



UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

The Graduate School
Grant and Contract Services

December 20, 1984

850-077

DOE University Research Instrumentation Program
Energy Program and Support Division
U. S. Department of Energy
Oak Ridge Operations
P.O. Box E
Oak Ridge, Tennessee 37831

85-02-077

Ladies and Gentlemen:

Enclosed are three proposals prepared by Professor Cornelius Rosse and Professor Karen A. Holbrook, Department of Biological Structure, and Research Associate Professor Barbara M. Hickey, School of Oceanography, requesting support under the "DOE University Research Instrumentation Program 1985."

Funding is requested as follows:

Dr. Rosse, \$103,703 for the period of July 1, 1985 through June 30, 1986;
Dr. Holbrook, \$160,710 for the period July 1, 1985 through June 30, 1986;
and Dr. Hickey, \$278,855 for the period May 31, 1985 through April 30, 1986.

A proposal for a fourth investigator, who was selected under the guidelines, is planned to be forwarded soon.

To provide complete information requested on the cover page of the application, we have attached a list of all University of Washington D.O.E. awards active as of December 1, 1984. We hope this information is helpful as you review these proposals.

It is a pleasure to submit these proposals for your consideration.

Sincerely,

BEST COPY AVAILABLE

Donald W. Allen
Donald W. Allen, Director
Grant and Contract Services

DWA/th
Enclosure

Please indicate Principal Investigator's name on all correspondence.

REPOSITORY Oak Ridge Operations
COLLECTION Records Holding Area
BOX No. B-41-7 Bldg. 2714-H
FOLDER 85-02-077

U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

COVER PAGE
(THIS PAGE MUST BE THE FIRST PAGE OF THE APPLICATION)

8507077

1. Name of Institution: University of Washington
2. Address: City Seattle 3. State WA 4. ZIP 98195
5. Principal Investigator: Karen A. Holbrook, Ph.D.
6. Department: Biological Structure
7. Telephone: Area Code 206 Office: 543-1860 Home: [REDACTED]
8. Title of Application: Image Analysis Microscope
9. Research Area: Health and Environmental Impact of Energy-Related Chemicals
10. Total DOE Funding for Research in Selected Area, (During the last two calendar years): \$ 432,378
11. Estimated Purchase Cost of Equipment: \$ 200,762
12. Amount Requested from DOE: \$ 160,710

List all Federal agencies which are currently considering proposals from the institution involving the same or similar equipment.

13. Agency: none Agency Proposal Number: N.A.
14. Agency: none Agency Proposal Number: N.A.

NOTE: The institution is responsible for informing DOE if a proposal involving the same or similar equipment is submitted to a federal agency prior to the announcement of DOE's URI awards.

15. List any federal agency which has provided funds to the institution during the past two years for the same or similar equipment.

Agency: none Amount of Funds: none

16. Please check one of the following :

I authorize external review of this proposal.

I do not authorize external review of this proposal.

Signature of Principal Investigator: Karen A. Holbrook Date: 12/19/84

Name and Title of Institutional Official
(President or Designee)

Donald W. Allen, Director

Grant and Contract Services

Signature: Donald W. Allen

Date: 12/20/84

Name and Title of Financial Officer

Donald W. Allen, Director

Grant and Contract Services

Signature: Donald W. Allen

Date: 12/20/84

U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

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Grant and Contract Services

Signature: Donald W. Allen

Date: 12/19/84

1076108

U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Share (1)	Other Federal Funds (2)	TOTAL
A. Purchase Price (3)	<u>160,710</u>	<u>39,552</u>	<u> </u>	\$ <u>200,262</u>
B. Estimated Cost Sharing (Shipping, installation, etc.)	X X X X	<u>500</u>	<u> </u>	<u>500</u>
C. TOTAL	<u>160,710</u>	<u>40,052</u>	<u> </u>	\$ <u>200,762</u>

NOTES: (1) Non-Federal funds only - 20% of equipment cost

(2) Estimate funds to be obtained from other Federal agencies for purchasing the instrument, user charges, etc.

(3) Only the purchase price of the instrumentation is eligible for DOE funding through this program.

A. Purchase Price (Detail)

Description	Quantity	Total Estimated Unit Price	Total
1. Magiscan 2 A Image Analysis System Complete Basic Unit as Per the Attached Specifications and additional software update	1	\$ 103,000	\$ 103,000
2. Banded Chromosome Software Program Product No. 79655	1	30,000	30,000
3. Nikon Research Microscope "Microphot" Estimated price, based on configuration	1	25,000	25,000
4. Motorized Stage, Product No. 77702	1	6,500	6,500
5. Focus Module, Product No. 79624	1	3,600	3,600
6. Motor Controller, Product No. 79623	1	6,000	6,000
7. Dage SIT, Product No. 79629	1	8,000	8,000
8. IEEE 488 Interface, Product No. 79625	1	3,500	3,500
		Subtotal	\$185,600
		Sales Tax WSST (.079)	14,662
Subtotal:			<u>\$200,262</u>

B. Estimated Cost Sharing (Detail)

	Institution's Share	Other Federal Funds	Total
1. Shipping/Handling	<u>\$ 500</u>	<u> </u>	<u>\$ 500</u>
2. Installation	<u> </u>	<u> </u>	<u> </u>
3. Building/Laboratory Renovation	<u> </u>	<u> </u>	<u> </u>
Subtotal:	<u>\$ 500</u>	<u> </u>	<u>\$ 500</u>

DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
CONTRACT SUMMARY FORM

Contract/Subcontract No.	Principal Investigator	Title	Contract Dates		Total Award Value	\$ AMT Awarded for period 19 to 19	DOE Technical Monitor/Location
			MO YR	FM TO MO YR			
AT06-79EV10270, MD10	Cornelius Rosse	Marrow-Tumor Interactions, The Role of Bone Marrow in Controlling Chemically Induced Tumors	01 84	12 85	450,378		
AT06-82ER60069, MD1	Lawrence A. Loeb	DNA Damage in Human Cells	09 82	08 85	277,707		
AT06-81ER10879, MD4	Benjamin D. Hall	Genetic Engineering of Yeasts for Fermentation of Xylose to Ethanol	04 84	03 86	70,000		
AT06-80-ER10680, MD3	Mary E. Lidstrom	Genetics in Methylo-trophic Bacteria	02 84	01 87	70,000		
					\$868,085		

*There are the DOE-Sponsored Grants and Contracts pertaining to Health and Environmental Impacts of Energy Related Chemicals in the School of Medicine, University of Washington. 81 additional Grants and Contracts are sponsored by DOE at the University of Washington.

RELATED FEDERAL AGENCY CONTRACTS

Agency	(Grant No.) Contract No.	Title	Contract FROM MO YR	Dates TO MO YR	Total Award Value
NIH	CA 20823	Cornelius Rosse, MD DSc Lymphocyte Production and Traffic in the Bone Marrow	4 80	3 85	\$ 394,424
NIH	AI 19696	Lai-Ming Ching, PhD Cytotoxic T Lymphocyte Precursors In Vitro	1 83	12 85	103,700
NIH	AG 04360	Andrew Farr, PhD Age Dependent Modulation of T Cell Function	7 83	6 86	175,473
March of Dimes	5-455	Brian Hamilton, MD PhD Bone Marrow Transplantation- Effect of Graft-versus Host Disease on Immunologic Reconstitution	9 84	8 86	50,000
NIH	CA 32757	Brian Hamilton, MD PhD Mechanisms of Minor-H Antigen GVHD	7 82	6 85	107,500
NIH	CA 39889	Brian Hamilton, MD PhD Marrow Transplantation: Immune Dysfunction in GVH	1 85	12 87	285,509
NIH	CA 38189	Minako Lee, MD Bone-Bone Marrow Interaction	6 84	5 87	187,707
NIH	CA 32553	Sylvia Pollack, PhD Specific Anti-Tumor Activity of Armed Lymphoid Cells	12 83	11 87	353,448
NIH	CA 37006	Sylvia Pollack, PhD Regulatory Interactions of NK Cells with B Cells	3 84	2 87	204,254

RELATED FEDERAL AGENCY CONTRACTS

Agency	Contract No.	Title	Contract FROM MO YR	Dates TO MO YR	Total Award Value
NIH	EY 04542	John I. Clark, Ph.D. Cell Aging and the Early Stages of Cataract Development	6 84	5 85	82,551
NSF	PCM-8409156	Marilyn S. Hamilton, Ph.D. Oncofetal Immunogens of Preimplantation Embryos	8 84	1 86	84,000
NIH	GM 22759	Robert P. Bolender, Ph.D. Analytical Morphology and Biochemistry of Hepatocytes	5 84	4 85	53,938
NIH	HD 17664	Karen A. Holbrook, Ph.D. Fetal Skin Biology	4 84	3 85	201,179
NIH	AM 21577	Karen A. Holbrook, Ph.D. Interdisciplinary Basic Research in Dermatology	12 84	11 85	64,386
NIH	EY 01208	Anita E. Hendrickson, Ph.D. Primate Visual System Development and Deprivation	1 84	12 84	116,049
NIH	AM 28154	Mark Nameroff, M.D., Ph.D. Control of Differentiation in the Myogenic Lineage	4 84	3 85	83,330
NIH	HL 29761	John W. Prothero, Ph.D. Fiber Pathways in Hearts	1 84	12 84	81,634
NIH	AG 01751	John W. Prothero, Ph.D. Gene Action in the Pathobiology of Aging	8 84	7 85	39,075

SUMMARY

a. This application requests funding for the purchase of a NIKON/MAGISCAN 2A IMAGE ANALYSIS MICROSCOPE manufactured by Nikon/Joyce-Loebl, Ltd. The equipment is needed to provide a new dimension to research programs of cellular and molecular biology located in the Department of Biological Structure, School of Medicine, University of Washington. Fifteen of these programs are described in this application pertaining to the areas of Cellular and Developmental Biology, Experimental Immunology and Hemopoiesis, Neurobiology, Reproductive Biology and Stereology and Morphometrics. All of the programs are federally supported; three by DOE, the others by NIH or NSF.

b. The image analysis microscope will be used for solving six types of basic problems, many of which overlap several of the fifteen programs described in this application.

i. Textural analysis of short-range order in cytoplasmic proteins that contribute to the development of transparency in the cells of the lens and cornea of the developing eye or to the development of cytoplasmic opacity during cataract formation.

ii. Quantitation of changes in gene products expressed in the cytoplasm or the cell membrane during development or during cell differentiation and maturation in renewing cell systems. The cell systems that will be investigated include fetal skin, various hemopoietic cells and tissues, lymphocytes of the various subclasses, muscle cells, gametes, and early embryos. Quantitative analysis will be based on fluorochrome or immunoperoxidase labeled antibodies specific for gene products in these systems.

iii. Cell kinetic studies relying on radioautographic detection of labeled DNA precursors through grain counts. The technique will be used for defining cell lineage relationships in the myogenic system, in the fetal epidermis, in the maturation and differentiation program of T lymphocytes and natural killer cells.

iv. Quantitation of distinct karyotypes in cell transfer and cross circulation experiments designed to study cellular traffic and precursor-progeny relationships. The distinct shapes of the T6T6 marker chromosomes in CBA mice are ideal for automated image

analysis of chromosome spreads and will be employed in cellular immunology, tumor immunobiology, experimental bone marrow transplantation, and experimental hematology.

v. Clonal analysis of myogenic and connective tissue cell precursors combined with radioautography and fluorochrome labeled antibody detection of gene products.

vi. Quantitation of changes in tissue mass or cell size and the distribution of cellular and subcellular elements caused by various types of cellular injury (chemical, pharmacological, immunological, or neoplastic injury). The biological systems investigated will include hemopoietic tissues and bone, the thymus, lung, liver, pancreas and the retina.

c. Acquisition of the NIKON/MAGISCAN 2A image analysis microscope will have a very positive impact on both the quantity and quality of research performed in cellular and molecular biology, neurobiology, reproductive biology, experimental immunology and hemopoiesis, and stereology and morphometrics. The instrument will replace slow and error-prone manual data collection from images and will provide the capability for quantitating various gene products in relation to the three-dimensional architecture of cells and tissues, a task which is not possible to accomplish with other methods.

d. Graduate student participation as research assistants is an integral part of all the programs described. The number of graduates from Biological Structure is shown in Appendix II. There are 14 students currently enrolled whose work will immediately benefit from the acquisition of the image analysis microscope.

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NARRATIVE

Commitments, Accomplishments and Potential of the Program in the Area of HEALTH AND

ENVIRONMENTAL IMPACTS OF ENERGY-RELATED AGENTS

Commitments. The Department of Biological Structure at the University of Washington has held a research contract with the Department of Energy and its predecessors (ERDA, AEC) uninterruptedly since 1957. Until 1979 the Principal Investigator of the successively renewed contracts was Dr. Newton B. Everett, Chairman of the Department of Biological Structure. The focus of the "DOE"-funded research was lymphocyte biology and hemopoietic stem cell-related research. The present Chairman of the Department, Dr. Cornelius Rosse, became involved with this research as a co-investigator in 1968; in 1979, following the death of Dr. Everett, he assumed the responsibility for the then current contract. In 1980 a new contract was awarded by DOE to Dr. Rosse with the new focus of elucidating cellular defense mechanisms of the host that are engaged in the recognition and neutralization of environmentally induced neoplasms. Under Dr. Rosse's leadership this program has grown significantly. The growth is reflected in the recent establishment of a Biological Structure Cancer Research Laboratory constructed with funds obtained from NCI and the School of Medicine, University of Washington, as well as the recruitment of faculty with specific interests in the area of the DOE-sponsored research.

This brief account of the long history of DOE-sponsored research in the Department of Biological Structure should indicate not only the impressive growth of the program but also the substantial, long-lasting and tangible commitment of the Department and of the School of Medicine to this program.

Accomplishments. The Department of Biological Structure has distinguished itself among anatomy departments through both its teaching and research programs. Research in the Department consists of six multi-investigator programs: 1) cellular and developmental biology, 2) experimental immunology and hemopoiesis, 3) reproductive biology, 4) neurobiology, 5) molecular structure and 6) morphometrics and computer

modeling. In each of these areas of science the Department has gained national and international prominence. To name a few of these accomplishments, one may point to:

1) The pioneering use of radioactive DNA precursors in the study of hemopoietic and immune cell production and lifespan through the DOE sponsored program. The same program has focused attention on the central role of the bone marrow in the production of lymphocytes and other cells that participate in host defense against cancer.

2) The program in fetal skin biology has taken a multifaceted approach to establish standards for the diagnosis of congenital skin abnormalities through fetal skin biopsy.

3) Studies using direct observation and cinematographic record of live gametes have contributed greatly to the understanding of such basic processes in reproduction as gamete maturation, mechanisms of gamete transport and in vitro fertilization.

4) The Department has been a leader in developing techniques for examining biological material with electron microscopic techniques and our recent emphasis of quantitating morphological data (stereology, morphometry) has provided a new aspect to this work which is widely recognized.

5) The structure of metalloproteins and other biologically relevant molecules has been solved by X-ray diffraction and refined analysis relying on computer modeling.

Potential. Because of the focus of the DOE-sponsored program on such fundamental phenomena as cell differentiation, cell production, cell migration and cell interactions, DOE-sponsored research exerts a significant influence on departmental research programs concerned with the cell biology of the intercellular matrix, fetal skin biology, cell differentiation in skeletal muscle and connective tissue, mechanisms of cataract formation in the lens of the eye, reproductive biology and macromolecular structure.

The overall environment in the Cancer Research Laboratory, as well as in the whole Department, is very conducive to innovation and to the adaptation and exploitation of

new techniques and approaches. Many members of the faculty are only just beginning to realize their potential in research and academia; this is being borne out by the continuing success in obtaining funding for newly submitted grant applications.

Description of Equipment Requested and the Need for It

This application requests funding for the purchase of a fully automated image analysis system to be used primarily in research programs of cellular and molecular biology located in the Department of Biological Structure, University of Washington. The instrument of choice is the NIKON/MAGISCAN 2A manufactured by Nikon/Joyce-Loebl. A detailed description and the specifications of this unit are attached (Appendix I).

The potential and usefulness of automated image analysis for the quantitative study of biological structure has been recognized and advocated during the past decade (5,32). Only recently have programs become available on the market that are adaptable to the varied research programs in the Department of Biological Structure (9).

In many of the research programs in this Department, data collection from images is the rate-determining step. Many experiments have been ruled out at present because of the large investment of time data collection requires. Collecting data and performing the first stages of analysis with a fully automated image analyzer such as the NIKON/MAGISCAN 2A will greatly accelerate this research and will extend its scope by providing the capability for the quantitative analysis of biological structure not afforded by other approaches.

The NIKON/MAGISCAN2A has several features that make it ideally suited for the broad application it will have in this Department. The MAGISCAN 2A is based on a high-speed, central processing unit that is custom designed for image analysis, whereas other systems we have considered are based on conventional microprocessors not optimized for this task. Since the machine will be used by many researchers with diverse needs, a key requirement is that the operating system be based on a high-level language. This will enable our programmers to efficiently generate sophisticated and accurate software. The

system software that controls the MAGISCAN 2A is based on the standard, high-level UCSD Pascal operating system. Software is already available from Nikon/Joyce-Loebl to perform several of the analyses that we need. Other software will be developed by us and by other users. Finally, the MAGISCAN 2A can accept a full range of options and attachments to allow for a broad range of data acquisition and communication. It can directly control the motorized stage and focus on an optical microscope fitted with a camera, as well as acquire images from photographic prints and films or other image sources. For data communications from other computers, it can be equipped with both a standard RS-232C serial port and a high-speed IEEE-488 data channel. It is well designed to collect and analyze biological images quickly and flexibly and to transmit such data efficiently to other machines for further analysis.

The Department will be purchasing a VAX 11/780 computer (Digital Equipment Corporation) to which much of the image data will be sent for further analysis. An automatic image analyzer is optimized for the collection of images and performing manipulations on whole images. A VAX 11/780 is suited for high speed mathematical and statistical analysis. By using both capabilities in parallel we will be able to effectively use the image datasets that the image analyzer will generate. This ability to perform exhaustive analysis on images (impossible without both an automatic image analyzer and a computer) will greatly enhance the research programs in this Department.

Description of Research Sponsored by DOE and Other Federal Agencies

The six areas of research in the Department of Biological Structure (described in the section "Accomplishments") deal with the differentiation and production of diverse cell types in a number of biological systems. Although the experimental approaches and the cell types investigated are quite varied, essentially all research programs conducted in the Department are concerned with obtaining quantitative data from images. Many experiments rely on images generated by various modalities of light and electron microscopy that contain, in addition to structural information, silver grains

(radioautographs) and reaction products (histochemical, cytochemical, immunochemical), chromosome spreads (for karyotyping) and cell clones. The recent development of a number of biological reagents such as ligands and monoclonal antibodies has made accessible for structural analysis a number of biologic - and especially developmental - processes that could not be contemplated until quite recently. The possibility for the automated analysis of microscopic images gives a new dimension to these studies because it makes it feasible to quantitate various gene products and biological molecules in relation to the three-dimensional architecture of cells and tissues.

The confines of this URI application permit only a sampling of the specific projects that would immediately become feasible through the establishment of a departmentally operated Image Analysis Facility. The 15 projects described below have been selected to illustrate both the breadth of the research and the varied applications of the image analysis microscope that would bring about a qualitative change in the research program of the Department of Biological Structure.

Research Program No. 1: Development of Morphometric Methods for Evaluating the Effects of Drugs, Toxins, and Trauma on Cells.

Principal Investigator: Robert P. Bolender, Ph.D.

The overall objective of this program is to use methods in quantitative morphology (stereology and serial section reconstructions) to describe - as reliably as possible - structural changes in cells (4). In turn, mathematical models are used to combine morphometric and biochemical data in order that relationships of structure to function can be defined with equations. Structure-function equations are used to access the effects of drugs, toxins, and trauma on cells and to evaluate treatment and recovery protocols. The model systems investigated in this program are focussed primarily on the liver and pancreas. The work is supported by two grants from NIGMS (P.I. Bolender).

To obtain accurate ultrastructural information from cells, the number of cells in a tissue or organ must be known. Obtaining cell frequencies requires information coming

from stereology and serial section reconstructions. Using specially stained sections, nuclear counts will be collected with the MAGISCAN 2A, sorted, and analyzed. Such digitized information will be used for reconstructing the tissue architecture from sections and determining the number of nuclei in a given volume of tissue. These reconstructions are currently being done manually for most of the studies on the liver and pancreas, which limits both the scope of the analysis and the number of parameters that can be built into the study.

One of the methods for assessing responses of cells to experimental settings is to determine the distribution of nuclear profiles in sections, using methods of two- and three-dimensional pattern analysis. Since the MAGISCAN 2A can give x-y coordinates for nuclear profiles in sections, quantitative information about the distribution patterns of nuclei will be readily obtained.

The MAGISCAN 2A is well suited for these studies and data collected with this system can be easily integrated into our existing computer programs. Moreover, the MAGISCAN 2A will allow us to expand our data bases and to do studies that are not currently practical because they are too labor intensive without an automatic image analyzer.

Research Program No. 2: Lung Morphology in Health and Disease

Principal Investigator: John F. Bertram, Ph.D

Co-Investigator: Robert P. Bolender, Ph.D

The aim of this research is to characterize in quantitative terms the cellular morphology of the normal mammalian lung and then assess how this morphology is modified following injury (sepsis, acid aspiration). Our morphological studies form part of a Program Project awarded by the NIH. The broad purpose of this project is to investigate aspects of lung trauma from a cell biological perspective.

In addition to morphometric (quantitative morphologic) information, biochemical and physiological data are also being collected from the lungs. Integration of structural and functional data will provide a detailed description of how modification of

metabolism and function in specific cell populations translates into dysfunction of the total organ, as exemplified by conditions such as pulmonary fibrosis and edema.

The principal morphometric approach we have used is stereology (3). An image analysis system will not only accelerate the collection of data, but will also enable us to measure parameters which cannot be measured by any other means. With a large data base, univariate and multivariate statistics will be used to identify subtle morphological differences between normal and pathological tissues.

Fully automated image analysis systems have previously been used by Dr. Bertram in studies of pulmonary morphology at the University of Western Australia and were found to be extremely efficient. In projected studies at the light microscopic level, an image analyzer will be used to measure the harmonic mean thickness of the interalveolar septum, mean alveolar diameter (known to change in emphysema) and the number of nuclear profiles per unit area of tissue (to investigate cellular hyperplasia). In studies of tracheal, bronchial and bronchiolar epithelium, measurements will be made of the volume proportion (density) of mucus (known to be altered in chronic bronchitis), and the number of nuclear profiles per unit area of sectioned epithelium. These data will then be integrated with biochemical and physiological data.

We will also assess the ability of the image analyzer to quantify transmission electron micrographs. Previous analyzers have had difficulty discriminating between the various organelles resolved by electron microscopy. With the improved optics and software of the NIKON MAGISCAN 2A we will, for the first time, be able to make a serious attempt to automatically quantitate electron micrographs of biological tissues. A great deal of software development will be required for this task. The development of such software is quite feasible with the expertise and resources of our research group.

Research Program No. 3: Development of Cytoplasmic Transparency and Opacity in the Lens of the Eye

Principal Investigator: John I. Clark, Ph.D.

The overall objective of this program is the understanding of factors that lead to and maintain lens transparency in the eye. Two approaches are being used: 1) transformation of the opaque embryonic lens into a transparent structure; and 2) prevention of cataract formation in postnatal lenses. The work is supported by a grant from the Eye Institute, NIH. (P.I. Clark).

Transparent cells have a uniform structure and opaque cells have inhomogeneous structure which appears as rough texture in the light microscope. The quantification and localization of the transparent and opaque regions of developing lenses can be accomplished using automated image analyzing light microscopy which allows scans of sections of embryonic lenses. For development of lens cell transparency, short range order must be established in cytoplasmic proteins. An accurate measurement of short range order can be obtained with textural analysis, using second order grey level statistics and transforms. The basis of this approach is to plot optical density versus area profile. In previous work this program has demonstrated that temperature dependent phase transitions of lens cell cytoplasm can lead to reversible changes of lens transparency in vitro (6). It has been assumed that such phase transition-induced losses of lens transparency involve loss of short range order by cytoplasmic proteins. Densitometric analysis of cells subjected to conditions inducing phase transitions will generate the first quantitative and temporal data regarding this process.

The MAGISCAN 2A is particularly well suited for these studies because it can recognize 256 different density levels and can generate optical density profiles on line scans of operator-defined length. Correlating these data with biochemical, biophysical and electron microscopic information will provide a valuable data base for defining the alterations in adult lens cells that lead to opacification and cataract formation.

Lens sections will be reconstructed into three dimensional representations of whole lenses at different stages of development. This approach will allow the staging of the development of transparency in human embryonic lenses. For the 3-D reconstructions, the output of the MAGISCAN 2A will be interfaced with the VAX 11/780 computer and MORPHO software. The phase contrast, interference contrast and polarizing capabilities of the NIKON microscope integrated with the image analysis system will be a great asset in this research program.

Research Program No. 4: Cellular Aging in the Visual System

Principal Investigator: Christine A. Curcio, Ph.D.

Co-Investigator: Anita E. Hendrickson, Ph.D.

The major goal of this research program is to find a cellular basis for the declines in visual function which occur in the latter half of the human lifespan. Therefore, two specific projects have been initiated to determine to what extent the neural retina may be involved in these events: 1) a quantitative description of the distribution of rods and cones in the normal adult human retina; and 2) an investigation of whether quantitative changes in the photoreceptor population occurs with advanced age. The work is supported by the National Eye Institute and the Lions Sight Conservation Foundation.

That quantifying photoreceptors is an exceptionally arduous task when done manually is indicated by the fact that it has been done exhaustively for only one human eye (24). A major problem for a human observer studying histological sections of the retina is the geometric regularity of the receptor mosaic. Thus, observer error due to tedium and disorientation during counting is substantial. Interesting and important questions pertaining to individual variability, receptor density along the vertical meridian, and the possibility of a human visual streak remain unexplored to date because of the large amount of tissue required for a satisfactory answer.

The advent of Nomarski differential interference contrast optics (DIC) allows the photoreceptor mosaic to be visualized in a fresh-fixed, flattened retina mounted in

glycerol. This preparation has several advantages: 1) the retina can be preserved and analyzed in its entirety rather than in small histological samples; 2) tissue distortions introduced by histological processing which render interpretation of spatial data difficult are virtually eliminated; 3) this tissue remains available for a variety of other preparations such as electron microscopy or stained whole mount. Thus, the acquisition of a microscope with Nomarski DIC optics, such as the proposed NIKON, will greatly increase the quality of the information that can be gained from each retina.

The nature of the Nomarski image of the tightly packed photoreceptor mosaic suggests that automated separation of the individual cells may be difficult, but the capacity of the MAGISCAN 2A system for higher order analysis of grey levels is likely to allow us to treat the mosaic as a texture amenable to Fourier transforms. The outcome of such an analysis will be the size of individual cells from which receptor density (cells per mm^2 of retina) can be derived. Thus, the proposed image analysis system will allow us to sample more tissue for each retina in order to generate a more accurate density function.

The proposed image analysis system has several features which will allow us to analyze and display information about photoreceptor density in a much more informative way than previously achieved. Traditionally these kind of data are placed into a retinal map by building a model of the retina, which is not portable and does not allow quantitative comparisons among several eyes. We wish to generate a digital map of photoreceptor density. Thus, it is important that the location of each density sample be preserved. The MAGISCAN 2A system reads x-y stage position in digital form which can be entered into the computer. These Cartesian coordinates can then be transformed into a foveocentric polar coordinate system more meaningful for analyzing retinal topography. Furthermore, it will be important for us to be able to transmit these data to other computers for off-line analysis, such as the VAX 11/780 for coordinate transformation and

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graphical display of the photoreceptor map. The MAGISCAN 2A has an interface to support such telecommunications.

The understanding of the distribution of photoreceptors in the adult and aged human eye can only be approached with an automated image analysis system and the versatility of the NIKON microscope and the MAGISCAN 2A make the task experimentally feasible.

Research Program No. : The Biology of Fetal Skin

Principal Investigator: Karen A. Holbrook, Ph.D.

Co-Investigators: Clara Riddle, Ph.D. Mark Bressler, M.D.
Rich Frederickson, Ph.D. Carolyn Foster, M.S.
Jackie Bickenbach, Ph.D. Carole Johnson, B.S.
Chris Fisher, Ph.D. Eugene A. Bauer, M.D.
Lynne Smith, Ph.D. (Washington University)
Virginia Sybert, M.D.
Mary L. Williams, M.D. (UCSF)

The objectives of this program are to define the normal structural and biochemical markers, and the functional properties of human embryonic and fetal skin which characterize specific stages of in utero development. These markers in the normal fetus provide baseline data for the prenatal diagnosis of certain inherited skin disorders which cannot be diagnosed by techniques other than skin biopsy (16). The program is supported by two grants from the NIH (P.I. Holbrook).

Acquisition of an automated or semi-automated image analysis system will greatly enhance several projects in this program because: 1) It will provide the capability for quantitating, on their structural substrate, a variety of proteins, receptor molecules, antigens and cell types as they appear or disappear at specific stages of skin development. 2) It will permit experiments to be designed for analyzing epidermal cell kinetics using the technique of quantitative autoradiography. 3) Through stereologic analysis, it will allow for quantitative assessment of processes such as vascularization and for identification of cell populations such as those found in the amniotic fluid.

Some concrete examples are discussed below to illustrate the types of studies that

will be made possible by the automated image analysis facility.

1. Epidermal growth factor (EGF) receptors appear in high densities on the surfaces of cells which are mitotically active. We have obtained a specific antibody to EGF receptors on human cells and will use this in immunoperoxidase labeling of skin sections from fetuses at progressive stages of gestation. We are interested in identifying populations of epidermal cells which might be precursors to epidermal appendages and to determine whether differences in receptor densities on epidermal cells can distinguish rapidly and slowly cycling cells. The quantitative data obtained from the labeling studies will be compared with autoradiographic data which we will obtain by incubating frozen sections of the same tissue with radioactively labeled-EGF and analyzing the amount of label taken up in culture. Ongoing studies rely on qualitative assessment of the brown reaction product and hand counting of grains in the radioactive emulsion. Both grain counts and the reaction product lend themselves for quantitation. For reasons explained earlier, automated image analysis will greatly improve the data.

2. Epithelia which transport ions have the enzyme Na^+/K^+ -ATPase in the plasma membrane and show morphologic modifications of the membrane which are identified in freeze-fracture preparations as clusters of intramembranous particles. We have already obtained the morphological evidence that the pre-keratinized fetal epidermis has the properties of an iontransporting epithelium and now wish to correlate these data with biochemical evidence for the presence of the enzyme in the cells and with immunohistochemical data which will allow us to localize and quantitate the amount of the enzyme. We have developed an antibody that is specific for the enzyme and this will be used on sections of embryonic/fetal skin at progressive stages of gestation. The amount of label in the sections will be quantitated using the analytic microscope.

3. It has been hypothesized that the development of many collagenous connective tissues is patterned first in a template formed from type III collagen and this is reinforced and/or replaced with new deposits of type I collagen. Type V collagen is

present in fetal skin but its structure and distribution are unknown. It is possible to test in fetal skin biopsies the validity of this hypothesis and use the data as an additional criterion in diagnosis. Sections of skin from human fetuses at progressive stages of gestation will be labeled with types I, III and V anticollagen antibodies. The amount of each antibody in each region of the dermis will be quantitated using the MAGISCAN 2 and compared with the biochemical data of collagens obtained using parallel samples of the same tissue. Similar studies will be performed using antibodies against specific glycosaminoglycans. The data on these two components of the extracellular matrix will help us to understand the conditions for matrix interactions which support or promote the transition of a watery, mesenchymal matrix of embryonic skin to the densely fibrous dermis of late-stage fetuses.

4. We will attempt to use quantitative data on collagenase expression in fetal skin to support our morphologic assessment of a fetus affected with recessive dystrophic epidermolysis bullosa (RDEB). RDEB is a severely disfiguring and debilitating, blistering disease in which the epidermis, mucous membranes and cornea are sloughed with friction. Healing results in severe scarring and contractures. The diagnosis is still made ultimately on the basis of skin morphology which would be highly enhanced by quantitating the collagenase in the fetal biopsy samples. We will establish the normal range with an anti-collagenase antibody in normal tissue and then compare these data with skin obtained from a fetus at risk for RDEB.

5. Langerhans cells of the skin are among the accessory cells which function in antigen presentation to T-lymphocytes. They have a number of characteristic surface receptors and antigens (OKT6, Fc, IgG, HLA-DR, Mg⁺-activated ATPase, C3, S-100, etc.). Langerhans cells appear early in fetal skin, but it is not known whether they have the receptors and antigens when they enter the epidermis or whether they are acquired in situ. Preliminary studies have shown the feasibility of labeling Langerhans cells with antibodies to the various antigens and receptors in epidermal sheets from human embryos

and fetuses. The cells are currently counted by hand and it has been impossible to measure the amount of label. This project will be enhanced immeasurably with the MAGISCAN 2A.

6. There are a number of inherited scaling and blistering diseases of the skin which are diagnosed in utero on the basis of the morphology of fetal skin samples. We hypothesize that blistering and excessive scaling will cause desquamation of sheets of epidermal cells into the amniotic fluid and that this might occur with enough frequency to alter the numbers of cells in various populations of amniotic fluid. We have many such fluids from both normal and affected fetuses and are counting the different cell populations by tedious morphometric measurements (grid counting). Using the MAGISCAN 2, it will be possible to automate these counts and obtain data on both normal and abnormal populations more efficiently with the anticipation that this may be a viable alternative in the future to prenatal diagnosis of certain disorders, thus saving the family from the more specialized, less available, more invasive procedures of fetoscopy and skin biopsy.

Research Program No. 6: Sperm and Egg Interactions

Principal Investigator: Penny Gaddum-Rosse, Ph.D.

Co-Investigator: David Battaglia, M.S.

One of the aims of this research is to understand the molecular basis of sperm-egg interactions during fertilization.

Studies of fertilization events employ numerous fluorescent and radioactively labeled probes to identify certain membrane and cytoplasmic domains. However, careful statistical analysis of the relationships of labeled molecules to specific sperm or egg structures during fertilization has not been accomplished. Quantitation of the relative amounts of certain constituents in the mammalian egg has been difficult due to the limited quantities of eggs that are available for biochemical analysis. Moreover, most biochemical techniques destroy spatial organization. Hence, any system that allows for

quantitation using light microscopy would be extremely advantageous to reproductive biology.

Of several experiments that would be made possible by quantitative image analysis, only one will be mentioned. Actin has been implicated as having a major role in sperm penetration, movement of the cortical granules, approximation of the two pronuclei and subsequent segmentation. Fluorescent probes have already been employed to identify f-actin in mouse and rat eggs, however, the distribution of actin appears to be complex (2). It is important to define the relationship of this protein in quantitative and spatial terms to the structures that execute the movements germane to the fertilization process.

Phase contrast, polarizing and interference contrast capabilities of the Nikon microscope are ideal for the study of these living cells. The MAGISCAN 2 image analysis system provides the potential for writing specific software for quantitation of the probes at the cellular and subcellular levels and generate information that cannot be obtained by other means.

Research Program No. 7: Immunogens of Sperm and Preimplantation Embryos

Principal Investigator: Marilyn S. Hamilton, Ph.D.

The overall objective of this research is to study immune responses to early embryos and sperm. Such responses are known to occur during pregnancy or after vasectomy. It is hypothesized that naturally occurring immune responses during human pregnancies may be associated with infertility and spontaneous abortion. This work is supported by grants from the National Science Foundation and the March of Dimes. (P.I. M.S. Hamilton)

We have developed hybridomas from the lymphoid tissues of animals that have experienced pregnancy or vasectomy. We have obtained a number of monoclonal antibodies from these hybridomas which bind to the cell surface of sperm and/or early embryos; some interfere with in vitro fertilization and early embryonic development (14). In the analysis of how these monoclonal antibodies disrupt fertilization and development, it is

important to quantitate changes in the corresponding antigens on sperm and early embryos and to determine quantitatively how antigen expression by these cells is altered in the presence of antibodies. Furthermore, the sperm membrane is incorporated into the zygote and can be detected during preimplantation embryo stages. Quantitation of specific antigens associated with the sperm membrane in the embryo will provide insights into the mechanisms by which antibodies to sperm alter early embryonic development. The phase contrast and fluorescence capabilities of the Nikon MICROPHOT optical microscope combined with the image analysis capabilities of the MAGISCAN 2 system will permit the required quantitative analysis of a significant sample size. The possibilities for improved and more efficient collection of quantitative data will permit an analysis of this dynamic system.

Research Program No. 8: Control of Differentiation in the Myogenic Lineage

Principal Investigator: Mark Nameroff, Ph.D.

The long-range goal of this program is an understanding of the cellular mechanisms that control the differentiation of skeletal muscle cells. This program is supported by a grant from the Arthritis Institute of the NIH.

There are currently two general hypotheses or models for skeletal muscle differentiation. In one model, myogenic precursors are a uniform cell population that are induced by the microenvironment to differentiate into postmitotic, end-stage muscle cells. In the second model, myogenic cells are a heterogeneous but lineally related cell population in which terminal differentiation is the consequence of passing through the various stages of the lineage. The latter model hypothesizes that the microenvironment affects division rates in the lineage and may affect decisions by a myogenic stem cell (28,29).

Testing predictions made by these two models is carried out by tissue culture studies of individual cells and their progeny in clones established from embryonic myogenic tissue. Clones are grown in culture by seeding 50 to 100 cells into 60 mm dishes. At various times after setting up the cultures, the clones are exposed to

^3H -Thymidine to determine by autoradiography which of the cells are in cycle. The same clones are also examined for the presence of various muscle-specific proteins by use of either indirect immunofluorescence or peroxidase-conjugated antibodies against these proteins. Testing of the models requires that we determine the number of cells (in each clone) that contain muscle proteins, the number that do not contain such proteins, and the number of cells that are in cycle. Currently, this procedure is carried out manually and requires many hours to obtain the data from one clone. Thus, for statistical significance, hundreds of hours are needed to examine clones under just one set of conditions. Fatigue and inaccuracies are a serious limitation in such analyses. The automated microscope with its quantitating abilities will not only speed up this work by several orders of magnitude but will also increase the reliability of the resulting data. It will also permit examination of clones from several species of animals (including human) in order to determine the general validity and scope of the models of myogenesis. The capabilities of MAGISCAN 2A are well suited for the type of analysis this program requires. The appropriate modifications of the software will be accomplished through collaboration with the scientific team responsible for running the Image Analysis Facility.

Research Program No. 9: Age-Related Clonal Attenuation.

Principal Investigator: John Angello, Ph.D

Co-Investigator: John W. Prothero, Ph.D.

This program investigates the mechanisms which underlie the phenomenon of clonal attenuation. The experimental approach involves a quantitative analysis of the factors which influence the rate of clonal attenuation. The work is supported by a program project funded by the National Institute of Aging.

A population of normal diploid cells grown in vitro undergoes a limited number of doublings, after which the cells become postmitotic. There is a progressive decline in the potential for cell division throughout the life of the culture which is known as

clonal attenuation and this is correlated with the age of the donor animal, suggesting that clonal attenuation and aging are related. Understanding the mechanisms by which various agents, such as growth factors, alter the rate of clonal attenuation in vitro may throw light on the nature of the mechanisms which influence clonal attenuation in vivo.

Cells grown in vitro are passaged just before reaching confluency. At each passage a proportion of the cells are plated at low density, so that the multiplication of individual cells can be monitored. This involves fixing the clones at the end of some specified period after plating, and counting the number of cells in each clone. From the data obtained in this way one plots clone size distribution curves, which express the proportion of cells that give rise to clones of various sizes. By a careful analysis of the clone size-distribution-curves, derived at different times in the history of the culture, it proves possible to make inferences as to the factors which govern the rate of clonal attenuation (1). We are investigating the effects of agents which are known to alter the rate of clonal attenuation (i.e., serum and mitogen concentrations; promoters). The limiting factor in our ability to carry out these experiments is the enormously time-consuming task of determining clone size by visual counts which introduces many errors. What is needed critically for these studies is an automated image analysis system capable of determining the number of cells after staining in individual clones. This would make our studies more accurate and very much more manageable in practical terms. We anticipate that the proposed image analysis system will collect as much information in an afternoon as we currently achieve in a month.

Research Program No. 10: Tumor Influence on Bone Metabolism

Principal Investigator: Minako Y. Lee

The overall objective of this research program is to investigate the modulatory influence certain neoplasms exert on hemopoiesis and on bone metabolism. This research is supported by a contract from the Department of Energy (Marrow Tumor Interactions, P.I.

Cornelius Rosse) and a research grant from the National Cancer Institute (Bone-Bone Marrow Interactions, P.I. Lee).

In this program an experimental model has been developed in mice which permits a systematic analysis of two clinically well known phenomena: leukemoid reactions (severe granulocytosis) and hypercalcemia which develop in response to a variety of extramedullary tumors. In the course of our studies concerned with the hemopoietic effects of the CE-1460 mammary carcinoma, we have discovered that in addition to causing severe neutrophil hyperplasia - coupled with the suppression of erythropoiesis and lymphocytopoiesis - the tumor also caused activation of osteoclasts and an increase in the metabolic turnover of bone tissue manifested by hypercalcemia and increased urinary excretion of calcium and hydroxyproline. Much of our current work is concerned with defining the humoral factors that mediate these effects. Some of these factors are produced directly by the tumor and others by host cells under the tumor's influence.

Quite apart from its clinical relevance, the most interesting aspect of this experimental model is the potential it provides for investigating the close topographical association of bone and hemopoietic tissue (bone marrow). Some of our preliminary studies suggest that conditioned medium obtained from cultured tumor cells can increase granulocytopoietic activity and also bone resorption activity in vitro. Therefore, our present hypothesis is that stimulation of granulocytopoiesis also stimulates osteoclasts or their precursors and at the same time inhibits the development of osteoblasts. These stimulatory and inhibitory mechanisms may operate through the microenvironmental stroma of the bone marrow. Much has been learned about the influence of local stromal elements on hemopoietic cell differentiation, but essentially nothing is known about the nature of the microenvironmental interactions between bone and hemopoietic tissue that could explain their co-existence. Our quantitative studies have clarified to a great extent tumor influence on hemopoietic stem cells, progenitor cells, the increased production rate of neutrophils and decreased production rate of

lymphocytes (18-23), but the only way for beginning to approach bone-bone marrow interactions is to gain quantitative assessments of bone changes at the interface of hemopoietic tissue and bone. Such an approach is possible only with an automated image analysis microscope. The confines of this application permit only a sketchy description of one of the experiments to illustrate this point.

The quantitation of bone and bone marrow must be done by measuring the respective areas of bone and marrow in cancellous and hollow bones on histological sections. Such measurements must be correlated with the quantitation of osteoclasts and with the quantitative assessment of bone deposition and bone resorption. We have already worked out the methods for the histological preparation and serial sectioning of cancellous (vertebrae) and hollow (femora) bones (17) which we will combine with the histochemical reaction for acid phosphatase to identify osteoclasts. Since bone into which tetracycline has been incorporated emits fluorescence, we will quantitate the rate of bone deposition and resorption by measuring fluorescent bone in mice following a 7-day period of tetracycline treatment immediately before sacrifice or some time before sacrifice. Without describing a comprehensive experimental protocol, it should be apparent that using mice with or without transplanted tumors at ages when active skeletal growth is occurring or has already ceased, it will be possible to obtain accurate quantitation of the changes in bone turnover caused by the tumor or its products. It will be possible to correlate these changes with osteoclast numbers, the volume of hemopoietic marrow, biochemical data of calcium and hydroxyproline metabolism and with changes in hemopoietic cell populations (stromal cells, neutrophil progenitors, marrow sinusoidal spaces).

The Magiscan 2A Nikon Image Analysis System is ideally suited for performing the quantitative, histochemical and morphometric studies required in this complex project. The histochemical and fluorescent reactions will make the creation of a program very easy for automated measurements. Only through such quantitation will it be possible to

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assess unequivocally the biological effects of the tumor and its products. Such data are essential for formulating experiments in the future that will test the hypothesis we have proposed. The future experiments will also rely on the use of the image analysis facility, and will be primarily concerned with the reciprocal changes in bone and hemopoietic tissue under conditions in which one or the other is being primarily manipulated. Such studies will include the inhibition of granulocytopoiesis by drugs or irradiation during tumor-induced neutrophilia or the stimulation of osteoclasts in congenitally osteopetrotic mice transplanted with the tumor or infused with the appropriate factors released by the tumor.

Research Program No. 11: Cell Traffic in the Immune and Hemopoietic System

Principal Investigator: Cornelius Rosse, M.D., D.Sc.
Lai-Ming Ching, Ph.D.
Minako Y. Lee, M.D.
Jose Mejino, M.D.

The goal of the research in this program is to understand the cell exchange between various hemopoietic and lymphocytopoietic tissues with particular emphasis on the bone marrow and the thymus. The work is supported by a contract from DOE and a research grant from NIH (P.I. Rosse).

Establishing parabiotic union between two congenic strains of mice which possess distinguishable marker chromosomes (CBA/CAJ and CBA/T6T6) provides a useful experimental system for studying cellular migration streams because an effective cross circulation can be established between the two parabionts. The limiting factor in such experiments is the identification and typing of metaphase chromosome spreads in the cell populations of interest. The MAGISCAN 2A has the capability for generating a program that can distinguish between the normal set of 40 chromosomes of the CBA/CAJ strain and the karyotype that contains the two marker chromosomes of the CBA/T6T6 strain. In such a system it is possible to obtain answers to the following types of questions:

1) Are pre-T cells resident in the bone marrow a self-maintaining population or do they rely for their maintenance on an input of cells derived from the thymus?

2) Although it is known that an irradiated thymus can be repopulated by transplanted bone marrow cells, is there a steady stream of cells from the bone marrow to the normal thymus which is responsible for maintaining thymic T cell production?

3) It is known that pluripotent hemopoietic stem cells (CFU-S) and committed stem cells (e.g., CFU-C, BFU-E) enter the circulation but is there a continuous exchange of various stem cells between the blood and the hemopoietic tissues?

Our laboratory has considerable experience with this system but the types of questions we venture to ask and the number of studies we can undertake are severely limited by the time demanding analysis of chromosome spreads with conventional microscopy. The answer to the above questions could be readily obtained with the automated image analysis system. Following defined periods of parabiotic union, scoring of the proportion of CBA/CAJ and CBA/T6T6 chromosome spreads of pre-T cells in the bone marrow would give the answers with respect to:

1) The maintenance of pre-T cells in the bone marrow. These cells can be fractionated, enriched and induced to divide by PHA stimulation (27-31). If the population itself is self maintaining in the marrow, 100% of mitoses will be of the CAJ type in the CAJ mouse and of the T6T6 type in the T6T6 mouse, whereas the metaphases will be of mixed type if there is immigration.

2) After varying periods of parabiosis, varying proportions of metaphases will be of the opposite partners karyotype in the thymus if there is indeed continuous cell input into the thymus under homeostatic conditions.

3) Scoring of karyotypes prepared from spleen colonies or from in vitro colonies of various types derived from the bone marrow of one parabiotic partner will reveal to what extent there has been an exchange between the circulation and the bone marrow with respect to the cells assayed by spleen colonies or by in vitro colony formation.

Provided there is a sufficient sample size in each case, inference could be drawn about the self maintenance of various cell populations, and many questions could be

cleared up in the homeostatic system which, at the moment, can only be approached in experiments that rely on depletion of the hemopoietic or the lymphoid system by irradiation. These depletion procedures of necessity alter the normal pattern of cell migration and cell population maintenance. The scope of these studies requires automated image analysis.

Research Program No. 12: Identification of Bone Marrow Pre-T Cells

Principal Investigator: Lai-Ming Ching, Ph.D.

Co-Investigator: Cornelius Rosse, M.D., D.Sc.

The overall goal of this research is to understand how T lymphocytes differentiate from their immature bone marrow precursors (pre-T cells). The work is supported by two NIH grants (P.I. Ching and Rosse). Approval of another grant from NIH is pending (P.I. Ching).

Although it is known that bone marrow cells can repopulate the irradiated thymus, the identity of the bone marrow-derived pre-T cell remains unknown despite the fact that some plausible candidate cell populations have been proposed. One of the cell populations we are investigating is the putative pre-T cell which is Thy-1 negative and can be induced by PHA to proliferate and to express T cell differentiation antigens (27). To test whether these cells and these cells only repopulate the thymus of an irradiated mouse, cell populations with three different types of markers are needed. We will perform the experiment with congenic mice that carry marker chromosomes: CBA/CAJ (no marker), CBA/T6T6 (two markers) and (CBA/CAJ x CBA/T6T6) F_1 (one marker).

PHA responsive putative pre-T cells will be obtained by Percoll density gradient fractionation from CBA/T6T6 bone marrow. These cells will be mixed with F_1 bone marrow cells depleted by Percoll gradient fractionation of the PHA-responsive cells. The cell mixture will be injected into lethally irradiated CBA/CAJ mice. The scoring of karyotypes for one, two or no marker chromosomes in the thymus, 4-6 weeks after transplantation, will determine what proportion of thymocyte precursors are derived from PHA-nonresponsive, PHA-responsive or radiation resistant host cells.

Because we anticipate that fractionation will have to be refined and many fractions tested, the experiment can only be attempted if a sufficient number of karyotypes can be scored. This task is of sufficient magnitude to require automated image analysis, provided by MAGISCAN 2A.

Research Program No. 13: Intrathymic T Lymphocyte Differentiation

Principal Investigator: Andrew Farr, Ph.D.

The major aim of this research program is to understand the cellular interactions that control and influence T lymphocyte differentiation within the thymus. The work is supported by a grant from NIH (P.I., Andrew Farr).

Despite a large amount of early work, much uncertainty remains about the intrathymic maturation pathway of T cells. There is now a good possibility for resolving many of the uncertainties because of a number of recent developments: 1) The major histocompatibility antigens (Class II or Ia antigens) which are stimulatory for T lymphocyte precursors that express the receptor for these MHC antigens have been identified (7). 2) A monoclonal antibody has been developed which recognizes an allotypic determinant on the β chain of the T lymphocyte antigen receptor molecule (15) making it possible to follow the development of cells that will contribute to the functional extrathymic T lymphocyte pool. 3) A maturation sequence with respect to the cellular expression of the T cell receptor protein has been identified (7) and this is reminiscent of μ heavy chain expression by B lymphocytes during their maturation. The cytoplasmic and cell membrane expression of the T lymphocyte antigen receptor has, moreover, been correlated with the differential location of cells in the thymic cortex and medulla (7). 4) A labeled DNA precursor, ^3H -deoxycytidine, which is avidly incorporated by thymocytes and not reutilized, can now be substituted for ^3H -TdR which is poorly used by thymocytes and confounds the interpretation of cell kinetic data because of its rapid dilution in proliferating cells and its extensive reutilization due to intrathymic cell death on a large scale.

Our hypothesis is that lymphocytes developing in the thymus with nonsense rearrangements of the antigen receptor gene will become eliminated during intrathymic maturation, whereas cells with appropriate gene rearrangements that enable them to synthesize the receptor protein will attain functional maturity when they are discharged into the periphery. The developments described make it possible to obtain unequivocal information about the maturation of T cells in the thymus by correlating the quantitative expression of the T cell antigen receptor in the cytoplasm or in the cell membrane with cell size, location in the thymic cortex or medulla and with ^3H -deoxycytidine radioautography used to give data about the proliferative status, self-replicating ability and post-mitotic age of receptor-positive cells in the thymus.

Such a task is daunting without an automated image analysis system. The problems the experiment poses are ideal for the capabilities of an automated image analysis microscope because: 1) the cells of interest can be readily identified due to their fluorescence (the anti-receptor antibody has been fluoresceinated), 2) their size can be measured and 3) radioautographic silver grains over the fluorochrome positive cells can be recorded. Different staining techniques assure that only cytoplasmic or cell membrane positive cells react with the antibodies. A cell will be analyzed for size, immunofluorescence quantitation of the receptor and the number of exposed silver grains over the nucleus. Appropriate types of counts will be performed on sections or on smears prepared from cell suspensions. Depending on the schedule of ^3H -deoxycytidine administration, the following questions can be answered:

1. Which receptor-positive cells are proliferating and which are post-mitotic?
 2. What is the rate of turnover and production of cell populations of receptor-positive thymic lymphocytes?
 3. What is the cellular maturation sequence in receptor-positive thymic lymphocytes?
- Since only approximately 10% of thymocytes are positive for the receptor, the need for an automated image analysis system becomes even more imperative in order to collect a

sufficient data base.

Research Program No. 14: Immunobiology of Bone Marrow Transplantation

Principal Investigator: Brian L. Hamilton, M.D., Ph.D.

Co-Investigators: Hans D. Ochs, M.D.
Cornelius Rosse, M.D., D.Sc.

The goal of this research program is to define the immunologic mechanisms which result in graft-versus-host disease (GVHD) due to minor histocompatibility antigens (minor HA) and are associated with the development of an acquired immunodeficiency syndrome (IDS). The program is supported by two NIH grants (P.I. Hamilton) and a grant from the March of Dimes (P.I. Hamilton).

An experimental model has been developed in this program for bone marrow transplantation across minor HA barriers in mice which were selected for identity at the major histocompatibility complex antigens (11-13). This model is particularly well suited to study the immunobiology of GVHD including the mechanisms which result in the IDS associated with GVHD. Mice suffering from GVHD-IDS have impaired cell-mediated immunity and humoral immunity.

The image analysis microscope described in this proposal will be used extensively to test the hypothesis that the immunodeficiency results from the absence of specific cell populations (T cell subclasses, B cells) in the mice transplanted with minor HA incompatible bone marrow. The production of lymphocytes in the bone marrow, thymus and spleen has never been studied in mice undergoing GVHD due to minor HA, therefore, mice undergoing GVHD will be labeled with ^3H -TdR or ^3H -deoxycytidine using techniques routinely employed in this laboratory (30). At appropriate times after labeling, mice will be sacrificed and bone marrow, thymus and spleen cells will be prepared in single cell suspension. These cells will then be labeled with FITC-conjugated antibodies to immunoglobulin (to label B cells), to Thy-1 (to label all T cells) or antibodies that recognize subclasses of T lymphocytes. The Magiscan 2A program will then be used to identify and enumerate the FITC-labeled cells, quantitate fluorescence

and radioautographic grains over each cell to permit the computation of cell population kinetics. Along with appropriate controls, this experiment will determine whether the lymphoid depletion associated with GVHD is the result of decreased production of lymphocytes of the specific subclasses or to the elimination of host cells by transplanted immunocompetent cells.

Research Program No. 15: Maturation Program and Regulatory Interactions of Natural Killer Cells

Principal Investigator: Sylvia B. Pollack, Ph.D.
Co-Investigators: Cornelius Rosse, M.D., D.Sc.
Claire Robles, Ph.D.

The major role of the research in this program is to understand the maturation and differentiation of natural killer (NK) cells and to elucidate their roles in the regulation of immune and hemopoietic cellular responses. The work in this program is funded by two NIH grants (P.I. Pollack) and a contract from DOE (P.I. Rosse). Although it is known that NK cells are both bone marrow dependent and bone marrow derived (10), there is essentially no information about their maturation and differentiation program. It is not known, for instance, what stage of maturation these cells reach before they are discharged from the marrow, how their precursors relate to other hemopoietic progenitor cells and how their production in the marrow is regulated. These problems are being attacked by two experimental strategies:

1) Based on preliminary data we hypothesize that NK cells in the bone marrow acquire the ability to bind to appropriate targets (YAC-1) before they develop the capacity to lyse the targets as well (25). We are using well established techniques of cellular kinetics to label DNA synthesizing NK precursors with ^3H -TdR in vivo (30) and determine on radioautographs the proliferative status and turnover of cells that bind or bind and lyse NK-sensitive targets. 2) We have developed antisera that recognize antigens expressed by NK cells (26). These antibodies are used for surface labeling of NK cells in combination with cell kinetic studies that rely on radioautography with

³H-labeled DNA precursors.

The bottleneck in both these strategies is the analysis of radioautographs for labeled cells and grain counts. In the first strategy the Magiscan 2A will be employed in a user-directed manner. The system will be programmed to automatically identify doublets, triplets, quadruplets of cell aggregates and locate these for the observer. The observer will then close down the field on the cell bound to the target and obtain automated readout of cell size and labeling intensity in terms of grain count on the individual bound cells. In the second strategy, the image analysis system will be used entirely in an automated mode. The system is ideal for identifying fluorochrome-labeled cells, determine their incidence, size, quantitate their fluorescence and also determine their grain count. Information in the two strategies will be readily correlated since the data for the various parameters will be recorded for individual cells.

The image analysis system will also help us to define targets of NK cells among differentiating hemopoietic and immune cells. It is now known that natural killer cells can recognize and lyse not only sensitive tumor cells but also certain normal cell types in the bone marrow and thymus. Recently we and others have demonstrated that NK cells regulate the response of B cells to antigenic stimulation. We hypothesize that NK cells keep B cell proliferation and differentiation within homeostatic limits. The major question is whether differentiation associated antigens on hemopoietic cells (e.g., B cells) serve as the targets for NK cells. In these studies, known differentiation antigens of hemopoietic cells will be labeled with fluoresceinated antisera and the binding of NK cells will be determined for the appropriate cell populations. Quantitation of the fluorescence data is essential to determine the relationship of antigen expression to recognition by NK cells. We have tried to approach this question with the Fluorescence Activated Cell Sorter but could not obtain the resolution of the target binders necessary for this type of analysis. These experiments will only be possible if we can quantitate differentiation antigens on a per cell basis, a possibility provided only by the image analysis microscope.

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Summary of Major Accomplishments

Some earlier accomplishments of DOE and other federally sponsored research in the Department of Biological Structure have already been described in the first section of this narrative. More recent accomplishments can be summarized in terms of the six major departmental research areas:

1. Cellular and Developmental Biology. In addition to the establishment of the program in fetal skin biology, two new laboratories have been set up, one dealing with the molecular biology of the intercellular matrix and the other with the biology of lens cell transparency and cataract formation. Myogenic stem cells have been discovered not only in embryonic but in adult skeletal muscle.

2. Experimental Immunology and Hemopoiesis. A cell population has been discovered in the bone marrow which greatly enhances the tumor neutralization potential of tumor antigens specific T lymphocytes. Experimental model systems have been developed for studying: a) the influence tumors exert on hemopoiesis, lymphocytopoiesis and bone metabolism, b) the factors that regulate T lymphocyte differentiation in the thymus and in the periphery, c) the maturation of natural killer cells in the bone marrow and d) an immunodeficiency syndrome that develops as a consequence of graft-versus-host disease in minor HA bone marrow transplantation chimeras.

3. Reproductive Biology. In addition to the development of monoclonal antibodies that have been generated against differentiation antigens expressed by gametes and early embryos, direct observations of in vitro fertilization events have defined several components of this process, including cytoskeletal elements involved in sperm penetration and segmentation.

4. Neurobiology. The analysis of visual pathways and visual cortical areas has provided new information about how visual information is processed. A major survey of human retinal development has been completed and the types of thalamic neurons that process somatosensory information have been characterized.

5. Molecular Structure. The tertiary structure of several metalloproteins has been determined and a computer graphics program has been established for displaying the dynamic behavior of large, biologically relevant molecules.

6. Morphometrics and Computer Modeling. Several refinements have been made for the stereologic assessment of cellular changes caused by drugs, toxins and trauma. A program has been initiated for modeling the development of myocardial fiber patterns in the heart.

Qualifications of the Faculty

The one page resumé for the principal members of the scientific staff mentioned in this application are presented in Attachment 1. Seven of these investigators are at the Assistant Professorial rank, five at the Associate Professorial rank, three are Professors and four hold junior research faculty appointments. With four exceptions, each of these twenty investigators is a PI of at least one peer-reviewed NIH grant and several hold more than one federally supported research grant or contract.

It is noteworthy that several members of the faculty have gained recognition for the quantitative methods they have developed for the study of biologic structure; others have recognized expertise in computer science and have written software for collecting data relevant to the analysis of biological structure (some of the latter are not listed in this application). Dr. John Bertram has used the Quantimet, an image analysis system, in his research before he joined the Department and he will be partially responsible for running the NIKON/MAGISCAN 2A facility. To meet the specific needs of the research programs described in this application, a significant amount of software will have to be created based on the system software of MAGISCAN 2A. Drs. Dean Pentcheff, Ronald Stenkamp, and Keith Watenpaugh (not named in the programs described in this application) as well as Drs. Bertram, Bolender, Curcio and Prothero, have the knowledge, interest and motivation to accomplish this in collaboration with members of the faculty whose prime interest is in biology.

Institutional Cost Sharing

There are two elements with respect to institutional cost sharing:

1. The School of Medicine, University of Washington has provided the matching funds for the NCI construction grant that rebuilt the laboratory which accommodates the DOE-supported research program in Biological Structure. We moved into our new laboratory complex in February, 1984. Six of the fifteen projects described in this application are accommodated in this research facility.

2. The University of Washington will commit funds to provide 20% of the cost of the equipment.

Institutional Experience with Major Research Equipment

Our electron microscopes provide the best example of major research equipment that is used by several programs in the Department of Biological Structure. A newly constructed electron microscope suite houses the following electron microscopes which serve departmental and some affiliated programs: Philips 201 TEM, Philips 300 TEM, ETEC SEM, and Philips 420 S(TEM).

The Philips 420 S(TEM) was purchased in 1983 by investigators in Biological Structure on a BRSO shared instrumentation grant awarded by NIH. In addition, the Department has a Balzers 301 freeze-fracture unit and other major pieces of equipment (ultramicrotomes, ultracentrifuges, computing spectrophotometers, gamma and beta counters, etc.) which are maintained in departmental equipment rooms for shared use by departmental programs. All this equipment is available to and is extensively used by our postdoctoral fellows and graduate students who are active participants in departmental research programs. The equipment is maintained by service contracts the cost of which is shared by the principal users.

Maintenance

The NIKON/MAGISCAN 2A image analysis microscope will be established and maintained as a central departmental facility. The facility will be located in room I-501 in close

vicinity of the research laboratories of all programs described in the application. Drs. John Bertram and Andrew Farr will be primarily responsible for supervising and maintaining the facility. Dr. Bertram has had previous experience with the Quantimet image analysis system; Dr. Farr's expertise is in immunohistochemical and immunocytochemical techniques, the most widely used methodology for the studies that will rely on the image analysis facility. Dr. Dean Pencheff will coordinate the expansion of the software library and will work together with investigators to help them meet the specific needs of their projects with the MAGISCAN 2A menu. In this respect, he will be working together with Drs. Bolender, Stenkamp and Watenpaugh. In addition, a programmer and research technologist will be hired to look after the day-to-day operational aspects of the image analysis facility. Part of the salary of these individuals will be provided by the research grants of the user investigators and part by departmental funds.

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ATTACHMENT 1

FACULTY RESUMES

Principal Investigator: Karen A. Holbrook, Ph.D.
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J.C. Angelo, Ph.D. Research Associate	A.E. Hendrickson, Ph.D. Professor
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L.M. Ching, Ph.D. Research Associate Professor	N.D. Pentchoff, B.S. Systems Programmer
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C.A. Curcio, Ph.D. Fellow-Research Associate	J.W. Prothero, Ph.D. Associate Professor
A.G. Farr, Ph.D. Assistant Professor	C. Rosse, M.D., D.Sc. Professor and Chairman
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1963-1966 Teaching Assistant, Zoology, University of Wisconsin, Madison, WI

1976-1979 Senior Fellow, Dermatology, University of Washington, Seattle, WA

REPRESENTATIVE PUBLICATIONS

- Holbrook, K.A. and V.P. Sybert 1984 Animal models of ichthyosis and other forms of aberrant keratinization. In: Animal Models in Dermatology. N. Lowe and H. Baden, Eds. Karger, Basel. (In Press).
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1982-1983	Senior Fellow, Department of Biological Structure, University of Washington, Seattle, WA
1981-1982	Research Officer, Departments of Pathology and Pharmacology, University of Western Australia
1977-1981	Demonstrator in Histology, Cytology, Cell Biology, Gross Anatomy, Unit of Human Morphology, Flinders University of South Australia, Adelaide
1976	Demonstrator in Histology, Cytology, Electron Microscopy, Gross Anatomy and Neuroanatomy, Department of Anatomy and Human Biology, University of Western Australia, Perth

REPRESENTATIVE PUBLICATIONS:

- Westrum, L.E., R.C. Dunn and J.F. Bertram 1984 A morphometric study of the effects of maturation and aging on synaptic patterns in the spinal trigeminal nucleus of the cat. *Brain Res.* (Submitted)
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1965-1970 Predoctoral Fellow, Department of Anatomy, Harvard University
1963-1965 Teaching Assistant and Lecturer, Department of Zoology, Columbia University
1960-1963 High School Science Teacher, Elwood School District, Long Island, New York

REPRESENTATIVE PUBLICATIONS

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REPRESENTATIVE PUBLICATIONS

- Ching, L. M., A. G. Farr, S. Hosier, C. Rosse and S. K. Anderson 1984 Myeloid-lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown in vitro. Submitted.
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1976-1982 Research Associate, Department of Physics, Massachusetts Institute of Technology, Cambridge, MA
1976 Instructor, Gross Anatomy, Harvard Medical School, Boston, MA
1974-1976 Research Associate, Department of Chemistry, University of Massachusetts, Boston, MA
1974-1976 Research Fellow in Anatomy, Department of Anatomy, Harvard Medical School, Boston, MA

REPRESENTATIVE PUBLICATIONS

- Kissinger, C.R., E.T. Adman, R.E. Stemkamp and J.I. Clark Crystal structure of sorbinil. *Acta Cryst.* (Submitted).
- Clark, J.I. and M.E. Danford Low temperature and acrylamide inhibit lens opacification caused by calcium. *Ophthal. Res.* (Submitted).
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- Clark, J.I., M. Delaye and G.B. Benedek 1981 Laser light scattering and cataract. In Proceedings of the Technical Conference on Electro-Optics and Lasers. Industrial and Scientific Conference Publications, Chicago, IL, pp. 365-370.

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REPRESENTATIVE PUBLICATIONS:

Curcio, C.A., N.A. McNelly and J.W. Hinds. Variation in longevity of rats: evidence for a systematic increase in lifespan over time (Submitted).
Curcio, C.A. and T. Kemper Nucleus raphe dorsalis in senile dementia: Neurofibrillary changes and neuronal packing density (Submitted).
Buell, S.J., L. Magagna, P.D. Coleman and C.A. Curcio Stability of dendritic length and branching in cortical barrels of aging mice (Submitted).
Curcio, C.A. and J.W. Hinds 1983 Stability of synaptic density and spine volume in dentate gyrus of aged rats. *Neurobiol. Aging* 4: 77-87.
Curcio, C.A. and P.D. Coleman 1982 Stability of neuron number in cortical barrels of aging mice. *J. Comp. Neurol.* 212: 158-172.
Curcio, C.A., S.J. Buell and P.D. Coleman 1982 Morphology of the aging nervous system: not all downhill. In: J.A. Mortimer, F.J. Pirozzolo and D.J. Maletta (Eds.). *The Aging Motor System. Adv. Neurogerontol.* 3: New York, Praeger, pp. 7-35.
Curcio, C.A. and K.R. Sloan, Jr. 1981 A computer system for combined neuronal mapping and morphometry. *J. Neuroscience Methods* 4: 267-276.
Curcio, C.A. and J.K. Harting 1978 Organization of pulvinar afferents to Area 18 in the squirrel monkey: evidence for stripes. *Brain Res.* 143: 155-161.

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1979-1980 Postdoctoral Fellow, Dept. Medicine, National Jewish Hosp.
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1972-1975 Laboratory Demonstrator in Histology, Univ. Chicago

REPRESENTATIVE PUBLICATIONS:

- Farr, A. G. and S. K. Anderson 1984 In situ ultrastructural demonstration of cells bearing Ia antigens in the murine pancreas. Diabetes (Submitted)
- Ching, L. M., Hosier, S., Anderson, S., Rosse, C. and Farr, A. G. 1984 Myeloid lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown in vitro. Blood (Submitted)
- Ching, L. M., Farr, A. G., Rosse, C., and Hosier, S. 1984 The ontogeny of a post-thymic precursor T-cell which generates cytotoxic T lymphocyte precursor cells in colonies grown in vitro. Eur. J. Immunol. (Submitted)
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- DeBruyn, P. P. H. and Farr, A. G. 1980 Lymphocyte-reticuloendothelial system interactions and their fine structural correlates. In: The Reticuloendothelial System: A Comprehensive Treatise. M. Escobar, H. Friedman and S. Reichard, Eds., Plenum Press, New York, 1980.
- Farr, A. G., Cho, Y., and DeBruyn, P. P. H. 1980 The structure of the sinus wall of the lymph node relative to its endocytic properties and transmural passage of cells. Am. J. Anat. 157: 265-284.
- Farr, A. G., Wechter, W. J., Kiely, J. M. and Unanue, E. R. 1979 Induction of cytotoxic macrophages following in vitro interactions between Listeria immune T cells and macrophages. J. Immunol. 122: 2405-2412.
- Farr, A. G., Kiely, J. M. and Unanue, E. R. 1979 Macrophage-T-cell interactions involving Listeria monocytogenes. Role of the H-2 gene complex. J. Immunol. 122: 2395-2404.

1076158

BIOGRAPHICAL SKETCH

PENELOPE GADDUM-ROSSE

BORN:

[REDACTED], Mombasa, Kenya

EDUCATION:

APPOINTMENTS: 1981-present Associate Professor, Department of Biological Structure, University of Washington, Seattle, WA
1974-1981 Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1974-1975 Professional Leave: Royal College of Surgeons, London
1972-1974 Research Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1969-1972 Research Associate, Department of Biological Structure, University of Washington, Seattle, WA
1966-1968 Postdoctoral Fellow, Department of Biological Structure, University of Washington, Seattle, WA

REPRESENTATIVE PUBLICATIONS:

- Brenner, R.M. and P. Gaddum-Rosse 1984 The oviduct. In Comparative Primate Biology. Vol. V, Endocrinology, Reproduction and Development. W.R. Dukelow, Volume Ed. Alan R. Liss, Inc., New York. (In Press).
- Graney, D.O. and P. Gaddum-Rosse 1984 Female reproductive tract. In Sexually Transmitted Diseases. K.K. Holmes, P.A. Mardh, P.F. Sparling and P.J. Wiesner, Eds. McGraw-Hill Book Co., New York, pp. 145-161.
- Battaglia, D.E. and P. Gaddum-Rosse 1984 Rat eggs normally exhibit a variety of surface phenomena during fertilization. Gamete Res. 10: 107-118.
- Gaddum-Rosse, P., R.J. Blandau, L.B. Langley and D.E. Battaglia 1984 In vitro fertilization in the rat: observations on living eggs. Fertil. Steril. 42: 285-292.
- Lee, W.I. and P. Gaddum-Rosse 1983 Studies on sperm motility by laser-light scattering. In The Sperm Cell. J. André, Ed. Martinus Nijhoff, Boston, pp. 321-327. (Proc. IV Intern. Symp. Spermatology, Seuilac, France, 1982).
- Odor, D.L., P. Gaddum-Rosse and R.E. Rumery 1983 Secretory cells of the oviduct of the pig-tailed monkey, Macaca nemestrina, during the menstrual cycle and after estrogen treatment. Am. J. Anat. 166: 149-172.
- Gaddum-Rosse, P., R.J. Blandau, L.B. Langley and K. Sato 1982 Sperm tail entry into the mouse egg in vitro. Gamete Res. 6: 215-223.
- Lee, W.I., P. Gaddum-Rosse, W.D. Smith, M. Stenchever and R.J. Blandau 1982 Laser light-scattering study of the effect of washing on sperm motility. Fertil. Steril. 38: 62-67.
- Lee, W.I., P. Gaddum-Rosse and R.J. Blandau 1981 Sperm penetration into cervical mucus in vitro. III. Effect of freezing on estrous bovine cervical mucus. Fertil. Steril. 36: 209-213.
- Gaddum-Rosse, P. 1981 Some observations on sperm transport through the uterotubal junction of the rat. Am. J. Anat. 160: 333-341.
- Odor, D.L., P. Gaddum-Rosse, R.E. Rumery and R.J. Blandau 1980 Cyclic variations in the oviductal ciliated cells during the menstrual cycle and after estrogen treatment in the pig-tailed monkey, Macaca nemestrina. Anat. Rec. 198: 35-57.
- Gaddum-Rosse, P., R.J. Blandau and W.I. Lee 1980 Sperm penetration into cervical mucus in vitro. II. Human spermatozoa in bovine mucus. Fertil. Steril. 33: 644-648.
- Gaddum-Rosse, P., R.J. Blandau and W.I. Lee 1980 Sperm penetration into cervical mucus in vitro. I. Comparative studies. Fertil. Steril. 33: 636-643.
- Blandau, R.J., P. Gaddum-Rosse and W.I. Lee 1978 Letter to the Editor. On the use of cow cervical mucus in the human. Fertil. Steril. 29: 707.
- Rumery, R.E., P. Gaddum-Rosse, R.J. Blandau and D.L. Odor 1978 Cyclic changes in ciliation of the oviductal epithelium in the pig-tailed macaque (Macaca nemestrina). Am. J. Anat. 153: 345-366.

BIOGRAPHICAL SKETCH

BRIAN L. HAMILTON

BORN: [REDACTED] Portland, Oregon

EDUCATION: [REDACTED] [REDACTED]

APPOINTMENTS: 1982-present Assistant Professor, Department of Biological Structure,
University of Washington, Seattle, Washington
1982-present Adjunct Assistant Professor, Dept. Pediatrics, University
of Washington, Seattle, Washington
1981-1982 Research Assistant Professor, Dept. Biological Structure,
Univ. Washington School of Medicine, Seattle,
1978-1981 Fellow in Pediatric Immunology, Children's Hospital Medical
Center, Harvard Medical School, Boston, MA
1976-1978 Intern and Resident in Pediatrics, Children's Medical Center,
Dallas, TX

REPRESENTATIVE PUBLICATIONS:

- Hamilton, B. L. 1984 Absence of correlation between cytolytic T lymphocytes and lethal murine graft-versus-host disease in response to minor histocompatibility antigens. *Transplantation* 38: 357-360.
- Hamilton, B. L. and Harris, D. 1984 Prevention of graft-versus-host disease using antibody to Thy-1: A role for complement in vivo. *Transplantation* (In Press).
- Mauch, P., Lipton, J. M., Hamilton, B. L., Obbogy, J., Kudisch, M., Nathan, D. G. and Hellman, S. 1984 Acute graft-versus-host disease: Modification with allogeneic cultured donor cells. *Blood* 63: 1112-1119.
- Mauch, P. J., Lipton, J. M., Hamilton, B. L., Obbogy, J., Nathan, D. G. and Hellman, S. 1984 Reduction of lethal graft-versus-host disease: Transplantation of murine cultured cells across minor histocompatibility differences. *Blood* (In Press).
- Hamilton, B. L. and R. Parkman 1983 Acute and chronic graft-versus-host disease induced by minor histocompatibility antigens in mice. *Transplantation*. 36: 150-155.
- Kudisch, M., B. L. Hamilton and J. M. Lipton 1983 Early detection of hematopoietic engraftment in murine bone marrow transplantation utilizing hemoglobin electrophoretic differences. *Transplantation* 35: 515-518.
- Duprez, V., Hamilton, B. L. and Burakoff, S. J. 1982 The generation of cytotoxic T lymphocytes in thymectomized irradiated and bone marrow-reconstituted mice. *J. Exp. Med.* 156: 844-859.

BIOGRAPHICAL SKETCH

MARILYN S. HAMILTON

BORN: [REDACTED]

EDUCATION: [REDACTED]

APPOINTMENTS: 1981-present Research Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1980-1981 Research Associate in Medicine, Children's Hospital Medical Center, Boston, MA
1980-1981 Research Associate in Pediatrics, Harvard Medical School, Boston, MA
1978-1980 Research Fellow, Department of Surgery, Transplantation Unit Massachusetts General Hospital, Boston, MA
1976-1978 Postdoctoral Fellow, Department of Cell Biology, University of Texas Health Science Center, Dallas, TX

REPRESENTATIVE PUBLICATIONS

- Hamilton, M.S., R.B. Vernon and E.M. Eddy A monoclonal antibody, EC-1, derived from a syngeneical multiparous mouse alters in vitro fertilization and development (Submitted).
- Kessler, D.L., W.D. Smith, M.S. Hamilton and R.E. Berger Infertility in mice after unilateral vasectomy. Fert. Steril., (In Press).
- Vernon, R.B., M.S. Hamilton and E.M. Eddy Effects of in vivo and in vitro fertilization environments on the expresion of a surface antigen of the mouse tail. Biol. Reprod. (In Press).
- Naz, R.K., N.J. Alexander, M.A. Isahakia and M.S. Hamilton 1984 Monoclonal antibody to a human germ cell membrane glycoprotein that inhibits fertilization. Science 225: 342-344.
- Hamilton, M.S. 1983 Antigens of gametes. In: Reproductive Immunology. S. Isojima and W.D. Billington, Eds., Elsevier, Science Publications, Amsterdam
- Anderson, D.J., P.H. Adams, M.S. Hamilton and N.J. Alexander 1983 Antisperm antibodies in mouse vasectomy sera cross-react with embryonal teratocarcinoma antigens. J. Immunol. 131: 2908-2912.
- Hamilton, M.S. 1983 Review: Maternal immune responses to oncofetal antigens. J. Reprod. Immunol. 5: 249-264.
- Hamilton, M.S. and D.J. Anderson 1982 Antibodies to antigens on teratocarcinoma cells are associated with parity in mice. Biology of Reproduction 27: 104-109.
- Singh, B., R. Raghupathy, A.R.E. Shaw, D.G. Tews, M.S. Hamilton and T.G. Wegmann 1982 Characterization and cross-reactivity of human and mouse oncofetal antigens: Use of a new solid phase assay for detection of cell surface antigens. Transplantation 33: 156-162.
- Hamilton, M.S., R.C. Burton and H.J. Winn 1981 Natural killing and antibody dependent cellular cytotoxicity of tumor targets are mediated by the same effector cells: A genetic and serological study. Transplan. Proc. 13: 787-789.
- Hamilton, M.S. and R.E. Billingham 1979 The privileged status of the subcutaneous site for skin allografts in rats. Transplantation 28: 199-202.
- Hamilton, M.S., R.D. May, A.E. Beer and E.S. Vitetta 1979 The influence of immunization of female mice with F9 teratocarcinoma cells on their reproductive performance. Transplan. Proc. 11: 1069-1072.
- Hamilton, M.S. and I. Hellstrom 1978 Selection for histoincompatible progeny in mice. Biol. Reprod. 19: 267-270.
- Head, J.R., M.S. Hamilton and A.E. Beer 1978 Maternal hamster immune responses to alloantigens of the fetus. Fed. Proc. 37: 2054-2056.

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BIOGRAPHICAL SKETCH

ANITA E. HENDRICKSON

BORN: [REDACTED] LaCrosse, WI

EDUCATION: [REDACTED]

APPOINTMENTS: 1984-present Professor, Department of Biological Structure, University of Washington, Seattle, WA
1981-present Professor, Department of Ophthalmology, University of Washington, Seattle, WA
1975-present, Affiliate, Child Development and Mental Retardation Center, University of Washington, Seattle, WA
1972-present Affiliate, Regional Primate Research Center, University of Washington, Seattle, WA
1975-1976 Visiting Associate Professor, Neuropathology, Harvard Medical School, Boston, MA
1975-1978 Adjunct Associate Professor, Department of Psychology, University of Washington, Seattle, WA
1973-1981 Associate Professor, Department of Ophthalmology, University of Washington, Seattle, WA
1969-1973 Assistant Professor, Department of Ophthalmology, University of Washington, Seattle, WA
1967-1969 Instructor, Department of Ophthalmology, University of Washington, Seattle, WA
1965-1967 Research Instructor, Department of Biological Structure, University of Washington, Seattle, WA
1964-1965 Research Associate, Children's Memorial Hospital, Chicago, IL
1964-1965 Instructor, Department of Anatomy, Northwestern Medical School, Chicago, IL

REPRESENTATIVE PUBLICATIONS

- Dineen, J.T. and A.E. Hendrickson Overlap of retinal and prestriate cortical pathways in the primate resectum. *Brain Res.* (In Press).
- Pagon, R.A., S.K. Clarren, D.F. Milam, Jr., and A.E. Hendrickson 1983 Autosomal recessive eye and brain anomalies: Warburg syndrome. *J. Ped.* 102: 542-546.
- Hendrickson, A.E., M.P. Ogren, J.E. Vaughn, R.P. Barber, and J.-Y. Wu 1983 Light and electron microscopic immunocytochemical localization of glutamic acid decarboxylase in monkey geniculate complex: evidence for GABAergic neurons and synapses. *J. Neurosci.* 3: 1245-1262.
- Humphrey, A.L. and A.E. Hendrickson 1983 Background and stimulus-induced patterns of high metabolic activity in the visual cortex (area 17) of the squirrel and Macaque monkey. *J. Neurosci.* 3: 345-358.
- Dineen, J.T., A.E. Hendrickson and G. Keating 1982 Alteration of retinal inputs following striate cortex removal in adult monkeys. *Exp. Brain Res.* 47: 446-456.
- Creel, D., A.E. Hendrickson and A. Leventhal 1982 Retinal projections in tyrosine-negative albino cats. *J. Neurosci.* 2: 907-911.
- Hendrickson, A.E. 1982 The orthograde axoplasmic transport tracing technique and its implications for additional striate cortex neuroanatomical analysis. *In: Cytochemical Methods in Neuroanatomy* (V. Chan-Palay & S. Palay, Eds.), Alan Liss, New York, pp. 1-16.
- Abramov, I., J. Gordon, A.E. Hendrickson, L. Hainline, V. Dobson and E. LaBoissiere 1982 The retina of the newborn human infant. *Science* 217: 265-267.

1076162

BIOGRAPHICAL SKETCH

MINAKO YOSHIOKA LEE

BORN: [REDACTED] Tokyo, Japan
Japanese (Permanent U.S. visa)

EDUCATION: [REDACTED]

APPOINTMENTS: 1979-present Research Assistant Professor, Depts. Medicine and Biological Structure, Univ. Washington School of Medicine, Seattle
1977-1979 Acting Instructor, Division of Hematology, Dept. Medicine, Univ. Washington School of Medicine, Seattle
1975-1977 Research Fellow, Division of Hematology, Dept. Medicine, Univ. Washington School of Medicine, Seattle
1970-1972 Resident in Clinical Pathology, VA Hospital, Minneapolis,
1969-1970 Resident in Pathology, Northwestern Hospital, Minneapolis,
1967-1968 Resident in Pathology, Mayo Clinic, Rochester, Minnesota
1966-1967 Resident in Medicine, Northwestern Hospital, Minneapolis
1965-1966 Internship, Cook County Hospital, Chicago,
1964-1965 Resident in Medicine, U.S. Air Force Hospital, Tachikawa, Japan,
1963-1964 Internship, U.S. Air Force Hospital, Tachikawa, Japan

REPRESENTATIVE PUBLICATIONS: (From a total of 22)

- Lau, K. H. W., Lee, M. Y., Linkhart, T. A., Mohan, S., Vermeiden, J. and Baylink, J. Hypercalcemia induced by a mouse CE mammary carcinoma is mediated by a humoral factor which stimulates local prostaglandin in bone. *J. Biolog. Chemist* (Submitted)
- Lee, M. Y. and Lottsfeldt, J. L. 1984 Augmentation of neutrophilic granulocyte progenitors in the bone marrow of mice with tumor-induced neutrophilia: Cytochemical study of in vitro colonies. *Blood* 64: 499-506.
- Lee, M. Y. and Wolf, N. S. 1984 Effect of a neutrophilia inducing tumor on hemopoietic stem cells in mice. *Int. J. Cell Cloning* 2: 185-199.
- Lee, M. Y. and Baylink, D. 1983 Hypercalcemia, excessive bone resorption, and neutrophilia in mice bearing a mammary carcinoma. *Proc. Soc. Exp. Biol. Med.* 172: 424-429.
- Lee, M. Y., Uvelli, D. A., Agodoa, L. C. Y., Scribner, B. H., Finch, C. A. and Babb, A. L. 1982 Clinical studies of a continuous extracorporeal cyanate treatment system for patients with sickle cell disease. *J. Lab. Clin. Med.* 100: 334-344.
- Lee, M. Y. and Rosse, C. 1982 Depletion of lymphocyte subpopulations in primary and secondary lymphoid organs of mice by a transplanted granulocytosis-inducing mammary carcinoma. *Cancer Res.* 42: 1255-1260.
- Pootrakul, P., Hungsprenges, S., Baylink, D., Thompson, E., English, E., Lee, M., Burnell, J. and Finch, C. 1981 Relationship between erythropoiesis and bone metabolism in thalassemia. *New Engl. J. Med.* 304: 1470-1473.
- Lee, M. Y., Sperlin, A. and Dale, D. C. 1980 Distribution of granulocytopenic committed stem cells in mice with tumor induced neutrophilia. *Exp. Hematol.* 8: 249-255.
- Finch, C. A., Price, T. H. and Lee, M. Y. 1979 Neutrophil kinetics in man. *In Granulocyte Physiology, Function and Dysfunction*, an American Association of Blood Banks Publication, pp. 9-33.
- Lee, M. Y. and Rosse, C. 1979 Replacement of fatty marrow by active granulocytopenic bone marrow following transplantation of mammary carcinoma into mice. *Anat. Rec.* 195: 31-46.
- Lee, M., Durch, S., Dale, D., and Finch, C. A. 1979 Kinetics of tumor-induced murine neutrophilia. *Blood* 53: 619-632.

BIOGRAPHICAL SKETCH

MARK A. NAMEROFF

BORN: [REDACTED] Philadelphia, PA

EDUCATION: [REDACTED]
[REDACTED]

APPOINTMENTS: 1975-present Associate Professor, Department of Biological Structure, University of Washington, Seattle, WA
1970-1975 Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1969-1970 Instructor, Catholic University, Washington, D.C.
1967-1970 Staff Member, Laboratory of Skeletal Muscle Research, Armed Forces Institute of Pathology, Washington, D.C.
1965-1967 Instructor, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA
1965-1967 USPHS Pre- and Postdoctoral Fellow, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia
1962-1964 Life Insurance Medical Research Fund Fellow, Department of Anatomy, School of Medicine, University of Pennsylvania

REPRESENTATIVE PUBLICATIONS:

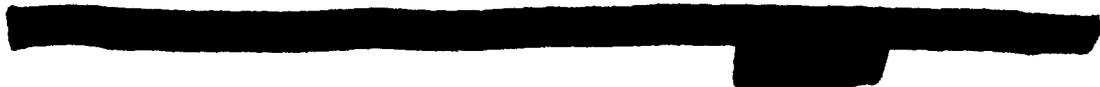
- Quinn, L.S., H. Holtzer and M. Nameroff 1984 Chick skeletal muscle cells are generated in groups of sixteen from stem cells. *Nature* (In Press).
- Quinn, L.S., M. Nameroff and H. Holtzer 1984 Age-dependent changes in myogenic precursor cell compartment sizes. Evidence for the existence of a stem cell. *Exptl. Cell Res.* 154: 65-82.
- Robinson, M.M., L.S. Quinn and M. Nameroff 1984 BB creatine kinase and myogenic differentiation. Immunocytochemical identification of a distinct precursor compartment in the chicken skeletal myogenic lineage. *Differentiation* 26: 112-120.
- Quinn, L.S. and M. Nameroff 1983 Analysis of the myogenic lineage in chick embryos. IV. Effects of conditioned medium. *Differentiation* 24: 124-130.
- Quinn, L.S. and M. Nameroff 1983 Analysis of the myogenic lineage in chick embryos. III. Quantitative evidence for discrete compartments of precursor cells. *Differentiation* 24: 111-123.
- Nameroff, M. 1981 Skeletal myogenesis: Evidence for a programmed cell lineage. *In* Proc. 2nd Intl. Congress on Cell Biology, H.G. Schweiger, Ed., Springer-Verlag, New York, pp. 539-542.
- Kligman, D. and M. Nameroff 1980 Analysis of the myogenic lineage in chick embryos. II. Evidence for a deterministic lineage in the final stages. *Exptl. Cell Res.* 127: 237-247.
- Kligman, D. and M. Nameroff 1980 Analysis of the myogenic lineage in chick embryos. I. Studies on the terminal cell division. *Exptl. Cell Res.* 125: 201-210.
- Nameroff, M. 1977 Fusion, phospholipase C and myogenesis. *In*: Regulation of Cell Proliferation and Differentiation, Cellular Senescence and Somatic Cell genetics Series, Vol. 2, W.W. Nichols and D.G. Murphy, Eds., Plenum Press, New York, pp. 139-142.
- Trotter, J.A. and M. Nameroff 1976 Myoblast differentiation *in vitro*: Morphological differentiation of mononucleated myoblasts. *Develop. Biol.* 49: 548-555.
- Nameroff, M. and E. Munar 1976 Inhibition of cellular differentiation by phospholipase C. II. Separation of fusion and recognition among myogenic cells. *Develop. Biol.* 49: 288-293.

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BIOGRAPHICAL SKETCH

N. DEAN PENTCHEFF

EDUCATION:



APPOINTMENTS:

1983-present	Research Technician, Department of Biological Structure, University of Washington, Seattle, WA
1983-present	Computer Programmer, Center for Bioengineering, University of Washington, Seattle, WA
1981	Systems Analyst and Programmer, Duke University Marine Laboratory, Beaufort, NC
1981	Computer Programmer, Duke University Marine Laboratory, Marine Biomedical Research Center, Beaufort, NC
1980	Systems Analyst, CIBA-GEIGY Corporation, U.S. Headquarters, Ardsley, NY
1976-1978	Research Assistant (summers), Bermuda Biological Station, St. Georges, Bermuda.

REPRESENTATIVE PUBLICATIONS:

Bolender, R.P., N.D. Pentcheff 1984 A Tutorial Introduction to Stereology: Tutorials, Basics, and PCS System I Programs. (In Press).

Pentcheff, N.D., R.P. Bolender 1984 PCS System I: Point counting stereology programs for cell biology. Computer Programs in Biomedicine (Submitted).

Larsen, M.P., N.D. Pentcheff, R.P. Bolender 1983 ASSESS: A simulation system for evaluating stereological sampling. Acta Stereologica (Suppl. I & II), I: 41-46.

Larsen, M.P., E.A. Pederson, N.D. Pentcheff, J.F. Bertram, R.P. Bolender 1983 PCS-I and PCS-II: Point counting stereology programs. Acta Stereologica (Suppl. I & II), I: 95-98.

BIOGRAPHICAL SKETCH

SYLVIA BYRNE POLLACK

BORN: [REDACTED] Ithaca, New York

EDUCATION: [REDACTED]

APPOINTMENTS: 1981-present Research Associate Professor, Dept. Biological Structure,
Univ. Washington School of Medicine, Seattle,
1979-1981 Associate Member, Fred Hutchinson Cancer Research Center,
Seattle
1977-1981 Research Associate Professor, Dept. Microbiology and
Immunology, Univ. Washington, Seattle
1975-1979 Assistant Member, Fred Hutchinson Cancer Research Center,
Seattle
1973-1977 Research Assistant Professor, Dept. Microbiology and
Immunology, Univ. Washington School of Medicine, Seattle
1968-1973 Research Associate, Dept. Microbiology, Univ. Washington
School of Medicine, Seattle
1967-1968 Instructor, Dept. Anatomy, Women's Medical College of
Pennsylvania, Philadelphia

REPRESENTATIVE PUBLICATIONS: (From a total of 46)

- Pollack, S. B. and C. Rosse 1984 Production of natural killer cells in bone marrow. In The Reticuloendothelial System. S. Reichard, Ed. Alan R. Liss, Inc., New York. (In Press)
- Robles, C. P., P. Pereira, P. Wortley and S. B. Pollack 1984 Regulation of B cell responses by NK cells. In Mechanism for Cytotoxicity by NK Cells. R. B. Herberman, Ed., Academic Press, New York. (In Press)
- Pollack, S. B. 1984 Phenotype, functional heterogeneity and lineage of natural killer cells. In Immunobiology of Natural Killer Cells. E. Lotzova and R. B. Herberman, Eds., CRC Press (In Press)
- Emmons, S. L. and S. B. Pollack 1984 Murine NK cell heterogeneity: Subpopulations of C57BL/6 splenic NK cells detected by NK-1.1 and NK-2.1 antiserum. Submitted.
- Pollack, S. B. 1983 In vivo functions of NK cells. Survey and Synthesis of Pathology Research. 2: 93-106.
- Brooks, C. G., R. C. Burton, S. B. Pollack and C. S. Henney 1983 The presence of NK alloantigens on cloned cytotoxic T lymphocytes. J. Immunol. 131: 1391-1395.
- Pollack, S. B. and S. L. Emmons 1982 NK 2.1: An NK-associated antigen detected with NZB anti-BALB/c serum. J. Immunol. 129: 2277-2281.
- Lotzova, E., C. A. Savary and S. B. Pollack 1983 Prevention of rejection of allogeneic bone marrow transplants by NK 1.1 antiserum. Transplantation, 35: 490-494.
- Pollack, S. B. and L. A. Hallenbeck 1982 In vivo reduction of NK activity with anti-NK 1 serum: Direct evaluation of NK cells in tumor clearance. Int. J. Cancer 29: 203-206.
- Tam, M. R., S. L. Emmons and S. B. Pollack 1980 Analysis and enrichment of murine natural killer cells with the fluorescence-activated cell sorter. J. Immunol.
- Pollack, S. B. 1982 Direct evidence for anti-tumor activity by NK cells in vivo: Growth of B16 melanoma in anti-NK 1.1 treated mice. In NK Cells and Other Natural Effector Cells. R. B. Herberman, Ed., Academic Press, pp. 1347-1352. 124: 650-655.
- Pollack, S. B., M. R. Tam, R. C. Nowinski and S. L. Emmons 1979 Presence of T cell-associated antigens on murine NK cells. J. Immunol. 123: 1818-1821.

1076166

BIOGRAPHICAL SKETCH

JOHN W. PROTHERO

BORN: [REDACTED] Toronto, Ontario, Canada

EDUCATION: [REDACTED]

APPOINTMENTS: 1970-present Associate Professor, Department of Biological Structure, University of Washington, Seattle, WA
1967-1970 Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1965-1967 Acting Assistant Professor, University of Washington, Seattle, WA
1965 Research Associate, Department of Biology, Massachusetts Institute of Technology
1961-1965 Medical Research Fellow, Medical Research Council, Canada (Royal Institution, London, 6 months; and Laboratory of Molecular Biology, Cambridge, England)

REPRESENTATIVE PUBLICATIONS:

- Prothero, J. and K. Jurgens The model of daily torpor in homeotherms (Submitted)
Angello, J. and J. Prothero Clonal alteration in chick fibroblasts: Experiments, model and computer simulations (Submitted).
Prothero, J.W. and J. Sundsten Folding of the cerebral cortex in mammals: A scaling model. Submitted.
Prothero, J.W. 1984 Organ scaling in mammals: The kidneys. *Comp. Biochem. Physiol.* 77A: 133-138.
Sundsten, J.W. and J.W. Prothero 1983 Three dimensional reconstruction from serial sections: II. A microcomputer-based facility for rapid data collection. *Anat. Rec.* 207: 665-671.
Prothero, J. 1984 Scaling of standard energy metabolism in mammals: I. Neglect of circadian rhythms. *J. Theor. Biol.* 106: 1-8.
Prothero, Jeffrey and J.W. Prothero 1982 Three-dimensional reconstruction from serial sections. I. A portable microcomputer-based software package in Fortran. *Computers Biomed. Res.* 15: 598-604.
Mannard J., A. Lindsay, J. Sundsten and J. Prothero 1982 A plotted-to-tabular data conversion program for microcomputers. *Int. J. Bio-Med. Computing* 13: 369-373.
Prothero, J. 1982 Organ scaling in mammals: The liver. *Bomp. Biochem. Physiol.* 71A: 567-577.
Prothero, J.W. and J. Gallant 1981 A model of clonal attenuation. *PNAS* 78: 333-337.
Gallant, J. and J.W. Prothero 1980 Testing models of error propagation. *J. Theor. Biol.* 83: 561-578.
Prothero, J.W. 1980 Control of stem cell proliferation: a density-dependent commitment model. *J. Theor. Biol.* 84: 725-736.
Prothero, J.W. 1980 Scaling of blood parameters in mammals. *Comp. Biochem. Physiol.* 67A: 649-657.
Prothero, J.W. 1979 Heart weight as a function of body weight in mammals. *Growth* 43: 139-150.
Prothero, J.W. 1979 Maximal oxygen consumption in various animals and plants. *Comp. Biochem. Physiol.* 64A: 463-466.
Martinez, A.O., T.H. Norwood, J.W. Prothero and G.M. Martin 1979 Evidence for clonal attenuation of growth potential in HeLa cells. *In Vitro* 14: 996-1002.
Cummings, F.W. and J.W. Prothero 1978 A model of pattern formation in multicellular organisms. *Collective Phenomena* 3: 41-53.
Prothero, J.W., M. Starling and C. Rosse 1978 Cell kinetics in the erythroid compartment of guinea pig bone marrow: A model based on ³H-TdR studies. *Cell Tissue Kinet.* 11: 301-316.

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BIOGRAPHICAL SKETCH
CORNELIUS ROSSE

BORN: [REDACTED] Hungary

EDUCATION: [REDACTED]

APPOINTMENTS: 1981-present Professor and Chairman, Department of Biological Structure, University of Washington School of Medicine
1975-1981 Professor, Department of Biological structure, University of Washington School of Medicine
1974-1975 Visiting Professor, Department of Anatomy, Royal College of Surgeons, University of London, Postgraduate School
1970-1975 Associate Professor, Biological Structure, University of Washington School of Medicine
1967-1970 Assistant Professor, Biological Structure, University of Washington School of Medicine
1965-1967 Demonstrator in Anatomy, University of Bristol, England
1965 House Physician to J. E. Cates, Royal Infirmary, University of Bristol, England
1964-1965 House Surgeon to Professors R. Milner-Walker and A. G. Riddell, Royal Infirmary, University of Bristol, England

REPRESENTATIVE PUBLICATIONS

- Pollack, S. B. and C. Rosse 1984 Production of natural killer cells in bone marrow. In Proc. 10th RES Congress, S. Reichard (Ed.) (Submitted)
- Ching, L. M., A. G. Farr, S. Hosier, S. K. Anderson, and C. Rosse 1984 Myeloid-lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown in vitro. (Submitted)
- Ching, L. M., A. G. Farr, S. Hosier and C. Rosse 1984 The ontogeny of a post-thymic precursor T-cell which generates cytotoxic T lymphocyte precursor cells in colonies grown in vitro. (Submitted)
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- Rosse, C. 1980 Perspectives of lymphocyte production and cellular traffic in bone marrow. In The Handbook of Cancer Immunology. H. Waters, Ed., Garland ATPM Press, Washington, D.C., Vol. 6, p. 250.

BIOGRAPHICAL SKETCH

RONALD E. STENKAMP

BORN: [REDACTED] Bend, Oregon

EDUCATION: [REDACTED]

APPOINTMENTS: 1981-present Research Assistant Professor, Department of Biological Structure, University of Washington
1978-1981 Research Associate, Department of Biological Structure, University of Washington, Seattle, WA
1977 American Cancer Society Postdoctoral Fellow, Department of Biological Structure, University of Washington, Seattle, WA
1975-1977 American Cancer Society Postdoctoral Fellow, Postdoctoral Associate, Sessel-Anonymous Fellow with Dr. T.A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT
1974-1975 Senior Fellow, Department of Biological Structure, University of Washington, Seattle, WA
1974-1975 Research Assistant with Dr. Lyle H. Jensen, Department of Biological Structure, University of Washington, Seattle, WA
1970-1973 Teaching Assistant, Department of Chemistry, University of Washington, Seattle, WA

REPRESENTATIVE PUBLICATIONS:

- Stenkamp, R.E. and L.H. Jensen 1984 Resolution revisited. *Acta Cryst.*, (In Press).
Essig, M.G., F. Shafizadeh, T.G. Cochran and R.E. Stenkamp 1984 The crystal structure of a septanose derived from levoglucosenone, *J. Carb. Research.*, (In Press).
Essig, M.G., T.T. Stevenson, F. Shafizadeh, R.E. Stenkamp and L.H. Jensen 1984 1S-(1 α 4 α , 5 β , 5a β , 6 β , 8 β , 9 β , 9a β)- octahydro-5,8-dihydroxyl-1,4-epoxy-6,9-methano-3-benzoxepin-7(2H)-one (a levoguclosenone derivative). The product of a 1,4-hydride shift. *J. Org. Chem.* (In Press).
Essig, M.G., F. Shafizadeh, T.G. Cochran and R.E. Stenkamp 1984 The crystal structure of a septanose derived from levoglucosenone. *Carbohydrate Research* (In Press).
Stenkamp, R.E., L.C. Sieker and L.H. Jensen 1984 Binuclear iron complexes in methemerythrin and azidomethemerythrin at 2.0Å resolution. *J. Am. Chem. Soc.*, 104: 618-622.
Perkins, C.M., N.J. Rose, R.E. Stenkamp, L.H. Jensen, B. Swinstein and L. Pickart 1984 The structure of a copper complex of the growth factor glycyL-L-histidyl-L-lysine at 1.1Å resolution. *Inorganica Chimica Acta*, 83: 93-99.
Stenkamp, R.E., L.C. Sieker and L.H. Jensen 1983 Restraints in the refinement of methemerythrin and azidomethemerythrin at 2.0 Å resolution. *Acta Cryst.*, B39: 697-703.
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Stevenson, T.T., M.G. Essig, F. Shafizadeh, L.H. Jensen and R.E. Stenkamp 1983 The crystal structure of an epoxide of a levoglucosenone-cyclopentadiene adduct. *Carbohydrate Research*, 118: 261-268.
Stevenson, T.T., R.H. Furneaux, D. Pang, F. Shafizadeh, L.H. Jensen and R.E. Stenkamp 1983 The crystal structure of a nonalkenic, cyclic trimer of levoglucenone. *Carbohydrate Research*, 112: 179-187.
Stenkamp, R.E., L.H. Jensen, T.B. Murphy and N.J. Rose 1982 Structure of a benzene solvate of alpha-furildioxime. *Acta Cryst.*, B38: 1169-1172.
Stenkamp, R.E. and R.P. Ko 1982 1,2-Dimethyl-5-trifluoracetyl-2- H-cyclopenta [d] pyridazine. *Acta Cryst.*, B38: 994-996.

1076169

BIOGRAPHICAL SKETCH

KEITH D. WATENPAUGH

BORN: [REDACTED], Amarillo, Texas

EDUCATION: [REDACTED]

APPOINTMENTS: 1978-present Research Associate Professor, Department of Biological Structure, University of Washington, Seattle, WA
1972-1978 Research Assistant Professor, Department of Biological Structure, University of Washington, Seattle, WA
1969-1972 Research Associate, Department of Biological Structure, University of Washington, Seattle, WA
1966-1969 Senior Fellow, Department of Biological Structure, University of Washington, Seattle, WA

REPRESENTATIVE PUBLICATIONS:

- Watenpaugh, K.D. 1984 Overview of phasing by isomorphous replacement. In: Methods in Enzymology. Wyckoff, Hirs and Timasheff, Eds. Academic Press, New York, (In Press).
- Alden, R.A., G. Bricogne, S.T. Freer, S.R. Hall, W.A. Hendrickson, P. Machin, R.F. Munn, A.J. Olson, G.N. Reeke, Jr., S. Sheriff, J.M. Stewart, J. Sygush, L.F. TenEyck and K.D. Watenpaugh 1983 Cooperative programming in crystallography. Comput. Chem. 7, 137-148.
- Ludwig, M.L., K.A. Pattridge, W.W. Smith, L.H. Jensen and K.D. Watenpaugh 1982 Comparisons of Flavodoxins. In: 7th International Symposium on Flavins and Flavoproteins, Ann Arbor, Massey and Williams, Eds. Elsevier, Amsterdam, pp. 19-27.
- Watenpaugh, K.D., L.C. Sieker and L.H. Jensen 1980 Crystallographic refinement of rubredoxin at 1.2Å resolution. J. Molec. Biol., 138: 615-633.
- Watenpaugh, K.D., L.C. Sieker and L.H. Jensen 1979 The structure of rubredoxin at 1.2Å resolution. J. Molec. Biol., 131: 509-522.
- Hanson, J.C., K.D. Watenpaugh, L.C. Sieker and L.H. Jensen 1979 A limited-range step-scan method for collecting x-ray diffraction data. Acta Cryst., A35: 616-621.
- Watenpaugh, K.D., T.N. Margulis, L.C. Sieker and L.H. Jensen 1978 Water structure in a protein crystal: Rubredoxin at 1.2 Å resolution. J. Molec. Biol., 122: 175-190.
- Fitzgerald, A., R.E. Stenkamp, K.D. Watenpaugh and L.H. Jensen 1977 The crystal and molecular structure of dihydroxo (1,2,3,4,5,6,7,8-octaethylporphinato) antimony (V) perchlorate monoethanol solvate: A crystal structure that exhibits a subcell. Acta Cryst., B33: 1688-1696.
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- Adman, E.T., K.D. Watenpaugh and L.H. Jensen 1975 NH...S hydrogen bonds in P. aerogenes ferredoxin, C. pasteurianum rubredoxin and Chromatium high potential iron protein. Proc. Nat. Acad. Sci. (USA), 72: 4854-4858.
- Watenpaugh, K.D., L.C. Sieker and L.H. Jensen 1975 A crystallographic structural study of the oxidation states of Desulfovibrio vulgaris flavodoxin. In: 5th International Symposium on Flavins and Flavoproteins, San Francisco, T.P. Singer, Ed., Elsevier, Amsterdam, 405-410.
- Watenpaugh, K.D., L.C. Sieker and L.H. Jensen 1974 Flavin mononucleotide conformation and environment in flavodoxin from Desulfovibrio vulgaris. In: 4th Steenbock Symposium on Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions, Madison, Wisconsin, 431-439.
- Watenpaugh, K.D., L.C. Sieker and L.H. Jensen 1974 Anomalous scattering in protein structure analysis. In: I.U.Cr. Intercongress Conference on Anomalous Scattering Transactions, Madrid, 393-405.

ATTACHMENT 2
ASSURANCE OF COMPLIANCE

1076171

U.S. DEPARTMENT OF ENERGY
ASSURANCE OF COMPLIANCE

Nondiscrimination in Federally Assisted Programs

The University of Washington

(Hereinafter called the "Applicant")

(Name of Applicant)

HEREBY AGREES to comply with Title VI of the Civil Rights Act of 1964 (Pub. L. 88-352), Section 16 of the Federal Energy Administration Act of 1974 (Pub. L. 93-275), Section 401 of the Energy Reorganization Act of 1974 (Pub. L. 93-438), Title IX of the Education Amendments of 1972, as amended, (Pub. L. 92-318, Pub. L. 93-568, and Pub. L. 94-482), Section 504 of the Rehabilitation Act of 1973 (Pub. L. 93-112), the Age Discrimination Act of 1975 (Pub. L. 94-135), Title VIII of the Civil Rights Act of 1968 (Pub. L. 90-284), the Department of Energy Organization Act of 1977 (Pub. L. 95-91), and the Energy Conservation and Production Act of 1976, as amended, (Pub. L. 94-385). In accordance with the above laws and regulations issued pursuant thereto, the Applicant agrees to assure that no person in the United States shall, on the ground of race, color, national origin, sex, age, or handicap, be excluded from participation in, be denied the benefits of, or be otherwise subjected to discrimination under any program or activity in which the Applicant receives Federal assistance from the Department of Energy.

Applicability and Period of Obligation

In the case of any service, financial aid, covered employment, equipment, property, or structure provided, leased, or improved with Federal assistance extended to the Applicant by the Department of Energy, this assurance obligates the Applicant for the period during which Federal assistance is extended. In the case of any transfer of such service, financial aid, equipment, property, or structure, this assurance obligates the transferee for the period during which Federal assistance is extended. If any personal property is so provided, this assurance obligates the Applicant for the period during which it retains ownership or possession of the property. In all other cases, this assurance obligates the Applicant for the period during which the Federal assistance is extended to the Applicant by the Department of Energy.

Employment Practices

Where a primary objective of the Federal assistance is to provide employment or where the Applicant's employment practices affect the delivery of services in programs or activities resulting from Federal assistance extended by the Department, the Applicant agrees not to discriminate on the ground of race, color, national origin, sex, age, or handicap, in its employment practices. Such employment practices may include, but are not limited to, recruitment, recruitment advertising, hiring, layoff or termination, promotion, demotion, transfer, rates of pay, training and participation in upward mobility programs, or other forms of compensation and use of facilities.

Subrecipient Assurance

The Applicant shall require any individual, organization, or other entity with whom it subcontracts, subgrants, or subleases for the purpose of providing any service, financial aid, equipment, property, or structure to comply with laws cited above. To this end, the subrecipient shall be required to sign a written assurance form, however, the obligation of both recipient and subrecipient to ensure compliance is not relieved by the collection or submission of written assurance forms.

Data Collection and Access to Records

The Applicant agrees to compile and maintain information pertaining to programs or activities developed as a result of the Applicant's receipt of Federal assistance from the Department of Energy. Such information shall include, but is not limited to, the following: (1) the manner in which services are or will be provided and related data necessary for determining whether any persons are or will be denied such services on the basis of prohibited discrimination; (2) the population eligible to be served by race, color, national origin, sex, age and handicap; (3) data regarding covered employment including use or planned use of bilingual public contact employees serving beneficiaries of the program where necessary to permit effective participation by beneficiaries unable to speak or understand English; (4) the location of existing or proposed facilities connected with the program and related information adequate for determining whether the location has or will have the effect of unnecessarily denying access to any person on the basis of prohibited discrimination; (5) the present or proposed membership by race, color, national origin, sex, age and handicap, in any planning or advisory body which is an integral part of the program; and (6) any additional written data determined by the Department of Energy to be relevant to its obligation to assure compliance by recipients with laws cited in the first paragraph of this assurance.

The Applicant agrees to submit requested data to the Department of Energy regarding programs and activities developed by the Applicant from the use of Federal assistance funds extended by the Department of Energy. Facilities of the Applicant (including the physical plants, buildings, or other structures) and all records, books, accounts, and other sources of information pertinent to the Applicant's compliance with the civil rights laws shall be made available for inspection during normal business hours on request of an officer or employee of the Department of Energy specifically authorized to make such inspections. Instructions in this regard will be provided by the Director, Federally Assisted Programs Division, Office of Equal Opportunity, U.S. Department of Energy.

This assurance is given in consideration of and for the purpose of obtaining any and all Federal grants, loans, contracts (excluding procurement contracts), property, discounts or other Federal assistance extended after the date hereto, to the Applicant by the Department of Energy, including installment payments on account after such date of application for Federal assistance which are approved before such date. The Applicant recognizes and agrees that such Federal assistance will be extended in reliance upon the representations and agreements made in this assurance and that the United States shall have the right to seek judicial enforcement of this assurance. This assurance is binding on the Applicant, its successors, transferees, and assignees, as well as the person whose signature appears below and who is authorized to sign this assurance on behalf of the Applicant.

December 17, 1984
(Date)

University of Washington

Seattle, Washington
(Name of Applicant)

(Address) Donald W. Allen

(Authorized Official) Donald W. Allen, Director
Grant and Contract Services

(206) 543-4043
(Applicant's Telephone Number)

1076173

APPENDIX I

1. Description and Specifications of NIKON/JOYCE-
LOEBL MAGISCAN 2A and NIKON Research
Microscope MICROPHOT
2. MAGISCAN 2 Image Analysis System
3. MENU - Magiscan Analysis Program



INSTRUMENT DIVISION

NIKON INC.
201 LINCOLN CENTRE DR
FOSTER CITY, CA 94404
(415) 871-1321

Quotation

Dr. Cornelius Rose, Chairman
Department of Biological Structure
Mail Code SM20
University of Washington
Seattle, Washington 98195

Date November 30, 1984

Reference No.

Attention

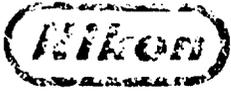
We are pleased to submit the following quotation. (Page 1)

Catalog No.	Quantity	Description	Unit Price	Amount
I		<p><u>NIKON/JOYCE-LOEBL MAGISCAN 2 A WITH WINCHESTER DRIVE</u></p> <p>Image processing/analysis system, designed for the analysis of images obtained from either micro or macro specimen sources, consisting of:</p> <p><u>SCANNER:</u></p> <p>Low light level Newvicon CCTV instrumentation camera with isolated external transformer, CCIR standard 625 line scan, Camera Tube: Newvicon, frontplate illumination, geometric distortion 1%, linear distortion 0.5%, resolution 800 lines, lens mounting standard "C" mount.</p> <p><u>VIDEO INPUT:</u></p> <p>CCIR standard video signal: 0.7V P-P with 0.3V sync.</p> <p>Scanner is driven from -4.0V + -30% into 75 ohms mixed sync. signal digitally generated from master oscillator crystal.</p> <p>Analog/Digital converter: flash converter 6 bit - 64 grey levels. Conversion rate: 13.333MHz.</p> <p>Input lookup table provides video rate grey point transformation and scaling into any contiguous group of image memory planes.</p>		

1076175

REV. SEPT. 1, 1984

(continue)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR
FOSTER CITY, CA 94104
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 2)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p><u>IMAGE STORE:</u></p> <p>Image store of modular bit plane design: expandable from standard 1024 X 1024 X 8 bits to optional 1024 X 1024 X 16 bits.</p> <p>Store Access Time: 300NS.</p> <p>Store addressing and control performed by an independent memory address processor, giving pipeline access with neighborhood addressing and variable resolution display and loading of images (512 X 512 and 256 X 256).</p> <p>Organization of store is determined dynamically by software. No restriction is placed on the number of bits in stored grey level images or in number of binary planes, subject only to capacity of image memory.</p> <p><u>VIDEO OUTPUT:</u></p> <p>Output map selects any contiguous group of bit planes for grey image display with any number of superimposed binary overlays for text and graphics. Partial image overlays can also be used.</p> <p>8 bit D/A converter: conversion rate 13.333MHz synchronized with A/D converter for direct or stored video display.</p> <p>High resolution integral black and white monitor: P4 whit phosphor, 12" diagonal, video amplifier bandwidth: DC to 15 MHz.</p>		

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(continued)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR.
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 3)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p>Pseudo color display with 14" diagonal high resolution color monitor, complete with RGB hardware and operational software.</p> <p><u>PROCESSOR:</u></p> <p>Custom designed microprogrammable 16 bit central processor, implemented using LSI and MSI SCHOTTKY TTL. The architecture is optimised for image processing and comprises:</p> <ul style="list-style-type: none"> 512 registers Arithmetic logic unit 8 X 8 LSI multiplier Hardware shift and mask logic <p>Instruction time: (Minimum - 150 nSec)</p> <p>128K bytes macro memory for program and data storage.</p> <p><u>MICROPROGRAM CONTROL UNIT:</u></p> <p>An LSI controller independently fetches the next micro-instruction from the microprogram memory, executing conditional branch and branch to subroutine instructions.</p> <p>512 X 48 bit initialization and primary bootstrap ROM containing self testing procedures automatically executed at power up and reset.</p> <p>4K X 48 bit user writable RAM contains the PASCAL interpreter and control store for image processing routines. Specific microcode units for an application are loaded at run time and can be overlaid from disk, to increase the virtual microcode store space.</p>		

1076177

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INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR.
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 4)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p><u>I/O CONTROLLER:</u></p> <p>Provides INTEL "8085 A" style byte oriented bi-directional data and address bus for interfacing to peripheral devices, both internal and external.</p> <p>On board devices:</p> <ul style="list-style-type: none"> a. Keyboard interface b. Character generator - may be programmed with specific character set for user language and symbols c. Control switches and lamps d. Disk Drives- <ul style="list-style-type: none"> Integrally mounted, 5 1/4" Winchester Hard disk drive with 16.77 Mb capacity. Will store 63 images with load time of 5 sec. and store time of 5 sec. 4 surfaces, 4 read/write heads, 512 cylinders 2048 tracks, with 32 sectors/track Software formatted at 256 bytes per sector Access time: track - track 10 msecs. Latency 8.5 msecs average 5 1/4" mini floppy disk drive, double sided, double density 80 cylinders with 16 sectors/cylinder Software formatted at 512 bytes/sector Formatted capacity - 655 K bytes Modified Frequency Modulation (MFM) Recording technique 		

1076178

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INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No:

Attention

We are pleased to submit the following quotation. (Page 5)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p>e. Serial interface- Standard EIA - RS232 C interface - voltage driven Baud rates selectable either by initial switch settings or under software control (110-9600 Baud)</p> <p>f. Parallel interface - Extension of I/O Controller bus as above</p> <p>g. Programmable interval timer.</p> <p><u>PHYSICAL:</u></p> <p>Desktop console containing processor, image memory, disk drives and display. Dimensions: 700 X 520 X 345mm. Weight: 50 Kg.</p> <p>Full QWERTY detachable keyboard plus soft keys plus PASCAL editing keys. Dimensions: 520 X 240 X 95mm. Weight: 4 Kg.</p> <p>Lightpen connected to electronics unit. Single picture point resolution.</p> <p><u>ENVIRONMENT:</u></p> <p>Electrical supply: 110 volts 50-60Hz Power consumption 500VA Ambient temperature: 10 - 25'C Relative humidity: 20% - 80% non condensing Noise: acoustical rating of fans - 2. each at NC - 28.</p>		

1076179

(continued)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR.
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 6)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p><u>SOFTWARE:</u></p> <p>"MENU" Analysis program featuring: Easy, menu driven operation Split screen display showing results of processing Wide range of standard morphological measurements Comprehensive data analysis and display.</p> <p>UCSD PASCAL language with extensive software library.</p> <p><u>IMAGE PROCESSING:</u></p> <p>Shade correction using stored correction matrix.</p> <p>Mapping function with linear density, histogram or arbitrary look up table.</p> <p>Local operators for smoothing band pass. Gradient and Laplacian filtering.</p> <p>Segmentation by grey level with tracking and 2D-Edge lock using automatic or interactive threshold setting.</p> <p>Binary operations for Dilation and Erosion with Arbitrary structural element, cleaning logical AND, OR, NOT editing and boundary finding.</p> <p>Manual image editing with the single picture point (pixel) lightpen allows LINE add or remove and REGION accept or reject.</p> <p>Geometrical measurements on field, regions, or features include area, (area excluding holes), horizontal feret diameter, vertical feret diameter, center of gravity, maximum length, breadth, perimeter, orientation, count, integrated optical density, field area, total detected area.</p>		

1076180

(continued)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 7)

Catalog No.	Quantity	Description	Unit Price	Amount
3605		<p>Densitometric measurements provide histograms of grey levels with first and second order texture parameters, either within specified regions or along image lines.</p> <p>Output Processing gives labelled or tabulated results, statistical analysis, graphical, histogram, bi-varient distributions, and scattergrams.</p> <p><u>Printer:</u></p> <p>Printer with RS-232 serial interface. Dot matrix with 160 CPS print rate, 9X9 to 18X18 matrix. Full 96-character with ASCII with descenders with additional special characters. Buffer size 2K, bit image graphics, selectable and programmable 80 or 132 characters per line. Built-in self test mode. Net weight 17 lbs.</p>		94000
		TOTAL		94000

1076181



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 8)

atalog No.	Quantity	Description	Unit Price	Amount
II		Nikon Research Microscope "Microphot" Estimated price, based on configuration		\$18-25,000
III		Motorized stage powered by precision stepper motors is available to scan 8 3"X1" slides. This stage is normally supplied with the Chromosome System on a Nikon Biophot. This is a custom-made item and is a special order requiring eight to twelve weeks A.R.O. Product No. 77702		6,500 00
IV		<u>Focus Module (Z Direction)</u> To work in conjunction with microscopes fitted with motorized stages. Requires fitting of precision stepper motor to fine focus control shaft on microscope. Can be installed on new or existing microscope bases. Will require stage to be custom machined (extra) at Garden City unless installed on new base prior to delivery. Product No. 79624		3,600 00
V		<u>Motor Controller</u> To power motorized stages (X,Y Direction) and the Focus Module (Z) Direction. Used with III & IV above. Controlled from Magiscan 2 via lightpen. Product No. 79623		6,000 00
VI		Dage SIT (Silicon Intensified Target) CCTV Instrumentation Camera with isolated external transformer, DCIR standard 625 line scan, Camera Tube: intensified silicon diode, sensitivity: .00001 fc frontplate illumination, geometrical distortion 3%, linear distortion 0.5%, resolution 600 lines, lens mounting standard "C" mount. Product No. 79629		8,000 00

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(continued)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR.
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

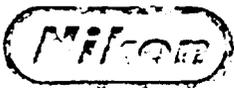
Attention

We are pleased to submit the following quotation. (Page 9)

Catalog No.	Quantity	Description	Unit Price	Amount
		<u>OPTIONAL:</u> <u>IEEE 488 Interface</u> For high speed parallel data transfer between the Magiscan 2 and a host computer or a SEM (Scanning Electron Microscope) also requires a P.V.I. This interface allows Magiscan 2 to communicate on the IEEE 488 as talker, listener or controller. Can be installed in the field. Product No. 79625		3,500 00
		Banded Chromosome Software Program. Product No. 79655		30,000 00
		<u>OPTIONAL MAINTENANCE AND SERVICE PROGRAMS AVAILABLE</u> A. Basic maintenance with 2 visits per year.		1,000 00
		B. All inclusive, unlimited calls and parts per year.		5,000 00
		C. Maintenance & on-call travel and hourly service charges as needed, when needed.		to be billed
		D. Software subscription provides updates and revisions of programs supplied to user. Includes documentation and magnetic media per year.		3,000 00

1076183

(continued)



INSTRUMENT DIVISION

NIKON INC.
804 LINCOLN CENTRE DR
FOSTER CITY, CA 94404
(415) 571-8320

Quotation

Date November 30, 1984

Reference No.

Attention

We are pleased to submit the following quotation. (Page 10)

Catalog No.	Quantity	Description	Unit Price	Amount
		<p><u>NOTE:</u></p> <p>The Magiscan system is warranted for one year after date of installation when electrical power conforms to computer industry standards with regard to groundings, surges, spikes, EMI & RFI.</p> <p>Nikon Inc., Instrument Division has responsibility for equipment terminating at the IEEE 488 parallel port and the RS-232 serial port, except when the Nikon supplied printer is connected to the RS-232 serial port.</p> <p style="text-align: center;">REV. Oct. 29, 1984</p>		

Prices quoted are firm for 30 days.

Terms: Special

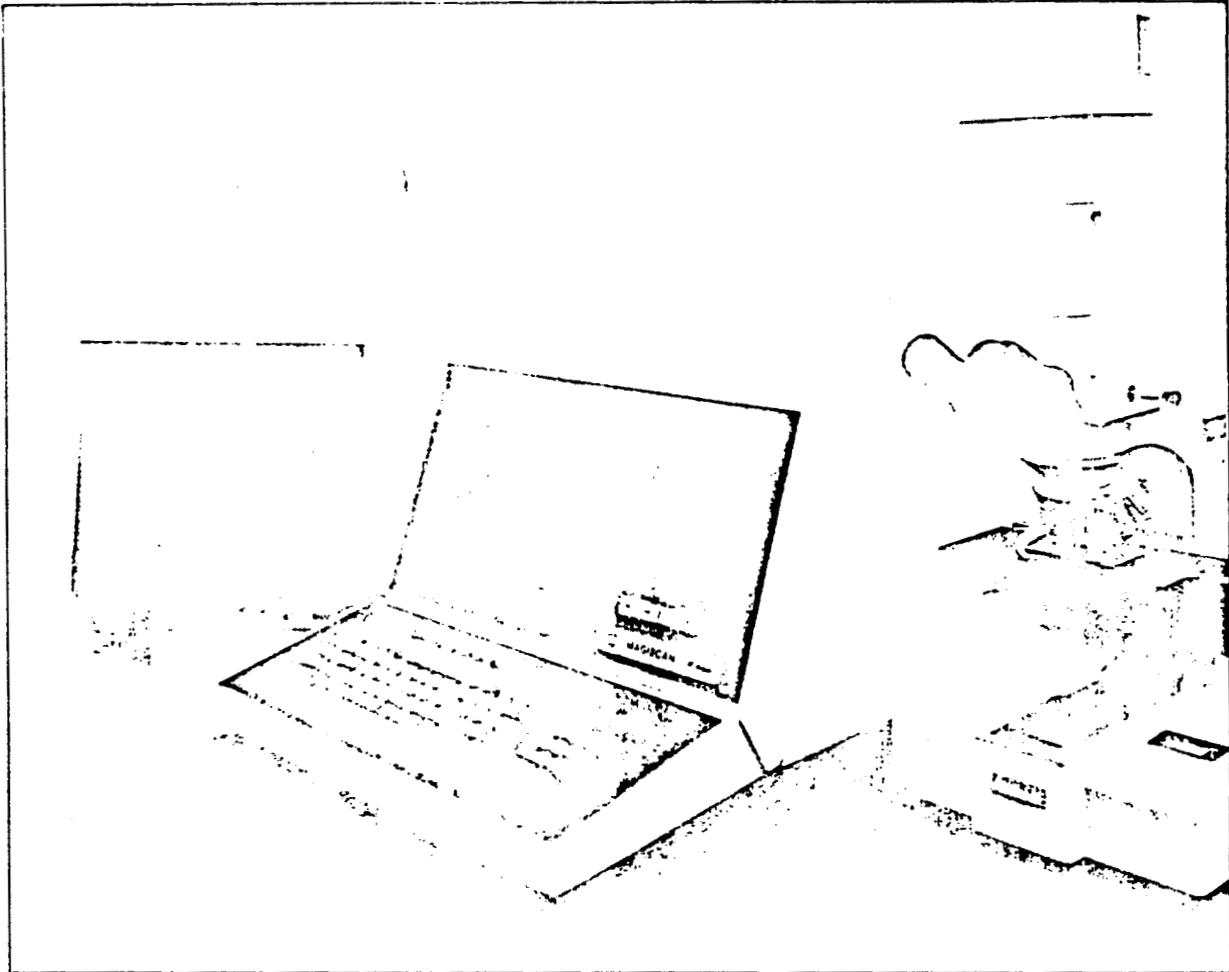
Delivery Date: 45 - 60 Days A.R.O.

F.O.B. Point: Garden City, New York
cc: Meridian Instrument, Jan Strelow
1076184 Nikon Ins. Group, Connie Hewitt

NIKON INC., INSTRUMENT DIVISION

By K. Hendricks
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Sales Representative

MAGISCAN 2™ IMAGE ANALYSIS SYSTEM



- CCTV & SEM INPUT
- REAL TIME & STORED IMAGE PROCESSING
- B&W & COLOR OUTPUT
- EASY TO USE
- COMPACT, PROGRAMMABLE, COMPLETE

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Magiscan is a Trademark of Joyce-Loebl, Gateshead, England.

NIKON INTRODUCES A COMPLETE, EASY-TO-USE SYSTEM

Magiscan 2 is a computer-based user programmable system that can analyze and quantify real-time or digitally stored images. With Magiscan 2, technicians and researchers can observe manipulate and quickly make decisions regarding images obtained with the special CCTV Camera.

The image is stored as an array of 512 x 512 or 256 x 256 picture elements (PIXELS) within the 1024 x 1024 x 8 bit (expandable up to 16 bit) modular bit-plane image store. Magiscan 2 uses a custom designed microprogrammable processor to handle both image and data analysis. Working in serial mode, it executes high level instructions on feature data held in the main 128-K byte memory. For critical image processing routines, it switches to the pipeline mode, accessing the image store by means of a separate address processor. The modular design of the image store, together with input and output mapping units, allows maximum flexibility in storing multiple grey, binary or graphical images.

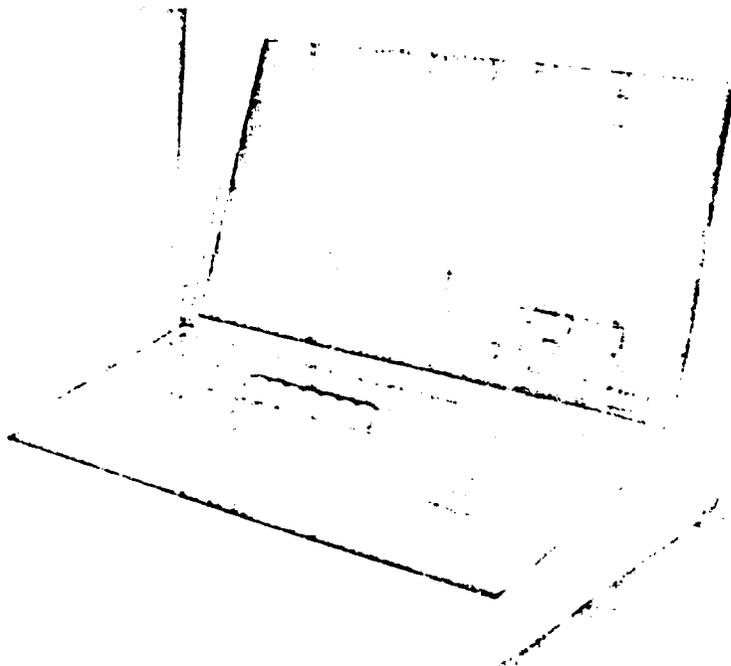
Information is displayed on the integral 12-inch black-and-white monitor and in pseudocolor on an accompanying 14-inch color monitor. Using the included light pen, the user can conveniently and quickly edit image content.

The fully programmable Magiscan 2 is housed in a compact desk top unit with built-in display and detachable keyboard.

The Standard Magiscan 2 MENU Program systematically guides the user through the image analysis sequence from image acquisition, processing, subsequent analyzing to a complete presentation of results.

In addition to the special application programs, Magiscan 2 can be programmed to perform a wide range of user defined tasks by employing the UCSD PASCAL software library of processing and analysis routines.

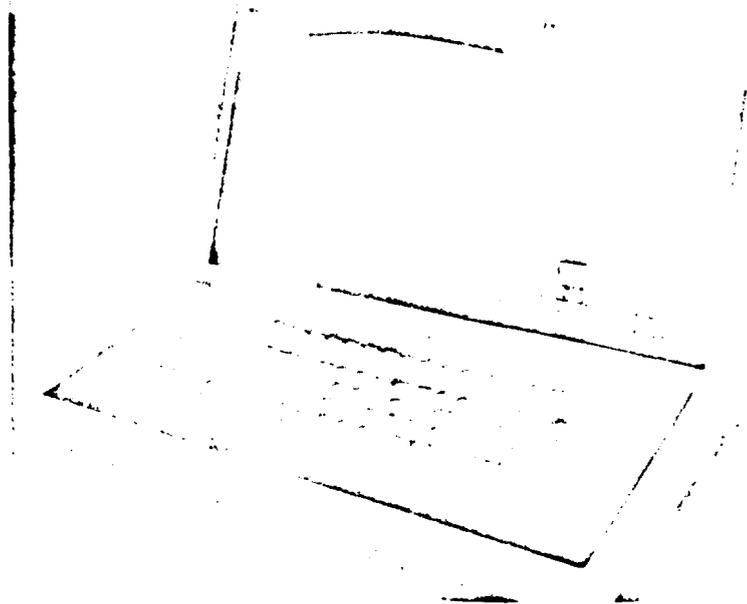
The Magiscan 2 system and its versatile accessories allow today's scientist to meet both current and future challenges.



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Wide Range of Applications

- | | |
|-------------------------------|--------------------------|
| • Space Technology | • Biochemistry |
| • Petroleum Geology | • Parasitology |
| • Hydraulics | • Pharmacology |
| • Power Systems | • Ophthalmology |
| • Mining | • Hematology |
| • Textiles | • Radiology |
| • Chemistry | • Dentistry |
| • Polymer Sciences | • Entomology |
| • Geophysics | • Anatomy |
| • Oceanography | • Astronomy |
| • Food Technology | • Wood Technology |
| • Ceramics | • Plant Science |
| • Structural Sciences | • Microbiology |
| • Electronics | • Ecology |
| • Metallurgical
Minerology | • Veterinary
Medicine |
| • Nuclear Systems | • Nutrition |
| • Filtration | • Botany |
| • Autoradiography | • Zoology |
| • Pharmaceuticals | • Neurology |
| • General Geology | • Genetics |
| • Seismology | • Pollution |
| • Animal Science | • Contamination |
| • Agronomy | • Particle Analysis |



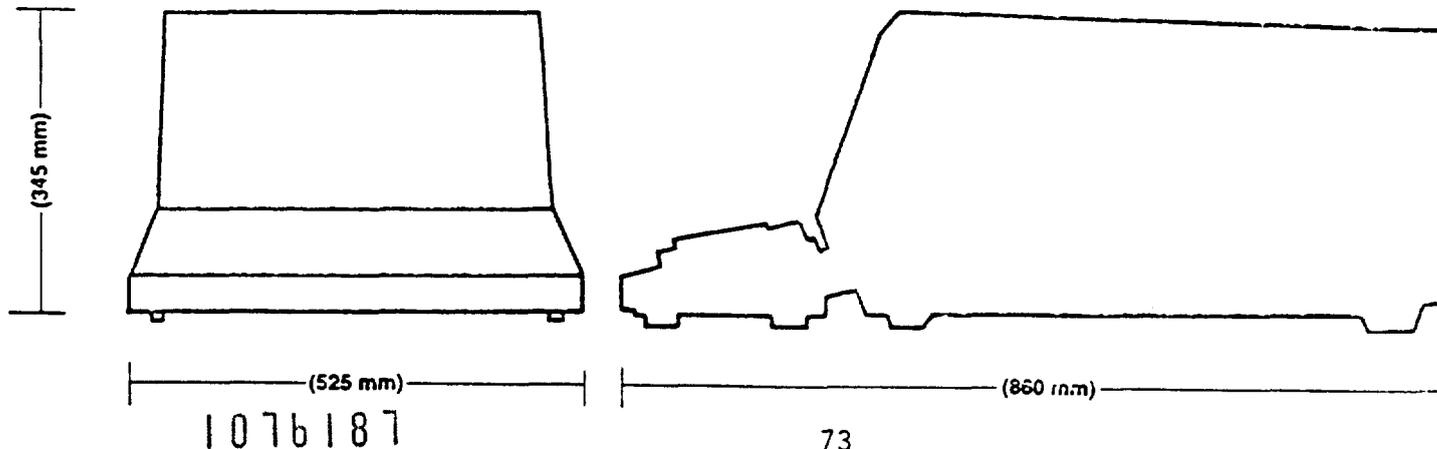
SPECIAL APPLICATION PROGRAMS

Human chromosome analysis—for banded and homogeneous samples, automatic location of good quality metaphase cells, automatic axis and centromere fitting, interactive control of analysis and measurements, chromosomes classified into a Karyogram. Provides visual and printed results.

ASTM Metallurgy—automatic inclusion and grain boundary detection, growing of incomplete boundaries, interactive editing facilities, presentation of development data for areas and grain size distribution.

Circle Fitting—separates agglomerates of circular particles. Interactive MENU-style operation, extensive editing facilities, statistical data and particle size distribution histogram provided.

ACCESSORIES Macro System with 55-mm Micro Nikkor Lens, IEEE Interface, Programmable Video Interface for SEM, etc. Hard Copy Printers, X-Y Stage Controller, Automatic Focus Module, Custom Nikon Microscopes and Image Store Expansion Modules.



SPECIFICATIONS

Scanner

Low light level Newvicon CCTV instrumentation camera, CCIR standard 625-line scan, lens mounting standard "C" mount.

Video Input

- Scanner signal digitally generated from master oscillator crystal.
- Analog digital converter: flash 6-bit A/D converter with 64 grey levels, 13.333 MHz conversion rate.
- Input lookup table provides video rate grey point transformation and sealing into any contiguous group of image memory planes.

Image Store

- Image store of modular bit plane design: expandable from standard 1024 x 1024 x 8-bits to 1024 x 1024 x 16-bits.
- Store access time: 300 ns.
- Store addressing and control performed by an independent memory address processor, giving pipeline access with neighborhood addressing and variable resolution display and loading of images (512 x 512 and 256 x 256 pixels).
- Organization of store is determined dynamically by software. No restriction is placed on the number of bits in stored grey level images or in number of binary planes.

I/O Controller

- Intel 8085-style byte-oriented bidirectional data and address bus.
- On board devices:
 - a. Keyboard Interface
 - b. Character generator that may be programmed with specific character set for user language and symbols
 - c. Control switches and lamps
 - d. Disk drive
 - Twin 5.25 in. minifloppy disk
 - Double-density, 77 track formatting
 - Formatted capacity—434 K bytes per drive
 - e. Serial interface
 - Standard EIA and RS-232-C interface, voltage driven with selectable baud rates
 - f. Parallel interface extension of I/O controlled bus as above
 - g. Programmable interval timer

Image Processing

- Shade correction using stored correction matrix.
- Grey-level mapping function with linear density, histogram or definable look-up table.
- Local operators for smoothing band pass. Gradient and Laplacian filtering.
- Segmentation by grey level with tracking and 2-D edge lock using automatic or interactive threshold setting.
- Binary operations for dilation and erosion, cleaning logical AND, OR NOT editing and boundary finding.
- Manual image editing with single pixel lightpen allows LINE add or remove and REGION accept or reject.

- Geometrical measurements of field, regions, or features including area, detected area, horizontal Feret diameter, vertical Feret diameter, center of gravity, maximum length, breadth, perimeter, orientation, count, integrated optical density, field area, and total detected area.

Video Output

- Output map selects any contiguous group of bit planes for grey image display with any number of superimposed binary overlays for text and graphics. Partial image overlays can also be used.
- 8 bit D/A converter with 13.333 MHz conversion rate synchronized with A/D converter for direct or stored video display.
- High-resolution integral 12-in. diagonal black-and-white
- Pseudocolor display with 14-in. diagonal high-resolution color monitor.

Processor

- Custom designed microprogrammable 16-bit central processor, implemented using LSI and MSI Schotky TTL logic. Architecture is optimized for image processing with 256 registers, arithmetic and logic unit, 8 x 8 LSI multiplier, and hardware shift and mask logic.
- Instruction time: 150 ns minimum
- 128-K bytes macro memory for program and data storage.

Microprogram Control Unit:

- An LSI controller independently prefetches the next microinstruction from the microprogram memory.
- 4-K x 48-bit user-writable RAM contains the Pascal interpreter and control store for image processing routines.

Physical

- Desktop console containing processor, image memory, disk drives and display. Dimensions: 700 x 520 x 345 mm (27.55 x 20.47 x 13.58 in.).
- Full QWERTY detachable keyboard plus soft keys and Pascal editing keys. Dimensions: 520 x 240 x 95 mm (20.47 x 9.44 x 3.74 in.).
- Lightpen connected to electronics unit. Single-pixel resolution.

Environmental

- Electrical supply: 110 volts, 50-60 Hz.
- Power consumption: 500 VA
- Ambient temperature: 10°-25°C
- Relative humidity: 20%-80% noncondensing

Software

- Menu control of basic functions.
- Output Processing gives labeled or tabulated results, statistical analyses, graphical, histogram, bi-varient distributions, and scattergrams.
- UCSD PASCAL language with extensive software library.
- Densitometric measurements provide histograms of grey levels with first and second order texture parameters, either within specified regions or along image lines. Specifications subject to change without notice.

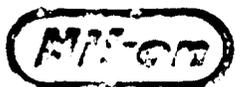
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M E N U

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MAGISCAN ANALYSIS PROGRAM

- EASY, MENU DRIVEN OPERATION
- SPLIT SCREEN DISPLAY SHOWS RESULTS OF PROCESSING
- WIDE RANGE OF STANDARD MORPHOLOGICAL MEASUREMENTS
- COMPREHENSIVE DATA ANALYSIS AND DISPLAY

INTRODUCTION

The Analysis Program consists of a series of Menus organized to guide the operator through the procedures necessary to perform image analysis on the MAGISCAN 2. Each page in the Menu Program displays a limited number of operator selectable options related to one aspect of the analysis. The operator selects the appropriate option by pressing a single alphanumeric key. The instrument will either execute the selected operations, or display a new page of the Menu prompting for the necessary information required to perform the operation.

This organization eliminates operator confusion, minimizes the possibility of errors and simplifies the operation of the MAGISCAN 2 enabling it to be used by personnel with no previous experience of computers or program writing.

CONTROL MENU:

ENVIRONMENT: Select Menu to input system environment information.
 PROGRAM: Select Menu to input program variables.
 EXECUTE: To run the current program sequence.
 RESULTS: Select Menu to choose output presentation of results.
 QUIT: To terminate current analysis session.

ENVIRONMENT MENU:

DISPLAY: Set true to display analysis sequence (i.e. monitor operation). Set false for fast non-display mode of operation.
 SINGLE STEP: Set true to include pauses between each operation. Set false for continuous analysis sequence.
 CALIBRATE: Perform numerical calibration of output results in any system of units. Scale values can be preset or can be defined absolutely with reference to a graduated scale in the field of view.
 FRAME: Define a rectangular measuring frame. Frame Size and position are variable and can be adjusted using the lightpen.
 STAGE: Define raster scanning pattern with linearly interpolated autofocussing for a microscope fitted with autostage and autofocus.
 COLOR: Set true to allow color processing options.



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PROGRAM MENU:

During selection of the Program, the display is split into quadrants to show the effect of each step in the current sequence.

VIDEO INPUT: Select Menu of video input modes.
 GREY IMAGE OPERATORS: Select Menu of grey level operators.
 SEGMENTATION: Select Menu of thresholding.
 BINARY IMAGE OPERATORS: Select Menu of binary operators.
 EDIT: Set true for manual lightpen editing during runtime.
 FEATURE SELECT: Set Menu of feature select/delete factors.
 MEASUREMENTS: Select Menu of measurements.
 PROGRAM MANAGEMENT: Allows for the display, saving and loading of program sequences.

VIDEO INPUT MENU:

A list of options for loading the input map look-up tables to perform contrast enhancement and the output map for pseudo color display. Either one or two images may be stored independently.

LINEAR: Linear input/output transform.
 DENSITY: Optical density transform.
 EQUALIZATION: Histogram equalization transform.
 CONTRAST: Contrast enhancement transform.
 INVERSE: Select inverse of current transform.
 SHADE CORRECT: Shade correction using a stored matrix of coefficients.

If the system incorporates a color display, the following additional functions are available:

BRIGHTNESS: Peak white level indication to allow the correct setting of sample illumination.
 COLOR WEDGE: Interactive setting of pseudo color display of the stored image.

GREY IMAGE OPERATOR MENU:

Can be defined to operate in 3*3 or 5*5 pixel regions. Any number of grey transforms may be applied to the stored image in sequence.

MEAN SMOOTHING
 GAUSSIAN SMOOTHING
 X GRADIENT OPERATOR
 Y GRADIENT OPERATOR
 LAPLACIAN OPERATOR
 NON-LINEAR EDGE ENHANCEMENT
 OPERATOR (eliminates "halo" effect)
 ARITHMETIC OPERATORS (ADD, SUBTRACT, MULTIPLY)



SEGMENTATION MENU:

Segmentation is the process of thresholding the output result from any grey image operator or sequence of operators, and reducing this to a simple grey level threshold or slicing of the processed grey image. For ease of use, an additional segmentation option is presented which combines the edge operator with adaptive thresholding in one operation. Each segmentation option offers the choice of automatic or interactive setting of the high and low thresholds for each phase.

BINARY IMAGE OPERATOR MENU:

Any number of binary operators may be used sequentially on the stored binary images.

- DILATE
- ERODE
- OPEN (Erode followed by dilate)
- CLOSE (Dilate followed by erode)
- LOGICAL (ADD, OR NOT)

EDIT MENU:

Uses single picture point (pixel) lightpen.

- LINE: Add pixels to binary image.
- ERASE: Remove pixels from binary image.
- REJECT: Eliminate objects or regions from image prior to measurement.
- ACCEPT: Accept objects or regions from image for further analysis.

FEATURE SELECT MENU:

Select/omit features from inclusion in measurement routines by setting high and low limits to various form factors.

- SIZE: (Area)
- CIRCULARITY: $(4 * \pi * \text{AREA} / \text{PERIMETER}^2)$
- ELONGATION: (Length/breadth)

MEASUREMENT MENU:

Multiple measurements can be made on each feature.

- AREA: Area excluding holes
- HORIZONTAL FERET DIAMETER
- VERTICAL FERET DIAMETER
- CENTER OF GRAVITY
- MAXIMUM LENGTH
- BREADTH
- PERIMETER
- ORIENTATION
- COUNT
- INTEGRATED OPTICAL DENSITY
- FIELD AREA
- TOTAL DETECTED AREA

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PROGRAM MANAGEMENT MENU:

NEW: Deletes current program sequence in preparation for entering a new sequence.
 LOAD: Load a program from disk.
 SAVE: Save a program to disk.
 DISPLAY: Display current program states.

RESULTS MENU:

Measurements may be individually presented or used in arithmetic expressions and the results presented.

FEATURE MARK: Identify features included in measurements by boundary delineation in overlaid binary image.

TABULATE: List individual results.

STATISTICS: Field of view count, feature count, range, minimum, maximum, standard deviation, variance, skewness, kurtosis, arithmetic mean, geometric mean.

HISTOGRAM: By count or measurement or measurement expression:

- programmable and/or automatic range selection
- linear scale
- logarithmic scale
- cumulative linear scale
- cumulative logarithmic scale

SCATTERGRAMS: Displays the correlation between any two expressions together with the "least squares fit" straight line and correlation coefficient.

EXECUTE:

- Executes current program sequence on full image.
- Results are automatically buffered to output disk file. 100,000 results maximum on one diskette.

The Nikon/Joyce Loeb1 policy of continuous improvement means that specifications may be changed without prior notice.

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APPENDIX II
Graduate Students Benefiting from DOE-Sponsored
Research in the
Department of Biological Structure

A. Students Who Have Benefited From DOE-Sponsored Research and Have Obtained M.S. or Ph.D., Degree in Biological Structure or Pathology

1. Averill, Lynn Ph.D.
Thesis Supervisor: Dr. N.S. Wolf
Current Position
Post-doctoral Fellow
Case Western Reserve University
Cleveland, Ohio 44106
2. Bourdage, Robert J., Ph.D.
Thesis Supervisor: Dr. Sheridan Halbert
Current Position
Assistant Professor
Department of Anatomy
Texas College of Osteopathic Medicine
Camp Bowie at Montgomery
Fort Worth, TX 76107
3. Bottomly, Helen K. Ph.D.
Thesis Supervisor: Dr. Roy Schwarz
Current Position
National Institute of Allergies and Infectious Diseases
Institute of Immunology
NIH
Bethesda, MD
4. Brankovan-Ercegovac, Vera Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Virginia Mason Research Center
1000 Seneca Street
Seattle, WA 98101
5. Cantino, Marie Ph.D.
Thesis Supervisor: Dr. Dale Johnson
Current Position
Post-doctoral Trainee
Department of Bioengineering
University of Washington
Seattle, WA 98195
6. Cusick, Catherine G. Ph.D.
Thesis Supervisor: Dr. Raymond Lund
Current Position
Research Associate
Department of Psychology
Vanderbilt University
Nashville, TN
7. Dorshkind, Kenneth A. Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Assistant Professor
Division of Biomedical Sciences
University of California, Riverside
Riverside, CA 92521-0120

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8. Gong, Daniel M.S.
Thesis Supervisor: Dr. E.M. Eddy
Current Position
Unknown
9. Jaeger, Christine B. Ph.D.
Thesis Supervisor: Dr. Raymond Lund
Current Position
Postdoctoral Fellow
Dept. of Neurology
New York Hospital, Cornell Medical Center
New York, NY
10. Jen, Ling-Sun Ph.D.
Thesis Supervisor: Dr. Raymond Lund
Current Position
Assistant Professor
Dept. of Anatomy
Hong Kong
11. Kligman, Douglas Ph.D.
Thesis Supervisor: Dr. Mark A Nameroff
Current Position
Laboratory of Biochemical Genetics, Bldg. 36-1C06
National Institutes of Health
Bethesda, MD
12. Landreth, Kenneth Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Senior Research Scientist
Cancer Research Program
Oklahoma Medical Research Foundation
825 N.E. 13th
Oklahoma City, OK 73104
13. McKay, John Ph.D.
Thesis Supervisor: Dr. Mark A. Nameroff
Current Position
Post-doctoral Fellow
Fred Hutchinson Cancer Research Center
Seattle, WA 98104
14. Mulé, James J. Ph.D.
Thesis Supervisor: Dr. N.B. Everett
Current Position
D.C.T., N.C.I.
Dept. of Human & Health Sciences
Public Health Service
National Institutes of Health
Bethesda, MD 20205

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15. Patton, Dorothy L. Ph.D.
Thesis Supervisor: Dr. Sheridan A Halbert
Current Position
Research Assistant Professor
Dept. of Obstetrics and Gynecology
University of Washington
Seattle, WA 98195
16. Press, Oliver, W. Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Instructor
Dept. of Medicine
University of Washington
Seattle, WA 98195
17. Quinn, LeBris Smith Ph.D.
Thesis Supervisor: Dr. Mark A. Nameroff
Current Position
Senior Fellow
Dept. of Pathology
University of Washington
Seattle, WA 98195
18. Scuderi, Phillip Jr. Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Research Scientist
Carver Genetic Physics, Inc.
Seattle, WA 98105
19. Seiker, Larry C.
Thesis Supervisor: Dr. Lyle Jensen
Current Position
Research Assistant Professor
Dept. of Biological Structure
University of Washington
Seattle, WA 98195
20. Steiner, Verena M.S.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Residency Program in Pathology
University of Vermont
Burlington, VT

B. Students Currently Benefiting from DOE-Sponsored Research in the Department of Biological Structure

1. Battaglia, David
2. Bohannon, Nancy Jones
3. Bramson, Rachel
4. Brewitt, Barbara A.
5. Davidson, David A.
6. Foster, Carolyn A.
7. Johnson, Lonnie
8. Kissinger, Charles R.
9. Liu, Hwan-Wun
10. Newman-Gage, Helen
11. Smith, Lynne
12. Stroehel, Virginia
13. Tupper, Joan C.
14. Vernon, Robert