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ABSTRACT

A new fiber optic probe is developed to study different parts of the eye. The probe positioned in front of an eye, delivers a low power light from a laser diode into the eye and guides the light which is back scattered by different components (aqueous humor, lens, and vitreous humor) of the eye through a receiving optical fiber to a photo detector. The probe provides rapid determination of macromolecular diffusivities and their respective size distributions in the eye lens and the gel-like material in the vitreous humor. We report α -crystalline size distributions, as a function of penetration depth, inside the lens and hyaluronic acid molecular size distribution in the vitreous body. In a clinical setting, the probe can be mounted on a slit-lamp apparatus simply by using a H-ruby lens holder. The capability of detecting cataracts, both nuclear and peripheral, in their early stages of formation, in a non invasive and quantitative fashion, has the potential in patient monitoring and in developing and testing new drugs or diet therapies to "dissolve" or slow down the cataract formation before surgery is necessary. The ability to detect biochemical and macromolecular changes in the vitreous structure can be very useful in identifying certain diseases of the posterior chamber, e.g., posterior vitreous detachment.

1. INTRODUCTION

The motivation for present study is two fold. First, to help understand how cataracts are formed and what makes them grow? Second, to help unravel the mysteries of how the vitreous is composed and what role it plays in normal physiology of the eye? One must, therefore, in order to achieve these goals, develop a mean to diagnose different regions of the eye non-invasively and quantitatively.

Cataracts remain the major cause of blindness affecting about 50 million people each year worldwide. It is estimated that over \$5 billion will be spent this year in treating cataract patients in the United States alone¹. There is no medical treatment to prevent or halt the progression of a cataract; nor is there any way to reverse a cataract once it has formed. The only known treatment is surgical. However, a medical treatment could be possible if we understand how a cataract forms and what makes it grow¹. In order to find a medical treatment for cataracts, first, we must be able to detect a growing cataract in its early stages of formation. The ability of such a detection will be useful in patient monitoring and in the development and testing of possible "anticataract" drugs or "diet" therapies. The National Eye Institute (NEI) of the National Institutes of Health (NIH) in the USA predicts that by the year 2000 new drugs will slow the progression of cataracts which is the leading cause of blindness worldwide². Most recently Sardi³ has strongly argued in favor of using multivitamins, antioxidants, and nutritional supplements in eradicating cataracts. We expect our approach to be helpful in screening the efficacy of such methods.

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The technique of dynamic light scattering (DLS) or quasi-elastic light scattering (QELS) was first applied to the study of cataractogenesis in the pioneering work of Tanaka and Benedek⁴. An extensive review of using QELS to study cataracts has been given by Bursell et al⁵. DLS/QELS being non-invasive and quantitative, seem to hold promising potential in its use as a routine ophthalmic device. But, its commercial scope as an ophthalmic diagnostic tool in clinical settings has remained limited. This is because of elaborate instrumentation, bulk optics and associated optical alignment problems, statistical errors in data analysis, multiple scattering problems associated with mild and severe cataracts, the polydisperse nature of the cataract itself, and the patient radiation exposure levels.

In the past few years DLS instrumentation has embraced several new and innovative technological advances. The immediate impact of these have resulted in the miniaturization of DLS instrumentation⁶. Recently a lens-less back-scatter fiber optic probe was developed to study concentrated particulate dispersions by Dhadwal et al.⁷ Subsequently, Ansari et al⁸. and Dhadwal et al⁹. have shown the utility of their probe to the studies of cataractogenesis in excised bovine and cadaver eye lenses, and in live animals¹⁰. Unfortunately these preliminary studies have certain limitations and pose severe constraints in probing different parts of the eye. One of the major limitation of the lens-less probe is its close proximity (almost touching) to the corneal surface. This is because of a large scattering volume and short penetration depth into the sample (see figure 3b of reference 7). Since the effective depth of the scattering volume is ~ 5 mm and the maximum thickness (anterior-posteriorly diameter)¹¹ of an adult human lens is ~ 5 mm, it is very difficult to accurately pinpoint the desired location in the scattering volume as the entire lens is illuminated by an expanding incident laser light. Our experimental data also shows that when the probe is moved away from the sample/air interface beyond ~ 2.5 mm, the effect of backreflection causes a significant amount of distortion in the collected scattered light. This backreflection results in increasing the photon count rate and in reducing the spatial coherence (β) values. Because of these limitations, in-vivo measurements can only be performed in the front part (anterior cortex) of the lens. The lens nucleus, posterior cortex, and the vitreous body cannot be probed or accessed reliably by the lens-less probe. Therefore, because of these limitations, one cannot use the lens-less probe to utilize the full potential of DLS as an eye diagnostics device.

1.1. Why this study?

The newly developed DLS probe alleviates some of the major concerns as an ophthalmic diagnostics device and is much superior in performance when compared with the earlier reported work⁸⁻¹⁰. We have shown in paper number 2629-23 of this proceedings that the new probe out performs the older (lens-less) probe⁷ by providing accurate size determination of variety of colloidal dispersions in 5 seconds at extremely low laser power levels. Furthermore, the major problem of probing different parts of the eye has been solved. Since the penetration depth ("focusing distance") in our new probe can be changed anywhere from 0 to 17 mm, we can now easily study the anterior chamber, the lens, and the posterior chamber of the eye without the possibility of touching any part of the eye. In the present study we have employed 100% solid state technology for both launching the laser light from a laser diode into the eye and detecting the scattered light signal using a highly sensitive avalanche photodiode (APD) detector. When combined with extremely small scattering volume ($\sim 2 \times 10^{-5} \text{ mm}^3$) offered by the new probe, it has resulted in significantly reducing laser power levels, and thus enhancing the safety of our method. The most widely used clinical apparatus for visual inspection of the eye is a slit-lamp microscope. This apparatus, however, lacks the sensitivity and accuracy to detect small cellular and biochemical changes¹² in the eye. Thus it cannot be used to detect subtle changes in the anterior chamber (aqueous humor), lens (nuclear and peripheral cataractogenesis) or the molecular changes in the posterior chamber (vitreous humor) of the eye. It is, therefore, our desire to help characterize these parts of the eye non-invasively and quantitatively. In this paper we apply this new probe to show its feasibility and safe use on live laboratory animals as a precursor to a detailed on-going planned tests on animal models and human patients in a clinical environment.

1.2. Physical and Biochemical Characteristics of the Eye

A flow diagram, shown in Figure 1, represents major components and their compositions in a human eye. Since α -crystallines are the largest molecules, they are the strong scatterers of laser radiation in a DLS experiment. When these protein molecules are agglomerated, they give rise to lens opacities. This has been shown by earlier experiments using QELS⁸⁻¹⁹. The lens gradually becomes cloudy hindering the light transmission and the ability to focus a sharp image on the retina at the back of the eye. A review on lens is given by Worgul²⁰ and the subject of cataracts is covered by Datiles²¹, and Leske and Sperduto²². The vitreous humor is the largest structure within the eye. It occupies ~80% of the total volume in an eye ball and consists of hyaluronic acid molecules, collagen fibrils, and water. It transmits 90% of the light of wavelength 670 nm used in the experiments reported in this paper. However, the vitreous composition, morphology, and function and its role in disease is the least understood than any other part of the eye. The subject of vitreous is covered in detail in an excellent book by Sebag²³.

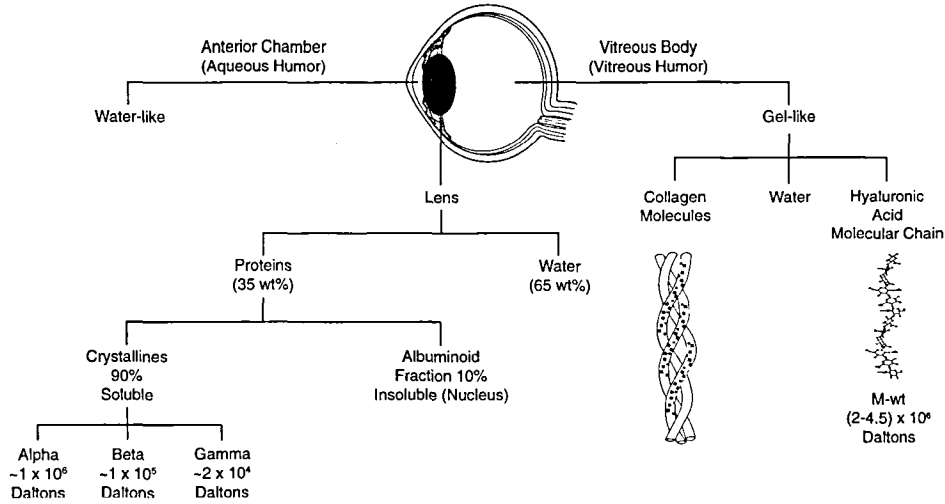


Figure 1.—Eye Composition Schematic

2. DATA ANALYSIS

In our experiments, visible light of 670 nm wavelength from a laser diode is focused into a small scattering volume ($\sim 2 \times 10^{-5} \text{ mm}^3$ diameter) inside the eye. The detected signal is processed via a digital correlator to yield a time autocorrelation function (TCF). For dilute dispersions of spherical particles the slope of the autocorrelation function provides a quick and accurate determination of the particle's translation diffusion coefficient, which can be related to its size via a Stokes-Einstein equation, provided the viscosity of the suspending fluid, its temperature, and its refractive index are known. For concentrated suspensions and for dispersions containing more than one scattering species in the suspending medium, however, the data analysis and interpretation of the autocorrelation function becomes more difficult.

The self-beating experiments reported here measure a normalized intensity-intensity temporal autocorrelation function $g^2(\tau)$ which is related to the normalized electric field temporal autocorrelation function of the fluctuations in the scattered light amplitude $g^1(\tau)$ via the Siegert relation²⁴,

$$g^2(\tau) = A[1 + \beta |g^1(\tau)|^2] \quad (1)$$

where $A = \langle i \rangle^2$ is the average d.c photocurrent or the baseline of the autocorrelation function, and β ($0 < \beta < 1$) is an empirical experimental constant and is a measure of the spatial coherence of the scattering geometry of the collection optics which can be related to signal-to-noise (S/N) and hence considered a measure of the efficiency of a DLS spectrometer. The effect of the number of coherence areas on the character of the fluctuations is given by Ford (Figure 4, page 41)²⁵.

Earlier studies of protein diffusivity in eye lenses using QELS by Benedek et al.²⁶ has established the presence of two decay constants. Equation 1 can be written in terms of a fast and a slowly decaying component due to alpha crystalline and its larger aggregates diffusion respectively,

$$g^1(\tau) = I_\alpha \exp(-D_\alpha q^2 \tau) + I_{\alpha A} \exp(-D_{\alpha A} q^2 \tau) \quad (2)$$

where I_α and $I_{\alpha A}$, D_α and $D_{\alpha A}$ are the scattering strengths, and corresponding translational diffusion coefficients due to small α crystallines and their larger aggregates in an eye lens respectively and q is the magnitude of the scattering vector. The Stokes-Einstein relation²⁵ together with the knowledge of the scattering strengths from each size species leads to a particle size distribution. Similar reasoning can be applied to the vitreous humor comprising two components (hyaluronic acid chains and collagen molecules) dispersed in water. For the rabbit eye system a value for the viscosity ($\eta=1.331$ centipoise) and a refractive index of 1.333 at a body temperature of approximately 40°C was used to extract macromolecular sizes. The autocorrelation functions of the animal eye lenses were analyzed using the commercial data inversion routines supplied by the Brookhaven Instruments Corporation (Holtsville, NY). These include a double exponential²⁷ and a CONTIN²⁸ program. The molecular weight of the scattering species can be estimated using the following formula²⁹.

$$M = \rho(4/3)\pi r^3 N_A \quad (3)$$

where ρ is the density of α -crystallines assumed by Libondi et al.³⁰ to be 1.50 gm/cm³, r is the radius of the scattering species calculated from the diffusion data using equation 1, and $N_A = 6.02 \times 10^{23}$ molecules/gm-mole is the Avagadro's number.

3. INSTRUMENTATION

3.1. Design considerations

One of the most important criteria in a DLS or QELS experiment is the sensitivity of its detection optics. This is related to a coherence area (A_c) inside the scattering volume or the number of coherence areas (N_c) detected by the detector. Ford²⁵ has shown that the smaller the N_c , the greater the signal-to-noise ratio (or the spatial coherence β). The conventional DLS instrumentation relies upon lenses and apertures in their launch and detection optics to control the coherence conditions. A greater coherence area can be attained by focusing the incident laser beam into a sample as tightly as possible. Unfortunately, in such systems this arrangement also contributes to optical alignment problems.

The optical alignment problems have been circumvented in the design of a lens-less fiber optic probe⁷. Unfortunately, the lens-less probe is severely limited in controlling the coherence conditions. This is because a laser beam emanated at the tip of the lens-less probe has an expanding Gaussian beam profile illuminating a large cross sectional area ($\sim 5 \text{ mm}^2$) inside the scattering volume. According to Brown³¹, the use of monomode optical fibers in DLS measurements do not have any detrimental effects in altering the coherence conditions at the detector. In this way the optical fibers act as wave guides or light pipes. Hence the only controlling factor is the sizes of the laser beam and the scattering volume inside a sample. In designing our probe we made use of the basic principles of conventional DLS systems by incorporating two micro gradient index (GRIN) lenses individually onto two separate monomode optical fibers in such a way that a well defined focused beam illuminates a very narrow scattering volume $\sim 2 \times 10^{-5} \text{ mm}^3$. Using Ford's²⁵ equations, we calculated A_c and N_c for the two systems. These are reported in Table 1.

TABLE 1. Coherence parameters

SYSTEM	R (mm)	a (mm)	$A_c (\text{mm}^2)$	$A_{\text{det}} (\text{mm}^2)$	N_c
Old (lens-less) probe (Dhadwal et al.) ⁷	6.5	0.7	1.232×10^{-5}	9.08×10^{-6}	0.74
New (two-lens probe (this paper)	22	0.025	0.111	9.08×10^{-6}	8.21×10^{-5}

$A_c = \lambda^2 R^2 / \pi a^2$, where A_c is the coherence area, $\lambda = 670 \text{ nm}$ is the wavelength of the laser diode, R is the distance between the scattering volume and the detector, and a is the beam radius. The detector area, $A_{\text{det}} = \pi b^2$, $b = 1.7 \text{ }\mu\text{m}$ being the radius of the monomode optical fiber (detector/receiver).

In our approach, the coherence conditions are improved by almost four orders of magnitude. This improvement is reflected in higher spatial coherence (β) values. This can be seen in Figure 2 where DLS measurements were performed on a sample of aqueous dispersion (0.1 wt.%) of 52 nm diameter polystyrene latex spheres. We employed both a He-Ne gas laser (Aerotech LSR5R) and a laser diode (Toshiba TOLD 9215) in these measurements. A avalanche photo-diode (APD) module (PCS-2, EG&G Canada) was used to detect the scattered light from the scattering particles. In order to provide a fair comparison between the older (lens-less) probe and our new probe, we kept the scattered count rate consistent (~ 300 -600 Kc/s) by setting the output power at 1.0 mW and 0.01 mW for the lens-less probe and the new probe respectively. The new probe's photon detection efficiency is significantly higher. We can also see that gas lasers have certain advantage in producing higher β values. As shown in Figure 2, there is a gain in the spatial coherence (β) values when a gas laser is used. This is no surprise because gas lasers have larger coherence lengths compared with laser diodes. But by achieving a good control of the scattering volume and hence the coherence area, our new probe gives excellent β values with both a semiconductor laser and a gas laser. Using a laser diode, a β value of 0.75 for the new probe is significantly higher. It is six times greater than the β value obtained by the lens-less probe under identical experimental conditions.

In the new (two lens) probe a penetration depth of 0-17 mm in the sample can be achieved by simply changing the position of the probe in front of the sample. In contrast, the older (lensless) probe does not have this capability. The older probe has a penetration depth of only $\sim 2 \text{ mm}$. In the older (lens-less) probe design it is difficult to pin point with accuracy the location of the scattering volume because a large cross-sectional area of 5.5 mm^2 in the sample volume is illuminated. We show, in Figure 3, the effects of bringing the older (lens-less) probe up to 3 mm away from the sample. When the older probe is positioned around $\sim 2 \text{ mm}$, a sharp increase in the scattered light occurs due to back-reflection. This mixing of the reflected light from the cuvette surface with the scattered signal from the sample results in reducing the β values and in generating erroneous particle size measurements. The experimental findings are tabulated in Table 2.

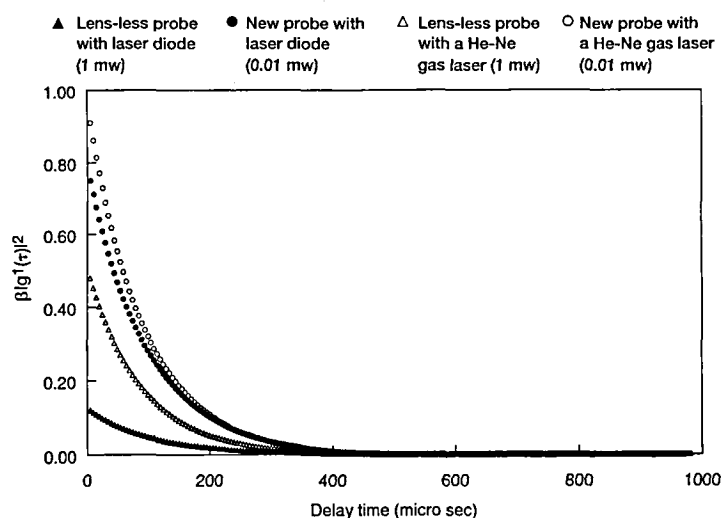


Figure 2.—Performance comparison of two probes.

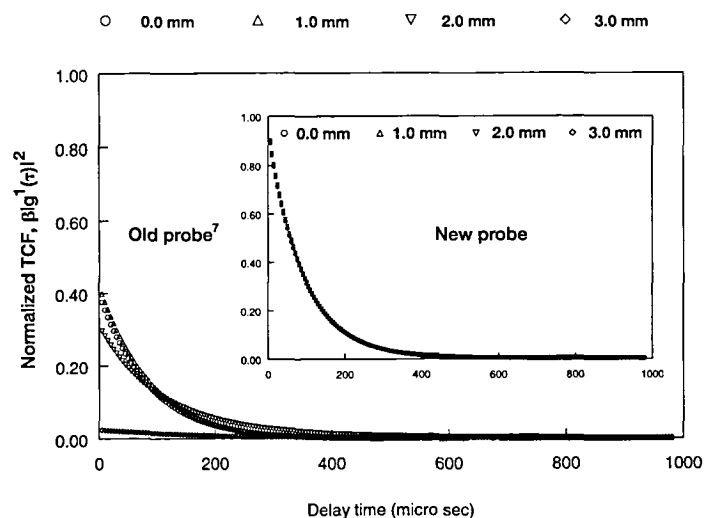


Figure 3.—Effects of penetration depth up to 3 mm. The new probe is shown in the inset. Note significantly higher β values.

TABLE 2. Effect of changing penetration depth up to 3 mm: DLS results using 52nm polystyrene standards (0.1 wt%) aqueous dispersion. The new probe makes reliable and accurate-size measurements up to a penetration depth of 17 mm.

Penetration Depth (mm)	Older (lens-less) Probe (Laser power used 1 mW)				New (two-lens) Probe (Laser power used 0.01mW)			
	Count Rate (Kc/s)	Spatial coh. β	Particle Diameter (nm)	Poly-disper-sity	Count Rate (Kc/s)	Spatial coh. β	Particle Diameter (nm)	Poly-disper-sity
0.0	561	0.38	54.6	0.005	323	0.89	56.7	0.020
0.5	543	0.40	55.7	0.005	339	0.89	56.3	0.044
0.1	557	0.40	55.8	0.005	344	0.89	56.8	0.037
1.5	583	0.41	59.9	0.005	368	0.89	55.5	0.063
2.0	778	0.30	67.5	0.190	356	0.90	55.9	0.087
2.5	1231	0.22	85.3	0.200	374	0.89	54.9	0.103
3.0	7358	0.02	98.8	0.245	394	0.90	56.1	0.071

3.2. Experimental Set up:

The laser light out of a laser/detector module is transmitted by a compact backscatter fiber optic probe to the eye. Depending upon the position of the scattering volume, Brownian motion of the particles in the aqueous humor, protein crystallines inside the lens and the macromolecules in the vitreous humor are monitored. The scattered signal goes back to the laser/detector module through the probe where it is amplified, discriminated, and converted into TTL logic pulses.

A fiber optic probe comprising two monomode optical fibers and two GRIN micro lenses, as illustrated in Figure 4, provides a compact and remote means of studying the dynamical characteristics of the macromolecules in the eye. A 13 mm diameter fiber optic probe contains the necessary optics to perform DLS measurements at a scattering angle of 161.5° . The probe is non-invasive and is conveniently positioned in front of the eye (cornea), but having no physical contact with any part of the eye. Two monomode optical fibers, each housed in a stainless steel ferrule, are mounted into a separate stainless steel housing. An air gap (0-0.5 mm) is intentionally left between the fiber housing and the lens housing in order to produce a tightly focused spot in the scattering volume. The two optical fibers in their housings are aligned and fixed into position off-axis with the GRIN lens. The two housings are placed inside a third (outer) housing made of stainless steel, and the back end of the housing is covered with a heat-shrink tubing. The two free ends of the optical fibers were terminated with FC/PC-type male connectors for easy mating with the laser/detector module.

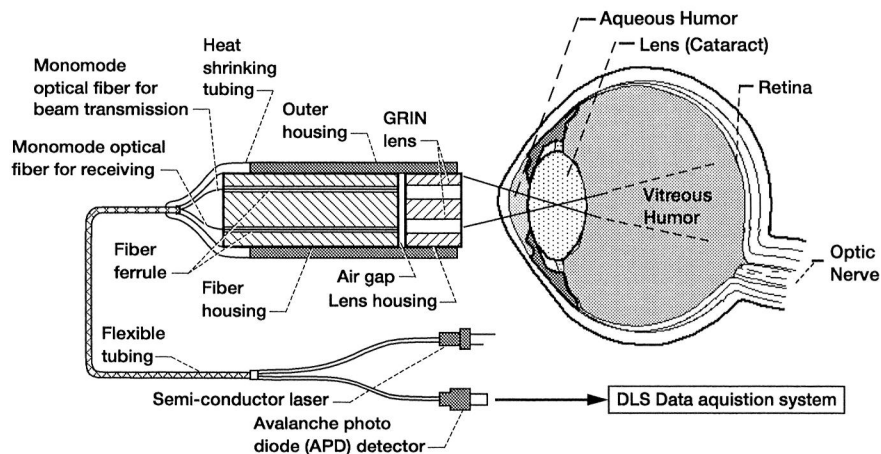


Figure 4.—New DLS fiber optic probe design.

For in-vivo measurements, the probe as shown in Figure 5 was mounted on a V-clamp of a manipulator arm of a stereotaxic device. The position of the probe can be easily controlled by a micrometer stage of the manipulator arm. This arrangement provides precise positioning and location of the scattering volume in any substantially transparent region of the of the entire eye.

The in-vivo rabbit studies were conducted in accordance with the U.S. Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the institutional review board of the Drexel University for animal studies. Animals were sedated with Ketamine (5 mg/Kg) and Xylazine (1 mg/Kg). The eyes were dilated by the instillation of topical anesthetic (tetracaine) and followed by a mydriatic agent (1% Mydracyl). Upon full dilation, the corneal surfaces were observed as well as the posterior fundi using a Welch Allen direct ophthalmoscope. Before directing the fiber optic probe to a rabbit eye, an additional drop of anesthetic was applied. After the measurements, the corneal surfaces and the fundi were then re-examined. No significant differences from the initial and terminal examinations were found. Antibiotic ointment was then placed in the inferior cul-de-sacs and the animals were returned to their cages for recovery.

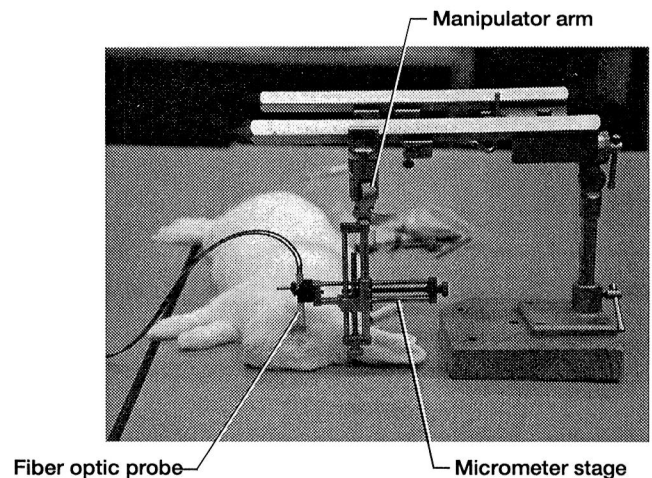


Figure 5.—Experimental setup for in-vivo DLS measurements in a live rabbit

4. RESULTS AND DISCUSSION

We performed in-vivo DLS measurements on two live rabbits; one; 2 months old and the other; 6 months old. DLS measurements were performed on both left and right eyes as a function of the penetration depth from the corneal surface. This is shown in Figure 6. There are several features of this result that require explanation. The difference in the age of the two rabbits do not show any appreciable difference in the average particle size values. The measured α -crystalline sizes are also similar in both left and right eyes. In the anterior chamber (aqueous humor) of the eye, we did not detect a considerable number of scattered photons and therefore conclude no presence of any substantial suspended particulate matter in this region. However, we find a specific trend as we move inside the lens and away from the anterior cortex. We see a gradual increase in the particle size as we move from the anterior cortex to the nucleus of the lens. Within the nucleus the size roughly remains constant, and then it gradually decreases as we move out of the nuclear region and into the posterior cortex. The first point in the anterior cortex and the last point in the posterior cortex are almost identical. This result shows detailed structure of the lens in terms of α -crystalline size and its distribution in the lens tissue. This is also consistent with earlier observations that alpha crystalline size or the extent of opacity is greater in the nucleus than in the anterior or posterior chambers of the lens³⁰. The approximate markers in Figure 6 for the aqueous, lens, and the vitreous regions are consistent with that of a schematic rabbit eye given by Hughes³².

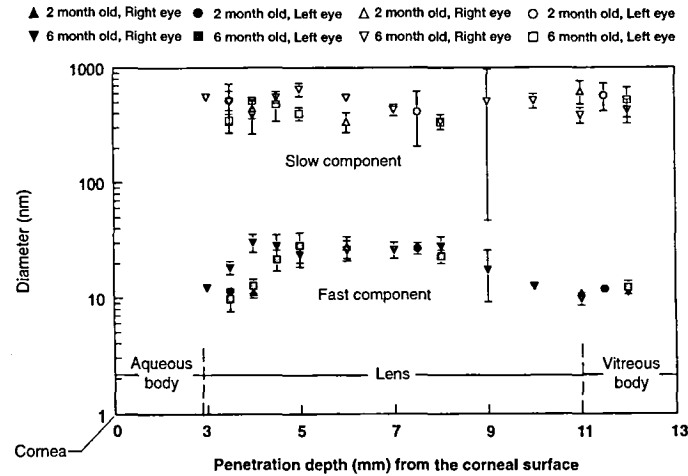


Figure 6.—In-vivo DLS results for two live rabbits.

The in-vivo intensity-intensity time autocorrelation functions (TCF) in live animal eyes were measured according to equation 1. Typical in-vivo normalized TCF profiles in the lens of the older rabbit are presented in Figure 7. For comparison purposes, autocorrelation data from an aqueous dispersion of polystyrene latex spheres (0.5 wt.%) of radius 10 nm showing a single exponential decay is also included. Five TCF runs are included here to show their reproducibility in the cortex and the nucleus of

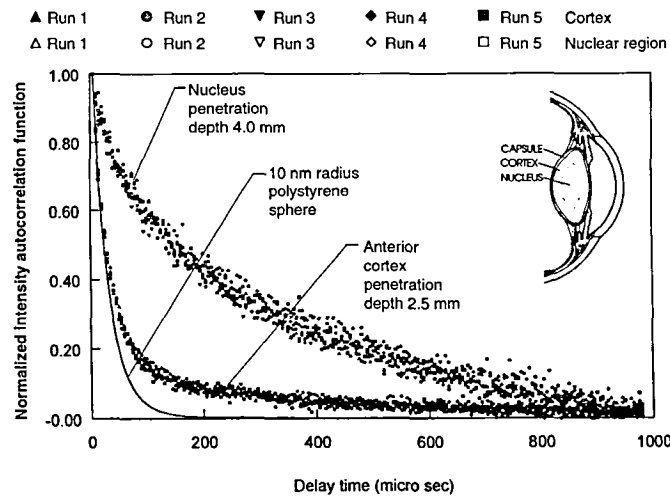


Figure 7.— TCF profiles: 6 month old rabbit—left eye.

the lens. Each TCF comprising 196 channels was collected for a duration of 15-20 seconds with a sample delay time of 5 μ sec. The α -crystalline sizes measured from the TCF's are reproducible within 5-10%.

The shape of the autocorrelation functions reveals bimodal (two exponential) distributions (equation 2). We believe the fast decaying component in the rabbit eye is caused by the Brownian motion of small particles (α -crystallines) and the slowly decaying component is due to the presence of larger protein aggregates.

The two sets of five correlation functions at two different locations; one in the anterior cortex and another in the nuclear region, clearly show distinct α -crystalline characteristics. The steep slope of the TCF in the anterior cortex is an indication of smaller size species while a shallower slope is indicative of the larger size particles in the nuclear region of the lens. This corroborates the earlier observations of Libondi et al.³⁰. We further analyzed our data using the double exponential method. The result is plotted in Figure 8.

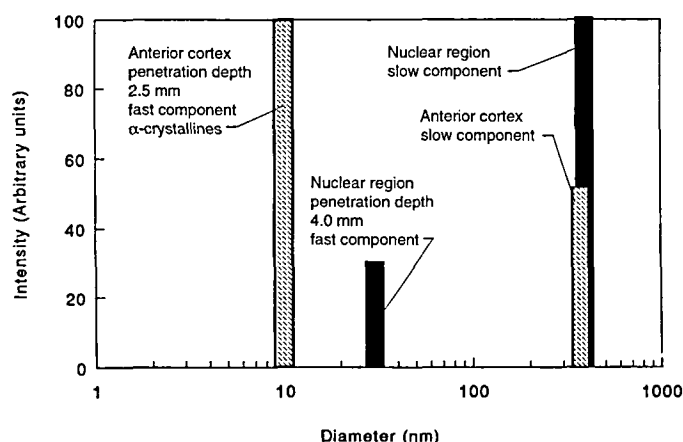


Figure 8.— α -crystalline size distribution in a 6 month old live rabbit (left eye).

We determined an average size of 10 nm in the anterior cortex and 30 nm in the nucleus. The slower component is \sim 400 nm and it remains almost identical for both the anterior and the nuclear regions of the lens. However, we note an important feature. This feature is the difference in the relative amplitude of the scattered intensities from the scattering species located in the anterior cortex and the nucleus of the lens. In the anterior cortex, the dominant source of the scattered light is α -crystallines while in the nuclear region bigger aggregates contribute a significant portion of the scattered light. Hence the relative population of smaller α -crystallines decreases in the nuclear region and their aggregate size increases.

We further investigated the difference in the protein size in the nucleus of a younger (2 month) and an older (6 month) rabbit. We found slight differences in the behavior of α -crystalline size. We have shown earlier in Figure 6 that the differences are really subtle. In Figure 9 we present two-component analysis in the lens nucleus and a comparison between the older and the younger rabbit. The in-vivo DLS measurements were made in the right eye. The fast components for both rabbits have almost identical values (\sim 30 nm in diameter). The molecular weight, using equation 3, is calculated to be $\sim 5 \times 10^6$ daltons. The slower components differ slightly (\sim 400-600 nm). However, the greatest difference is in the population of smaller particles in the older rabbit. In younger rabbit, the scattering intensity by small particles is stronger almost by a factor of 6. We therefore conclude that small molecular changes do occur as a function of aging and that these subtle changes and the early onset of cataractogenesis can be detected by the new probe.

The vitreous humor of the eye is the least understood part of an eye²³. We present preliminary DLS measurements made in vivo. The vitreous body shows strongly two-exponential behavior consistent with its gel-like properties. We see almost constant value for the fast component in this region. We ascribe this fast component due to the diffusion of hyaluronate molecular coils in water and the slow diffusion component due to the collagen-fibril network. We investigated this region a little more by getting a size distribution of the vitreous using CONTIN²⁸. This is shown in Figure 10. Using equation 3 we calculated the molecular weight for the fast component to be 3.78×10^6 daltons. This is consistent with the range ($2-4.5 \times 10^6$) of values given by Balazs and Delinger³³. More in-vivo experimental work is being performed at this time in our laboratory to fully understand the structure of the vitreous humor. We have recently concluded three dimensional (3-D) scans of the bovine vitreous using the new probe. This will be reported elsewhere³⁴.

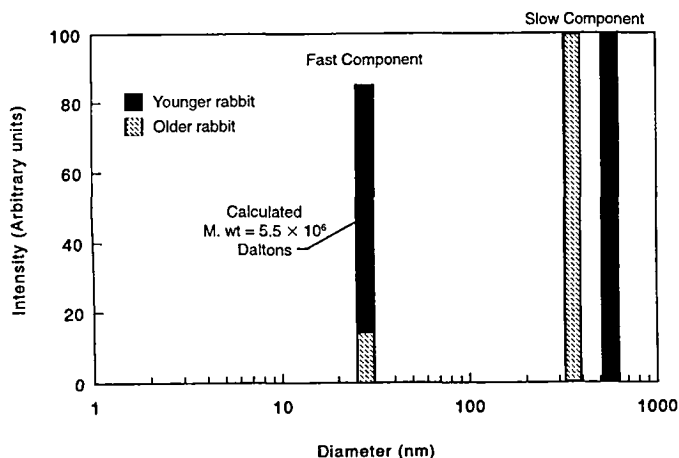


Figure 9.—Size distribution in the lens nucleus of two live rabbits. DLS measurements were made on a Right-Eye at a penetration depth of 5.0 mm from the corneal surface.

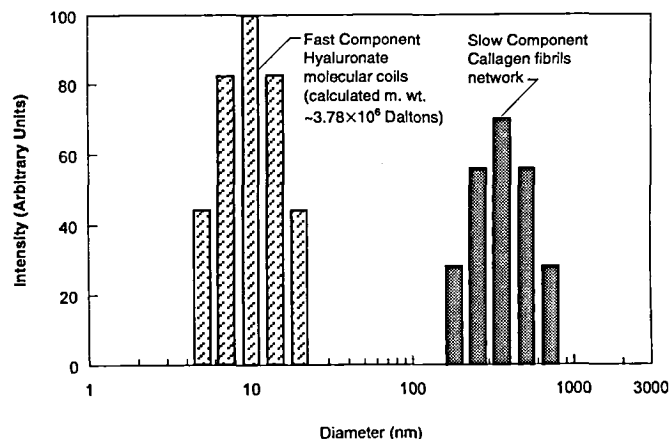


Figure 10.—In-vivo vitreous humor size distribution (CONTIN Analysis) of a 6 month old rabbit (Right Eye). Penetration depth from the Corneal Surface is 11.0 mm.

It is a typical belief that older people develop some type of a senile cataract compared to their younger counterparts. However, it is also an observation, although not very common, that an older person can be found without a cataract. We encountered such a situation. We obtained an intact lens of a 73 year old male cadaver. We performed DLS measurements as a function of penetration depth from the front surface of the lens. These are shown in Figure 11.

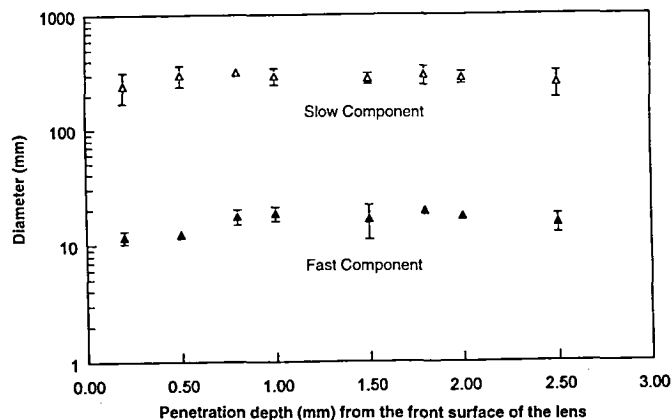


Figure 11.—Dynamic light scattering (DLS) results of a 73 year old (cadaver) human intact eye (Physical Lens appearance: Extremely Transparent).

The data was analyzed using the double exponential method (equation 2). The size of the fast component (α -crystallines) varies slightly. It is lower in the anterior portion of the lens, increases in the nuclear region, and then drops slightly in the posterior section of the lens. This slight variation is consistent with the structures of the anterior, the nucleus, and the posterior cortex as we indicated earlier in Figures 6-9. The slower component shows approximately similar behavior. The interesting feature of this data is the very small size of α -crystalline aggregates (~ 20 nm in diameter) in the lens nucleus considering the old age of the donor. A visual examination of the lens by one of us (MAD; an experienced ophthalmologist), revealed an extremely transparent nature of the lens. We have never seen such a transparent lens before in a older subject. The very transparent nature of the lens is consistent with our findings of small particle size for the α -crystallines. This is also consistent with a question that Dr. Schulman, in his book¹, asks why some people develop cataracts while others do not ?

5. CONCLUSION

In this paper we have reported a new DLS probe to non-invasively and quantitatively diagnose various parts of the eye. For clinical use we have mounted the DLS fiber optic probe on a H-ruby lens holder. The entire assembly can easily be mounted on a slit-lamp apparatus as shown in Figure 12. In addition to routine eye examination with a slit-lamp apparatus, our method provides early detection of cataracts, the characterization of hyaluronic acid chains and the collagen protein fibrils in the vitreous humor, and though not demonstrated here, the characterization of protein molecules and the metabolic waste product in the aqueous fluid in the anterior chamber of the eye.

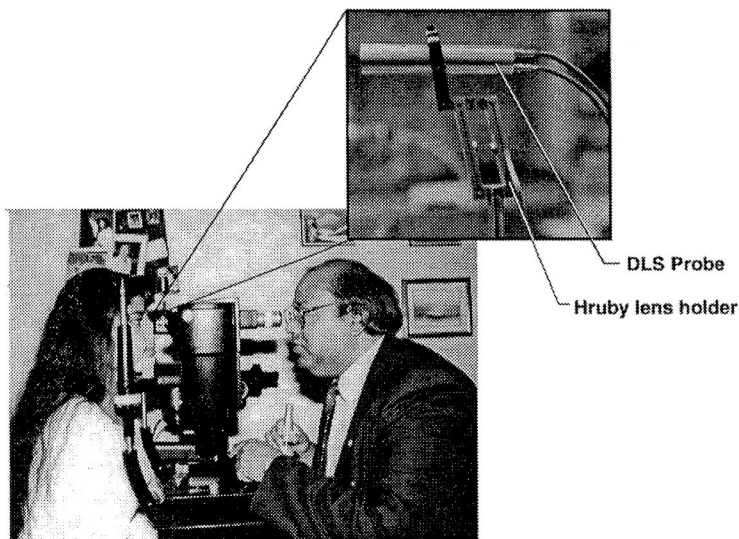


Figure 12.—A conventional slit-lamp apparatus can be easily modified to incorporate the new DLS probe for complete eye diagnostics.

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