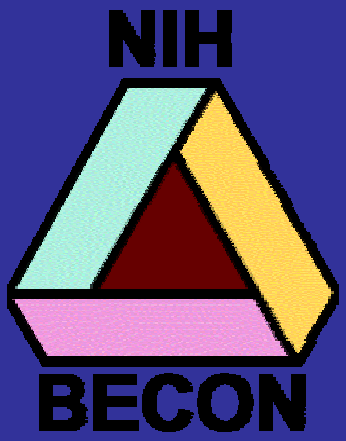
# Bioengineering Research Partnerships
# First Annual Grantee Meeting




March 23, 2001

Bioengineering Consortium
National Institutes of Health
Bethesda, MD

BIOENGINEERING CONSORTIUM

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

Welcome to the First Annual Bioengineering Research Partnership Grantee Meeting.

Since its establishment in 1997, the Bioengineering Consortium (BECON) has provided a focus for biomedical engineering research and training activities at the National Institutes of Health (NIH). Active participation by all of the NIH centers, offices, and institutes and other federal agencies has resulted in the realization of the substantial benefits to be derived from the application of engineering, physical, and computational science principles and techniques to address problems in biology and medicine. The importance of this field is demonstrated by the fact that the total annual budget for bioengineering research has increased steadily over the past four years.

To facilitate the development of the field of biomedical engineering, the BECON has coordinated trans-NIH initiatives aimed at encouraging and supporting multi-disciplinary and integrative approaches to biomedical research and training. One of the most successful and visible of these research initiatives is the Bioengineering Research Partnership (BRP) Program which was first announced in October 1999. The partnerships that have developed in response to these grants are examples of the types of collaboration between the biomedical and allied technical disciplines that can provide significant advances for improving human health. To date, 34 BRP awards have been made for a total investment of about $130 million by twelve NIH research institutes and centers.

This meeting is the first time that the BRP grantees, BECON members, and NIH institute/center representatives will get together to discuss common concerns and the program in general. Your comments and suggestions concerning partnership experience, program efficacy, needs and directions in biomedical engineering research and training, and the format for future BRP grantee meetings are solicited. Also, please take this opportunity to meet with your NIH institute/center representative to discuss progress and concerns for your project.

I hope that the BRP Grantee Meeting is valuable and enjoyable to you. All of the NIH program, grants, and review staff involved in the BRP program look forward to your participation and comments.

Dr. Wendy Baldwin, Chair
Bioengineering Consortium

# Table of Contents

First Annual Bioengineering Research Partnership Grantee Meeting
March 23, 2001

## MEETING AGENDA

**7:30AM** **Meetings with NIH institute representatives and grantees**
*– Rooms C, D, F1, F2 and G*

**8:30** **Welcome and meeting overview** (Richard Swaja) - *Room E*

**8:40** **Perspectives on the BECON and BRP Program** (Wendy Baldwin)

**8:55** **Introduction of BECON members** (Richard Swaja)

**9:00** **National Institute of Bioimaging and Bioengineering** (Donna Dean)

**9:30** **Break**

**9:45** **Grantee presentations**
*William Heetderks (Room E) and Laurence Clarke (Room D)*

**11:30** **Summary of issues and working lunch groups** (Greg Milman) - *Room E*

**12:00** **Working lunch with breakout sessions** - *Rooms C, F1, F2 and G*
C – Management Issues
F1 – Intellectual Property, Commercialization and Publication Issues
F2 – NIH Issues
G – Future Issues for NIH Bioengineering

**1:30 PM** **Technology Transfer, Intellectual Property, and Conflict of Interest**
Experience and Requirements at the NIH (Theodore Roumel) – *Room E*

**2:00** **Reports of breakout groups and discussion** - *Room E*

**2:45** **Break**

**3:00** **Continued reports and discussion**

**3:45** **Meeting summary** – *Room E*

**4:00** **Adjourn or meetings of IC representatives and grantees**

*Room C will also be used as a designated hospitality room.*

# BRP GRANTEE PRESENTATIONS

## Session I - Room E

**Devices, Biomechanics, Molecular Biology, and Drug Delivery**

***Bill Heetderks, Chair***

Berns
Frazier
Langer
Olsen
Soper
Weiss
Koller
Brown
Ley
Fredberg
Jain
Stephanopolous
Crandall
Sklar
Chien
Huse
Sweeney

## Session II - Room D

**Neural Applications, Imaging, and Materials**

***Laurence Clarke, Chair***

DeLuca
Gilbert
Greenberg
Long
Peli
Rylander
Halpern
Hirschl
Hoffman
Izatt
Jacques
Westenskow
Majumdar
Intaglietta
Ratner
Shain
Snyder

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# Department of Energy
# Advanced Medical Instrumentation Program

## Physics, Chemistry, and Engineering to Solve Problems in Human Health

Medical engineering integrates principles of physics, chemistry, engineering, and mathematics and applies them to solve problems in human health. Its goal is to develop innovative biologics, materials, processes, implants, devices, and informatics approaches for the prevention, diagnosis, and treatment of disease, for patient rehabilitation and for improving health. Medical engineering has been an important and continuing research activity in the DOE Laboratories since the inception of the Atomic Energy Commission in 1947. The most notable contributions of the Laboratories in this regard have been the development of radiotracers and imaging instrumentation, which are routinely used in the practice of medicine worldwide. In January 1999, BER compiled and published an inventory of medical engineering research projects ongoing in the DOE National Laboratories (*Biomedical Engineering at DOE National Labs*). There were more than 230 projects with considerable strengths in the following scientific areas: biomaterials, sensor technology, lasers, and imaging instrumentation.

During the past two decades, the medical engineering programs in the National Laboratories have been the beneficiaries of the rapid advances made in nuclear physics, engineering, chemistry, and molecular biology. Research on the medical applications of synchrotron light sources, lasers, mass spectrometry high field magnets, micro-fabricated machines, bio-sensors, and DNA chips, to name a few, is ongoing in many of the Laboratories. The large number of talented scientists and extraordinary technologies has made the National Laboratories a national resource for medical engineering. The unique resources at the National Laboratories allow medical research that cannot be done at universities or industry.

In FY 1999, BER initiated a new Advanced Medical Instrumentation (AMI) research program. The AMI Program supports basic engineering research that utilizes the unique resources and expertise at DOE National Laboratories to develop new innovative medical technology. The overall goal of this Program is to support basic research and technology development that will ultimately lead to the development of medical instruments that can be transferred to the National Institutes of Health for clinical testing or to industry for further commercial development. The AMI Program supports multi-disciplinary, multi-institutional research projects that address ***high-risk*** medical technology problems. The focus of the Program is to further develop basic technologies developed in other DOE Programs, such as Defense, Environmental, and Physics into technologies that will have medical applications. To date, 17 projects at approximately $2.0 million per year have been funded in biomaterials, medical sensors, medical photonics, imaging, and informatics. A second AMI announcement was recently released to the National Laboratories requesting innovative multi-disciplinary, multi-institutional (National Laboratories and University) research proposals in medical engineering. The main research interest of this announcment is in biomedical imaging (in-situ real-time small animal imaging) however, other research areas in medical sensors, medical photonics, smart medical instruments will also be supported. It is anticipated that up to $6 million will be available to support this effort. Research success is being realized in four important medical engineering areas:

1. **Medical Imaging**: Researchers at the DOE Thomas Jefferson National Accelerator Facility, in collaboration with Johns Hopkins University, the University of Virginia, George Washington University, and Dilon Technologies are using accelerator detector technology to develop a compact Scintimammography Gamma Camera for early detection of breast cancer. In contrast to full sized gamma whole body cameras, this new camera can be placed very close to breast, allowing physicians to detect small tumors that would be difficult or impossible to see on mammography or standard gamma cameras.

2. **Medical Biosensors***:* In collaboration with The Wilmer Eye Institute at Johns Hopkins School of Medicine and Oak Ridge National Laboratory, significant progress has been made in the development of advanced sensors for artificial retina for the blind. This highly technical project utilizes many of the National Laboratories unique resources in biosensor, micro-machining, and computer modeling to develop components for an artificial retina.

3. **Computational Modeling**: *PEREGRINE*, a radiation therapy targeting system, combines state-of-the-art Monte Carlo radiation transport techniques and the most comprehensive nuclear and atomic databases to produce very accurate dose calculation for patients undergoing radiation therapy. The *PEREGRINE* system, which capitalizes on advances made in computer modeling for defense, was the recipient of a 1999 R&D 100 Award and recently licensed to NOMOS Corporation of Sewickley, PA, for commercialization.

4. **Advanced Biomaterials**: Peripheral nerve damage resulting in the loss of function of limbs is a serious consequence of traumatic injuries. Researchers at the Ames Laboratory are developing a novel biodegradable polymer that will act as a support and a guide for nerve cell regeneration. This biocompatable polymer will stimulate nerve cell regeneration and steer the growing nerve cell to the severed ends of the nerve fiber.

The AMI Program works closely with other Federal Agencies, especially the National Institutes of Health, to help coordinate and focus the medical engineering research efforts at the National Laboratories. This includes participation on the National Institutes of Health Biomedical Engineering Consortium (BECON) and the Multi-Agency Tissue Engineering Science (MATES) Working Group. It is recognizing among the various Federal Agencies that the DOE National Laboratories fill a unique and undisputed niche with respect to biomedical engineering research. (NIH funding is traditionally hypothesis driven and disease-related.) The DOE is recognized as the Federal Agency with a primary mission in supporting the basic sciences forming the foundation of medical engineering: physics, chemistry, and engineering.

The AMI Program is the major source of support for medical engineering research at the National Laboratories and thus, serves to nurture collaborations between the DOE National Laboratories and leading medical schools and teaching hospitals. These collaborations leverage the National Laboratories' unique resources and expertise in the biological, physical, chemical, engineering, and computing sciences to provide innovative and high-risk solutions to medical application problems dealing with the diagnosis, prevention, and treatment of disease. The AMI Program is a natural extension of the nuclear medicine field that BER has invented and supported for over half a century.

# National Science Foundation
# Biomedical Engineering

NSF supports biomedical engineering (BME) research, education, and new product innovation through (1) individual investigator research grants made by the NSF BME program, the NSF program for Research to Aid Persons with Disabilities (RAPD), and other NSF programs, (2) cooperative research agreements with NSF Engineering Research Centers (ERCs) and Science and Technology Centers (STCs), (3) Integrative Graduate Education and Research Traineeships (IGERT), (4) Combined Research and Curriculum Development (CRCD) awards, (5) SBIR and STTR grants, and also other awards made through various NSF programs. BME areas supported include, but are not limited to, tissue engineering, metabolic engineering, genomic engineering, biochips (microarrays), bioinformatics, post-genomic quantitative systems biotechnology, biophotonics, bioimaging, biosensors, nanobiotechnology, controlled release, biomaterials, biomechanics, artificial organs, medical devices and medical instrumentation, therapeutic bioagent processing, bioprocess control, biocomplexity, and mathematical and computational biology.

Detailed information on these NSF programs is available at the following web addresses and associated links:

(1) BME/RAPD programs: www.eng.nsf.gov/bes/Programs/programs.htm
(2) ERCs: www.eng.nsf.gov/eec/erc.htm
(3) STCs: www.nsf.gov/od/oia/programs/stc/start.htm
(4) IGERT: www.nsf.gov/home/crssprgm/igert/start.htm
(5) CRCD: www.eng.nsf.gov/eec/crcd/crcd.htm
(6) SBIR/STTR: www.eng.nsf.gov/sbir/

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**PROJECT TITLE:** Integrated Platform for Chemical Analysis of Live Cells

**PARTNERS' NAMES AND AFFILIATIONS:** (no commercial partners)

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G. P. Li, Department of Electrical and Computer Engineering and Center for Biomedical Engineering, University of California Irvine.

Vasan Venugopolan, Department of Chemical & Biochemical Engineering and Material Sciences

**GRANTING NIH INSTITUTE/CENTER:** National Center for Research Resources

**ABSTRACT:**

The overall aim of this proposal is to design, build and test an integrated optical and microfluidics system that will enable the performance of novel biochemical assays in single living cells. The device will be tested in biomedical systems relating primarily to cancer, and growth and development, though it will have wide application in the areas of molecular medicine and drug development. This project is submitted in response to the PAS-99-010 to support Bioengineering Research Partnerships (BRP's). It involves a close collaboration amongst three academic research labs, each with a very different focus and expertise: (1) photonics/microscopy (Berns & Venugopalan); (2) BioMEMS/microfluidics (Li & Bachman); (3) analytical chemistry/cell biology (Allbritton & Sims).

The project's specific aims are: (1) the development of a laser microscope platform for single cell manipulations and analysis; (2) development of a multipurpose, modular microfluidics chip for single cell assays; (3) development of a broad range of analytes which can be assayed in single cells.

Development of the microscope platform will involve, (a) basic studies to characterize and optimize the physical mechanisms of laser interaction with cells and BioMEMS materials, and (b) development of a fluorescence module for detection of substrates and analytes in the integrated microfluidic system. Development of the microfluidic system will involve (a) basic engineering of the MEMS microfabrication process, (b) design engineering of different chip configurations, and (c) biomaterials compatibility studies. In addition, the BioMEMS microfluidics systems will be integrated with both the microscope platform and the chemical analysis systems described below. The third specific aim involves further perfection if a unique new bio-assay system through the development of new enzyme assays for activation of kinases and proteases, and the transfer of these assays to the integrated device. A multidisciplinary approach is required to accomplish the tasks involved in the development and integration of this system. The achievement of the technological objectives interfaced into one platform will provide an enabling technology with a wide variety of applications in molecular medicine and biomedical research.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Progress has been made in all three major objective areas: chemical analyte development, microscope platform development, and biomems systems development. A major commercial partner in the form of a company founded by one of the co-investigators has been established to commercialize the integrated platform and chemical analytes. In addition, a second corporate partner relationship is being developed to spin-off a secondary technology from this project.

**ISSUES:** There are no issues.

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**PROJECT TITLE:** Nonlinear Computational Biomechanics of the Hip

**PARTNERS' NAMES AND AFFILIATIONS:**
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**GRANTING NIH INSTITUTE/CENTER:** NIAMS

**ABSTRACT:**
Disorders of the hip comprise a substantial fraction of current musculoskelatal disease burden. Complex nonlinear mechanical phenomena pervade many aspects of treatment of hip disease and injury, including total hip arthroplasty, intra-articular fractures, osteonecrosis, and developmental dysplasia. While bioengineering capabilities exist – in principle – to quantify key mechanical factors influencing treatment outcomes in these areas, contemporary clinical decision making still rests almost entirely on subjective empirical experience. This Bioengineering Research Partnership (BRP) brings together the capabilities of an experienced computational biomechanics research group, four senior orthopaedic hip surgeons, a veterinary research orthopaedist, and an industry-based materials scientist, in order to advance the state of the art in biomechanically-grounded management of disorders and injuries of the human hip. The central focus of the research Partnership lies in applying nonlinear finite element formulations to address as-yet-unquantified mechanical phenomena that are clinically recognized as being crucial to patient outcome. Building on previous and ongoing finite element work, new computational formulations will be developed to tackle nonlinearities currently limiting the accuracy of numerical simulations in five clinically important areas of hip surgery. The first two areas involve leading complications of total hip arthroplasty. First, as regards abrasive wear of polyethylene, we propose to incorporate local directionality of femoral head counterface motion in computing wear rates with a sliding-distance-coupled contact finite element formulation. Second, as regards dislocation, we propose to introduce soft tissue tethering into a large-displacement sliding contact model of resistance to dislocation. The third area involves intra-articular fractures of the acetabulum: estimating residual cartilage contact stress elevations accompanying attempts at surgical restoration of articular surface congruity. The fourth area involves osteonecrosis: computationally characterizing a new animal model (the emu) which unlike previous animal models progresses to human-like femoral head collapse, and using that model for *in-vivo* testing of computationally optimized placement of a novel head-preserving implant device. The fifth application area involves surgical management of developmental hip dysplasia: using novel mesh pre-processing techniques to quantify improvements of intra-articular contact stress achieved by pelvic osteotomies. This Partnership will bring together a critical mass of engineers and surgeons, to achieve clinically-grounded advances in nonlinear numerical simulations of surgery of the hip.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Our scientific work is moving along very well, particularly as regards computer code development for voxel-based contact finite element analysis, and energy-based assessment of fracture severity. Things also are going well on patient-specific preprocessing to zone central acetabular fractures and osteotomies for developmental hip dysplasia. And, most of the code is in place for direction-dependent THA wear, with pilot testing underway. Our work with the emu model has concentrated thus far on technical improvements for cryogen delivery. This involves about an 8-week turn-around time for each new trial, owning to needing to wait about 4 weeks for histologically apparent osteonecrosis to develop, and another 4 weeks for decalcified bone histology. We think/hope we are pretty close to our "final" cryogen probe design, but this has been a tedious development/evaluation process, and we have no good alternative but to continue in this fashion to see it through to completion, prior to going into "production" in creating osteonecrosis lesions in the emu model.

**ISSUES:**

Our industrial partner, Implex Corporation, was enlisted for collaboration owing to a unique under-development product, a surgical implant for structural grafting of femoral head osteonecrosis, made of their proprietary Hedrocel® porous tantalum foam. Our collaboration with Implex has moved along well, in that we have collaboratively designed and they have fabricated "emu-sized" versions of these devices, plus the necessary specialized operative instrumentation. And, we have piloted these devices in some of our emus (sham surgeries, not osteonecrosis), and they seem to be functioning well. However, in the fall of 2000, Implex signed a joint distribution agreement for Hedrocel® with Zimmer, Inc., a large orthopaedic manufacturer. We understand that there are now negotiations for Zimmer to purchase Implex, and if this goes forward, it is an open question as to how supportive Zimmer will be of continuing collaborative work in this area. Moreover, this the Zimmer Corporation itself is being divested from Bristol-Myers Squib, and the nature of subsequent acquisition/merger is uncertain. Thus, our longstanding collaborative/semi-informal working relationship with Implex through Dr. Poggie may or may not need to be restructured.

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**PROJECT TITLE:** Absorption Mechanisms of Peptide/Protein Drugs via Lung

**PARTNERS' NAMES AND AFFILIATIONS:**
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**GRANTING NIH INSTITUTE/CENTER:** National Heart, Lung and Blood Institute

**ABSTRACT:**
Our long-term goal is to elucidate the mechanisms for absorption of peptide and protein drugs across the alveolar epithelium that offers most of the surface area available for drug absorption in the lung airspaces. We will utilize primary cultured rat alveolar epithelial cell monolayers as an in vitro model to investigate absorption mechanisms of the alveolar epithelium and delineate possible ways to enhance transport rates of peptide/protein drugs. Various di-/tri-peptides, granulocyte-colony stimulating factor (G-CSF, 18.8 kDa) and human growth hormone (hGH, 22 kDa) will be used as model drugs. We propose to [1] delineate the mechanisms and pathways (i.e., paracellular diffusion, fluid-phase transcytosis, receptor-mediated and/or adsorptive transcytosis) for transepithelial transport of model protein drugs across the alveolar epithelial barrier, [2] investigate the mechanisms underlying stimulation of protein drug absorption via transcytosis across the alveolar epithelial barrier, [3] investigate how di-/tri-peptide drugs are absorbed across the alveolar epithelium, and [4] determine the stimulatory effects of physicochemical variables (e.g., the hyperosmotic gradient imposed by dissolution of peptide/protein drug in the thin alveolar lining fluid, acidic pH of the lining fluid, and controlled release mechanisms that may be related to a specific formulation (e.g., large porous carrier particles) of the peptide/protein drugs) on alveolar epithelial absorption of protein and di-/tri-peptide drugs. Through the collaborative investigation of pulmonary protein/peptide drug absorption among four different laboratories utilizing experimental approaches spanning from cell biology/physiology to bio(chemical)engineering, we will provide new information on how alveolar epithelium handles peptide/protein drugs. These studies will be useful for devising new drug formulation and delivery methodologies to improve the bioavailability of poorly absorbed peptide/protein drugs via the alveolar epithelial barrier.

**STATUS OF RESEARCH AND PARTNERSHIP:**
**Projects 1 and 4** *(Elucidation of transcytotic pathways / Effects of biophysical parameters (e.g., pH, osmolality) and soluble factors (e.g., hormones) on protein transport across alveolar epithelium):* We are studying the mechanisms and regulation of IgG transport across primary cultured rat alveolar epithelial cell monolayers (RAECM) grown on tissue culture-treated polycarbonate filters. The apparent permeability ($P_{app}$) of IgG in the apical-to-basolateral (ab) direction is ~5 times greater than that (~2 x$10^{-8}$ cm/sec) in the opposite (ba) direction, yielding net IgG absorption. Both ab and ba fluxes saturate with increasing IgG concentration in respective upstream fluids. Apparent half-max concentration of ~11 nM and max flux of ~2 fmol/cm$^2$/hr for *ab* fluxes, and 25 nM and 0.7 fmol/cm$^2$/hr for *ba* fluxes, are found at 35$^o$C. In the presence of 20x excess unlabeled Fc (but not Fab, F(ab')$_2$ or IgY) in upstream fluid, labeled IgG flux in the ab (or ba) direction

decreases by ~85%, suggesting saturable flux in either direction is mediated by receptors that recognize the Fc portion of IgG. Fluxes decrease by ~75% and ~100 % in ab and ba directions at 4°C. After 48h, exposure to 1 M dexamethasone decreases ab IgG flux > 50%, while ba flux is unchanged. These results are consistent with net absorption of IgG across rat alveolar epithelium via FcRn-mediated transcytosis.

**Project 2** *(Studies of alveolar epithelial di-peptide transporters):* Our laboratories previously reported saturable ab transport of Gly-L-Phe across alveolar epithelial cell monolayers. On the basis of these results, uptake experiments and RT-PCR (PepT1, PepT2 and GAPDH) have been undertaken to investigate peptide transporters in rat alveolar epithelial cells. Using $^{3}$H-Gly-Sar, a metabolically stable dipeptide transporter substrate, uptake is greater from apical than basolateral fluid of cell monolayers on days 5-6. RT-PCR for PepT1 conducted using 1µg of total RNA extracted from RAECM on days 1 and 5, with intestine and kidney as positive controls, show a strong band in intestine, a slight band in kidney, and no band for alveolar epithelial cells on either day 1 or 5. On the other hand, PepT2 is positive in alveolar epithelial cells on both days 1 and 5. The intensity of GAPDH is similar across specimens, assuring equal loading of samples. The RT-PCR product obtained from alveolar epithelial cells matches fully the predicted sequence for PepT2 (but not PepT1).

**Project 3** *(Transferrin receptor (TfR)-mediated modulation of protein transport across alveolar epithelium):* TfR expressed on basolateral cell surface of RAECM grown on permeable polycarbonate filter membranes, estimated by specific binding of $^{125}$I labeled transferrin, on days 3 and 7 is 9.85 and 1.49 fmol/cm$^2$. Total intracellular TfR (soluble TfR) on day 1 is 23.2 fmol/cm$^2$ (69.5 pg TfR/µg protein), declining to 0.94 fmol/cm$^2$ (5.3 pg TfR/µg protein) by day 7. These data suggest that day 7 (type I-like) cells synthesize less TfR than those (type II-like) at day 3. Based on our previous report on keratinocyte growth factor (KGF) preserving type II cell phenotype and morphology, we investigated the effect of KGF on TfR expression. Cell monolayers are grown in the absence (control) or presence of KGF (10 ng/mL) from day 1 onward. KGF causes retention of basolateral surface TfR expression through day 7 (8.67 fmol/cm$^2$), while control monolayers demonstrate a greatly reduced level by day 7 (1.23 fmol/cm$^2$). Establishment of tight alveolar cell monolayers that retain high levels of TfR expression has the potential to serve as a model system to study transcytosis of protein and peptide drugs across alveolar type II cells.

**ISSUES:**

(1) Project leaders participate in monthly meetings to develop overall strategy, develop further collaborations, and plan optimal directions for the four projects. Quarterly scientific meetings of all personnel involved in the projects are held, where each group presents its latest research findings.

(2) Monthly seminar series sponsored by the project leaders is held under the auspices of the USC Center for Drug Design/Delivery. Recent speakers include: Curtis T. Okamoto, Ph.D. (Assistant Professor of Pharmaceutical Sciences, USC, Los Angeles, CA); David Edwards, Ph.D. (Adjunct Professor at Harvard University and CEO of A-I-R, Inc., Cambridge, MA); Sung-Wan Kim, Ph.D. (Professor of Pharmaceutical Technology, University of Utah, Salt Lake City, UT); and Francis Szoka, Ph.D. (Professor of Pharmaceutics, University of California, San Francisco, CA).

(3) We are planning to establish an in-situ/in-vivo small animal facility for pulmonary drug delivery studies to complement the mechanistic studies described above using our in vitro model.

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**PROJECT TITLE:** Harnessing Motoneuron Activity: From Lab to Clinic

**PARTNERS NAMES AND AFFILIATION:**
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**GRANTING NIH INSTITUTE/CENTER:** National Center for Medical Rehabilitation Research

## ABSTRACT:

We propose to develop an automatic system for decomposing the electromyographic (EMG) signal into the constituent action potentials corresponding to the firing of individual motor units activated by motoneurons. The system will be an outgrowth of our existing rudimentary system which, over the past 20 years, has enabled us to perform various novel investigations that have provided a variety of new insight into motor control. However, the current system suffers from many limitations, which curtail its usefulness as a research tool and has never been useful as a Clinical Tool. The new system will have a dramatically enhanced performance: 1) decomposition time for typical contractions will be decreased from dozens of hours to a few minutes, 2) the automatic decomposition accuracy will be increased from 60 % to 95% - with provisions for assisted editing to reach 100% accuracy, 3) it will be able to decompose signals from dynamic as well as static contractions (which is a current limitation), 4) it will weigh less than 10 kg, and have a notebook computer configuration, and 5) most importantly the decomposition algorithms will be completely rewritten using a newly developed knowledge-based Artificial Intelligence language blackboard platform developed by us. This platform has been used successfully to decompose polyphonic signals and radar spread spectrum signals having a complexity comparable to that of the EMG signal.

The proposal is composed of 5 projects. The first and dominant project will be Design Driven. It describes the design and development of the new system, which has at its heart, Knowledge-Based algorithms for decomposing the signals. The other four projects will be hypotheses-based and will address basic science questions and clinical applications that will reveal the utility of the new system. These projects will also be used to test and improve the evolving design of the new system. Project 2 will address the modifications, which occur in the firing of motor units as a function of Aging. Project 3 will address the phenomenon of motor unit substitution, which will be useful in Ergonomics work environments and in the Rehabilitation of patients with Peripheral Nerve Injury and Spinal Cord Injury. Projects 4 and 5 are two Clinical Studies. Project 4 will explore the use of quantified neuromotor activity for developing prognostic indicators for determining denervation and re-enervation of Paralyzed Laryngeal Muscles. Project 5 will study patients with acute ataxia following a Cerebellar Stroke to explore the manifestation of CNS disorders in the firing characteristics of the motoneurons.

## STATUS OF RESEARCH PARTNERSHIP:

The program began on June 1, 2000. This report covers the work performed in the first eight and one half months. As indicated in the time line of the proposal, we began Project 1 and Project 2. The second project, which was originally proposed to investigate the modifications of the motoneuron firing patterns as a consequence of aging and exercise, was curtailed due to budget cuts. It is now limited to investigating the effects of aging.

Project # 1 – Decomposition of the EMG Signal -- The main aspect of this project is to re-design a current outdated inefficient system and to construct a prototype for decomposing the EMG signal into the constituent motor-unit action potentials. There are two main components to this project: the software and the hardware.

The development of the software is on schedule. We have designed, implemented, and tested a significant portion of the new EMG decomposition system. The new design is based on the IPUS architecture from the field

of Artificial Intelligence. Its implementation, using C++, is modular, object-oriented and targeted at the Windows platform, although it is easily portable to other environments. The new algorithms are capable of detecting and classifying multi-channel motor unit firings at speeds two or more orders of magnitude greater than that of the previous algorithms. We are close to achieving near real-time performance – a designated goal of the project. The increased speed is largely due to improved software design, more efficient use of signal processing resources, and the compatibility of the new software with faster computing platforms. The code for the software is rustic and needs to be refined and hardened. The current algorithms need to be developed to incorporate the knowledge base for decomposing the signals that we are developing. In terms of functionality, the new system creates "hooks" that hold intermediate results for later use in knowledge-based analysis and correction of the signal processing outputs. Once the design and implementation of the analysis and correction phase is complete, the decomposition algorithms are expected to provide the 95%+ accuracy that previously could only be provided through a laborious time-consuming manual editing process. We will focus on this aspect during the next year.

The development of the hardware is on schedule. We have made progress in three areas:

*Electronic Signal Conditioning hardware* -- We performed tests on the existing decomposition system under a variety of conditions to determine the design specifications of the input stage. This included a determination of the electrical source characteristics for both wire and needle electrodes and selection of desired signal bandwidth and sampling rate for data acquisition. Based on these specifications, a signal conditioning stage was designed incorporating the additional safety and performance specifications required for the design of a medical device. A prototype is currently being evaluated.

*Computer Systems and Data Acquisition Hardware* -- The computer hardware has been divided into two modules. One module consists of a stand-alone Single Board Computer configured to handle data acquisition and control subject display. A second module consists of a laptop computer to handle decomposition and operator interaction. A 100 Base-T Ethernet LAN will carry out the communication between the two processors. The main advantage of this configuration is that it presents a small form-factor with a high degree of flexibility in selection of CPU, storage components and displays. This is relevant because the data acquisition hardware and signal conditioning hardware would share the same enclosure. A flat screen LCD display will be used as the subject monitor. This has two advantages: it can be placed separately from all other data acquisition hardware, and medical grade flat LCD displays are available. Preliminary tests on the computer data acquisition system have been done using two desktop computers to determine the needed computational power.

*Software associated with low level Hardware control and User Interface* -- The software development is divided into two parts: Hardware Control and Graphical User Interface. For this we use a Virtual Instrumentation application software. We have developed software modules that allow us to test and verify important characteristics of the hardware. Thus far, the required acquisition rate has been verified, we have successfully streamed the collected data to disc, displayed the data in real time, collected digital signals, verified the analog output, and created non-buffered communication between the two computers. The user interface is gradually being developed as new functions are added.

Project # 2- Aging -- The NCMRR has been notified that the project has been modified. It is on schedule. We have modified an existing exercise chair making it suitable to test the subjects for this project. It can now accommodate elderly subjects with ease. We set up a laboratory to perform the experiments. Five healthy young subjects (aged 21 to 32 years) were recruited and tested. Voluntary isometric contractions at 20 and 50 % of maximal voluntary effort were measured. The subjects traced target trajectories by attempting to extend the knee against a rigid force transducer. Visual feedback on the actual force generated was provided to the subjects. Intramuscular EMG from the Vastus Medialis muscle, and surface EMG signals from the Vastus Medialis, Vastus Lateralis, Rectus Femoris, and Biceps Femoris muscles were recorded along with the force produced. The processing of acquired data using the existing decomposition program is under way. Data from two of the subjects have been decomposed and qualitatively investigated to ensure that the current set-up and protocol yield reliable data. We are now in the process of recruiting sedentary elderly subjects.

**ISSUES:** None

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**PROJECT TITLE:** Integrated Sample Preparation For Genomic Analysis in Micro Device Format

**PARTNER'S NAMES AND AFFILIATIONS:**

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**GRANTING NIH INSTITUTE/CENTER:** National Institute of Environmental Health Sciences

**ABSTRACT:**

It has been astutely pointed out by those speculating on the potential impact of miniaturization technology for analysis, that the revolution caused by microelectronics was not miniaturization per se, but that microelectronics became an elegant solution to a problem of complexity. If miniaturization technology for bio-analysis becomes revolutionary, it will be because it provides an elegant solution to the complexity of bio-analysis. The globally stated goal of miniaturized analysis systems is to provide a sample-in-answer-out solution (e.g. completely integrated on-chip sample preparation, separation and detection) for the end user, and promises a ready solution to high throughput, as well. The integrated sample preparation system is a vital component of the micro total analysis system envisioned by many researchers. Integrated micro scale approaches will be investigated in this BRP for preparing whole blood through the complete sample preparation steps required for subsequent analysis in more selective separation/detection systems. The long-term goal of the proposed research is to produce an integrated sample preparation micro-analytical device for performing genomic analysis from blood samples. The strategy is to develop a front-end micro sample preparation system, μ-SPS, for use as a research tool with the flexibility to be integrated with a number of downstream analysis platforms, i.e. either sequencing or genotyping. The μ-SPS is composed of three main micro-compartments including: 1.) Sample introduction, combined with cell sorting and selection; 2.) Cell lysis, recovery of the nucleic acid material of choice (e.g. DNA or mRNA), and sample clean up via solid phase extraction or affinity capture; and 3.) Elution of the material to an amplification μ-compartment, and subsequent amplification (e.g. PCR or rtPCR). The specific aims focus on the underlying studies necessary to realize the various μ-compartments of an integrated sample preparation system for genomic analysis. Studies of the μ-compartment prototypes are paralleled by an investigation of techniques for integrating the μ-compartments. The final specific aim is the realization of a prototype μ-SPS breadboard.

**STATUS OF RESEARCH & PARTNERSHIP:**

Micro Electrophysiological Analysis Systems for Single Cells. The Frazier group has recently micro fabricated and tested a micro analytical device for routing, sorting, and lysing blood cells. Single cell and populations of cells are manipulated within micro channels and reservoirs using acoustic energy sources. The blood cells are sorted and concentrated into cell populations using an integrated electrophysiological detection system based on impedance spectroscopy. The μ-EAS incorporates small rectangular channels with typical cross-sectional areas of 30 $\mu m^2$. Epoxy-based photoresist was used to form cell-suspension reservoirs and the sides of the micro channels. Gold microelectrodes were electroplated to instrument the channel with a multiple detection zones.

Micro Solid Phase Extraction (SPE). The Landers group recently has demonstrated the capability to assemble micro-column devices with a relatively high surface-to-volume ratio for SPE of analytes. The micro-SPE device is designed for the direct extraction of DNA from crude mixtures, including whole blood, on the μl and sub-μl scale. Testing has shown, not only could purified DNA be adsorbed to the μSPE tip and desorbed effectively for PCR amplification when pre-purified DNA was used, but DNA could also be extracted directly from WBCs and amplified. When WBCs were simply lysed and used directly as a matrix for PCR amplification, PCR failed due to the presence of inhibitors in the WBCs. The purified DNA was evaluated for its PCR-readiness.

Continuous DNA Filtration. The Austin group recently demonstrated the use of the two dimensional micro array formats as a basis for a continuous DNA filtration and pre-separation. The micro fluid system uses orthogonally generated electrical fields and controlled sample velocity rates to realize filtration and separation of DNA based the strand lengths.

Integration Technologies for Bio-Analysis Systems. The BRP team has focused attention on the fabrication of the integrated sample preparation system in polymers. Polymer μ-analytical devices have the potential advantages of using high volume fabrication methods, such as ablation, embossing, or molding. This provides a wide variety of substrate materials of different chemical and mechanical properties using fabrication techniques that are amenable to creating high definition μ-compartments in continuum. The potential advantages of using polymeric substrates for μ-analytical devices have been documented in Hewlett-Packard patents US 5,500,071, 5,671,410 and 5,658,413. A μ-capillary electrophoresis (μ-CE) device fabricated using excimer laser ablation in a polyimide sheet material has been successfully integrated into hardware with thermal, fluid, and detection interfacing, allowing for fully automated device actuation and data acquisition. Additionally, the μ-CE device has an integrated UV detection μ-compartment, with the longest path length (250 μm) reported for on-column/device detection. This provides improved sensitivity vs. conventional μ-column techniques.

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**PROJECT TITLE:** Micromechanics of Airway Smooth Muscle Cells in Culture

**PARTNERS NAMES AND AFFILIATION:**

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| Geoffrey Maksym, Ph.D. | Dalhousie U., Canada |

**GRANTING NIH INSTITUTE/CENTER:** NHLBI, Division of Lung Diseases

**ABSTRACT**:

Acute narrowing of the airway lumen in asthma is driven by myosin motors that exert their mechanical effects within a cytoskeletal scaffolding that is both deformable and in a continuous state of remodeling. The mechanical properties of that scaffolding are not well defined. This BRP application describes a multi-disciplinary design-directed bioengineering project to fill that gap of knowledge. We propose to develop a micromechanical technology to measure the rheological properties of adherent living airway smooth muscle cells in culture, and the time-course of mechanical changes that occur in response to contractile stimuli or after genetic manipulation of cytoskeletal proteins. Ligand-coated ferromagnetic microbeads are bound to the cytoskeleton, and oscillatory mechanical torques are then applied to the bead by a sinusoidally-varying external magnetic field. Resulting oscillatory bead motions deform the cell, and can be determined by measuring changes of the remanent magnetic field due to bead rotations or, alternatively, by direct observation of oscillatory bead displacements using light microscopy; these are complementary detection methods each with special advantages. This technology becomes, in effect, a micro-rheometry system that can probe – in cell culture conditions – contractile responses and underlying cellular rate processes over time scales as short as tens of milliseconds to as long as hundreds of seconds. Thus, it measures mechanical properties of cells using deformation times (and stress magnitudes) that span the physiological range. We propose to develop this technology and then use it to test the hypothesis that the contractile response of human airway smooth muscle cells in culture is attenuated by overexpression of heat shock protein 27 (HSP27) dominant negative mutants. This hypothesis bears upon a question whose importance has been identified only recently, namely, the stability of the cytoskeleton of the airway smooth muscle cell and the role of CSK stability in airway narrowing in asthma.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Instrumentation development is progressing as planned

**ISSUES:**

Using a prototype of the technology under development, we have made what would appear to be an important basic discovery concerning the rheology of living cells. We measured the rheology of cultured mammalian cells (airway smooth muscle, fibroblasts, macrophages, neutrophils) from 0.01 to 1000 Hz. Data for all cell types, frequencies and biological interventions studied could be scaled onto universal master curves. This scaling identifies these cells as soft glassy materials existing close to a glass transition, and implies that cytoskeletal proteins may regulate cell mechanical properties mainly by modulating the effective noise temperature of the matrix. The practical implications are that the effective noise temperature is an easily quantified measure of the ability of the cytoskeleton to deform, flow and reorganize. These factors come into play during cell contraction, spreading, division, locomotion, extravasation and invasion.

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**PROJECT TITLE:** Imaging Activity in Visual Cortex at the Cellular Level

**PARTNERS" NAMES AND AFFILIATIONS:**
Winfried Denk, Max-Planck Institute for Medical Research, Heidelberg, Germany
Roger Y. Tsien, Howard Hughes Medical Institute, University of California, San Diego

**GRANTING NIH INSTITUTE/CENTER:** National Eye Institute

**ABSTRACT:**
We are combining advances in optics, molecular probes and gene therapy techniques to monitor neural activity in the visual cortex of awake, behaving animals, with single cell resolution. Two-photon imaging makes it possible to visualize fluorescent cells lying several hundred microns under the cortical surface and to minimize the photodynamic damage to the cells. Fluorescent proteins allow one to visualize the details of cell morphology, and their fluorescence can be linked to measures of neural activity. Adenoviral vectors enable us to insert genetic constructs that code for these proteins into the genome of cortical cells, allowing one to label large numbers of cells with a sparse distribution and with minimal damage to the cells. We are merging the instrumentation for imaging with the equipment for animal recording and behavior, inserting constructs into viruses, and increasing the temporal resolution of the 2-photon microscope for monitoring activity tied to visual stimuli and animal behavior. Being able to record from the same identified cells over an extended period allows one to study experience dependent changes in the functional properties and morphology of cells. The approach will have a wide range of applications, including the study of morphological changes in cells, the biophysics of neuronal integration, the neural basis of learning and higher order cognitive function, and patterns of gene expression in the intact brain.

**STATUS OF RESEARCH AND PARTNERSHIP:**
2-photon imaging has several advantages in imaging fluorescently labeled structures in the intact brain. It relies on the principle that a fluorophore can be excited by the simultaneous collision of two photons at twice the wavelength of its single photon excitation. At the higher excitation wavelength, in the infrared, there is much less light scatter and further penetration of light into the tissue, allowing one to image structures deep below the cortical surface. Furthermore, the technique is tomographic, providing images from a very narrow depth plane.

The project is being developed along several lines simultaneously. First, we have designed and contracted the construction of a new scanning head that allows one to obtain images with high temporal resolution. This combines a slow scanner with a fast scanner, using a technology known as resonance scanning. The scanning pattern is characterized as a "moving postage stamp", where the laser beam will be guided to the locations of labeled cells, quickly scanning a small rectangular area to include the entire cell body, and skipping from cell to cell, to scan in the order of 20 cells. The scanner is designed to handle about 1000 postage stamps per second, therefore scanning each cell, depending on the total number, at about 50Hz.

A second area of progress is the integration of imaging technology with the awake, behaving monkey preparation. We have built a structure and a positioning gantry that will accommodate a

chair in which the animal is trained to sit with its head fixed, to fixate a stimulus on a computer monitor with its eyes, and to perform visual discrimination tasks. The structure supports and positions the imaging hardware over a recording chamber, which is implanted in the animal's skull. We have designed and machined chambers with windows that allow one to image the cerebral cortex through a clear artificial dura. We have integrated the software for behavioral control with the software for imaging. While the 2-photon scanning heads are being developed, we are using a video camera to do intrinsic signal optical imaging of the cortex, a technique that allows one to image the functional architecture of the visual cortex.

A third area is the development of genetically encoded fluorophores that are sensitive to neural activity. The advantage of using genetically encoded probes is that one can label large numbers of neurons via the use of viral vectors, which is much more practical than intracellular injection. The probes that have been developed by Roger Tsien rely on the principle of fluorescence resonance energy transfer, where two fluorophores, when brought in close proximity by a conformational change of a third molecule, can transfer a photon. Light excites the first fluorophore, which emits a photon that excites the second fluorophore. The currently available probes, including "cameleons" and "camgaroos" are calcium sensors, and therefore provide a good measure of neuronal activity. The Tsien lab is developing new versions of these sensors that will provide stronger signals. Our current results delivering standard GFP (green fluorescent protein) with adenoviral vectors in the monkey visual cortex gives strong labeling of the finest details of neuronal structure. Even this level of structure labeling will be invaluable in allowing us to monitor structural changes in the intact cerebral cortex following changes in visual experience.

Finally, we have made great progress in developing the experimental model to which the new technology will be applied. We have developed the primary visual cortex as a model for studying the neuronal basis of perceptual learning, and for revealing the role of top-down influences of perceptual task and expectation in shaping the response properties of cells at early stages of visual processing. The ability to monitor the activity of many identified neurons simultaneously, and to look at changes in their function and structure over time, will be invaluable in this study.

**ISSUES**:

The several technologies that are to be merged in this project are rapidly developing. More sensitive probes will be needed to provide signals that are stronger and that follow more closely the time course of the cellular processes. Better viral vectors will be needed to maintain expression of the probes for longer periods of time.

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**PROJECT TITLE:** Development/Testing of Artificial Retinas for the Blind

**GRANTING NIH INSTITUTE/CENTER:** National Eye Institute

**ABSTRACT:**

Our research for this partnership grant is to develop a long-term implantable retinal stimulator for patients blinded by outer retinal degenerations. Using technologies developed by the Alfred E. Mann group of companies over the past 30 years for implantable stimulators, we will develop a chronic retinal stimulator and associated external hardware for use both in research and as a clinical device.

In order to achieve this goal, several areas of research are still needed. In this bioengineering research partnership, academia will collaborate with industry to accomplish the basic research necessary to make a chronic retinal prosthesis a reality. Areas of basic research that we are focusing on include:

- Electrode geometry and electrode material selection
- Surgical attachment of the retinal implant
- Low power electronic circuit design
- Hermetic packaging

Each of these areas needs additional research for the creation of an optimal chronic retinal prosthesis which will enable persons blinded by outer retinal degenerations to regain the most important loss they have suffered--the loss of mobility. The aim of this five-year proposal is to complete the design and manufacture of a retinal prosthesis and associated external hardware and test it chronically in animals, so that an investigational device application can be made to the FDA in preparation for a clinical trial.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The partnership has made significant progress in its first year with many of the year one milestones already met.

Over a dozen existing candidate electrode materials have been tested so far and found to perform worse than the industry standard, platinum, when chronically stimulated. We have developed several new candidate materials which are currently under test. Also a setup to test electrode geometries in-vivo has been constructed and will be used to optimize electrode geometries.

In the area of surgical attachment, custom retinal tacks have been designed and fabricated for this application. This approach to surgical attachment has so far shown to be the most chronically reliable means for attaching the electrode arrays. However, alternative attachment methods are still being pursued.

One major objective of the first year was to test the effects of local heating on the retina. Acute and chronic experiments in 18 dogs with custom-made focal heaters revealed that as low as 50mW caused retinal damage when in contact with the retina. However, power as high as 500mW caused no retinal damage acutely or chronically when placed in the middle of the vitreous cavity. This has important implications for the design of the retinal prosthesis since electronics which consume more than 50mW cannot be placed on the surface of the retina, but can be placed in the vitreous.

Several preliminary hermetic implants have also been constructed with a limited number of electrodes. These wireless implants are presently being chronically tested in dogs and, though crude, are the world's first chronic fully implantable functionally active retinal prosthetics. Previous devices have suffered from not enough power output or electronics failure in the salt water environment of the body. Several more will be built for testing in other animal models as well.

Low power implant electronics continue to improve with a fourth generation integrated circuit designed, built and currently being tested. Other designs and components (ex. Hermetic packaging and electrodes) for a practical retinal prosthetic (ie. higher electrode number) are also being developed and tested.

**ISSUES:**

1. Electronic tools to increase communication between remote collaborating sites.

2. Intellectual property and 'teaming' agreements in collaborations.

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**PROJECT TITLE:** Magnetic Resonance Guided Electrophysiology Intervention

**PARTNER' NAMES AND AFFILIATIONS:**
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2) Guy Clatterbaugh: Johns Hopkins Applied Physics Laboratory
3) Albert Lardo: Surgivision, Incorporated
4) Erez Nevo: Robin Medical, Incorportated
5) Steve Sagon: Bard Electrophysiology, Incorporated

**GRANTING NIH INSTITUTE/ CENTER:** National Heart, Lung and Blood Institute

**ABSTRACT:**

Ventricular tachyarrhythmias and atrial fibrillation are the most important arrhythmias affecting patients. They are the most frequently encountered tachycardias, account for the most morbidity and mortality, and despite much progress, remain therapeutic challenges. Invasive electrical studies of the heart (electrophysiologic studies) are often used in the diagnosis and therapy of arrhythmias, and many arrhythmias can be cured by selective destruction of critical electrical pathways with radiofrequency (RF) catheter ablation. A major limitation in studying arrhythmias in patients, however, is the lack of ability to accurately correlate anatomical and electrical information. Anatomy is derived from x-ray images, which are two-dimensional and have substantial anatomic ambiguity. Another major limitation is the lack of ability to visualize ablated areas of myocardium during catheter ablation procedures, making it difficult to confirm the presence of ablated lesions in the desired locations. We have developed ways of combining the anatomic information from magnetic resonance imaging (MRI), with electrophysiologic testing and catheter ablation.

We hypothesize that magnetic resonance imaging, with transesophageal receivers, intracardiac receivers and MRI-compatible (non-magnetic) electrode catheters, can (1) provide accurate navigation of catheters without radiation, (2) provide the ability to visualize ablated lesions, and (3) aid in producing more accurate electrical maps. As a prototype for the development of new approaches to electrophysiologic testing and catheter ablation, this proposal addresses atrial fibrillation primarily. The imaging technologies developed in this project, should however, be broadly applicable to using MRI to guide interventional procedures in the heart in general, as well as in other organ systems.

**STATUS OF RESEARCH AND PARTNERSHIP:**

There have been no changes in the partners. New technologies developed include 1) improved receiver antennas, 2) steerable MRI compatible electrode catheters, and 3) new sensors for tracking the tips of catheters. We have used the new receiver antennas for studying pulmonary veins in dogs. The improved receivers have at least a 2-fold improvement in signal-to-noise ratio. The studies in the pulmonary veins have shown that with the new receivers, resolution is enhanced, and that muscle sleeves in the pulmonary veins can be visualized. The importance of visualizing muscle sleeves is that they likely participate in the generation and sustaining of atrial fibrillation, and that ablating them may lead to elimination of atrial fibrillation. We have used the steerable MRI compatible electrode catheters to perform electrophysiological studies and catheter ablations in animals to gain experience for use of these catheters in humans. The steerablility is important in positioning the catheters into arbitrary sites within the heart. We developed miniaturized sensors that are suitable for placement in a catheter tip, that are suitable for tracking the tips of catheters. The sensors have been interfaced with the GE cardiovascular MRI system, and we are currently having them incorporated into the tips of electrode catheters. In addition to the above technological developments, we have determined that there are MR imaging sequences available that do not cause significant heating of the electrode catheters.

We also have studied the anatomic details of the subeustacian isthmus (SEI) region and the location and spatial extent of radiofrequency ablation lesions in humans using standard magnetic resonance imaging (MRI) following catheter ablation for atrial flutter. Twelve patients underwent atrial flutter ablation consisting of a line across the SEI and a line from the coronary sinus to the tricuspid valve annulus. MRI was performed pre and post-ablation using a standard external thoracic coil and a T2-weighted edema sensitive fast spin echo imaging sequence. Anatomic details of the entire SEI region could be quantified in short axis and right anterior oblique anatomic views in all patients (SEI length = 2.4±0.4cm, width = 3.3±0.5cm, depth = 2.8±0.3cm). SEI lesion cluster depth measured from right anterior oblique views was 1.2±0.6 cm. Thus, we demonstrated that the spatial extent of radiofrequency ablation lesions at the SEI can be visualized in humans following catheter ablation for atrial flutter. These data indicate that a large subeustacian isthmus lesion depth is required to achieve bidirectional conduction block and suggests that the use of interactive MRI in future ablation procedures may help facilitate lesion placement, reduce the number of RF applications and enhance success.

It was hypothesized that focal sources of atrial fibrillation located in the pulmonary veins (PV) may be treatable by electrical isolation of the PV's. We developed and tested a novel fiberoptic balloon catheter designed for production of circumferential lesions in the PV's. A total of 8 circumferential lesions were created in the superior pulmonary veins of 5 healthy mongrel dogs. Continuous-wave, near-infrared (l = 1.06 mm) Nd:YAG laser radiation was delivered radially through a 2-cm-long diffusing optical fiber tip, enclosed in a balloon. The PV ostia were successfully ablated using a laser power of 50 W and an irradiation time of 90 s. In 8 out of 10 superior pulmonary veins (80%), lesions were observed. Of these 8 lesions produced, 7 lesions were continuous and transmural (87.5%). By gross and histologic examination, there was no evidence of endothelial disruption. Thus, it was demonstrated that continuous circumferential lesions were created in the pulmonary vein ostia using a new fiberoptic balloon catheter. The data from use of lasers for ablation of pulmonary veins shows that myocardial necrosis can be achieved following a single application without endothelial disruption. This elimination of endothelial disruption may lead to reduction in pulmonary vein stenosis, which is a severe complication of pulmonary vein ablation using RF application.

Most recently, we are using MRI for aiding ablations of the pulmonary veins in humans. We are obtaining MR images pre ablation to determine the geometry of the pulmonary veins, and we are then obtaining MR images immediately post ablation and one month post ablation. The scan immediately post ablation is to assess whether lesions can be identified, and the scan at one month is to determine if anatomical complications, such as pulmonary vein stenosis is present. We have studied two patients, and have found the MR images critical to understanding the pulmonary vein anatomy during the ablation procedure.

In the coming year, technological development will continue. We are nearly ready to test an improved, deployable receiver coil. This coil, rather than the loopless receivers, should enhance signal-to-noise ratio by close to an order of magnitude. We will also continue development on the MRI tracking system. This system is being integrated into catheters, and initial testing in the scanner should commence shortly. Further enhancements will include using the data from the tracking system to dynamically manipulate the imaging plane to keep it in the plane of the catheter tip. We have also obtained a high-performance graphics workstation, and we plan to develop enhanced software to allow real-time or near-real time display of cardiac structures in 3-dimensions during interventions, with superimposed catheter-tip-localization and electrical maps.

We will continue to investigate MRI guidance for electrophysiologic studies and catheter ablation in an animal model. We plan to start the use of the atrial fibrillation - canine model to investigate linear and pulmonary vein ablations in canines. Since we have shown that electrode catheters can be safely placed and used in an MRI environment, we will be applying to the FDA for an investigational device exemption (IDE) for placing catheters in patients undergoing MRI. We will then be performing electrophysiologic studies in patients under MRI guidance and comparing those results to comparable studies performed under standard Xray guidance. Once those studies are complete, we will begin studies of ablation under MRI guidance.

**ISSUES:**

Since funding of partners is through contracts, the partners' indirect costs become direct costs in the PI's budget. This is a disincentive for having partners.

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**PROJECT TITLE:** Total Liquid Ventilation: A Bioengineering Partnership

**PARTNERS' NAMES AND AFFILIATIONS:**
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**GRANTING NIH INSTITUTE/CENTER:** National Heart, Lung and Blood Institute

**ABSTRACT:**
ARDS is a frequently lethal pulmonary process that occurs in approximately 150,000 patients each year. Total liquid ventilation (TLV), in which the lungs are filled with perfluorocarbon and ventilated with a device which oxygenates and removes carbon dioxide from the perfluorocarbon, has great potential to effectively treat patients with ARDS. The clinical principal investigator has been performing studies in liquid ventilation over the last 8 years. Through our laboratory effort, we have generated data that demonstrate the efficacy of TLV in improving gas exchange, pulmonary function, and oxygen delivery, as well as in reducing acute lung injury. The bioengineering principal investigator has been performing studies in biofluid mechanics and transport of the pulmonary system for many years. This proposal addresses several fundamental physiological and bioengineering issues that underlie the progress toward establishing TLV as a clinical tool: 1) the optimal means for administering the liquid into the lungs; 2) the effect of ventilation parameters upon gas exchange; and 3) the expiratory flow limitation which restricts the effectiveness of the technique. The current research proposal is, therefore, directed at developing a new partnership between a clinician scientist and a bioengineer in the investigation of these issues which involve principles of fluid delivery and distribution, gas transport, and flow limitation during expiration. Specifically, our investigation will assess the distribution of the perfluorocarbon with regard to rate of fill, position during filling, and the characteristics of the perfluorocarbon. Secondly, we intend to investigate and to model the parameters which affect gas exchange during TLV, such as tidal volume, respiratory rate, and lung distension, and to model local flow patterns within the airways and alveoli. Finally, we plan to assess the relationship of flow limitation during expiration to the rate of flow and the state of inflation of the lungs and to investigate strategic means of manipulating parameters which determine flow limitation. A thorough understanding of these issues and solutions to these problems will be critical to the clinical application of this new and exciting technology.

**STATUS OF RESEARCH AND PARTNERSHIP:**
Our research in the above specific aims has progressed well. We have developed data evaluating the effect of rate of perfluorocarbon administration upon the homogeneity of the distribution of perfluorocarbon in the lungs. We have also characterized the effect of perfluorocarbon flow rate and lung volume upon the development of flow limitation and identified the location of flow limitation along the longitudinal distribution of the airways. We have also begun to define predictors of the onset of flow limitation which will allow servoregulation of liquid drainage from the lungs and avoidance of airway collapse.

**ISSUES:**

We hold conferences involving all members of the partnership, along with trainees, every 2 to 4 weeks. Ongoing research is discussed and data presented at these meetings with vital input contributed from both the bioengineering and clinical investigators. These conferences, with the associated integration of expertise, have resulted in a broader approach to our research. Data on projects which have bioengineering/clinical relevance, other than those associated with the current BRP, are frequently presented at this conference to obtain input from investigators. In fact, presentation of such data regarding work on a total artificial lung became a routine agenda item at our meetings and resulted in submission of a BRP to develop a clinically applicable, implantable lung replacement.

We have numerous trainees involved in the partnership including three M.D. research fellows, one medical student, two bioengineering Ph.D. research fellows, three bioengineering Ph.D. candidates, and one bioengineering masters/undergraduate student. One of the bioengineering Ph.D. research fellows spends a predominance of his time in the animal laboratories of the clinical partner, learning to perform animal experiments and to apply biologic data to his research. These trainees have been critical to sustaining the cross-fertilization and integration that has been valuable to this partnership.

We have no specific problems with the partnership.

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**PROJECT TITLE:** Image and Model Based Analysis of Lung Disease (R01 HL64368)

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**GRANTING NIH INSTITUTE/CENTER:** National Heart Lung and Blood Institute

**ABSTRACT:**

With the emergence of therapeutic interventions for many common lung diseases, there is a critical need for sensitive and objective measures of regional lung status both for detection of disease and for outcomes analysis. X-ray CT remains the imaging modality of choice for comprehensively evaluating the lung. Significant advances are being made in both temporal and spatial resolution. Helical scanners are now capable of sub second (300msec and faster) data acquisition speeds. With the recent introduction of 2-D detector arrays on spiral scanners, true volumetric imaging is on the horizon. Scan apertures are at sub-cardiac cycle time frames, allowing for the imaging of not only anatomy but also ventilation and perfusion, providing structure-to-function correlations. This proposal brings together a multi-disciplinary team of investigators to focus on improving lung imaging, developing and validating imaging and image analysis protocols for quantitation of anatomic and physiologic lung features, and to develop a model of the normal human lung based upon these new measures. The unique measures that will be made possible by this research will be integrated into a model of the normal human lung. This model will provide for an atlas of the lung, lobes sub lobar segments and airway and vascular branching structure of the lung and attached to each level of this structure will be the normal range of the CT-based measures of regional lung physiology including ventilation, perfusion, and specific compliance as well as quantitative anatomic features including regional tissue texture and airway and vascular geometry. This model of the normal human lung, developed for three decades of adult age, will provide the comparative basis for detecting and quantitating pre-clinical and clinical distribution of disease. To focus our evaluation of the utility of the methodologies to be developed, we will concentrate on smoking related lung disease, specifically emphysema and lung cancer.

As the core of this partnership, we will establish a state-of-the-art CT research scanning center which will allow the partners of this proposal access to the tools needed to investigate the basic principals of CT imaging and to engineer the methodology necessary to extract unprecedented detail of regional lung structure and function. For these purposes, we have forged an agreement with Marconi Medical Systems (formerly Picker International) who will provide this group with a state-of-the-art multi-slice spiral CT scanner and an agreement to maintain this engineering scanner with alpha level hardware and software upgrades over the full five years of the project. We expect the scanner to achieve true dynamic volumetric imaging of the lung with speeds below the R-R interval of the cardiac cycle, a spatial resolution of better than 24 line pairs per centimeter, and low dose radiation protocols.

## STATUS OF RESEARCH AND PARTNERSHIP:

**1) Imaging**

a) Establish a scanning facility (State of the art, high speed CT to be used as a tool for the assessment of both multi-slice and dynamic imaging strategies for the advancing field of X-ray CT) *Status: Up and Running in temporary building and scanner is soon to be upgraded from 4 to 16 detector system with rotation speed decreasing from 0.5 to 0.3 sec per rotation. The University of Iowa has committed to build a dedicated 3,500 square foot CT Imaging research addition to the hospital complex and construction is projected to begin in spring of 2001. The partnership team has monthly conference calls with members of Marconi Medical and all partners make regular trips to the CT scanning facility. Marconi has sponsored two meetings at Marconi Headquarters in Cleveland with all partners in attendance.*

b) Establish methods for physiologic control and monitoring subject during scanning to assure standardization of scanning protocols *Status: Simultaneous cardiac and respiratory gating has been implemented.*

c) Improve tomographic reconstruction methods for superior temporal and spatial resolution without any significant beam hardening and scattering effects. *Status: A mathematical model of the normal human lung from bronchus to alveoli has been implement, a CT scanning simulator has been developed, and the system is being evaluated to develop method for imaging the lung volumetrically and dynamically simultaneously via spiral scanning.*

**2) Image segmentation**

a) Lungs, Lobes, Sub-Lobar Segments: *Status: Lung and lung lobe segmentation has been achieved*

b) Airways (central and peripheral) *Status: Airway segmentation out to 7 generations has been achieved with sub voxel accuracy of airway boarder identification achieved via modeling of scanner point spread function.*

c) Vascular Tree *Status: Under Development*

d) Tissue characterization *Status: Adaptive Multiple Feature Method (AMFM) and histogram based methods implemented as an integrated system on PC with analysis times brought down to 10-15 minutes with standardized report generation implement in an access data base and anatomic distribution of tissue types displayed in near real time via volume rendering methodology.*

**3) Image matching**

*a)* Image Registration based upon the shape of the lung and lung lobes, airway and vascular branching structure. *Status: lung and lobar registration implemented with need for refinement taking into account internal landmarks.*

**4) Physiologic analysis**

a) Perfusion: In isolated and intact canine lungs and under various conditions of stress: Characterize the flow models used to calculate parenchymal perfusion from CT-based time intensity curves *Status: Integrated, PC-based package implemented to deconvolve flow curves for assessment of micro vascular flow parameters. Regional Flow measured successfully in a pig model via multi slice Marconi Scanner. Flow Phantom has been developed and is being studied.*

b) Ventilation: Characterize Xenon washout measures of regional ventilation and compare with measures of specific compliance in animals and *humans Status: 3D measure of regional ventilation in a pig model completed successfully via a combined xenon washin-washout method. Interactive analysis software integrated with PC version of blood flow software.*

c) Evaluation of the utility of the physiological measures, along with tissue characterization from 2-d by following early progression of lung pathology in a dog model of emphysema. *Status: sheep model of emphysema established and methods for tracking pulmonary function simultaneous to CT scanning have been implemented.*

**5) Construction of the lung model**

a) Development of the data-structure linking lung structure to function in normal male and female humans *Status: Data Structure for the establishment of the lung model is under investigation*

b) Provide standard, global measures of lung function to be linked to the CT-based Normal Lung Model

i) $FEV_1$, FVC, DLCO, Lung Volumes (use already established values for the normal lung)

ii) Exhaled Nitric Oxide (use values gathered from scanned normal subjects)

iii) Broncho-alveolar-lavage (BAL) (use already established values for the normal lung)

Status: These will be available when human scanning commences

**6) Application of the model to detection and quantification of pathologic processes**

a) Correlation of in-vivo measures with ex-vivo measures of lung lobes surgically removed for solitary pulmonary nodules *Status: to be initiated within 6-9 months.*

b) Assessment of the early effects of smoking (through scanning of non-smokers and smokers with normal ranges of pulmonary function test parameters ) *Status: scans to begin within 2-3 months.*

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**PROJECT TITLE:** Bioengineering Design of Artificial Blood (R24-HL 64395)

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**GRANTING NIH INSTITUTE/CENTER:** National Heart Lung and Blood Institute

**ABSTRACT:**
We plan to design, develop and produce an economic oxygen carrying plasma expander based on modified molecular human hemoglobin engineered with properties that insure the maintenance of microvascular function, leading to improved survival and tissue oxygenation relative to blood, for treatment of trauma victims within 48 hours of injury. The program is vertically integrated, including production of purified hemoglobin from red blood cells by means of a modified, self contained plasma fractionation centrifuge that directly produces the necessary molecular modifications and a unit of artificial blood ready for use. This approach eliminates the need for fabrication of large, clean facilities and reduces cost. Furthermore it allows for production in remote areas. Our bioengineering design principle is that when a blood replacement fluid is introduced in the circulation it should cause the resulting blood viscosity to remain sufficiently elevated to insure the adequate generation of shear stress at the blood/tissue interface, which we have demonstrated to be necessary to provide normal capillary blood flow, the key determinant of tissue survival. According to this principle even small amounts of cell-free hemoglobin (1-2 g/dl) are very effective in improving survival after hemorrhage. Due to this low-dose effect at least two units of product can be obtained from each unit of collected normal blood. The molecular modification to be pursued is surface modification with polyethylene glycol (PEG), and other modifications that will result in molecules with large radius. Different formulations of product are envisioned including a low volume concentrated solution that will restore blood volume by autotransfusion. The program encompasses all aspects of artificial blood production from obtaining the raw materials to the final commercial product and is aimed at establishing a blood transfusion technology that delivers a blood replacement biomaterial that is cost effective and as efficacious as blood. The envisioned artificial blood will be universal, requiring no typing, will have long shelf life and will be easy to store.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The focus of the initial activity has been the implementation of the research and development plan leading to the design of an effective product that can be manufacture at and delivered at costs that are competitive with blood. The problem of effectiveness was addressed by establishing a control baseline in terms of existing products. An array of microvascular tests were made at UCSD to determine the microvascular transport properties of an oxygen carrying bovine molecular hemoglobin solution manufactured by Biopure Inc. presently marketed for veterinary applications. It was found that when red blood cells were substituted with this product for a total hemoglobin concentration of 6 g/dl, tissue oxygen was zero. By comparison reduction of red blood cells concentrations to the same level with colloidal plasm expanders (starch, dextran) improved tissue oxygenation to above normal values. These findings were corroborated by the studies conducted at Sangart Inc. in pigs, utilizing a polyethylene glycol modified hemoglobin whose performance in terms of resuscitation from hemorrhagic shock was found to be superior to that obtained in control animals perfused only with normal blood. Preliminary findings confirmed the central hypothesis of this BRP that an effective article blood can be obtained though the formulation of a product that has comparatively high viscosity, high oncotic pressure and high oxygen affinity. We have shown that these properties can be obtained by reacting human hemoglobin with polyethylene glycol (PEG). Since both hemoglobin and PEG are comparatively costly products, a significant effort is underway at Sangart Inc., Northeastern University and UCSD to lower the concentration of these components by achieving the appropriate viscosity and oncotic pressures with colloids such as dextran or starch, and exploring the optimal PEG molecular characteristics.

**ISSUES:**

In terms of issues that have come into focus as the BRP work was started it has become apparent that a project of this nature must be directly tied to the plans for manufacture and marketing of the product. This appears to be particularly relevant for a blood substitute because it presents a unique combination of sourcing, processing, transportation and delivery method that can fundamentally affect the final cost, and therefore the economic effectiveness of the results. In this context the critical issue is to determine what is the most effective quantity of product to be assembled in any given setting.

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**PROJECT TITLE:** Partnership for Research in Optical Coherence Tomography

**PARTNERS' NAMES AND AFFILIATIONS:**

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| Peter K. Kaiser, M.D. | Cleveland Clinic Foundation, Cole Eye Institute (CCF) |
| Raj Shekhar, Ph.D. | Cleveland Clinic Foundation, Biomedical Engineering (CCF) |
| Cynthia A. Toth, M.D. | Duke University, Duke Eye Center (DU) |
| Matthew R. Glucksberg, Ph.D. | Northwestern University, Biomedical Engineering Department (NU) |
| Jeffery W. Kiel, Ph.D. | U. Texas Health Science Ctr. San Antonio (Consultant to NU) |

**GRANTING NIH INSTITUTE/CENTERS:** National Eye Institute (lead) and National Cancer Institute

**ABSTRACT:**

This Biomedical Research Partnership proposal represents a multidisciplinary approach to advance the state of the art in diagnostic anatomical and functional imaging *in situ* at the micron scale. This will be achieved by developing fundamental advances in the technology of Optical Coherence Tomography, validating new techniques using animal models, and employing new technologies in pilot clinical studies. Optical Coherence Tomography (OCT) is a novel imaging technique based on infrared light reflectometry, which is capable of achieving micron-scale spatial resolution imaging non-invasively in human tissues. The initial clinical applications of OCT in ophthalmology have been successful, however, significant advances in OCT are now possible such that this nascent technology is on the threshold of finding applications with much wider clinical impact, particularly in minimally invasive early cancer detection. In addition, we will develop and apply novel technologies for structural and functional imaging in ophthalmic applications. Our Partnership includes biomedical engineers and clinicians from five institutions with demonstrated leadership in the transfer of optical diagnostic technologies to clinical practice. The **specific aims** of the proposal and the institutions involved in each (abbreviations defined above) are: **1.** To enhance and expand the clinical utility of Optical Coherence Tomography by developing the following core technologies: i) high frame rate imaging, ii) ultrahigh resolution imaging (<5 microns), iii) minimally invasive endoscopic and ophthalmic delivery systems, and iv) imaging of physiological function including blood flow and tissue hydration (CWRU-BME). **2.** To apply these technologies for pilot studies of early cancer detection in the gastrointestinal tract (CWRU-GI, CWRU-BME). **3.** To apply these technologies for studies of chemoprevention and early cancer detection in the lung (BCCA, CWRU-BME). **4.** To improve the accuracy and safety of keratorefractive surgery by developing OCT technology to measure the corneal epithelial remodeling response, structural stability, and hydration changes following laser in-situ keratomileusis (LASIK) (CCF, CWRU-BME). **5.** To improve imaging of retinal, sub-retinal, and vitreous pathologies with increased resolution and reduced motion artifacts (DU, CCF, CWRU-BME). **6.** To develop and test technologies for quantitative detection of blood flow in the retina and choroid of animals, and to apply these technologies to monitor patients with vascular complications of diabetes, glaucoma and retinal occlusive disease (NU, DU, CCF, CWRU-BME).

**STATUS OF RESEARCH AND PARTNERSHIP:**

Specific Aim #1: Develop core technologies for OCT The CWRU-BME group has developed several advances in OCT technology which are undergoing clinical trials at CWRU and Partner institutions. High-speed OCT imaging technology has been adapted for real-time analysis of the ocular anterior segment in living patients using a novel hand-held OCT probe. Due to the fast acquisition rate of 8 frames/sec, positioning of the probe using real-time image-based feedback is intuitive and motion artifact is greatly reduced. Ongoing clinical studies using this technology include development of a normative database of anterior segment structure dimensions at CWRU and quantitative comparison of OCT and ultrasound biomicroscopy at CCF. The CWRU group has also developed novel signal processing approaches for color Doppler OCT (CDOCT), which allow for cross-sectional visualization of retinal vessels and monitoring of retinal hemodynamics. Using a newly developed system, vessels on the order of 10 microns in diameter were imaged in living human skin, with a relative frequency sensitivity less than $5 \times 10^{-5}$. These results represent the highest velocity resolution ever obtained with CDOCT. The CWRU-BME group has also developed a novel fiber-based polarization-sensitive optical coherence tomography system for applications in retinal nerve fiber layer thickness analysis. In contrast to other PS-OCT systems which have been described, the CWRU unit can be implemented as a simple retrofit to conventional OCT scanners.

Specific Aim #2: Endoscopic OCT (EOCT) for early cancer detection in the gastrointestinal (GI) tract. This specific aim was not funded by NCI. However, real-time EOCT scanning technology has been developed to the point of international multi-center trials, led by the CWRU-GI team. To date, EOCT has been demonstrated for identification of normal microscopic sub-surface structures in all endoscopically accessible GI tissues, and shows promise for differentiation of dysplasia from normal tissue and precancerous tissue in the esophagus and colon.

Specific Aim #3: Bronchoscopic OCT (BOCT) for studies of chemoprevention and early cancer detection in the lung. Funding for this work from NCI commenced in the second year of the project. Construction of the first-generation BOCT device (~3mm diameter probe) is underway at BCCA for anticipated in vitro and in vivo studies. Design work on next-generation probe technology (<1mm diameter probe) is proceeding with evaluation of components, in particular micro bearings and micro-machined ceramic and optical components. Meanwhile, early success has been achieved in the development of instrumentation for confocal microendeoscopy (work originally slated for the later years of the partnership) using newly available micro-electro-mechanical technology.

Specific Aim 4: Corneal OCT following LASIK. An arc-scanning OCT system was developed at CCF for corneal imaging. The corneal OCT unit was used to image corneal pathologies such as corneal scar and post-surgical ectasia. It was also used to provide quantitative measurements on anatomic changes related to laser in-situ keratomileusis. Preliminary results show that pre-operative OCT corneal thickness measurements agrees closely with ultrasound pachymetry. Image processing algorithms were developed to automatically measure the thickness profiles of the corneal epithelium, LASIK flap, and cornea.

Specific Aim 5: OCT imaging of retinal, sub-retinal, and vitreous pathologies. Dr. Toth and her colleagues have continued laboratory and clinical studies of OCT imaging of ocular tissues and macular disease and have numerous presentations and publications resulting from NIH funding. The most notable finding this year, has been the association of vitreomacular traction with tearing of the retinal pigment epithelium and with the active growth of subretinal blood vessels. This work will continue in subsequent years. The DU and CWRU-BME teams are collaborating to implement an improved high-speed retinal OCT scanner.

Specific Aim 6: Development of Doppler OCT for retinal and choroidal blood flow. The NU group has implemented Doppler OCT to image large choroidal vessels caused by red blood cell motion. This experiment demonstrates the feasibility of detecting RBC motion in choroidal vessels despite being approximately 300 microns beneath the vitreal-retina interface. In collaboration with CWRU-BME, the NU team is implementing a rapid scanning optical delay to increase velocity sensitivity in order to more accurately detect RBC velocity in the choroidal capillary network. The NU team is also exploring possible biocompatible contrast agents that are more highly scattering at the source wavelength.

**ISSUES:**

Our Partnership has benefited from good communication between the lead institution (CWRU) and Partner institutions, including several reciprocal visits by research/technical personnel and frequent telephone/e-mail communications. However, Partner institutions have been reluctant to collaborate directly due to competitive issues. In addition, it has been difficult to schedule regular meetings of the entire collaboration.

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**PROJECT TITLE:** Biomedical Optics for Medical Research and Clinical Care

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| William Horton, MD | OHSU (Molecular and Medical Genetics) |
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| Manfred Baetscher, PhD | OHSU (Transgenic Facility, Comparative Medicine) |
| Ron Sakaguchi, DDS, PhD | OHSU (Biomaterials and Biomechanics) |
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**GRANTING NIH INSTITUTE/CENTER:** National Cancer Institute, 1R24 CA84587

**ABSTRACT:**
Biomedical Optics is a field using light to interrogate tissues for diagnostic purposes and to treat disease and assist surgery, with applications in both biomedical research and clinical care. Oregon has a network of bioengineering laboratories specializing in biomedical optics and two medical centers with clinical activities using biomedical optics and medical lasers. This proposal establishes a Biomedical Optics Laboratory on the campus of the Oregon Health Sciences University (OHSU) as a core research facility to support the interface of new optical technologies from the bioengineering laboratories with projects in the medical research and clinical care activities at OHSU. The proposal provides funding for the bioengineering laboratories to initiate new projects for translation into the medical center. The design-directed projects of the bioengineering laboratories are organized around hypothesis-driven projects of biomedical investigators and clinicians at the medical centers. The initial projects fall under two broad themes: (1) tissue engineering and biomaterials development with initial emphasis on bone regeneration and biomaterial implants, and (2)

cancer detection and treatment with initial emphasis on optical imaging of dysplasia and superficial cancer, optical fiber devices for dosimetry and treatment evaluation during light-activated chemotherapy (photodynamic therapy or PDT), and PDT as a tool in basic medical research. Management and outreach activities of the program include an annual review by an external advisory board, an annual symposium at the annual Oregon Academy of Sciences meeting, and a website with extensive database and educational materials. The research program complements a Biomedical Optics graduate school curriculum.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The status of the research and partnership is that the design, renovation and initial establishment of the Biomedical Optics Lab has been a major task. Nevertheless, six of eight projects have been initiated and are showing progress:

1. Transcutaneous confocal imaging of GFP expression in bone and cartilage
2. Light-activation of apoptosis in cancer cells
3. Novel Optical Coherence Tomography techniques
4. Atmospheric LIDAR techniques applied to tissues
5. Laser speckle assessment of mechanics in genetically modified collagen
6. Optical spectroscopic needle for guiding tissue biopsy

The remaining two projects will begin in the second year:

7. Photolithography with adhesion molecules to guide cell repopulation of artificial tissue implants
8. Photo-ablation of resident DNA in stem cell blastocyst embryo to enable transgenic DNA transfer without chimeras.

**ISSUES:**

There have been no problematic issues. Rather, the program has provided a catalyst for integrating the community of investigators involved in biomedical optics in Oregon. Two of the institutions, OHSU and OGI, have agreed to officially merge in July 2001 and establish a School of Engineering within the OHSU system. The Biomedical Optics Laboratory is the initial example of collaboration between the two institutions and the intramural showcase for integration of engineering and medical research.

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**PROJECT TITLE:** Integrative Biology of Tumor Angiogenesis, Invasion and Metastasis

**PARTNERS' NAMES & AFFILIATIONS:**

**Project 1: Vascular Angiogenesis**
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Paul L. Huang, MGH/Harvard

**Project 2: Invasion**
Yves Boucher and Kevin Burton, MGH
Michael Klagsbrun, Children's Hospital
Bruce Zetter, Children's Hospital

**Project 3: Hematogenous Metastasis**
Lance L. Munn, MGH
Josh Fidler, MD Anderson
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**Project 4: Lymphangiogenesis and Lymphatic Metastasis**
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**GRANTING NIH INSTITUTE/CENTER:** National Cancer Institute

**ABSTRACT:**
Now that numerous important genes associated with tumor angiogenesis, invasion and metastasis have been discovered, the grand challenge is to understand their function in **intact animals**. The second major challenge is to **integrate and apply** this knowledge to cancer prevention, detection and treatment. In the proposed BRPG, we will meet these challenges with a new, more precise, *quantitative*, *integrative* and *multi-disciplinary* bioengineering approach. This new bioengineering approach builds on unique and innovative techniques such as 1) genetically engineered mice to visualize gene expression, 2) *in vivo* models to visualize molecular and cellular events, 3) computer-assisted *in vivo* microscopy to quantify gene expression *and* function continuously and non-invasively at high (1-10 m) resolution in intact animals, 4) mathematical modeling to integrate the resulting information. Using this powerful technology, we will investigate four critical aspects of tumor metastasis: angiogenesis, invasion, hematogenous metastasis, and lymphangiogenesis & lymphatic metastasis. In the first year we will achieve several significant **milestones**: i) critically test the long-standing but unproven hypothesis that angiogenesis facilitates metastasis by increasing cell shedding; ii) establish a quantitative link between cell traction force and invasion through the tissue matrix; iii) demonstrate that stress generated by proliferating cancer cells can collapse lymphatic vessels in tumors; iv) provide the quantitative relationship between nitric oxide (NO) and angiogenesis in

vivo. In the second and third years we will build on these findings to provide deeper quantitative insight into expression and function of three genes (NO synthase, VEGF-A, VEGF-C) considered critical to these four aspects of metastasis. Years four and five will see integration of these data in a unified framework and identification of strategies for clinical translation. The proposed BRPG offers a **new paradigm** for integrative studies of the **dynamics** of gene expression and function in cancer. With this new paradigm available to our collaborating partners working at the forefront of genomics and proteomics, this BRPG will facilitate **translation** of knowledge about the molecular biology of cancer into effective cancer prevention, detection and treatment strategies.

**STATUS OF RESEARCH AND PARTNERSHIP: (July 1, 2000 - present)**

We have made significant progress in all four partnership projects.

**Project 1:** Vascular Angiogenesis: By using NOS knockout mice and NO measurement technology developed by Drs. Paul L. Huang and Donald G. Buerk, respectively, we have, discovered that eNOS plays a predominant role in VEGF-induced angiogenesis (Fukumura *et al*, PNAS, 2001).

**Project 2:** Invasion: By using cellular and molecular reagents developed by Drs. Michael Klagsbrun and Bruce Zetter, we have shown that VEGF plays a dose-dependant role in cancer cell mobility, Traction forces have been measured in several cancer cell lines for the first time and reveal that low force production does not directly correlate with rapid migration. Inhibition of myosin II, the molecular motor responsible for traction force, has been shown to reduce invasion of collagen gels by cancer cells.

**Project 3:** Hematogenous Metastasis: By using cellular and molecular reagents developed by Drs. Josh Fidler and Brian Seed, we have established an orthotopic model of renal cell carcinoma in mice that allows us to measure the rate of cell shedding by a renal tumor.

**Project 4:** Lymphangiogenesis and Lymphatic Metastasis: In collaboration with Dr. Kari Alitalo, we have discovered that intratumor lymphatics do not function despite the presence of the lymphangiogenic molecule VEGF-C and its receptors VEGFR2 and R3 in tumors (Leu *et al*, Cancer Research, 2000). Furthermore, in collaboration with Dr. Peter Carmeliet, we have shown that VEGF-C increases angiogenesis and growth in tumors without altering leukocyte-endothelial interaction (Kadambi *et al*, Cancer Research, 2001).

Publications

D. Fukumura, T. Gohongi, A Kadambi, J. Ang, C. Yun, D.G. Buerk, P.L. Huang and R.K. Jain, "Predominant Role of Endothelial Nitric Oxide Synthase in VEGF-induced Angiogenesis and Vascular Permeability," *Proceedings of the National Academy of Sciences, USA* (2001*).*

A.J. Leu, D.A. Berk, A. Lymboussaki, K. Alitalo and R.K. Jain, "Absence of Functional Lymphatics within a Murine Sarcoma: a Molecular and Functional Evaluation," *Cancer Research*, **60**: 4324-4327 (2000).

A. Kadambi, C.M. Carreira, C.-O. Yun, T.P. Padera, D.E.J.G.J. Dolmans, P. Carmeliet, D. Fukumura and R.K. Jain. "Vascular endothelial Growth Factor (VEGF)-C Differentially Affects Tumor Vascular Function and Leukocyte Recruitment: Role of VEGF-Receptor 2 and Host VEGF-A," *Cancer Research* (2001*).*

**ISSUES:**

The Bioengineering Research Partnership is an ideal and innovative program to integrate bioengineering with molecular biology and molecular medicine, and to facilitate translation of new knowledge from genomics and proteomics to improving health care and quality of life.

Other programs at the NIH need to embrace and emulate this approach in this era of "integrative biology," As articulated in the Feb. 1, 2001 editorial in Nature [Editorial "Post-genomic cultures", *Nature*, 409: Page 545, (2001)].

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**PROJECT TITLE:** Laser Cell Processing for Basic and Clinical Research

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Helen Heslop, M.D., Baylor College of Medicine (Houston, TX)
Leroy E. Hood, M.D., Ph.D., Institute for Systems Biology (Seattle, WA)

**GRANTING NIH INSTITUTE/CENTER:** National Center for Research Resources

**ABSTRACT:**
Photosis™ is a technology platform that incorporates high-speed optical scanning of biological samples, image analysis, and computer-controlled laser-irradiation of specific targets within the sample for the purpose of inducing a biological response. Specific cells to be treated within a mixed population are identified by parameters such as size, shape, fluorescence, or other distinguishing features. Once identified, individual cells are targeted with a laser to induce a desired response, such as cell death, optoporation (for gene transfer), or even inactivation of a specific mRNA transcript within the cell. The current β1-prototype system can process hundreds of millions of cells in an hour under sterile conditions, making it useful for several research and clinical applications. In fact, this prototype has several advantages over other methods of cell processing such as flow cytometry, and this conclusion is supported by preliminary data shown within. Photosis has many potential uses, and this proposal brings together a number of institutions and researchers to investigate and define the possible applications of this novel technology. In its current configuration, the instrument uses a single color for cell detection and a laser to induce necrosis in every targeted cell. These capabilities enable the first application which is tumor cell purging from autologous NHL stem cell transplants, because such contaminating tumor cells are known to contribute to disease relapse. Additional applications will be developed, some of which will require modifications to the system design and building of new prototypes. The prototypes will be placed at four partnership sites where the basic and clinical applications research will be carried out, including: (1) in vivo study of purified stem cell subpopulations in the xenogeneic fetal sheep transplant model; (2) human clinical trials to assess NHL purging in autologous stem cell transplantation; (3) purification of genetically-modified stem cells and T-cells expressing a selectable transgene, as well as selective transduction of specific cells in a mixture via optoporation; and (4) accurate mRNA expression profiling from purified primary human prostate cancer cell populations. The proposed work will result in several types of novel bioengineering instrumentation for advancing the start-of-the-art in cell processing. These instruments will be used in this program to advance basic and clinical research in stem cell biology, cancer, immunology, and genomics. Once developed, the resulting technology will be useful in other areas as well, some of which are described within.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The first phase of the project is underway. This involves the definition of instrument requirements for each of the partner sites, and the initiation of instrument development to meet those requirements. The collaboration with the first site (Dr. Bachier) is fully in place, and the development of the Photosis instrument for a pilot clinical trial in tumor purging is nearly complete. FDA contacts have been made in preparation for an IDE submission for this indication. Two sites (Drs. Zanjani and Hood) will be served by the second type of instrument to be developed. This instrument will draw heavily from the tumor purging instrument design, but with two critical additions: multi-color excitation and detection, and a more flexible user interface to facilitate research applications. Requirements definition for this instrument is nearly complete, and development will now quickly accelerate. The final site (Dr. Heslop) requires a somewhat different type of instrument in order to achieve opto-injection of genes into individual cells. One of our SAB members (Dr. M. Berns) has considerable experience in the area of opto-injection, and has offered to collaborate on the project to speed the development of this final instrument. This instrument, which is a future year task, will likely be based on the multi-color instrument design. Per the revised budget, activity at the partner sites is minimal in year one until the instruments are available for the respective sites to use.

**ISSUES:**

The original grant application had identified a partner site (Dr. Heslop, who is practicing gene therapy) for the use of the opto-injection instrument. We have since become more acquainted with Dr. Berns who has considerable experience in the theory and implementation of opto-injection. It now appears as though an early collaboration with Dr. Berns to develop the opto-injection instrument would substantially accelerate the process, whereas Dr. Heslop's contribution will still be the use of the instrument to implement gene therapy. Consequently, additional funds must now be identified in order to support Dr. Berns' involvement on the project, if that is to occur.

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**PROJECT TITLE:** Microchip Drug Delivery System

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**GRANTING NIH INSTITUTE/CENTER:** National Institute of Allergy and Infectious Diseases

**ABSTRACT:**

It is well known that the method by which a drug is delivered can have a significant effect on the drug's therapeutic efficacy. Most drugs have a concentration range in which they have maximum efficacy. Conventional drug delivery regimens result in sharp changes in systemic drug levels that can be toxic. Controlled drug delivery can alleviate the problems associated with conventional therapy by providing stable drug bioavailability in a therapeutically meaningful range and in addition can be used to localize the therapy to the tissue site of interest. We have shown that it is possible to fabricate a solid state silicon microchip in which a number of chemicals or drugs can be stored in individual micro-reservoirs and released on demand by electrochemically dissolving the gold cap in saline solutions with an external trigger. One advantage of this novel controlled release system is that it allows for simultaneous release of multiple drugs in complex release profiles. One can potentially develop a device that can be pre-programmed to deliver combinations of drugs in a pre-determined fashion. We believe that this novel delivery technology has broad utility in the biomedical areas such as local delivery of anesthetics for pain management, sub-dermal delivery of vaccines, peridontal delivery of antibiotic and anti-inflammatory agents, localized delivery of anti-tumor and neoplastic agents, gene delivery, delivery of antiarrhythmic agents. Based on the above mentioned rationale and our preliminary results we propose the following specific aims: (1) Development of an active, silicon based microchip for controlled release of drugs that can operate autonomously based on the electrochemical dissolution of a membrane over a drug containing reservoir, (2) Development of a passive, polymeric chip for the controlled release of drugs based on biodegradation of a polymeric membrane over a drug containing reservoir, (3) Evaluate the biocompatibility of active and passive microchip delivery device and (4) Evaluate the drug release both *in vitro* and *in vivo*, specifically: (a) show that predictable drug release is possible from both active and passive microchips (b) study a pathology such as brain tumors that may be better treated by combination therapy (c) evaluate the efficacy of these devices in the tumor model.

**STATUS OF RESEARCH AND PARTNERSHIP:**

During the past year we have investigated numerous aspects of the micro-reservoir drug delivery devices. Devices were used to release dye into serum *in vitro*, a much closer analogue to biological fluids than the saline solution used in the previous studies, and a study is in progress to evaluated the release of dye subcutaneously in a rat model. Additionally, gold films were electrochemically corroded subcutaneously in rats as part of the test of the biocompatability of gold corrosion products. Membranes electrochemically dissolved more slowly in serum than in saline, and dye was released more slowly from the reservoirs in serum. The differences between membrane opening behavior in serum and saline seem to be due to the differences in ionic strength and chloride ion concentration. The slower release kinetics in serum are probably due to a protein gel which forms on the surface of the membrane as it dissolves. The gel forms from denatured proteins in the presence of high concentrations of the reaction products.

The biocompatability of the electrochemical dissolution of gold membranes was tested using the cage implant system, in which samples of the material tested are placed inside wire mesh cages and the fluid inside is sampled periodically for cell counts and types. Devices were prepared so that the dissolution of a macroscopic piece of gold film could be performed inside a cage and the resulting cellular response monitored. Preliminary results from this study indicate that body's acute response to macroscopic gold dissolution is similar to the response from the initial wound, and that the chronic response is resolved much faster than that from the initial wound. The dissolved gold chloride acts as an anti-inflammatory agent, which is why it was used in the treatment of rheumatoid arthritis. The main cellular response seems to be due to the application of a voltage and the creation of new surface. Control samples which used the same electrical profile passes through inert platinum electrodes exhibited the same cell counts as the gold samples. The design of the device and its electrical operation will be modified based on the final results of this study.

Several types of electrical profiles have been compared to determine the best profiles for corrosion in saline and in serum. These will be compared with future studies to optimize voltage profiles for both membrane dissolution and biocompatability.

Nondestructive testing of the mechanical integrity of the membranes will be important for device manufacturing and membrane optimization. We have begun using a laser rangefinder in conjunction with a vacuum to test the deformation of membranes with the application of stress.

Significant progress has been made in the area of the passive microchip. A robust fabrication process has been developed that enables the microchips to be compression molded on a die plate from various polymers, including poly (lactic acid) and copolymers of lactic acid and glycolic acid. Reservoir caps have been fabricated out of cholesterol, polyanhydride, and various copolymers of poly(lactic-co-glycolic acid). The prototypes currently being used for *in vitro* experiments consist of a poly(lactic acid) substrate with poly(L-lactic-co-glycolic acid) reservoir caps. *In vitro* experiments have demonstrated the release of sodium fluorescein, a model chemical, from these devices. Experiments currently underway involve the release of additional chemicals (sodium fluorescein labeled dextran, tetramethylrhodamine labeled dextran, green fluorescent protein, and carbon 14 radiolabeled dextran and iodoantipyrine) in order to investigate the effect of light, pH, and molecular size on the release kinetics and detection accuracy. We hope that this will enable us to better understand our previous experimental results. In the near future, work will focus on improving the device sealing method, as well as releasing multiple chemicals from a single device, and releasing chemicals at different times from a single device.

**ISSUES:**

Microinjection is used to fill the microreservoirs with solutions, and the solvent evaporates leaving behind the active substance which is then hermetically sealed within the reservoirs. We have found that microscale drying is able to preserve the activity of proteins which normally are degraded by drying and must be lyophilized. This allows for the delivery of moisture sensitive biological molecules that cannot be delivered in alternative controlled release devices.

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**PROJECT TITLE:** Biomechanics of Leukocyte Adhesion Molecules

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**GRANTING NIH INSTITUTE/CENTER:** National Heart, Lung and Blood Institute

**ABSTRACT:**

This application proposes interdisciplinary bioengineering research in the area of molecular biomechanics. Leukocyte and endothelial adhesion molecules govern the trafficking of cells in inflammation, immunity, cancer metastasis and other processes. Some adhesion molecules, among them the selectins, are specialized to mediate adhesion in the presence of blood flow. Pressure-driven blood flow is associated with a shear stress exerted on the vessel wall, which results in a force on leukocytes and other cells trying to adhere to the endothelium. It is believed that adhesion under shear stress requires adhesion molecules with rapid association rates (on-rates), resulting in rapid formation of bonds. *In vitro* experiments and modeling studies indicate that the selectins also have high rates of bond dissociation (off-rates). Preliminary data suggest that the off-rates of selectins vary systematically with the shearing force exerted on the cell bound by the selectin (reactive compliance or tensile strength). In addition, the release of at least one of the selectins is accelerated by proteolytic cleavage by a surface-bound or membrane integral metalloproteinase. The current proposal has four specific aims. (1) To measure the bond lifetimes and apparent off-rates of L-, P- and E-selectin bound to their natural ligands. (2) To determine the role of L-selectin shedding in regulating leukocyte adhesion and selectin kinetics. (3) To determine the impact of the selectin length and their cytoplasmic tail for the biomechanics of adhesion under shear flow. (4) To design and build beads, liposomes and gas-filled bubbles (ultrasound contrast agents) that use leukocyte adhesion molecules to bind to vessel walls under shear stress. Each of these aims is approached in a three-pronged fashion. We propose to use laser trapping technology to directly measure biomechanical and kinetic parameters of selectin bonds, use single cells on sparse substrates to understand the biomechanics of selectins in an *in vitro* flow chamber, and use intravital microscopy to study selectin biomechanics in the context of the living organism. We propose to use molecular biology techniques to manipulate cDNA, cells, and mice to isolate each molecular mechanism. We will use the insights gained to design liposome-based targeted drug delivery systems and ultrasound contrast microbubbles for delivery in the vascular system under shear flow. At the end of the first year, we plan to have measurements of selectin off-rates, taking into account selectin shedding, and have tested selectin-containing liposomes for their ability to adhere under shear. Milestones for the following year are listed in the timeline.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The partnership is in progress. We exchange reagents (antibodies, transfected cell lines) and technical expertise. Several manuscripts have resulted from the partnership and several more are in preparation. We have organized the First Virginia Colloquim on the Biomechanics of Adhesion Molecules in 2000 and have sent out invitations for the second colloquium to be held April 20 and 21, 2001, featuring two keynote speakers (Dr. W. Weis of Stanford and Dr. D. Hammer of U. of Pennsylvania).

**ISSUES:**

The partnership is functioning very well. Dr. L. Karns, the original director of the molecular biology core, has meanwhile left the University of Virginia. However, the necessary functions of the core facility are covered by technical personnel. The progress in specific aim 4 (targeted microbubbles) was extraordinarily rapid, resulting in three published (Circulation, Circulation Research) and one submitted manuscript. It was not expected that this aim would be a focus area early on.

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**PROJECT TITLE:** Blind Pedestrians' Access to Complex Intersections

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Ron Hughes, David Harkey
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**GRANTING NIH INSTITUTE/CENTER:** National Eye Institute, National Institute of Nursing Research

**ABSTRACT:**

The goal of this partnership is to use a multidisciplinary team of engineers, psychologists, and rehabilitation professionals to determine ways to enhance the accessibility of complex intersections for pedestrians who are blind and visually impaired. The pedestrian travel environment has become increasingly complex as transportation engineers have designed roadways to carry more traffic in less time. Wide arterial roads, actuated signalization, continuous flow designs such as slip lanes and roundabouts, and irregular intersection geometries are examples of intersection features that have made street crossings more challenging for pedestrians. The research of the partnership focuses on improving access to the information that is necessary to negotiate intersections with features such as these, with an emphasis on information access by people who are blind and visually impaired.

The Western Michigan University team is continuing to lead the research program on pedestrian access at roundabout intersections. They are currently evaluating blind individuals' use of hearing to detect gaps in traffic at roundabouts, as well as the frequency with which drivers yield to long cane and dog guide users standing at roundabout crosswalks. The WMU team also is refining the prototype anti-veering training device that was developed several years ago at WMU. This device provides feedback to blind users about their amount and direction of veer as they walk. Its usefulness as a training tool will be evaluated in subsequent project years.

Minimizing veering is particularly important when crossing very wide streets. Beginning in Project Year 2, the WMU team will collaborate with the Boston College team to investigate various aspects of the design and installation of underfoot detectable warnings.

The BRP team at Vanderbilt University's Bill Wilkerson Speech and Hearing Center is investigating how well individuals can locate moving sound sources and determine the trajectory of sounds. The research is addressing the effect of variables such as the height of a sound from the ground, and its frequency, intensity and directionality, on sound localization. This line of research is relevant to our understanding of the use of hearing to monitor traffic at intersections, and ultimately to the development of effective simulations of situations that pedestrians may encounter.

Much of the work at Vanderbilt will complement research to be conducted by the team at Boston College, which is focusing on determining optimal signal characteristics for accessible pedestrian signals. This research will lead to ways to improve performance on tasks such as determining which crosswalk has the walk signal, establishing initial alignment for a street crossing and reducing veering during a crossing. Conducting laboratory and complementary "on the street" research is one of the hallmarks of this bioengineering research partnership.

The team at the Maryland School for the Blind is working collaboratively with researchers at the Wilmer Eye Institute of John Hopkins University. They are investigating the eye gaze and eye movement strategies of pedestrians with low vision as they negotiate complex intersections. This research is made possible by recent advances in eye tracking technologies that allows researchers to investigate factors such as one's direction and duration of gaze during travel. Ultimately, the research will help guide the development of training strategies and environmental interventions that improve the accessibility of complex intersections for people with low vision.

The work of each of the teams is supported by a group of researchers at the University of North Carolina's Highway Safety Research Center. This team aids in developing collaborative relationships with traffic engineers throughout the US, and will assist in developing and disseminating results of the partnership's work to transportation engineers and policy makers.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Each research team is on schedule to achieve its goals for Project Year 1. The teams supported each other in designing the Year 1 research activities and in sharing data collection and analysis resources. The teams also have taken advantage of opportunities to pursue pedestrian research opportunities related to the BRP activities. A technical bulletin on access to roundabouts was written this year by BRP staff and will be published by the US Access Board. Presentations on BRP-related research were given at conferences in the U.S. and in Great Britain.

**ISSUES:**

We are interested in discussing the evaluation criteria for BRP's, and the extent to which other partnerships are using web and other technologies to ensure that research groups in various locations have an opportunity for frequent interaction in support of the BRP's research goals. We also continue to be interested in strategies for increasing interaction among the partners.

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**PROJECT TITLE:** Morphological and Functional Musculo-skeletal Imaging

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**GRANTING NIH INSTITUTE/CENTER:** National Institute of Aging

**ABSTRACT:**

In response to the announcement, PA number: PAS-00-006, participants from the University of California San Francisco (UCSF), Lawrence Berkeley National Laboratories (LBNL) and Industry (Focus Imaging, Exponent Failure Analysis, General Electric) propose to form a Bioengineering Research Partnership (BRP) focussed on the systematic study of the morphology and function of the musculoskeletal system in disease and health. In addition, resources from existing research relationships with General Electric Medical Systems, SUN computers and IBM will be combined and utilized to rapidly evaulate and disseminate the developments of the BRP. The aim of this consortium is to improve medical care through bioengineering developments, and to facilitate close interactions between bioengineers, computer scientists, clinical investigators, basic scientists and corporate partners. This effort will expedite the development of clinically-relevant quantitative imaging tools and propel the technical advances from the laboratories into the operating rooms and clinics. We hypothesize that high resolution, fast magnetic resonance imaging techniques and positron emission tomography, combined with quantitative image analysis, processing and visualization, can provide new insights and clinically viable and relevant methods for objective evaluation of disorders of the musculo-skeletal system. The long-term objective of this partnership is to understand the link between morphology, function, biochemical changes and clinical symptoms in the musculo-skeletal system. An immediate objective is to develop, implement and optimize novel non-invasive imaging methods (magnetic resonance imaging: MRI and positron emission tomography: PET) that will allow us to depict the musculo-skeletal system, quantitate morphology, function, provide unique 3D visualization and graphical representations of function and morphology, as well as correlate these with biochemistry and c linical status. This research partnership is aimed at quantitating early degenerative changes in two clinical areas of emphasis: the knee and the spine. The first phase of the partnership will be technique development, followed by testing, and ultimately evaluation in case control studies in symptomatic patient populations. The specific goals are: (i) *to develop quantitative morphological and functional markers for degenerative diseases of the spine, (ii) to develop quantitative morphological and functional markers for the degenerative changes in the knee and osteoarthritis.* The focus of this partnership is consistent with the mission of the National Institute of Aging, and National Institute of Arthritis and Musculoskeletal Diseases.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The partnership has been established and initial interaction between the sites have been initiated. A web page has been set up, experiments have been planned, some results are already available.

**ISSUES:**

The primary issue has been the communication between institutions, not at the investigator level, but at the administrator level. This is a new concept, often with subcontracts etc. which takes a long time to get in place as it works through many large institutions.

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**PROJECT TITLE:** Magnetically Suspended Rotor Blood Pump, 1 RO1 HL64378-01

**PARTNERS' NAMES AND AFFILIATIONS:**
Utah Artificial Heart Institute
MedQuest Products, Inc.
University of Virginia

**GRANTING NIH INSTITUTE/CENTER:** National Heart, Lung, and Blood Institute

**ABSTRACT:**

The objective of this effort is to develop a novel ventricular assist device for patients suffering from congestive heart failure (CHF). The system will differ from current technology as follows:

1. A totally magnetically suspended impeller will be developed to eliminate contact bearings
2. A responsive physiologic controller will be developed to match system output to patient need without the use of sensors

This system will provide the following benefits to CHF patients:

1. Significantly improved system reliability and durability relative to other devices in clinical use or in advanced development
2. Significantly improved patient quality of life by using the unique characteristics of the magnetic bearings to provide system output based on physiologic needs
3. Anticoagulation needs will be reduced, lowering the post-operative cost to the patient and reducing the likelihood of anticoagulation-related complications

The following specific objectives will be met:

1. A completely implantable continuous flow VAD configured for human use will be ready for transition to manufacturing
2. The system will include transcutaneous energy transfer (TET) system, batteries, and sealed implantable controller housing
3. One year in vitro reliability testing on complete pre-production systems will be completed
4. At least 6 complete system animal implants with a minimum 60 day duration will be completed
5. The pre-clinical readiness Design History File and Design Review will be completed
6. A partnership with a commercial funding partner will be in place to prepare an Investigational Device Exemption (IDE) application, manufacture the system, and perform clinical evaluation

The continuous flow ventricular assist device (CFVAD) will be intended initially as a bridge to transplant, but the reliability characteristics will ultimately facilitate development of a longer term bridge to recovery and/or permanent device. By successfully completing this project, we can provide a superior treatment option at a lower cost for those who suffer from CHF. Developing novel means of addressing reliability and physiologic control issues will significantly improve the state of the art in circulatory devices, stimulating new development in acute circulatory support devices and in acute and chronic total artificial heart applications.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Two new partners have been added to the project team, Magnetic Moments, LLC (Goleta, CA) and Antakamatics, Inc (Pittsburgh, PA). Both partners are working under subcontract to MedQuest Products and are former developers of a competing maglev blood pump. Magnetic Moments is responsible for magnetic suspension design, development, and fabrication and Anatakamatics is responsible for flow path design optimization.

**ISSUES:**

Progress: Feasibility studies performed early in the project demonstrated a need to simplify the system design to meet the development schedule goals and to facilitate manufacturing. Prototypes were built based on a multidisciplinary design optimization model and bench testing and 2 animal implants have been performed. The next step is to further optimize and then freeze the design in preparation for validation and pre-IDE testing. We intend to freeze the design in late 2001 and begin clinical trials in 2002.

Simulation and Modeling: A system redesign was performed based on establishing system specifications and extensive system modeling and simulation. This optimization model considered magnetic suspension, fluid dynamics, and motor performance theory, enabling rapid evaluation of numerous design configurations. Intermediate analyses elucidated relationships between design parameters and performance, and helped the designers to balance tradeoffs, adjusting system constraints and objective weights. By combining localized optimization with a global scheme, the program furnished optimal dimensions, operating parameters and design parameters that were translated into component solid models and drawings. Comparing the prototype performance to that predicted allowed us to refine our simulation model. The value of the performance simulation modeling vs the traditional design-build-test-modify cycle was demonstrated by the rapid (9 month) progression from concept to prototype and the degree to which the first prototype met the design specifications.

Parallel Effort: This project has a clear objective of reducing this technology to practice in the form of a commercial device for clinical application to maximize patient benefit. It was recognized at the outset that the traditional serial research, product development, manufacturing development, quality, regulatory, and marketing steps would result in an unacceptable time to clinical application and was subject to product shortcomings due to the lack of early input from each of these functional areas. Further, it was recognized that world-class performance was required in each of those disciplines. Rather than accept a longer timeline and convert researchers to product developers and then to each subsequent discipline, we have chosen to assemble a complete, highly qualified and experienced team early in the development process and develop the complete clinical package as a parallel effort. We expect that this will drastically decrease our time to clinical trials.

Additional Funding: The funding provided by this grant was recognized to be less than half of the total investment required to bring this technology to clinical trials. MedQuest Products committed to obtaining additional funding as needed. The credibility bestowed by NIH recognition via BRP funding was a critical success factor in our effort to secure additional funding for the system development. Approximately $7 million in additional private funds have been committed.

Project Management: Management of a multi-center, multi-discipline partnership of academic, non-profit, and for profit organizations to develop a medical device for ultimate commercialization continues to be a challenge. We regularly encounter issues not normally addressed in purely commercial or purely academic collaborations such as the following:

- Changing roles of partner organizations
- Understanding and complying with new and changing regulatory demands
- Academic goals and standards vs. for-profit business goals and expectations

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**PROJECT TITLE:** Engineering Approaches to Low Vision Rehabilitation

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**GRANTING NIH INSTITUTE/CENTER:** National Eye Institute

**ABSTRACT:**
This project applies novel engineering approaches to the problems of low vision rehabilitation. We are building prototype devices based on solid theoretical foundations that, eventually, will become marketable rehabilitation products. The devices, designed and built with the help of our engineering partners, will be tested critically using diverse patient populations, with the help of the clinical partners to determine the effects on function and on the quality of life.

We shall develop and test both optical and electronic devices that implement three specific engineering approaches aimed at restoring (at least in part) the important interplay of central (high-resolution) and peripheral (wide-field) vision. The three engineering approaches that we will explore are multiplexing, dynamic control of display, and image enhancement. Also, we will show that various combinations of these approaches are possible and likely to be beneficial. In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory, and on-the-street evaluation for real-life determination of the effect and usefulness of the devices and techniques.

**STATUS OF RESEARCH AND PARTNERSHIP:**
The project has two major components: device development and device evaluation. Both components have commenced. Work has commenced on the three mobility aids: a paper describing the development and clinical use of one of the novel visual aids, the peripheral prisms for hemianopia, has been published; pilot studies have commenced on the other optical device, the trifield for restricted peripheral visual field and a local manufacturer has

made a sample device; and a prototype of the electronic device, augmented vision for monocular restricted peripheral visual field, has been tested. The zoom-and-roam device, which will be used to enhance television viewing by people with central visual field loss, has been delivered

Our Project Manager is meeting with all team members and Partners as he develops his project over-sight system. This includes critical path identification and appointment of a single responsible person for each sub-project. Weekly written reporting has been implemented at SERI and we will request that Partners provide regular written reports in the near future. We have a prototype web-based system for communication, information exchange and bookings (subjects, research space and personnel). The booking system, available to all Partners, is an essential feature as the device evaluation scheduling is complex involving carefully sequenced subject visits at more than one center and shared facilities and equipment. Systems have been implemented for document and software revision control with controlled sharing and access.

The most complex task at SERI, the development of a novel virtual-reality system that can monitor position of gaze, has begun. Most of the research for the most appropriate equipment is completed and orders have been placed. Software development has begun at SERI and one Partner. Weekly meetings of developers and users will begin son and will push this important project forward.

**ISSUES:**

Not all Partners have yet become engaged in the project so far. One device development Partner cannot conduct work on the project until we have completed certain work (algorithm development) at SERI. That Partner will need to reassign existing personnel to meet our needs. One device evaluation Partner is behind schedule with constructing a national, virtual-reality facility (National Advanced Driving Simulator). Their latest expected opening date is March 2001. For the next three months this is not a problem as we are not ready to initiate our work on their system. In case their system is not ready by the time we need to begin, we are investigating alternatives. We would have to involve a new Partner as the project budget does not include sufficient resources to develop a virtual-reality system with sufficient capabilities on our own. We have had very preliminary discussions with the owners of potential systems.

Three of the device evaluation studies involve driving on (local) open roads under carefully controlled conditions. Since one group of subjects cannot hold a driving license in Massachusetts, we are involved in negotiations with the Registry of Motor Vehicles. Alternatives for that study include a closed-road course or involving a new Partner in The Netherlands.

One of the problems that we have encountered is that the reductions in the proposed budget made by the NIH, that primarily reduced personnel at our main site (SERI), have left us with manpower that will be insufficient to complete aspects of the project in a timely manner. This will impact on our Partners as the project progresses. A positive note is that the interaction on this project with one device development Partner has led to work on an associated research proposal.

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**PROJECT TITLE:** Engineered Cardiac Morphogenesis: Stem Cells & Scaffolds

**PARTNERS' NAMES AND AFFILIATIONS:**
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Hope Heart Institute, Seattle, WA
University of Toronto, Toronto, Ontario CANADA

**GRANTING NIH INSTITUTE/CENTER:** National Heart, Lung and Blood Institute

**ABSTRACT:**
The long term aims of this project are to produce tissue engineered ventricular wall patches for myocardial repair, ventricular assist devices, and eventually replacement ventricles. Our team from academia and industry has expertise in biomaterials, bioreactors, tissue biomechanics, embryonic and somatic stem cells, muscle development, vasculogenesis, extracellular matrix, cardiac injury and regeneration, animal and human heart transplantation. This team will collaborate across three research foci: **1) "Instructive" tissue scaffolds**. Advanced biomaterial fabrication will be used to engineer biodegradable matrices and meshes with controlled pore dimensions, modified with receptor specific molecules. Matrices will be optimized to instruct cell attachment, orientation, migration, proliferation, differentiation, and overall tissue organization. **2) Cell and developmental biology**. Primary and stem cell-derived muscle and vascular cells will be studied on modified scaffolds to determine the optimal conditions for producing functional muscle tissue and vascular networks. Engineered tissues will be subjected to mechanical stresses to direct maturation toward *in vivo* phenotypes. Bioreactors will be developed to implement these requirements on a useful scale. **3) Clinical science and animal models**. Contractile ventricular patches will be tested in an injured heart model. Integration with host tissue and restoration of contractile function will be evaluated. A tubular cardiac assist organ comprised of vascularized myocardium and endocardium will also be developed. The "tube hearts" will be conditioned in pulsatile flow circuits, assessed for mechanical performance in vitro, and eventually grafted into aortas of syngeneic rats for in vivo evaluation. Progress toward these goals should establish design principles necessary for constructing more complex ventricular devices

**STATUS OF RESEARCH AND PARTNERSHIP:**
This BRP comprises six major laboratories at the University of Washington, two at the Hope Heart Institute, one at the University of Toronto, and laboratories at Advanced Tissue Sciences as well as collaborations with Advanced Polymer Systems. The program is currently in its first year of funding.
Status of the Partnership: The partnership is active and robust. The UW laboratories are highly interdisciplinary and include units from both the College of Engineering and the School of Medicine. The participation of the Hope Heart Institute in this program has been enthusiastic. The partners from Toronto, San Diego, and San Jose have willingly expended the time, effort and money to travel to Seattle for several strategic planning meetings and research. Five extensive plenary meetings have been held to strengthen the research interactions between the major laboratories and to unify research objectives.
Status of the Research: The project has three broad concerns which support the overall goal of achieving a tissue engineer: advanced tissue engineering scaffolds, basic research in cell and developmental biology, and animal/clinical models. Within these three area are ten specific aims. For the most part, work in this first year has concentrated on specific aims dealing with materials, and with cell biology. The animal model work is just now beginning.
Three-dimensional interconnected porous structures with defined (5μm and 20μm) pore size based on poly (HEMA) have been synthesized. These novel porous materials have progressed to various in vitro cell seeding and toxicity assays as well as preliminary in vivo testing. Initial work with a novel degradable polyurethanes (PEUs) provided to this project under subcontract from the U. of Toronto is promising; we are able to spin meshes and fibers from this material, and it too has progressed to in vitro cell experiments. (Spec. Aim#1: novel scaffolds).
Preliminary data included at the time of application demonstrated our ability to pattern cells in two dimensions via the microcontact printing of various adhesive proteins. This work has progressed under this project to include a variety of adhesive proteins and substrates, including PLGA and the degradable PEUs described above. This work has been submitted for publication. Further, strategies and experiments to approach pre-organized co-cultures of

cardiomyocytes with endothelial and other vascular cells are underway. For example we are using time-lapse microscopy to follow the utility and fate of Type I collagen in such patterned endothelial cell cultures, to assess tendencies for three dimensional luminal formation. The patterned cell culture system is also used to study differentiation of embryonal P19 carcinoma cells.

Work has progressed on the modification of existing fiber spinning and electrospraying apparatus for use with the novel polyurethanes and other potential scaffold materials. This has been successful and micron diameter fibers have been formed, coated with the adhesive protein laminin, seeded with myocytes, and cultured to form engineered myofiber-like structures. Growth and differentiation of the myocytes are studied in this system under static conditions as well as under cyclic stretching load, to mimic cardiac contraction. Such fibers may be amenable to assembly in a hierarchical manner, possibly with cultures of additional cell types, to form three dimensional myofiber, myofibril, and ultimately muscle fiber-like structures.

We have started studies to investigate the attachment of rat primary cardiomyocytes to several of the porous mesh structures described above. Preliminary results indicate attachment – but these studies have highlighted technical challenges in embedding and sectioning, which are being addressed. Control and stimulation of cell proliferation will be important in our construct. We have investigated the control of skeletal myoblast proliferation via the FGF receptor as follows. A chimera of the FGF receptor with the FKBP binding domain has yielded an FGFR that can be selectively dimerized (and thus turned "on") with the addition of a small, soluble synthetic FKBP ligand. Transfection of the chimera into skeletal myoblasts was successful and stable. Addition of the ligand mimics engagement of the FGFR and results in stimulated proliferation, inhibited differentiation, and activation of the MAP kinase pathway. We also have developed a system for generation of cardiomyocytes from mouse ES cells and continue investigating techniques for guided differentiation in this system. We have developed a screening system to test human mesenchymal stem cells (marrow) for cardiac differentiation markers. This work supports Spec Aim #3,4 (response of cells to scaffolds)

Work done with mostly skeletal muscle cells includes the following: Growth and differentiation studies were conducted on patterned laminin lines of varying width and spacing. Time lapse videomicroscopy was used on these same patterns to study cell migration. Cells were seeded onto laminin coated microfibers, and cell growth and differentiation studied. Myoblast penetration into porous PLA was evaluated. Permanent cell lines of mouse myoblasts carrying the Lac-Z and GFP markers were constructed for use in future co-culture experiments with connective tissue and vascular cells. Methods (BMP2, FGF4) to induce P19 embryonal carcinoma cells to commit to a cardiogenic lineage were developed. This process is currently being analyzed with respect to gene expression. Methods (endothelin) for inducing chick embryonic cardiomyocytes to commit to a Purkinjie cell lineage have been successful and this method is now being tested with cardiomyocytes grown on micropatterned substrates. Cardiac specific promoter / GFP constructs are being made and tested for use in FACS sorting of cardiogenic cells derived from stem cell populations.

In our focus on vasculogenesis, we have developed a Boyden Chamber-like migration assay to study the response of bovine aortic endothelial cells to various chemotractants, e.g. VEGF, through a porous membrane structure. This assay now will be employed with the various engineered porous scaffolds.

In our focus on extracellular matrix, we have studied the production of extracellular matrix by myocytes cultured on scaffolds by staining for type IV collagen, laminin, and perlecan in cultures of myocytes on the micron diameter fiber system (above), the patterned surfaces (above) and other porous scaffolds.

In effort to provide cell signals, we have utilized a modified AAV vector to explore transduction in endothelial cells. This represents an expansion of Aim#2 with the goal of providing growth factors or survival signals to cells in situ (eventually either in a bioreactor or in the implanted construct itself.). The AAV approach demonstrates much improved transfection efficiency and promises reduced immunogenicity. These experiments will be extended to myocytes, with VEGF-165 and/or hemoxygenase-1 as genes for delivery.

**ISSUES:**

Most of the challenges of the program have involved the normal issues associated with starting and administering a large inter-institutional research program. Mechanisms for dealing with intellectual property exist, but are cumbersome and drawn out. The high visibility of the program led to significant interactions with the lay media. The travel cost to ensure a healthy and participatory interaction between the partners has been significant, and these costs have largely been borne from outside the NIH funding. Finally, although we propose no human species work at this time, we foresee the upcoming decisions on federal support of stem cell research having a significant impact on the work of our field in general.

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**PROJECT TITLE:** Polarization Sensitive Retinal Tomography for Glaucoma

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**GRANTING NIH INSTITUTE/CENTER**: National Eye Institute, glaucoma and lens/cataract

**ABSTRACT:**
We report results of a study using polarization sensitive optical coherence tomography (PSOCT) to measure physical properties of the retina and to create images of retinal microstructure. Our instrument incorporates a mode-locked Ti:$Al_2O_3$ laser and achromatic polarization optics to record high resolution images. High-resolution B scans (two–dimensional images) of the in-vivo rhesus monkey retina have been recorded in the optic disk, peripapillary area and macula. Images of the peripapillary area allow measurement of the retinal nerve fiber layer (RNFL) thickness and calculation of the Stokes parameters of light back-scattered from the retina. Results of our study indicate: 1) PSOCT may be utilized to measure RNFL thickness; 2) PSOCT may be used to measure areas of birefringent tissue in the retina; and 3) selection of a scan pattern surrounding the optic nerve should account for the relatively large radial RNFL thickness gradient. Moreover, since glaucoma manifests in a destruction of the RNFL, PSOCT may be useful as a screening and diagnostic modality.

Figures 1a and 1b show images of two Stokes parameters (I and Q) of a segment of primate retina superior to the optic nerve across the superior peripapillary bundle. The scan was recorded temporal to nasal. Figure 1a is an intensity image of light back reflected from the retina. Figure 1b is the Q parameter. .

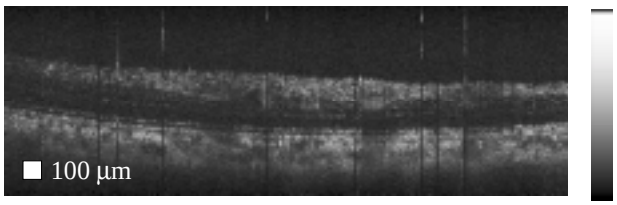


Figure 1a: I PSOCT Image of RNFL. Units are dB.

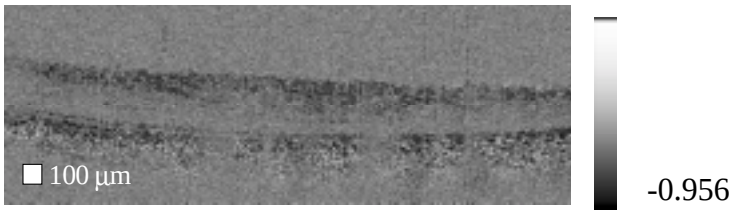


Figure 1b: Q PSOCT Image of RNFL. Units are ratio of 45 degree to –45 degree polarization state of light.

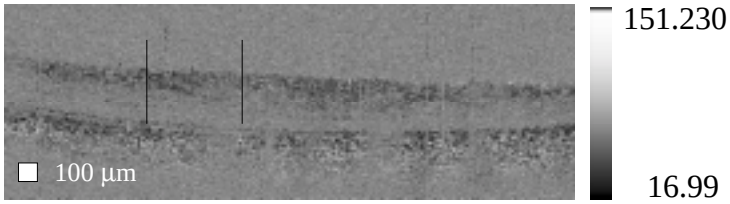


Figure 1c: Phi PSOCT Image of RNFL. Units are degrees

Measuring the average phase retardation between the anterior and posterior boundaries of the RNFL in the region indicated by the vertical bars in Figure 1c gives a value of $\phi_2$-$\phi_1$ = 22.55°. Using this value it is possible to calculate the birefringence for the RNFL layer using the values $d_{opt}$ = 180 microns, n = 1.36 and $\lambda_0$ = 845nm. These values give $\Delta n_{eff}$ = 1.995 * $10^{-4}$ .

The long term objective of our research is to obtain serial PSOCT images over time of the peripapillary retina to determine if the depth-resolved birefringence of the RNFL changes as glaucoma progresses. It is well documented that the thickness of the RNFL decreases in patients with glaucoma but it is not known whether birefringence or RNFL thickness is the more sensitive measure of glaucoma progression. Only a controlled analysis of many serial PSOCT images obtained simultaneously during the progression of the disease can answer that question. Any suggestions about the design of the animal studies would be helpful.

The research group at UT Austin is responsible for the basic science research which is shared by all members of the BRP partnership. Specifically, the UT Austin team is building three instruments for animal research: a high resolution PSOCT system, a very fast OCT system built with active pixel array detectors from EPFL, and a low-coherence optical reflectometer for functional neural imaging.

Dr. Johannes DeBoer recently moved from Beckman MLI to MIT/Harvard. He is responsible for implementing the clinical PSOCT instrument. He is collaborating with both Coherent and Humphrey-Zeiss. His move will require some reorganization of his clinical research team.

EPFL sent the active pixel array detectors to us on time and the detectors are being used by the UT Austin group and the MIT/Harvard group.

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**PROJECT TITLE:** Brain Prostheses: Tissue Compatibility & Integration

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**GRANTING NIH INSTITUTE/CENTER:** National Institute of Neurological Disorders and Stroke

**ABSTRACT:**
Nanofabricated prosthetics provide tremendous potential for furthering our understanding of central nervous system (CNS) function and treating CNS disease and injury. Such devices will permit precise localization and control of neuron function. Presently the success of these devices is limited by reactive biological responses. Our hypothesis is that successful performance of prostheses requires development of biologically interactive devices that will promote stable incorporation into the CNS permitting development and maintenance of low resistance connections with surrounding neurons. Our previous findings have lead us to further hypothesize that there are two discrete reactive responses — an early response associated with injury following prosthesis insertion and a prolonged response continuously promoted by tissue-prosthesis interactions. The principal goals of this proposal are to build upon this work, identify cellular processes responsible for reactive responses, and advance strategies to enhance biocompatibility by including drug delivery to control reactive responses. We will use rat and mouse models to study reactive responses in three-dimensional tissue samples permitting cell identification, descriptions of changes in cell-cell and cell-prosthesis interaction, and characterizations of the synthesis and deposition of cell products. Prostheses will be made using nanofabrication techniques, surfaces will be modified by chemical, biochemical, and physical (topographic) methods. Pharmacological intervention will be tested by systemic application as well as incorporating microfluidic elements into prosthetic devices. Results from these experiments will provide important new information for the intelligent design of improved biomaterials and micro-devices to control dynamic biological events in the CNS and insure the successful long-term performance of neural prostheses.

**STATUS OF RESEARCH AND PARTNERSHIP:** ongoing

**ISSUES:** none

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**PROJECT TITLE:** 7 TMR Drug Discovery, Microfluidics & HT Flow Cytometry

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Assistant Professor of Cell Biology and Physiology, UNMHSC

Co-PI, Hy D. Tran, PhD
Asst. Prof. Mechanical and Electrical Engineering, UNM

Co-PI, Andrea Mamoli, PhD
Asst. Prof. Mechanical and Electrical Engineering, UNM

**ABSTRACT:**

High throughput (HT) screening is integral to drug discovery. While flow cytometry is known for its ability to measure cell responses, its power in the homogeneous analysis of ligand binding or molecular assembly and its potential for high throughput are not well-recognized. The possibility of displaying virtually any molecule in a format compatible with particle-based analysis as well as the novel approach of plug-flow flow cytometry for sampling times ~1 sec could make flow cytometry a powerful alternative for the real-time analysis of molecular interactions. Thus, we propose four projects that bring together expertise in bioengineering and biomaterials, receptors and cell biology, and flow cytometry instrumentation. The first two projects concern biomaterials. In the first project, we propose to express the proteins relevant to signal transduction and termination (seven trans-membrane receptors – 7TMR, receptor tails, G protein sub-units, arrestins, and receptor kinases) in forms appropriate for flow cytometry. These proteins will have epitope tags suitable for homogeneous attachment to beads as well as fluorescent groups suitable for detection by conventional flow cytometry. In the second project, we will employ biomaterial display and detection strategies compatible with flow cytometric analysis. Beads will be used as platforms to display the molecules, to analyze molecular assemblies, to examine enzymatic activities, and to examine inhibition by combinatorial drug libraries. Projects 3 and 4 will involve instrumentation development, fluidics, micro-machines, and automation. In the third project, we will develop fluid handling approaches for cells and beads. We will target throughput rates of 1 sample per second, or near the industrial standard of 100,000 samples per day, using commercial fluid handling components for the types of assays described in Projects 1 and 2. In the fourth project, we will develop and implement micro-fluidic sample handling approaches compatible with flow cytometry

using novel elastomer-based micromachine technology. We have set a goal of 10 samples per second or 864,000 samples per day, exceeding the industrial throughput standard by nearly an order of magnitude. By integrating bioengineering, biomaterial, molecular, cellular and flow cytometric expertise, we expect to develop test platforms for high throughput analysis of molecular interactions with commercial potential in drug development. The resulting technological advances will allow us at the same time to define mechanistic details of cell activation through 7TMR mediated pathways.

**STATUS OF RESEARCH AND PARTNERSHIP:**

We have made considerable progress in generating biomaterials and integrating these materials into high throughput assays bead-based assays (see Figure 1). We are now at a stage where we can begin to study the molecular assemblies in these assays in three areas of applications: molecular mechanisms, drug discovery with combinatorial libraries, and proteomics. We have devised high throughput flow cytometry sample handling as proposed (see Figure 2), and devised on-line micro-mixing approaches for HTS. For our prototype instrumentation to be most useful, we now need to proceed into the arena of instrument control and integration. This would allow communication between the flow cytometer and sample handling system for longer periods of unattended operation. It is fair to say that we have in place a consortium of biomedical and bioengineering researchers developing complementary biology and instrumentation tools. While these tools for drug discovery presently focus on G-protein coupled, seven transmembrane receptors, they can be extended to other molecular interactions.

**ISSUES:**

Given the progress in our work, it has become necessary to consider their commercial impact. There are more than 10,000 flow cytometers worldwide in labs that span basic research, clinical diagnostics, and drug discovery. Thus it has become important for our institution to understand the legislation that governs Federal awards and to convey to industry the relevance of that legislation. The types of interactions that we have with industrial partners have been collaborations, licensing agreements, and research contracts, with funds from both NIH and industry. We have several different offices at UNM that work together to handle these relationships:1) the controller's office that deals with budgets for research contracts; 2) the UNM counsel who deals with the language of research contracts; and the Science and Technology Corporation and Patent Office that deals with intellectual property. So far, it has been very common to have our industrial partners want to license technology and at the same time to collaborate to move the technology to a platform that they can use or sell. The types of questions that come up routinely involve the following areas: 1) the rights to intellectual property which come from our environment where NIH grants develop technology and industrial partners support applications of such technology for proprietary instrumentation or biology: and 2) the implications of exclusive and non-exclusive licenses as they affect cost and access to the research community at large.

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**PROJECT TITLE:** Biomedical Applications of Electroactive Polymers

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**GRANTING NIH INSTITUTE/CENTER:** National Heart Lunge and Blood Institute

**ABSTRACT:**
The objective of the Bioengineering Research Partnership program is to refine materials and establish methods for application of electroactive polymers in prosthetics and interventional medical devices. Electroactive materials are materials that change shape when exposed to an electric field. They are attractive as actuators because of their high energy density – the amount of energy that can be imparted to a load for a given volume or mass of active material. Electroactive materials, chiefly the piezoceramics, have found important uses in a variety of industrial, consumer and military systems, as well as in ultrasonic transducers for medical imaging, flow measurement and therapy. The piezoceramics have not been successfully applied, however, as actuators in other medical devices.

A new class of electroactive polymers has recently been discovered which makes possible the development of devices using forms that would not previously have been practical. These materials remain flexible, can readily be formed into a variety of shapes, and provide much larger shape changes than do previously available materials.

Two target application areas have been chosen: (1) next-generation prosthetic blood pumps for treatment of end-stage heart disease, and (2) advanced instrumentation for minimally invasive surgery, particularly for use in confined spaces such as the thorax. These disparate applications share the need for very compact, efficient and uncomplicated means of actuation. Both suffer today from the need for bulky actuation mechanisms that must remain physically distinct from the parts which pump blood or manipulate tissue. The technology to be developed under this program will blur the lines between structure and actuator, leading to modes of therapy that are not currently available.

The Materials Research partner will work to optimize electroactive polymers, which have been developed thus far for military applications, for use in the target medical devices, and develop methods for fabrication of the required multilaminate actuator materials. As these materials are fundamentally different from the active materials of actuating mechanisms used by engineers in the past, the Mechanical Engineering partner will work to develop new design methodologies for use with the new materials. The Bioengineering partner will develop prototype devices to demonstrate the potential of the technology and lay the ground work for full development of new devices. Device development is staged so that simpler, proof-of-concept designs are built in the first two years, as optimized materials and more sophisticated design tools are being developed. By year five, devices will begin to demonstrate the full promise of the technology.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The program's first six months have proceeded as planned. In the surgical manipulator area, actuator materials have been produced for the first demonstration device, and machined parts are in process. Design of the second demonstration device is underway. The Mechanical Engineering partner has begun work as planned on finite element-driven design methods, with a focus on both blood pump actuation and instrument steering/manipulation applications. The Materials Science partner continues to refine fabrication processes for existing active polymers, while pursuing further improvements in actuation properties.

**ISSUES:**

No serious issues regarding the management or organization of the partnership have arisen. While electronic communication is obviously valuable, we have found it extremely useful to devote a full day to assessment of progress and technical problems, and we have begun to schedule these every 60 days. We believe that this is particularly important in a partnership, where input from each area of expertise can help keep work on track. We are developing an electronic repository of data and reference material for use by all of the partners, and would appreciate learning of other groups' experience with such efforts.

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**PROJECT TITLE:** The Design and Fabrication of Novel Micro-Instrument Platforms for Performing Genetic-based Analyses

**GRANTING NIH INSTITUTE/CENTER:**

**ABSTRACT:**

The focus of this Bioengineering Research Partnership (BRP) is to bring together a multi-disciplinary team to fabricate novel micro-instrument platforms targeted for detecting mutations in genes associated with certain cancers (colorectal cancer). Specifically, we will be developing microfluidic devices for PCR/LDR in collaboration with Prof. Francis Barany (Cornell Medical College), which can detect point mutations at a level of 1 mutant DNA in approximately 1000 normal DNAs. Our design approach is novel in that we will build modules that will carry out the following specific functions: (1) PCR amplification of the target genes (*K-ras* genes); (2) ligation detection reactions; (3) micro-capillary electrophoretic separation of the ligation reaction products; (4) addressable zipcode arrays for detection of ligation products; (5) synthetic preparation of ultra-bright near-IR fluorescent dyes and; (5) fabrication of fluorescence readout scanners composed of simple diode lasers and avalanche photodiodes. These modules can then be assembled to allow the automated processing tissue biopsy samples (obtained from Prof. Pat Paty, Sloane Kettering Memorial Cancer Institute) for screening *K-ras* mutations. The devices proposed in this application will be fabricated using high aspect ratio micromachining in plastics (polymethylmethacrylate, PMMA) via *LIGA* processing. Our expertise in *LIGA* and the extensive micromachining resources located on-site will allow us to make microstructures with exquisite dimensions (< 1 μm, lateral dimensions). In addition, the choice of substrate material in which we will be fabricating our devices offers a great deal of flexibility in both micromachining techniques (X-ray lithography, hot embossing, injection molding) as well as chemistries for immobilizing DNAs to the material, which is not obtainable using conventional glass-based microfluidic devices. For example, using PMMA as the substrate material for DNA micro-arrays, amide-type immobilization chemistries are used, which produces a bond that is very robust and stable toward most thermal and chemical denaturation steps used in hybridization-based assays. Also, PMMA can be used for electrophoretic analyses of DNAs without the special requirement of wall coatings, extending the operational lifetime of the micro-device. And most importantly, PMMA can be fabricated using simple injection molding, which will allow the fabrication of devices at minimal costs and at high production rates (~ 5 min per device). The ability to effectively use these substrate materials is driven by the use of near-IR fluorescence readout, which alleviates high background signals arising from autofluorescence of the substrate or matrix interferences and is conducive to miniaturization. Since the ability to fabricate such devices depends on a number of different expertise, we have assembled a team of researchers (Chemists, Engineers, Life Scientists) that can meet the challenges of this diverse research venture.

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**PROJECT TITLE:** Linking Genomics to Function via Metabolic Phenotyping

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**GRANTING NIH INSTITUTE/CENTER:** National Institute of Diabetes and Digestive and Kidney Diseases

**ABSTRACT:**
Recombinant strains with well defined genetic backgrounds are often found to exhibit small functional differences despite specific changes at the genetic level while in other cases, single gene alterations result in profound phenotypic variations. Although a first step in explaining such macroscopic differences is to probe the full detail of the expression phenotype by genome-wide expression measurements, transcription data alone are insufficient to elucidate the actual metabolic state of a cell and its functions. The latter require information about intracellular metabolic fluxes, which constitute fundamental determinants of cell physiology and excellent metrics of cell function. "Metabolic phenotyping" is the process and methods of determining intracellular fluxes as determinants of the cellular metabolic state. Combined with transcription data, the investigators provide a complete framework for analyzing the effect of drugs and studying disease. This application integrates the expertise of three participating laboratories for the purpose of combining metabolic and expression phenotyping to elucidate central carbon and lipid metabolism in model mouse hepatoma and hepatocyte cultures. Determination of intracellular fluxes will follow a systems approach termed metabolic reconstruction whereby the entire metabolic network is configured such as to best represent macroscopic rate and isotopic label distribution measurements made by GC-MS. Of particular attention are issues of observability, redundancy, and solution stability to ensure method feasibility and accuracy of the results. Differential transcription data will be obtained by DNA microarrays for mouse genes involved in central carbon metabolic, gluconeogenic and lipid biosynthetic pathways, as well as for other genes with particular expression variability that will be identified in the course of the research. Bioinformatics methods and programs, developed over the past 12 years will be deployed for this purpose. The general goal of the research is to identify relationships between the metabolic phenotype as defined above and the transcriptional state as defined by expression data of consequence in pathways important to diabetes. Specific aims will focus on flux quantification in mouse hepatoma and hepatocyte cultures to elucidate glutamine metabolism and lipogenesis, other central metabolic pathways and cholesterol synthesis, the effect of nutrients, hormones and drugs like Metformin and finally, pleiotrophic effects generated by altering the normal expression of a single gene, such as over-expressing the truncated version of sterol-regulatory element binding protein-1a in transgenic mice. The broader contribution of this research is to extend the paradigm of holistic transcriptional investigation introduced by DNA microarray technologies to the study of metabolic level processes by metabolic phenotyping. As such, it holds the promise of identifying most, if not all points in metabolism affected by the action of drugs or genetic modifications thus guiding future programs of drug development and gene therapy.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Experimental work is currently focused on the first aim of the project: To quantify fluxes in mouse hepatoma central carbon and lipid metabolism and to determine the relationship between these fluxes and gene expression profiles. This aim illustrates the interaction of the three partners in the project. Dr. Kelleher is primarily responsible for coordinating mammalian metabolic flux studies. Dr. Gullans is focused on the development and validation of the cDNA microarrays for transcriptional analysis of mouse mRNA. Dr. Stephanopoulos is responsible for bioinformatics and integration of metabolic and flux data.

**ISSUES:**

Educational component. We have recruited 3 Ph.D. candidates in Chemical Engineering at MIT to participate in the project. These students bring strong engineering problem solving skills to biomedical research. The BRP projects represent an excellent environment for graduate and postdoctoral training. We suggest more attention to the educational aspects of the BRP. Perhaps the BRP members could establish a network for communications focused on improving bioengineering interdisciplinary Ph.D. education.

Structure of BRP in terms of PI status. The most significant aspect of the BRP is the "partnership between "biomedical scientists and engineers. These projects involve substantial efforts from each of the partners. Academic institutions place great weight on NIH PI status. To encourage the participation in the BRP, NIH should find a mechanism for funding these projects that grants PI status to more than one partner.

Role of Engineering in Biomedical Research. Traditionally, engineering has focused on equipment and device design, manufacturing and optimization. While these topics continue to occupy the attention of a significant fraction of the engineering profession., it should be noted that the defining features of engineering, namely, *integration* and *quantification*, are applicable to many different systems and environments that extend beyond devices and equipment. The above attributes have become very important in the post-genomic era, due to the increased emphasis on data handling, analysis and integration. However, meaningful data analysis requires intimate familiarity with the system and seamless participation of engineers in the design and interpretation of experiments, a situation that presently is the exception rather than the rule in biomedical research at the cellular and molecular level. BRP's should foster truly collaborative environments that extent the role of engineering beyond its traditional confines.

Data Driven Hypotheses: The great majority of research is presently driven by specific well-defined hypotheses. These hypotheses reflect all available knowledge on a particular topic that is the result of many years of painstaking research. The outcome of most research efforts is to extend the envelope of knowledge through the validation of such hypotheses and the occasional discovery of, until then unknown, new molecules, genes or enzymes. While this paradigm has served science well in the past, it needs to be amended to accommodate an emerging different approach to scientific research catalyzed by genomics and genomics-derived technologies. The spectrum spanned by the data generated by such technologies extends far more broadly than any specific hypothesis can possibly encompass. Yet, the analysis of such data can lead to specific hypotheses that would be impossible to formulate in the absence of these data. There is no doubt that the investigation of such hypothesis will accelerate the pace of scientific discovery, however, the absence of a hypothesis diminishes the prospect of funding of a research proposal. Efforts should be made to allow for research that is not based on a specific hypotheses but will help generate valid *testable* hypotheses form the base of data generated. Such d*ata driven hypotheses* is a most efficient mechanism to help us think out of the box.

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**PROJECT TITLE:** BRP directed at Muscular Dystrophies

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**GRANTING NIH INSTITUTE/CENTER:** NIAMS

**ABSTRACT:**

The goal of this BRP is to utilize a number of bioengineering approaches in developing understanding, tools, and therapeutics for the ultimate treatment and monitoring of muscular dystrophies. The project is a collaboration between three investigators and includes the following areas of bioengineering: 1) cell and tissue engineering, 2) imaging, and 3) therapeutics. Collectively we will dilneate factors that when expressed in muscle may slow the rate of degeneration that is concomitant with either complete (Duchenne muscular dystrophy) or partial (Becker muscular dystrophy) loss of dystrophin. Closely related factors, eg. other membrane proteins, will also be studied. At the single molecule level, we will study the adhesive and mechanical properties of dystrophin and related constructs and complexes. At the cell and animal level, we will utilize the *mdx* mouse as the model for dystrophin-deficiency with other transgenics and cell lines developed and used as needed. The long-term goal is to gain understanding and tools necessary to develop gene therapy (eg. AAV-based) for muscular dystrrophies, particularly Duchenne and Becker. Three parallel lines of investigation are being pursued: 1) dissection of the mechanical and adhesive contributions of dystrophin and muscle adhesion proteins by Dennis Discher; 2) assessment of the functional effects of restoring missing molecules to dystrophic muscle using recombinant gene delivery; and 3) development of non-invasive imaging methods for monitoring therapeutic benefits of gene transfer.

**STATUS OF RESEARCH AND PARTNERSHIP:**

Since Sept.2000 (start date), we have begun to make significant progress in all three areas of investigation. We have successfully developed novel cell-patterning methods that allow us to assess the adhesiveness of individual differentiated myocytes. Adhesive defects in dystrophic myocytes are being quantitated in terms of peeling rates under set forces. At the single molecule level, we have demonstrated an ability to measure molecular compliance and strengths using AFM (*PNAS* 98:1565, 2001) and are just beginning to express and AFM-probe new constructs relevant to therapeutics in dystrophies. Similar constructs, notably truncated dystrophins, are being expressed in mice and studied at several levels of function. In addition, with dystrophic gamma-sarcoglycan deficient mice, we have found T2 changes indicative of muscle damage, and shown that AAV-delivered constructs, locally-injected, can be shown by these non-invasive methods to correct defects. Systemic delivery and larger animal experiments are just beginning.

**ISSUES:**

No issues of immediate concern have arisen.

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**PROJECT TITLE:** Development of Q-dots as Biological Probes

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**GRANTING NIH INSTITUTE/CENTER:** NCRR (lead), NIGMS, NCI

**ABSTRACT:**

The long-term goal of this Bioengineering Research Partnerships is to develop semiconductor nanocrystals fluorescent probes (Q-dots) technology that will provide biomedical research with better tools for diagnosis of diseases and biomedical techniques and instrumentation necessary for basic research of cellular and molecular structure and fundamental life processes. This includes Q-dot probe synthesis, bio-conjugation techniques, dedicated optical instrumentation and unique imaging methodologies. We will develop optimized protocols for Q-dot synthesis with desired optical, physical and chemical properties. Various spectroscopic and structural measurements will be used to fully characterize Q-dots. This information will be fed back into the synthesis for optimization of the desired properties. Bio-conjugation schemes and labeling protocols will be developed for biomolecules in fixed and living cells.

The utility and the new possibilities opened-up by Q-dot technology will be demonstrated by studying protein trafficking and assembly in living cells and by physically mapping genes. The movements of secretory granule membranes during recycling will be tracked in living cells. Actin-based locomotion and mitotic spindle assembly will be imaged in real-time in cell-extracts. Molecular mechanism of synaptic transmitter release will be studied by following vesicle dynamics and protein trafficking in the synaptic apparatus. We will also physically map large number of distinct markers on chromosomes and combed DNA molecules and monitor the kinetics of chromosome pairing during meiotic prophase. All these demonstrations rely on the unique photophysical properties of q-dots, enabling new experiments and measurements to be performed and significant new biology to be revealed.

**STATUS OF RESEARCH AND PARTNERSHIP:**

*Optical instrumentation:*

Construction of two instruments have been completed: (1) Multi-color scanning stage confocal microscope and (2) Time correlated single photon counting scanning stage confocal microscope. These instruments were used for demonstrating multi-color imaging and time-gated detection of Q-dots in fixed cells. These works are described in two papers, one published, one submitted.

*Bioconjugation:*

We developed an improved silanization protocol and characterized the product by a verity of methods. This work is described in a submitted paper. An alternative coating chemistry is also being explored. Biotinilated Q-dots were produced and oligonucleotides were conjugated to Q-dots.

*Photophysics:*

Extensive photophysical studies based on simultaneous recording of photon pair correlations (antibunching) and time correlated single photon counting (fluorescence lifetime) are currently in progress. We anticipate that these studies will lead to better understanding of intermittency (blinking), photobrightening and photodegradation phenomena in Q-dots.

*Experiments:*

**ISSUES:**

Funding for this project started 8 month ago. Most of the work so far has been done in the PI's lab, concentrating on synthesis, stability, and photophysics of Q-dots. It is anticipated that the partners will start to work with Q-dots later this years. No special issues have been encountered so far.

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**PROJECT TITLE:** Integration and Visualization of Physiologic Data

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**GRANTING NIH INSTITUTE/CENTER:** National Heart Lunge and Blood Institute

**ABSTRACT:**
This project seeks to develop new displays for visually representing physiologic variables, to enhance a clinician's ability to see and rapidly respond to critical events. Unexpected incidents are common; anesthesiologists face them during 20 percent of all anesthetics. One quarter of these incidents pose significant danger to patients. Human factors research has shown that graphic displays improve an anesthesiologist's ability to detect and identify critical events. Groups which observed graphic displays saw changes 3.1 minutes sooner than those observing traditional displays, erroneous decisions were reduced from 4.1% to 1.4% and human response times (the time used to correct the problem) averaged 28 sec less.

The research plan is to develop a physiologic display that is comprehensive, interpretive, functional and integrative. The display is to provide a comprehensive view of the surgical patient's physiologic state. If some of the variables, which define that state and drive the display, are not being monitored, the display will use models to predict "population normal" values, thus completing the physiologic picture. The physiologic icons in the display will be organized to enhance the vision of the interrelationship between organ system functions. The display will highlight input/output relationships, by showing drug concentrations and accompanying physiologic changes. Simulated sounds, which accompany the display, will be data driven, to enhance the serial interpretation of monitored variables. The display design process will be iterative with five cycles of design, computer implementation, evaluation, critique, and redesign.

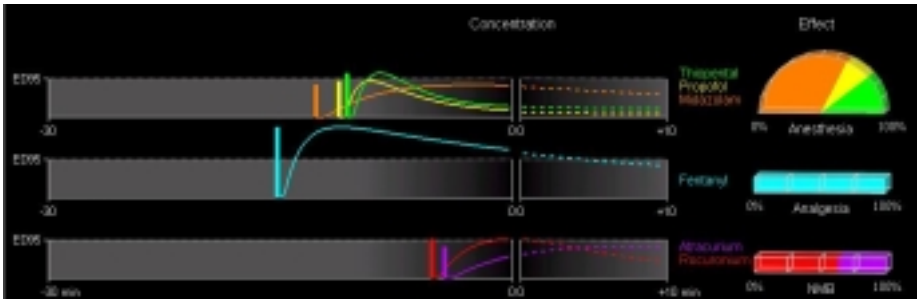
The display will be evaluated in a full-scale patient simulator environment. Twenty-four anesthesiologists will be asked to treat simulated patients in cases where 12 critical events occur. They will be asked to think aloud and treat the critical event so that four stages of situation awareness can be detected. At the conclusion of the six simulation scenarios, the anesthesiologist will be asked a set of 12 questions to assess their situation awareness. Each display will be evaluated with eye-tracking to identify the parts of the display that are most useful in decision-making.

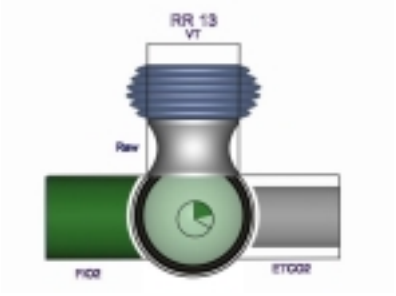
The anesthesiologist "sees" the patient on the operating table through the monitoring display. The proposed research will identify the type of display that best helps the anesthesiologists to rapidly identify the patient's physiologic state, to interpret the data and

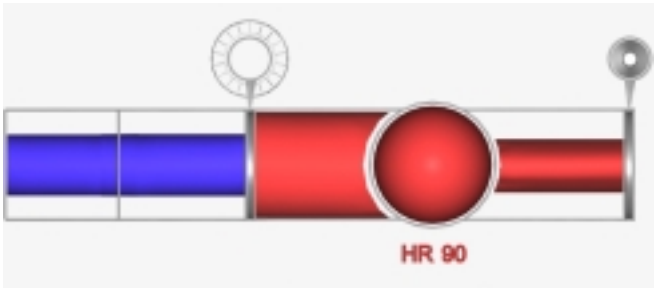
make accurate diagnostic decisions. The final display should enhance patient safety during anesthesia.

**STATUS OF RESEARCH AND PARTNERSHIP:**

The display design team has produced the displays shown in Figures 1-3. Figure 1 shows the anesthetic drugs administered during a surgical procedure and the effect these drugs have on the patient's state of anesthesia, analgesia and neuromuscular blockade. Figure 2 shows the patient's pulmonary physiologic state. Figure 3 is a mapping of the cardiovascular system variables.




The drug and cardiac physiologic displays have undergone extensive user evaluation. The elements of the displays are now "intuitive" to senior anesthesia staff. Residents understand the elements after a very short explanation. Thus, we have achieved the first goal of the project: to design a display that shows key physiologic variables in a fashion that is intuitive to practicing anesthesiologists.

When we compared the new display with the traditional display, medium level situation awareness was higher with the new display. During 63% of the simulated scenarios, reliable differences were found in favor of the new display.

The team members each bring their own perspective to the design process. Bioengineering members focus on quantitative measures, mathematical models and explanatory displays. Members from Human Factors focus on the functional nature of the display and its fit with the anesthesiologist's mental representation of the patient. Architecture focuses on the display's aesthetic properties and qualitative representations. The Bioengineering Partnership has resulted in a design that is intuitive and interesting to observe and one that rapidly conveys the most relevant information, because it is founded in principles from all three disciplines. When the display is introduced in the operating room, anesthesiologist's performance can be expected to improve, because of the interdisciplinary design efforts.