# Chapter III

## Biological Physics of the Eye and Vision

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## 1. Basic Anatomy, Physiology and Pathophysiology of the Visual System

The visual system may be thought of as consisting of two distinct yet interconnected systems. The first is the eyeball. The second is the retina, which resides within the eyeball, but which is in fact developed from and is connected to the brain by the optic nerve which leads from each eye to two peanut-size regions of cells called the lateral geniculate bodies, and thence by fibers to the back of the brain to the striate cortex or the primary visual cortex.

The eyeball at first glance appears to be simply a passive structure, an optical device, whose function is to form a sharp image of a visual field upon the retina. A more thoughtful analysis reveals the binocular eye system to be a remarkable stereoscopic tracking device, capable of self-focusing, and self-cleaning, with an extraordinary dynamic range for light intensity detection. It contains in its "film plane," a retina which is in effect a parallel processing, color sensitive computer of great sophistication (Hubel, 1988). A useful, detailed, and modern treatment of the structure and function of the eye is to be found in the book by C.W. Oyster (1999).

### 1.1 The Eyeball

We begin by discussing the eyeball, recognizing that its function is to form clear focused images on two separate retinas. These two images must fall on corresponding regions in each of the two retinas to within a few minutes of arc. The arc is measured by lines drawn through the center of rotation of the eye.

The eye is held in its orbit, or socket, by six muscles called the extraocular muscles. These are present in three balanced pairs: the medial and lateral recti, the superior and inferior recti, and the superior and inferior oblique muscles. These three pairs permit motion of the eyes to track left, or right or control twisting during tracking. It is

important to realize that the tracking motion is achieved by exquisite coordination in the tension and the relaxation of each pair of the extraocular muscles. Also it must be recognized that there must be a match of this pair of relaxation and tension as between both eyes! This control of the two eyes is directed from neurons in the brain stem. This control of each pair of the six oculomotor muscles must produce accurate spatial and temporal correlation in the positions of both eyes. This correlation is in part an unconsciously learned process. Children whose extraocular neuromuscular system is not sufficiently coordinated will see two images in the visual field. This corresponds to a displacement of the positions of the images in the two retinas. Unless this condition "diplopyia" is corrected surgically, the brain will learn to reject one of the two retinal images. As a result the normal neural processing in the rejected pathway will atrophy and in effect the vision will become impaired in one eye even though a perfectly clear image is presented to the retina. This condition is called "amblyopia"

We turn next to the structures at the surface and within the eye globe. These are illustrated schematically in Fig. 1.1 below. The outer coating of the eye consists of the transparent cornea and the opaque sclera, which join at the region called the limbus. The visual axis connects the object sudied with the center of the fovea, which is the region of the retina possessing the highest density of photo receptors.

## 1.2 The Cornea

The approximately spherically shaped cornea is the major factor in providing the refractive power of the eye. Three-fourths of this refractive power is provided by the cornea.

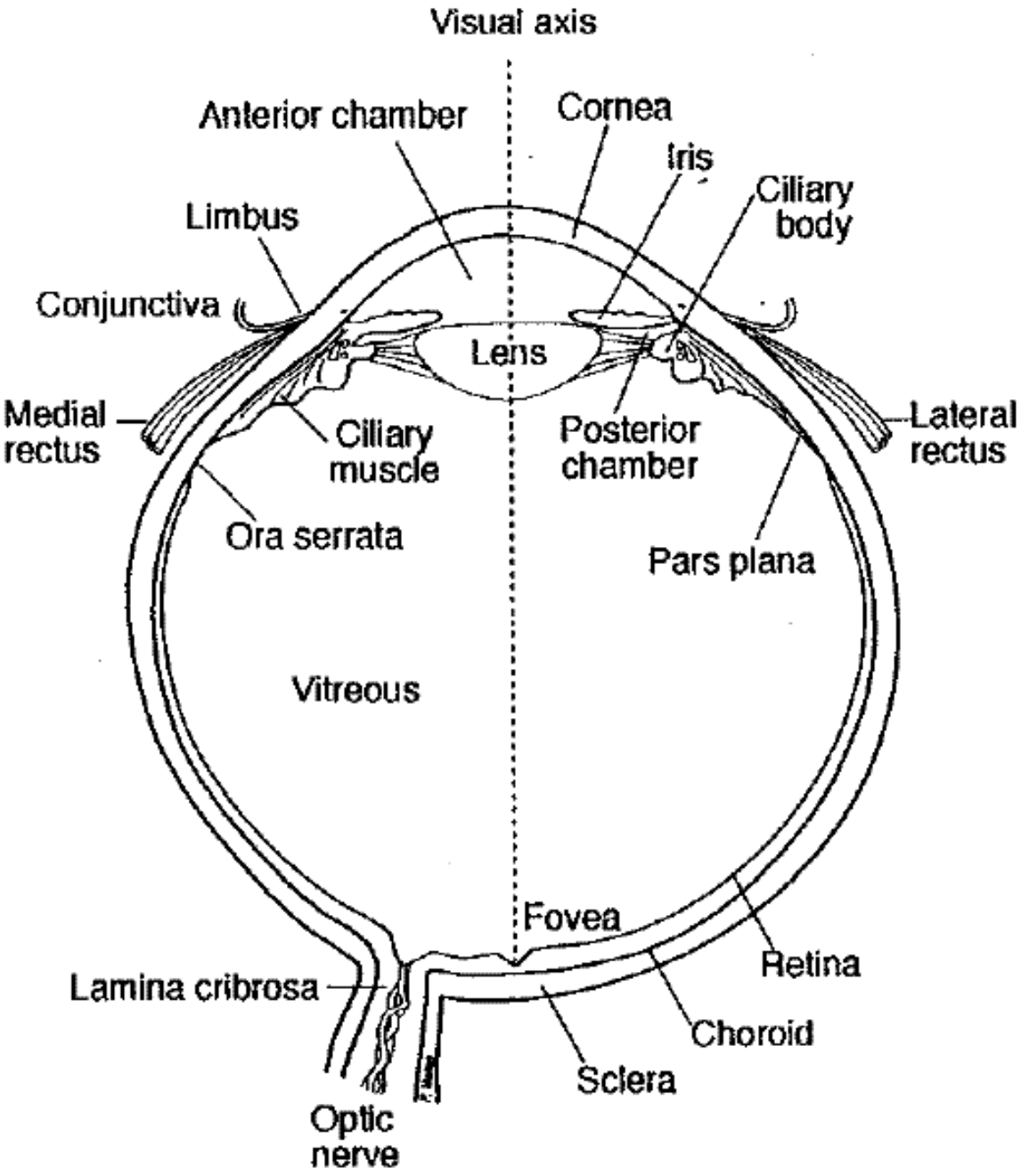


Fig. 1.1: Diagram of a meridional cross section of the right eye showing the principal internal structures as well as the outer sheath of sclera and cornea. Adapted from E. Wolff (1954).

The refractive index of the cornea is 1.377 in man. The refractive index of the aqueous is 1.33. The average radius of curvature of the human cornea is ~ 7.86 mm. The entire globe of the human eye has an average axial length of ~ 26 mm. The average horizontal diameter of the cornea is 11 - 12 mm, and its thickness is ~ 0.52 mm. The structure of the cornea consists of five layers parallel to its surfaces (D. Maurice 1984). The transparency of the cornea is a topic of great practical and theoretical importance. In subsequent sections we shall provide a theoretical analysis of the nature of the corneal microstructure which allows this tissue to be transparent. We shall also analyze the changes which produce opacification of the cornea. To do this it is important to understand the composition, structure, and function of each of the five layers in the cornea. In Fig 1.2 we show a schematic diagram of the corneal cross section.

**i) The corneal epithelium**

The outermost layer of the cornea consists itself of five layers of epithelial cells. At the top are flat, overlapping squamous cells ~ 45 μm wide, beneath this are found "wing" cells in the middle of the epithelium and finally at the bottom are "columnar" cells. The total thickness of the corneal epithelium is ~ 0.05 mm, i.e. 10% of the corneal thickness.

**ii) Bowman's zone, or Bowman's membrane**

This zone lies at the very bottom of the epithelium and serves as the transition between the epithelium and the underlying stromal layer. In man and primates the Bowman's zone is a cell free sheet about 12 μm thick. Electron microscope examination shows this zone to be made of collagen fibrils whose orientation and spacing seems quite random. The diameter of the fibrils in Bowman's zone is roughly 2/3 of that of fibrils found in the stroma.

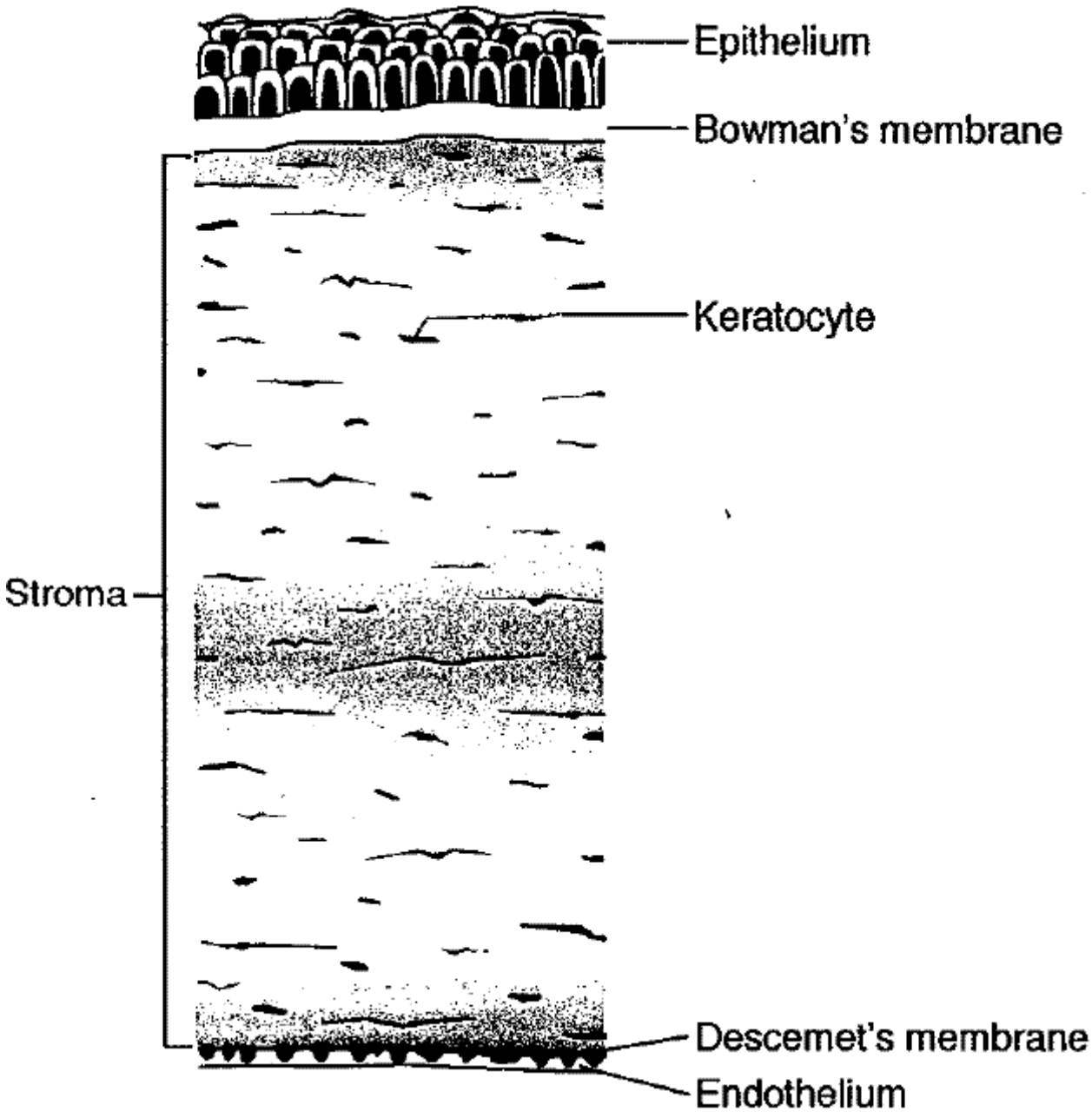


Figure 1.2: Schematic representation of the corneal showing the epithelium, Bowman's zone, stroma, Descemet's membrane and endothelium.

**iii) The corneal stroma**

The stroma constitutes about 90% of the corneal thickness. It is made up of sheets or lamella constituted of collagen fibrils. In each lamella the fibrils are aligned nearly in parallel. On passing from one lamella to the next however, the orientation of these fibrils changes discontinuously. The overall number of lamellae at the center of the human cornea is about 300. The mean diameter of each collagen fibril is ~ 22.5 nm. See Figure 1.3 for an artist's version of the fibril orientation in the stromal lamellae. For further electron microscope photographs, see D. Maurice (1984), and M. A. Jakus (1961). Between the lamellae and sometimes within a single lamella are found a few modified fibroblasts called keratocytes. These constitute only about 2% of the total stromal volume. The stromal keratocytes appear to be connected with the slow turnover of the mucopolysaccharides: keratan, and chondroitin sulfate, which together with water make up the material between the collagen fibrils.

**iv) Descemet's membrane**

This is a cellular layer which serves as the basement membrane of the endothelium. It is 7 μm in thickness at the posterior of the stroma.

**v) The endothelium**

Posterior to Descemet's membrane is a single layer of hexagonally shaped cells which completely cover the posterior surface of the cornea. The endothelial layer has the very important function of providing ion pumps which control the hydration of the cornea. The endothelial cells do not undergo mitosis. If a few are damaged, neighboring cells will invaginate into the affected cells. However, if a sufficient number of cells are damaged, this mechanism fails. Human endothelium cannot regenerate itself. Corneal swelling, clouding and opacification can result from the failure of the endothelial pumps.

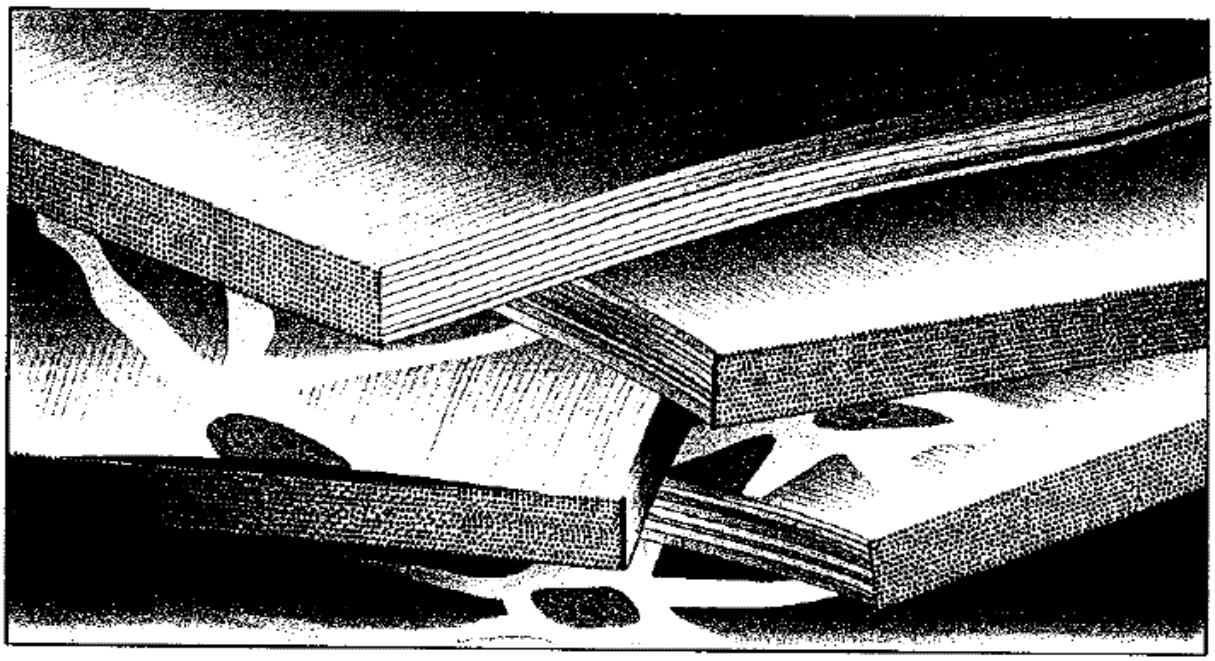

Fig. 1.3: Artist's rendering of the lamellar structure and arrangement of collagen fibrils in the corneal stroma.

## 1.3 The Ciliary Body, The Canals of Schlemm and Aqueous Humour Dynamics

The image forming function of the eye requires that its overall dimension be controlled. This is accomplished by the production of aqueous fluid or aqueous humour. The aqueous humour also provides nutrition to the a-vascular lens and cornea. The production of aqueous humour takes place at the ciliary body which is located within the eye behind the limbus, which marks the transitional zone between the transparent cornea and the opaque sclera. In Figure 1.4 we show a schematic diagram of this antero-nasal portion of a horizontal section through the right eye. This is a region where many important ocular functions are performed and is worthy of discussion. Aqueous humor is produced by special epithelial cells on the ciliary body. The mean aqueous flow rate produced by these cells is ~ 2 μl/min. This corresponds to ~ 1.5% of the aqueous volume/min. The inflow of aqueous is balanced by aqueous outflow through the trabecular meshwork and canals of Schlemm. This outflow is carried into the vascular supply by junctions with aqueous veins (see H. Davson 1969). In Figure 1-5 we see the schematic representation of the production of aqueous from the ciliary body in the posterior chamber behind the lens. Aqueous flows from the posterior chember through the zonules past the iris into the anterior chamber in front of the lens.

The continual production of aqueous humor, coupled with its outflow through resistive channels produces an intra ocular pressure (IOP) within the globe of the eye which induces a tension in the cornea and sclera and fixes the size of the globe. The intra ocular pressure shows a broad distribution within the population. The mean value of the I.O.P is ~ 16 mm Hg. The half width of the distribution of normal persons being about 5 mm Hg.

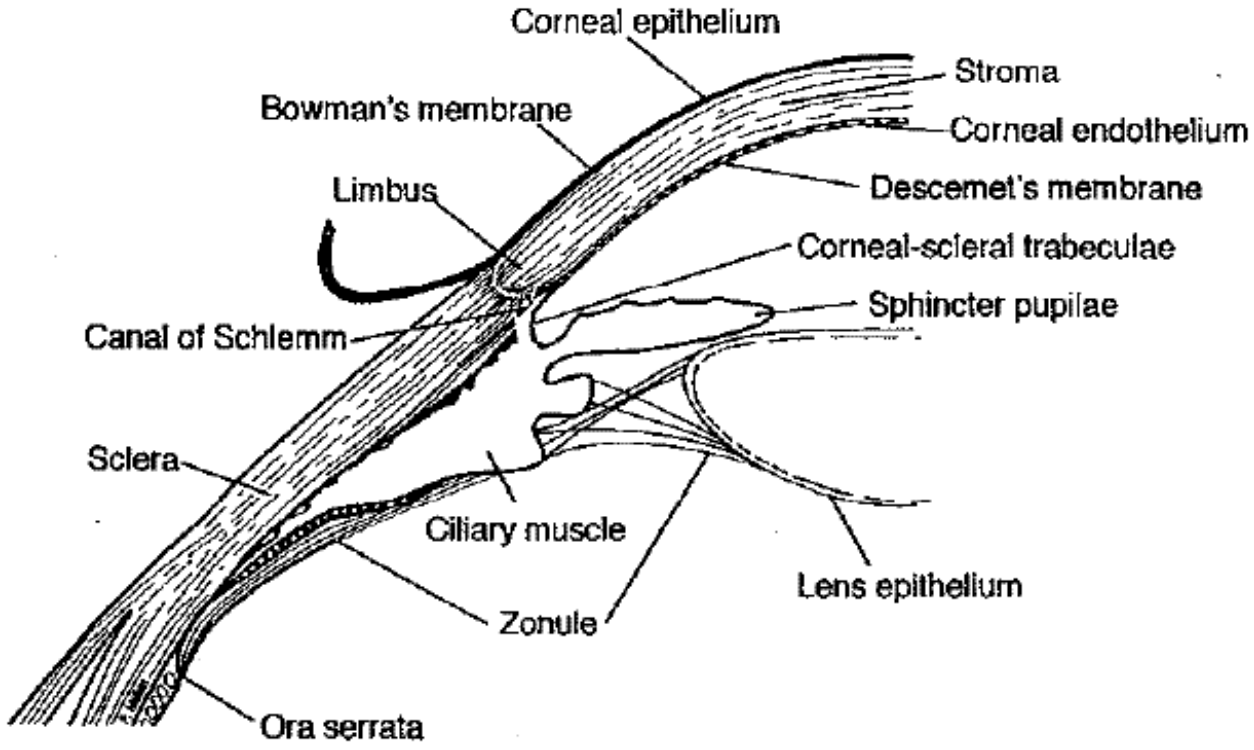


Fig. 1.4: Horizontal section of the right eye showing the anterior-nasal region of the eye beneath the limbus. Modified from Salzmann (1912).

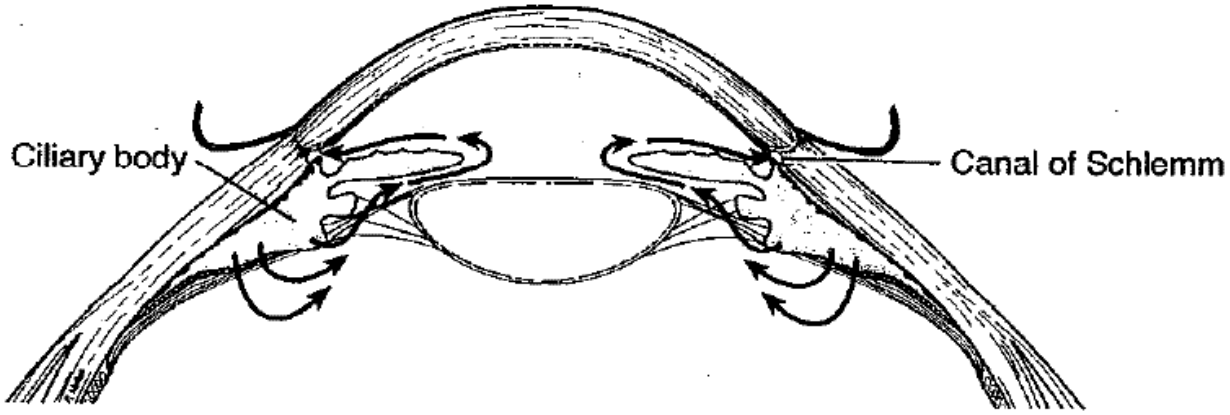


Fig. 1.5: Schematic illustration of the production of aqueous humor from the ciliary body and its flow to the canal of Schlemm. Adapted from H. Davson, **The Eye** Vol. 1, Scd. Ed. pg. 69, published by Academic Press, NY (1969).

The study of the physiology and biochemistry which underlie the mechanisms of aqueous secretion, the hydrodynamic resistance of the trabecular meshwork, and the analytical mathematical relations which describe the combined functioning of these systems is called aqueous humor dynamics. This field, whose development has occurred in the past 50 years, is one of considerable medical importance because of the disease known as glaucoma. In glaucoma the patient experiences scotoma: domains generally peripheral to the visual axis, where objects in the visual field simply cannot be seen. This occurs even though the cornea and lens form a clear image on the retina. As the disease progresses, more and more scotoma develop, until much of the peripheral vision is lost. The patient can see objects in a small solid angle about the visual axis, but the mean angular size of the visual field is successively diminished. Usually, the onset of these peripheral scotoma is not noticed by the patient as he becomes acclimated to use of vision in a small zone about the visual axis. Ultimately however, this small domain also fails and the patient becomes totally blind. One to two percent of persons in the United States are blind as a result of glaucoma.

The loss of vision in glaucoma is the result of loss of retinal ganglion cells. This is connected with damage to the optic disc at the posterior of the eye (see Figure 1-1). The optic disc is the region where the axons of the retinal ganglion cells merge and leave the eye. It is also the location where the retinal artery and vein bring blood to and from the retinal circulation. All these blood vessels and nerves pass through a sieve-like mesh of collagenous fibers called the lamina cribrosa which derive from the sclera. In glaucoma, the optic disc, as observed in the slit lamp microscope, appears depressed and shows "cupping." The lamina cribrosa meshwork beneath the disc becomes thicker and more constrictive. The sieve pores in the lamina cribrosa narrow and the nerve bundle fibers are damaged. Blood flow through the retinal arteries and veins can be successively constricted.

A summary of the 1994 status of understanding of glaucoma is to be found in Vision Research: A National Plan 1994-1998 (1994). It had long been widely believed that increase in intraocular pressure, associated with an increase in outflow resistance compared to aqueous humour production was the prime factor responsible for optic disc damage and death of retinal ganglion cells. Today it is thought that increased intraocular pressure (I.O.P.) is but one of the "risk factors" attendant upon the disease. Indeed, it is known that significant numbers of persons suffer from glaucoma in the absence of elevated I.O.P. (low tension glaucoma). Also, many persons with high intraocular pressure never develop glaucoma. Nevertheless, in order to help delay the course of this disease a variety of drug medications are used by ophthalmologists to control the level of I.O.P. There are approximately six broad classes of such pharmacologic agents. These either decrease aqueous humour production, or increase aqueous outflow facility. It should be emphasized that such medications can have annoying or sometimes serious side effect. Typically when pharmacologic intervention ultimately fails to stop the loss of ganglion cells and loss in the visual field, various surgical methods are employed. As a result of such surgery, the outflow facility can be improved or the aqueous production diminished.

It should be kept in mind that even with reduction of intraocular pressure, the pathogenesis at the disc and lamina cribrosa may still persist. Thus, while this disease remains uncured, it is clear that we must seek a scientific understanding of the precise cellular and molecular factors that control both aqueous humor dynamics and pathogenesis at the optic disc and lamina cribrosa.

## 1.4 The Iris, Ciliary Muscles, the Zonules and the Lens

In subsequent sections we shall provide analysis of the physical and chemical basis for the transparency and the opacification of the lens. It is therefore appropriate to provide an introduction to the anatomy and physiology of this and surrounding organs. We begin first by discussing the iris whose opening and closing modulates the size of the pupil and hence both the intensity of light and the sharpness of the image on the retina.

### i) The iris

As seen in Fig. 1.1 and in Fig. 1.4 the iris lies anterior to the lens and is bathed in aqueous humour on both surfaces. The iris emerges from the ciliary body and its central circular aperture is the pupil. The posterior surface cells are formed of the same epithelial layer as the pigmented cells at the base of the retina. This pigmentation of the iris serves to block the passage of light. The dimension of the pupil is controlled by two sets of fibers. Reduction in the diameter of the pupil is produced by circular fibers near the margin of the pupil. These fibers are called the sphincter pupillae. Increase of the diameter of the pupils is produced by radial fibers called dilator pupillae which pass from the margin to the periphery of the iris. Both these fibers are supplied by two sets of nerves. Just as the extraocular muscles are controlled by a feedback system involving the brain, so must the pupillary diameter be part of a feedback and control system involving the retina and the brain.

**ii) Ciliary muscles and the zonules**

Posterior to the iris is to be found the zonules, which are composed of fibers which attach on one side to the posterior surface of the ciliary processes. On the other side these fibers attach to the capsule surrounding the lens. The points of attachment are principally at the anterior surface of the lens near the margin but also attach to the lens at its margin and a bit posterior to the margin. The zonules attached anteriorly are called the suspensory ligament of the lens. This holds the lens in its place in the so-called hyaloid fossa. This is a deep concavity on the anterior surface of the vitreous. The vitreous is a transparent gel constituted largely of hyaluronic acid which fills fourth-fifths of the eyeball. It is bounded anteriorly by the lens, the ciliary zonules, the ciliary body, and posteriorly by the sclera and retina.

The zonules and the ciliary muscles are responsible for the focusing action (accommodation) of the lens. Reviews of the current understanding of the mechanisms responsible for changing the focal length of the lens are to be found in the works of Koretz (1988), and Koretz (2000). The former contains very clear illustrations of the attachments between the zonules and the lens capsule, and the changes in the positions of the lens and the ciliary body associated with accommodation.

A broad view of the role of the zonules in accommodation was initially proposed by the physicist-physiologist Hermann von Helmholtz (1821-1894) (see Helmholtz, H. 1855). According to this idea, when the eye is "at rest", i.e. adapted for long distance viewing, the zonules fibers are under maximal tension. The tension is a result of a relaxation of the ciliary muscle, which in the relaxed state, produces a maximum diameter of the ciliary muscle ring. This large diameter ring pulls on the zonules and flattens the lens, which is then said to be "unaccommodated". Under these conditions, the lens focuses images beyond six meters distant from the eye onto the retina. In this unaccommodated state, the lens has its largest focal length.

To form a clear image on the retina of relatively close objects, the ciliary muscles contract, thereby pulling the ciliary process anteriorly. This decreases the diameter of the ciliary muscle ring, thereby reducing the tension in the zonules and in the capsule. This allows the elastic lens to "ball up", i.e. decrease its radius of curvature, thereby reducing the focal distance.

As the human eye ages, the size of the lens increases. According to Koretz (1988 and 2000), this alters the angles of attachment of the zonules to the lens capsule, and thereby changes the stress distribution on the lens produced by the capsule. As a result, the "balling up" of the lens, upon reduction of the tension of the zonules, becomes diminished. With aging, the lens thus becomes less able to accommodate and the "near point" for viewing moves further and further from the eye. The inability of the lens to accommodate is known as presbyopia, from the Greek word meaning “old eye”.

It should be kept in mind that the process of accommodation in the young eye, which occurs in viewing nearby objects, requires neuromuscular feedback control loops from the brain to the ciliary muscles. Presumably, these are connected with the loops controlling convergence of the eyes, and the dilation of the pupil.

### iii) Emmetropia, presbyopia, myopia hyperopia, and the development of spectacles

The condition in which the cornea and the lens together form a sharp image on the retina is called emmetropia. Even for a person, having such a perfect correlation between the cornea and the lens, there comes a time, generally at age ~ 45, at which the accommodation of the lens by the zonules no longer functions effectively. Thus, even a person who during youth has perfect vision, (an emmetrope) will lose his ability to "see" small objects (such as the printed word) placed at the normal viewing distance.

Of course, in some individuals, the curvature of the cornea and lens relative to the size of the eyeball may be such that even with the accommodation provided by the

ciliary muscles and the zonules, a clear image is not formed on the retina. The condition in which rays parallel to the optic axis are focused at a point anterior to the retina is called myopia, or near sightedness. On the other hand, the condition in which rays parallel to the optic axis are focused on a point posterior to the retina is called hypermetropia, also hyperopia, or far sightedness.

Today we take for granted the use of eye glasses or spectacles to correct such refractive errors in the focusing properties of the eye. It is interesting to reflect briefly upon the history of the discovery and use of spectacles as well as the historical origins of our current knowledge of the structure and function of the eye. A useful short account of such history is to be found in the book of J. R. Levene (1977).

The manufacture of colored and nearly transparent glass was already flourishing in Egypt and the Aegean by the 14th century B.C. The art of glass blowing was invented about 50 B.C. This assisted in the search for clear transparent glass. According to Levene (1977) specific reference to the action of lenses are not to be found until the 11th century A.D., where they are discussed in the work of Alhazen (Ibn al-Hitham) (962-1028) entitled "Optical Thesaurus" (Latin translation 1572). He was an Arab mathematician born in Basra and gave the first account of atmospheric refraction and reflection from curved surfaces. He constructed spherical and parabolic mirrors (Chambers 1968). Based on the work of Alhazen and Robert Grosseteste (1175-1253), Roger Bacon (1214-1292) provided an account of magnification. See Levene (1977) and references therein, which shows that Bacon understood the usefulness of convex lenses as an aid to "weak sight."

According to Rosen (1956), eyeglasses were first invented in Italy probably about 1286. In this connection it is to be kept in mind that in Italy, Venice was an important center for the manufacture of glass by about the middle of the 11th century. By 1278 a guild system for glass artisans with an apprenticeship of eight years was in operation. The Venetian glassblowers located themselves in the island of Murano to help preserve

the secrets of their work. The first depiction of eye glasses is to be found in an Italian fresco dated 1352. The painter was Tommasso da Modena, and is a portrait of Cardinal Ugo di Provenza. This mural is in the Sala di Capitolo at the Seminary of San Nicolo in Treviso (Levene 1977). The great poet Petrach (1304-1374) in his "epistle to Posterity" writes that his eyesight had been good till the age of about 60 years, after which he needed the assistance of eye glasses. By the middle of the 15th century, progress in spectacle making trade was organized into guilds.

With the advent of lenses it became conceptually possible to understand the phenomenon of myopia, presbyopia and hypertropia. The first person to contribute to this understanding was Francesco Maurolyco (1495-1575). His description of myopia is as follows (Barck 1907).

> "Since the smaller the refractive sphere, the shorter the distance within which the rays unite, it thus happens that those who are equipped with a deeply curved pupil (crystalline lens) are, as indicated above, short sighted; for in these individuals the visual rays, hurrying on toward coincidence, are not all able to reach the more remote objects to be distinguished."

It remained for the great Johannes Kepler (1571 - 1630) to discover that the eye is a "camera obscura" or dark chamber in which the cornea and the lens act to form an inverted image upon the retina. Prior to Kepler, it was believed that the site at which vision occurred was the lens itself. Grons (1975), in his discussion of the history of the lens points out that Felix Platter (1536-1614) in his book "De Corporis humani structura" had previously called the lens: "perspicillum nervi visionii;" the spectacle (lens) of the optical nerve. Kepler's understanding of the role of the lens and retina was confirmed by Christopher Scheiner (1575-1650) a Jesuit from Bavaria. Scheiner succeeded in actually observing the image upon the retina by removing the sclera from the region of the posterior pole of the eye. In Scheiner's book Oculus (1619) is to be found a remarkably realistic representation of the correct anatomy of the eye.

It is interesting to carry the consequences of the development of spectacles for the improvement of human vision a bit further. In early 1608 a Dutch spectacle maker of Middleburg named Hans Lipperhey applied for a patent for a telescope. No patent was granted, but Lipperhey received a grant to construct three such instruments for the government on the conditions that his method be kept secret. The secrecy did not last long, a newsletter carried notice of the new "spyglass" by November 1608. Galileo Galilei heard about the telescope and learned more from a letter of Jacques Badovere probably by July 1609. We quote below from Galileo's "Starry Messenger" early in 1610 (S. Drake 1978).

> "About ten months ago a report reached my ears that a certain Fleming had constructed a spyglass by means of which visible objects, though very distant from the eye of the observer, were distinctly seen as if nearby. Of this truly remarkable effect several experiences were related, to which some persons gave credence while other denied them. A few days later the report was confirmed to me in a letter from a noble Frenchman at Paris, Jacques Badovere, which caused me to apply myself wholeheartedly to inquire into the means by which I might arrive at the invention of a similar instrument. This I did shortly afterwards, my basis being the theory of refraction. First I prepared a tube of lead, at the ends of which I fitted two glass lenses, both plane on one side while on the other side one was spherically concave and the other convex. Then, placing my eye near the concave lens, I perceived objects satisfactorily large and near, for they appeared three times closer and nine times larger than when seen with the naked eye alone. Next I constructed another one, more perfect, which represented objects as enlarged more than sixty times."

By August 21 Galileo had constructed an 8 power telescope whose capabilities were demonstrated from the Campanile in Venice. Below is a description of this event in a letter to Galileo's brother-in-law Benedetto Landucci at Florence (S. Drake 1978).

> "...all my hope of returning home is taken away, but by a useful and honorable event. It is nearly two months since news spread here that in

> Flanders there had been presented to Count Maurice a spyglass.....As it appeared to me that it must be founded on the science of optics, I began to think about its construction, which I finally found, and so perfectly that one which I made far surpassed the reputation of the Flemish one. And word having reached Venice that I had made one, it is six days since I was called by the Signoria, to which I had to show it together with the entire Senate, to the infinite amazement of all; and there have been numerous gentlemen and senators who, though old, have more than once climbed the stairs of the highest campaniles in Venice to observe at sea sails and vessels so far away, coming under full sail to port, two hours and more were required before they could be seen without my spyglass. For in fact the effect of this instrument is to represent an object that is for example fifty miles away as large and near as if it were but five.
>
> Now having known how useful this would be for maritime as well as land affairs, and seeing it desired by the Venetian government, I resolved on the 25th of this month to appear in the College and make a free gift of it to his Lordship [the Doge]. And having been ordered to wait in the room of the Pregadi, there appeared presently the Procurator Priuli, who is one of the governors of the University. Coming out of the College, he took my hand and told me how that body . . . would at once order the honorable governors that, if I were content, they should renew my appointment for life and with a salary of one thousand florins per year. . . .to run immediately. . . .Thus I find myself here, held for life, and shall have to be satisfied to enjoy my native land sometimes during the summer months.

As is well known Galileo went on to use his telescope to study the surface of the moon and an improved version with a 20 power magnification to discover the moons of Jupiter. Thus, we see how the technical development of lenses to improve human vision lead to the discovery and the development of telescopes and an extraordinary expansion of the science of astronomy.

The growing understanding of the optics of lenses also led to the microscope. In 1624 Galileo presented a microscope to Cardinal Zollern for the Duke of Bavaria. Faber, who was one of the society of Lincei observed a fly under this "occhialino" "and was so astonished that he said that Galileo was a kind of Creator, having exhibited

something no one before had known to have been created." (S. Drake 1978). Until the problem of chromatic aberration was solved microscopes having a single high power lens provided the requisite high resolution. Anton van Leeuvenhoek (1632-1723) used such a single lens microscope with great precision and skill. His microscope had a magnification of 500, and had a resolving power of 1 micron. With this device he discovered spermatozoa, bacteria, protozoa and blood corpuscles. He also made important contributions to the role of the capillaries in the circulation of blood. Here again we see that the practical development of the art and science of spectacle lenses led to a scientific instrument whose skillful use has provided the basis for major advances in biology and medicine.

Let us return briefly at this point to the further development of spectacles. Though, as we have seen previously, the invention of spectacles took place in about 1286, it took much longer until bifocal spectacles were invented. Benjamin Franklin is generally credited with the invention (Levene 1977). The first evidence for this is to be found in a letter Franklin wrote from Passy in France to George Whately in London dated August 21, 1784. In this letter Franklin writes

> "I cannot distinguish a Letter even of Large Print: but am happy in the invention of Double Spectacles, which serving for distant objects as well as near ones, make my Eyes as useful to me as ever they were: If all the other Defects and Infirmities were as easily and cheaply remedied, it would be worth while for Friends to live a good deal longer."

When Whatley received this letter he drew this invention to the attention of the master optician and instrument maker Peter Dolland (1730-1820). Dolland expressed the view that "they can serve for particular eyes, not in general." Upon learning of this response Franklin wrote to Whately on May 23, 1785 a long letter which in part contained the following:

> "By M. Dolland's Saying, that my double Spectacles can only serve particular Eyes, I doubt he has not been rightly informed of their

> Construction. I imagine it will be found pretty generally true, that the same Convexity of Glass, through which a Man sees clearest and best at the distance proper for reading, is not the best for greater distances. I therefore had formerly two Pair of Spectacles, which I shifted occasionally, as in travelling I sometimes read, and often wanted to regard the Prospects. Finding the Change troublesome, and not always sufficiently ready, I had the Glasses cut and half of each kind associated in the same Circle. By this means, as I wear my Spectacles constantly, I have only to move my Eyes up or down, as I want to see distinctly far or near, the proper Glass being always ready. This I find more particularly convenient since my being in France, the Glasses that serve me best at Table to see what I eat, not being the best to see the Faces of those on the other Side of the Table who speak to me; and when one's Ears are not well accustomed to the Sounds of a Language, a Sight of the Movements in the Features of him that speaks helps to explain; so that I understand French better by the help of my Spectacles."

On reading this letter one must marvel at the precision and clarity of Franklin's writing and thought.

It may be of interest to note that Peter Dolland's father: John Dolland (1706-1761), was originally a silk weaver and became an optician. John Dolland invented the achromatic lens. This lens is the basis of modern high resolution microscopes and refracting telescopes. The optical firm founded by John and Peter Dolland continued in existence at least into the 20th century.

Trifocal spectacles were invented by John Isaac Hawkins (1772-1855). Hawkins was an exceedingly ingenious and versatile inventor responsible for improvements in the mechanism and design of pianos notably the invention of the upright piano, which attracted the attention of Thomas Jefferson. Hawkins also invented a paper ruling machine, a portable polygraph (for simultaneous copying of handwriting) and methods for waterproofing shoes, raincoats and improvements in the distillation of liquors (Levene 1978). The trifocal spectacles were invented about 1826. According to Hawkins their purpose was to provide a lens

"so that the eye is never distressed by being strained to accommodate itself to a new focus, with every change of distance, there being always a magnifying power in the proper situation for looking at all distances"

....."persons whose focus like mine, grown longer and longer, until it has ceased to exist with the natural eye, can have nothing worthy to be called a comfortable use of the eyes without adopting trifocal spectacles" (Levene 1978).

**iv) The lens**

The normal eye lens is a biconvex, transparent, refractive body lacking any blood vessels or nerves. The index of refraction of the human lens increases from 1.386 near the surface to 1.406 at the center (G. Westheimer 1972). The magnitude and spatial gradient of the index of refraction results from the fact that the lens is made up of spherical laminae of tightly packed hexagonal fiber cells each containing a highly concentrated aqueous solution of proteins. In the rat, this protein concentration can be as high as 75% by weight at the center. In man the protein concentration is ~ 35% by weight at the center. In Figure 1-6 below we show a schematic diagram of a meridional section of a dog lens. This figure shows in one plane the extremely long fiber cells emerging from the lens epithelial cells. This figure is taken from the article by Maisel *et al*. in Bloemendal (1981). The entire lens is covered by a non-cellular capsule made up

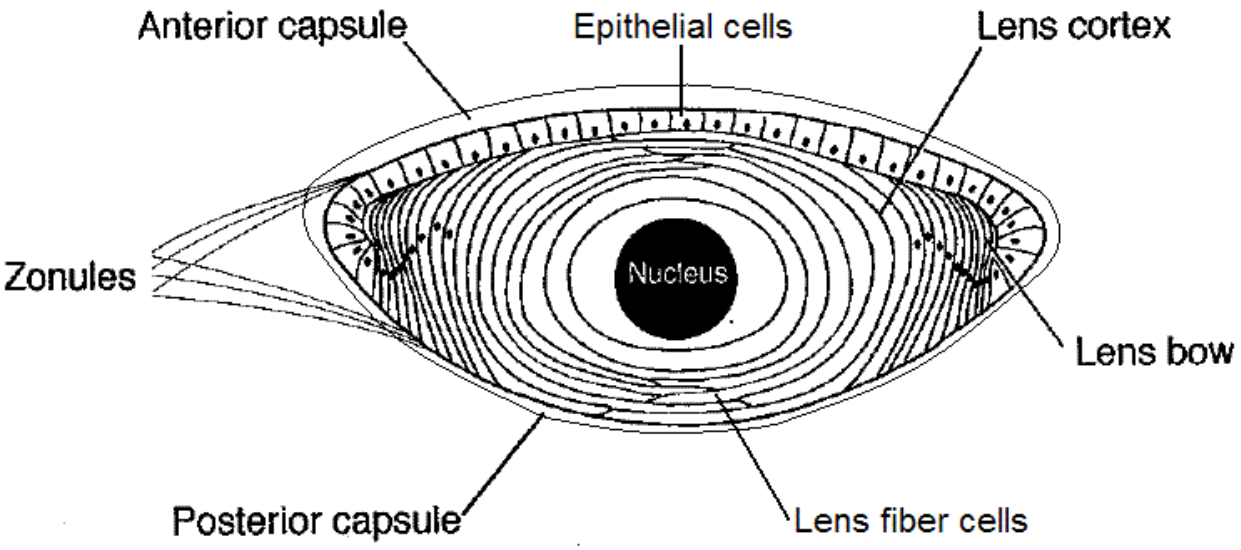


Fig. 1-6: Diagrammatic representation of a cross section of a dog lens. From H. Maisel, C.V. Harding, J. Alcala, J. Kuszah, R. Bradley, Cellular and Molecular Biology of the Eye Ed. by H. Bloemendal (1971).

of collagen filaments, and non collagenous glycoproteins. The zonular fibers responsible for accommodation extend from the ciliary body and connect to the lens capsule as anterior, posterior and equatorial zonules (H. Maisel *et al.* in Bloemendal 1981).

Directly beneath the anterior capsule is found a single layer of epithelial cells. The cells of the central anterior zone do not normally undergo mitosis. Only when one reaches the cells near the equator and the bow region does germination of lens cells occur. These germinatory cells are responsible for the continuous production of fiber cells. As a result of action of the germinating epithelial cells, layer upon layer of fiber cells are added to the underlying fiber cells. The lens weight and thickness increases throughout life. Between the ages of 10 years and 90 years the human lens mass increases from ~ 150 mg to ~ 250 mg (Harding 1977). There is no turnover of the lens cells. Thus the lens contains all the proteins which have been produced over the entire lifetime. As we shall see, in order to maintain the transparency of the lens these proteins must be exceptionally "stable." Despite the fact that they are present in very high concentration, to maintain transparency, they must not significantly aggregate with one another throughout life. If the homogeneous distribution of proteins within the lens cells is sufficiently disturbed, light, on passing through the lens, will be scattered, and opacification and cataract can result. We will discuss this problem in detail subsequently.

The eye lens cells contain a highly concentrated solution of lens specific proteins, the so-called crystallins. As mentioned above, the protein concentration varies from ~250 to 400 g/liter going from the lens cortex to the lens nucleus (see Fig. 1-6). The

mammalian crystallins can be divided into three distinct classas: α crystallins with molecular weight in the range from ~ 700,000 to ~ 1,200,000 Dalton, β crystallins with molecular weight in the range from ~ 50,000 to 300,000 and the γ crystallins with molecular weight ~ 20,000 Daltons. The γ crystallins are a family of single subunit proteins with high sequence homology between family members. The α and β crystallins are multisubunit proteins. The relative proportion of the various crystallins vary with mammalian species, age and position within the lens. The spatial and temporal variation is connected with differential synthesis during development. Also post-translational modifications can lead to alterations in the net energy of interaction between proteins. Such alterations can produce aggregation and/or phase separation within the lens fiber cells. We present below a table showing the composition of the various crystallin proteins in both the cortex and the nucleus of the calf lens (Siezen *et al.* 1985).

**Table 1-1**

Composition of soluble crystallins from calf lens cortex and nucleus

(from Siezen *et al.* 1985)

| Soluble Crystallin | Crystallin Composition % | |
|---|---|---|
| | Cortex | Nucleus |
| Total crystallin composition | | |
| α crystallin | 40 | 40 |
| β crystallin | 43 | 30 |
| γ crystallin | 17 | 30 |
| γ crystallin composition | | |
| $\gamma_B$ | 43 | 34 |
| $\gamma_C + \gamma_D$ | 28 | 31 |
| $\gamma_E$ or $\gamma_F$ | 22 | 29 |
| $\gamma_I$ (poorly defined) | 7 | 6 |

## 2.0 The Theory of Transparency of the Eye

We reviewed above the hard won knowledge of the structure of each of the elements of the eye. We also reviewed the intellectual achievements which have given us an understanding of the function of the eye as an instrument capable of presenting a neural representation of the outside world to the brain. The visual function of the eye and brain however depends on the maintenance of the transparency of the ocular tissues: principally the cornea and the lens. Until relatively recently the connection between the microscopic structure of these tissues and their transparency has not been understood. Thus, it had not been conceptually possible to understand the microscopic basis for the loss of transparency in blinding diseases such as corneal dystrophy and cataract.

At present the treatment for opacification of these tissues is surgery. In the case of an opaque cornea the method involves a corneal transplant. In the case of the lens the surgical method involves removal of the lens, and frequently the installation in its place of a prosthetic, plastic lens. Such surgical methods are costly, subject to complications and sometimes unavailable, particularly in third world countries. In order to develop medical and pharmaceutic methods to block, inhibit or perhaps even reverse opacification, it is necessary to understand the physico-chemical basis of the transparency of the eye, particularly the most vulnerable tissues: the cornea and the lens. We present below an analysis of the transparency of the cornea and the lens based on the paper of G. B. Benedek (1971).

## 2.1 The Transparency of the Cornea

As we have indicated in Section 1 above, the major portion of the cornea, the stroma, is made up of lamellae within which collagen fibers are laid down approximately parallel to one another and are surrounded by a mucopolysaccharide ground substance. The indices of refraction of collagen and the ground substance are respectively 1.55 and

1.354 (Maurice 1957 and 1969). On passage of light through the stroma there is very little *absorption* of light by the collagen or mucopolysaccharides. On the other hand, because of the difference in index of refraction between collagen and ground substance, each fiber will *scatter* light. In fact, Maurice (1957) calculated the amount of light scattered by each fiber, and showed that if each fiber radiated independently of the others, approximately 90% of the light would be scattered out of the incident beam. Thus, the cornea would in fact appear quite opaque. Maurice concluded that the assumption of independent scatterers was untenable and that it was necessary to take into account the correlation of the phases of waves scattered from each collagen fibril. The phase of each scattered wavelet is determined by the position of the corresponding fibril. If the fibrils were arranged in a perfect lattice, as occurs in the scattering of x-rays from a crystal, the position of each scatterer is known exactly. In such a case, it becomes straightforward to sum up the scattered wavelets. Indeed it was well known that, for a perfect crystal lattice, the scattered wavelets interfere destructively for all scattering directions except for a few specific directions corresponding to "Bragg reflections." In fact, these Bragg reflections occur provided that the wavelength of the x-rays is small compared to the spacing of the planes of atoms in the crystal. In the case that the incident radiation is light, whose wavelength is much larger than the spacing between atom planes, there will be complete destructive interference in *all* directions except that corresponding to that of the incident beam. Thus, such a perfect lattice will scatter no light to the side and the medium will appear perfectly transparent. In view of this, it was natural for Maurice to suggest that a similar perfect regularity existed in the corneal stroma. Indeed he stated so:

> "For a tissue to be transparent, it is necessary that its fibrils are parallel, equal in diameter, and have their axes disposed in a lattice." (Maurice 1957).

This doctrine was widely adopted and became part of the education of ophthalmologists.

Despite the mathematical appeal of a perfect lattice, it is not necessary that the fibrils be arranged in a lattice to obtain transparency. Lattice regularity is neither required theoretically nor is it found experimentally. In 1967 a vivid experimental case in point was published (J. Goldman and G. Benedek 1967). Dr. Goldman had observed that, in the transparent Bowman's zone of the shark, the collagen fibers were arranged in apparently complete disorder, their axes being oriented in all directions, see Fig. 2-1. Also, the Bowman's zone was so thick that a calculation, based on the assumption of independent scatterers, showed sufficient scattering so as to make this zone opaque. These findings represented a vivid challenge to the lattice theory of transparency.

To explain the transparency of this disordered tissue, Goldman and Benedek observed that over dimensions comparable to the wavelength of light, there were very many collagen fibrils. Under these conditions the medium could be regarded as having a mean index of refraction with spatial fluctuations in the index about its mean. The theory of the scattering of light from such random fluctuations shows that light is scattered only by those fluctuations in index of refraction whose wavelength is larger than one half the wavelength of light in the medium. In the normal shark Bowman's zone the fluctuations in fiber density over this scale of distance were small and the tissue was consequently transparent.

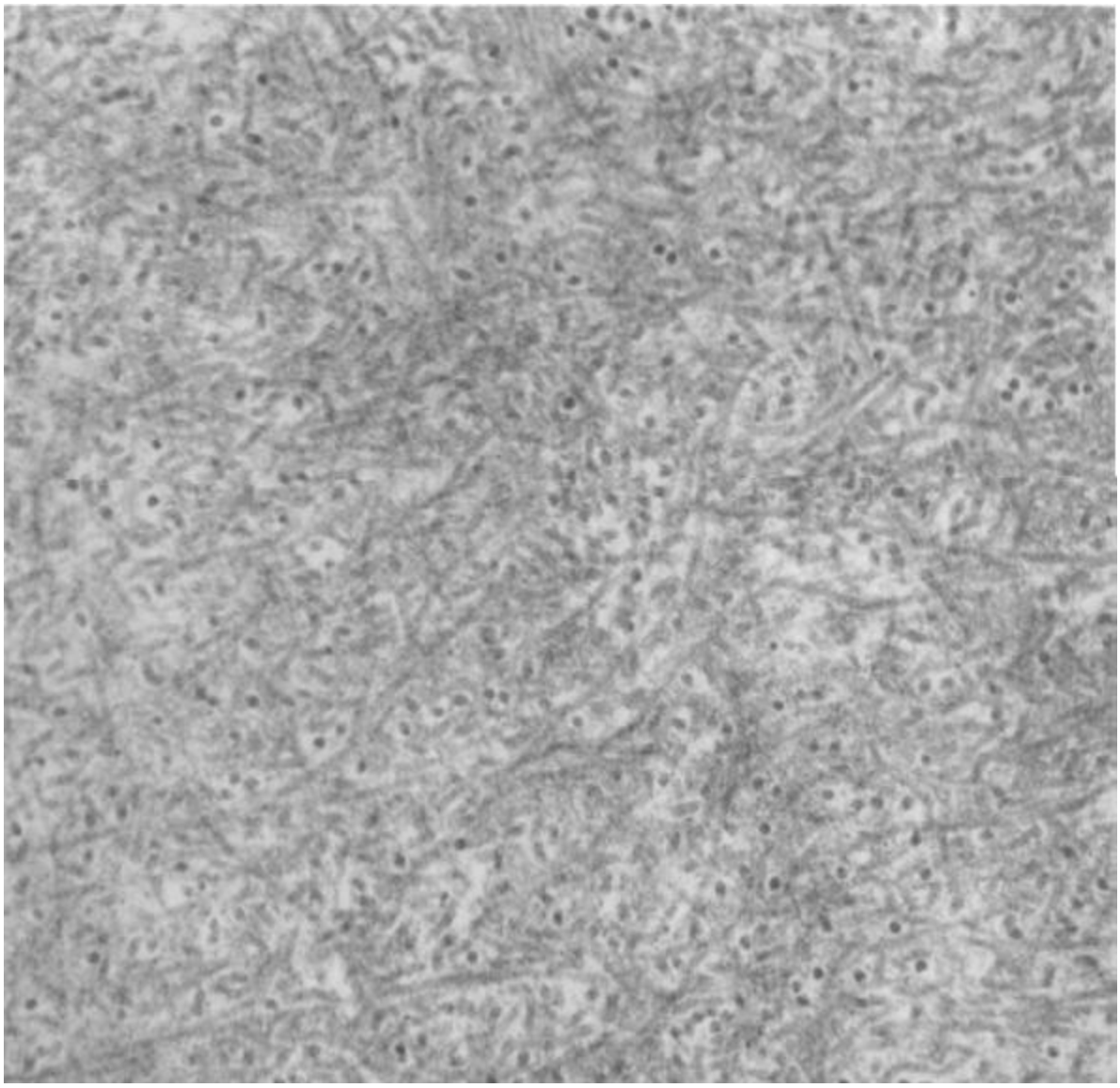

Fig. 2-1: Electron microscope photograph of the transparent Bowman's zone of the shark showing the disordered arrangement of collagen fibrils (from Goldman and Benedek 1968).

In the following we shall present a detailed analysis of the scattering of light which is appropriate for a stochastic (random) distribution of scattering centers. We shall establish the principle stated above for the Fourier components of the fluctuations responsible for scattering in a particular direction. We shall also show how one can quantitatively account for the actual turbidity of the cornea from measurements of the statistical properties of the experimentally observed spatial distribution of collagen fibrils. In a real sense, this analysis transcends the particular problem of corneal transparency. The theory we present will provide insight into the subtle spatial correlations which determine, in general, the transparency or turbidity of all non absorbing media.

**i) The scattering of light from the normal corneal stroma**

We begin by considering the scattering of light by the collagen fibrils in a single lamella. The scattering from many lamellae follows simply from that of a single one. Let the corneal collagen fibers be arranged so that their axis are parallel to the $z$ axis as shown in Fig. 2-2 below. In this figure, 0 is the arbitrary origin of a rectangular coordinate system. The incident light is taken as a plane wave propagating in the $x$ direction. The two dimensional vectors $\vec{R}_j$ from the origin 0 to the intersection of each fiber with the $x$ $y$ plane specifies the position of each fiber. The incident light is taken to have the form of a plane wave $E_o e^{i(\vec{k}_o \cdot \vec{r} - \omega_o t)}$. Here $k_o$ is the wave vector of the incident light wave in the medium, i.e. $k_o = 2\pi/(\lambda/n)$ where $n$ is the mean index of refraction. $\omega_o$ is the angular frequency of the light wave. The incident light induces an oscillatory dipole moment at each point in the medium. If the corneal collagen had an index of refraction $(n_c)$ equal to that of the ground substance $(n_i)$, the fields radiated by all the induced dipoles would sum to zero in all directions other than the forward direction.

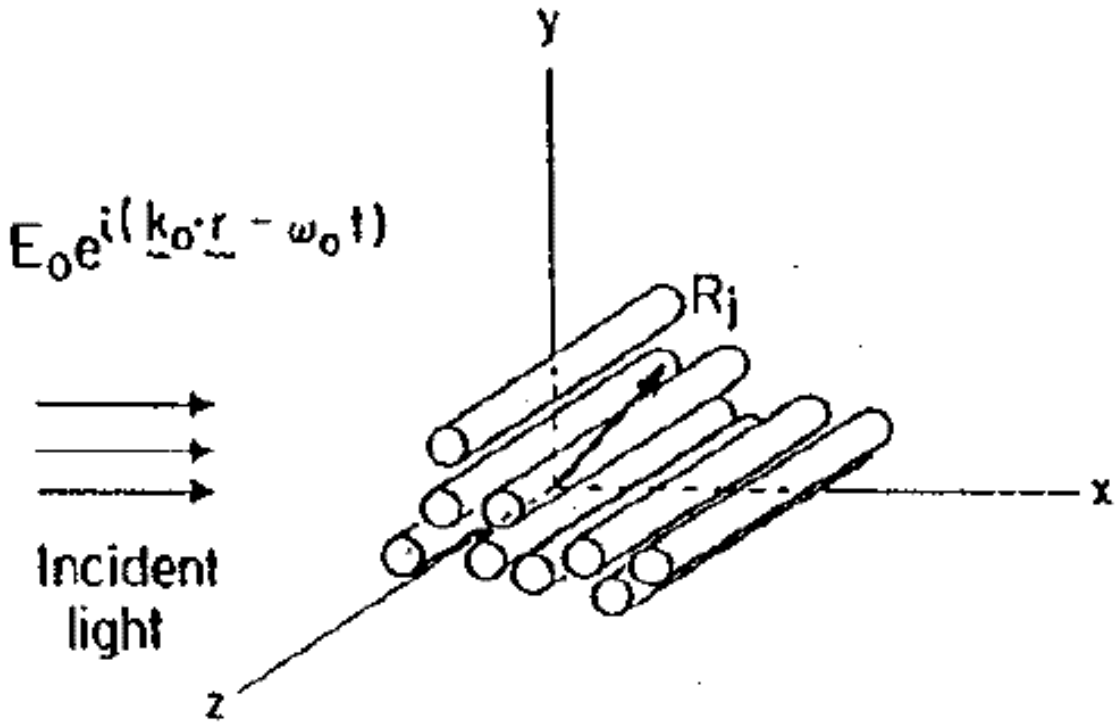


Fig. 2-2: Schematic diagram of corneal collagen fibers in a lamella. The incident light is shown propagating in the *x* direction (from Benedek 1971).

Because of the difference between the indices $n_c$ and $n_i$ we may regard each collagen fiber as being the source of a scattered wavelet. The amplitude $E'_{o_j}$ of the electric field in this wavelet originating from the *j* th fibril is proportional to $\left(n_c^2 - n_i^2\right)$. In Figure 2-3 below we show the positions of the observation point (or field point) $\vec{\mathcal{R}}$ and the source points $\vec{R}_j$ for fibers in a lamella. We assume $\vec{\mathcal{R}} >> \vec{R}_j$.

- The Field Scattered by Each Collagen Fiber

The electric field radiated to the field point from a single fiber at position $\vec{R}_j$ is given by:

$$E_j(\mathcal{R},t) = E'_{o_j} e^{i(k_o \mathcal{R} - \omega_o t)} e^{i(\vec{k}-\vec{k}_o)\cdot\vec{R}_j} \qquad (2.1\text{-}1)$$

The factor $\exp i(k_o\mathcal{R} - \omega_o t)$ is the same for all the fibers and represents the fact that the radiated field is a wave having the same wavelength and frequency as the exciting field.

The phase factor $e^{i(\vec{k}-\vec{k}_0)\cdot\vec{R}_j}$ in Eqn. (2.1-1) merits careful consideration. Here, $\vec{k}_0$ is the wave vector of the incident light, and $\vec{k}$ is the wave vector of the scattered light wave $\vec{k}_0$ and $\vec{k}$ have the same magnitude $\left(2\pi/\left(\lambda/n\right)\right)$. $\vec{k}_0$ points in the direction of the incident light, while $\vec{k}$ points from *0* to the observation point $\mathcal{R}$. This exponential represents the effect on the scattered field of the difference in path between rays scattered from the fiber at $\vec{R}_j$ and a fiber at *0*. We can see this in Fig. 2-4. The ray emanating from *0* and moving in the scattered direction $\theta$ travels a distance $R_j \cos\theta'$ further in its path to the observer of the scattered field than the ray scattered from *j*. In units of the optical phase this difference is $\left(2\pi/(\lambda/n)\right)\left(R_j \cos\theta'\right)$, where $\lambda/n$ is the wavelength of light in the stroma. It must be observed, however, that the oscillation induced by the incident field at the point $\vec{R}_j$ took place at a time later than that at *0*.

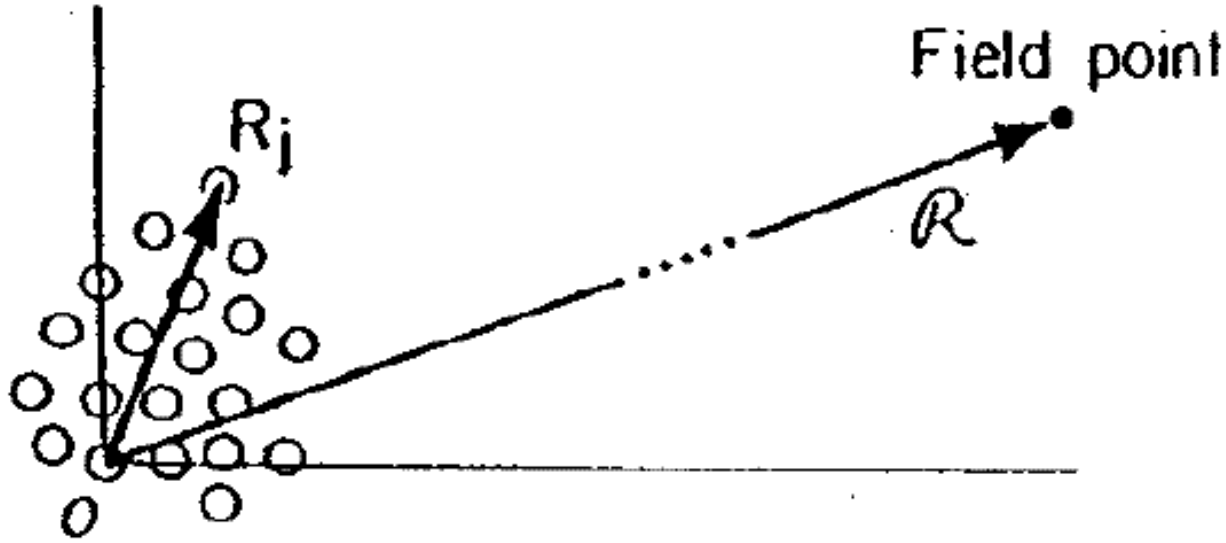


Fig. 2-3: Schematic diagram showing the field point $\vec{\mathcal{R}}$ and the source points $\vec{R}_j$.

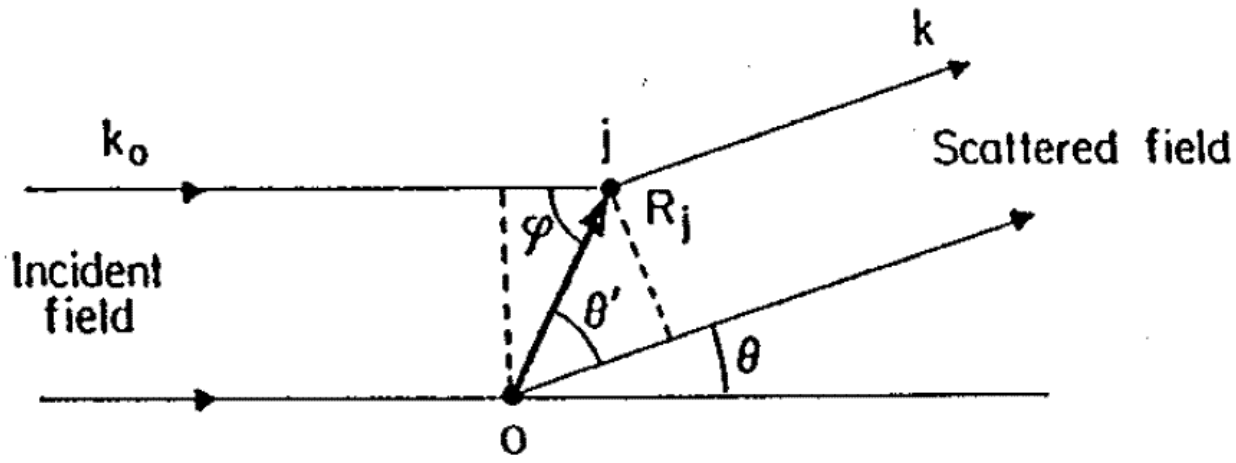


Fig. 2-4: Geometric representation of the difference in optical path between scattering from a fiber situated at the origin 0, and one situated at the source point $\vec{R}_j$. The scattered direction is specified by the scattering angle $\theta$.

This introduces a factor $(2\pi/(\lambda/n))R_j\cos\varphi$ to describe this retardation. The net difference in optical phase between rays scattered from 0 and *j* then is

$$\Delta\Phi = [2\pi/(\lambda/n)]\ R_j(\cos\theta' - \cos\varphi). \tag{2.1-3}$$

It is convenient to express this in terms of the wave vectors $\vec{k}_0$ and $\vec{k}$. Using the dot product notation; *viz.*,

$$\vec{k}_0 \cdot \vec{R}_j = (2\pi/(\lambda/n))\ R_j\cos\varphi \tag{2.1-4}$$

$$\vec{k} \cdot \vec{R}_j = (2\pi/(\lambda/n))\ R_j\cos\theta', \tag{2.1-5}$$

we see that we may write the phase difference $\Delta\Phi$ in terms of the difference vector $\vec{k} - \vec{k}_0 = \vec{K}$. Here $\vec{K}$ is called the scattering vector:

$$\Delta\Phi = (\vec{k} - \vec{k}_0)\cdot\vec{R}_j = \vec{K}\cdot\vec{R}_j. \tag{2.1-6}$$

The scattering vector ***K*** will enter our discussion continually, so let us examine its properties at this point. Since $\vec{K}$ is the difference between $\vec{k}_0$ and $\vec{k}$ we see from Fig. 2-5 that its magnitude is

$$K = 2k_0\sin(\theta/2) = (4\pi/(\lambda/n))\ \sin(\theta/2). \tag{2.1-7}$$

This figure also shows clearly that the direction of $\vec{K}$ is perpendicular to the plane bisecting $\vec{k}_0$ and $\vec{k}$.

In the subsequent analysis the amplitude factor $E'_{oj}$ in Eqn. 2.1-1 for the scattering from a single particle will not be consequential. Nevertheless for the sake of completeness we write below the magnitude and direction of $\vec{E}'_{oj}$ if the incident field $\vec{E}_o$ is polarized as shown in Fig. 2-6. The scattered field is a function of the distance *r* in the *xy* plane and the angle $\theta$ between the *x* axis and the direction of *r*. As indicated in Fig. 2-4, *r*, is the distance in the *xy* plane from the axis of the fiber to the observation or field point. We break $\vec{E}'_{oj}$ into a component in the *z* direction and a component in the *xy* plane in

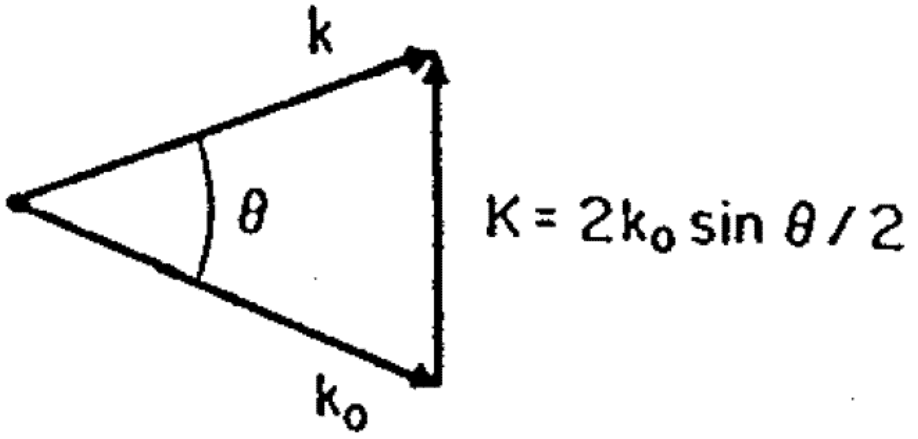


Fig. 2-5: Geometry of the scattering process. The wave vector of the incident light is $\vec{k}_0$, the wave vector of the scattered light is $\vec{k}$ The scattering vector is the difference vector. Its length is $2k_0 \sin(\theta/2)$.

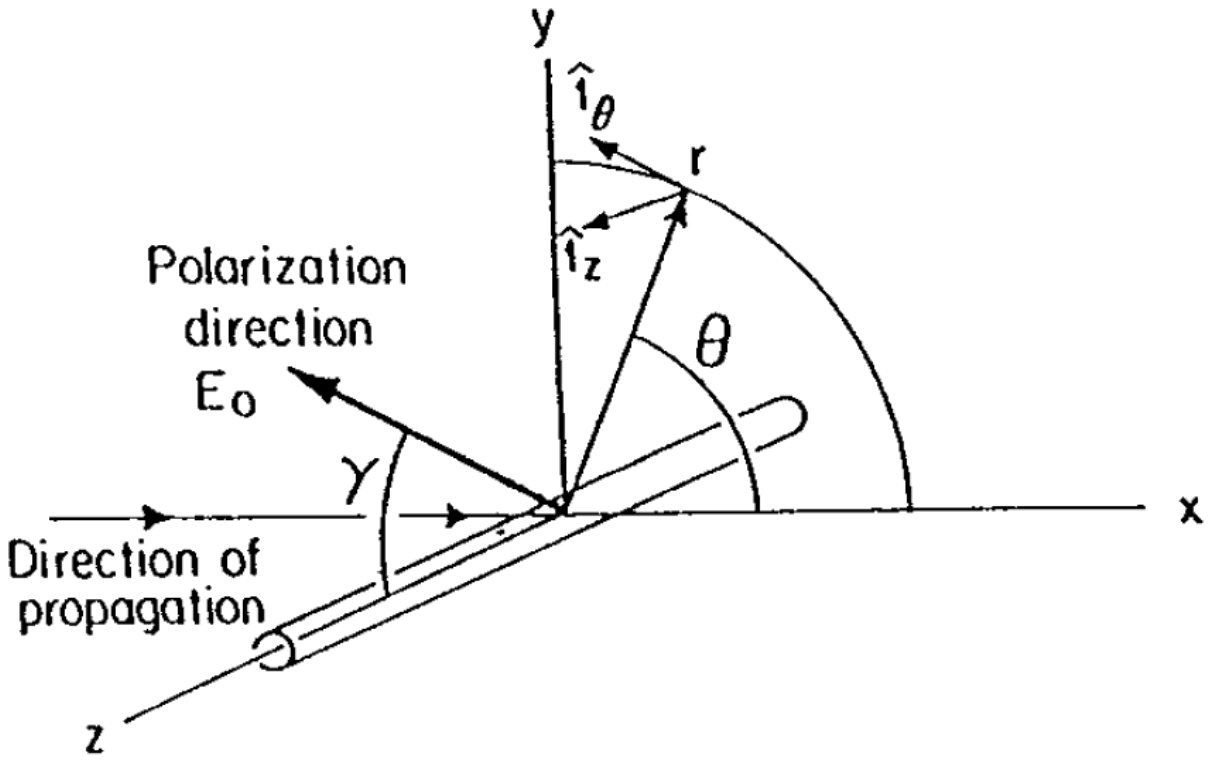


Fig. 2-6: Direction of the polarization vector of the incident light field $\vec{E}_o$ and incident propagation direction ($x$). Also shown are the unit vectors $\hat{1}_z$ and $\hat{1}_\theta$ that are used to specify the polarization of the scattered field as observed a distance $r$ from the axis of a single scattering collagen.

terms of the unit vectors $\hat{1}_z$ and $\hat{1}_\theta$. In the case that the radius of the fiber $r_0$ is small compared to the wavelength of the light $\lambda$ in the medium outside the fiber, and if all the fibers are within a region small compared to the distance $\mathcal{R}$ to the field point, then $\vec{E}'_{oj}$ is essentially independent of the position $\vec{R}_j$ of fiber $(\vec{E}'_{oj} = \vec{E}'_o)$ and is given by

$$\vec{E}'_{oj} = \frac{E_o}{4}\left(\frac{\lambda}{r}\right)^{1/2}\left(\frac{2\pi r_0}{\lambda}\right)^2 \left(m^2 - 1\right)\left[\hat{1}_z \cos\gamma + \hat{1}_\theta \left(\frac{2}{m^2+1}\right)\sin\gamma\cos\theta\right], \qquad \text{2.1-2}$$

where $m = n_c / n_i$. This result states that when the incident field is polarized along $z$, the scattered field is polarized entirely along the $z$ direction and is independent of $\theta$. However, when the incident field is polarized along $y$, the field is in the $xy$ plane in the direction of $\hat{1}_\theta$ and its amplitude varies as $\cos\theta$ in accordance with dipole radiation. The amplitude of the scattering from each fiber is proportional to the square of the fiber radius and is inversely proportional to $r^{1/2}$ because each fiber is regarded as an infinite cylinder. Because of the cylindrical symmetry the radiated field propagates only in the plane of the vector $\vec{R}_j$. This plane is the $xy$ plane.

- The Field and Intensity of Light Scattered From Many Fibers

We now calculate the total electric field scattered by all the fibers. If the distance to the observation point $\vec{\mathcal{R}}$ is much larger than the thickness of the lamella, then $\vec{E}'_{o_j}$ is independent of fiber position (*j*) so that we can write the total field $\vec{E}_{\text{tot}}\left(\vec{\mathcal{R}},t\right)$ from all the fibers as:

$$\vec{E}_{\text{tot}}\left(\vec{\mathcal{R}},t\right)=\sum_{j=1}^{N}\vec{E}_j\left(\vec{\mathcal{R}},t\right)=\vec{E}'_o\;\; e^{i\left(k_0\mathcal{R}-w_0t\right)}\sum_{j=1}^{N}\;e^{i\vec{K}\cdot\vec{R}_j} \tag{2.1-8}$$

Here *N* is the total number of fibers in the illuminated region of the lamella. The last term in Eqn. (2.1-8) represents the sum of the individual waves, each of which has the phase factor $e^{i\vec{K}\cdot\vec{R}_j}$. It is convenient to define the interference function $I(\vec{K})$.

$$I(\vec{K})=\sum_{j=1}^{N}\;e^{i\vec{K}\bullet\vec{R}j}. \tag{2.1-9}$$

In the present problem the $\vec{R}_j$'s are points in a plane, the spatial distribution of these points determine the magnitude of the scattered field. We now examine how the statistical properties of this spatial distribution determines the magnitude of *I* and hence the magnitude of the scattered field and scattered intensity.

Let us write the interference function in an alternative form which utilizes the properties of the well known delta function $\delta\left(\vec{R}-\vec{R}_j\right)$. This function has the property that its integral

$$\int_A d^2R\;\delta\left(\vec{R}-\vec{R}_j\right)$$

is unity if the area A includes the point $\vec{R}_j$ and is 0 if the area does not include $\vec{R}_j$. These $\delta$ functions can be used to locate the position of the points $\vec{R}_j$, and we may therefore write *I* as an integral over an area A which contains the *N* particles viz.

$$I(\vec{K})=\int_A d^2R\;e^{i\vec{K}\cdot\vec{R}}\;\sum_{j=1}^{N}\delta\left(\vec{R}-\vec{R}_j\right) \tag{2.1-10}$$

It may now be observed that, if the density of particles is sufficiently large that there are many particles in regions of dimension $(1/K)\times(1/K)$, then since the factor $e^{i\vec{K}\cdot\vec{R}}$ is

essentially constant in such small areas, we can describe the sum of delta functions as continuous number density $\rho(R)$. Thus we may write

$$\sum_{j=1}^{N} \delta\left(\vec{R} - \vec{R}_j\right) = \rho\ (R). \tag{2.1-11}$$

It is useful to regard this density of particles as having a mean value $<\rho>$ independent of position plus a spatial fluctuation in density about the mean viz.:

$$\rho\ (\vec{R}) = \ <\rho> + \Delta\rho\ (\vec{R}). \tag{2.1-12}$$

The mean value is given by $<\rho> = (N/A)$. If we use this expression for the spatial variation of the density into (2.1-10), we find

$$I = \int d^2R\ e^{i\vec{K}\cdot\vec{R}} <\rho> + \int d^2R e^{i\vec{K}\cdot\vec{R}} \Delta\rho\ (\vec{R}). \tag{2.1-13}$$

The first integral in Eqn. (14) has the property that it is equal to zero, if the area A is large compared to the light wavelength for all values of $\vec{K}$ other than *K* = 0. Thus a uniform spatial distribution of scattering elements scatters no light away from the incident beam. The scattering of light to the side is produced entirely by the spatial fluctuations in the density as is seen clearly in the second term of Eqn (2.1-13). This term however contains information about the precise nature of the density fluctuation responsible for scattering in the direction of $\vec{K}$. We can obtain this information by regarding the spatial fluctuation $\Delta\rho(\vec{R})$ as being composed of sinusoids having a distribution of wave vectors $\vec{q}$. Indeed the Fourier decomposition of $\Delta\rho(\vec{R})$ can be written as

$$\Delta\rho\ (\vec{R}) = \frac{1}{2\pi} \int d^2q\ e^{-i\vec{q}\cdot\vec{R}} \Delta\rho\ (\vec{q}). \tag{2.1-14}$$

Here the $\Delta\rho(\vec{q})$ is the Fourier amplitude of wave vector $\vec{q}$ in the decomposition. The Fourier amplitude $\Delta\rho(\vec{q})$ can be obtained by the Fourier inversion (2.1-14) viz.:

$$\Delta\rho\ (\vec{q}') = \left(\frac{1}{2\pi}\right) \int_A d^2R\ \ e^{iq'\cdot R}\Delta\rho(\vec{R})\,. \tag{2.1-15}$$

We may now see that $I(\vec{K})$ has a very clear physical meaning. If we substitute Eqn. (2.1-14) into Eqn. (2.1-13), we find, since the first term in (2.1-13) is zero, that

$$I(\vec{K}) = \left(\frac{1}{2\pi}\right) \int d^2q\ \Delta\rho(\vec{q}) \int_A d^2R\ e^{i(\vec{K}-\vec{q})\cdot R}\,. \tag{2.1-16}$$

But the second integral has the property that

$$\int_A d^2R\ e^{i(\vec{K}-\vec{q})\ \cdot\ R} = (2\pi)^2\,\delta\left(\vec{K}-\vec{q}\right) \tag{2.1-17}$$

where $\delta(K-q)$ is the delta function in wave vector space. Thus we find simply that

$$I(\vec{K}) = 2\pi\ \Delta\rho\ (\vec{K})\,. \tag{2.1-18}$$

This result signifies that of all the wave vector components in the fluctuation $\Delta\rho(\vec{R})$, only one component, that for which $\vec{q} = \vec{K}$ is responsible for the scattering in the direction $\vec{K}$. Furthermore, the amplitude of the scattered field is directly proportional to the Fourier amplitude $\Delta\rho(\vec{K})$ in the spatial fluctuation.

This condition is, in fact, equivalent to the famous Bragg reflection condition in the x-ray case. This is seen as follows: Consider first the magnitude of the wavelength $\lambda_f$ of the scattering fluctuations:

$$\left(\frac{2\pi}{\lambda_f}\right) = K\,. \tag{2.1-19}$$

But according to Eqn. (2.1-7)

$$K = 2k_o \sin\theta/2 = 2\left(\frac{2\pi}{(\lambda/n}\right)\sin(\theta/2)\,.$$

Thus

$$\lambda_f = \frac{(\lambda/n)}{2\sin(\theta/2)}. \qquad (2.1\text{-}20)$$

This is equivalent to the Bragg reflection condition. In the present context it states that the fluctuation responsible for scattering light an angle $\theta$ from the direction of $\vec{k}_o$ to that of $\vec{k}$ has a wavelength $\lambda_f$. This $\lambda_f$ has its smallest value for backward scattering where $\theta = \pi$. Here $\lambda_f$ is equal to one half the wavelength of the light in the medium. For smaller angles the wavelength of the scattering fluctuations increases as $(\sin(\theta/2))^{-1}$. Note further that the direction of the scattering fluctuations $\hat{1}_K$ corresponds to wave fronts of the fluctuation bisecting the angle between the wave vectors $\vec{k}_o$ and $\vec{k}$. That is to say, the scattering fluctuation "reflects" the incident wave vector into the scattered wave vector.

We may see more vividly the connection between Eqn. 2.1-20 and the Bragg reflection condition for the diffraction of x-rays from a crystal lattice. Figure 2-7 shows radiation incident from the left upon a 3-dimensional lattice of atoms. We seek to find the direction $\theta$ of the scattered beam for which constructive interference can take place. We see from the diagram that the condition for constructive interference is that the path length difference $(2\delta)$ between the incident and scattered beams must be an integral number of wavelengths, viz:

$$2\delta = m\lambda = 2d \cos\ \theta'$$

Note from the Figure that

$$\theta + 2\theta' = \pi$$

Thus

$$\theta' = \frac{\pi}{2} - \frac{\theta}{2}$$

Hence

$$\cos\ \theta' = \sin\left(\theta/2\right)$$

Thus, the condition for constructive interference becomes:

$$m\lambda = 2d\sin\left(\theta/2\right)$$

But $d$ in the crystal lattice corresponds to the wavelength of the fluctuation in the continuous medium. Thus:

$$d = \lambda_f = \frac{m\lambda}{2\sin\left(\theta/2\right)}$$

This is to be compared with

$$K = \left(\frac{2\pi}{\lambda_f}\right) = 2k_0\sin\left(\theta/2\right)$$

where $k_0 = \left(2\pi/\lambda\right)$ or

$$\lambda_f = \frac{\lambda}{2\sin\left(\theta/2\right)}$$

Thus, the result obtained for the fluctuations in the continuous medium are the same as that found in the Bragg scattering case of x-rays from a regular lattice except for the presence of higher order of diffraction in the lattice case.

Thus we have established in Eqn. 2.1-20 the result that for light to be scattered by a quasi-continuous medium, it must contain a spatial fluctuation in the particle density. Of all the Fourier components in this spatial fluctuation, only that having the scattering vector $\vec{K}$ is responsible for the scattering into direction $\theta$. Electron microscope photographs, of course, resolve structures much smaller than the light wavelength. In examining such photographs for the source of opacity of, for example, the ocular media one must search for these features that contain Fourier components comparable to or larger than the light wavelength.

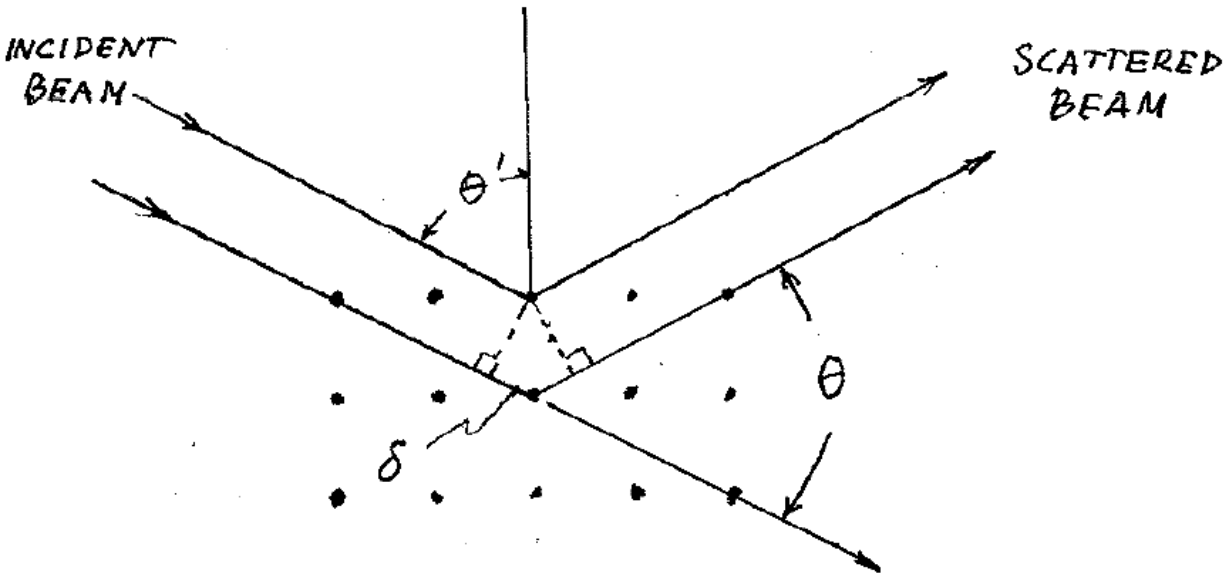


Fig. 2-7: Schematic diagram showing the condition for constructive interference in scattering of x-rays from a lattice.

We now show quantitatively why the Fourier amplitude $\Delta\rho(\vec{K})$ is in fact sufficiently small to allow transparency in the normal corneal stroma. The intensity of the light scattered is proportional to the square of the electric field. This is:

$$E^2_{\text{Tot}}(\mathcal{R},t) = \left|E'_o\right|^2 \left| I \right|^2 \tag{2.1-21}$$

To calculate the experimentally observed light intensity one, in fact, does not need to know the precise relative location of each of the scattering fibrils. Indeed, since the cornea is composed of a superposition of many lamellae, we see that the desired scattered electric field is the *ensemble average* over many statistically equivalent lamella. This ensemble average, denoted by < >, involves both an average over all orientations of fibers in the lamellae relative to the polarization of the incident light, and an ensemble average over the distribution of positions of the fibrils. We may therefore write the average total scattered field as:

$$\left\langle E^2_{\text{Tot}}(\mathcal{R},t)\right\rangle = \left\langle \left|E'_o\right|^2 \right\rangle \left\langle \left| I \right|^2 \right\rangle \tag{2.1-22}$$

where the first bracket represents the average of the scattered field amplitude over all orientations of the fibrils and the second bracket represents an average over distribution of fiber centers in a plane. Thus we see that the experimentally observed scattered light intensity depends not on the exact locations and orientations of the fibers, but only on a statistical average over the distribution of orientations and positions. The important term in (2.1-22) is the average of the square of the interference function $\left\langle \left| I \right|^2 \right\rangle$. This can be evaluated as follows. We see from Eqn. (2.1-9) that

$$\left\langle \left| I \right|^2 \right\rangle = \left\langle \sum_{j=1}^{N} \sum_{k=1}^{N} e^{i\vec{K}\cdot(\vec{R}_j - \vec{R}_k)} \right\rangle. \tag{2.1-23}$$

Here, $N$ is the total number of illuminated fibers. If we again introduce the delta function representation, which marks the positions of the scattering elements, we see

that $\left\langle \left| I \right|^2 \right\rangle$ can be expressed as an integral over the two dimensional area A comprised of the centers $\vec{R}_j$ of the scattering fibrils:

$$\left\langle \left| I \right|^2 \right\rangle = \int_A d^2\vec{R} \int_A d^2\vec{R}'\ e^{i\vec{K}\cdot(\vec{R}-\vec{R}')} \otimes \left\langle \sum_j \sum_k \delta(\vec{R}-\vec{R}_j)\,\delta(\vec{R}'-\vec{R}_k) \right\rangle. \tag{2.1-24}$$

The double sum in the ensemble average can be decomposed into diagonal terms $(j=k)$ and off diagonal terms $(j \neq k)$:

$$\left\langle \sum_j \sum_k \delta\left(\vec{R}-\vec{R}_j\right)\delta\left(\vec{R}'-\vec{R}_k\right) \right\rangle = \left\langle \sum_{\ell=1}^{N} \delta\left(\vec{R}-\vec{R}_\ell\right)\delta\left(\vec{R}'-\vec{R}_\ell\right) \right\rangle + \left\langle \sum_{\substack{j\neq k \\ j,k}}^{N} \delta\left(\vec{R}-\vec{R}_j\right)\delta\left(\vec{R}'-\vec{R}_k\right) \right\rangle. \tag{2.1-25}$$

The second term on the right hand side of (2.1-25) is composed of $N$ rows, each of which contains ($N$ – 1) terms. The terms in the row corresponding to $j$ = 1 are

$$\left\langle\, j=1 \,\right\rangle = \left\langle \delta\left(\vec{R}-\vec{R}_1\right)\left\{\delta\left(\vec{R}'-\vec{R}_2\right)+\delta\left(\vec{R}'-\vec{R}_3\right)+\cdots\delta\left(\vec{R}'-\vec{R}_N\right)\right\}\right\rangle. \tag{2.1-25a}$$

The ensemble average over each row gives the same result as the first row. Hence, the entire off diagonal sum in (2.1-25) is $N$ times the sum over the single row exhibited in Eqn. (2.1-25a). We can understand this sum if we recognize that it is to be integrated over the space $\vec{R}'$. With this in mind we see that $\left\langle\, j=1 \,\right\rangle$ can be expressed in terms of a conditional number density distribution $\rho\left(\vec{R}'\,|R_1'\right)$ viz.:

$$\left\langle\, j=1 \,\right\rangle = \delta\left(\vec{R}-\vec{R}_1\right)\rho\left(\vec{R}'\,|\vec{R}_1\right). \tag{2.1-25b}$$

Here $\rho\left(\vec{R}'\,|R_1\right)$ has the property that $\rho\left(\vec{R}'\,|R_1\right)d^2\vec{R}'$ is the mean number of particles in area element $d^2\vec{R}'$ around $\vec{R}'$ provided that there is certainly a particle at position $\vec{R}_1$. We can now place (2.1-25b) into (2.1-24), and observe that, because of the properties of the delta function,the integrals in Eqn. (2.1-24) produce the following form for $\left\langle \left| I \right|^2 \right\rangle$:

$$\left\langle \left| I \right|^2 \right\rangle = N + N\int_A d^2\vec{R}\int_A d^2\vec{R}'\ e^{i\vec{K}\cdot(\vec{R}-\vec{R}')}\delta\left(\vec{R}-\vec{R}_1\right)\rho\left(\vec{R}'\,|\vec{R}_1\right)$$

which on integrating over $R$ gives

$$\left\langle \left| I \right|^2 \right\rangle = N + N \int_A d^2\vec{R}'\ e^{i\vec{K}\cdot(\vec{R}_1 - \vec{R}')} \rho\left(\vec{R}' \mid \vec{R}_1\right). \tag{2.1-26}$$

Since the conditional number density distribution is a function only of the distance $\left|\vec{R}_1 - \vec{R}'\right|$, it is convenient to define a new variable $\vec{R}'' = \left(\vec{R}_1 - \vec{R}'\right)$. In terms of $\vec{R}''$ we can write (2.1-26) as

$$\left\langle \left| I \right|^2 \right\rangle = N + N \int_A d^2R''\ e^{i\vec{K}\cdot\vec{R}''} \rho\left(R'' \mid 0\right). \tag{2.1-27}$$

We may give a simple physical interpretation of this result for $\left\langle \left| I \right|^2 \right\rangle$. The first term corresponds to what may be called "independent particle scattering." It is the contribution of the *individual* scattering elements to the total scattered intensity without regard to the correlation in the phases of waves from near by pairs of particles. The second term takes into account the very important effect of the interference of waves scattered by all possible pairs of particles. The factor $e^{i\vec{K}\cdot\vec{R}''}$ describes the effect of this interference for a pair separated a distance $\vec{R}''$, and the conditional number distribution $\rho\left(R'' \mid 0\right)$ essentially enumerates the number of pairs that are to be found spaced apart a distance $R''$.

Since the conditional number density distribution $\rho\left(R'' \mid 0\right)$ plays a central role in determining the magnitude of $\left\langle \left| I \right|^2 \right\rangle$, we now examine the features of this distribution. We represent in Fig. 2-8 below an arrangement of scattering elements in a plane. Choosing any element as an origin, circumscribe two circles of radius $R''$, and $R'' + \delta R''$ as shown. Within this annulus one counts the number $\delta n(R'')$ of scattering centers. By carrying this process out, using many starting points as the center of the annulus, one finds the mean value $\left\langle \delta n(R'') \right\rangle$. This number, which can in principle be determined from electron microscope photographs of the distribution of scattering centers, enables the determination of $\rho\left(R'' \mid 0\right)$ according to the relation

$$\rho\left(R'' \mid 0\right) = \left(\frac{1}{2\pi R''}\right)\frac{\left\langle \delta n(R'')\right\rangle}{\delta R}. \qquad (2.1\text{-}28)$$

A perfectly similar situation applies for a three dimensional distribution of scattering elements. In that case the two dimensional annulus is replaced by spherical shells of radius $R''$ and thickness $\delta R''$.

If one imagines carrying out the construction shown in Fig. 2-8, it becomes clear that the distribution $\rho\left(R'' \mid 0\right)$ has the properties shown in Fig. 2-9. This figure shows the following features of $\rho\left(R'' \mid 0\right)$.

1. For $R'' << R_c$ $\quad \rho\left(R'' \mid 0\right) = 0$. This is due to the finite size of the scattering centers and the repulsion between particles at close distance of approach.

2. For $R'' \sim R_c$ a second particle is likely to be found surrounding the particle at the origin.

3. For $R'' \geq R_c$ it is possible that $\rho\left(R'' \mid 0\right)$ can show decreasing oscillation above and below the value of $\left\langle \rho \right\rangle$. The maxima in these few oscillations correspond to locations of shells of first, second and perhaps even third nearest neighbors. In the case of the collagen fibers in normal corneal stroma, electron micrographs show that after about two shells of near neighbors, there is no longer any significant correlation in the position of pairs of particles.

4. For $R'' >> R_c$ $\quad \rho\left(R'' \mid 0\right) = \left\langle \rho \right\rangle = (N/A)$. For distances large compared to the so-called correlation range $R_c$, there is no longer any effect of the presence of a particle at the origin 0. Thus the number of particles in differential area $d^2R''$ is $\left\langle \rho \right\rangle d^2R''$, where $\rho = (N/A)$.

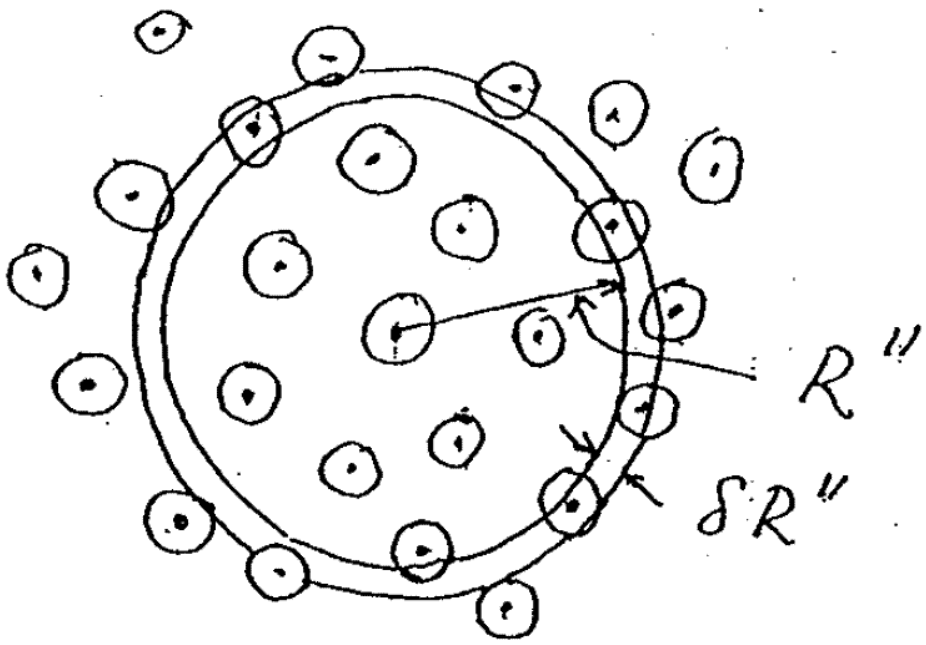


Fig. 2-8 Schematic representation of the determination of $\rho\left(R'' \mid 0\right)$ from the spatial distribution of scattering elements in a plane.

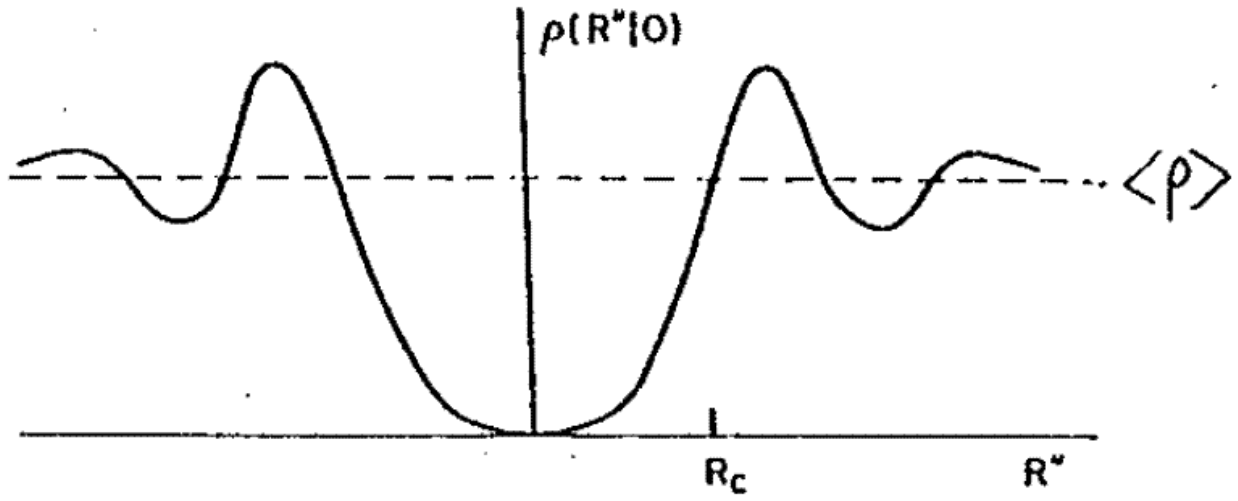


Fig. 2-9 Representation of the general features of $\rho\left(R'' \mid 0\right)$, the conditional number density distribution function.

To see more clearly the effect of the conditional probability distribution on the scattered intensity it is useful to introduce a function $f\left(\vec{R}'' \mid 0\right)$ which is defined by:

$$\rho\left(R'' \mid 0\right) \equiv \left\langle \rho \right\rangle \left(1 - f(R'')\right). \tag{2.1-29}$$

Clearly, $f(R'')$ has the properties:

$$f(R'') \to 1 \qquad R'' < R_c \tag{2.1-29a}$$

and

$$f(R'') \to 0 \qquad R'' > R_c. \tag{2.1-29b}$$

In Fig. 2-10 below we show schematically the form of $f(R'')$. Clearly, $f(R'')$ roughly has the form of a pulse which represents a region where second particle is *not* likely to be found. If we now replace $\rho\left(R'' \mid 0\right)$ by $f(\vec{R}'')$ using Eqn. (2.1-29) in Eqn. (2.1-27) for $\left\langle \left| I \right|^2 \right\rangle$, we find:

$$\left\langle \left| I \right|^2 \right\rangle = N\left(1 - \left\langle \rho \right\rangle \int_A d^2\vec{R}'' f(\vec{R}'')\ e^{i\vec{K}\cdot\vec{R}''}\right). \tag{2.1-30}$$

In obtaining this result we have used the fact that the term:

$$\langle\rho\rangle \int_A d^2\vec{R}''\ \ e^{i\vec{K}\cdot\vec{R}''} = 0 \qquad\qquad \text{for } \vec{K} \neq 0$$

provided that the illuminated area A is much larger than the wavelength of light.

Equation (2.1-30) gives physical insight into the precise factors which control the intensity of the scattered light, and hence the transparency or the turbidity of the scattering medium. To gain this insight let us first consider a condition that often occurs experimentally, namely that the correlation range $R_c$ is small compared to the wavelength of the light. To be more specific we shall assume that $KR_c << 2\pi$. Under these conditions the factor $\exp i\vec{K}\cdot\vec{R}''$ in Eqn. (2.1-30) can be set equal to unity. Thus, if we define a correlation area $A_c$ as:

$$A_c = \int_A d^2\vec{R}''\ \ f(\vec{R}'') \tag{2.1-31}$$

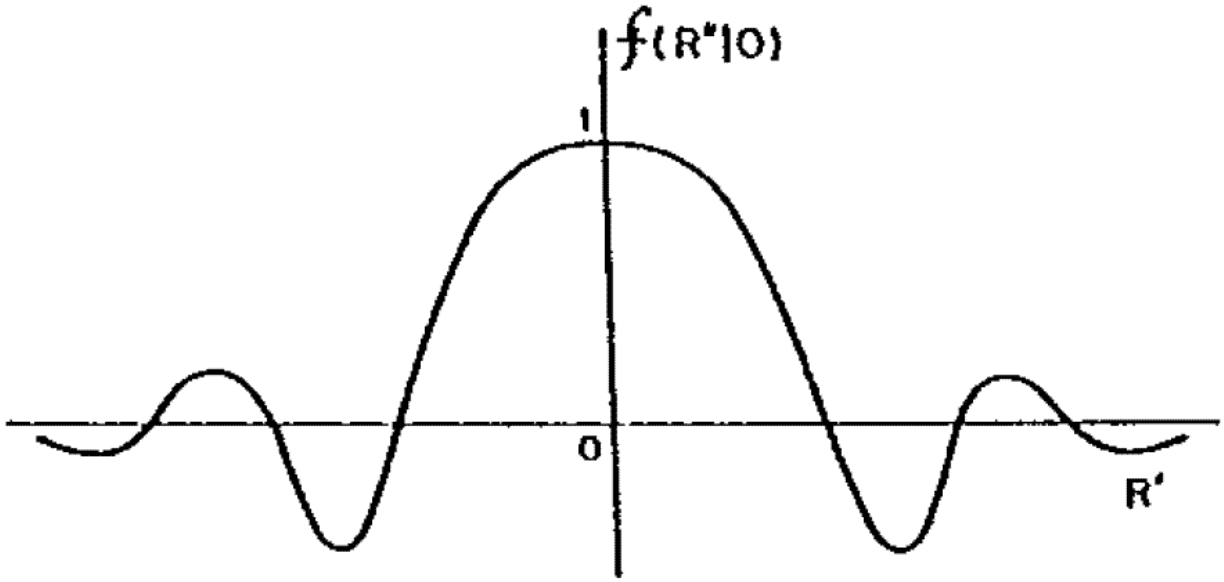


Fig. 2-10. A plot of the function $f(R'') = \Big(\Big\langle\ \rho\ \Big\rangle - \rho(R'' \mid 0)\Big)\Big/\Big\langle\ \rho\ \Big\rangle$ which represents the regions where a second particle in a pair is not likely to be found.

then, we find that:

$$\left\langle \left| I \right|^2 \right\rangle \cong N\left(1 - \left\langle \rho \right\rangle A_c\right). \tag{2.1-32}$$

Now we can write:

$$\langle \rho \rangle = \frac{1}{(A/N)} = \frac{1}{A_o}. \tag{2.1-33}$$

Here, $A_o = (A/N)$ is the average area available to each particle. $A_c$ on the other hand is effectively the area around a particle wherein a second particle is not to be found. In terms of $A_c$ and $A_o$ we then see that $\left\langle \left| I \right|^2 \right\rangle$ has the following form:

$$\left\langle \left| I \right|^2 \right\rangle = N\left[1 - \left(\frac{A_c}{A_o}\right)\right]. \tag{2.1-34}$$

We observe that the scattered intensity in this case is independent of the direction of scattering, i.e. the scattering becomes isotropic. Furthermore, Eqn. (2.1-34) shows how we can understand the magnitude of the scattered intensity.

Suppose we start with "point particles," for which the correlation area $A_c$, within which a second particle cannot be found, becomes very small compared to $A_0$. In this case $(A_c / A_o) << 1$, and $\left\langle \left| I \right|^2 \right\rangle \cong N$. This is the limit which applies when there is in effect no correlation in the phases of waves scattered from the particle. The scattered intensity is *N* times that produced by a single particle. In the case of the corneal stroma this scattering would be so great that ~ 90% of the incident beam would be scattered; the cornea would be opaque.

Consider next the case in which the particles have finite correlation range and the density becomes sufficiently large that we approach the close packed state. This is the case in which the correlation area $A_c$ becomes comparable to $A_0$, the average area available per particle. In this case $(A_c / A_o) \to 1$ and the interference factor $\left\langle \left| I \right|^2 \right\rangle \to 0$. In this case there is a great correlation in the relative position of the scattering particles. Hence the waves scattered from the individual scattering centers interfere destructively with one another. Very little light is then scattered and the medium is observed to become transparent. Thus, depending on the precise

magnitude of $(A_c / A_o)$, we can traverse the entire domain from complete transparency to opacity.

We see from the above analysis that long range order in the arrangement of scattering elements is by no means needed for a medium to be transparent. Indeed, if the positions of particles show short range order over distances comparable to the mean spacing between particles, then this suffices to produce destructive interference between all the scattered wavelets and hence transparency.

We may now examine the magnitude of the terms in Eqn. (2.1-30) and Eqn. (2.1-34) to determine the magnitude of $\left\langle \left| I \right|^2 \right\rangle$. In the case of normal corneal stroma, electron microscope photographs indicate that $R_c \sim 400\,\text{Å}$. Thus $K R_c = 2\pi\left(2R_c \sin(\theta/2)\right)\big/(\lambda/n) = 2\pi(1/5)$ for light of $(\lambda/n) \sim 6000\,\text{Å}$. Thus we can regard $\exp i\vec{K}\cdot\vec{R}''$ as being approximately unity over the integral in Eqn. (2.1-30). Under these conditions Eqn (2.1-34) applies. We can crudely estimate the magnitude of $A_c / A_o$ in comparison to unity. $A_c \sim \pi R_c^2 \sim 0.5\times10^6(\text{Å}^2)$. The photographs also indicate that $A_o = 1/\langle\rho\rangle \cong 0.3\times10^6(\text{Å}^2)$. Thus a crude estimate of these quantities show that the ratio $(A_c / A_o)$ is ~ 1.7 which is comparable to unity. Thus the correlation in the positions of the fibers plays a very important role in determing the amount of scattering. To obtain a more accurate estimation of the integral $\langle\rho\rangle\int f(R'')d^2R''$, we may use the results obtained by Hart and Farrell (1969) who constructed the form of the conditional number density distribution $\rho\left(R'' \mid 0\right)$ using electron microscope photographs. They kindly informed me (Benedek 1971) that $\langle\rho\rangle\int f(R'')d^2R''$ is between 0.8 and 0.95. Thus, the actual scattering from the cornea is between 0.2 and 0.05 times smaller than the estimate of 90% computed by Maurice on the basis of independent particle scattering, This dramatic reduction in scattered intensity occurs as a result of a correlation in the relative position of particles which persists only over the distance of second nearest neighbors.

Hart and Farrell (1969) have also computed the full qauntity:

$$\langle\rho\rangle\int f(R'')\ e^{i\vec{K}\cdot\vec{R}''}d^2\vec{R}''$$

including the effect the phase factor $e^{i\vec{K}\cdot\vec{R}''}$. They found that this integral has the value 0.87. Thus, with this value we find that the scattered light intensity falls to a value ~ 8 times smaller than that corresponding to independent scatterers. This small light scattering which amounts to about 10% of the incident light intensity is in fact the physical basis for the usefulness of the slit lamp microscope; a basic diagnostic instrument widely usd by ophthalmologists. This instrument sends a vertically oriented slit of light onto the cornea. The light scattered to the side by the ocular media: the aqueous, lens and vitreous, is picked up, magnified by the collection optics, and observed by the ophthalmologist. In this way the turbidity of these media can be assessed qualitatively and early detection of corneal dystrophy or cataract becomes possible. If the cornea were perfectly transparent, no light whatever would be scattered to the side and the normal cornea could not be seen at all.

The analysis of the interference function presented above for a two dimensional array of scatterers can readily be extended to the case of three dimensions. In the three dimensional case Eqn. 2.1-30 still applies provided that $\langle\rho\rangle$ becomes the three dimensional number density $\langle n\rangle = \left(N/V\right)$. $f\left(R''\middle|0\right)$ becomes three dimensional, and $d^2R''$ is replaced by $d^3R''$ viz.

$$\left\langle\left|I\right|^2\right\rangle = N\left(1-\left\langle n\right\rangle\int d^3R''f\left(R''\middle|0\right)e^{i\vec{K}\bullet\vec{R}''}\right)$$

In the case that the correlation range $R_c$ is small compared to the light wavelength, the exponential factor in the integral can be set equal to unity. Furthermore, as in the two dimensional case, we can define a correlation volume $v_c$ as

$$v_c = \int d^3R\ f\left(R''\middle|0\right)$$

This is the volume within which a second particle cannot be found, if there is a particle at 0. Thus, we may write $\left\langle \ |I|^2 \right\rangle$ as

$$\left\langle \ |I|^2 \right\rangle = N\left(1 - \langle n \rangle V_c\right)$$

The scattered intensity per unit volume is proportional to $\left\langle |I|^2 \right\rangle \Big/ V$ and is given by

$$\frac{\left\langle \ |I|^2 \right\rangle}{V} = \langle n \rangle \left(1 - \langle n \rangle V_c\right)$$

With this result, we can readily see how the scattered light intensity per unit volume changes with the mean density $\langle n \rangle$. At low number density, i.e. the case of the dilute gas for which $\langle n \rangle << \left(1/V_c\right)$, we find that the scattered intensity increases in direct proportion to the particle density. In this domain, the independent particle scattering approximation will apply. However, for molecules of finite size, as the particle density approaches $\left(1/V_c\right)$, the factor $\langle n \rangle V_c$ approaches unity. Indeed, in the case that the gas condenses into a fluid, we can expect that with close packing of the particles that $\langle n \rangle V_c$ will become quite close to unity and $|I|^2 \Big/ V$ will approach zero.

Thus we can see that as the density of particles in a scattering volume increases, the intensity of light scattered will at first increase in direct proportion to the number density. However, as the density approaches the close packing domain, the intensity of the scattered light will actually decrease as a function of the number density. The medium will become more transparent as the correlation volume becomes comparable with the volume available per particle. This phenomenon can be understood to be the result of a decrease in the fluctuations in the number of particles in the illuminated region, as we now shall demonstrate.

- Connection between the Scattering of Light and Spontaneous Fluctuations in the Number of Illuminated Particles.

Let us consider a reservoir which contains $N_0$ particles in total volume $V_0$ and let the total energy of the particles in the reservoir be $E_0$. Within this reservoir, let there be a small subsystem $S$ of volume $V << V_0$. This volume is fixed by the region of illumination. The system $S$ is open in the sense that there can be exchange of particle number $N$, and energy $E$ between system $S$ and the entire reservoir. Thus, in general, the number $N$ of particles in $S$ can range between $0 \le N \le N_0$ and the energy of the particles in $S$ can range between $0 \le E \le E_0$. On average, the number $N$ has the value $\langle N(V) \rangle = V\left(N_0 / V_0\right)$. If however, one has an ensemble of identically prepared reservoirs each having a subsystem $S$, then the actual number $N$ in each subsystem in the ensemble will be different from $\langle N \rangle$. We shall now obtain expressions for the mean square fluctuation in $N$ using two different lines of analyses.

In the first method, we will regard the variable $N$ as a stochastic variable and relate the mean square fluctuation $\left\langle \left(N - \langle N \rangle\right)^2 \right\rangle$ to the conditional number density distribution equivalent to $\rho\left(R'' \middle| 0\right)$ found previously.

In the second method, we shall use thermodynamic fluctuation theory to calculate $\left\langle \left(N - \langle N \rangle\right)^2 \right\rangle$ in terms of the equilibrium thermodynamic susceptibility relevant to the system examined. This susceptibility is the isothermal compressibility for a gas of particles or the osmotic compressibility for a solution of macromolecules.

We begin with the first method, in which we regard the position $\vec{R}_j$ of each of the particles as being a stochastic quantity. A different pattern of $\vec{R}_j$'s will exist for each member of an ensemble containing many reservoirs each having system $S$ defined by an illuminated volume $V$. From this point of view, the number density of particles $n(\vec{R})$ in a region around position $\vec{R}$ can be written as

$$n(\vec{R}) = \sum_j \delta\left(\vec{R} - \vec{R}_j\right). \tag{2.1-35}$$

The number of particles in volume $V$ is given by

$$N(V) = \int_V d^3\vec{R} \ \sum_{j=1} \delta\left(\vec{R} - \vec{R}_j\right). \tag{2.1-36}$$

$N(V)$ is of course a statistical quantity which fluctuates from one member of the ensemble to the next. We may define the ensemble average of $N(V)$ as:

$$\left\langle N(V)\right\rangle = \int_V d^3\vec{R} \left\langle \ \sum_{j=1} \delta\left(\vec{R} - \vec{R}_j\right) \ \right\rangle \tag{2.1-37}$$

If the total volume of the reservoir is $V_0$, and the total number of particles in system plus reservoir is $N_0$ then,

$$\left\langle N(V)\right\rangle = \left(\frac{V}{V_o}\right) N_o = \langle n\rangle V \tag{2.1-38}$$

where $\langle n\rangle = (N_o / V_o)$ is the mean number density of particles in the reservoir and system S. We now examine the fluctuations in $N(V)$ about this mean:

$$\left\langle \Delta N^2 \right\rangle \equiv \left\langle \left(N(V) - \left\langle N(V)\right\rangle\right)^2 \right\rangle = \left\langle N^2(V)\right\rangle - \left\langle N(V)\right\rangle^2. \tag{2.1-39}$$

Using Eqn. (2.1-37) we see that

$$N^2(V) = \int_V d^3\vec{R}' \int_V d^3\vec{R} \left( \sum_j \delta\left(\vec{R} - \vec{R}_j\right) \sum_k \delta\left(\vec{R}' - \vec{R}_k\right)\right) \tag{2.1-40}$$

or

$$N^2(V) = \int d^3R' \int d^3R \left( \sum_{\ell=1} \delta\left(\vec{R} - \vec{R}_\ell\right) \ \delta\left(\vec{R}' - \vec{R}_\ell\right) + \sum_j \sum_{\substack{k \\ j \neq k}} \delta\left(\vec{R} - \vec{R}_j\right) \ \delta\left(\vec{R}' - \vec{R}_k\right)\right). \tag{2.1-41}$$

If we now take the ensemble average of $N^2(V)$, we may follow the same line of reasoning as in our consideration of Eqn. (2.1-25a, b). This gives:

$$N^2(V) = \langle N(V)\rangle\left(1 + \int d^3\vec{R}' \int d^3\vec{R}\,\delta\left(\vec{R} - \vec{R}_1\right) n\left(R' \mid R_1\right)\right). \qquad \text{(2.1-42a)}$$

Here

$$\delta\left(\vec{R} - \vec{R}_1\right) n\left(R' \mid R_1\right) = \left\langle \sum_{\substack{k \\ k \neq 1}} \delta\left(\vec{R} - \vec{R}_1\right)\delta\left(\vec{R}' - \vec{R}_k\right)\right\rangle. \qquad \text{(2.1-42b)}$$

Thus, $d^3\vec{R}'\, n\left(R' \mid R_1\right)$ is the total number of particles, on average, which is to be found in differential volume $d^3R'$ around $R_1$ provided that there is a particle at $R_1$. Thus $n\left(R' \mid R_1\right)$ is a conditional number density distribution in three dimensional space. If we carry out the integration over $d^3R$, choosing $R_1$ to be a particle in the volume $V$, we obtain

$$\left\langle N^2(V)\right\rangle = \langle N(V)\rangle\left(1 + \int d^3\vec{R}''\, n(R'' \mid 0)\right) \qquad \text{(2.1-42c)}$$

Here we have noted that $n(R' \mid R_1)$ is a function only of $R'' = \left| R' - R_1 \right|$. We now introduce the three dimensional function $f(R'')$ as

$$n(R'' \mid 0) = \langle n\rangle\left(1 - f(R'')\right). \qquad \text{(2.1-43)}$$

Here

$$f(R'') \to 1 \qquad R << R_c$$
$$f(R'') \to 0 \qquad R >> R_c.$$

since for $R'' >> R_c$, the correlation distance, $n(R'' \mid 0)$ must approach the mean density $\langle n\rangle$, and for $R'' << R_c$, $n(R'' \mid 0)$ must approach zero. Here $f(R'')$ describes a spherically symmetric region about any particle where a second particle is not to be found. If we insert (2.1-43) into (2.1-42c) we find

$$\left\langle N^2(V)\right\rangle - \left\langle N(V)\right\rangle^2 = \langle N(V)\rangle\left(1 - \langle n\rangle \int d^3\vec{R}''\; f(\vec{R}'')\right). \qquad \text{(2.1-44)}$$

This result is to be compared with the form of the mean squared interference function which represents the superposition of phases of waves scattered from a three dimensional distribution of scattering centers. In this case one obtains:

$$\left\langle \left| I \right|^2 \right\rangle = \langle N(V) \rangle \left( 1 - \langle n \rangle \int d^3 R'' \, e^{i\vec{K}\cdot\vec{R}''} f(R'') \right). \qquad \text{(2.1-45)}$$

Upon comparing Eqns. (2.1-45) and (2.1-46) we see that if the correlation range $R_c$ is small compared to the wavelength of light, i.e. $KR_c << 1$, then Eqn. (2.1-44) and (2.1-45) have the same form. As before, $R_c$ corresponds essentially to the distance around any given particle within which a second particle is *not* to be found. We thus may call $f(R'')$ an exclusion function. The comparison between Eqn. (2.1-45) and Eqn. (2.1-44) shows that the intensity of light scattered from an illuminated volume $V$ is directly proportional to the mean square fluctuation in the number of scattering particles in that volume. This statement of course requires the proviso that the wavelengths of the fluctuations studied are sufficiently long compared to the correlation range $R_c$.

We now turn to the second method of analysis of the fluctuations in $N(V)$ that is: thermodynamic fluctuation theory. Let us return to the ensemble of reservoirs, each of which contains the system $S$ of volume $V$, which is free to exchange particles and energy with other particles in the reservoir. The exchange of energy and particle numbers between the system $S$ and the reservoir assumes that at equilibrium the temperature ($T$) of the system $S$ is the same as that of the reservoir. Furthermore, at equilibrium, the chemical potential $(\mu)$ of a particle in the system $S$ is the same as a particle in the reservoir. For such a system in equilibrium with a reservoir, all equilibrium properties can be described in terms of the so-called grand potential $\mathcal{G}(T,V,\mu)$. It is well known that the grand potential can be obtained from the grand

canonical partition function $\mathcal{Z}\left(\beta,V,\mu\right)$ where $\beta \equiv (1/kT)$. This grand canonical partition function is in turn the normalizing factor for the grand canonical probability distribution which describes the probability that the system $S$ will have an energy $E$ and contain $N$ particles within the $V$. This probability distribution has the form:

$$P_S(E,N) = g_S(E,N)\ e^{-\beta(E-\mu N)} \Big/ \mathcal{Z}(\beta,V,\mu) \tag{2.1-46}$$

In this expression $g_S(E,N)$ is the multiplicity of the system when it has $N$ particles and energy *E*. The normalizing factor $\mathcal{Z}(\beta,V,\mu)$ is given by

$$\mathcal{Z}(\beta,V,\mu) = \sum_N \left( \sum_E g_S(E,N)\ e^{-\beta E} \right) e^{\beta\mu N} \tag{2.1-47}$$

The quantity in round brackets above is the canonical partition function $Z(B,V,N)$, i.e.

$$Z(B,V,N) = \sum_E g_S(E,N)\ e^{-\beta E} \tag{2.1-48}$$

In the grand canonical ensemble we can calculate both $\langle N(V)\rangle$ and $\left\langle \left(N(V) - \langle N\rangle\right)^2 \right\rangle$ from the grand potential $\mathcal{G}(\beta,V,\mu)$. Indeed, it follows directly from the definition of $\langle N\rangle$ that

$$\langle N(V)\rangle = \left( \frac{\partial \mathcal{G}(\beta,V,\mu)}{\partial \mu} \right)_{\beta,V} \tag{2.1-49a}$$

Here $\mathcal{G}\left(\beta,V,\mu\right)$, the grand potential, is related to the grand canonical partition function $\mathcal{Z}$ by

$$\mathcal{G} = kT \ln \mathcal{Z}(\beta,V,\mu). \tag{2.1-49b}$$

Here $\mathcal{Z}(\beta,V,\mu)$ is given by Eqn. (2.1-47).

One can similarly compute the mean square fluctuation in *N*(*V*) starting from the grand canonical probability distribution to show that

$$\left\langle N^2(V)\right\rangle - \left\langle N(V)\right\rangle^2 = kT\left(\frac{\partial\langle N\rangle}{\partial\mu}\right)_{\beta,V} \tag{2.1-50}$$

It will be left as a homework exercise in thermodynamics to show that

$$\left(\frac{\partial\langle N\rangle}{\partial\mu}\right)_{\beta,V} = \left(\frac{\langle N\rangle}{V}\right)^2 V\,\beta_T \tag{2.1-50a}$$

where $\beta_T$ is the compressibility of the gas

i.e. $$\beta_T = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T = \frac{1}{\langle n\rangle}\left(\frac{\partial\langle n\rangle}{\partial P}\right)_T \tag{2.1-50b}$$

where here $\langle n\rangle$ = the density of the gas. Thus we see that the mean square fluctuation in the number of particles in the volume $V$ is directly proportional to the compressibility of the gas at temperature $T$ and having mean density $\langle n\rangle$. Indeed, we see that:

$$\left\langle N^2(V)\right\rangle - \left\langle N(V)\right\rangle^2 = \langle N\rangle\left(\frac{\langle N\rangle kT}{V}\right)\beta_T\,. \tag{2.1-51}$$

This result, combined with the fact that this mean square number fluctuation is directly proportional to the scattered light intensity, shows that one can in fact determine the compressibility of a gas by measuring the intensity of light scattered by that gas. A perfectly similar result obtains for a protein-water solution. In this case the scattered light is proportional to the mean square fluctuation of the number of proteins in the illuminated region. This quantity is proportional, in the protein water mixture, to the osmotic compressibility $(1/c)(\partial c/\partial\pi)_T$ where *c* is the protein concentration and $\pi$ is the protein osmotic compressibility.

It is instructive to carry this analysis a bit further to show how the existence of a correlation in the relative position of pairs of particles affects the compressibility.

Consider the simplest case: that of an ideal gas. In this case the integral $\int d^3R''\ f(R'') \to 0$. Thus, from Eqn. (2.1-47) we expect that $\left\langle \Delta N^2\right\rangle = \langle N\rangle$. This result is borne out exactly in the thermodynamic formulation in Eqn. (2.1-51). For an ideal gas

$$P = \left(\frac{\langle N\rangle kT}{V}\right).$$

Thus: $$-V\left(\frac{\partial P}{\partial V}\right) = \frac{\langle N\rangle kT}{V} = \beta_T^{-1}.$$

Thus we see from Eqn. (2.1-51) that $\left\langle \Delta N^2 \right\rangle = \langle N \rangle$ once again in the case of the ideal gas. This result is consistent with the statement that, in the absence of interaction between particles, the distribution of particles in a region *V* of space obeys the Poisson statistics. If we compare Eqn. (2.1-44) and Eqn. (2.1-51), we may obtain a very useful general linkage between the compressibility of a gas and the spatial correlation asociated with pairs of particles. This relation is:

$$\beta_T = \left(\frac{V}{\langle N\rangle kT}\right)\left\{1 - \langle n\rangle \int_V d^3R''\ f(R'')\right\}. \qquad (2.1\text{-}52)$$

This result shows that as one approaches close packing of particles with hard sphere repulsion, the compressibility can become quite small, the fluctuations $\left\langle \Delta N^2 \right\rangle$ decrease and the medium scatters less light. On the other hand, attractive interactions which produce long range correlations in the relative positions of particles, as occur in a fluid near its critical point, can produce a dramatic *increase* in the compressibility of the gas. In this case $\int f(R'')d^3R''$ becomes negative and the correlation range can become quite large, resulting in a large *increase* in $\beta_T$, and an increase in scattered light intensity.

It is worthwhile mentioning that since the compressibility of a non ideal gas such as the van der Waals gas can be obtained analytically as a function of density and temperature, one can employ Eqn. (2.1-52) to deduce at each $\langle n\rangle$ and *T*, the corresponding integral $\int f(R'')d^3R''$ describing the correlation in position of particle pairs.

### ii) The scattering of light from swollen pathologic corneas

In the previous section we showed how short range correlations in the position of pairs of collagen fibers produces destructive interference between waves scattered from collagen fibers in the normal cornea. This interference effect is responsible for the transparency of the normal cornea. We now examine the case of the swollen or edematous cornea which, depending on the degree of swelling, can entirely lose its transparency. We seek in fact to identify the structural alterations in collagen fibers arrangement which are responsible for the opacification of the cornea.

The swelling of the cornea is associated with damage to the corneal endothelium which is located at the interface between the aqueous and the corneal stroma. The endothelium contains active pumps whose function is to inhibit the flow of aqueous into the corneal stroma. Failure of the endothelium can lead to inflow of aqueous, and consequent swelling of the cornea. Associated with this swelling is an increase of the optical turbidity and eventually opacification of the cornea and blindness. In Figure 2-11 below we show an electron microscope photograph of a cross section of a swollen pathologic cornea taken from Goldman *et al.* (1968). The photograph shows that, at this stage of swelling, the cornea contains "lakes," irregular domains, where there is no collagen present at all. These are taken to have the form of long cylinders of irregular cross section. The size of these lakes vary considerably, from dimensions smaller than the light wavelength to dimensions large compared to the light wavelength. Clearly, these lakes represent regions where the index of refraction is different from the mean corneal refractive index. Thus, the lakes represent a fluctuation in the refractive index and thereby provide a mechanism for the strong scattering of incident light.

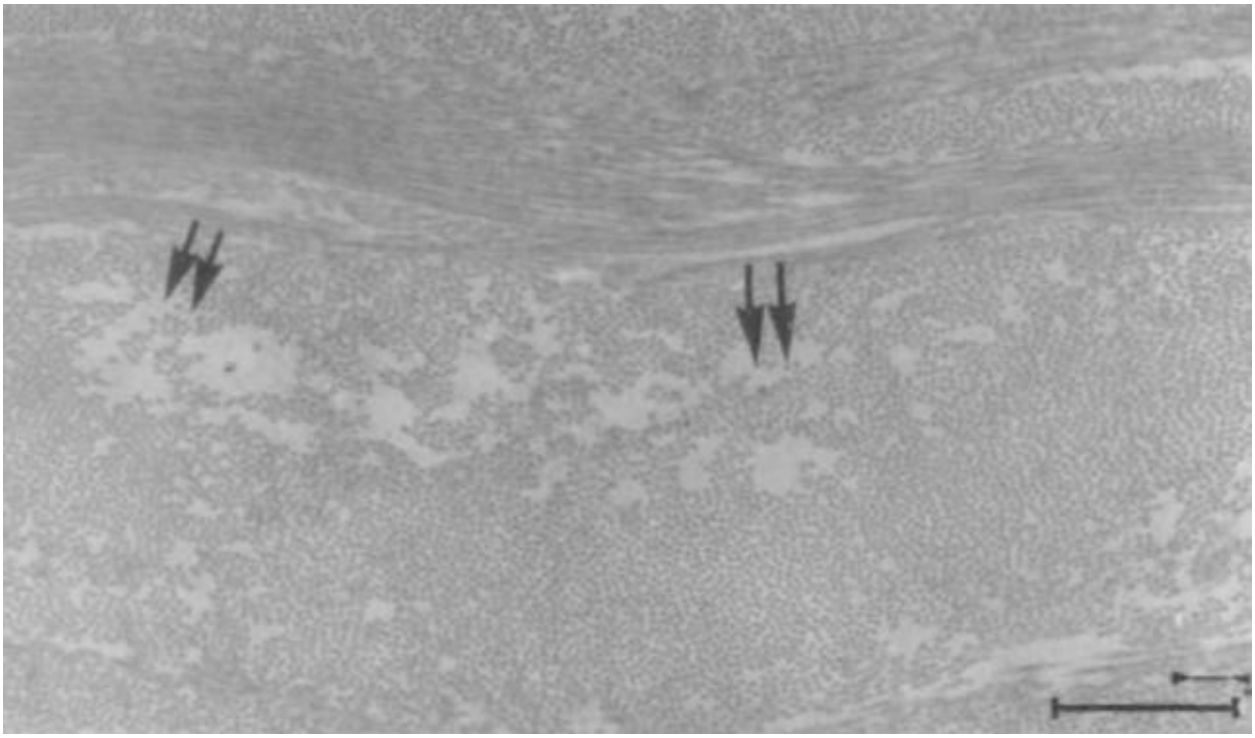

Fig. 2-11: Electron microscopic photograph of a swollen pathologic cornea showing the presence of lakes where no collagen is present. The short scale marker has length 2000 Å. (Taken from Goldman *et al.* 1968.)

It is possible to consider quantitatively the scattering effect of these lakes by making a simple conceptual modification of the analysis presented previously for normal corneal stroma. In Figure 2-12a we plot the index of refraction of the stroma as one moves along some particular direction in the swollen cornea. Here the index of refraction ($n$) shows sharp bumps at the position of each fiber by an amount equal to the difference between the index of the fibers ($n_c$) and the index ($n_i$) of the micropolysaccharide ground substance. Also, of course, there are gaps in this irregular comb-like pattern in the lake regions where the collagen is absent. The scattered electric field produced by the pattern of collagen fibrils shown in Fig. 2-12a can be regarded as originating as the sum of two arrangements of scattering sources represented in Figs. 2-12b and 2-12c. The first arrangement Fig. (2-12b) represents the distribution of fibrils which would be present in the absence of the lakes. The second arrangement, Fig. 2-12c, consists of fibers, each of which has a negative scattering amplitude, located at positions within the lakes where the fibers in 2-12b were placed. The scattered field produced by 2-12b and 2-12c will be the same as that from the actual arrangement exemplified in 2-12a. If we call $E_b$ and $E_c$ the fields scattered from the patterns represented in Figs. 2-12b and 2-12c respectively, we see that $\left\langle \left|E_{\text{tot}}\right|^2 \right\rangle$, i.e. the ensemble average of the square of the scattered field is given by

$$\left\langle \left|E_{\text{tot}}\right|^2 \right\rangle = \left\langle \left|E_b\right|^2 \right\rangle + \left\langle \left|E_c\right|^2 \right\rangle + 2\left\langle \left|E_b E_c\right| \right\rangle . \tag{2.1-53}$$

We can estimate $\left\langle \left|E_{\text{tot}}\right|^2 \right\rangle$ in a simple way if we assume that the size of each lake is smaller than, or of the same order as the light wavelengths. Under these conditions it can be shown (see homework problems) that $\left\langle \left|E_b E_c\right| \right\rangle \cong 0$. Also, from our analysis above we saw that

$$\left\langle \left|E_b\right|^2 \right\rangle = NE_o'^2 \left[1 - \langle\rho\rangle \int f\left(R''\right) e^{i\vec{K}\cdot\vec{R}''} d^2R''\right] \tag{2.1-54}$$

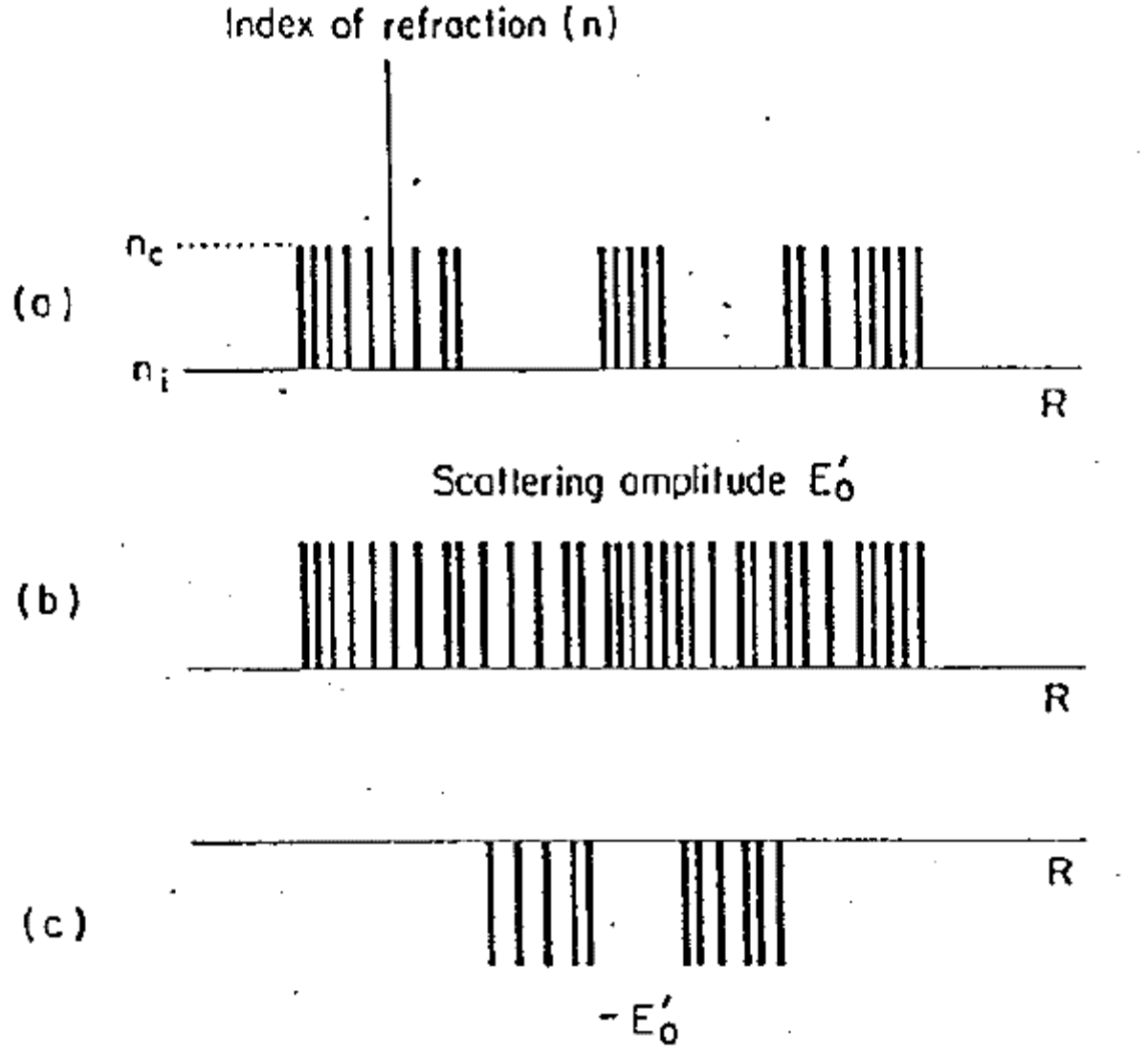


Fig. 2-12: Characterization of the scattering from lakes. In (a) we represent the fluctuation in index of refraction as a function of position in a lamella containing lakes. Each line represents a collagen fiber and the gaps represent the lakes. In (b) and (c) we represent an arrangement of scattering amplitudes which will radiate the same field as would be radiated from the fiber arrangement in (a). In (b) the missing fibers are randomly replaced with the average fiber density in the region of the lakes. In (c) fibers with negative scattering amplitudes cancel the field radiated by the replaced fibers. The field radiated by the sum of the configuration (b) plus (c) is the same as that radiated by the original swollen cornea represented in (a).

where $f(R'')$ and $\langle\rho\rangle$ refer respectively to the exclusion function and mean fiber density in the swollen stroma, in regions where there are no lakes. $N$ is the mean number of fibrils in the illuminated region in the reconstructed case 2-12b. $\left\langle |E_c|^2 \right\rangle$ can be estimated approximately as follows. If the lakes are not too large in comparison with the light wavelength, then the field scattered from the $\alpha^{\text{th}}$ lake is $\left(-N_\alpha E_o'\right)$, where $N_\alpha$ is the number of fibers missing in the lake labeled with index $\alpha$. Since we assume no correlation in the position of the lakes, each radiates independently of the other and hence

$$\left\langle |E_c|^2 \right\rangle = \left\langle \sum_{\alpha=1}^{p} N_\alpha^2 \; E_o'^2 \right\rangle. \tag{2.1-55}$$

Here $p$ represents the total number of lakes in the illuminated region. If we define the mean square value of the number of fibers per lake as $\left\langle N_\alpha^2 \right\rangle$, we have

$$\left\langle N_\alpha^2 \right\rangle = \frac{1}{p} \sum_{\alpha=1}^{p} N_\alpha^2 \,. \tag{2.1-56}$$

Thus

$$\left\langle |E_c|^2 \right\rangle = p \left\langle N_\alpha^2 \right\rangle E_o'^2 \tag{2.1-57}$$

and hence

$$\left\langle |E|_{\text{tot}}^2 \right\rangle = N E_o'^2 \left\{ 1 - \langle\rho\rangle \int f(R'') \; e^{iK\cdot R''} d^2R'' + \frac{p}{N} \left\langle N_\alpha^2 \right\rangle \right\}. \tag{2.1-58}$$

We have previously discussed the first two terms in the braces on the right hand side of this equation. The first represents the effect on the scattering of the individual collagen fibers. The second terms represents the reduction in this scattering produced by interference between particles whose relative positions are correlated. In the swollen cornea there may well be a change in this correlation in position of pairs of collagen fibers. This change could certainly affect the degree of cancellation between the first two terms which normally reduces the intensity of the scattered light. If lakes however are present to the extent shown in Fig. 2-10, then these lakes will play a very important role in the scattering. We can see this quantitatively in the following way. Let us

denote the third term, the term corresponding to the scattering from the lakes as *e*, the opacification factor where:

$$e = \frac{p}{N}\left\langle N_\alpha^2 \right\rangle. \tag{2.1-59a}$$

This can also be written as

$$e = \frac{p\left\langle N_\alpha \right\rangle}{N} \quad \frac{\left\langle N_\alpha^2 \right\rangle}{\left\langle N_\alpha \right\rangle}. \tag{2.1-59b}$$

Our previous analysis shows that if the entire term in curly brackets in Eqn. (2.1-58) is ~ 1, then ~ 90% of the incident light will be scattered and hence the cornea will be opaque. Thus it is clear that if *e* alone is $\approx 1$, then opacity will result. It is now possible to form an estimate of *e* using the information contained in the electron microscope photographs of Fig. 2.1-11. The quantity $p\left\langle N_\alpha \right\rangle / N$ represents the fraction of stroma occupied by the lakes. This fraction is about 0.1 as can be seen from Fig. 2.1-11. Furthermore, the quantity $\left\langle N_\alpha^2 \right\rangle / \left\langle N_\alpha \right\rangle$ in Eqn. (2.1-59b) is crudely equal to the mean number of fibrils missing in the average lake. This quantity is easily between 20 and 40 as can be seen from Fig. 2.1-11. Thus the quantity *e* is roughly between 2 and 4. As mentioned previously, the correlation of the phases of light waves scattered from correlated pairs of collagen particles can result in the first two terms in the braces of (2.1-58) adding to a number small compared to unity. However, in the presence of lakes if the opacification factor *e* approaches or exceeds unity, this produces very strong and even multiple scattering and hence the cornea will be opaque.

In the analysis above we have made the assumption that the size of the lakes is smaller than or comparable to the light wavelength. It is possible to extend the analysis to consider the effect of lakes whose dimensions are significantly larger than the light wavelength, and to generalize the form of *e* so as to include the effect of such large lakes (see Benedek 1971).

It is appropriate to close this discussion of the factors that control the transparency of the cornea by applying our analysis to features of the normal and diseased eye. Surrounding the clear, transparent cornea we find the "white of the eye": the opaque sclera. We can understand the reason for the opacity of the sclera when we recognize that both the diameter of the collagen, and the spacings between collagen fibers are comparable to the wavelength of light. The corresponding spatial fluctuations in index of refraction produce large values for the interference function $I(K)$ and hence strong scattering of light, which corresponds to opacity of the scattering medium.

In the case of corneal disease, it is important to recognize that there are a variety of dietary, environmental, or infectious factors which can damage the corneal epithelium and lead to ulceration of the corneal stroma. Indeed, corneal opacification is a leading cause of blindness, particularly in third world nations. At present the treatment for corneal opacity is a surgical corneal transplant. From a fundamental physico-chemical point of view there are two alternative strategies. One strategy involves a facilitation of the molecular factors that control the spacing of collagen fibers during wound healing so as to reduce fluctuations in collagen fiber spacing which contribute to the pair correlation further. A second strategy is based on the fundamental fact that the scattered light intensity proportional to the difference of the squares of the index of refraction of the collagen $(n_c)$ and the ground substance $(n_i)$. Normally, $n_c$ = 1.55 and $n_i = 1.35$ (D. Maurice, 1969). In principal, it may be possible to introduce into the ground substance a second medium which would reduce the difference $\left(n_c^2 - n_i^2\right)$. If this difference were reduced sufficiently, opaque scar domains should become transparent. To the knowledge of the writer this fundamental strategy for the clarification of the cornea has as not yet been seriously investigated.

## 2.2 The Transparency of the Lens and Cataract Disease

### i) The scattering of light from the normal lens

Having examined the physical basis for the transparency or opacity of the cornea, it is natural to extend our considerations to the eye lens whose molecular and histological features have been previously discussed in Section 1.4 - (iv). Each region of the lens interior consists of densely packed fiber cells whose cytoplasm contains a high concentration of the lens proteins. These are a mixture of three families of proteins: the so-called $\alpha$, $\beta$ and $\gamma$ crystallins. We consider first the physical basis for the transparency of the cytoplasm of these normal fiber cells. Each such cell is, in effect, a highly concentrated aqueous-protein solution. The index of refraction of a typical protein molecule is different from that of the aqueous solvent, and hence will scatter light incident on the solution. If each protein scattered light independently of the others, i.e. if there is no correlation in the relative position of pairs of protein molecules, then the scattered light intensity will be directly proportional to the number concentration of the proteins. Indeed this linear proportionality is the basis of conventional turbidimetric methods used by the biochemist to measure protein concentration in dilute solutions. Already in the early 1960's it was understood that if one extrapolated the behavior of a dilute solution to the high concentrations appropriate to the lens cell cytoplasm, the solution would be quite opaque. This is perfectly analogous to the scattering of light by the protein collagen in the cornea as described previously. Clearly, to understand either the transparency of the cornea or the lens, it became necessary to include the effect of the correlation in the position of pairs of proteins. Just as Maurice in 1957 proposed a perfect lattice to describe the correlation in position of collagen fibers, so in 1962 S. Trokel proposed that the lens proteins were arranged in a very well ordered structure - a paracrystalline state - in which the relative positions of proteins is precisely regular over very large distances (Trokel 1962). In fact, no such long-range correlation is needed theoretically nor is it found experimentally. The scattering of

light, as we have seen quite generally above, is produced by microscopic fluctuations in the index of refraction. These fluctuations must be of such a spatial extent that their Fourier components have a wavelength comparable with or larger than the wavelength of light. In a protein solution this fluctuation is produced by fluctuations in the number density of proteins. In the normal lens, the fluctuations in the number density of protein molecules over dimensions comparable to the light wavelength is small because the proteins are densely packed. The high density of proteins in fact imposes a concomitant short-range correlation in the relative positions of pairs of protein molecules. This short-range correlation, as expressed in the pair correlation function $\rho(R \mid 0)$, is sufficient to produce sufficient destructive interference between waves scattered from the proteins so that little light is scattered out of the incident beam: hence the medium is transparent. This principle was first applied in 1971 to explain the transparency of the normal lens (Benedek 1971). Later, this line of reasoning was confirmed experimentally (Delaye and Tardieu 1983). These authors used small angle x-ray scattering to characterize the turbidity of aqueous solutions of the lens proteins as a function of the protein concentration. At low protein concentration the turbidity was found to increase in linear proportion to the protein concentration. However, when the concentration increases to the point that short-range interprotein repulsion begins to produce a correlation in the positions of near neighbor protein, the turbidity becomes at first independent of and then decreases with protein concentration. Beyond this maximum in turbidity the dense protein solutions become ever more transparent as the concentration increases. These workers also provided a detailed theoretical model for the form of pair correlation function of the proteins using a hard sphere model of the proteins.

### ii) Protein aggregation and lens opacification

Our discussion above has established that the transparency of the lens requires that spatial variations in the index of refraction be small over dimensions comparable to or greater than the wavelength of light. We now consider those physico-chemical changes in the uniform distribution of lens proteins which may be responsible for the loss of lens transparency. Medically the term "cataract" is used to designate a lens which contains cloudy, turbid regions. It can be demonstrated experimentally that even in a "normal" lens, the process of aging results in a continual increase in lens turbidity. Acceleration of the formation of lens inhomogeneities can produce so much scattering of light in various domains of the lens, that those domains become "milky." Such turbid domains can so strongly deform the wave fronts of the incident light that the lens can no longer form a sharp image on the retina. In addition, such turbid domains can serve as strong "glare sources" which can obscure vision in the presence of bright light (Miller and Benedek 1973). When the scattering from the lens becomes so great as to obscure vision, the cataractous lens in effect has blinded the eye. At present, the only remedy for blinding cataract is surgical removal. It is important to recognize that with increasing age, lens cataract becomes increasingly probable. Thus, as the population ages, increasingly great resources must be devoted to the costs of cataract removal. We should also point out that in third world nations the supply of trained ophthalmic surgeons is quite small compared to the incidence of cataract. Even in the developed nations cataract surgery produces a significant percentage of "complications" which compromise vision. Clearly, it is very important to understand the molecular and cellular factors responsible for the scattering of light from the lens. Such knowledge could lead to pharmaceutical agents which can inhibit or block the process of lens opacification.

In 1962 S. Trokel pointed out that aggregates of the lens proteins could have a significant effect on lens transparency. In 1971 Benedek independently undertook a

quantitative analysis of the effect of protein aggregates on the turbidity of the lens. The idea behind that analysis (Benedek 1971) is as follows. Let us suppose that attractive interactions between lens proteins leads to the formation of globular aggregates randomly distributed throughout the lens cell cytoplasm. If these globular aggregates have an index of refraction $n_a$ which differs from the mean index $n_\ell$ of the surrounding protein background, then they will scatter light in proportion to the number of such aggregates. The existence of such aggregates is supported by the fact that biochemical separation of the proteins in cataractous lenses reveals the presence of a significant fraction of insoluble proteins called the "albuminoid fraction."

It is possible to estimate quantitatively the molecular weight of such aggregates. These aggregates play a role in the lens quite analogous to that of the lakes in the cornea. Let us, for convenience, assume that the size of each aggregate is small compared to the light wavelength. Under these conditions the turbidity of the lens cytoplasm is equivalent to the turbidity of an aqueous solution of protein macromolecules. It is well known that the turbidity $\tau$ of such a solution for unpolarized light is given by (McIntyre and Gornick 1964):

$$\tau = 24\pi^3 \ \xi^2 \ N_a \ V_a^2 \ / \lambda^4 . \qquad \text{(2.2-1a)}$$

Here

$$\xi = \frac{n_a^2 - n_\ell^2}{n_a^2 + 2n_\ell^2} \qquad \text{(2.2-1b)}$$

$N_a =$ number of aggregate molecules/cc
$V_a =$ volume of each aggregate molecule
$\lambda =$ wavelength of light.

The turbidity, which has the units of $cm^{-1}$, measures the attenuation of the incident beam of intensity $I_o$ on passing a distance $z$ through the scattering media. If $I_z$ is the intensity of the beam at $z$, then the relationship:

$$I(z) = I_o \; e^{-\tau z} \tag{2.2-2}$$

defines the turbidity $\tau$.

We may express the turbidity $\tau$ in terms of the molecular weight $M_a$ of each aggregate (in units of gms/mole), and the fraction $\zeta$ of the protein mass in the aggregates. If $\rho$ is the average mass density of protein in the lens cytoplasm in gm/cc, then:

$$N_a = \frac{\zeta \rho}{(M_a / N_o)} \tag{2.2-3}$$

where $N_o$ is Avogadro's number. Also the volume $V_a$ of the aggregate can be related to its molecular weight $M_a$ through the partial specific volume $\overline{v}$ for proteins. $\overline{v}$ is the volume of solvent excluded per gram of dispersed solute protein. Thus:

$$V_a = \left( \frac{\overline{v} \, M_a}{N_o} \right). \tag{2.2-4}$$

If we use (2.2-3) and (2.2-4) in Eqn. (2.2-1a) for the turbidity, we find:

$$\tau = \frac{24\pi^3}{\lambda^4} \; \xi^{\,2} (\overline{v})^2 \; \zeta \rho \left( \frac{M_a}{N_o} \right) \tag{2.2-5}$$

The quantity $\zeta \rho$ is the mass density of protein in aggregated form in (gm/cc). For fixed values of $\zeta \rho$, the turbidity is directly proportional to the molecular weight of the aggregates. This direct proportionality between turbidity and molecular weight, for fixed mass density of protein in solution is the basis of the light scattering method for the determination of molecular weight of macromolecules. We may evaluate numerically the relationship between $\tau$ and $M_a$ provided we know $\zeta$, and $\xi$. In the cataractous lens $\zeta$ can become as large as 0.20. If we also use $n_a$ = 1.60 and $n_\ell$ = 1.39 where $n_a$ is a typical value of the index of refraction of pure proteins and $n_\ell$ is the mean index of refraction of the lens, then $\xi \cong 0.10$. Using also $\lambda = 5000 \mathring{A}$, $\overline{v} \cong 0.7 \text{ cc/gm}$, $\rho = 330 \text{ mg/cc}$, we find that

$$\tau = (0.64 \times 10^{-7})\, M_a \qquad \text{cm}^{-1} \tag{2.2-6}$$

where $M_a$ has units of grams per mole. From Eqn. (2.2-6), we see that $M_a$ can be determined if $\tau$, the turbidity, is known. To estimate the turbidity we note that if $z_o$ is the characteristic distance over which the incident beam is attenuated significantly, then $\tau \sim 1/z_o$. Hence

$$M_a = \left(\frac{15.6 \times 10^6}{z_o(\text{in cm})}\right)\left(\frac{\text{gm}}{\text{mole}}\right). \tag{2.2-7}$$

The overall thickness of the lens is ~ 0.5 cm. Thus, $z_o$ must be smaller than this. In the case of cataracts which cloud the nucleus of the lens, since the size of the nucleus region is about one third the thickness of the lens, we may estimate crudely that $z_o$ = (1/6) cm. If we use this rough estimate for $z_o$, we conclude that the molecular weight of the hypothesized protein aggregation is $\sim 90 \times 10^6$ gm/mole.

It is worth noting that at the time at which the reasoning above was first presented (Benedek 1971), there was no experimentally established molecular mechanism for the turbidity of the cataractous lens. The theoretical analysis above had two galvanizing effects on biochemical investigations. First, it proposed that cataractous lenses should contain high molecular weight protein aggregates and second, that the molecular weight of such aggregates could be expected to be in the range of 50 - 100 $\times 10^6$ gm/mole. Such predictions were subject to biochemical testing. Indeed approximately six months after the publication of the theory, Spector *et al.* (1971a, b) showed that large size aggregates do indeed occur and increase with age in the bovine lens. Subsequent investigation of the human lens were conducted to establish experimentally, the fraction of protein in such aggregates as a function of position, age and degree of cataract, and also to determine quantitatively the molecular weight of the aggregates. This was carried out by Jedziniak *et al.* (1973), (1975). These workers found that the concentration of heavy molecular weight aggregates increase strongly with age rising to about 10 - 15% in lenses aged 75 years old and in cataractous lenses. The aggregates

were localized principally in the lens nucleus. In fact 80% of all the aggregates in the aging lens were found in the nucleus. The molecular weight of these aggregates was found to fall in the range 100 - 300 $\times$ $10^6$ gm/mole in good agreement with the theoretical estimate.

In the ensuing years many studies have been made to identify the specific chemical reactions which take place on the protein surface connected with the formation of the aggregates. Indeed a wide range of chemical modifications associated with the aggregates has been reported (Bloemendal 1981, Harding 1991). The observed chemical modifications include oxidation, glycation, racemization, and carbamoylation It is difficult to imagine how to control the variety of metabolic pathways which produce such a broad range of protein modifications. There is, however, an alternative strategy for the inhibition of aggregate formation which will emerge as we examine further evidence relating to non-binding attractive interactions between the lens proteins. Such evidence first began to emerge from measurements of the Brownian movement of the lens proteins in the intact lens which we present in the next section.

## 2.3 Observation of the Brownian Movement of Proteins in the Intact Eye Lens

It is natural to inquire if the protein aggregates found by biochemical analysis of lens extracts can be observed in the whole intact lens. The answer to this question is yes, as was first shown by Tanaka and Benedek (1975) using the method of quasi-elastic light scattering spectroscopy. By this method it is possible to detect and measure quantitatively the Brownian motion, and hence the diffusion coefficient of proteins in aqueous solution or the whole lens. If a laser light beam passes through a protein solution, the intensity of the scattered light will exhibit temporal fluctuations as a result of the Brownian movement of the proteins in the illuminated region. The correlation time of these random fluctuations can be measured quite accurately and hence the diffusion coefficient of the proteins can be determined. According to the famous Stokes-Einstein relation, the diffusion coefficient can be connected with the hydrodynamic radius or the size of the protein. Aggregation of proteins can readily be detected using this method since such aggregates scatter light strongly, and exhibit a considerably reduced Brownian movement in comparison with the unaggregated proteins.

The use of quasielastic light scattering to investigate protein aggregation has led to the development of ophthalmic instruments which can detect and quantitatively characterize the early development of cataract in the living human eye. The findings of Tanaka and Benedek (1975) also revealed a new hitherto unexpected molecular mechanism for lens opacification. We shall presage our discussion of these experiments by providing physical insight into the method of quasi-elastic light scattering (QLS) spectroscopy, which is being widely used to quantitatively detect the self-assembly, association, aggregation and phase separation of a wide range of synthetic and biological macromolecules.

**i) The electric field of light scattered by a solution of macromolecules**

In Figure 2-13 we show the geometric elements associated with the scattering of an incident plane wave of light by solute particles each of which is located at position $\vec{r}_j(t)$, relative to a fixed origin 0 in the solvent. The electric field of the incident wave has the form $E_o \exp i(\vec{k}_o \cdot \vec{r} - \omega_o t)$. The total scattered electric field $E_s(t)$ passing through a small field aperture a distance *R* away from the origin 0, and having wave vector $\vec{k}_s$ is the superposition of the fields scattered by each of the scattering particles whose position at time *t* is $\vec{r}_j(t)$. Each of these particles changes its position as a result of the Brownian movement. The total scattered field $E_s(t)$ passing through a field aperture is given by the superposition of the electric fields scattered by each of the macromolecules in the illuminated region.

$$E_s(t) = \sum_{j=1}^{N} E_j(t) = \sum_{j=1}^{N} E_o' \; e^{i(\vec{K}\cdot\vec{r}_j(t) - \omega_o t)}. \tag{2.3-1}$$

Here:
$$\vec{K} = \vec{k}_o - \vec{k}_s \tag{2.3-2}$$

is the scattering vector. The quantity $\vec{K} \cdot \vec{r}_j \equiv \phi_j(t)$ represents the phase of the outgoing wave scattered by the $j^{\text{th}}$ macromolecule. Equation (2.3-1) is simply the generalization to three dimensions of results we have previously presented in connection with scattering from collagen fibers in the cornea in Section 2.0 above. The scattering vector $\vec{K}$ has length:

$$|K| = 2k_o \sin(\theta/2) \tag{2.3-3}$$

This is illustrated in the inset in Fig. 2-13. $k_o = 2\pi/(\lambda/n)$ where $\lambda$ is the wavelength of the incident light in vacuo and *n* is the index of refraction of the medium. We observe that the phase $\phi_j(t)$ of the scattered wave changes only if the particle moves

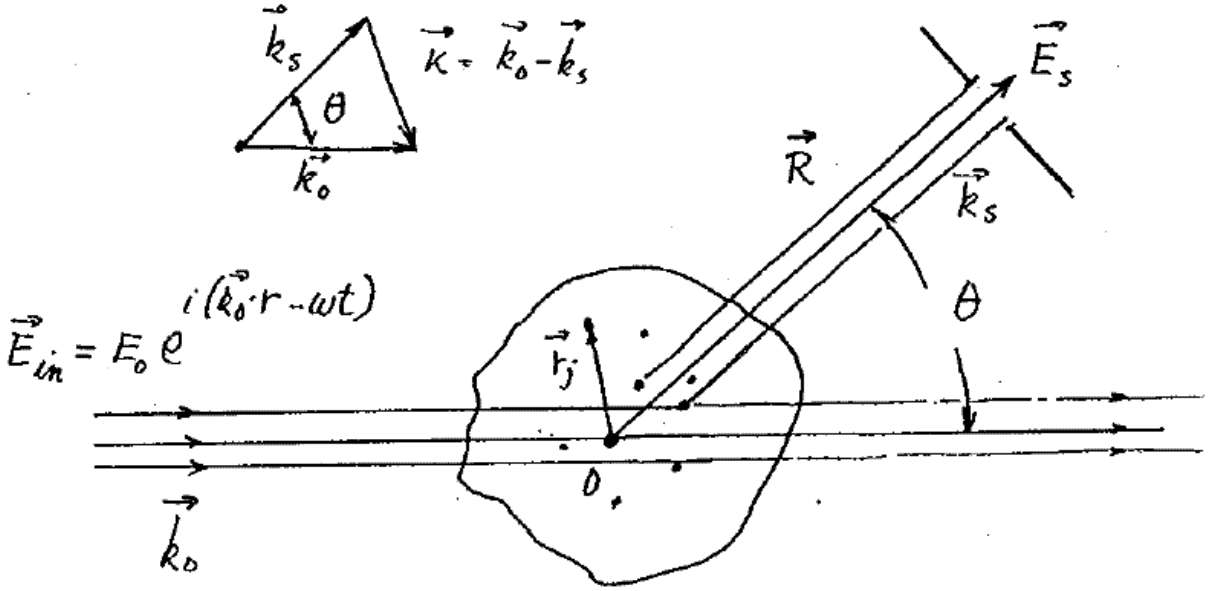


Fig. 2-13: Incident light of wave vector $\vec{k}_o$ is incident upon a medium containing suspended particles whose position $\vec{r}_j(t)$ is a function of time as a result of Brownian motion. The scattered light field $\vec{E}_s$ having wave vector $\vec{k}_o$ is collected by a field aperture.

along the direction of the scattering vector $\vec{K}$. Motion perpendicular to $\vec{K}$ does not affect the phase of the wave. Furthermore, in order that the phase of the wave scattered by a particle change by $2\pi$, that particle must move a net distance $\Delta r_j = (2\pi / K)$ along the direction of $\vec{K}$. The amplitude $E_o'$, of the electric field scattered by a single particle into the field aperture is given by

$$E_o' = E_o \left[ e^{i\vec{k}_s \cdot \vec{R}/|R|} \right] \left( \frac{\omega}{c} \right)^2 (\alpha - \alpha_o) V \sin \Phi . \tag{2.3-4}$$

Here $\Phi$ is the angle between the direction of polarization of $E_o'$ and the direction $\vec{k}_s$ of propagation of the scattered wave. $(\alpha - \alpha_o)$ is the difference between the dipole polarizability of the macromolecule and the solvent, and $V$ is the volume of the scattering particle. In order to see quantitatively the information contained in the scattered electric field, it is convenient to rearrange Eqn. (2.3-1) to the form

$$E_s(t) = \delta E_s(t)\ e^{-i\omega_o t} \tag{2.3-5a}$$

where

$$\delta E_s(t) = E_o' \sum_{j=1}^{N} e^{i\vec{K} \cdot \vec{r}_j(t)} = E_o' I\left(\vec{K}, t\right) \tag{2.3-5b}$$

Thus we see that the scattered electric field consists of a carrier wave oscillating at the incident light frequency. However, the amplitude $\delta E_s(t)$ of this carrier wave shows random fluctuations in time as the scattering particles execute their random Brownian movement. The method of quasi-elastic light scattering spectroscopy consists in determining the characteristic correlation time $\tau$ of these random amplitude fluctuations.

It is possible to estimate this correlation time in a simple manner. At any instant of time the various phase factors in (2.3-5b) will sum up to give some amplitude $\delta E_s$. To estimate $\tau$ we need to find the time required for the phase factors $\phi_j$ to change roughly by a factor $\sim \pi$. In this case, the random movement of each of the particles will

certainly produce a new superposition of phase factors which will give an amplitude $\delta E_s(t+\tau)$ which is essentially uncorrelated with that at time $t$. Since $r_j(t)$ is a random variable with zero mean, so is the phase $\phi_j(t)$. In order that the root mean square fluctuation in $\Delta\phi_j(t)$ be of the order $\sim\pi$, we must have:

$$\left\langle \Delta\phi_j^2 \right\rangle = |K|^2 \left\langle (\Delta r_j^2)_K \right\rangle \sim \pi^2 . \tag{2.3-6}$$

It can be shown that the isotropic random walk of a particle produces a mean square displacement of the particle along any given direction which is linearly proportional to the elapsed time $\tau$ (see, for example, Villars and Benedek 1974), i.e.

$$\left\langle \left(\Delta r_j^2\right)_K \right\rangle = 2D\tau \tag{2.3-7}$$

where $D$ is the diffusion coefficient of the diffusing particles. If we insert this into Eqn. (2.3-6) we find that an approximate connection between $\tau$ and the three dimensional diffusion coefficient $D$ is

$$\left(\frac{1}{\tau}\right) \sim DK^2 . \tag{2.3-8}$$

We shall presently establish accurately the numerical factor connecting $(1/\tau)$ and $DK^2$. At present it suffices to point out that an experimental measurement of the correlation time $\tau$ immediately enables a determination of the macromolecular diffusion coefficient since the scattering vector K is known from the scattering geometry according to Eqn. (2.3-3).

The diffusion coefficient $D$ provides valuable information on the size of the scattering elements by virtue of the famous Stokes-Einstein relation (Einstein 1905) viz.:

$$D = k_B T / 6\pi\eta R_H . \tag{2.3-9}$$

Here $k_B$ is Boltzmann's constant, $\eta$ is the viscosity of the solvent, and $R_H$ is the hydrodynamic radius of the diffusing particle. Thus, a measurement of $D$ provides a

direct measure of the size of the particle and hence permits an essentially non invasive means of detecting the aggregation of protein.

Our basic result for the electric field scattered from an incident light beam of constant amplitude $E_o$ is given in Eqn. (2.3-5a,b). It is useful to re-express this result in such a way that it expresses in a clear way the connections between the scattered field and the fluctuations in particle position. This can be done by a simple extension to three dimensions of the analysis given previously for the cornea in Section 2.1. Let us define an interference function $I(\vec{K})$, appropriate to the three dimensional case now considered, as follows:

$$I(\vec{K},t) = \sum_{j=1}^{N} e^{i\vec{K}\cdot\vec{r}_j(t)} . \tag{2.3-10}$$

Here *N* is the total number of particles in the illuminated region which can scatter light into the collection optics. Let us now designate as $\rho(r,t)$ the local number density of particles in solution viz.:

$$\rho(r,t) = \sum_{J=1}^{N} \delta(r - r_j(t)) . \tag{2.3-11}$$

Here $\delta(r - r_j)$ is the Dirac delta function. If we use Eqn. (2.3-11), we see that the interference function can be expressed as:

$$I(\vec{K},t) = \int_V \rho(r,t)\ e^{i\vec{K}\cdot\vec{r}} d^3r . \tag{2.3-12}$$

If the number density of particles is sufficiently large so that there are many particles in a spatial domain whose size is small compared to (1/*K*), we see that $\rho(r,t)$ can be regarded as a continuous function of the position *r* and time *t*. If we now note that $\rho(r,t)$ can be expressed as the mean particle density $<\rho>$ plus a fluctuation about the density, we write then the continuous density function as

$$\rho(r,t) = <\rho> + \delta\rho(r,t) . \tag{2.3-13}$$

If we substitute (2.3-13) into (2.3-12), we observe that the integral over the mean density $< \rho >$ gives zero provided that the illuminated region (volume V) is large compared to the $(1/K)$: the light wavelength. Then, the interference function $I(K)$ is determined entirely by the fluctuation $\delta\rho(r,t)$ of the number density about its mean value, that is:

$$I(K,t) = \int_V \delta\rho(\vec{r},t)\, e^{i\vec{K}\cdot\vec{r}} d^3r\,. \tag{2.3-14}$$

But the integral on the right hand side of this equation is directly proportional to the instantaneous $K^{\text{th}}$ Fourier amplitude $\delta\rho(K,t)$ of the density fluctuation. Indeed the Fourier amplitudes $\delta\rho(K,t)$ is defined as:

$$\delta\,\rho(K,t) = \frac{1}{(2\pi)^{3/2}} \int_V \delta\,\rho(\vec{r},t)\, e^{i\vec{K}\cdot\vec{r}} d^3r\,. \tag{2.3-15}$$

The spatial variation in the density fluctuation $\delta\rho(r,t)$ is simply a sinusoidal superposition of Fourier amplitudes $\delta\,\rho(K',t)$ in accordance with the equation

$$\delta\,\rho(r,t) = \frac{1}{(2\pi)^{3/2}} \int \delta\,\rho(\vec{K}',t)\, e^{i\vec{K}'\cdot\vec{r}} d^3K'\,. \tag{2.3-16}$$

This result follows directly from Eqn. (2.3-15), if we make use of the fact that:

$$(2\pi)^3\,\delta(K-K') = \int_V e^{i(\vec{K}-\vec{K}')\cdot\vec{r}}\, d^3r\,. \tag{2.3-17}$$

Thus we see that we can write the scattered field $E_s(t)$ in Eqns. (2.3-5a, b) in the alternate form:

$$E_s(t) = \delta\,E_s(t)\, e^{-i\omega_o t} \tag{2.3-18a}$$

where

$$\delta\,E_s = E_o'\,(2\pi)^{3/2}\,\delta\rho(\vec{K},t)\,. \tag{2.3-18b}$$

Thus we see that the field scattered into the collection optics with wave vector $\vec{k}_s$ has an amplitude and a time dependence which is directly proportional to the Fourier

amplitude of that density fluctuation which has wave vector $K = \vec{k}_o - \vec{k}_s$. Physically this corresponds, in the present three dimensional case, to the Bragg reflection, by the scattering fluctuation, of the incident light into the scattering direction, as shown previously for two dimensions in Eqn. (2.1-20). Eqn. (2.3-18) also states that the random temporal fluctuation in the amplitude of the scattered field mirrors exactly the temporal fluctuation in the Fourier amplitude of the scattering fluctuations. The correlation time of these random fluctuations provides a direct measure of the diffusion coefficient of the macromolecules in solution.

**ii) Estimate of the spectrum of the scattered light: Ultra-high resolution spectroscopy using optical mixing**

The amplitude modulation of the scattered electric field implies that the optical spectrum of the scattered field consists of a line centered at angular frequency $\omega_o$, but which has a line width $\Delta\omega \sim (1/\tau)$. We may estimate this line width using our previous estimate that $(1/\tau) \sim DK^2$. If we use $D \sim 10^{-7}$ cm$^2$/sec as applies for example in the case of the protein ovalbumin and a scattering vector $K \sim 2 \times 10^5$ cm$^{-1}$ corresponding to scattering angle ~ 90°, we see that $(1/\tau) \sim 4 \times 10^3$ radians/sec. Thus, if the incident light has frequency $5 \times 10^{14}$ Hz, the spectral width of the scattered light will be ~ 600 Hz. This extremely narrow spectral width signifies that the incident light photons are nearly elastically scattered. Hence the term "quasi-elastic light scattering."

It is clear that to resolve such a narrow spectral line one requires a spectrometer of exceptionally high resolving power. Indeed, to get good resolution of this spectrum, the resolving power $\mathcal{R} \sim (\nu_o / \delta\nu)$ should be greater than $10^{13}$. Grating spectrometers have resolving power $\mathcal{R} \leq 8 \times 10^5$, while the best spherical Faby-Perot spectrometer have $\mathcal{R} < 5 \times 10^7$. Thus the resolving power required is about six orders of magnitude greater than that which is available using the best conventional optical spectrometer.

It is in fact possible to devise a spectrometer with the required ultrahigh resolving power using the techniques of optical mixing spectroscopy (Benedek 1969). The essential feature of this technique is to transfer the spectral information centered on the

optical frequency to a much lower frequency where conventional electrical filters, or electronic autocorrelators can be used to analyze the spectral line shape. One means to accomplish this shift in frequency is to superpose the scattered light with some of the incident light on the surface of a photo multiplier. Because of the photoelectric effect the output photocurrent is proportional to the square of the total electric field on the photo-emissive surface. Thus the photocurrent will contain the spectra of all the difference frequencies between the signal (scattered light) and the local oscillator (incident beam). This is the so-called heterodyne beat method (Cummins, Knable and Yeh 1964). A second means to down-shift the optical frequency is achieved in the so-called "self beating spectrometer" (Ford and Benedek 1965). Here, scattered light alone falls on the surface of the photo tube. The process of "squaring" which occurs on the photo tube surface results in a photocurrent which is proportional to fluctuations in the short term averaged intensity of the scattered light. Thus, the carrier frequency is completely removed insofar as the photocurrent fluctuation are concerned. In effect the photoelectric effect produces all possible pairs of beat notes between the spectral components in the scattered light. As a result, the spectrum of the photocurrent in the "self beat" spectrometer is twice the width of the heterodyne beat spectrometer for Lorentzian spectral line shapes. The terms quasielastic light scattering spectroscopy, or optical mixing spectroscopy used here are but two of the terms introduced in the literature to designate this method of ultimate spectral resolution. Equivalent terms are "intensity fluctuation spectroscopy, dynamic light scattering spectroscopy, and photon correlation spectroscopy."

### iii) The temporal autocorrelation function and the spectrum of the intensity fluctuations in the scattered field

Light incident upon the photo emissive surface of a photomultiplier tube produces a photo current $I(t)$ in direct proportion to the square of the absolute magnitude of the incident light field $\left| E(t) \right|^2$. Thus, as a result of the photoelectric effect the carrier wave frequency in the light wave is in effect removed, and the photocurrent is directly proportional to the intensity of the incident light wave. Fluctuations in photocurrent, therefore, accurately represents the time dependence of the fluctutations in the intensity $I(t) \equiv \beta \left| E(t) \right|^2$ of light incident on the photosurface. Here $\beta$ is the coefficient of proportionality connecting $I$ and $\left| E \right|^2$.

We now seek to examine quantitatively the specific information contained in the random temporal fluctuations in the intensity of the light scattered from a macromolecular solution. According to the theory of stochastic processes, if the random fluctuations are characterized as a Gaussian random process, all the information in the fluctuation is to be found in the so-called temporal autocorrelation function.

In the present case, the random variable is the intensity $I(t)$ which fluctuates in time about some non-zero mean value $\overline{I}$, i.e. $I(t) = \overline{I} + \delta I(t)$. Here $\delta I(t)$, on average, is zero, but it fluctuates randomly in time above and below zero. The information contained in the temporal fluctuations in $I(t)$ or $\delta I(t)$ can be found from the so-called temporal autocorrelation function for $I(t)$ which is defined as:

$$R_I(\tau) = \langle I(t) I(t+\tau) \rangle \qquad \text{2.3-19a}$$

or

$$R_I(\tau) = \langle \delta I(t) \delta I(t+\tau) \rangle + \left( \overline{I} \right)^2 \qquad \text{2.3-19b}$$

Here, the time average $\langle\ \rangle$ is taken over all possible starting timees for the random process. One can envision the meaning of the autocorrelation function $R_{\delta I}(\tau) \equiv \langle \delta I(t)\, \delta I(t+\tau) \rangle$ as follows. Imagine an ensemble of recordings of the temporal fluctuation for $I(t)$, each member of the ensemble corresponding to a different starting time for the random process. Consider one such member of the ensemble. For its time record, one multiplies the value of $I(t)$ by its value at some later time $\tau$. This corresponds to multiplying the time record $\delta I(t)$ by an identical record in which there is a displacement $\tau$ in the time position of the two records. One then averages $\langle\ \rangle$ the product $\delta I(t)\, \delta I(t+\tau)$ over all the members of ensemble. It is clear that for times long compared to some "correlation time $(\tau_c)$ of the temporal fluctuation that the ensemble average $\langle \delta I(t)\, \delta I(t+\tau) \rangle$ will be zero because beyond $\tau_c$ there will on average be no correlation between the fluctuations at $t \text{ and } t+\tau \text{ where } \tau \gg \tau_c$. On the other hand, for $\tau \ll \tau_c$ there will be a great correlation in the values of $\delta I\left(t\right) \text{ and } \delta I\left(t+\tau\right) \text{ and } \left\langle \delta I\left(t\right) \delta I\left(t+\tau\right) \right\rangle \rightarrow \left\langle \delta I^2 \right\rangle$. From the form of the dependence of $R_{\delta I}(\tau)$ on the delay time $\tau$, one can obtain the physically important information on the magnitude of the correlation time $\tau_c$ and the microscopic mechanism for the dynamics of these fluctuations.

In the present context, $I(t) \equiv \beta \left| E(t) \right|^2$. Thus, the autocorrelation function of the scattered light intensity is given by:

$$R_I(\tau) = \beta^2 \left\langle \left| E_S(t) \right|^2 \left| E_S(t+\tau) \right|^2 \right\rangle \qquad \text{2.3-20}$$

This temporal autocorrelation function is also related to the spectrum $S_I(\omega)$ of the intensity fluctuations by the Wiener-Khintchine equation viz.:

$$S_I(\omega) = \int_{-\infty}^{\infty} e^{i\omega\tau} R_I(\tau) d\,\tau \qquad \text{(2.3-21a)}$$

and its converse:

$$R_I(\tau) = \frac{1}{2\pi} \int_{-\infty}^{\infty} S_I(\omega') e^{-i\omega'\tau} d\,\omega'. \qquad \text{(2.3-21b)}$$

From these equations we see that a spectrometer can be designed so as to measure either the spectrum or the autocorrelation function of the photocurrent. The information contained in one is equivalent to that contained in the other. In actual experimental measurements, the autocorrelation function is usually preferred as it reduces considerably the time needed for measurement.

We now can calculate $R_I(\tau)$ in two ways depending upon whether we use Eqn. (2.3-5) or Eqn. (2.3-18) for $E_s(t)$. The former equation looks upon the fluctuation as the random walk of individual particles. The latter equation looks on the fluctuations as being produced by long wavelength fluctuations in a continuous density of solute molecules. It is instructive to calculate $R_I(\tau)$ in both ways. (Dubin, Lunacek, Benedek 1967 and Clark, Lunacek and Benedek 1970).

- Molecular Theory: Brownian Movement

In this approach we use Eqn. (2.3-5) to express $E_s(t)$ and Eqn. (2.3-20) for $R_I(\tau)$. This gives:

$$R_I(\tau) = \left|E_o'\right|^2 \beta^2 \times \left\langle \sum_m \sum_j \sum_k \sum_\ell \exp i\left\{\phi_m(t) - \phi_j(t)\right\} \times \exp i\left[\phi_k(t+\tau) - \phi_\ell(t+\tau)\right] \right\rangle \qquad \text{(2.3-22a)}$$

where the phase factors $\phi_k$ are given by:

$$\phi_k = \vec{K} \cdot \vec{r}_k(t)\,. \qquad \text{(2.3-22b)}$$

The statistical independence of the positions of different particles implies that of all the possible terms in the quadruple sum only the following terms are non zero. There are $N^2$ terms for which $m = j$ and $k = \ell$. Each such term contributes unity to the sum. Also, there are $N^2 - N$ terms for which $m = \ell$, and $j = k$ but $m \neq j$ corresponding to correlation in the phases of an individual particle at two times differing by *t*. There are $N(N-1)$ such terms each having the form

$$\left\langle \exp i\left[\phi_1(t)-\phi_1(t+\tau)\right]\exp -i\left[\phi_2(t)-\phi_2(t+\tau)\right]\right\rangle .$$

Finally, we have terms for which $m=k$ and $j=\ell$ but $m\neq j$ but $m\neq j$. These terms give zero on ensemble averaging over the initial position of all the particles. Thus, if we neglect $N$ in comparison with $N^2$, we find:

$$R_I(\tau)=N^2\left|E_o'\right|^4\beta^2\times\left(1+\left\langle \exp i\left[\phi_1(t)-\phi_1(t+\tau)\right]\exp -i\left[\phi_2(t)-\phi_2(t+\tau)\right]\right\rangle\right). \tag{2.3-23}$$

Since each particle is independent of the others, we may write this as:

$$R_I(\tau)=N^2\left|E_o'\right|^4\beta^2\left(1+\left|\left\langle \exp i(\phi(t)-\phi(t+\tau)\right\rangle\right|^2\right). \tag{2.3-24}$$

We may now determine the autocorrelation of the phases, provided we know the conditional probability distribution $P(\Delta r,\tau\,|\,0,0)$ which is the probability that a particle at $t$ = 0 at position $r$ = 0 will be found at time $\tau$ later at position $r=\Delta r$. It is well-known (see Villars and Benedek 2000 or Wang and Uhlenbeck 1945) that if a particle undergoes an isotropic random walk of step length $L$, with time $T$ between steps, then the diffusion coefficient $D$ of the particle is defined as:

$$D=L^2/6T \tag{2.3-25}$$

and the probability distribution $P(\Delta r,\tau\,|\,0,0)$ is given by

$$P(\Delta r,\tau\,|\,0,0)=\left(4\pi D\tau\right)^{-3/2}\exp(-\Delta r^2/4D\tau). \tag{2.3-26}$$

Since $\left(\phi(t)-\phi(t+\tau)\right)=\vec{K}\cdot\Delta\vec{r}(\tau)$, the correlation in the phase of a wave scattered by a particle at two times differing by $\tau$ is given by:

$$\left\langle \exp i\left(\vec{K}\cdot\Delta\vec{r}(\tau)\right)\right\rangle=\int P(\Delta r,\tau\,|\,0,0)\left[\exp\left(i\vec{K}\cdot\Delta\vec{r}(\tau)\right)\right]d^3\Delta r. \tag{2.3-27}$$

If we use Eqn. (2.3-26) in (2.3-27) and integrate over all the illuminated volume, we find immediately that

$$\left\langle \exp i\left(\vec{K}\cdot\Delta\vec{r}(\tau)\right)\right\rangle = e^{-D|K|^2\tau}. \tag{2.3-28}$$

Thus, the intensity autocorrelation function is given by

$$R_I(\tau) = N^2\left|E_o'\right|^4\beta^2\left(1+\exp-2DK^2\tau\right). \tag{2.3-29}$$

Or since $R_I(0) = N\left|E_0'\right|^4\beta^2$ is the value of the autocorrelation function for $\tau = 0$, we see that normalized autocorrelation function $\left(R_I(\tau)/R_I(0)\right)$ has the form

$$\left(R_I(\tau)/R_I(0)\right) = \left(1+\exp-(\tau/\tau_c)\right). \tag{2.3-30}$$

Thus, the intensity autocorrelation function dies off exponentially with time *t*, with a characteristic correlation time $\tau_c$ given by

$$\left(1/\tau_c\right) = 2DK^2. \tag{2.3-31}$$

Thus, a measurement of the autocorrelation function of the photocurrent gives the diffusion coefficient of the moving particles according to Eqn. (2.3-30, 31). We observe that the intensity fluctuations no longer have any reference to the freqency $(\omega_o/2\pi)$ of the incident light wave. The squaring process which occurs on the surface of the photomuptiplier tube in effect removes the carrier wave and shifts the information contained in the random amplitude modulation down to a much lower frequency domain where its information content can be captured by an electronic autocorrelator or spectrum analyzer.

The spectrum of the intensity fluctuation can be found from the autocorrelation function using the Wiener-Khintchine theorem, Eqn. (2.3-21a). Thus:

$$S_I(\omega) = N^2 \left| E_o' \right|^4 \beta^2 \int \left(1 + \exp(-|\tau|/\tau_c\right) e^{i\omega\tau} d\tau \cdot \qquad \text{(2.3-32a)}$$

Thus,

$$S_I(\omega) = N^2 \left|E_o'\right|^4 \beta^2 \left\{ 2\pi\delta(\omega) + \frac{(2/\tau_c)}{\left(\omega^2 + (1/\tau_c)^2\right)} \right\}. \qquad \text{(2.3-32b)}$$

We observe that the spectrum of the photocurrent contains a delta function part corresponding to the mean d.c. scattered intensity. In addition the photocurrent contains a Lorentzian line whose center is at zero frequency. This Lorentzian line decreases in amplitude such that the width at half height corresponds to a frequency $2\pi(\Delta\nu)_{1/2} = (1/\tau_c) = 2DK^2$. Using these methods of spectroscopy, optical lines whose natural width are ~ 50 Hz or even smaller can be readily resolved.

In the discussion presented above, we have not discussed the fact that fluctuations in the scattered field are spatially correlated over small coherence areas. The field apertures which determine the solid angles of collection of the scattered light are designed, in the interest of optimal signal to noise ratio, to collect only a few such coherence areas. There is no correlation in the fluctuation of the intensity of light scattered from different coherence areas, whose size is determined by the size of the illuminated region (see, for example, Clark, Lunacek and Benedek (1970)).

- Continuum Theory: The diffusion equation

We have seen previously that the scattered light may be regarded as being produced by Fourier components of the fluctuations $\delta\rho(\vec{r},t)$ in the continuous number density of the solute molecules. This is expressed in equation (2.3-18 a,b). If we use the expression for $R_I(\tau)$, we find that

$$R_I(\tau) = \left|E_o\right|^4 \beta^2 (2\pi)^6 \left\langle \left| \delta\rho(K,t) \right|^2 \left| \delta\rho(K,t+\tau) \right|^2 \right\rangle. \qquad \text{(2.3-33)}$$

If the process which gives rise to $\delta\rho$ is a Gaussian random process, then we can relate the correlation function for $(\Delta\rho)^2$ to that of $\Delta\rho$ according to the relation:

$$\left\langle \left|\delta\rho(K,t)\right|^2 \left|\delta\rho(K,t+\tau)\right|^2 \right\rangle = \left\langle \left|\delta\rho(K,t)\right|^2 \right\rangle^2 + \left| \left\langle \delta\rho^*(K,t)\delta\rho(K,t+\tau) \right\rangle \right|^2 . \quad (2.3\text{-}34)$$

The first term on the right hand side of this equation corresponds to the mean intensity of the scattered light. The second term essentially relates the correlation function of $\delta\rho^2$ to the square of the correlation function of $\delta\rho$ (Benedek 1969).

We now must determine the time dependence of the correlation function $R_{\delta\rho}(\tau) = \langle \delta\rho(K,\tau)\delta\rho(K,t+\tau) \rangle$. We can do this using the diffusion equation which governs the space time variation of an initially imposed density fluctuation. The diffusion of independent solute molecules is governed by the famous equation

$$\frac{\partial}{\partial t}\rho(\vec{r},t) = D\nabla^2\rho(\vec{r},t) . \quad (2.3\text{-}35)$$

Since the fluctuation responsible for the light scattering has wave vector $\vec{K}$, we may use this diffusion equation to determine the temporal evolution of a macroscopic sinusoidal spatial fluctuation given by

$$\Delta\rho(r,t) = \Delta\rho(0)e^{i\vec{K}\cdot\vec{r}} f(t) \quad (2.3\text{-}36)$$

where $f(t) = 1$ at $t = 0$. If we use this in Eqn. (2.3-35), we see that

$$\left(\frac{\partial f}{\partial t}\right) = -DK^2 f(t)$$

thus,

$$f(t) = e^{-DK^2 t} . \quad (2.3\text{-}37)$$

Thus, according to the diffusion equation, a sinusoidal density fluctuation will relax to zero exponentially at a rate given by $DK^2$. Physically this relaxation takes place as a result of the random walk of solute molecules on balance preferentially from regions of high concentration to regions of low concentration. What is the connection between the *random* thermal fluctuation in $\delta\rho(K,t)$ and the time dependence of the macroscopic smoothed quantity $\Delta\rho(K,t)=\Delta\rho(K,0)e^{-DK^2t}$? Onsagar (1931 a,b) has concluded in his hypothesis of the regression of fluctuations that the decay of the correlation function of the random variable has the same time dependence as the decay of the macroscopic fluctuation as predicted from the continuum hydrodynamic or diffusion equations (see also Felderhof 1966). Thus we can write:

$$\left\langle \delta\rho^*(K,t)\,\delta\rho(K,t+\tau)\right\rangle = \left\langle \left|\Delta\rho(K,t)\right|^2\right\rangle e^{-DK^2\tau}. \tag{2.3-38}$$

If we use this in the equation for $R_I(\tau)$ viz. (2.3-33), we find:

$$R_I(\tau)=\left|E_o'\right|^2\beta^2(2\pi)^6\left\langle\left|\delta\rho(K,t)\right|^2\right\rangle^2\left(1+\exp-2DK^2\tau\right). \tag{2.3-39}$$

This result has precisely the same form as we obtained previously using the temporal and spatial probability distribution for an ensemble of particles undergoing random Brownian movement. Once again we see, now using a continuum point of view, that the intensity correlation function again has a correlation time $\tau_c$ given by $(1/\tau_c)=2DK^2$.

The continuum method employed above has the advantage that it can be applied to a broad class of thermodynamic fluctuations for which a microscopic probability distribution is not readily available. In a continuous medium, light is scattered by spontaneous thermal fluctuations which produce concomitant changes in the dielectric constant. Examples of such fluctuations are pressure, temperature, density and entropy fluctuation. The correlation time for such fundamental thermodynamic fluctuations can be calculated theoretically using the Navier-Stokes equation or the corresponding

Boltzmann transport equation. Using light scattering spectroscopy it has proven possible to quantitatively measure both the amplitude and the time dependence of these fluctuations which are fundamental in statistical thermodynamics. See for example the following references: Clark (1975), Fleury and Boon (1974), Berne and Pecora (1976), Boon and Yip (1980), and Weiss and Müller (1995).

**iv) Quasielastic light scattering from intact calf and human lenses**

We are now in a position to describe and understand the experiments of Tanaka and Benedek (1975). They used the method of quasielastic light scattering spectroscopy to study the spectrum, or the temporal autocorrelation function of light scattered quasielastically from the proteins diffusing within the lens fiber cells. This enabled the determination of the diffusion coefficient of these proteins as described in the preceding sections. Fig. 2-14 below shows schematically the experimental set up. In these experiments it was possible to vary the temperature of the lens over the range 0°C < $T$ < 50°C.

In the case of the calf lens it had been well known previously that on lowering the lens temperature to the region of 17°C, the turbidity of the nucleus rapidly increases with decreasing temperature, producing an opaque lens nucleus. This opacity can be reversed, indeed, the lens becomes quite clear when the temperature is raised above ~ 17°C. This phenomenon is known as reversible "cold cataract." Prior to the work of Tanaka and Benedek the physico-chemical basis of this phenomenon, which also exists in rat, mouse, and rabbit eyes, was not understood. In Fig. 2-15 we illustrate this temperature induced cataract. The essential clue to the basis of this phenomenon is to be found in the data on the mean diffusivity $\overline{D}$ versus temperature at five different positions within the calf lens (see Fig. 2-16).

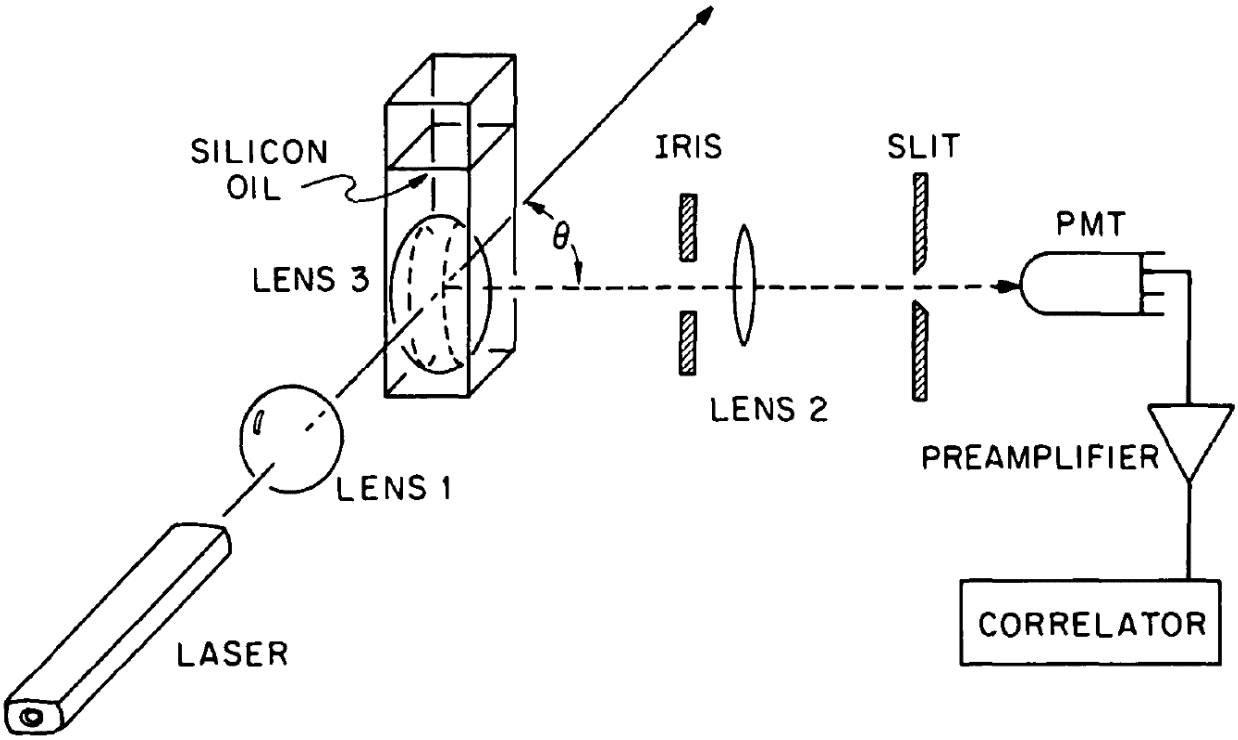


Fig. 2-14: Experimental set up of quasielastic light scattering spectroscopy of the intact lens (lens 3) shows a human or a calf lens

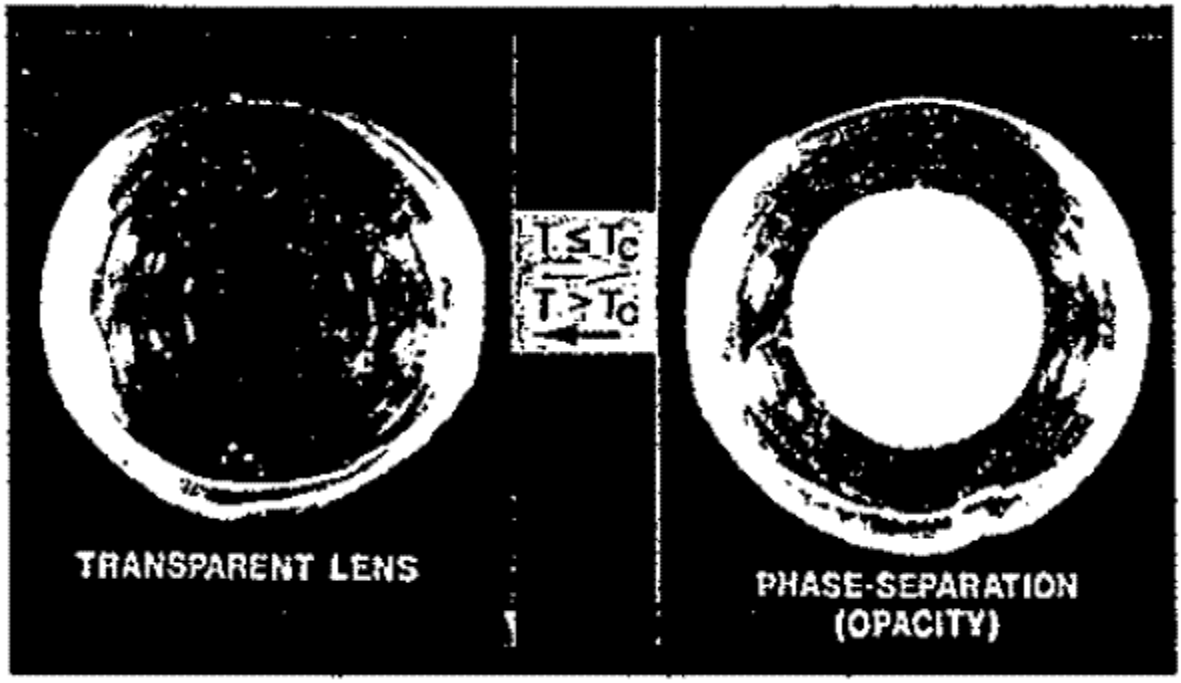


Fig. 2-15: The cold cataract phenomenon. Two photos of the same lens. The one on the left is at temperature $T$ = 25°C. The one on the right is at temperature $T$ = 4°C.

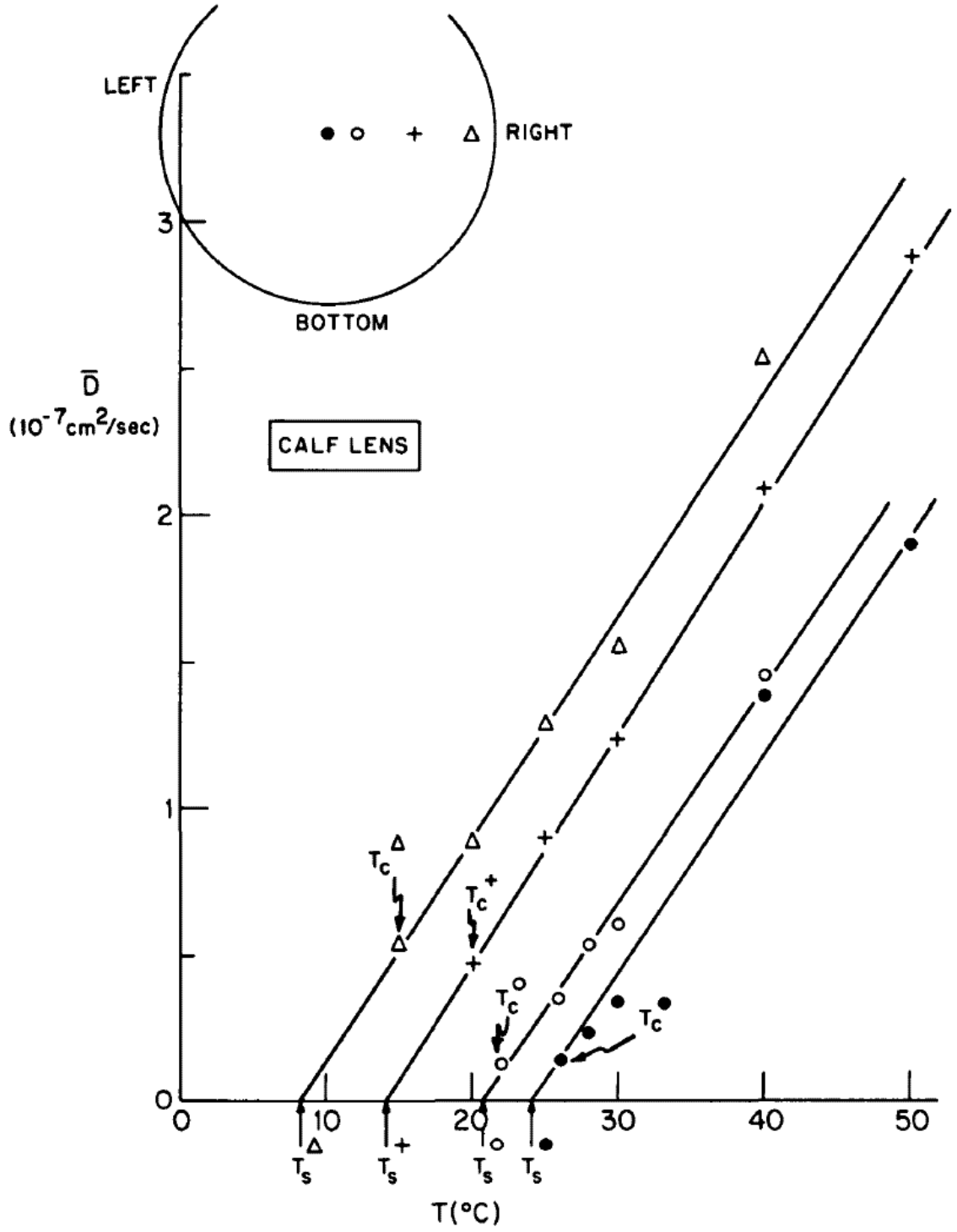


Fig. 2-16: Mean diffusivity $\overline{D}$ of a calf lens as a function of temperature for different positions within the lens.

The mean diffusivity $\overline{D}$ is essentially the average diffusion coefficient of the mixture of lens proteins within the lens cell cytoplasm.

Observe first that the mean diffusion coefficient $\overline{D}$ at 37°C is in the range $1 \times 10^{-7} < \overline{D} < 2.5 \times 10^{-7}$ cm$^2$/sec. This is about the same as that of the *a* crystallin in aqueous solution. This is consistent with the fact that of the three families of lens proteins, $\alpha$, $\beta$, $\gamma$, the $\alpha$ crystallins are by far the largest and therefore are principally responsible for the scattering of the light.

Fig. 2-16 also shows the striking decrease in the diffusivity $\overline{D}$ as the temperature is lowered. At each position in the lens, as the temperature decreases, one observes a certain temperature $T_c$ at which the lens is found to scatter light so strongly that the medium in effect becomes opaque. Also at each position by extrapolation one finds a temperature $T_s$ at which this diffusion coefficient would become zero. In Figure 2-17 we plot $T_c$ and $T_s$ as a function of position from the center to the edge of the lens. The results shown in Fig. 2-17 can be understood if we remember that the protein concentration in the calf lens varies as a function of position. The protein concentration in the center is ~ 40% while that at the periphery is ~ 27%. Thus, by changing the position in the lens we are in effect changing the protein concentration in the solution under examination. The temperature $T_c$ corresponds physically to the "cloud temperature" at which the mixture undergoes separation into coexisting phases. Indeed, the $T_c$ versus position curve corresponds to the ascending limb of the coexistence curve describing the boundary for liquid-liquid phase separation of the mixture. We see then that in these young calf lenses the phenomenon of cold cataract corresponds physically to the spontaneous, reversible separation of cytoplasmic proteins into coexisting protein-poor and protein-rich phases. The strong scattering of light which occurs as the temperature is lowered below $T_c$ results from the formation of droplets of condensed proteins in the cytoplasm.

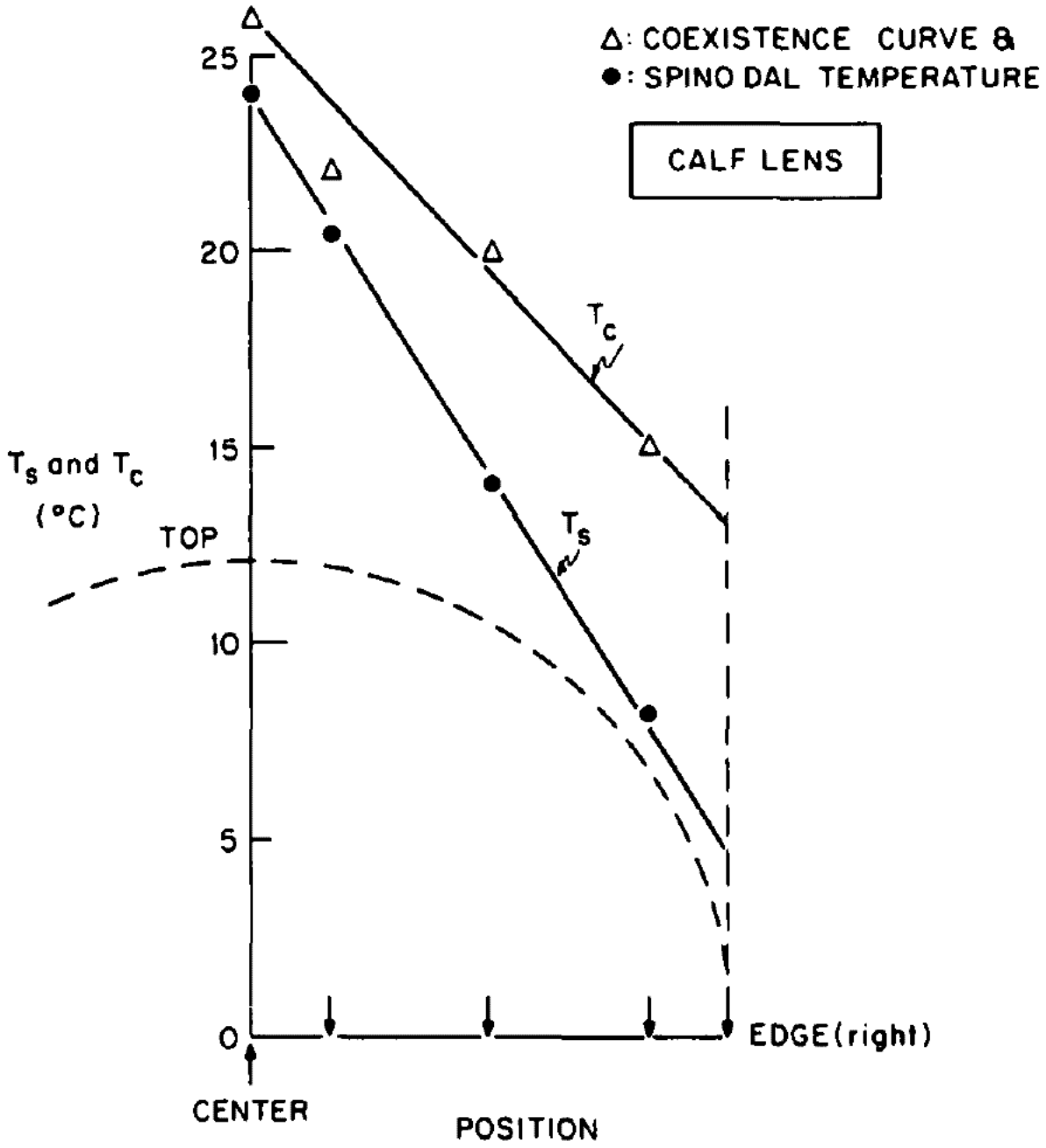


Fig. 2-17: Phase separation temperature $T_c$, and spinodal temperature $T_s$ as a function of the position in the calf lens.

Increased light scattering associated with crossing the coexistence curve is a well-known phenomenon in binary mixtures, which we discussed previously in the thermodynamics of mixtures in Chapter 1.

We now discuss the meaning of the temperature $T_s$ which lies beneath the phase separation temperature. It is known that in mixtures there is a so-called spinodal line representing the positions at which the osmotic compressibility $(\partial c / \partial \pi)$ diverges. This spinodal line denotes the limit of supercooling of the mixture. Since the diffusivity in general can be shown to be inversely proportional to the compressibility, we expect $\overline{D}$ to become zero along the spinodal line. The existence of both the coexistence curve and spinodal line are distinct hallmarks of liquid-liquid phase separation.

The diffusivity $\overline{D}$ was also studied for both the normal human lens, and a cataractous lens. In both normal and cataractous lenses $\overline{D}$ was found essentially independent of position implying greater spatial conformity of the protein concentration in the human lens as compared to the calf lens. At 37 °C the diffusivity $\overline{D}$ in the cataractous lens was found to be 5.5 times smaller than that in the normal lens. This signifies the presence of aggregates which, if globular, would have a hydrodynamic radius 5.5 larger than the unaggregated *a* crystallin. The molecular weight of such an aggregate is about $500 \times 10^6$ Daltons at 37°C. This is comparable to the value of $\sim 200 \times 10^6$ Daltons found as the result of biochemical separation of the heavy molecular weight aggregates. Thus, these *ex-vivo* experiments demonstrate that using the non-invasive methods of quasielastic light scattering spectroscopy it is possible to detect the presence and size of the aggregates responsible for opacification of the lens. More recent developments (Thurston *et al.* 1996 and Benedek *et al.* 1987) have shown that it is possible to develop instrumentation which can safely and accurately measure the development of the high molecular weight aggregates in the living human lens. This form of ophthalmic instrumentation has several useful

applications: 1) It can monitor quantitatively the earliest stages of cataractogenesis long before obvious opacification can be observed in the conventional slit lamp microscope. 2) It can be used to assess the effectiveness of putative anti-cataract drugs as a function of the mode of administration and the dose . 3) It can be used in epidemiological studies to evaluate the role of environmental factors and diet on the incidence of cataract in large populations.

The Tanaka - Benedek paper also showed clearly a second mechanism for lens opacification. Such opacification could be produced by the separation of the lens proteins into protein-rich and protein-poor domains. Subsequent studies by Tanaka and his co-workers (Tanaka *et al.* 1977, Tanaka *et al.* 1983) showed that this mechanism does in fact apply for a variety of animal cataract model systems. In these systems the cataractogenic insult has the effect of altering the net interprotein interaction energy so that the critical temperature rises above body temperature. Thus, the cytoplasmic proteins phase separate into protein-rich and poor domains and opacity results. This mechanism along with high molecular weight aggregate formation was also shown to be present in the case of cataract induced by x-irradiation (Clark, Giblin, Reddy and Benedek 1982).