be excellent substrates for degradation by the enzyme.[242] The membrane potential of the normal lens appears to decline with age, particularly after the age of 40 years.

The decline in voltage is accompanied by a decrease in membrane resistance, indicating that some channel mechanism is being activated in the aging lens.[243] This channel is present in lens membranes and appears to permit Na+, K+, and Ca2+ to pass.[244,245] It is interesting in this respect that the lens sodium and free calcium content also appear to increase after the age of 40.[243] There is a remarkable agreement between the relative increase in permeability to sodium and the increase in lens optical density measured at the wavelength of peak sensitivity of the eye. Both increase more rapidly after the age of 40 and again indicate a common mechanism between alterations in the ionic and structural protein contents of the human lens.

## *Lens Phospholipid Changes with Age and Cataracts*

Human lens membrane lipid composition is related to the membrane's organization,[246] structure,[247-251] and function.[252-256] Age-related changes in human lens lipid composition may serve as a marker for oxidative stress and may reflect systemic oxidative

insult, providing a window into the health of an individual.[257] Species-related phospholipid differences support the idea that humans have adapted so that their lens membranes have a high sphingolipid content that confers resistance to oxidation, allowing these membranes to stay clear for a relatively longer time than is the case in many other species.[257]

The changes observed in the phospholipid composition of the human lens with age and cataracta were substantial—greater than that reported for any organ or disease. Biochemical studies show that, upon maturation, the cholesterol-to-phospholipid ratio of lens membranes dramatically changes from 0.6-0.8 in superficial to over 5.0 in deep cortical and nuclear membranes.[258] All this indicates that lens membranes, apart from those in the most superficial cortex, deviate from most cell membranes in the body. This is in line with electrophysiological studies showing that deep cortical membranes are non-leaky, have a high resistance and low capacitance and have no or restricted cell-to-cell communication.[259,260]

The relative and absolute amount of sphingolipids (including dihydrosphingomyelin and sphingomyelin) increase with age, while glycerolipids (including phosphatidylcholine and two phosphatidylethanolamine-related phospholipids) decrease.[266] These changes are exacerbated by the presence of cataracts and are substantial—greater than the changes in lipid levels reported in any organ in association with any disease. The changes in the amount of lipids with age and cataracts support the idea that glycerolipids are selectively oxidized over lipids with fewer double bonds, such as sphingolipids. As a result of the elevation of sphingolipid levels with species, age, and cataracts, lipid hydrocarbon chain order (or stiffness) increases. Increased membrane stiffness may increase light-scattering, reduce calcium pump activity, alter protein-lipid interactions, and perhaps slow fiber cell elongation.[261-264] The cause of the changes may be due to lipid oxidation.

Lens glycerolipids are approximately three to four times more unsaturated than lens sphingolipid, and consequently they can be selectively oxidized more than unsaturated lipids. Conversely, de Vries[265] calculated that the amount of sphingolipid per wet weight of lens—a relatively unsaturated lipid—increases with age up to approximately 45 years. Because phospholipid and cholesterol synthesis do not change within the ages studied,[265] the relative and absolute changes between the sphingolipid and glycerolipid with age must be due to degradation. Recent research[266] showed that the relative amount of sphingolipids (dihydrosphingomyelin and sphingomyelin) increased from 48 percent at 22 years of age to 57 percent at 69 years of age, in agreement with previous studies.[261-264] With cataracts, the relative amount of sphingolipid increased to 78 percent. However, an increase in sphingolipid content in the human lens with age and cataract may indicate deleterious phospholipid oxidation. Human lens lipid composition versus age curves, exhibiting a plateau at 45 years, are remarkably similar to the curves of accommodative amplitude versus age[267] and human lens membrane cation passive permeability versus age.[268]

Correlation does not necessarily indicate causation—however, scenarios can be envisioned in which lens membrane stiffness induced by phospholipid composi-

tional changes directly or indirectly contribute to presbyopia and/or passive membrane permeability of cations. Recent studies suggest that, as a result of increased sphingolipid content in cataractous lenses compared with age-matched clear lenses, light-scattering increases.[269] Lipids scatter 2 to 95 times more light *in vitro* than do crystallin proteins, indicating that they may contribute to the light-scattering intensity of the lens *in vivo*.[269] Because lipids with ordered hydrocarbon chains have higher polarizabilities, they scatter 2.5 times more light than lipids with disordered hydrocarbon chains.[269]

An increase in lipid hydrocarbon chain order may also contribute to cataractogenesis indirectly by reducing the activity of the sarco/endoplasmic reticulum isoform of the calcium pump.[254] Reduced pump activity could cause an increase in lens calcium levels, wich is elevated in all cataracts,[272-275] and maintenance of the calcium homeostasis is essential to lens clarity. The higher sphingolipid content of cataractous lenses may also change protein-lipid interaction[276,277] and slow fiber cell elongation[261]—two factors that could contribute to cataracts.[252-261]

## *Age-related Changes in Ganglioside Composition*

Lens tissues are enriched in the plasma membranes and are known to contain a relatively high concentration of gangliosides among non-neural tissues.[278] Because gangliosides are mainly located at the outer leaflet of the plasma membranes, changes in their content and composition may disrupt the functions of the plasma membranes, such as ion transport, cell-to-cell interactions, transmembrane signaling, and so on.[279] Ogiso et al.[280] reported that human lens accumulates gangliosides in association with aging and senile cataract progression. Structural analysis reveals that gangliosides in human cataractous lenses were composed of ganglio-series gangliosides, such as GM3, GM2, GM1 and GDla, and sialyl-Lewis$^x$ containing neolacto-series gangliosides.[280] Although Lewis$^x$-containing, neolacto-series glycolipid was found to accumulate in association with aging and cataract progression, the sialyl-Lewis$^x$ gangliosides did not show much accumulation in individual lenses from subjects between 16 and 80-years of age.[281,282]

The content of sialyl-Lewis$^x$ gangliosides was about two to four times higher than that of Lewis$^x$ glycolipids, suggesting the possibility that the increase in Le$^x$ glycolipid is partly due to the desialylation of sialyl-Le$^x$ gangliosides.[282] On the other hand, the expression of ganglio-series gangliosides increased in an age-related manner.[282] The age dependent, cataract-related increase in ganglioside content in the human cataractous lens is largely derived from the increase in ganglioseries, GM3, GM1 and GD1a.[280] Age-related changes in some gangliosides and neutral GSLs, for example, GM3, GM1 and Gb3, appear to be attributable to the accumulation of lens fibers.[282] Thus, age-related changes in lens glycolipids may modify the cell-to-cell interaction induced by cell surface sugar chains, leading to the initiation and progression of cataract.[282]

## *Water Content Modifications in Lens with Aging*

The age-dependance of lens hydration has been studied by a number of techniques in human, as well as in animal, lenses. Lahm et al.[283] found a slight age-dependent increase in total water of the intermediate and nuclear regions of human lenses, even if none of these were statistically significant. Nunnari et al.[284] and Bours et al.[285] also reported no significant changes in the total water content of aging human lenses. On the other hand, the decrease in bound (nonfreezable) water as a percent of the total water with age was statistically significant in each segment.[283] This indicates that syneresis[286] is involved in aging.

In syneresis, bound water is released from the hydration layer of byopolymers and becomes free water.[286] The physical process itself has a number of potential consequences. In the eye, syneresis accounts for the liquid pocket formation in the aging vitreous.[287] The amount of bound water decreases with age, which supports the existence of syneresis as a factor in aging and in cataract formation[286] as inferred from light-scattering measurements,[288,289] and shown by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) techniques.[284-290]

In aging and cataractous lenses, irreversible syneresis contributes to the turbidity of the lens by increasing the amplitude of refractive index fluctuations.[291,292] Besides light scattering and thermal studies, the role of syneresis in cataractogenesis has been proven by NMR.[293-296] Recent research confirmed that the amount of bound water decreases with age, which supports the existence of syneresis as a factor in aging.[297] The implication is that in normal lenses without apparent turbidity, aging causes tighter packing of protein molecules, possibly leading to higher molecular species. The remaining bound water layer, however, becomes tighter, more immobilized, and therefore, potentially still a sufficient barrier to prevent aggregation and cataract formation.[297]

## *Oxidative Stress in the Aging Lens*

Due to its constant exposure to light and oxidants, oxidation is a major insult to the lens.[298-300] Oxidative stress corresponds to an imbalance between the rate of oxidant production and the rate of its degradation.[301] The complete four-electron reduction of oxygen occurs within the mitochondria, and the end product is water.

A partial reduction produces superoxide and various reactive oxidative intermediates (free radicals and reactive oxygen species, or ROS including hydroxyl radicals, singlet oxygen radicals, and hydrogen peroxide).[302] Besides these endogenous oxidants, other sources are food, air pollutants, tobacco smoke, exercise, ionizing radiation, IR and, of course, the sun.[303]

Although the organism adapts by preventing undesirable reactions with its endogenous and partly redundant antioxidant defense (glutathione superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and repairing

damaged molecules and tissues, the few molecules and undesirable reactions that are not prevented or repaired will accumulate over time and be deleterious in the long term.[303] All of these conditions will lead to the formation of excessive oxidants and oxidative stress.

Oxidative stress is countered by antioxidants that are defined as substances that, at low concentrations relative to the substrate, inhibit the damage to the structural and functional molecules of the body, namely proteins, lipids, carbohydrates, and DNA.[303] Antioxidants function by several possible mechanisms:

- Scavenging of free radicals involved in chain reactions; tocopherol acting in the lipid phase
- Regeneration of other antioxidants; ascorbate reduces tocopheryloxy radical to tocopherol by donating an atom
- Reacting with initiating radicals or oxidants (catalase with hydrogen peroxide)
- Chelating or sequestering transition metal catalysts which are pro-oxidants; albumin or polyphenols with cupric ion
- Inhibiting or activating an enzyme; tocopherol and polyphenols inhibit tyrosine kinase and ascorbate activates nitric oxide synthase[303]

There is a considerable body of evidence to indicate that the ability of the human lens to withstand oxidative attack actually declines with age because the overall level of glutathione decreases and the important enzyme glutathione reductase becomes less stable.[304]

Because it is well-known that lens nuclear cataracts involve protein oxidation,[305] there is now, therefore, the possibility that nuclear and cortical cataracts, with their totally different aetiology and morphological appearance, may both arise from oxidative mechanisms—one taking place primarily at the surface membranes, and the other within the nuclear proteins. This may help explain why the majority of senile cataracts are, in fact, mixed in form, with contributions from both nuclear and cortical changes.[306]

Recent epidemiological studies of cataracts do suggest that a high intake of antioxidants—either in the diet, or in the form of supplements—does confer a considerable protective effect.[307] Age-related nuclear (ARN) cataracts are associated with a loss of glutathione in the center of the lens and extensive modification of the nuclear proteins that include coloration, oxidation, insolubilization, and crosslinkin g.[308]Accumulation of oxidatively damaged proteins is causally related to the formation of cataracts[298,304,309,314] and many other age-related debilities.[315-317]

## Age-related Decline in Ibiquitin Conjugation

The extent of accumulation of oxidatively damaged proteins depends on both the rate of production and on the efficiency of removal of the oxidatively damaged proteins.[318,319] In most cells, intracellular proteolytic enzymes selectively remove the oxidized or damaged proteins.[320-324] Therefore, proteolytic capabilities are considered as secondary defense systems, which can avert or delay the accumulation of damaged proteins.[318,322,325,328]

The ubiquitin-dependent proteolytic pathway is a primary proteolytic system which is involved in the selective degradation of oxidatively damaged proteins in various types of cells or cell-free systems,[329-333] and a substantial amount of literature indicates that the ubiquitin-dependent proteolytic system functions in lens cells, as well.[332-334] Shang et al.[335] showed that:

- lenses—especially the nuclei of lenses—undergo dramatic changes with aging, including a decreased level of ubiquitin conjugates and decreased ubiquitin conjugation activity
- there is an increase in endogenous ubiquitin-protein conjugates and enhanced ubiquitin conjugation activity in response to oxidative stress in each developmental zone of lenses
- the ability to mount a ubiquitin-dependent response to oxidative stress decreases in the old lens—especially in the nucleus of old lenses

This attenuated ability to enhance the ubiquitin conjugation activity with oxidative damage may be associated with the observed accumulation of damaged proteins in old lenses.

The progression of cataractogenesis in the normal aging population can be characterized as a continual increase in the intensity of light scattered from the lens. An important molecular mechanism for such light scattering is, in fact, the condensation of protein into aggregates.[335] Protein insolubilization in human lenses during aging and cataracts is well documented.[336-339] Garner et al.[340] showed the association of gamma crystallin with the membrane protein component of human cataract lenses.

Recent studies[341,342] indicate that the fiber cell plasma membrane has a high capacity to bind a-crystallin in a nonsaturable manner. This association may play an important role in triggering the further interaction of crystallins with plasma membranes in normal aging and cataract formation, which results in massive protein insolubilization.[343] The basis for the great association of crystallins with lens membranes during aging and cataractogenesis is unknown, but might involve the interplay of two broad mechanisms. Modification of membrane structures could enhance its protein-binding characteristics, modification of crystallin structure could increase their affinity to bind, and a combination of altered membranes and crystallin structure might be important for association. This increase is an exponential function of age and has a *time constant* that, on average, is approximately 35 years.[344]

## *Hormonal Influence on Lens with Aging*

The human lens continues to grow throughout life and in all decades from 10 to 70 years—the male lens is heavier than its female counterpart.[345] These age-related differences between males and females are interesting because not only do their relative susceptibilities to cataract change with age, but their response to physical trauma also does.

A role for female hormones in protecting against cataracts has been suggested by recent epidemiological studies. Below the age of 50, the prevalence of cataracts seems to be similar in males and females,[346,347] but it increases in postmenopausal women.[346-348] Moreover, postmenopausal women on hormone replacement therapy, or younger women taking oral contraceptives, display a decreased prevalence and severity of cataracts.[349-352] In addition, the prevalence and severity of certain forms of cataracts are lower in postmenopausal women on hormone replacement therapy involving administration of estrogen, with or without progesterone, than in those who are not undergoing hormone replacement therapy.

Hales et al.[353] showed that transforming growth factor-β (TGFβ)—a multifunctional growth factor that is present in the aqueous and vitreous humours[354]—induces rat lenses in culture to develop opacities and other changes that have many features of human subcapsular cataracts. Hales also showed that estrogen protects against cataracts. Interestingly, lenses from male rats are more susceptible than those from female rats and, furthermore, the latter receive added protection from TGFβ if estrogen is also present in the medium.[355]

The molecular mechanisms underlying the cataractogenic effect of TGFβ are poorly understood, but TGFβ is known to induce transdifferentiation of lens cells so that they produce at least two types of foreign protein—smooth muscle actin and collagen types 1 and 3.[356] Neither of these is synthesized in significant amounts by normal lens cells, but can be detected in certain cataracts[122] and in cells, giving rise to PCO.[358] The TGFβ stimulated production of abnormal intracellular and extracellular proteins disrupts the homogeneous structure of the anterior epithelium, and light-scattering, multilayered cell aggregates are produced.[355]

Not only do male and female lenses differ in their relative sensitivity to TGFβ, but they also respond differently to mechanical stress. Weale[359,360] carried out a quantitative study of the birefringence of male and female lenses, and although the overall pattern is the same, the effect of external stress on the birefringence pattern measured *in vitro* is different in males and females. Weale[360] measured the greatest stress that could be given before an irreversible change in birefringence occurred and, although in both cases the magnitude of the reversible stress declines with age, the rate of decline appears to be steeper with female lenses. Furthermore, Weale identified a number of female lenses in which the merest mechanical stress induced irreversible birefringence changes, and he concluded that this pointed to a subtle structural difference between male and female lenses.[360]

## The Effect of Physical Agents on the Aging Lens

### *Ultraviolet Radiation*

Sunlight is the principal source of ultraviolet radiation (UVR) for most of the world's population. Depletion of the stratospheric ozone increases the intensity of UVR. UVR is considered one of the major risk factors for cataracts,[361-364] and

several studies have shown that sunlight increases the risk of cortical cataracts.[365-368] Effects of UVR may be analyzed from different perspectives (e.g., at the molecular, cellular, tissue, individual, population, and ecosystem levels).[369] UVR damages the lens by disturbing cell proliferation in the lens epithelium,[370] by altering kinetic properties of enzymes in the energy metabolism,[371] by increasing insoluble and decreasing soluble protein,[372,373] by inducing unscheduled DNA synthesis,[374] and by disturbing the sodium potassium balance and thereby the water balance in the lens.[375,376] One of the major difficulties in epidemiologic studies has been quantification of exposure to UVR from the sun.

The consequences of UVR exposure on the epithelium must be considered both in terms of mutagenic as well as cytotoxic effects.[377,378] The single layer of epithelium is the first physical and cellular (biological) defense against electromagnetic radiation in the ocular lens.[379] Some of the direct effects of UVR exposure on cultured cells have been reviewed in detail.[380] UVR exposure results in unscheduled DNA synthesis and repair.[381-383] The human lens epithelium accumulates insults due to UVR exposure in its genome over a period of time that are manifested in the aged lens.[384] There is, of course, an age dependence of UVR damage to different molecular species, including enzymes such as hexokinase, phosphofructokinase, isocitrate dehydrogenase, and malate dehydrogenase.[385] Loss of hexokinase[386] would result in the inability of the lens to produce NADPH and downstream antioxidants.

It is conceivable that proteins (such as $Na^+/K^+$) ATPase, cytoskeletal elements, membrane proteins), which are dependent on –SH function will be damaged by exposure to increased oxidants. In addition to intensity of sunlight, the ocular dose depends on other factors, such as the amount of time spent outdoors, the environment, the use of ocular protection, and the use of hats.[363,364,387-389] In earlier studies, safety limits for UVR-B induced cataract have been based on a dichotomous dose-response model, assuming that the outcome of UVR-B exposure is limited to a binary response: cataract/no cataracts.[390] In those studies, cataracts were measured qualitatively with a slit lamp, with a grading scale. It has recently been shown with quantitative measurements of cataracts, however, that UVR-B-induced cataracts has a continuous dose-response function.[391] For this reason, a new concept—maximum acceptable dose (MAD) for avoidance of UVR-B cataract—was developed for estimation of UVR-B toxicity in the lens.[392]

Based on the dose-response function, MAD is defined as the dose corresponding to a limit for pathologic forward light scattering. The limit for pathologic forward light-scattering is settled arbitrarily, based on the frequency distribution of light scattering in normal unexposed lenses. The limit is defined so that a certain fraction ($\alpha$) of normal unexposed lenses scatter light in the forward direction to an intensity above the limit. The magnitude of the fraction is a parameter that has to be settled and is given as an index to $MAD_{1-\alpha}$.

The high rate of cell division in the germinative zone in the young lens may render the young lens more sensitive to UVR-B–triggered DNA fragmentation. Further, the young lens requires more protein synthesis that includes a part of the young lens that is biologically more important than that of the older lens.[372,393]

Lerman[394] exposed young (first decade) and old (seventh decade) normal human lenses to low level ($<0.1\,kJ/cm^2$) broad band UVR-B (300–400 nm), and found that

the γ-crystallins were significantly affected by UVR-B in young lenses, while the aged lens proteins appeared to be relatively unaffected by this degree of UVR-B exposure. The finding that MAD for avoidance of UVR-B-induced cataracts strongly depends on age implicates that, in the future, age should be considered. Until better data are available, the current data should be considered in toxicity estimates for avoidance of UVR-B cataracts after exposure to the sun, as well as to artificial sources.[395]

## *Medications and Cataracts*

Oral corticosteroids are known to cause cataracts, but the role of many other systemic medications in cataract etiology is uncertain.[396] Several case reports suggest that allopurinol may cause cataracts,[397-399] but epidemiologic studies are inconsistent.[400-402] Case series of institutionalized patients suggest that phenothiazines are associated with cataract development,[403,404] but only one population-based study has examined these medications.[405] There have been several studies of diuretics and cataracts, with some finding a protective effect,[406] and others a harmful one.[407-409] In one case series,[410] a high proportion of patients on amiodarone had cataracts, but this association does not appear to have been studied by other investigators.

There are biological reasons why some drugs used to lower serum cholesterol might cause cataracts, but such an effect has not been shown.[411] Finally, the possibility that aspirin lowers the risk of cataracts has received a great deal of attention in recent years, but studies are far from consistent.[396] Different types of cataracts have different etiologies, and so it is important to distinguish between types of cataracts when studying cataract risk factors.

The Blue Mountains Eye Study is a large population-based study in which cataract diagnosis was based on grading of lens photographs.[412] An association between inhaled steroids and cataracts was found in this study population. Four medications were associated with increased cataract prevalence—phenothiazines were associated with nuclear cataract; amiodarone with cortical cataract; and aspirin and mepacrine (an antimalarial medication that was used extensively during World War II) were associated with posterior subcapsular cataract.[412] Aspirin is the only one of these four medications that is used extensively in the community. Most medications studied were not associated with cataracts, including allopurinol, cholesterol-lowering medications, thiazide diuretics, frusemide, beta blockers, calcium-channel blockers, benzodiazepines, and nonsteroidal antiinflammatory drugs.[412]

### Aspirin and Nonsteroidal Anti-inflammatory Drugs

There have been at least 15 previous studies of the association between aspirin use and cataracts,[396,414-427] including three randomized trials.[420-422] None of the randomized trials found any protective effect of aspirin. Of the 12 observational studies,

six found that cataracts were less frequent among aspirin users.[414-418] These positive studies had methodological flaws that could explain their findings, including failure to adjust for prior steroid use and other confounders, inadequate control groups, and use of cataract surgery cases. In the The Blue Mountains Eye Study, long-term aspirin use was associated with increased prevalence of posterior subcapsular cataract.[412]

At least three other studies have found a slightly higher risk of cataract in aspirin users.[405,426,427] Based on the combined evidence from nearly 20 years of research, it is possible to conclude that aspirin does not protect against the development or progression of cataracts. Hankinson et al.[428] found some evidence that nonsteroidal anti-inflammatory medications might be associated with increased risk of cataracts, but The Blue Mountains Eye Study found no such association. Interestingly, this study did find that persons with self-reported osteoarthritis were more likely to have had cataract surgery than persons without osteoarthritis.

## Diuretics and Antihypertensives

Harding and van Heyningen[406] reported that thiazide diuretics were used less frequently by patients who underwent cataract surgery than control subjects. More recently, the Beaver Dam Eye Study found that use of thiazides was associated with lower prevalence of nuclear cataracts and increased prevalence of posterior subcapsular cataract.[429] Several other studies have found that use of diuretics was associated with increased risk of cataracts.[407-409] The Blue Mountains Eye Study did not find convincing evidence of any harmful or beneficial effects of diuretics on the lens.[412] Although frusemide was associated with increased prevalence of cortical and posterior subcapsular cataracts in age- and gender-adjusted analyses, these associations appeared to be because of confounding.

The Blue Mountains Eye Study found that long-term users of potassium-sparing diuretics might be at increased risk of cataract. The Beaver Dam Eye Study also found a raised incidence for potassium-sparing diuretics, but this was not statistically significant.[429] A cataractogenic effect of potassium-sparing diuretics is biologically plausible, because these diuretics disturb sodium transport across the lens fiber membrane.[430,431]

The calcium-channel blocker nifedipine has been associated with increased risk of cataract extraction[406] and angiotensin-converting enzyme inhibitors with decreased risk of nuclear cataracts.[429] Neither of these medication types was associated with cataract in the Blue Mountains Eye Study. Confounding by hypertension and other cardiovascular conditions is a potential problem in studies of cataracts and antihypertensive medications,[409,429] including diuretics. The Blue Mountains Eye Study addressed this problem by using statistical techniques to check for history of cardiovascular disease, and by repeating analyses in normotensive persons. After confounding had been adjusted for, none of the antihypertensive medications studied were statistically significantly associated with cataracts.

### Cholesterol-lowering Medications

The normal lens membrane contains a very high concentration of cholesterol, most of which is actually synthesized in the lens.[411] Hence, drugs that reduce cholesterol synthesis could cause cataracts. Laboratory experiments have found that simvastatin has a particularly strong inhibitory effect on lens cholesterol synthesis.[411] The Blue Mountains Eye Study, however, found no association between any type of cataract and use of simvastatin (or any other cholesterol-lowering drug).[412] Previous epidemiologic studies of simvastatin and lovostatin have similarly found no association with cataracts.[411,432]

### Allopurinol

Several case series have noted that persons treated with allopurinol seem to have characteristic lens changes,[397-399] perhaps due to photobinding by allopurinol in the lens.[398] In the Lens Opacities Case-Control Study, persons using gout medications (most likely to be allopurinol) had increased prevalence of cataracts.[400] Two other epidemiologic studies found no relationship between allopurinol and cataract extraction.[401,402] In The Blue Mountains Eye Study, use of allopurinol for 10 or more years was associated with posterior subcapsular cataracts in the initial analyses, but there was no association after adjusting for confounders. Previously observed associations between the use of allopurinol and cataracts may have been because of the higher prevalence of risk factors for cataracts among these persons.

### Antimalarials

The Blue Mountains Eye Study found a strong association between posterior subcapsular cataract and use of mepacrine—a 9-aminoacridine that was used extensively for malaria prophylaxis by Australian soldiers in the Pacific during World War II—and these data support studies conducted in the 1950s, which reported a high prevalence of cataracts in persons taking chloroquine.[433]

### Phenothiazines

An association between the phenothiazine chlorpromazine and cataracts was first reported in the 1960s in patients living in psychiatric institutions.[434,435] Two epidemiologic studies have found that use of psychotropic medications is associated with cataracts, but these studies did not investigate specific classes of medications.[434,435] The only epidemiologic study to date of phenothiazines and cataracts among persons living in the community was conducted by Isaac et al.[405]

The increased frequency of cataracts among phenothiazine users in that study could have been caused by selection bias, because only cataract extraction cases were studied (phenothiazines have an anticholinergic effect that can cause blurred vision, which might lead to increased eye examinations and detection of cataract). In The Blue Mountains Eye Study, which avoided this selection bias by basing cataract diagnosis on grading of lens photographs, phenothiazines were associated with increased prevalence of nuclear cataracts.[412]

### Amiodarone

This antiarrhythmic drug was associated with an increased prevalence of cortical cataracts in the Blue Mountains Eye Study. This is consistent with reports by Flach et al.[410] of high rates of subcapsular cataracts in patients treated with amiodarone.

## A Clinical Approach to Lens Modifications with Aging

### *Introduction*

With the new instruments available today in clinical practice, it is possible to study the correlation between bio-densitometric changes, optical high-order aberrations (HOAs), and modulation transfer function (MTF) of the crystalline lens that take place during the aging process. We have presented a comprehensive study[436] in which these changes have been measured in different age groups of patients without cataracts, to evaluate ways in which morphology and optical performance of the human crystalline lens degrade with age. All the measurements are simple, objective, and performed quickly, requiring minimum cooperation from the subject.

### *Lens Bio-densitometric Changes Through Aging*

#### Scheimpflug Photography Features

To evaluate lens morphology and densitometric data, a Scheimpflug slit lamp (EAS 1000, Nidek, Japan) was used.[437] In this technique, slit-lamp photography measures light that is reflected anteriorly from the lens to the camera. To record a slit image, an alignment system is coupled to a television monitor, and a fixation light is placed to lie along the optical axis of the slit projection lens. A photograph is taken using a flash intensity of 200 W-seconds. Density is measured by optical density units that are EAS 1000-specific. The resulting cross-sectional image of the anterior chamber and lens is displayed on a monitor for evaluation by the operator. If satisfactory, the image can be transferred to the computer for analysis. To quantify nuclear lens density, linear densitometric analysis of the image was performed in

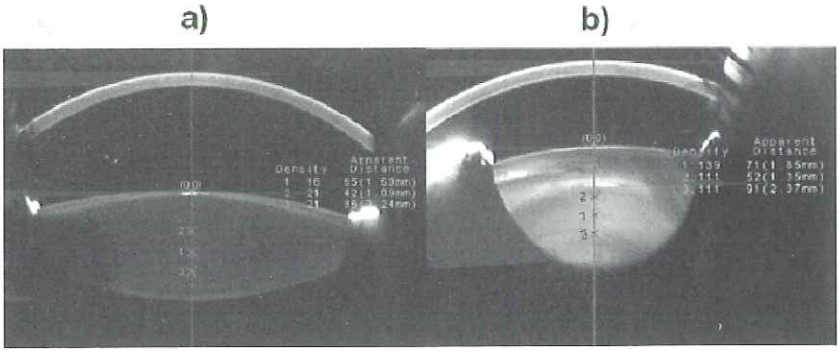


**Fig. 5.11** Photographs using a flash intensity of 200 W-seconds in a Scheimpflug slit lamp. (A) Eight-year-old subject. (B) Eighty-year-old subject. Linear densitometric analysis to quantify nuclear density. 1, 2, 3 = densities at the embryonic nucleus, anterior fetal nucleus, and posterior fetal nucleus, respectively

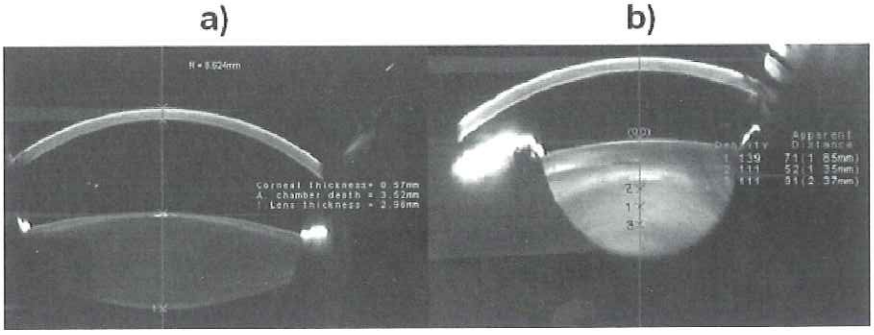


**Fig. 5.12** Photographs using a flash intensity of 200 W–seconds in a Scheimpflug slit lamp. (A) Eight-year-old subject. A. = anterior; R = radius. (B) Eighty-year-old subject. Axial biometric analysis to quantify lens thickness. 1, 2, 3 = densities at the embryonic nucleus, anterior fetal nucleus, and posterior fetal nucleus, respectively

our study. Density was measured at the embryonic, anterior, and posterior fetal nuclei (see Fig. 5.11). To quantify lens thickness, an axial biometric analysis of the image was performed (see Fig. 5.12).

## Results from Scheimpflug Photography

The correlation with age on nucleus density is represented in Figs. 5.13 to 5.15. Densities of embryonic, anterior fetal, and posterior fetal nuclei show a positive correlation with aging after the age of 40.

The scatterplots of embryonic and anterior fetal nuclei clearly show a turning point around the age of 40 years, after which densities of the nuclei show an increase with age. The relationship between age and crystalline lens thickness is shown in Fig. 5.16. As exhibited, crystalline lens thickness increases significantly with age in a linear mode.

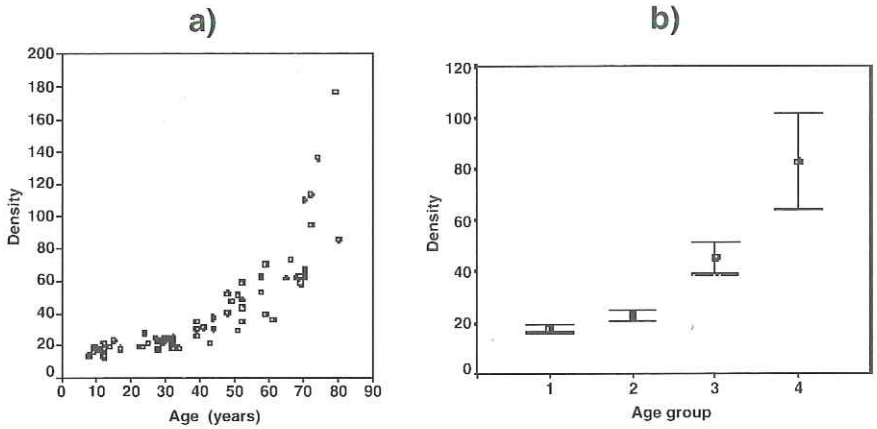


**Fig. 5.13** (A) Embryonic nucleus (flash intensity, 200 W–seconds) as a function of age. A positive correlation was found after the age of 40 years (r=0.762, P<0.0001). (B) Embryonic nucleus (flash intensity, 200 W–seconds) in four age groups. The mean difference using Bonferroni multiple comparison is statistically significant for Groups 2 and 3 (P<0.002) and for Groups 3 and 4 (P<0.0001). Error bars, minimum and maximum of the 95 percent confidence interval

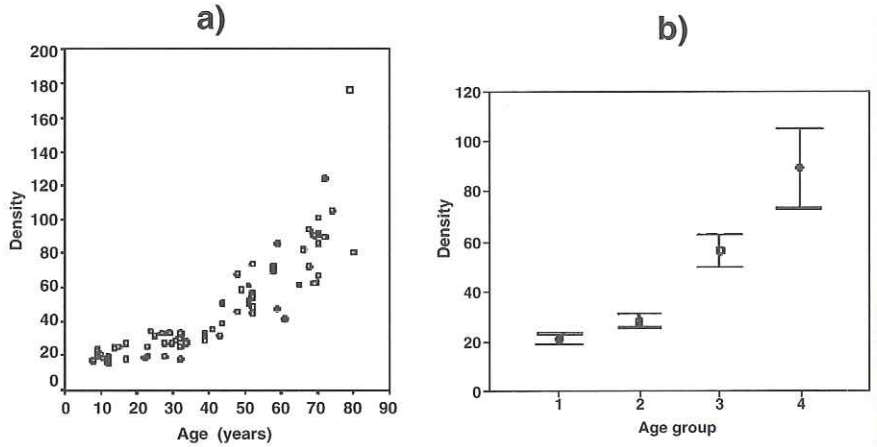


**Fig. 5.14** (A) Anterior fetal nucleus (flash intensity, 200 W-seconds) as a function of age. A positive correlation was found after the age of 40 years (r =0.764, P<0.0001). (B) Anterior fetal nucleus (flash intensity, 200 W-seconds) in four age groups. The mean difference using Bonferroni multiple comparison is statistically significant for Groups 2 and 3 (P<0.0001) and for Groups 3 and 4 (P<.0001). Error bars, minimum and maximum of the 95 percent confidence interval

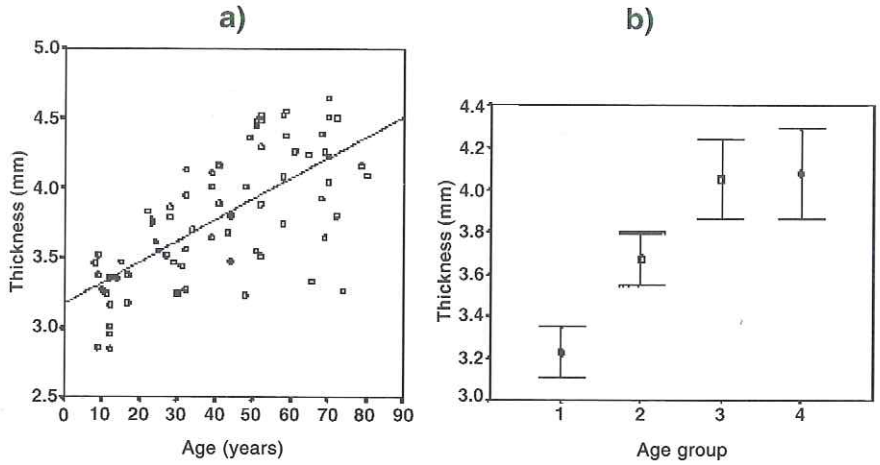


**Fig. 5.15** (A) Posterior fetal nucleus (flash intensity, 200 W-seconds) as a function of age. A positive correlation was found after the age of 40 years ($r = 0.756$, $P < 0.0001$). (B) Posterior fetal nucleus (flash intensity, 200 W-seconds) in four age groups. The mean difference using Bonferroni multiple comparison is statistically significant for Groups 2 and 3 ($P < 0.0001$) and for Groups 3 and 4 ($P < 0.0001$). Error bars, minimum and maximum of the 95 percent confidence interval

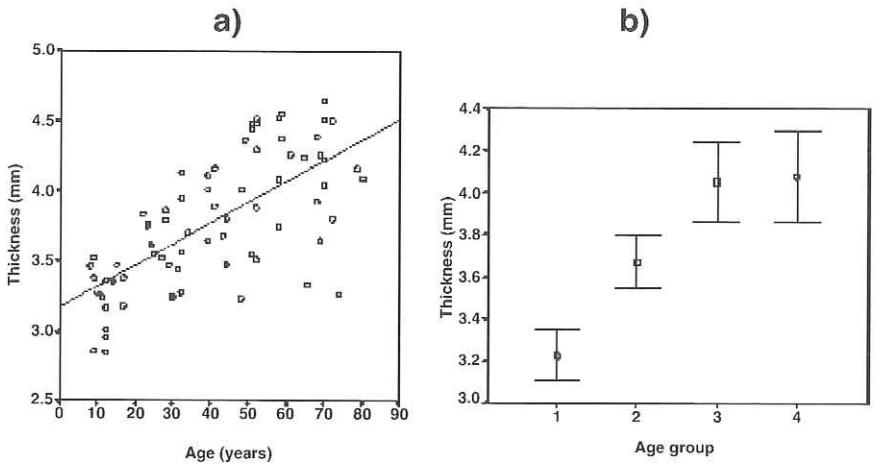


**Fig. 5.16** (A) Crystalline lens thickness as a function of age. A positive linear correlation was found ($r = 0.679$, $P < 0.0001$). (B) Crystalline lens thickness in four age groups. The mean difference using Bonferroni multiple comparison is statistically significant for Groups 1 and 2 ($P < 0.002$) and for Groups 2 and 3 ($P < 0.004$). Error bars, minimum and maximum of the 95 percent confidence interval

## *Optical Changes of the Human Crystalline Lens Through Life*

### Modulation Transfer Function

The MTF value quantitatively characterizes the performance of the optical system of the eye. For years, the photography industry has used MTF values to measure the optical quality of lenses. The MTF is the ratio of the image wave contrast to the object wave contrast. The higher the MTF value is, the higher the quality of the image is after it passes through a lens. Optical quality was studied using the MTF for monochromatic light. In our study, the MTF was measured[436] with the Optical Quality Analysis System (Visiometrics S.L., Terrassa, Spain)—a recent instrument based on the double-pass technique and developed to perform an objective optical quality-of-vision evaluation. The double-pass technique is based on recording images of a point source after reflection in the retina and a double pass through the ocular media.[438]

With this configuration, therefore, the ocular point-spread function (PSF) can be obtained. The point spread function (PSF) defines the propagation of electromagnetic radiation or other imaging waves from a point source or point object. The degree of spreading (blurring) of the point object is a measure for the quality of an imaging system.

From the point-spread function images, the MTF that yields the relationship between the contrast of an object and its associated image as a function of spatial frequency was obtained, computing the modulus of the 2-dimensional Fourier transformations of the point spread function. The 1-dimensional MTF was calculated as the radial projection (averaging over all orientations) of the 2-dimensional MTF (see Figs.5.17 to 5.19).

Measurements were done with a 5-mm pupil. Data at 0.5 MTF represent the spatial frequency (cycles per degree) in which the image contrast is degraded 50

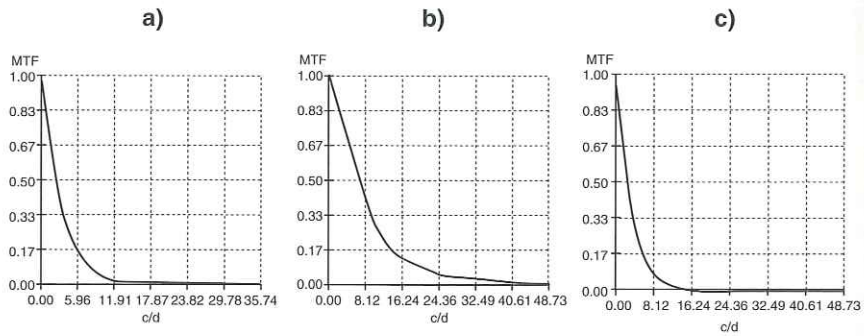


**Fig. 5.17** Curves of spatial frequency and modulation transfer function (MTF) obtained using the Optical Quality Analysis System in (A) an eight-year old subject, (B) a 30-year old subject, and (C) an 80-year old subject. c/d = cycles per degree

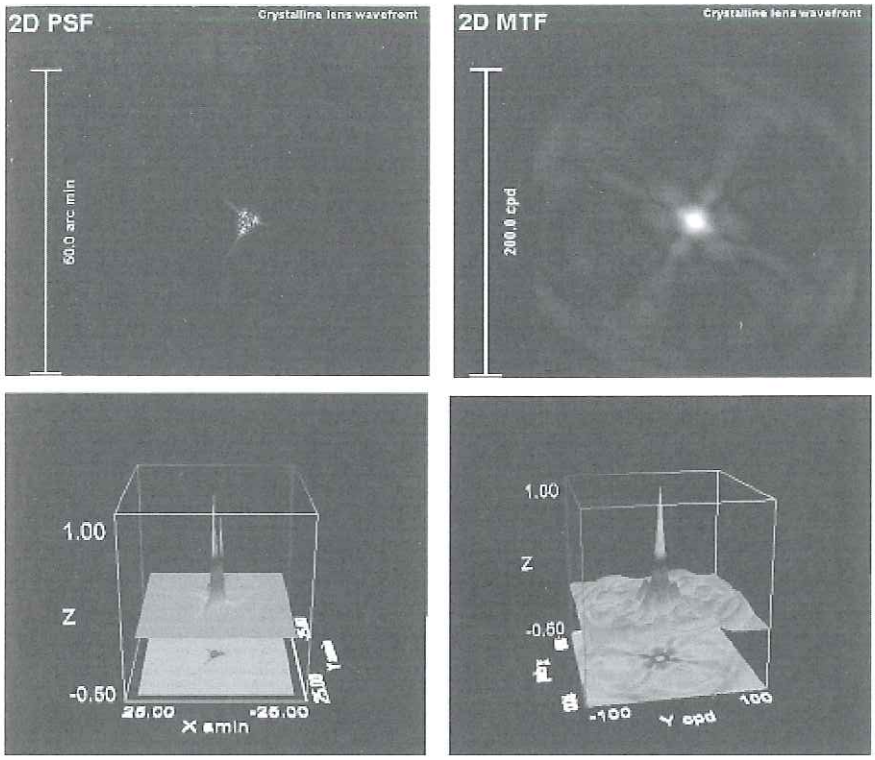


**Fig. 5.18** A graphic correlation between 2D - 3D PSF and MTF of the crystalline lens with the OQAS

percent relative to the object contrast. Data at 0.1 MTF represent the spatial frequency in which the image contrast is degraded 90 percent relative to the object contrast, and correspond to the maximum resolution of the optical system. The OQAS creates two- and three-dimensional retinal images (or maps) that describe a patient's total optical system (Fig. 5.20).

### MTF Results Measured with the Optical Quality Analysis System

The error bar graphs shown in Fig. 5.21 represent 0.1 and 0.5 MTFs in different age groups. The 0.5 MTFs are 4.317 for Group 1, 5.384 for Group 2, 3.501 for Group 3, and 3.046 for Group 4. A significant difference is seen between the age groups of 21 to 40 and 41 to 60 for 0.1 and 0.5 MTFs. The 0.1 MTF decreased with age from 18.557 to 10.100 cycles per degree.

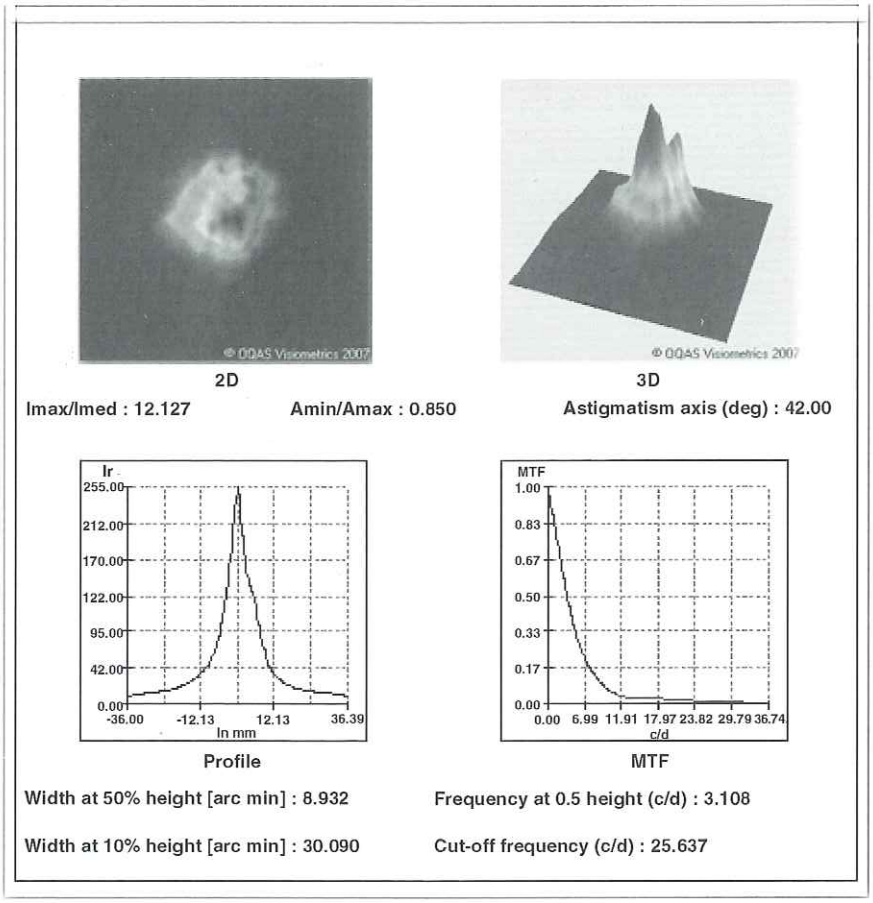


**Fig. 5.19** Graphic representation of two- and three-dimensional retinal images (or maps) that describe a patient's total optical quality in a cataractous eye

## Wavefront Analysis

Ocular and corneal wavefront errors were measured (see Fig. 5.22) with a Hartmann–Shack aberrometer (Wavefront Analyzer, Topcon, Tokyo, Japan). Measurements were taken for 4- and 6-mm pupils.

The Wavefront Analyzer gives us the total ocular and corneal aberrations for 4- and 6-mm pupils, coma-like Zernike polynomials ($Z_3^i+Z_5^i$) and $Z_4^i+Z_6^i$ for a 6-mm pupil, and ocular and corneal $Z_s$ for 4- and 6-mm pupils. Zernike mode $Z3^{-3}$ through $Z3^3$ plus a fifth-order $Z$ ($Z5^{-5}$ through $Z5^5$) corresponds to coma-like aberrations.

From ocular and corneal aberrations, intraocular aberrations can be obtained. Intraocular aberrations result from the difference between ocular and corneal aberrations, and they are due more to the crystalline lens and less to the posterior corneal surface.

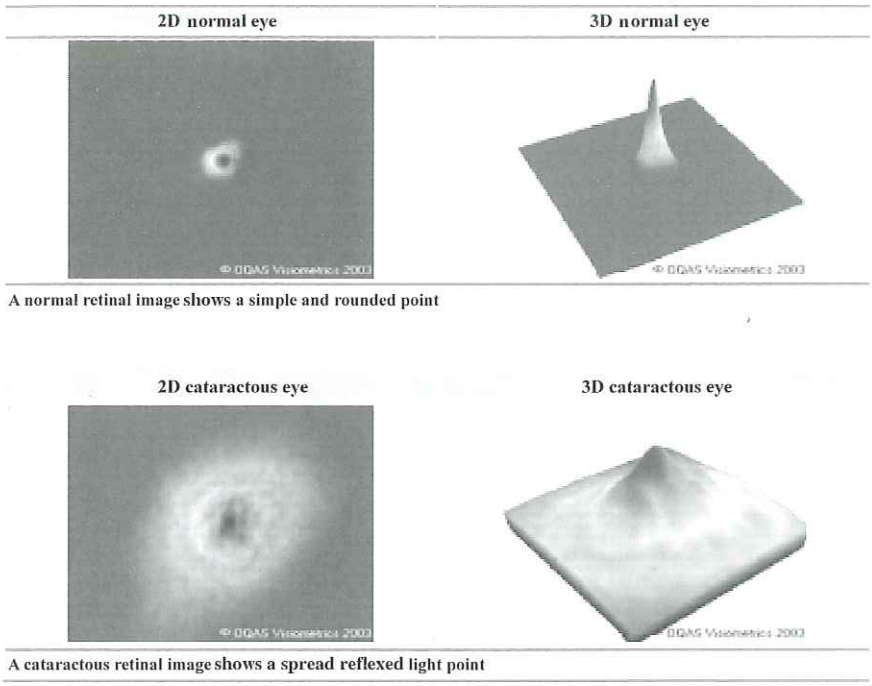


**Fig. 5-20** OQAS 2-D and 3-D representation of PSF in normal and cataractous eyes

### Results on Ocular and Corneal Wavefront Errors Measured with a Hartmann–Shack Aberrometer

Total ocular and corneal HOAs of a 6-mm pupil are measured as a function of age. Corneal HOA shows a weak statistically significant variation with age. Ocular HOA increases linearly with age. As shown in the scatterplot, ocular HOA is smaller than corneal HOA until 30 to 40 years of age. In the 40s, ocular HOA is similar to corneal HOA, and it increases in older subjects. The same result can be seen for ocular and corneal $Z_4^i+Z_6^i$ aberrations. Corneal coma-like aberrations were not statistically significant, and ocular coma-like aberrations show a positive linear correlation with age (see Fig. 5.23). Intraocular spherical aberration ($Z_4^0$) for a 6-mm pupil (see Fig. 5.24) shows a positive linear correlation with age. Intraocular coma aberration ($Z_3^{-1}$), on the contrary, shows a negative linear correlation with age (see Fig. 5.25).

## *Conclusions*

In our study, nucleus density showed a positive correlation with age, after 40 years, for embryonic, anterior fetal, and posterior fetal nuclei. When different age groups

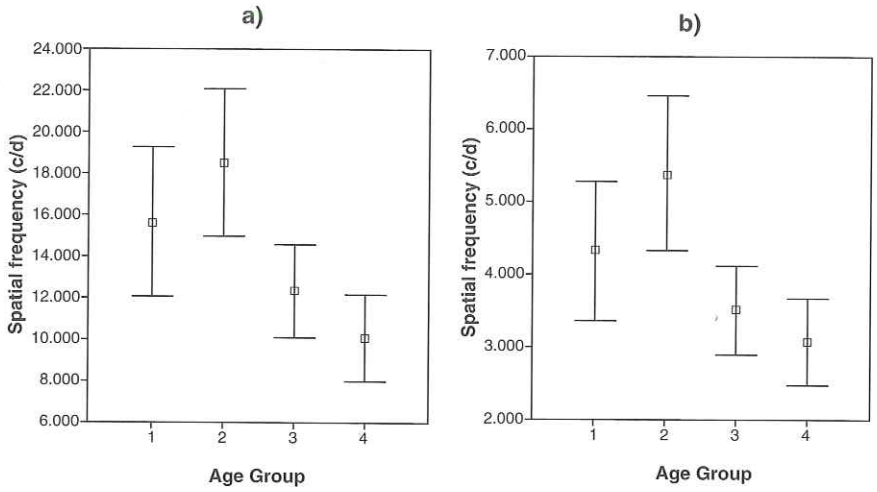


**Fig. 5.21** (A) Spatial frequency for 0.1 modulation transfer function (MTF) in four age groups for a 5-mm pupil. The mean difference with Bonferroni multiple comparison is statistically significant for groups 2 and 3 ($P<0.009$). (B) Spatial frequency for 0.5 MTF in 4 age groups for a 5-mm pupil. The mean difference using Bonferroni multiple comparison is statistically significant for groups 2 and 3 ($P<0.004$). c/d=cycles per degree. Error bars, minimum and maximum of the 95 percent confidence interval. (Group 1 included subjects from 8 to 20-years old (n=15); Group 2, subjects from 21 to 40 (n=20); Group 3, subjects from 41 to 60 (n=21); and Group 4, subjects from 61 to 80 (n=16)

are analyzed, we can see that nucleus density does not increase before the age of 40, after which nucleus density increases linearly with age. As a result of the continuous production of new fibers, the aging lens becomes thicker.

We found a correlation between age and overall lens thickness, as was also found by Kashima et al.[449] In our study, crystalline lens thickness increases from eight years of age to the age of 40, after which the increase in lens thickness is not statistically significant. Due to the anatomical changes that take place with aging, scattering and aberrations of the crystalline lens are expected to increase. The main contributors to the overall aberrations in the eye are the tears, anterior and posterior surfaces of the cornea, and crystalline lens. So, if the aberrations of the crystalline lens increase, total ocular aberrations will increase as well.

Several previous studies have reported an increase in overall eye aberrations with aging.[439-442] In our study, overall eye HOAs increased linearly with aging, as previously reported in the literature. This increment in overall ocular HOAs is not due to corneal HOA, which shows a very weak correlation with age. Before the age of 30 years, overall HOA and $Z_4^i+Z_6^i$ were significantly larger for the cornea than for the entire eye, which suggests that the lens compensates for part of the corneal aberrations. The corneal and lens aberrations show, in fact, a trend to compensate each other.[443] In our study, we found that this mechanism is disrupted in the older eye as a consequence of normal aging.

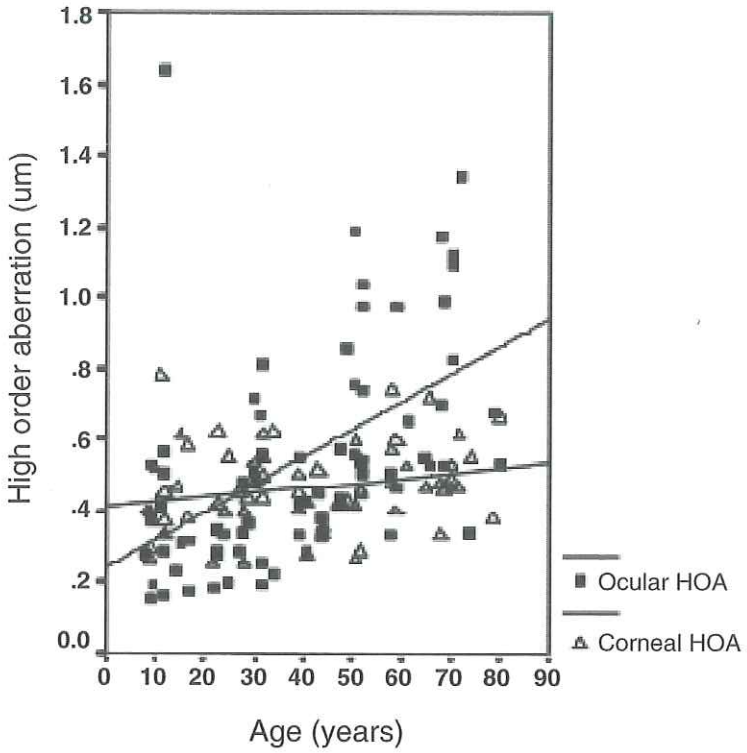


**Fig. 5.22** Total ocular and corneal high-order aberrations (HOAs) as a function of age for a 6-mm pupil. For ocular HOA, a positive linear correlation was found ($r=0.511$, $P<0.0001$). For corneal HOA, a weakly positive correlation was found ($r=0.248$, $P<0.036$)

According to our data, the turning point for the coupling of these two optical systems (cornea and the entire eye) seems to appear around 40 years of age. The changes in the optical performance of the crystalline lens with aging should be related to the anatomical changes (nucleus density and thickness) found. With previous studies, authors have investigated the correlation of the development of aberrometric changes with aging.[439-442] In such studies, the Zernike polynomials that were used differed from those analyzed in this study.

We found a linear correlation between intraocular spherical aberration and age. Because the main contributor to intraocular aberration is the crystalline lens, we can assume that spherical crystalline lens aberration increases with age. On the other hand, intraocular coma aberration ($Z_3^{-1}$) decreases with age. In this study, we investigated corneal, ocular, and intraocular HOAs in the same patient. We studied the overall corneal and ocular HOAs, corneal and ocular $Z_4^i+Z_6^i$, intraocular spherical aberration ($Z_4^0$), and intraocular $Z_3^{-1}$. We found a positive linear correlation for all the aberrations studied except for intraocular $Z_3^{-1}$, which shows a negative linear correlation. Our results confirm that the increase in corneal aberration is too small

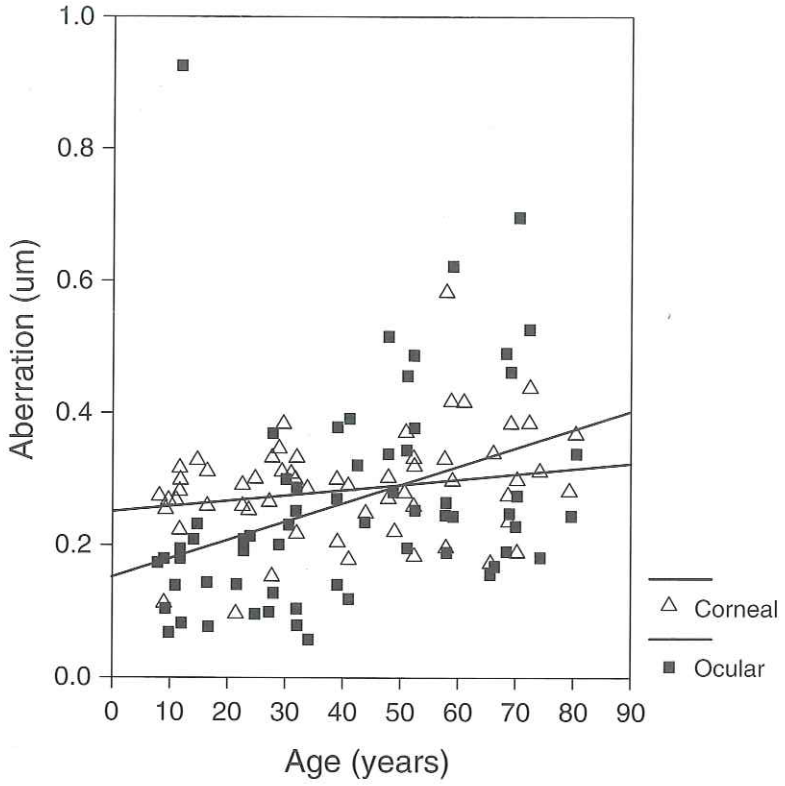


**Fig. 5.23** Ocular and corneal $Z_4^i+Z_6^i$ (Zernike polynomials) aberration as a function of age for a 6-mm pupil. For ocular aberration, a positive linear correlation was found ($r=0.368$, $P<0.001$). For corneal aberration, a weakly positive correlation was found ($r = 0.244$, $P<0.039$)

to account for the increase in ocular aberrations and support the theory that the crystalline lens must be responsible for the increase in the ocular aberrations that take place with aging.

To the best of our knowledge, the correlation between crystalline lens aberration changes and the increase in the densitometric values and thickness of the lens has not been previously reported. Changes in crystalline lens morphology are responsible for the degradation of the optical performance of the human eye through aging. Such anatomical changes are also related to the degradation of the MTF with age, as shown by double-pass imaging in the present study, in agreement with other previous reports on the subject.[447,448]

We observed degradation in the MTF in different age groups. The highest MTF is observed in Group 2 and corresponds to subjects between 21 and 40 years. Between 41 and 60 years, the MTF declines. The turning point for crystalline lens changes seems to be around the age of 40, when presbyopia appears. Nuclear crystalline lens density increases around the age of 40, and this anatomical change

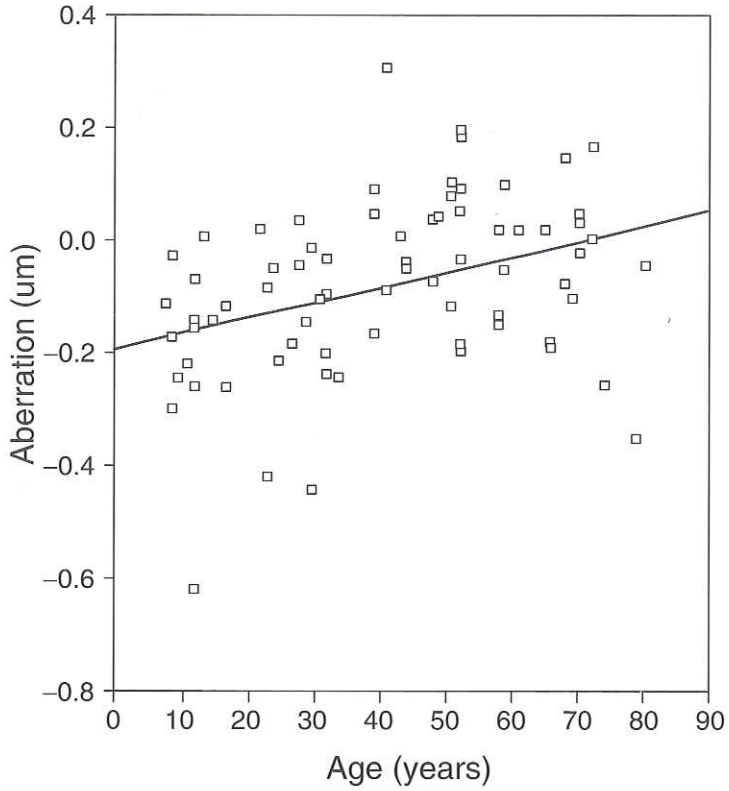


**Fig. 5.24** Intraocular spherical aberration ($Z_4^0$) as a function of age for a 6-mm pupil. A positive linear correlation was found ($r = 0.382$, $P<0.001$)

implies an increase in intraocular aberrations. The increase in scattering and aberrations around the age of 40 decreases the optical quality of the eye, which we measure in our study using the MTF.

We can conclude that there is a degradation of the optical function of the crystalline lens measured as changes in the MTF and in the aberration pattern through aging and, also, that those changes are associated with morphological changes in the thickness and density of the lens. The turning point for these changes is shown to be around the age of 40 years. Further morphological changes in the crystalline lens, and the consequent degradation in the eye's optical quality with aging, should decrease the normal performance of the human eye before the development of evident cataracts. Such visual deterioration would continue further with the development of cataract that is evident at a clinical level.[449]

The decrease in the eye's optical performance through aging, shown as a continuous process related to morphological changes at the level of the crystalline

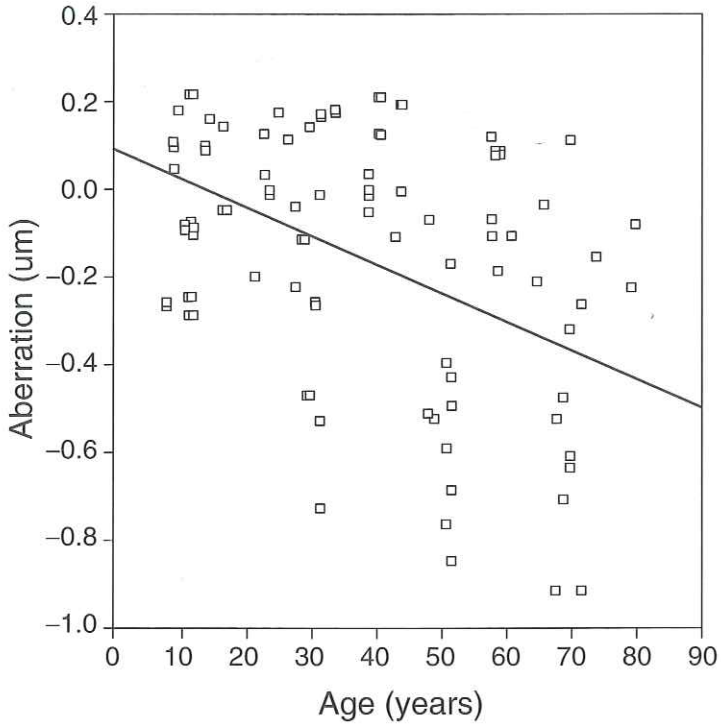


**Fig. 5.25** Intraocular coma aberration ($Z_3^{-1}$) as a function of age for a 6-mm pupil. A negative linear correlation was found ($r = -0.459$, $P < 0.0001$)

lens, may have clinical implications in the future. The use of Scheimpflug Photography and MTF striation can help to develop new guidelines for cataracts (see Fig. 5.26).

New intraocular lens (IOL) technology is being used to try to improve the optical performance of the eye using customized lens optical design.[450] The increase in spherical aberrations associated with aging that were observed by us can be compensated for either by the induction of negative spherical aberration at the corneal level, as in hyperopic excimer laser procedures, or by an adequately designed customized IOL.[451] If the optical performance of an eye that is implanted with a customized IOL reaches a level that is superior to that of an aged eye, crystalline lens substitution may have a clinical indication, especially if improvements in other lens functions (such as accommodation) can also be implemented and the complication rates for the surgery are minimal and acceptable. Future research in this area seems to be of utmost importance.

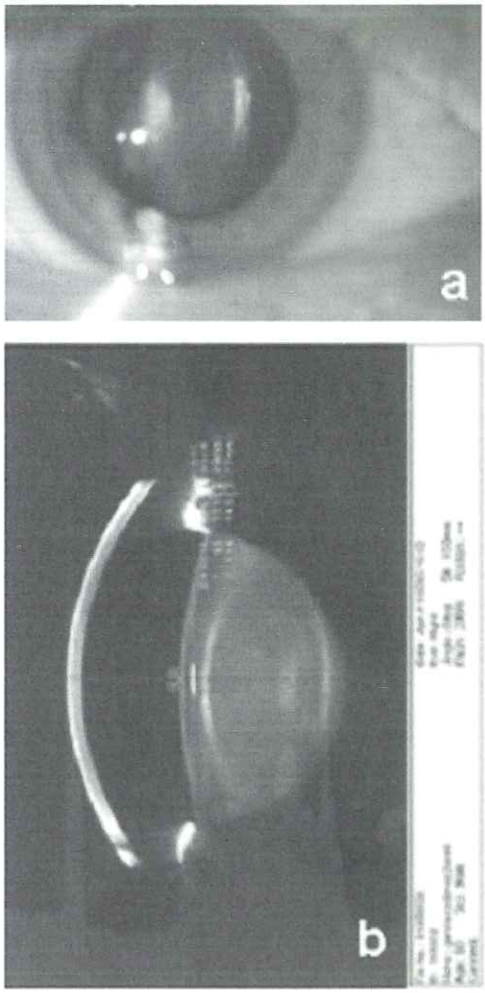


**Fig. 5.26** Scheimpflug Photography analysis provides objective data (B) compared with slit lamp image (A). A wide range of numeric information can help to develop new guidelines for cataracts

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