GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

# PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. G-33-A08 ~~GTRI~~/GIT DATE 4/29/83

Project Director: Nai-Teng Yu School/~~Lab~~ Chemistry

Sponsor: DHHS/PHS/NATIONAL EYE INSTITUTE

Type Agreement: Grant No. 2 R01 EY01746-08

Award Period: From 5/01/83 To 04/30/84 (Performance) 07/31/84 (Reports)

Sponsor Amount: Total Estimated: $ 80,917 Funded: $ 80,917

Cost Sharing Amount: $ 5,821 Cost Sharing No: G-33-349

Title: Comparative Raman Studies of Human and Animal Lenses

ADMINISTRATIVE DATA OCA Contact Frank Huff

1) Sponsor Technical Contact:

Henry N. Fukui, Ph.D.
Extramural Program Director
Cataract Program
National Eye Institute

2) Sponsor Admin/Contractual Matters:

Garry R. Sanders
Grants Management Specialist
Extramural Services Branch
National Eye Institute

(301) 496-5884

Defense Priority Rating: NA Military Security Classification: NA

(or) Company/Industrial Proprietary: _____

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: 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.

Equipment: Title vests with _____

COMMENTS:

Continuation of G-33-A07


COPIES TO:

Research Administrative Network
Research Property Management
Accounting
Procurement/EES Supply Services
Research Security Services
Reports Coordinator (OCA)
GTRI
Library
Research Communications (2)
Project File
Other Yu
Other I. Newton

FORM OCA 4:781 (Rev 982)

EORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date April 25, 1985

oject No. G-33-A08 School/~~Lab~~ Chemistry

cludes Subproject No.(s) N/A

oject Director(s) Nai-Teng Yu GTRC / ~~GTI~~

ponsor DHHS/PHS/National Eye Institute

itle Comparative Raman Studies of Human and Animal Lenses

ffective Completion Date: 4/30/84 (Performance) 7/31/84 (Reports)

rant/Contract Closeout Actions Remaining:

NOTE: Annual report submitted as part of Renewal Application for G-33-A09

- [X] None
- [X] Final Invoice or Final Fiscal Report - already submitted
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

ontinues Project No. G-33-A07 Continued by Project No. G-33-A09

OPIES TO:

oject Director
esearch Administrative Network
esearch Property Management
ccounting
rocurement/GTRI Supply Services
esearch Security Services
eports Coordinator (OCA)
egal Services

Library
GTRC
Research Communications (2)
Project File
Other Heyser

Jones

ORM OCA 69.285

G-33-A08

| SECTION IV<br>PROGRESS REPORT SUMMARY | GRANT NUMBER<br>EY01746-09 | |
|---|---|---|
| PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR<br>Yu, Nai-Teng | PERIOD COVERED BY THIS REPORT<br>FROM<br>05/01/83 | THROUGH<br>02/20/84 |
| NAME OF ORGANIZATION<br>Georgia Institute of Technology | | |

TITLE *(Repeat title shown in item 1 on first page)*
Comparative Raman Studies of Human and Animal Lenses

*(SEE INSTRUCTIONS)*

Publications:


1. Yu, N.-T., Bando, M. and Kuck, J. F. R., Jr. (1983) "Metabolic Production of a Blue-Green Fluorophor in Lenses of Dark-adapted Mice and Its Increase with Age" Invest. Ophthalmol Vis Sci 24, 1157-1161.

2. Bando, M., Yu, N.-T. and Kuck, J. F. R., Jr. (1984) "Fluorophors and Chromophores from Rat Lens Crystallins in UV with Hydroxykynurenine" Invest. Ophthalmol Vis Sci (in press).


Two copies each are provided with this application.

Progress Report:

1. General scientific goals of the project during the budget year: no change.

2. Concise description of the progress:

(a) We have taken a major step in the instrumental development for an automated Raman/fluorescence microprobe surface scanning system. The modified Zeiss Universal microscope has been coupled to a Spex 1870 spectrometer. Laser can be focused to less than 2 micrometer spots into cataractous lens specimen viewed under a video camera; microscope has digital-controlled X-Y stage. The Raman/fluorescence light is detected by a PAR model 1420 intensified photodiode array multichannel image detector, which is controlled by an IBM PC microcomputer. Two documents are attached: one describes the nature of the problem and the proposed solution and the other reports the accomplishments we have made in the development of the automated Raman/fluorescence microprobe system. Mr. Fred Thompson has played a major role and will continue the task until its completion. We expect to perform preliminary tests soon and hope to start collection of Raman/fluorescence profile data during the next grant period.

(b) We have investigated the effect of long wave UV light (in vivo) on the sulfhydryl profiles along the visual axis of mouse lenses. As shown in Fig. 1, we have identified the maximum effect occurring near the centers of both anterior and posterior cortex. The most interesting finding is that the effect on the posterior side is even slightly greater than that on the anterior side despite the fact that UV light enters the anterior side first before it strikes the posterior portion. Furthermore, the effect near the center of lens nucleus is very small or insignificant. Since the UV effect on the pre-existing lens crystallin in the nucleus is negligible, we conclude that the effect observed in both anterior and posterior cortex is probably caused by altered protein synthesis as a result of UV action on lens epithelium. Isoelectric-focusing analysis of control and UV-irradiated mouse lenses is now in progress.

(c) We have made non-invasive quantitative measurement of disulfide bonds by laser Raman optical dissection technique. We have obtained a series of disulfide profiles along the visual and equatorial axes of mouse lenses ranging in age from 26 days to 8 months. Two groups of cataract-resistant mice were employed: one group raised in a room with normal lighting conditions and the other under continuous near UV exposure. The bell-shaped profiles were obtained with maxima near the center of the lens nucleus.

(d) The lenses of guinea pig between 2 weeks and 4 years of age have been investigated. Although guinea pig is also a rodent, unlike mouse and rat lenses, there is no appreciable formation of disulfide bonds as a result of the normal aging process. Similar to human lens, the rate of -SH decrease is slow and there are no central minima in the -SH profiles.

3. Specific objectives for the coming year:

(a) To complete the development of our automated Raman/fluorescence microprobe digital scanning instrumentation.

(b) To obtain 2-dimensional Raman/fluorescence -SH and fluorophor profiles of normal and catactous human lenses.

(c) To implement an important collaborative research with Dr. Christine Slingsby (Birkbeck College, University of London) involving Raman studies on the reactivities of SH groups in bovine γ-II crystallin. Dr. Slingsby plans to visit my lab. at Georgia Tech to carry out the following experiments:

i) The spectrum of bovine γ-II after reduction with dithiothreitol followed by extensive dialysis. This should give a signal at 2580 $cm^{-1}$ equivalent to seven SH groups/molecule.

ii) A spectrum which is the same sample as (1) only having been left for approximately three weeks. This would prove whether or not an internal disulfide has formed.

iii) A spectrum of γ-crystallin II isolated under normal conditions yet not pre-treated with dithiothreitol. This would indicate whether or not the molecule had formed mixed disulfides with 2-mercaptoethanol.

iv) Spectrums (i), (ii) and (iii) will be compared with one from a sample of γ-II extracted and isolated with no reducing agents in buffers.

v) A spectrum of γ-II reacted with 2 or 3 equivalents of glutathione.

vi) A spectrum of γ-II reacted with glutathione, then ethyl mercury chloride, then dithiothreitol.

vii) A spectrum of "native" β $L_2$ dimer and a reaggregated dimer.

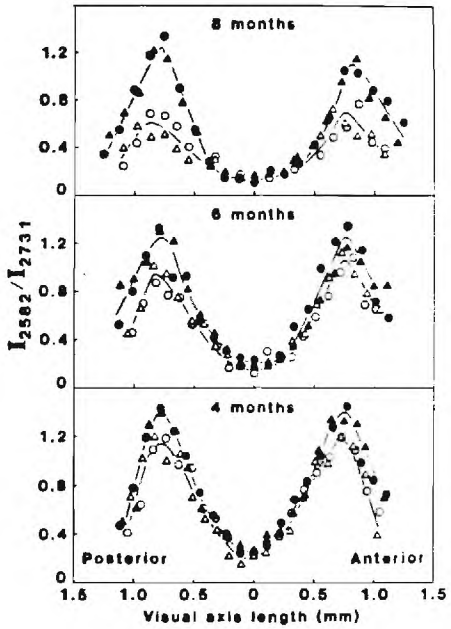


Figure 1

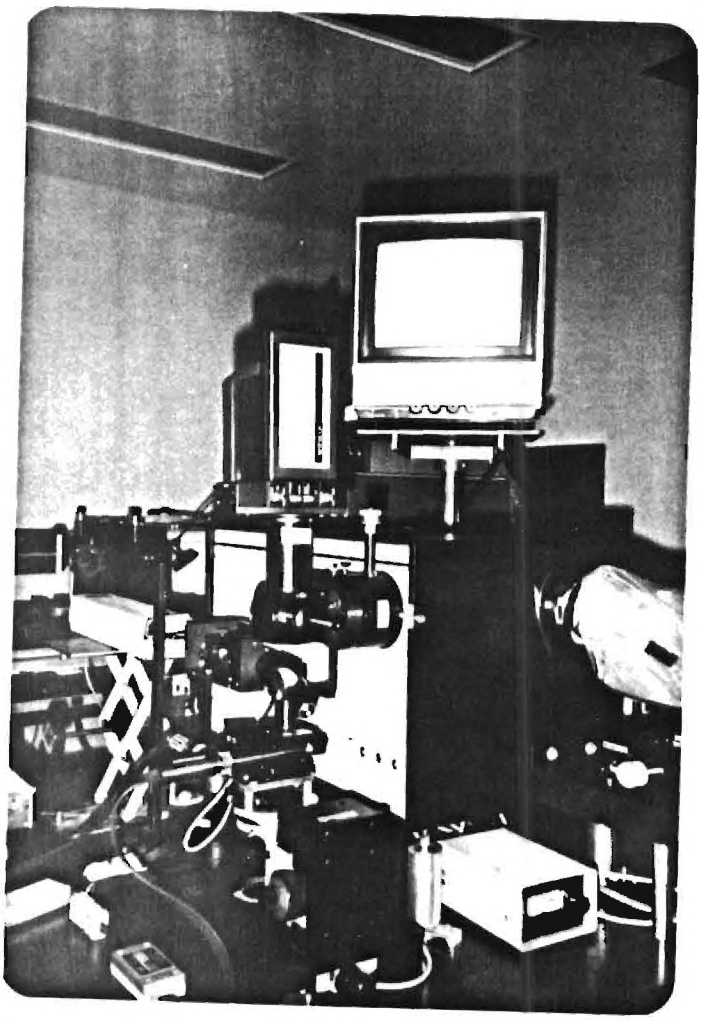

Figure 2
Automated Digital Scanning Raman/Fluorescence Microscope System. The IBM PC microcomputer is behind the spectrometer.

OMB No. 0925-0637

DEPARTMENT OF HEALTH AND HUMAN SERVICES

**PROTECTION OF HUMAN SUBJECTS ASSURANCE/CERTIFICATION/DECLARATION**

☐ ORIGINAL ☒ FOLLOWUP ☐ EXEMPTION (previously undesignated)

☒ GRANT ☐ CONTRACT ☐ FELLOW ☐ OTHER

☐ New ☐ Competing continuation ☒ Noncompeting continuation ☐ Supplemental

APPLICATION IDENTIFICATION NO. *(if known)*

EY01746-09

*POLICY: A research activity involving human subjects that is not exempt from HHS regulations may not be funded unless an Institutional Review Board (IRB) has reviewed and approved the activity in accordance with Section 474 of the Public Health Service Act as implemented by Title 45, Part 46 of the Code of Federal Regulations (45 CFR 46–as revised). The applicant institution must submit certification of IRB approval to HHS unless the applicant institution has designated a specific exemption under Section 46.101(b) which applies to the proposed research activity. Institutions with an assurance of compliance on file with HHS which covers the proposed activity should submit certification of IRB review and approval with each application. (In exceptional cases, certification may be accepted up to 60 days after the receipt date for which the application is submitted.) In the case of institutions which do not have an assurance of compliance on file with HHS covering the proposed activity, certification of IRB review and approval must be submitted within 30 days of the receipt of a written request from HHS for certification.*

1. TITLE OF APPLICATION OR ACTIVITY

Comparative Raman Studies of Human and Animal Lenses

2. PRINCIPAL INVESTIGATOR, PROGRAM DIRECTOR, OR FELLOW

Yu, Nai-Teng

3. FOOD AND DRUG ADMINISTRATION REQUIRED INFORMATION *(see reverse side)*

4. HHS ASSURANCE STATUS

☒ This institution has an approved assurance of compliance on file with HHS which covers this activity.

G0520 Assurance identification number _____ IRB identification number

☐ No assurance of compliance which applies to this activity has been established with HHS, but the applicant institution will provide written assurance of compliance and certification of IRB review and approval in accordance with 45 CFR 46 upon request.

5. CERTIFICATION OF IRB REVIEW OR DECLARATION OF EXEMPTION

☒ This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This certification fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device. *(See reverse side of this form.)*

September 29,1982 Date of IRB review and approval. *(If approval is pending, write "pending." Followup certification is required.)*
*(month/day/year)*

☒ Full Board Review ☐ Expedited Review Current review "Pending"

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by 45 CFR 46 will be reviewed and approved before they are initiated and that appropriate further certification *(Form HHS 596)* will be submitted.

☐ Human subjects are involved, but this activity qualifies for exemption under 46.101(b) in accordance with paragraph _____ *(insert paragraph number of exemption in 46.101(b), 1 through 5)*, but the institution did not designate that exemption on the application.

6. **Each official signing below certifies that the information provided on this form is correct and that each institution assumes responsibility for assuring required future reviews, approvals, and submissions of certification.**

| APPLICANT INSTITUTION | COOPERATING INSTITUTION |
|---|---|
| NAME, ADDRESS, AND TELEPHONE NO.<br>GEORGIA TECH RESEARCH INSTITUTE<br>Georgia Institute of Technology<br>Atlanta, GA 30332<br>404/894-4815 (Office of Contract Adm.) | NAME, ADDRESS, AND TELEPHONE NO. |
| NAME AND TITLE OF OFFICIAL *(print or type)*<br>Faith G. Costello, Contracting Officer | NAME AND TITLE OF OFFICIAL *(print or type)* |
| SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)*<br>2/24/84 | SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)* |

HHS 596 (Rev. 1/82)

*(If additional space is needed, please use reverse side under "Notes")*