GIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL
[ ] REVISION NO. ____

ect No. G 33-A06 DATE: 6-18-81

ect Director: N-T YU School/Lab Chemistry

sor: DHEW/PHS/NIH - National Eye Institute

Agreement: Grant No. 5-R01-EY01746-06

d Period: From 5-1-81 To 4-30-82 (Performance) 7-31-82 (Reports)

sor Amount: $ 63,828 Contracted through:

Sharing: $ 3,359 (G-33-365) ~~GTRI~~ (GIT)

e: Comparative Raman Studies of Human and Animal Lenses

NISTRATIVE DATA OCA CONTACT Don Hosty

ponsor Technical Contact: F. N. FUKUI, Ph D, Anterior Segment Diseases Branch, Cataract Program, National Eye Institute, Bethesda MD 20014

ponsor Admin./Contractual Contact: Ms GAYE LYNCH, Grants Management Office, Extramural Services Branch, National Eye Institute, Bethesda, Md 20014 Phone (301) 496-5884

rts: See Deliverable Schedule Security Classification: N/A

nse Priority Rating: N/A

RICTIONS

Attached NIH Supplemental Information Sheet for Additional Requirements.

el: Foreign travel must have prior approval - contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

pment: Title vests with G-IT, however, we are accountable for all equipment purchased.

ENTS: Follow-on Project To G 33-A05 (05 year)

ES TO:

nistrative Coordinator
earch Property Management
ounting Office
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Research Security Services
~~Reports Coordinator~~ (OCA)
Legal Services (OCA)
Library, Technical Reports
EES Research Public Relations (2
Project File (OCA)
Other: ____

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 7/11/83

Project Title: Comparative Raman Studies of Human and Animal Lenses

Project No: G-33-A06

Project Director: Nai-Teng Yu

Sponsor: DHEW/PHS/NIH - National Eye Institute

Effective Termination Date: 4/30/82

Clearance of Accounting Charges: 7/31/82

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

NOTE: Follow-on project (07 year) is G-33-A07

Assigned to: Chemistry (School/Laboratory)

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Other GTRI

FORM OCA 10:781

G-33-A06

APPLICANT REPEAT GRANT NUMBER SHOWN ON PAGE 1	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	EY01746-07

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
Yu, Nai-Teng	FROM	THROUGH
NAME OF ORGANIZATION	05/01/81	02/20/82
Georgia Institute of Technology		

TITLE (Repeat title shown in Item 1 on first page)

Comparative Raman Studies of Human and Animal Lenses G-33-A06/Yu

1 List all publications not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
2. Provide two reprints of publications not previously submitted to the awarding unit.
3 Progress Report (See instructions)

1. Manuscript accepted for publication:

Nai-Teng Yu, John F. R. Kuck, Jr. and Carl C. Askren "Laser Raman Spectroscopy of the Lens in situ, Measured in an Anesthetized Rabbit". Current Eye Res. (in press) Two copies are submitted with this application.

3. Progress Report:

(i) General scientific goals of the project during the budget year: No change.

(ii) We took a major step toward the development of laser Raman spectroscopy as an in situ structural probe of the ocular lens. We have succeeded in obtaining the first Raman spectrum from the lens of a live animal. A laser beam (514.5 nm; 15 mW) was directed into the eye of an anesthetized rabbit at 60° from the visual axis and Raman emission was collected at 90° from the incident beam. The power density at the retina was estimated at 0.5W/cm^2. The entire scattering column in the lens can be imaged on the entrance slit of a spectrometer with so little distortion that Raman "optical dissection" analysis (Askren, Yu and Kuck (1979) Exp. Eye Res. 29, 647) can be performed on the in situ lens. In addition, we have demonstrated that a low power He-Ne laser (632.8 nm; 0.78 mW) is a suitable excitation source for detecting the red fluorophor in a brunescent human lens.

There is a possibility that various fluorophors in aging and brunescent human lenses are formed by photoreaction between the lens proteins and some photosensitizers such as 3-OH kynurenine derivatives in the lens. Preliminary studies show that γ-crystallin treated with 3-OH kynurenine plus near UV for 12 hr. exhibits an enhanced fluorescence intensity in the red.

The results with excitation wavelengths at 406.7 and 514.5 nm are shown in Figs. 1 and 2. The findings may be important in understanding the mechanisms by which the red fluorophor is formed in brunescent human lens.

Specific objectives for the coming year:

1. To improve the technique for in situ Raman spectroscopy of the lens.
2. To determine the differences among the three crystallins in regard to the photosensitivity with 3-OH kynurenine.
3. To investigate the similarities and differences between the fluorophors formed by photoreaction with 3-OH kynurenine and those in order and brunescent human lenses by means of laser Raman/flurescence techniques.

(1) γ-crystallin treated with 3-OH kynurenine plus near UV for 12 hr. Protein = 0.414 mg/ml

(2) γ-crystallin treated with 3-OH kynurenine plus dark for 12 hr. Protein = 0.414 mg/ml.

(3) γ-crystallin treated with near UV only for 12 hr. Protein = 0.333 mg/ml.

(4) non-treated γ-crystallin. Protein - 0.522 mg/ml.

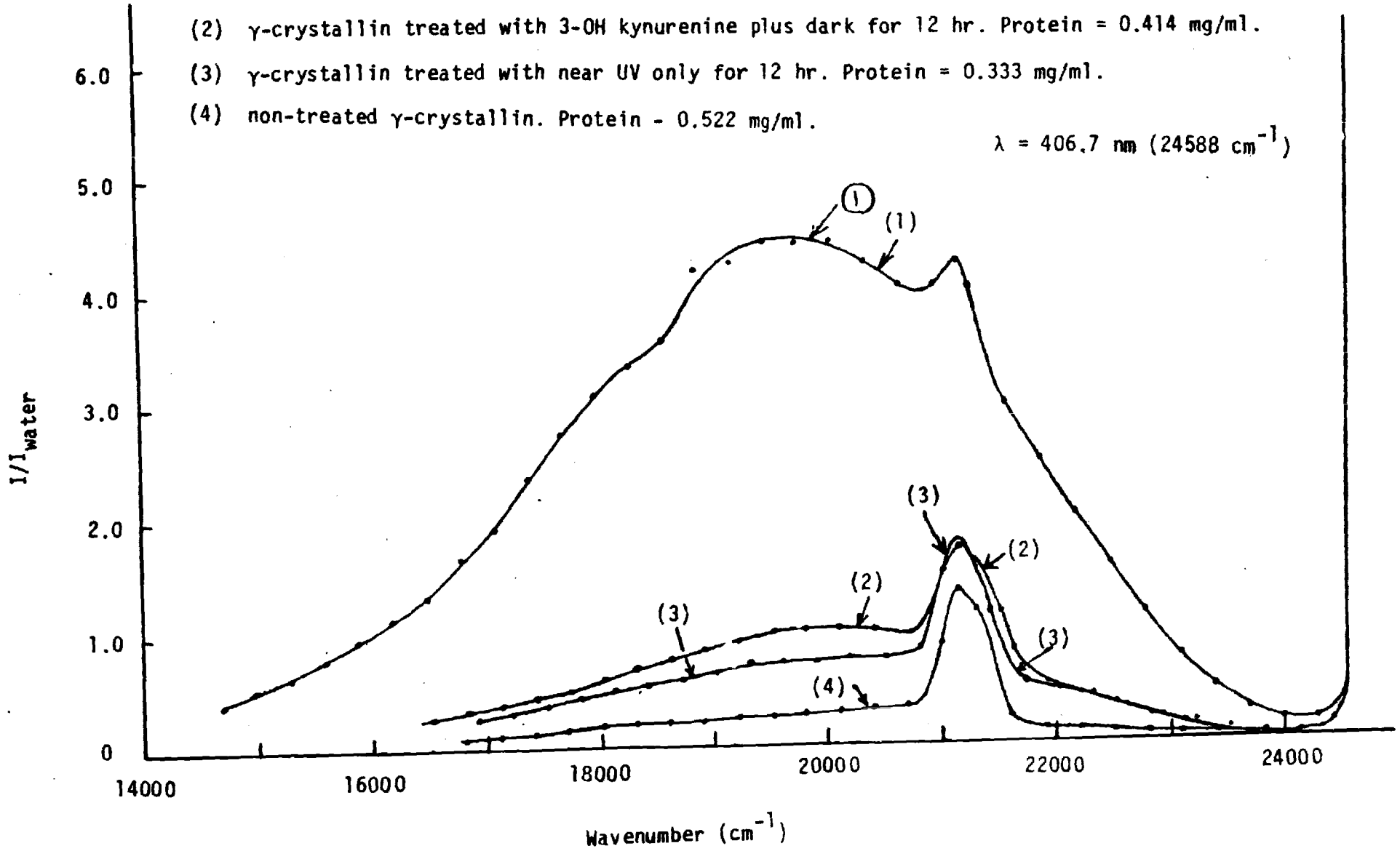


Figure 1

(1) γ-crystallin treated with 3-OH kynurenine plus near UV for 12 hr. Protein = 0.414 mg/ml.

(2) γ-crystallin treated with 3-OH plus dark for 12 hr. Protein = 0.414 mg/ml.

(3) γ-crystallin treated with near UV for only 12 hr. Protein = 0.33 mg/ml.

(4) non-treated γ-crystallin. Protein = 0.522 mg/ml.

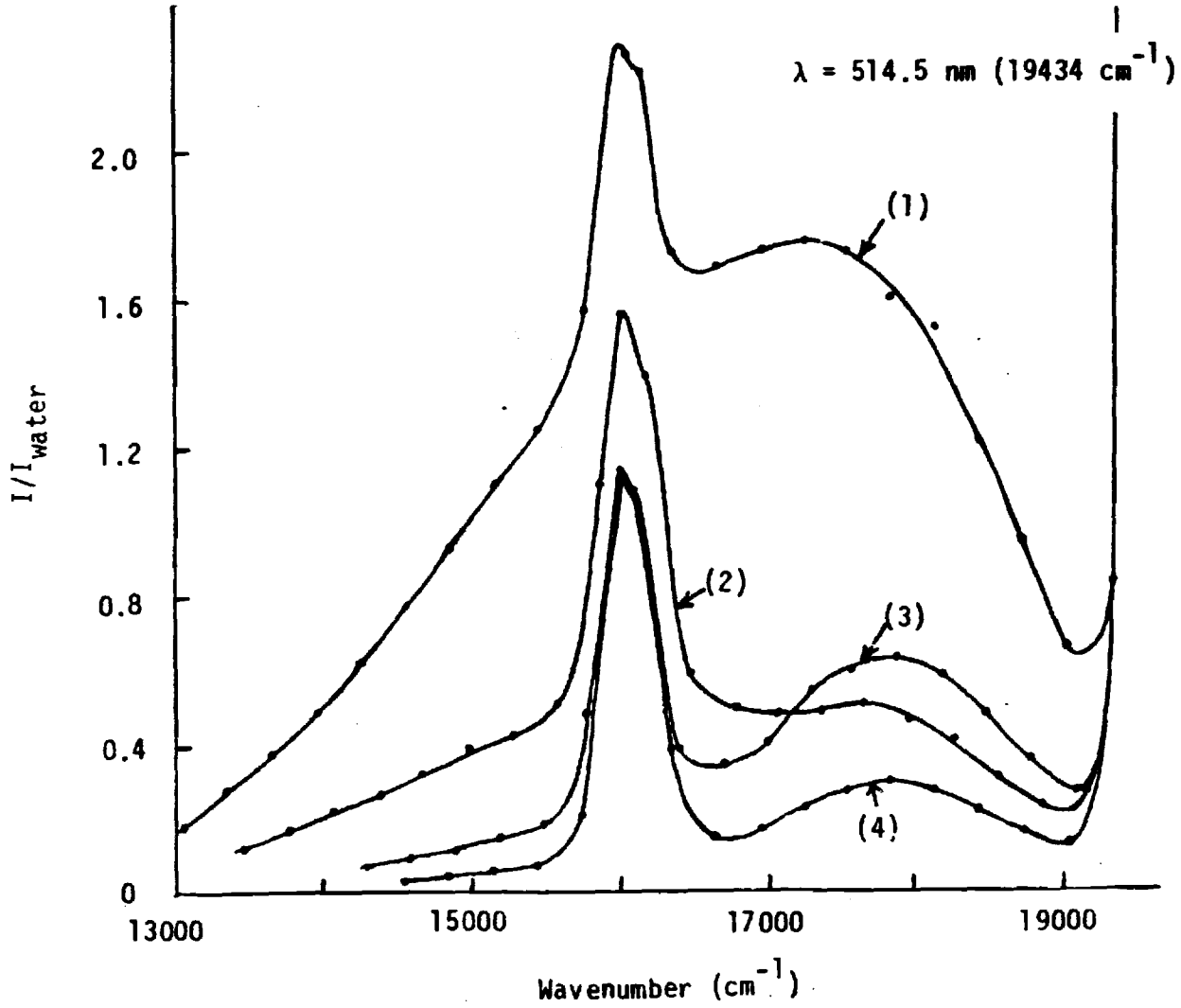


Figure 2

G-33-N06

Yu, Nai-Teng
585-28-9345

C. Progress Report/Preliminary Studies

Title of invention : Non-invasive early prediction of cataract by Raman/fluorescence

i) Period: May 1, 1979 - April 30, 1982

ii) Professional Personnel who have worked on the project:
Yu, Nai-Teng, Professor (Principal Investigator) 05/01/79-present (50%)
Askren, CarlC., Research Technician, 06/01/81-08/31/82, 100%
Graduate Res. Assist. 05/01/79-05/31/80, 100%
Pace, C., Research Associate, 05/01/70-04/30/80, 100%
Bando, M., Sr. Res. Assoc., 10/01/81-present, 100%

iii) Previous Application's Specific Aims: In previous applications we proposed to achieve the following specific aims: 1) To obtain quantitative experimental data to explain why cats rarely develop cataracts, while dogs frequently do; 2) To demonstrate that Raman spectra can be obtained from the lens of a living animal with laser power sufficiently low (~1 mW) to avoid retinal damage; 3) To follow the development of a mouse senile cataract using a Raman spectrometer with a cooled, silicon intensified target, and an optical multichannel analyzer (SIM-OMA); 4) To characterize by laser Raman scattering a late-appearing (senile) cataract in Emory mouse; 5) To investigate whether in vivo near UV irradiation will accelerate the oxidation of protein sulfhydryl in the aging rabbit lens; 6) To correlate Raman spectroscopy of γ-crystallin with available three-dimensional structure obtained from X-ray crystallography; 7) To determine if human senile cataract involves the oxidation of protein sulfhydryl to form disulfide bonds; 8) To perform Raman measurements on specific amino acids (Trp, Tyr, Cys) and "fluorophors" from human and animal lenses, and to determine if their profile changes with age; 9) To obtain "fingerprint" information on "fluorophors" found in the lens by means of coherent anti-Stokes Raman scattering (CARS); 10) To employ different Raman techniques for determining small conformational changes of proteins associated with aging or cold cataract.

iv) Summary of Progress and Important Findings: (a) We have developed a multichannel Raman system which was successfully employed to obtain the first Raman spectrum from the lens of a live rabbit (Yu, Kuck and Askren, 1982); (b) We discovered a red fluorophor with emission maximum at 672 nm (excited at 647.1 nm), which is absent in normal lenses younger than 70-year old (Yu, Kuck and Askren, 1979). We now have a sensitive indicator for monitoring the early development of brunescent cataract formation; (c) subsequent studies of brunescent cataracts with laser wavelength at 568.2 and 676.4 nm led to the discovery of near red (emission maximum at 633 nm) and far red (emission maximum at 707 nm) fluorophors which are also highly characteristic of brunescent cataract (Yu and Kuck, 1980); (d) Raman spectra of cataractous Emory mouse lens have been obtained. A pair of lenses from the same mouse (8-month old), one normal and the other partially opaque, were found to exhibit different -SH intensity at 2580 cm^{-1} (unpublished results); (e) We have demonstrated that fluorophors in mouse lens can be generated in the absence of light. Fluorescence profiles along the visual axis of aging dark-adapted mouse lenses have been obtained and compared with those of light-adapted mouse lenses; (f) Near red fluorophor (emission at

633 nm with excitation at 568.2 nm) can be artifically generated by incubating 3-OH kynurenine with rat γ-crystallin in the presence of near UV irradiation for 16 hrs (unpublished results); (g) With excitation wavelength at 676.4 nm we have been able to obtain high-quality Raman spectrum from a human lens as old as 70-year without fluorescence interference (unpublished results); (h) "Laser Raman Optical Dissection" technique (Askren, Yu and Kuck, 1979) was introduced to measure the variation of sulfhydryl level along the visual axis of the lens. The effects of aging on VA sulfhydryl profiles are quite different between human and rodents. This has been correlated with the derivation of albuminoid. The -SH concentration profiles of bovine, rabbit and chicken lenses of several ages have also been obtained (Kuck, Yu and Askren, 1982); (i) "Difference Raman" technique was employed to detect aged-related changes in the tertiary structure of crystallins in the nucleus of rat lens (Yu and Kuck, 1981). The nearly complete 2 SH → S-S conversion in rat lens nucleus without significant changes in the secondary structure has been interpreted in terms of the three-dimensional structure revealed by X-ray crystallographic studies of γ-crystallin (bovine); (j) We have completed the measurements of critical wavelengths in human lenses with ages between 0 and 80-year old. A normal aging curve was obtained and a brunescence zone was defined (unpublished); (k) A demonstration that red fluorophor in a brunescent human cataract can be detected in 1.68 sec. with only 0.6 mW of laser beam at 632.8 nm (unpublished results).

v) Detailed Progress Report:

(a) Multichannel Raman Spectroscopy of the Lens in situ, Measured in an Anesthetized Rabbit (with Kuck and Askren, Current Eye Res. in press).

We have obtained the first Raman spectrum from the lens of a live rabbit. A laser beam (514.5 nm; 15 mW) was directed into the eye of an anesthetized rabbit at 60° from the visual axis and Raman emission was collected at 90° from the incident beam (Fig. 1). The power density at retina was estimated at 0.5 W/cm^2. The entire scattering column in the lens can be imaged on the entrance slit of our spectrometer with little distortion so that Raman "optical dissection" analysis can be performed on the in situ lens.

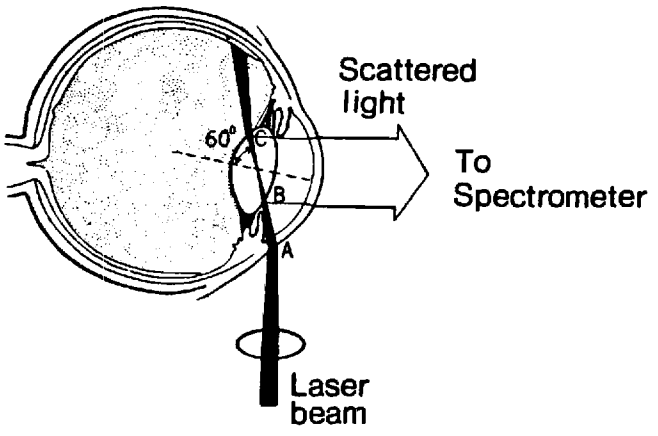


Fig. 1

For in situ Raman spectroscopy, a multichannel detector (500-700 channels) is superior to the conventional scanning single-

channel photomultiplier-photon counting detection modern. Since all the channels accumulate optical signals simultaneously, the variations in light output from the lens due to animal's eye movement are relatively inconsequential if the position of the eye in the laser beam is quickly realigned after movement.

(b) Discovery of Red Fluorescence Characteristic of Human Brunescent Cataract (with Kuck and Askren, Invest. Ophthal. & Vis. Sci., 18, 1278-80 (1979)).

We found a red fluorophor in the nucleus of the older human lens, whose accumulation appears to parallel the development of brunescent cataract. Its appearance may be regarded as a sign of incipient brunescence for four reasons: (1) it is not normally present before the seventh decade, (2) its concentration increases rapidly with age around the 7th decade, (3) its accumulation is remarkably higher ($\sim 10^2$) in brunescent lenses than in normal lenses of comparable age, and (4) its distribution has a maximum near the center of the nucleus. In these properties it differs from the blue fluorophor of the lens which is present in the normal lenses of all ages, and is only slightly elevated above normal in brunescent lenses. Red fluorophor does posses the important properties expected of a substance involved in nuclear pathology. The red fluorophor has an emission maximum at 672 nm with excitation at 647.1 nm.

(c) Comparison of Fluorescence between Normal Lens and Brunescent Cataract, With Varying Laser Wavelength (unpublished).

Careful comparison of emission properties of normal and brunescent lenses (both at 53-year-old) (Fig. 2) led to the discovery of two additional red fluorophors with excitation/ emission at 568/633 (near red) and 676/707 (far red). These two

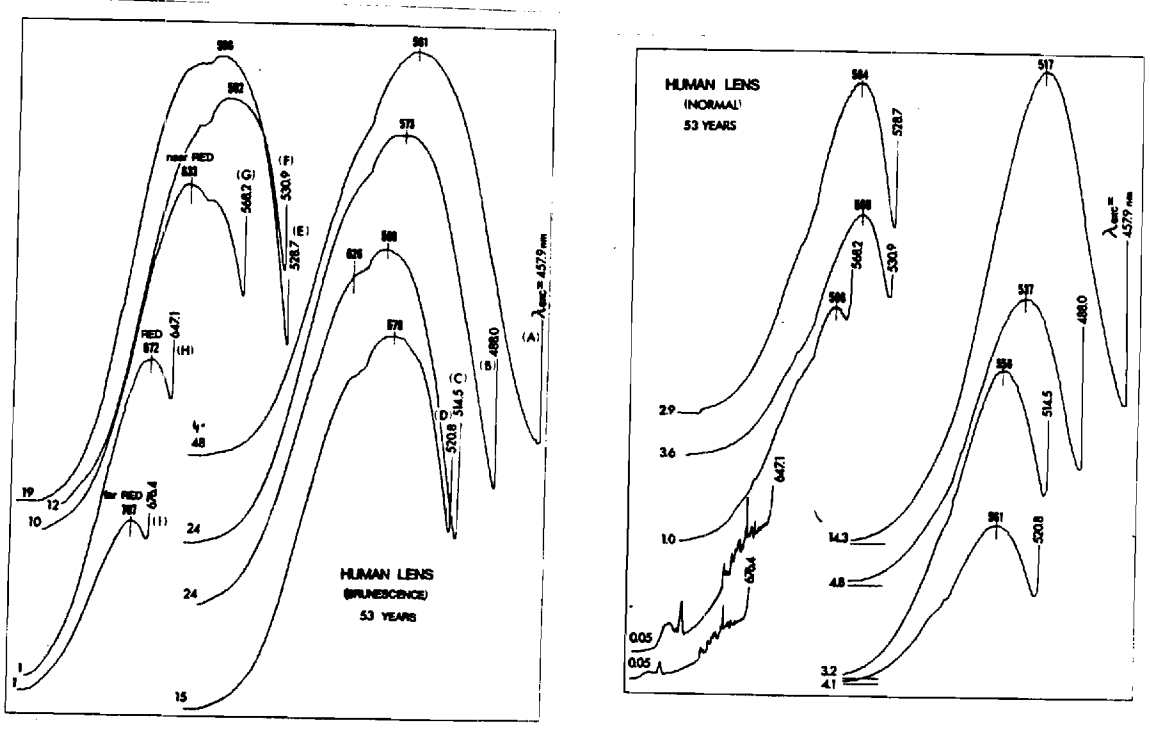


Fig. 2

fluorophors, along with the one at 647/672 (red), are quite distinct from those found in normal lenses. We believe that these signals at 633,672 and 707 nm may serve as probes for the in situ monitoring of brunescent cataract formation.

(d) Accelerated Changes in Sulfhydryl Accompany the Cataract Formation in Emory Mouse (with Kuck, unpublished).

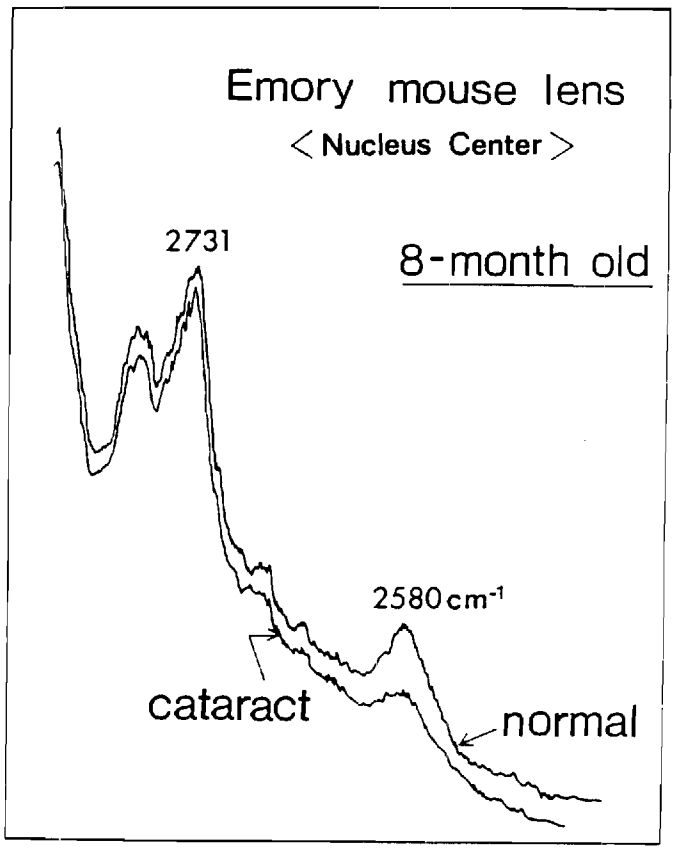


Fig. 3

We have established that the 2SH → S-S conversion is an important feature of normal lens aging in mouse and rat, without cataract formation. However, it has been shown that an accelerated rate of such a conversion does lead to lens opacity such as in UV-irradiated mice (East, Chang, Yu and Kuck, 1978). Recently, we compared the rates of disappearance of lens sulfhydryl between cataract-prone mouse (Emory mouse, a senile cataract model) and cataract-resistant mouse, and found that there was an acceleration for the former. More interesting is the finding that a pair of lenses from an 8-month old Emory mouse do not have the same rate of disappearance of lens sulfhydryl. As shown in Fig. 3, the normal lens exhibits a stronger -SH signal at 2580 cm^{-1}, compared to the cataractous one. This cataractous lens was only partially opaque so that Raman scattered light from the nucleus center could be transmitted.

(e) Metabolic Production of A Green Fluorophor in Mice(with Bando & Kuck)

Exposure of ocular lens to UV light can result in production of fluorescent materials, both in vivo and in vitro (Lerman et al., 1976a,b; Grover and Zigman, 1972; Borkman et al., 1977). However, we have demonstrated that fluorophors in the lens can also be generated in the absence of light as a result of aging. As shown in Fig. 4, the 2-week old mouse lenses (both dark-adapted and light-adapted) exhibit pratically no fluorescence with excitation at 407.6 nm. Quite interestingly, fluorescence intensity increases considerably in the nucleus of 35-week and 45-week old lenses. The fluorescent intensity in the lens of 45-week old light-adapted mouse lens is not significantly higher than that of the 45-week old

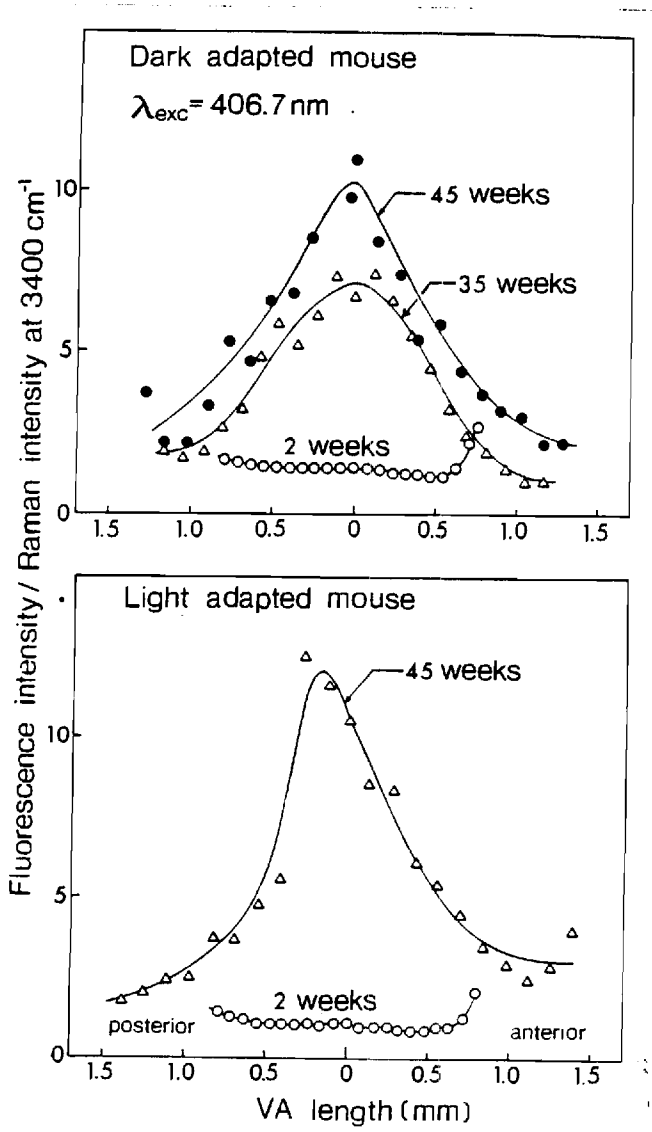


Fig. 4

dark-adapted mouse. We are continuing the measurements up to at least 2-year old to see if fluorophor concentration is higher in light-adapted mouse.

(f) Near Red Fluorophor Generated from 3-OH Kynurenine (with Bando and Kuck, unpublished).

We demonstrated that near red fluorophor at 633 nm can be artifically generated by incubating 3-OH kynurenine with rat γ-crystallin in the presence of near UV irradiation for 16 hrs (curve 1 of Fig. 5). The fluoroscent complex is apparently covalently linked to γ-crystallin because it cannot be removed by exhaustive dialysis. Incubation of γ-crystallin with 3-OH kynurenine without near UV produces no near red fluorophor Only γ-crystallin with or without near UV also exhibits no near red fluorescence (curves 2 and 3 of Fig. 5). Since human lens contains high concentration of 3-OH kynurenine O-β-glucoside (Bando, Nakajima and Satoh, 1981), this raises the possibility that near red fluorophor in brunescent cataract may indeed be generated by ambinent UV light. We are currently investigating if differences exist among the three crystallins (α-, β- and γ-) in regard to the photosensitivity with 3-OH kynurenine. Studies of artificially generated red and far red fluorophors are also in progress.

(g) Earlier Raman studies of human lenses were restricted to young lenses (20-year old) because older lenses had increased fluorescence which interfered with Raman measurements. Now we have overcome that deficiency by the use of exciting light of much longer wavelengths; this gives good Raman signals but does not excite the major fluorophors (Yu, Kuck and Askren, 1979). After we reported the Raman spectrum of a 58-year human lens (Kuck, Yu and Askren, 1982) with excitation at 647.1 nm, we have succeeded at obtaining interpretable Raman spectra from a 70-year

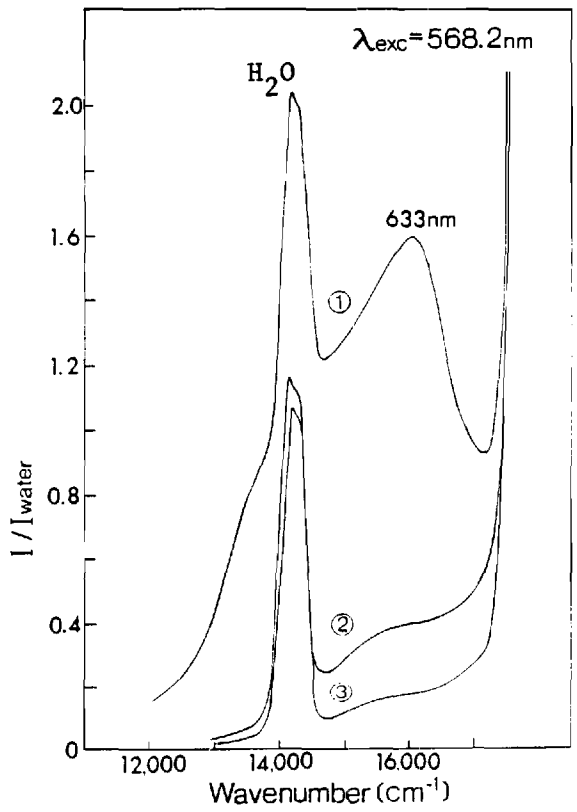
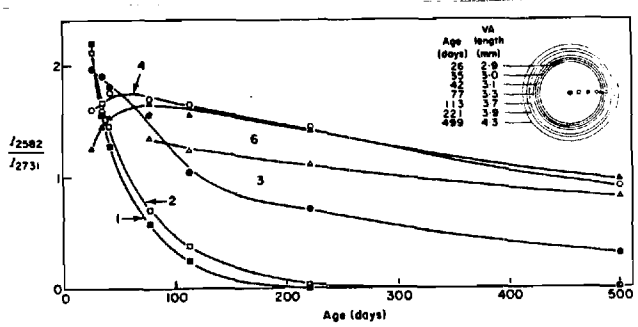


Fig. 5

old lens excited at 676.4 nm (unpublished results). Our SP-171 Kr^+ laser has a line at 752 nm (1.2 watts), which will be employed to obtain Raman spectra of even older human lenses.

(h) Laser Raman Optical Dissection Technique (with Askren and Kuck, Exp. Eye Res. 29, 647-654 (1979)). We have shown that Raman spectroscopy can be used as a unique optical dissection technique for obtaining the -SH concentration profile along the visual axis (VA) of ocular lenses. The VA length of each rat and mouse lens was measured using a translation stage micrometer and the laser scattering. The rat VA length varied from 2.85 mm (26 days) to 4.32 mm (16 months). Spectra were obtained from 20 increments along the VA. The results are presented as VA proriles. The salient features in the series of curves are two maxima in the cortex (one anterior, one posterior) and a central minimum. The youngest rat lens showed a bell-shaped curve. All curves were nearly symmetric for the rat the maxima slightly off center for the mouse. The two maxima of the second youngest rat lens were separated by a distance of 1.55 mm which increased to 2.95 mm in the oldest lens. In a 7½ month lens a 0.78 mm segment of the VA center contained too little sulfhydryl to be detected by this technique. This segment increased to 1.44 mm in the oldest lens. The apparant rate of decrease in SH, being quite pronounced in the nucleus, is different at other points along the VA. A plot of sulfhydryl level vs. age for several points at distance r from the center (VA midpoint) along the VA indicates a steady decrease in SH levels with age for r <1.2 mm (Fig. 6). For larger r, there is actually



Changes in rat lens sulfhydryl. The intensity ratios for anterior VA distances (in mm) of (1) r = 0·00 (■), (2) r = 0·40 (□), (3) r = 0·80 (●), (4) r = 1·20 (○), (5) r = 1·35 (▲), and (6) r = 1·60 (△) are presented as a function of age.

Fig. 6

an increase in SH. These results are interpreted in terms of 2SH → S-S conversion, changing rates of synthesis of the different crystallins of glutathione synthesis along the VA.

(i) Comparisons of Sulfhydryl Behavior in the Intact Lenses of Humans, Water Buffalo, Rabbit, Chicken, Rat and Mouse: Variations in the Nucleus and Along the Optical Axis During Aging (with Kuck and Askren, Exp. Eye Res. 34, 23-37 (1982)).

For the first time "laser Raman optical dissection" technique was employed to reveal the dramatic differences in sulfhydryl behavior among several species. As demonstrated in (h), the sulfhydryl concentration in the central nucleus of rat and mouse lenses falls precipitously with age. However, in the lenses of man and water buffalo, the -SH decreases at a much slower rate with age (see Fig. 7). The difference between the two groups appears to be correlated with the derivation of albuminoid: in the rodents it is chiefly γ-crystallin which gives rise to albuminoid while in human and bovine lenses albuminoid is related to α-crystallin. The sulfhydryl concentration profiles along the visual axis of human, rabbit and chicken lenses of several ages show that these species have profiles unlike those of rat and mouse lenses; the rabbit lens is more like the human lens while the chicken lens is in a class by itself due to the predominance of δ-crystallin in the nucleus and the consequent extremely low concentration of sulfhydryl. (see Figs. 8,9,10 & 11).

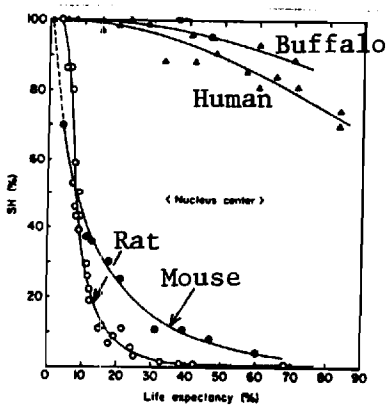


Fig. 7

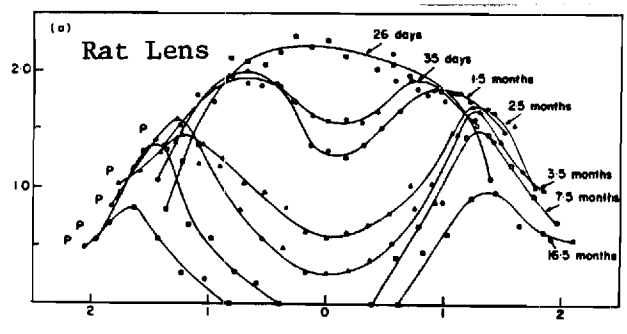


Fig. 8

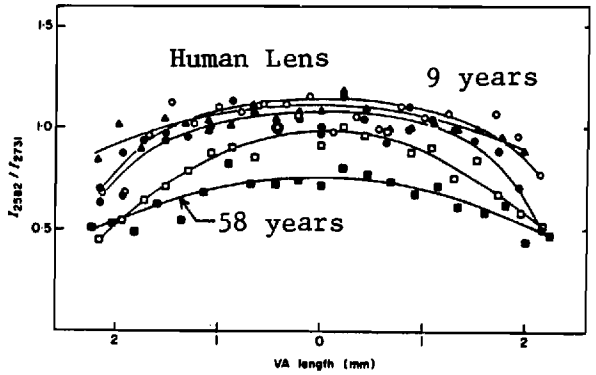


Fig. 9

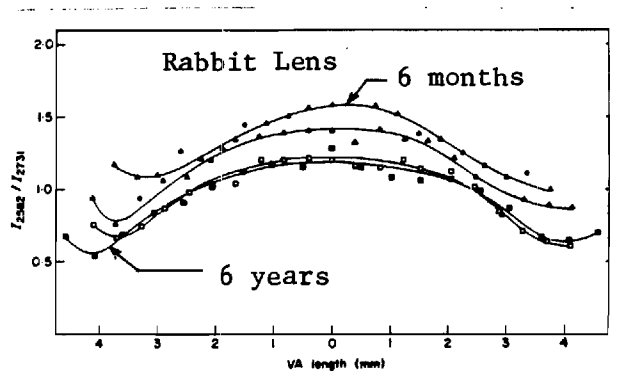


Fig. 10

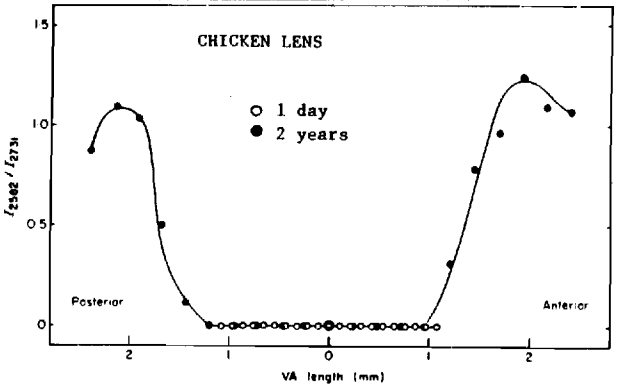


Fig. 11

(j) Difference Raman Technique Reveals Age-Related Changes in Rat Lens Protein Tertiary Structure (with Kuck, unpublished).

Raman studies on intact rat lenses have demonstrated that γ-crystallin in the central nucleus undergo almost complete oxidation of sulfhydryl to disulfides within 50% life expectancy. It is of interest to examine the effects of disulfide formation on secondary structures of γ-crystallins in the intact state. Raman "amide III" band near 1240 cm^{-1} reveals secondary structure, while Tyr "doublet" near 840 cm^{-1} and a Trp ring mode at 760 cm^{-1} reveal tertiary structure. Difference Raman spectra between young (1-month) and old (8-month) lenses display 3 positive bands betweetn 800-890 cm^{-1}, but complete cancellation of "amide III" singals indicating significant changes in tertiary structure but not in secondary structure. The absence of changes in secondary structure (predominately anti-parallel β sheet) implies that all the -SH groups must be clustered together and/or so arranged on the molecular surface that intra- and inter-disulfide crosslinks are readily formed during normal aging without involving protein unfolding. Spectral changes (450-800 cm^{-1}) caused by aging process are presented in Fig. 12. The signals at 663 and 725 cm^{-1} are due to the C-S stretching vibrations of -C-S-S-C- linkages and methionine, respectively.

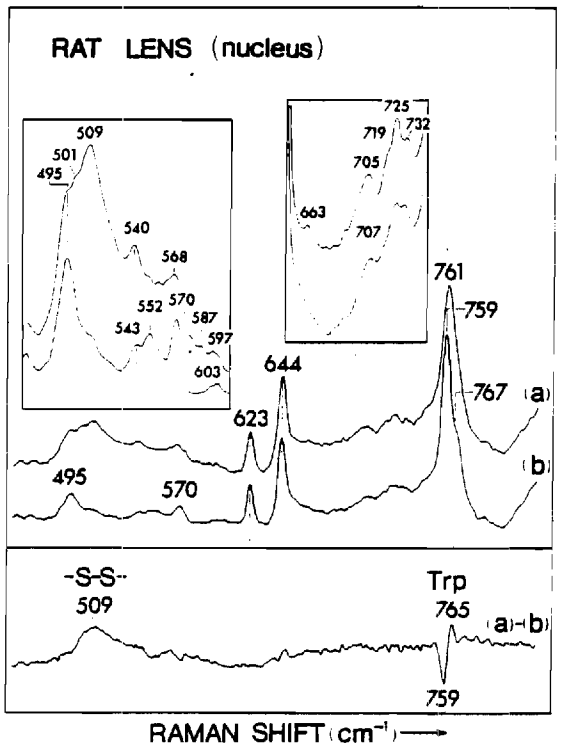
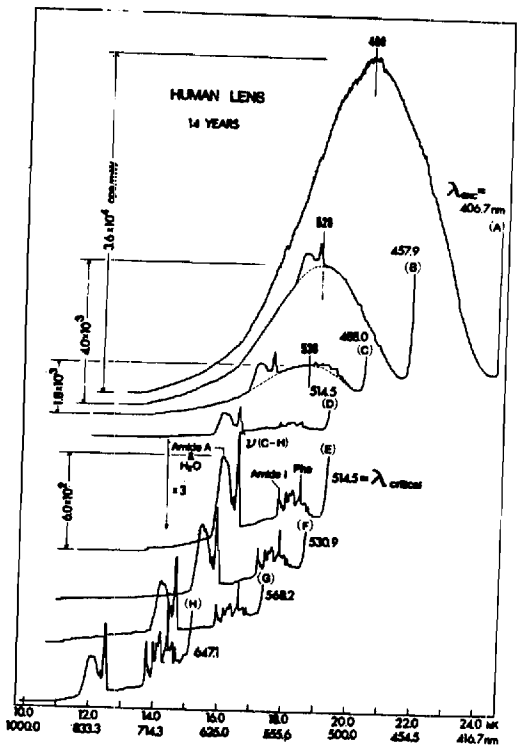


Fig. 12

(k) Measurement of Critical Wavelengths in Human Lenses (with Kuck, unpublished).

Human lenses exhibit strong fluorescence at all ages. However, as excitation wavelength increases, the intensity of fluorescence relative to Raman intensity decreases. A critical wavelength may be defined as the shortest excitation wavelength at which the fluorescence intensity vanishes relative to Raman signals. As shown in Fig. 13, the 14-year old lens has a λ (critical) at 514.5 nm. We have measured the λ (critical) vs. age (Fig. 14) which shows variations between 480 and 670 nm. A brunescence zone is reached if λ (critical) is longer than 670 nm.



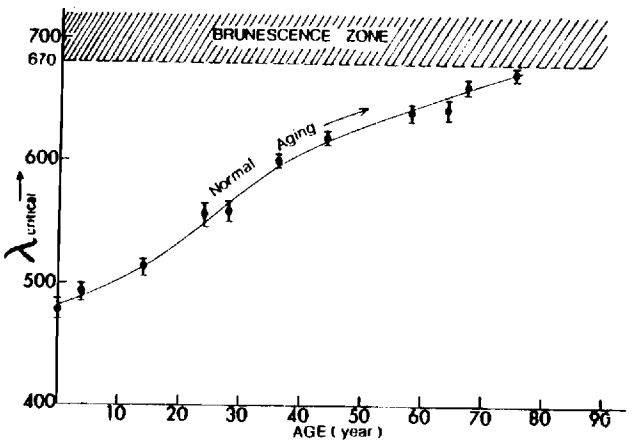


Fig. 14

Fig. 13

(1) A Demonstration That Red Fluorophor in A Brunescent Human Cataract (Extracted) Can be Detected in 1.68 Second With 0.6 mW Beam at 632.8 nm (unpublished results).

We have been interested in pursuing the idea that red fluoroscence in human lens may be used to monitor the early development of brunescent cataract. To reduce the laser power and time needed to record the entire red fluorescence spectrum, we have taken a major step in redesigning our light-dispersion system (Fig. 15). With a modified spectrometer (Spex 1870, through rental arrangements) the light paths are reduced from 9 to 5, and an optimization of dispersion and resolution, it is now possible to view the entire red fluorescence under the excitation of 632.8 nm beam (at 0.6 mW) in only 1.68 second. Fig. 16 shows a comparison between a 73-year old brunescent lens and a 32-year old normal lens. A total of 20,416 counts of signals could be accumulated at 672 nm (red) in only 1.68 seconds. Under the same conditions, the normal lens exhibited 367 counts.

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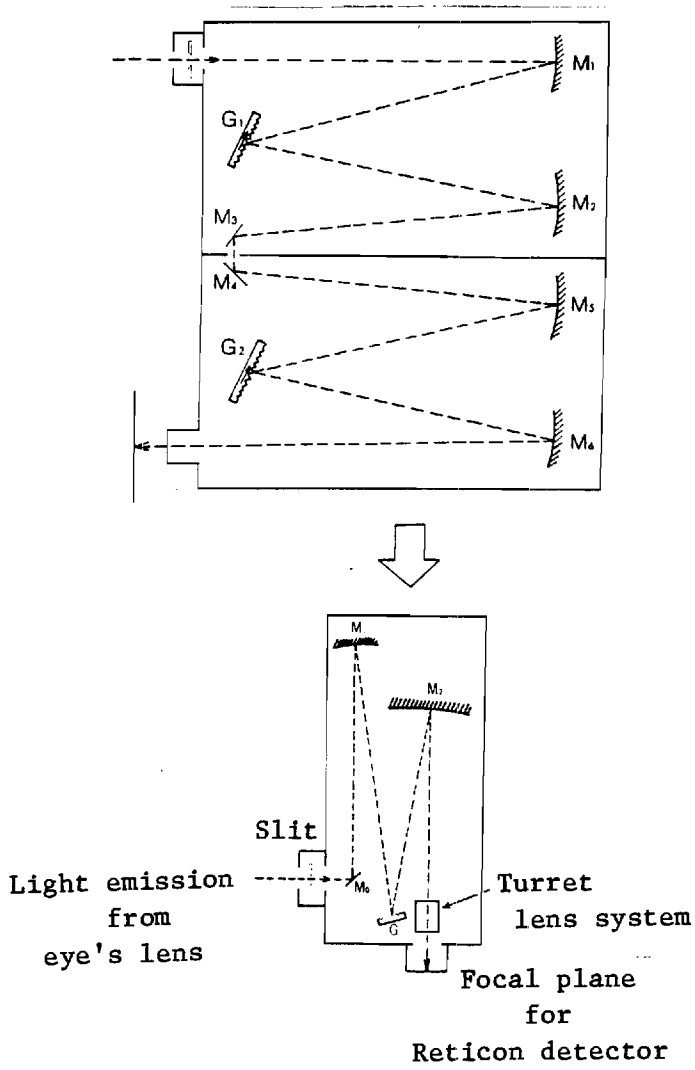

Fig. 15

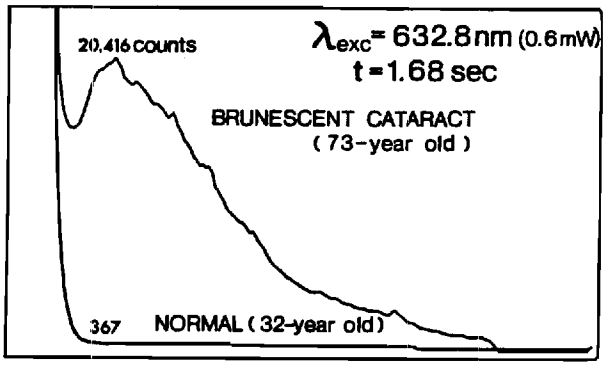

Fig. 16

Publications (1978-82) which acknowledge the support by EY01746 grant:

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3. Yu, N. T. and Kuck, J. F. R. (1978) "Focusing on Lenses with Laser Raman Spectroscopy" The Spex Speaker, Vol. 23, No. 3, pp. 1-8 (published by Spex Industries, Inc., 3380 Park Ave., Metachen, N. J. 08840).

4. East, E. J., Chang, R. C. C., Yu, N. T. and Kuck, J. F. R. (1978) "Raman Spectroscopic Measurements of Total Sulfhydryl Intact Lens as Affected by Aging and Ultraviolet Irradiation. Deuterium Exchange a as a Probe for Accessible Sulfhydryl in Living Tissue" J. Biol. Chem. 253, 1436.

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10. Yu, N. T. (1982) "Studies of Intricate Structure of Eye's Lens by Laser Raman Scattering" J. Chem. Education (in preparation).

11. Yu, N. T. and Kuck, J. F. R. (1982) "Age-Related Changes in Lens Protein Teritary Structure as Detected by a Sensitive Multichannel Difference Raman Technique" (in preparation).

12. Yu, N. T., Bando, M. and Kuck, J. F. R. (1982) "Metabolic Production of a Green Fluorophor in Lenses of Dark-Adapted Mice" (in preparation).