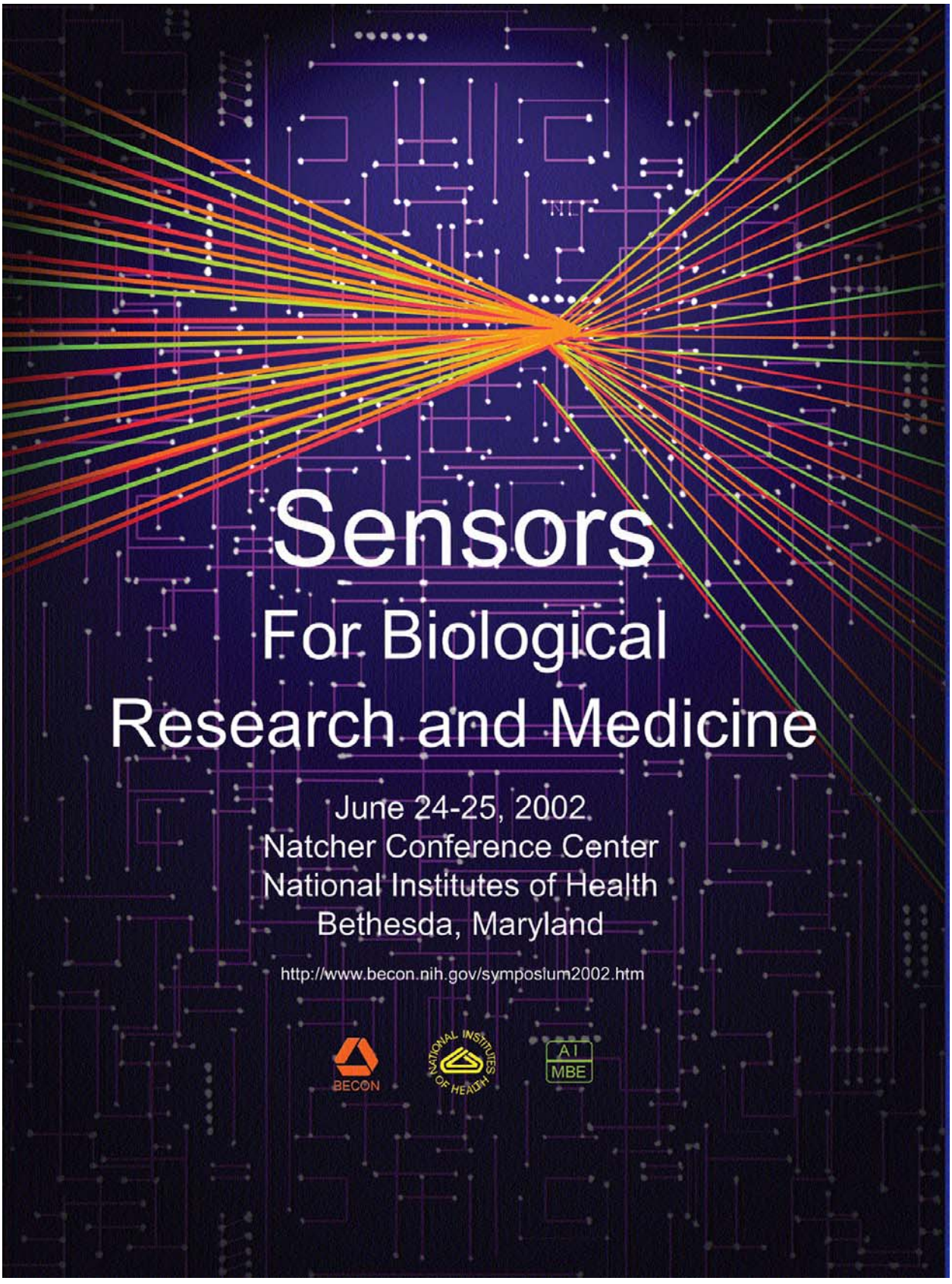
Sensors
For Biological
Research and Medicine
June 24-25, 2002
Natcher Conference Center
National Institutes of Health
Bethesda, Maryland
http://www.becon.nih.gov/symposium2002.htm
BECON
NATIONAL INSTITUTES OF HEALTH
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Sensors for Biological Research and Medicine

National Institutes of Health Bioengineering Consortium (BECON)

Nature Conference Center

June 24-25, 2002

Foreword

Biomedical sensors can be defined broadly as devices that detect specific molecules or biological processes and convert this information into a signal. Familiar sensors include blood pressure monitors and the glucose meters that are used to manage diabetes. The National Institutes of Health (NIH) has long been a leader in research of disease processes. With our expanding knowledge of the biological components of disease comes an urgent need for technologies that would allow us to detect them. In fact, we find ourselves at a very exciting time of rapid technological development in miniaturization, materials science, fluid mechanics, optics, and a host of other fields that should enable us to move our biological knowledge rapidly into the clinic to improve the health of the American public.

This merging of biology and technology has often brought about major medical advances, and NIH recognizes its responsibility to provide a forum where scientists from different fields can meet and exchange information. The symposium, "Sensors for Biological Research and Medicine" had nine plenary talks and ten breakout sessions to showcase important sensor technologies and examples of sensors being used in medicine and biological research, and to identify new applications. "Sensors for Biological Research and Medicine" was designed to provide a forum for biological and medical researchers to imagine new opportunities for using sensors in their own work, and where engineers, physicists, and materials scientists could discover new applications for their technologies. Panel discussions were used to explore the current needs for new sensors and potential solutions.

Recognition of the importance of engineering for the achievement of NIH's goals has resulted in several trans-NIH initiatives and has spurred the evaluation of how such applications are reviewed within the NIH system. BECON has promoted the use of Bioengineering Research Grants or Bioengineering Research Partnerships to support non-hypothesis, technology-driven projects.

Previous BECON symposia have helped shape the NIH research agenda and inspired collaborations between the participants. The three purposes of this symposium were:

- To provide a forum to showcase current biomedical sensor technology and applications, and to identify future biomedical needs and the emerging technologies that can meet them;

- To facilitate communication among physical and technical scientists, biomedical researchers, and clinicians interested in developing or applying sensor technology to research and medicine; and

- To provide advice to NIH concerning opportunities and needs in the field of biomedical sensors.

We are very grateful to the many people who worked to make this meeting a success. First to be thanked are the speakers and panel members who shared their exciting work and lead discussions to identify new opportunities in sensor technology and application. We want to especially thank the external advisors who met early in the planning process to define our goals, and to design a meeting built on the strengths of the NIH community. We were privileged with an excellent NIH organizing committee who spent a great deal of time, thought, and energy developing the program, identifying presenters, and designing our posters and Web site. We want to acknowledge the chairman of BECON, Dr. Jeffery A. Schloss of the National Human Genome Research Institute, who continues to support and expand bioengineering research at NIH in the tradition of the excellent leadership first provided by Dr. Wendy Baldwin, Deputy Director for Extramural Research at NIH.

External Co-Chairs

Warren S. Grundfest, M.D., F.A.C.S.
Milan Mrksich, Ph.D.

NIH Co-Chairs

Joan T. Harmon, Ph.D.
Maren R. Laughlin, Ph.D.

From left: Milan Mrksich, Joan Harmon, Ruth Kirschstein, Maren Laughlin and Warren Grundfest.

Contents

Executive Summary and Recommendations

The NIH's Bioengineering Consortium (BECON) held a two-day symposium titled "Sensors for Biological Research and Medicine" on June 24-25, 2002, at the Natcher Conference Center on the NIH Main Campus in Bethesda, Maryland. The conference was the fifth in a series of annual BECON symposia on emerging bioengineering topics, and attracted over 500 scientists, engineers, and clinicians. NIH Co-chairs for the symposium were Dr. Maren R. Laughlin of the National Institute of Diabetes and Digestive and Kidney Disease and Dr. Joan T. Harmon of the National Institute of Biomedical Imaging and Bioengineering. Extramural Co-chairs were Dr. Warren S. Grundfest of the University of California - Los Angeles (UCLA) and Dr. Milan Mrksich of the University of Chicago.

Goals

Specific goals of the meeting were

- To provide a forum to showcase current biomedical sensor technology and applications, and to identify future biomedical needs and the emerging technologies that can meet them;
- To facilitate communication among physical and technical scientists, biomedical researchers, and clinicians interested in developing or applying sensor technology to research and medicine; and
- To provide advice to NIH concerning opportunities and needs in the field of biomedical sensors.

Plenary Sessions

The conference consisted of a keynote address, eight plenary talks, ten topical breakout sessions, and scientific poster exhibits. Dr. Ruth L. Kirschstein, Deputy Director, welcomed participants on behalf of NIH. In his keynote address entitled "Sensors in Modern Medicine", Dr. John A. Parrish of Harvard University outlined the barriers and potential solutions to successful transfer of new technologies into medical practice, and discussed the paths to development of a new generation of sensors required for clinical practice in the future. The topics of the remaining talks were divided between examples of sensors used in research and medicine, new sensor technologies, and opportunities where new sensors are needed. Three speakers participated in a plenary session called "Critical Clinical Barriers to Diagnosis and Treatment of Disease". Lance Liotta of the National Cancer Institute discussed the use of protein microarrays to aid diagnosis by analyzing key signaling pathways in microdissected tissue biopsies from cancer patients. Allan J. Tobin of the University of California, Los Angeles discussed ways to measure neurotransmitters for neurobiology research and diagnosis of neurological disease, and Michele Follen from the University of Texas M.D. Anderson Cancer Center shared data demonstrating the use of optical spectroscopy for the in vivo diagnosis of cervical neoplasia. In a plenary session called "Biomedical Sensor Technology", Valentin Fuster of Mt.Sinai School of Medicine showed his work using MRI to study and characterize athero-thrombotic plaques in vivo. Jeffrey Borenstein of The Charles Stark Draper Laboratory showed how micro-electro-mechanical systems (MEMS) can be used to simultaneously and non-invasively detect a number of pathogens or disease markers in vivo, or in samples such as air, urine, saliva, blood and breath. George M. Whitesides of Harvard University discussed "New Tools for Bioanalysis", focusing on materials and fabrication methodology for engineering new surfaces

for cell growth and study. To showcase "Success in Sensor Technology", David R. Walt of Tufts University showed how coherent imaging fibers can be used to make fiber-optic chemical sensors for multianalyte determinations. Finally, Eric Rasmussen of the Center for Robot-Assisted Search and Rescue (CRASAR) gave a dramatic presentation called "Sensors in Today's World—Robotics in the World Trade Center Rescue" in which he showed how robots have been used to find and recover trapped people, and how sensors mounted on such robots could be used to monitor the environment and condition of disaster survivors, and aid in their rescue.

Breakout Sessions

Ten breakout sessions provided a forum for discussion between sensor technologists, physicians, and biologists regarding the state of the art of various aspects of sensors and their future applications in medicine and biological research. They identified the major barriers to advancement and opportunities for NIH to facilitate progress in sensor research. The specific topics were:

- Active disease management;
- Advanced technologies for biological and biomedical research;
- Diagnostic technologies;
- Informatics, validation, and computational applications;
- Technologies for predisposition;
- Biointerfaces and biomaterials;
- Biomedical microsystems, nanosystems, and integrated devices;
- Cell-based sensing;
- Emerging transduction technology; and
- Enabling concepts and materials for future biomedical sensor technology.

Research Funding Opportunities

The meeting closed with a plenary panel on "Federal Funding Opportunities for Sensor Research". Panel members representing several agencies and NIH Institutes and Centers presented their sensor needs and available research funding opportunities. The NIH was represented by Winnie K. Rossi, M.A. of the National Institute on Aging (NIA), Eleni Kousvelari, D.D.S., D.Sc. of the National Institute of Dental and Craniofacial Research (NIDCR), Maren R. Laughlin, Ph.D. of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Richard E. Swaja, Ph.D. of the National Institute of Biomedical Imaging and Bioengineering (NIBIB), and Gregory Milman, Ph.D. of the National Institute of Allergy and Infectious Diseases (NIAID). The U.S. Department of Energy (DOE) was represented by Dean Cole, Ph.D., the National Institute of Standards and Technology (NIST) was represented by Mrunal Chapekar, Ph.D., and the National Science Foundation (NSF) was represented by Sohi Rastegar, Ph.D. Finally, Jeffery A. Schloss, Ph.D. represented the NIH BECON committee.

Recommendations to NIH

Advice for NIH was collected from the speakers and panel chairmen. On the last day of the meeting, after most of the discussions and breakout sessions had taken place, this group of

advisors was asked to address how NIH can promote sensor technology and the use of new sensors in predisposition, diagnosis, and treatment of disease.

Sensor research is comprised of a wide array of technologies, some in their earliest infancy, others that are fairly mature and ready to be implemented, and others that are already in use. NIH has developed programs to support basic research in sensor technology. Now, NIH should also work to define the clinical problems that are most pressing, to actively recruit teams of engineers and biomedical researchers with specific expertise to work on these projects, to develop these sensors in the context in which they will be used, and to find ways to support movement of these devices down the developmental pipeline and into the clinical setting. The period of development between proof of concept and the market is particularly vulnerable because companies are reluctant to fund projects until clinical utility is proven. NIH should be aware that long periods of support may be needed: 5-10 years in some cases. In cases where there is little financial motivation for a company to develop a needed sensor product (e.g., third world diseases), the NIH should consider supporting the entire research and development effort.

Communication of clinical needs and disease biomarkers to the engineering community is an important role for NIH. Requests for applications and other initiatives can be used to alert engineers to a particular clinical need. However, these initiatives must be broadcast to these scientists in the publications they routinely read. Initiatives should be tailored for a specific clinical goal or technology, which are more likely than very general programs to result in responsive applications that can be appropriately reviewed and ranked. For instance, the resources, time, and risk associated with engineering chronic indwelling sensors are much greater than those associated with sensors meant for short-term use, making it difficult to rank these two types of projects. In addition, NIH should work to make sure that disease biomarkers (such as those discovered through large-scale projects that employ array technologies) are efficiently communicated to researchers working in sensor technology. In many instances, sensor technology that can be used to monitor these new disease markers exists or can be easily developed.

Finally, NIH should communicate to clinicians and clinical scientists the range of engineering capabilities available that are applicable to the clinical arena, and emphasize the need for both clinical/biological and engineering expertise when developing new sensors.

<u>Specific Opportunities for NIH</u>

- Interdisciplinary teams are essential and must be fostered from discovery to application;

- Strong collaborations between engineers, biomedical scientists and practicing clinicians are needed. These can be promoted by allowing students to trade environments; engineering students can spend time in the clinic, and medical trainees can spend time in engineering laboratories;

- Multi-year support mechanisms for students are needed to facilitate career development, to allow integration of thought processes, and to appreciate the knowledge to be gained from other disciplines;

- NIH should take on responsibility in translation of technology to the clinic or laboratory;

- Researchers working in sensor development need to be encouraged, even at early stages in their research, to use appropriate biological models. There should be awareness that the choice of a model can impact sensor function dramatically. In

particular, human subjects are be very different from animal models, and animal models are very different from isolated biological fluids, tissues or cells;

- NIH should encourage the utilization of complex mixtures, such as blood or saliva, in the design of a sensor will permit the measurement of chemical, biological and physical parameters;

- Encouragement should be given to field validation to ensure that a sensor works in the environments where it is needed (for rescue work, third world inaccessible populations, public health applications);

- The application of computer science to sensor research needs to be supported in areas of data acquisition, storage and analyses of dissimilar sets of data. Algorithm development, performance modeling, telehealth, and medical information systems are some additional areas that need further development;

- There are a number of ethical, legal and social issues related to the development and utilization of sensors. These issues include the security and transmittal of sensor information, an individual's privacy regarding insurance jeopardy and personal medical records, the ownership and accessibility of data, the data accuracy, the cost-benefit ratio especially with regard to underserved or remote populations, and population screening where it is essential that an individual's desire to avoid knowledge of present disease status or future disease potential is protected;

- New disease biomarkers are needed, followed by development of sensor technology to detect these new markers. NIH can aid this process by encouraging large-scale discovery projects for biomarkers (such as those that use array technologies). New disease markers or patterns of markers should be communicated to the sensor technology community so they can develop the needed sensors;

- NIH should encourage projects aimed at integrating available components into new sensors. This is often not considered innovative by review committees, yet is a vitally important activity that should be endorsed by the NIH. In order to promote this goal, NIH should alert study sections to the goals of the Institutes and Centers, especially in RFA reviews;

- NIH should encourage approaches that leverage existing technology platforms. This is particularly important for clinical and *in vivo* applications where regulatory issues often drive and limit the development of technology. By using existing (already FDA approved) technology platforms, the time to clinical use can be shortened;

- NIH should enhance support of sensor materials research. Specifically research is required to develop materials with short response times, with applicability to continuous measurement (resetting of sensor for multiple measurements), and with the ability to deliver drugs, sense environments, detect therapeutic efficacy and monitor physiology. Examples can be found among receptor-based, cell-based, and chemical-based materials;

- Non-invasive sensors should be developed by the application of imaging technologies. Imaging modalities require improvement of co-registration methods, development of high performance optics to enhance the depth of measurement while maintaining molecular

information. Imaging technologies should be developed for imaging at all levels, from the single molecule to the whole body;

- Many current technologies could be used immediately for population screening to substantial benefit. NIH should focus resources on diseases or conditions for which treatments are available, that have highest prevalence and cause the greatest harm (in suffering and treatment costs);

- Diagnostics which are simple to use, rapid, inexpensive, reliable and suitable for use in third world countries, inner city clinics and rural areas should be a major focus of the NIH; and

- NIH should support the creation of a database/clearing house for building research teams with relevant skills/knowledge.

Gaps exist in the following important areas in sensor development:

- The major barrier in sensor development and deployment is the loss of sensor function when put in contact with a biological system such as blood, saliva or interstitial fluid. This is referred to as biofouling. It is critical to overcome this natural protective barrier with research designed at understanding the interaction of proteins and cells with the sensor surface (immune reactions) and the subsequent process of fibrotic encapsulation of the sensor. New sensor surface materials may prevent this process by utilization of biosmart materials or materials that maintain sensor stability and function in spite of these interactions;

- Sensors that can function continuously in clinical or home health care environments present some of the biggest technological and scientific challenges. A sensor would optimally require a recognition event that doesn't require chemical or mechanical resetting (calibration). Extending continuous monitoring technologies requires new receptor designs that allow for highly selective and chemically reversible recognition events;

- Validation of sensor function is imperative for this technology to give a meaningful measurement. To accomplish this, standards and protocols are required. The specific parameters used for validation will determine the range over which the sensor results are meaningful and quantitative;

- 'Functional standards' that correlate to the desired phenomenon (such as disease presence or analyte concentration) are needed for technology development, especially prior to the stage where the technology can be tested in animals or people;

- Systems integration; combining the inputs from several sensors to yield useful integrated information; providing a working sensor or 'closed-loop' device from advances in miniaturization, materials, signal transduction, drug delivery, etc.;

- Almost every use of micro/nano systems will require the integration of multiple functions to achieve performance and cost advantages. Research is needed to explore the many possible paths to system integration with a focus on those with the most promise to meet the demanding needs of *in vivo* applications where true micro/nano scale integration will have the largest impact but also where integration is most challenging;

- Cell-based biosensors are differentially sensitive to environmental stimuli, such as temperature, G-forces, culture medium, and barometric pressure. Research is necessary to define methods for the manufacture and transport of these sensors considering the condition of the cells attached to the sensor at the final place of use; and

- Production of quantitative data is one of the most significant limitation to assays/sensors that involve immobilized recognition and/or tranduction events at interfaces. New approaches to produce quantitative results from a large array of multiplexed data are necessary. The responses of multiple chemicals from a non-specific array are impossible to completely deconvolute. Better computer algorithms and greater discrimination (orthogonally) between sensory elements of the array can limit the overlap of the response space of particular analytes and thereby allow for better quantification.

Keynote Address

Dr. John A. Parrish is the Chairman of the Department of Dermatology at Harvard Medical School (HMS), Chief of the Dermatology Service at Massachusetts General Hospital (MGH), Professor of Dermatology at HMS, and Professor of Health Science and Technology at Massachusetts Institute of Technology (MIT). Although his original training was in internal medicine, dermatology, and clinical research, he has spent the last 20 years conducting and directing basic research in photobiology, biological effects of lasers, and cutaneous biology.

Dr. Parrish, in collaboration with Thomas B. Fitzpatrick, M.D., developed a novel treatment of psoriasis (oral psoralen photochemotherapy, or PUVA) which is now used worldwide. His research group at MGH introduced laser lithotripsy of kidney stones, selective laser therapy of vascular birthmarks and lesions, and novel laser-based diagnosis and treatments of selective cardiovascular disorders and malignancies.

Dr. Parrish organized the first, and now the world's largest, multidisciplinary research group to systematically study the basic nature of laser effects on tissue—the Wellman Laboratories of Photomedicine at MGH of which he is Director. Dr. Parrish is also Director of the MGH-Harvard Cutaneous Biology Research Center (CBRC), a research center committed to fundamental research in cutaneous biology as broadly defined. Dr. Parrish is also Director of the Center for Integration of Medicine and Innovative Technology (CIMIT), a multidisciplinary research and clinical effort to introduce new therapeutic and diagnostic procedures to improve health care.

Dr. Parrish is a member of the Institute of Medicine of the National Academy of Sciences.

Dr. Parrish has over 300 publications, many of which describe new treatments and diagnostics. He has written eight books, most of which are textbooks, but include a book on baseball, a book on the Vietnam War, and a book on skin for the layman.

Sensors in Modern Medicine

Technology in biomedical sensors must be developed in concert with the clinicians and biologists who understand how these sensors can be used. The primary barriers to successful transfer of technology have to do with highly specialized knowledge and vocabularies; technologists lack a clear understanding of the clinical problems they are trying to solve, while clinicians lack knowledge about technical options for meeting their needs. These two groups of people rarely come in contact with each other naturally, and when they do, they can lack a shared vocabulary to discuss and develop technological solutions to medical problems. CIMIT, The Center for Integration of Medicine and Innovative Technology at MIT and Harvard, attempts to solve this basic problem by bringing together people from a “problem-rich environment” (Harvard Medical School and the Boston area hospitals) and a “solution-rich environment” (MIT and the Draper Laboratories). Besides the need for an appropriate team to work on biomedical sensors, other large barriers exist concerning intellectual property, funding, and evaluation of safety, efficacy and cost-effectiveness, and finally, widespread acceptance in clinical medicine for a new product.

Sensors will be increasingly important for medicine as we attempt to tailor therapies to the individual, as better treatments turn lethal diseases into chronic diseases, and as we move toward home care. We need smaller, cheaper, more portable sensors that can be used easily by the patient or are indwelling. These sensors must be very reliable, fast, and have a well-

defined failure mode. In addition, they must have adequate data storage, computing and communication ability. An example of a sensor application is the operating room where a large number of sensors are used, and medical personnel must evaluate the readings from each individually and mentally integrate these readings to follow a patient's physiology. An integrated processing system is needed that can acquire and process several signals into a coherent picture that can be quickly understood and used to make medical decisions.

A "wish list" of desired sensors and their capabilities includes "smart forceps" to improve surgery, the ability to predict total organ failure, a "deployable" ICU, a device to track skin lesions, a closed loop 'dosing system', such as that needed to deliver insulin in response to changes in blood glucose, and wireless technology for sensors. Collaboration of specialists with different expertise will be essential to attain this "wish list" as well as other applications.

Plenary Speakers

Dr. Lance Liotta is Chief of the Laboratory of Pathology and Chief of the Section of Tumor Invasion and Metastases in the Division of Clinical Sciences, National Cancer Institute, NIH. He is the former Deputy Director for Intramural Research, NIH. He received his undergraduate degree at Hiram College in 1969 and went on to complete an M.D./Ph.D. program at Case Western Reserve University in 1976. Dr. Liotta's Ph.D. is in Biomedical Engineering. Dr. Liotta served his residency training in anatomic pathology at the NIH in the Laboratory of Pathology. He became Chief of the same laboratory in 1982.

Dr. Liotta has invented technology in the fields of molecular markers, therapeutic molecules, immunoassays, microdissection, and proteomics. Dr. Liotta and colleagues in NICHD and the NCI invented Laser Capture Microdissection (LCM), which is commercialized and used in more than 1,000 labs worldwide. The technology has enabled investigators for the first time to develop cDNA libraries of normal epithelium, premalignant precursor lesions, invasive carcinoma, adjacent stroma, and metastasis, all from the same patient. LCM has been applied to make broad discoveries in genomics, functional genetics, and is now extending into tissue proteomics. In partnership with Dr. Emanuel Petricion of the FDA, Dr. Liotta initiated the first joint initiative between the FDA and the NCI to develop new technology for the discovery of proteins and the profiling of signal pathways in actual human tissue. They were the first to use "Artificial Intelligence"-type learning algorithms to discover disease-associated proteomic patterns in the serum of patients which correlates with the presence of early stage ovarian and prostate cancer (Lancet 2002, 359:572-577). Dr. Liotta's protein ligand microarrays have been used to analyze the protein signal pathways that are deranged during the evolution of invasive prostate cancer in human tissue. He has proposed that LCM combined with protein microarrays constitute a new paradigm for studying the mechanism of action of candidate pharmaceuticals. The technology is being applied to patient tissue biopsies conducted before, during, and after experimental therapy.

Protein Arrays for Clinical Proteomics: Personalized Molecular Medicine
Lance A. Liotta, M.D., Ph.D., Elise C. Kohn, M.D., and Emanuel F. Petricoin, Ph.D.,**
National Cancer Institute, CCR, National Institutes of Health and **Center for Biologics Evaluation and Research, FDA

The cause of most human disease lies in the functional disregulation of protein-protein interactions. Understanding the role that protein networks play in disease will create enormous clinical opportunities, since these pathways represent the drug targets of the next decade. In the future, entire cellular networks, not just one disregulated protein, will be the target of therapeutics. The next technologic leap will be the application of proteomic technologies at the bedside. We have developed protein microarray technology and are applying this technology to analyze the state of key signaling pathways in microdissected human tissue cells. In a series of ongoing clinical trials, using subject biopsies, we are currently analyzing the state of protein signal pathways in the disease-altered cells, before, during, and after therapy. This can herald the advent of true patient-tailored therapy.

Pathologic changes within an organ may be reflected in proteomic patterns in serum. We investigated whether such patterns exist and distinguish neoplastic and non-neoplastic disease within the ovary and prostate. Serum proteomic mass spectra (< 20,000 Da), were analyzed by a genetic algorithm linked to self organizing cluster analysis to discover the “fittest” pattern that

discriminates two training populations. A point in N space representing the pattern of an unknown sample is compared for its proximity to defined unaffected and cancer clusters.

Unknown Ovarian Cancer Sera: The bioinformatics tool correctly classified 50/50 ovarian cancers including 18/18 Stage I, and 63/66 (95%) of the benign and unaffected controls: Sensitivity=94%, Specificity=96%, and PPV=94% ($p < .001$). Unknown Prostate Cancer Sera: the method accurately predicted ($p< .001$) PrCa (22/24, 92%, 95% CI=88-100%), 17/18 with PSA values of 4-10 ng/ml (95% CI: 65-99%), while classifying 137/197, ($p2 < .001$ with histologic BPH as unique. Following radical prostatectomy 7/ 7 sera patterns reverted from cancer to a unique cluster ($p=0.016$).

Serum proteomic profiling may constitute a sensitive and specific surveillance tool for early diagnosis and provide a window into body physiology.

Dr. Allan J. Tobin is the Director of the UCLA Brain Research Institute. Dr. Tobin received his S.B. (1963) from MIT, in Humanities and Science, and his Ph.D. (1969) from Harvard, in Biophysics. He was an Assistant Professor of Biology at Harvard from 1971 to 1975 and a visiting scientist at the Institut Pasteur in 1982. Holder of the Eleanor Leslie Chair in Neuroscience, he is both Professor of Neurology in the UCLA School of Medicine and Professor of Physiological Science in the UCLA College of Letters and Science. He is also the Scientific Director of the Hereditary Disease Foundation and Co-Director (with Jack Judy) of the UCLA NeuroEngineering Training Program, supported by an NSF IGERT award, a joint effort of the Brain Research Institute's Neuroscience Program and the School of Engineering and Applied Science's Biomedical Engineering Program.

The hallmark of Dr. Tobin's work has been his ability to encourage researchers and students from different backgrounds to interact in unexpectedly creative ways. These interactions have led to unusual multidisciplinary collaborations on Huntington's disease, Parkinson's disease, epilepsy, and juvenile diabetes. As an educator, researcher, and prize-wining textbook author, he has consistently promoted the integration of molecular genetics, cell biology, neuroscience, and engineering.

Optical Biosensors for Neurotransmitters and Other Intercellular Signals

Jenna L. Rickus[1], Jeffrey I. Zink[2], Bruce Dunn[3], and Allan J. Tobin[4]
[1]Interdepartment Program for Neuroscience and NeuroEngineering Program, [2]Department of Chemistry and Biochemistry, [3]Department of Materials Science and Engineering, and [4]Brain Research Institute and Departments of Neurology and Physiological Science, University of California, Los Angeles, CA 90095.

Enzymes, encapsulated in the pores of the sol-gel derived glass, retain their spectroscopic properties and their biological activities. We have used one such encapsulated enzyme, glutamate dehydrogenase (GDH), to measure concentrations of glutamate, the major excitatory neurotransmitter in the central nervous system, with the goal of monitoring glutamate release with a temporal resolution of milliseconds and a spatial resolution of tens of micrometers. GDH catalyzes the oxidative deamination of glutamate to α-ketoglutarate, with NAD^+ serving as electron acceptor. To allow continuous monitoring, we have adopted a photochemical means of regenerating NAD^+ from NADH. The technology we have developed can be extended to other dehydrogenases, the largest class of redox enzymes, for one-time or real-time monitoring of other analytes.

Dr. Michele Follen received the B.A. degree the University of Michigan, Ann Arbor, in 1975, the M.D. degree from the University of Michigan Medical School in 1980, the M.S. degree in clinical research design from the University of Michigan in 1989, and the Ph.D. degree in Epidemiology from the University of Michigan in 2000.

She is a professor of Gynecologic Oncology and the director of the Biomedical Engineering Center at the University of Texas M.D. Anderson Cancer Center. She is the director of the Colposcopy Clinic at the M.D. Anderson Cancer Center and the Division Director of Gynecologic Oncology at the UT Health Science Center—Lyndon Baines Johnson Hospital, Houston, Texas. She is also a Professor of Biomedical Engineering at the University of Texas at Austin. Her research interests include the use of optical spectroscopy and imaging for detection of cervical precancer and treatment of preinvasive cervical neoplasia with chemo-preventive agents.

Optical Technologies for Cervical Neoplasia

We have a multidisciplinary group of optical engineers, physicians, cell biologists, statisticians, bio-mathematicians, behavioral scientists, and decision scientists to evaluate emerging optical technologies for the screening and detection of cervical neoplasia. We have followed the Littennberg model of technology assessment: evaluating biologic plausibility, technical effectiveness, clinical efficacy, patient and provider satisfaction, and cost-effectiveness and ethical implications. For 12 years we have been researching fluorescence and reflectance spectroscopy and now are pursuing large clinical trials of 800 and 1,000 patients appropriately stratified by the biologic variables of interest. We have modified equipment-based findings from both biologic-plausibility projects and patient-satisfaction studies and are now evolving a patient/provider interface based on provider feedback. We have performed cost-effectiveness modeling and are currently collecting primary cost data to be used in relative-value-base-unit research. We are now further extending our research on fluorescence and reflectance spectroscopy to be useful in the developing world. In the last 7 years, we have pilot tested Raman Spectroscopic devices and Optical Coherence Tomography, and in the last 3 years, we have developed a confocal microscope for *in vivo* optical detection, which is currently undergoing pilot testing in the clinic. We are combining interests with our large cervical biomarker program to begin exploring optical contrast agents. This work is nascent, but it appears very promising. The large team of collaborators and the nature of our collaborative work will be emphasized.

Dr. Valentin Fuster received his medical degree from Barcelona University and, after an Internship at Hospital Clinic in Barcelona, spent several years at the Mayo Clinic, first as a Resident, and finally as Professor of Medicine and Consultant in Cardiology before his departure in 1981 for the Mount Sinai School of Medicine as head of Cardiology. Between 1991 and 1994, Dr. Fuster was the Mallinckrodt Professor of Medicine at Harvard Medical School and Chief of Cardiology at the Massachusetts General Hospital. In 1994, he returned to Mount Sinai Medical Center as Director of the new Zena and Michael A. Wiener Cardiovascular Institute at the Mount Sinai School of Medicine and is presently the Richard Gorlin, M.D./Heart Research Foundation Professor of Cardiology. Dr. Fuster is Past President of the American Heart Association, former member of the National Heart, Lung and Blood Institute Advisory Council, and Chairman of the Fellowship Training Directors Program of the American College of Cardiology.

Dr. Fuster is the recipient of three major ongoing NIH grants. He has published more than 400 articles on the subjects of coronary disease, atherosclerosis, and thrombosis, and he has become the lead Editor of a major textbook on cardiology “The Heart” (previously edited by Dr. J. Willis Hurst). He contributed first hand to the launching of the new Forum for Young Investigators of the American Heart Association.

Dr. Fuster has been the recipient of the Andreas Gruntzig Scientific Award of the European Society of Cardiology, the Lewis A. Conner Memorial Award for scientific accomplishment by the American Heart Association, and the Distinguished Scientist Award for scientific accomplishment in cardiology from the American College of Cardiology. He is the recipient of nine honorary degrees (Honoris Causa) from distinguished universities throughout the world, and he was the recipient of the 1996 Principe de Asturias Award of Science and Technology, the highest award to Spanish-speaking scientists from the son of the King and Queen of Spain. In 1997, Dr. Fuster received the Distinguished Achievement Award from the Council of Clinical Cardiology of the American Heart Association. In March 2000, he received the Distinguished Service Award from the American College of Cardiology for his contribution to Medicine. In November 2001, during the National Scientific Sessions in Anaheim, California, Dr. Fuster received the James B. Herrick Award from the American Heart Association Council of Clinical Cardiology.

Most recently, Dr. Fuster was elected as a member of the Institute of Medicine of the National Academy of Sciences, and he was appointed president-elect of the World Heart Federation.

MRI of Vulnerable plaque and Complicated Thrombosis

V. Fuster, Z. A. Fayad, R. Corti, M. Poon, J. J. Badimon, Mount Sinai Medical Center, New York, NY

Our group is pursuing the vascular biology leading to the vulnerable plaque and complicated thrombosis as well as MRI imaging for their non-invasive identification in humans.

1. Vascular Biology Leading to the High-Risk Plaque and Complicated Thrombosis[1]

Disruption of a high-risk or vulnerable (type IV and Va lesions of the AHA) with a subsequent change in plaque geometry and thrombosis (type IV lesion) may result in an acute coronary syndrome (Fig. 1). In the coronary arteries, the high-risk plaques are traditionally called “vulnerable plaques.” They tend to be relatively small, but soft or vulnerable to “passive” disruption because of the high lipid content. In addition, a macrophage dependent “active” phenomenon of plaque disruption (related to matrix metalloproteinases or MMP) and thrombosis (related to tissue factor or TF) is evolving. Indeed, the continuing entry, survival, and replication

of monocytes, macrophages and lymphocytes within plaques are in part dependent on factors such as endothelial adhesion molecules (i.e., VCAM-1), monocytes chemotactic protein (MCP-1), monocyte colony stimulating factor (M-CSF), and interleukin-2 for lymphocytes. Macrophages, following what appears to be a defense mission by protecting the vessel wall from excess lipid accumulation, may eventually undergo apoptosis with release of MMP's and TF. In contrast with the lipid-rich "vulnerable" coronary plaques, the carotid plaques responsible for cerebrovascular events are less lipid-rich and more stenotic. Hence, the general term "high-risk plaques" rather than "vulnerable plaques" which specifically apply to the coronary arteries and aorta is used (see below).

Following the successful results of lipid lowering trials, and based on pathological and *in vitro* magnetic resonance imaging (MRI) observations, we postulated that when high LDL-C predominates over the influx, there is a decrease in the softness of the plaque and so, presumably in the "passive" phenomenon of plaque disruption; *in vivo* MRI observations in aortic arch disease and recently in coronary artery disease support this concept. These stabilized lesions appear to represent the Type Vb lesions (AHA classification). Furthermore, when low HDL cholesterol is increased experimentally, there is partial decrease in the number and activity of the macrophages and so, presumably, stabilization of the "active" phenomenon of plaque disruption and thrombus formation.

Work by our group and others, suggest that circulating blood TF associated to monocytes and white blood cells, may be involved in circulating blood thrombogenicity. Indeed, the predictive value of coronary events of *high titres of CRP* may be a manifestation of such activated blood phenomena. In fact, under conditions of lipid lowering with statins, correction of hyperglycemia in Diabetes Type 2 and discontinuation of cigarette smoking, we and others have found a decrease in systemic thrombogenicity, perhaps because platelet and/or circulating monocyte tissue factor activity becomes modified. Hence, the new concept of a "high-risk blood" has developed.

2. Magnetic Resonance Imaging in the Understanding and Diagnosis of Athero-Thrombotic Disease[2]

There has been increasing awareness of the importance of composition of athero-thrombotic plaque as a major risk factor for acute coronary syndromes.[1] Several invasive and noninvasive imaging techniques are available to assess athero-thrombotic vessels.[2] Most of the standard techniques identify luminal diameter or stenosis, wall thickness, or plaque volume; however, none are effective in determining the plaques that are unstable and vulnerable to thrombosis and proliferation. *In vivo*, high-resolution, multi-contrast, magnetic resonance imaging (MRI) holds the best promise of non-invasively imaging vulnerable plaques and determination of the different plaque components such as lipid core, fibrosis, calcifications and thrombosis deposits in all arteries including the coronary arteries.[2] The MR findings have been extensively validated against pathology in *ex vivo* studies of carotid, aortic, and coronary artery specimens obtained at autopsy.[3-6] Subsequent work on imaging carotid arteries *in vivo* in patients[7] (Fig. 2) referred for endarterectomy showed a high correlation with pathology and with previous *ex vivo* results. A recent study in patients with plaques in the thoracic aorta[8] (Fig. 3) showed that when compared to transesophageal echocardiography, plaque composition and size are accurately characterized and measured using *in vivo* MRI. Carotid and aortic athero-thrombotic plaque assessment by MRI may lend itself to use as a screening tool for prediction of future cardiovascular events and for the evaluation of therapeutic intervention benefits. These MR techniques have been also adapted for the study of plaques in different animal models.[9-20] A new *in vivo* study showed that MRI can characterize plaques in transgenic mice models.[15-18] Therefore, MRI can be used as an investigative tool to follow *in vivo* progression, regression and plaque stabilization in different transgenic (Fig 4) and non-transgenic animal models.[18] The

ultimate goal is imaging of plaque *in vivo* in human coronary arteries. Preliminary studies in a porcine model of athero-thrombosis showed that the major difficulties of MR coronary wall imaging are due to the combination of cardiac and respiratory motion artifacts, the non-linear course of the coronary arteries, as well as their relatively small size and location. Studies in an *in vivo* pig model[14] and in humans[21] suggest that MRI may soon be applicable to study and characterize athero-thrombotic plaques in human coronaries *in vivo* (Fig. 5). We have shown recently the utility of MRI in the study of treatment in humans. MR was used to measure the effect of lipid-lowering therapy (statins) in asymptomatic untreated hypercholesterolemic patients with carotid and aortic atherosclerosis (Fig. 6).[22] Finally, the potential of *in vivo* MRI to detect arterial thrombotic obstruction and define thrombus age has been very recently evaluated using black-blood T1W and T2W.[23] Carotid thrombi were induced in swine by arterial injury. Serial high-resolution *in vivo* MR images were obtained at 6 hours, 1 day and at 1, 2, 3, 6, and 9 weeks. Thrombus appearance and relative signal intensity revealed characteristic temporal changes in the MR images, reflecting histological changes in the composition.

In conclusion, the assessment of athero-thrombotic plaques by imaging techniques is essential for the identification of the high-risk or vulnerable plaques. *In vivo*, high-resolution, multi-contrast MRI holds the best promise of non-invasively imaging high-risk plaques and characterizing the different components in all arteries including the coronary arteries. MR allows serial evaluation assessment of the progression and regression of atherosclerosis over time. The use of specific MR contrast agents targeted for athero-thrombotic plaque imaging may enhance the plaque characterization.[24,25] Application of MRI opens new areas for diagnosis, prevention, and treatment (e.g., lipid-lowering drug regimens) of athero-thrombosis in all arterial locations.[26]

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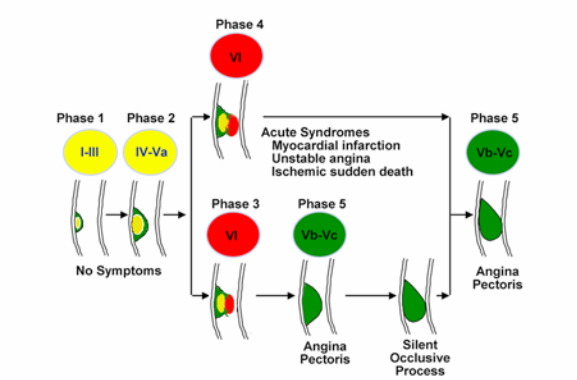

Figure 1: Phases and lesions morphology of coronary atherosclerosis. Progression is based on gross pathologic and clinical findings. An early lesion (phase 1) can become a fibrolipid plaque (phase 2). Phase 2 can progress into an acute phase (phase 3 or 4). Formation of thrombosis or hematoma may cause angina pectoris (phase 3) or an acute coronary syndrome due to occlusive thrombosis (phase 4). Phase 3 and 4 lesions can evolve into a fibrotic phase (phase 5) characterized by more stenotic plaques that may progress to occlusive lesions. Yellow indicates lipid accumulation, red indicates thrombosis and hemorrhage, and green indicates fibrous tissue. Roman numerals indicate the lesion types.
I–III=early lesions with isolated macrophage–foam cells (I), multiple foam-cell layers (II), or isolated extracellular lipids (III); IV–Va=advanced lesions (fibrolipid plaques with confluent extracellular lipid pools [IV] or fibromuscular tissue layers and atheroma [Va]); VI =advanced lesions (complicated plaques with surface defects, hemorrhage, or thrombi deposition); Vb–Vc=advanced lesions with calcifications (Vb) or fibrous tissue (Vc).

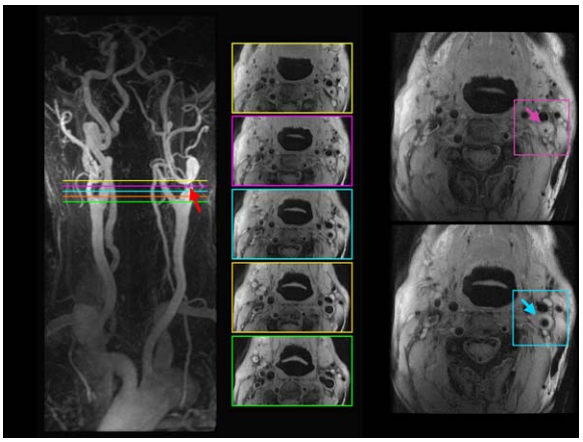

Figure 2: Carotid MR angiogram (left panel) showing a severe stenosis in the left internal carotid artery (red arrow). The MR angiogram is obtained with a contrast enhanced (Gd-DTPA) 3D fast gradient-echo and a carotid-aortic arch phased-array coil. Cross-sectional MR black-blood images of the carotid arteries are shown in the middle and right panels. Display of the MR slice positions are shown on the left panel (colored lines). Magnified views of some of the carotid plaques are shown in the right panel. The arrows indicate the carotid plaques.

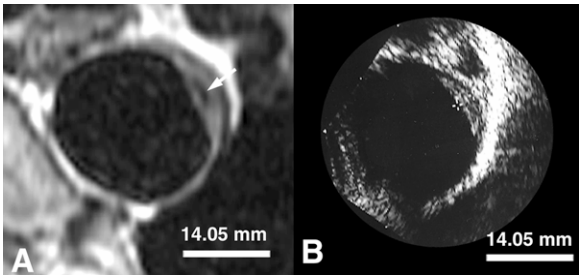

Figure 3: *In-vivo* magnetic resonance image from a patient with a 4.5 mm thick plaque in the descending thoracic aorta: T2-weighted MR image (panel A) with the corresponding transesophageal echocardiography image (panel B). The MR image shows an example of a plaque with a dark area in the center (arrow) identified on the MR image as a lipid-rich core (panel A). The lipid-rich core is separated from the lumen by a fibrous cap. Plaque characterization was based on the information obtained from T1-weighted, PDW, and T2-weighted MR images.

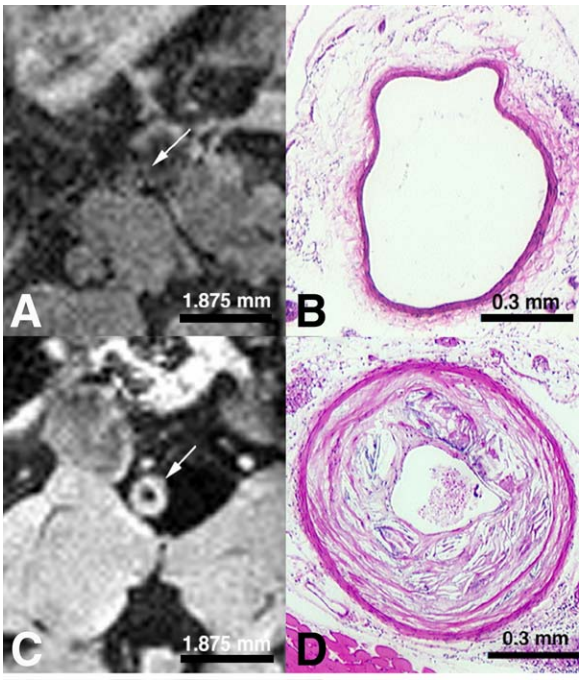

Figure 4*: In-vivo* MR image of the abdominal aorta (arrow) in a normal mouse and in an apolipoprotein E-knockout (apoE1$^{-/-}$) mouse showing differences between normal and atherosclerotic arteries. MR images in the wild-type mice are shown in **A** (magnified, see scale) and histopathology (**B**), as shown by the hematoxylin and eosin stain (original magnification x40). A large atherosclerotic lesion (arrow) that encircles the abdominal aorta of a 12 month-old apoE1$^{-/-}$ mouse is shown on the MR images in **C** (magnified). These findings correlated with histopathology as shown in **D**.

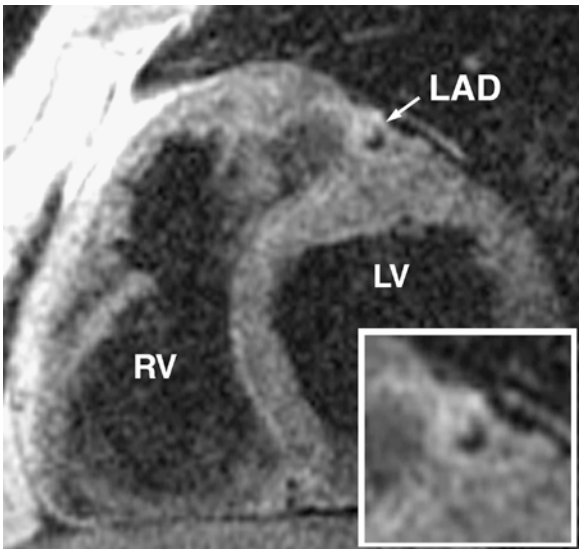

Figure 5: *In-vivo* cross-sectional MR image of a patient with a plaque (arrow) in the left anterior descending artery (LAD). The insert represents magnified view of the LAD plaque. The MR images are 4 mm thick with an in-plane spatial resolution of 750μm, acquired during suspended respiration (<16 seconds) using long echo train fast spin echo imaging with "velocity-selective" flow suppression. RV = right ventricle. LV = left ventricle.

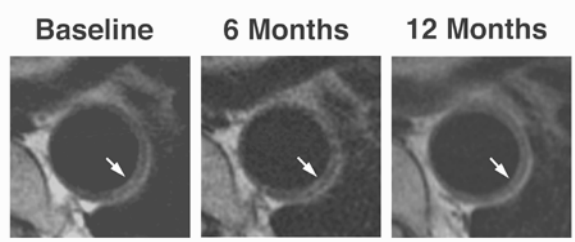

Figure 6: Serial T2-weighted MR images of the same patient at baseline, 6, and 12 months after lipid lowering therapy initiation. Maximal atherosclerotic plaque size changes in the descending aorta are indicated by the arrow.

Dr. Jeffrey Borenstein is currently Group Leader of the MEMS Technology Group at Draper Laboratory, and is an Associate Director for the Center for the Integration of Medicine and Innovative Technology (CIMIT). Dr. Borenstein has a Ph.D. in Physics and has 16 years of experience in microsystems technology and biomedical devices. Prior to joining Draper Laboratory, he worked at Mobil Corporation and North American Philips Corporation. Dr. Borenstein is co-Principal Investigator on a program aimed at developing tissue-engineered replacement organs for transplantation. He is also developing microfluidic and microfabrication technologies for drug delivery devices. In addition, Dr. Borenstein is leading a team of engineers and biologists in the development of sensors for clinical and biodefense applications. These sensors are capable of highly specific protein detection in clinical samples of blood and other fluids, as well as in drug discovery applications for new vaccines. In addition, Dr. Borenstein is developing sensors for the detection of trace concentrations of biowarfare agents in clinical and environmental samples. Dr. Borenstein has co-chaired symposia in the field of BioMEMS and has authored or co-authored over fifty publications and edited one book.

Applications of Microsystems-Based Technologies for Medicine

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The rapidly emerging field of MicroElectroMechanical Systems (MEMS), also known as microsystems technology, has penetrated a wide array of applications, in areas as diverse as automotives, inertial guidance and navigation, microoptics, and chemical and biological sensing. Commercial success has already been realized in automotive and industrial sensing applications; however, the most significant opportunity for microsystems lies in the domain of biomedical technology, most specifically in the field of biosensing. Advantages of MEMS sensors in this arena include the unprecedented level of precision realized by microfabrication, the equivalence of size scales with cells, the potential for multifunctional integration, low cost, and small size, which enables small sample volumes and implantable devices. In this paper, two microsystems-based technologies with applications in clinical diagnostics will be presented; a miniaturized ion mobility spectrometer, and a transducer array with functionalized surface chemistry.

The micromachined Planar High Field Asymmetric Waveform Ion Mobility Spectrometer (PFAIMS) developed at Draper Laboratory is a novel detector for chemical and biological sensing applications. The FAIMS method uses the non-linear mobility dependence of ions on high strength RF electric fields for ion filtering, and has a detection limit in the part-per-trillion regime. The FAIMS scales down without a loss in sensitivity, unlike conventional time-of-flight ion mobility spectrometers. Gas samples are introduced into the spectrometer and then ionized, and the ions are transported through a filter towards a detector by a carrier gas. The ion filter is electronically tunable and the ion species allowed to pass through the filter are selected by adjusting the RF and compensation electric fields applied between the ion filter electrodes.

Preliminary work with the PFAIMS spectrometer has been conducted for many promising biomedical applications. It is widely known that the presence of biogenic amines in human body fluids such as urine, saliva, and blood may reveal or suggest pathological conditions such as cancer. Chemical changes and degradation processes of cells after death are accompanied by the formation of molecular byproducts. For example, decarboxylation of ornithine and lysine produces putrescine and cadaverine respectively. Figure 1 shows PFAIMS spectra for a mixture containing both putrescine and cadaverine. The two peaks are well separated at -30 volts and -10 volts respectively.

Breath analysis has been utilized for centuries in the diagnosis and management of disease, with a wide spectrum of volatile organic compounds associated with particular conditions. These include ketones in ketoacidosis, feculent amines in bowel obstruction, and bacterial byproducts in anaerobic infections. Radioactive labeled metabolites are used in gastroenterology tests. Breath pentane, produced by peroxidation within cell membranes, has been found to be elevated in proportion to ischemia and inflammation in heart disease, and is a promising marker for reperfusion injury. Preliminary experiments with the PFAIMS indicate the potential for a simplified non-invasive breath analysis system. In these experiments, sample collection involved collecting a breath sample directly onto a solid phase micro-extraction (SPME) fiber assembly. The SPME assembly was inserted into a GC injector port and desorbed the sample from the fiber into the GC column. The PFAIMS was attached at the detector port of the GC. The resultant GC-PFAIMS plot in Figure 2 shows the chromatographic retention time on the y-axis and the PFAIMS compensation voltage plotted on the x-axis; differences between two subjects are highlighted in red.

Bioelectronics, specifically the detection and identification of biological material such as proteins, DNA, and microorganisms, has contributed significantly to rapid advances in the fields of genomics, proteomics, and biotechnology. Bioelectronics represents the merging of molecular biology and electronics to develop new products such as sensors. The impact of microbial pathogens on human health underscores the need for rapid, sensitive microbial pathogen detection and identification. Among the various health concerns and issues associated with microbial pathogens is the threat of biological weapons, such as *Bacillus anthracis,* and the need for a high-throughput screen of pathogens for development of vaccines and antibiotics (*Mycobacterium tuberculosis*.) Traditional approaches for pathogen detection include cultivation-based methods, monitoring of host immune response, and visual observation of typical microbial form in host. However, the detection and speciation of slow-growing organisms can take weeks (e.g., *M. tuberculosis*), and some visible microorganisms cannot be cultivated. Many newer approaches are based on either DNA/RNA analysis following amplification or affinity immunoassay. Miniaturized transducers such as SAW (Surface Acoustic Wave) devices and microcantilevers represent important microsystems-based products that are amenable to reduced costs and unattended operation. However, these devices are limited in that they do not perform accurately in a liquid medium. The goal of this work is to develop a microarray sensor technology that is capable of measuring a detailed signature profile of blood-borne, or other body fluid, pathogens in near real-time.

This recently developed microarray affinity detection system relies on both positive affinity of the microorganism for the specific ligands to establish a fingerprint of the microorganism, as well as the lack of affinity for a separate set of ligands to provide a high degree of uniqueness for the specific identification of the microorganism. The sensor approach utilizes a microfabricated, electronic solid-state chemical analysis array (μCANARY) with microfluidic addressing that utilizes small sample volumes, does not require amplification, and is rapid and rugged. Unlike many sensors, the μCANARY is capable of performing a multitude of biochemical assays simultaneously on a single silicon chip. The sensor chip is “activated” by the placement of receptor proteins to the analytes of interest onto the chip during its fabrication process, so no special label reagents (radioactive or fluorescent tags) are required; see Figure 3, where *E. coli* is attached to the sensor surface. Excitation and readout is performed with low voltage, low frequency signals, so commercial off-the-shelf electronic components can be used to create a small, inexpensive, rugged handheld-calculator-sized system. The sensitivity, small size, and low voltage of the μCANARY sensor make it potentially useful for *in-vivo* sensor applications such as implanted organ rejection monitoring. Fingerprinting of microbial pathogens with this sensor technology will ultimately shift diagnostic microbiology from current culture-based

methods to a detection/identification approach that discriminates pathogens with high specificity and with a technology platform than provides ultrasensitive measurements in near real time.

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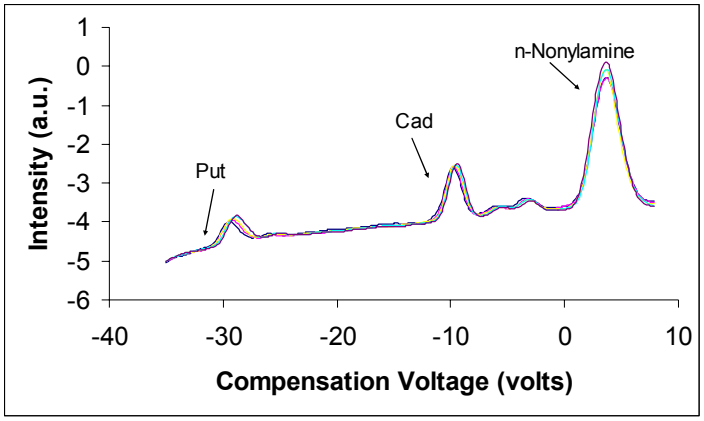


Figure 1. PFAIMS spectra showing Putrescine and Cadaverine clearly resolved from one another.

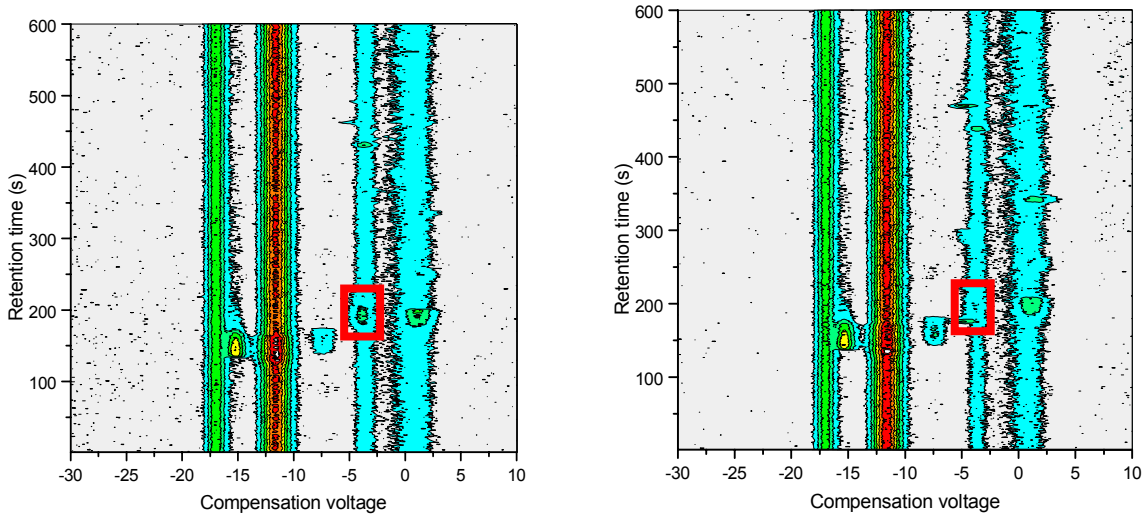


Figure 2. (a) GC-PFAIMS spectra obtained from subject #2. (b) GC-PFAIMS spectra obtained from subject #1. Compare size of peak at –3 V compensation (outlined in red).

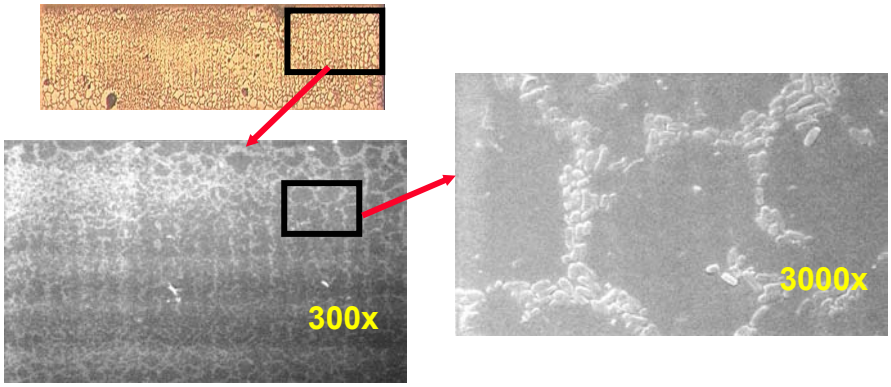


Figure 3. SEM images of *E. coli* binding to surface of MicroCANARY sensor array.

Dr. George M. Whitesides was born August 3, 1939, in Louisville, KY. He received an A.B. degree from Harvard University in 1960 and a Ph.D. from the California Institute of Technology (with J.D. Roberts) in 1964. He was a member of the faculty of the Massachusetts Institute of Technology from 1963 to 1982. He joined the Department of Chemistry of Harvard University in 1982, and was Department Chairman from 1986-9. He is now Mallinckrodt Professor of Chemistry at Harvard University.

He received an Alfred P. Sloan Fellowship in 1968; the American Chemical Society (ACS) Award in Pure Chemistry in 1975; the Harrison Howe Award (Rochester Section of the ACS) in 1979; an Alumni Distinguished Service Award (California Institute of Technology) in 1980; the Remsen Award (ACS, Maryland Section) in 1983; an Arthur C. Cope Scholar Award (ACS) in 1989; the James Flack Norris Award (ACS, New England Section) in 1994; the Arthur C. Cope Award (ACS) in 1995; the Defense Advanced Research Projects Agency Award for Significant Technical Achievement in 1996; the Madison Marshall Award (ACS) in 1996; the National Medal of Science in 1998; the Sierra Nevada Distinguished Chemist Award (Sierra Nevada Section of the ACS), the Wallac Oy Innovation Award in High Throughput Screening (Society for Biomolecular Screening), and the Award for Excellence in Surface Science (Surfaces in Biomaterials Foundation) in 1999; and the Von Hippel award (Materials Research Society) in 2000. In 2001 he received the World Technology Award for Materials from the World Technology Network and a doctorate honoris causa from the University of Twente (The Netherlands). He is a member of the American Academy of Arts and Sciences, the National Academy of Sciences, and the American Philosophical Society. He is also a Fellow of the American Association for the Advancement of Science, the New York Academy of Sciences, and the World Technology Network, and a Foreign Fellow of the Indian National Science Academy, an Honorary Member of the Materials Research Society of India, and an Honorary Fellow of the Chemical Research Society of India.

Recent advisory positions include:

- *National Research Council:* Board on Chemical Sciences and Technology (1984-9; Chairman, 1986-9); Naval Studies Board (1989-97; Vice Chairman, 1992-97); Board on Science, Technology and Economic Policy (1991-7); Board on Physics and Astronomy (1997-) Committee on Science and Technology for Countering Terrorism (2002)

- *National Science Foundation*: Chemistry Advisory Committee (1984-6; Chairman, 1986), Materials Research Advisory Committee (1991-3; Chairman, 1993), Review Panel for the Materials Research Laboratories (1993, co-Chairman); Advisory Committee for Mathematics and Physical Sciences (1993-6); NSF Senior Assessment Panel: International Assessment of U. S. Mathematical Sciences (1997)

- *Department of Defense*: Defense Advanced Research Projects Agency Defense Science Research Council (1984-); Defense Science Board (1993-); Threat Reduction Advisory Committee to the Defense Threat Reduction Agency (1998-)

- *National Aeronautics and Space Administration (NASA)*: Biological and Physical Research Maximization and Prioritization (REMAP) Task Force (2002)

- *Other*: M.I.T. Advisory Committee for Lincoln Laboratory (1985- ; Chairman 2000-); Scientific Advisory Committee for the Scripps Research Institute (1993-)

He is a member of the editorial boards of *Journal of Applied Biochemistry and Biotechnology*, *Bioorganic and Medicinal Chemistry Letters*, *Chemistry of Materials*, *Angewandte Chemie*,

Chemistry & Biology, *Langmuir*, *Nanotechnology*, *Colloids and Surfaces B: Biointerfaces*, and *Sensors and Actuators*.

Present research interests include materials science, biophysics, complexity, surface science, microfluidics, self-assembly, micro- and nanotechnology, and cell-surface biochemistry.

New Tools for Bioanalysis

Biology and biochemistry is facing a new generation of problems in analysis. The interest in analyzing the cell is extending from a primary focus on molecular structure to include broad interest in mechanical structure and phenotypic behaviors. Proteins are replacing nucleic acids as the most important targets for new types of analyses. ADME/Tox is increasingly important in efforts to improve the productivity of the pharmaceutical industry. Presymptomatic detection of disease is a possible approach to the improving the outcome in many diseases. All of these problems, and others, will require new types of analytical systems.

One approach to the development of new tools for bioanalysis is through a combination of the techniques of microfabrication with the problems of biomedicine. We have worked in one part of this problem: that is, the development of new, microfabricated tools for studying the behavior of cells in attached cell culture. A combination of five materials/techniques is providing these tools:

1. Self-assembled monolayers (SAMs) of alkanethiolates on gold, to control the character of interfaces;
2. "Inert surfaces" (surfaces that do not adsorb proteins and therefore do not allow cells to attach);
3. Surface plasmon resonance (SPR), a technique that makes it possible to observe the kinetics and infer the thermodynamics of adsorption of proteins and other biological macromolecules at the surface of SAMs;
4. Soft lithography, to pattern the interface in its plane;
5. Controlled, laminar flows in microchannels, which provide the basis for methods both of fabrication inside capillaries and for controlling the medium surrounding cells, and the shear they experience.

This talk will discuss the use of these tools in bioanalysis.

Leading references:
"Soft Lithography," Xia, Y.; Whitesides, G.M., Angew. *Chem. Int. Ed. Engl.* 1998, 37, 550-575.

“Soft Lithography in Biology and Biochemistry” Whitesides, G. M.; Ostuni, E.; Takayama, S.; Jiang, X.; Ingber, D.E. *Ann. Rev. Biomed. Eng.* 2001, 3, 225-373.

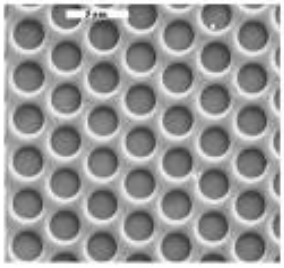
“Flexible Methods for Microfluidics” Whitesides, G. M.; Stroock, A. D. *Physics Today* 2001, 54, 42-48.

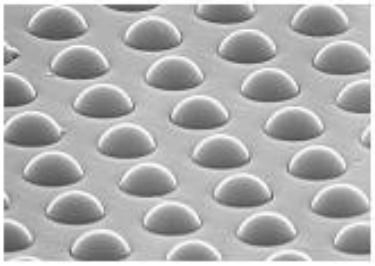
Dr. David R. Walt is Robinson Professor of Chemistry at Tufts University. He received a B.S. in Chemistry from the University of Michigan and a Ph.D. in Chemical Biology from SUNY at Stony Brook. After postdoctoral studies at MIT, he joined the chemistry faculty at Tufts. Dr. Walt served as Chemistry Department Chairman from 1989 to 1996. Dr. Walt serves on many government advisory panels and boards and chaired a National Research Council panel on New Measurement Technologies for the Oceans. He is Executive Editor of Applied Biochemistry and Biotechnology and serves on the editorial advisory board for numerous journals. Dr. Walt is the Scientific Founder of Illumina, Inc. He has received numerous national and international awards and honors recognizing his work including a National Science Foundation Special Creativity Award, The Biosensors and Bioelectronics Award, and the Samuel R. Scholes Award in Glass Science. He was elected a fellow of the American Association for the Advancement of Science in 2000. Funding for his work has come from the U.S. Department of Energy, National Science Foundation, National Institutes of Health, Office of Naval Research, DARPA, Environmental Protection Agency, as well as numerous foundations and corporations. Dr. Walt has published over 140 papers, holds over thirty patents, and has given hundreds of invited scientific presentations.

Success in Sensor Technology

Functionalized optical fiber sensors have emerged as alternatives to other conventional methods of analysis. Optical sensors not only have the adaptability for multiplexing and miniaturization, but also may be used for remote monitoring. Optical imaging fibers are suited for sensor arrays because multiple sensor elements can be incorporated onto the fiber's face. These sensor arrays may contain thousands of individual sensing regions on the end of a fiber with a diameter of only a few hundred microns.

We have used coherent imaging fibers to make fiber-optic chemical sensors. Sensors can be made with spatially discrete sensing sites for multianalyte determinations. We are investigating the limits of our ability to create high-density sensing arrays containing millions of microsensors and nanosensors. Micrometer- and nanometer-sized sensors have been fabricated by etching the cores of the optical imaging fiber to create wells. The wells can be filled with complementary-sized microspheres such that one microsphere is incorporated into one micro-well and each well is optically “wired”, i.e., sensory materials placed within the well can be individually interrogated.



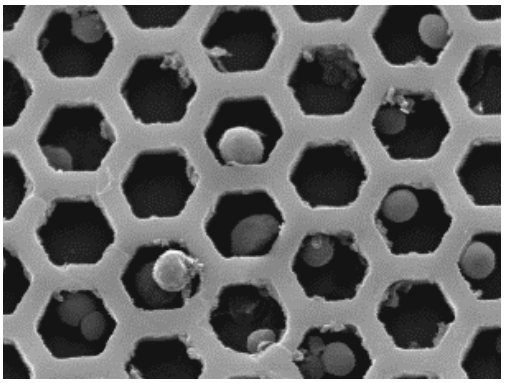


Fiber optic array sensors are based on attaching specific sensing elements to microspheres that fill each well, with random microsphere placement. An important consequence of the beads-in-wells process is that replicates of each bead type will be present in every array. This inherent redundancy provides two significant advantages. First, since repeat sensors must agree, their signals can be used to virtually eliminate both false positives and false negatives. Second, signal-to-noise ratios scale as the square root of the number of identical sensing elements, so examining identical microspheres in the array enhances sensitivity. By summing large numbers

of sensors, the measurements have improved precision, thereby enabling the detection of lower analyte concentrations. Several different types of arrays can be prepared using this approach:

Artificial Nose Sensors—The “artificial nose” sensors are patterned after the mammalian olfactory system such that complex, time-dependent signals from multiple sensors provide a “fingerprint” of each analyte. Microsphere-immobilized dye molecules on the distal end of the fiber give distinct fluorescence response patterns upon exposure to organic vapors. Solvatochromic dyes (fluorescent dyes sensitive to their environment) are incorporated into each of the different beads either through direct adsorption onto the substrate surface or by solvent swelling. The intrinsic chemical and physical nature of the various bead types, in conjunction with the solvatochromatic dye, give rise to unique responses to vapors. These different response patterns include spectral shifts, intensity changes, temporal responses and descriptive response contours that are influenced by the physical and chemical nature (polarity, shape and size) of the vapor and the polymer. Video images of the different temporal responses are captured as the input signals for a computational network vapor recognition program. Recent advances in terms of sensitivity, reproducibility, and the types of complex problems that can be addressed will be discussed.

Cell Arrays—Single cells can be loaded into the microwells. The microwells range in size from 3-25 micrometers such that the array can be used to accommodate different types of cells. By containing single cells in each individual microwell, the responses of all the cells in the array can be monitored simultaneously. Each microwell, containing a single living cell, can be used to monitor several physiological and genetic responses simultaneously. Cells can be genetically engineered to express different reporter molecules and used in simultaneous screens for drug candidates or environmental toxins.



DNA Arrays-Single-stranded oligonucleotide probes are attached to optically encoded microspheres (3 μm) and then multiple types of microbead sensors are mixed together. The mixed sensor population is then randomly distributed on the etched face of an optical imaging fiber to create a randomly ordered addressable high-density bead array. One such study incorporated twenty-five different oligonucleotide probes and was employed to detect twenty-five different sequences from disease states (lymphocyte and cytokine expression) and disease-related genes (oncogenesis, cystic fibrosis). Another study is aimed at typing different strains of bacteria. Finally, we are preparing nanoarrays containing combinatorial bead libraries for unbiased gene expression studies.

Some of this technology forms the basis for the biotechnology company, Illumina, Inc. A brief history describing the genesis of the idea through its commercialization will be discussed.

Dr. Eric Rasmussen spent 7 years enlisted in nuclear submarines before leaving the Navy to receive his undergraduate and medical degrees from Stanford University. After a period working in Haiti with the State Department, and on staff as a molecular biologist at Los Alamos National Laboratory, he completed a residency in Medicine and returned to the Navy as the Assistant Program Director within the Internal Medicine Department of the Navy Medical Center near San Francisco, California. From there he was selected as the director of Surface Fleet Medical Programs (Code-53) at the Navy's Medical Institute in Florida, and subsequently served as a physician-at-sea aboard the aircraft carrier USS Abraham Lincoln (CVN-72) and on deployment with the missile cruiser USS Yorktown (CG-48). He served three brief rotations on the ground in Bosnia, and during that period was appointed a Principle Investigator in Medicine for the Defense Advanced Research Projects Agency (DARPA). In 1996, he was awarded both a Certificate of Meritorious Achievement from DARPA and an appointment as a Fellow of the American College of Physicians. He was selected as the Fleet Surgeon for the US Navy's Third Fleet in 1997 and spent much of the subsequent four years focused on medical support within austere environments, with special attention to civil-military operations. His work included both international exercises that deeply incorporated UN relief agencies into the exercise development process, and the field evaluation of technologies specifically developed to improve integration at the civil-military boundary.

Dr. Rasmussen returned in mid-2001 to the medical faculty at the Naval Medical Center in San Diego, with a simultaneous appointment to the Regional Security Enhancement Program at the Naval Postgraduate School in Monterey and a teaching position within the United Nations Office for the Coordination of Humanitarian Affairs. He is a Visiting Scholar at the Rocky Mountain Institute, a Senior Research Scientist with the Department of Computer Science and the director for bioinformatics at the Center for Robot-Assisted Search and Rescue, both within the University of South Florida, and a Principle Investigator for both DARPA and for the National Science Foundation. He is published in wilderness ecology, biophysics, biochemistry, clinical medicine, humanitarian medicine, decision analysis, shipboard medical care, aerospace medicine, and trauma research. In addition to being a candidate within the World Health Organization's Master's Degree in Disaster Medicine, he holds several personal, unit, and theater military decorations and is qualified both in Submarines and as a Surface Warfare Medical Officer.

Biological Informatics in Urban Search and Rescue: Ground-Truth Lessons From The Pile

Eric Rasmussen, M.D., F.A.C.P., Commander, Medical Corps, U.S. Navy and Director for Bioinformatics, Center for Robot-Assisted Search and Rescue, University of South Florida

On the morning of September 11th, the Center for Robot-Assisted Search and Rescue (CRASAR) deployed robot teams from Colorado and Florida to the World Trade Center site for technical search, medical intervention, victim rescue, body recovery, and forensic analysis. Those teams arrived in Manhattan at about 6 p.m. on the 11th, and were searching the site by the very early hours of September 12th.

A total of 17 robots, of various types and with multiple sensors, were eventually present, and seven of those were used on the Pile. Assessing the available data, the robots were at least the equal of the two human FEMA Rescue teams in the detection of bodies. Regrettably, no team, human, robot, dog, or other, found anyone alive.

The robot teams remained an integral part of the rescue effort from September 12th until October 2nd, assisting the New York Fire Department, FEMA, and military rescuers in several

critical tasks. The tasks included the searching of voids, providing data on structural integrity of the slurry retaining wall for the Hudson River, and finding at least ten sets of human remains in places where technical search specialists using dogs and traditional tools could not reach. As the tempo shifted to recovery, the robot teams continued to work on the Pile daily until deploying home on October 2nd.

Prior to 9/11 there had been no real-world deployment of USAR robots. Even though the mission and protocols of the robots had been developed over many previous months, and the robots themselves had been developed over many years for other purposes, establishing a center for robot-assisted search and rescue research had taken place only a few days before on September 2nd.

We had developed guidelines for robot use based on our work with the National Institute for Urban Search and Rescue in Santa Barbara, California (www.niusr.org). The guidelines included a list of core responses, each of which was designed to mitigate risks to human rescuers while optimizing the non-human advantages offered by a machine in a potentially dangerous environment. Design objectives include support to victims of violent conflict and to victims of both natural and technical disasters; site assessments for worrisome, but unclear, circumstances; biometric determinations of life in a victim; life-sustaining rescue measures (including those designed *ad hoc* for the WTC response); and the manipulation of the immediate physical environment to reduce risk to both victims and rescuers.

During the presentation I will discuss the tools we brought to the 9/11 response, the needs we discovered as we worked with the technical rescue teams in one of the most difficult and complex urban environments imaginable, and the program for research and development we have designed as a consequence of the experience we have gained. We will focus on platforms, mobility, sensors, and synthetic integration, with particular attention to the medical aspects of sensors and sensing strategies, distributed control, and human-robot interactions.

Possibly the most pervasive lesson learned is that robots for USAR must be considered from a whole-system "information technology" perspective, where platforms, sensors, control schemes, networks, and interfaces must all be co-evolved to ensure the information extracted by the robots is truly usable by both the rescue community and the medical support behind them. That requires support to both Fire and Rescue, and to the medical providers at the next level of care. We have developed some strong relationships with national urban search-and-rescue teams, but we want a still deeper and more integrated relationship with the medical community.

A second important lesson is the need to develop a suite of site-specific medical payloads for USAR robots that can acquire and transmit bio-relevant signatures to appropriate locations for analysis and response. We will note that “bio-relevant” distinctly includes environmental sensing. During the search of an air conduit in WTC Tower Two, we lost the propulsion tracks off a robot because we had no method for determining ambient temperature. When the robot appeared to be misbehaving, we pointed the camera down at itself. We soon noted that the treads had melted off the tracks. Later investigation revealed that the air temperature in that space was over 350 degrees F due to aircraft fuel still burning on the other side of the chamber wall. A cooking thermometer placed within view of the camera lens, secured by a few inches of muffler tape, was a cheap, quick, and effective solution.

Many sensor problems are not so easily addressed. The assessment of air quality during industrial accident assessments and deliberate releases, for example, has proved quite challenging, as has the method for providing adequate ventilation to victims once inadequate air quality is found.

Another difficult problem is the binary decision between alive and dead. A live person is a rescue effort (as opposed to a “recovery”) and, as noted in Oklahoma City and in Gurjarat, India, their extrication may require an enormous effort from many people, and that effort is dangerous. In the Mexico City earthquake in September 1985, more than 130 rescuers died during the rescue efforts. Much of that risk can be reduced with the use of our smallest robots, carefully designed for victim localization and area assessment. CRASAR robots, for example, now can carry optical lights, infrared illumination, and two-way audio to aid in the assessment of a conscious victim and the determining of the victim’s surroundings, which are often otherwise dark and reportedly terrifying. However, since many victims are unconscious and difficult to assess by optical camera, a more reliable and sensitive determination of life signs, with methods for consequent life support as required, is an appropriate and desirable research effort.

One method for improving the determining of life signs is with experimentally enhanced video. From trial runs with the Florida Regional Search and Rescue Task Force, we have film showing an operator detecting even very shallow breathing in a favorably placed victim. However, in many cases the victim will be unfavorably presented and so better sensors, both contact and remote, can be of enormous benefit. Acoustic sensors, with very sensitive 3-D localization capabilities and digital noise-cancellation of the robot motors, would be valuable. Other current areas of evaluation include phased-array millimeter wave radar chips from Lawrence Livermore National Laboratory as "heartbeat detectors", hyper-spectral imaging developed for standoff physio-sensors from the Strong Angel “Vital-Touch” refugee assessments, and a search for best-of-breed external touch pulse-ox sensors for SpO_2. We actually need a suite of tools, with multiple configurations and, importantly, the ability to drop the sensor package, complete with homing beacon, lights, and two-way audio, off of the robot and leave it next to the victim. We can then go on to the next search, but without abandoning the first victim to the dark and the silence.

We should note that the major effort in a rescue is not the finding of the victim, though that is certainly important, but rather it is the extrication once the victim is found. FEMA statistics indicate that, once a trapped survivor is located, extrication takes between four and ten hours, and often there are ten volunteers associated with that single rescue. FEMA statistics indicate that, of the long-term survivors at a collapse, 80% were essentially on the surface, 15% were trapped in a space but unhurt, and only 5% of survivors were pinned in the interior. These 20% of interior survivors are often out of reach of direct medical support, a situation that robots can remedy. For example, robots at the WTC were prepared to carry flexible IV tubing for transporting drinking water, oxygen, or medicine (e.g. topical and oral anesthesia, oral antibiotics, diabetes and cardiac meds) to trapped victims.

Our current efforts incorporate the development of sustainable rescue-community readiness as we determine optimal states of preparedness and response capability. We are now integrated into the First Responder teams that will respond to most significant domestic disasters, including the release of Mass Casualty weapons, and robots are nearly ideal for such environments. We are also now a part of several search-and-rescue training programs and we have, therefore, the ability to determine the ground-truth utility of anything we design.

From our perspective, the potential capabilities robots can bring to search and rescue operations requires cutting-edge research in sensors, multi-sensor data fusion, perceptualization, and the consequent delivery of care within an austere environment. Each topic is independently valuable, but the synthesis is likely to accrue exponential benefits even before assessing the enhanced victim rescue potential or the improvement in safety and efficiency for First Responders.

Reports from Breakout Sessions

Active Disease Management

Moderator

Mauro Ferrari, Ph.D., The Ohio State University

Panelists

Gerard L. Cote, Ph.D., Texas A&M University
Ernest Carter, M.D., Ph.D., Howard University
Francis Moussy, Ph.D., University of Connecticut
Basil Swanson, Ph.D., Los Alamos National Laboratory

Broad Statement

Management of chronic diseases require continual monitoring, and monitoring in acute disease is important for evaluating therapy. Three frontiers of sensing technologies are important with regard to applications for active disease management. The first is the development and clinical deployment of *in vivo* chemical, physical, and biological sensors that are capable of long-term, independent monitoring of the onset and progression of pathological states. The second is the refinement of information-relaying telemetric technologies that will allow clinical deployment of sensors in an ambulatory population with the objective of timely, physician-provided therapeutic intervention. The third is the logic linkage of implanted sensors to *in vivo* actuators capable of delivering therapeutic intervention in a feedback loop, self-controlled fashion.

Vision

As palliative therapies are devised, more and more Americans are living for extended times with chronic disease and require new technologies to increase their quality of life and decrease the overall cost of their healthcare. In the future, patients will receive an implanted device that will measure an appropriate marker (glucose, in a diabetic patient) and automatically release an appropriate dose of the needed drug without the active involvement of the patient or physician.

Objectives

Single-platform systems will be developed that feature sensing elements logically linked to therapeutic actuators, such as drug-delivery implants. These assemblies will enhance the body's self-healing capabilities, in a largely self-regulated monitoring-to-therapy feed-back loop fashion. Pathologies that will most benefit from sensor-based disease monitoring include coronary artery disease, pulmonary fibrosis, cancer, diabetes, hypertension, congestive heart failure, obesity, trauma, gastro-intestinal, and ocular diseases. The development of these novel generation devices will require highly collaborative interactions between life scientists, clinicians, and experts in nanotechnology, biomaterials, bioinformatics, the physical sciences, and engineering.

Obstacles and Challenges

Opportunities for breakthrough developments exist in several strategic directions, which are prioritized as follows:

- The development of totally implantable and minimally-invasive sensors has been hampered by poor lifetime and reliability mostly caused by tissue reactions. To address this issue, basic research in the general area of biomaterials is necessary especially with regard to understanding organic/inorganic interface reaction, the use of biologically-inspired approaches to mimic nature's function, the development of biocompatible and biodegradable materials, and the development of sensor materials that can be integrated with a patient's organ tissue for long-term *in vivo* applications;

- A major opportunity for a shift in paradigm lies in the development of biofouling-insensitive sensing strategies. These include sensors that detect *physical properties* of cells, tissues, or molecular assemblies such as temperature and pressure, as opposed to molecular concentrations;

- The development of the logic linkers between sensing and therapeutic actuation is believed to be well within the reach of current technology. Major deficiencies however exist in the 'smart' (time-controlled, and/or biologically guided) delivery of biopharmaceutical compounds;

- Sensor-based management of chronic diseases at home is an important future application of sensor systems. This application will require a new breed of computer-based sensors and therapeutic instrumentation based on novel molecular and tissue engineering coupled with micro- and nano-scale robotic technology. The integration of tele-, computer-, and biomaterials-based systems is needed for this application to be realized;

- Several sensing modalities including near-infrared, fluorescence, Raman, and absorption or scattering spectroscopy and polarimetry offer opportunities for diagnosis and monitoring, but all have some problems (e.g., invasiveness, specificity, temperature and pressure performance, optical scattering limitations, and power requirements) that need to be studied. Specific areas that need to be studied include non-invasive ("on-vivo") and invasive optical technologies, micro- and nano-probes, multi-modality approaches, and optical imaging systems.

Recommendations

- Support the development of anti-biofouling approaches (organic/inorganic interfaces, surface modifications) to extend the useful life of implantable sensors;

- Support the development of innovative biofouling-indifferent technologies;

- Support the development of drug-delivery and other therapeutic actuators, to be logically linked to implantable sensors, to provide single-platform diagnostic and therapeutic in-vivo systems;

- Support interdisciplinary research and development of (1) micro- and nano-based probes for inter- and extra-cellular monitoring, (2) totally implantable sensors including non-invasive technologies and hybrids, (3) multi-modality approaches (e.g.; ultrasound/optical, fluorescence/polarizations, etc.), (4) image-based sensing, (5) optimal sensing target identification, and (6) multisensor arrays;

- The application of computer science and technologies to sensor research needs to be supported in the areas of algorithm development, data presentation, telehealth, performance modeling, and medical information systems;

- Animal models need to be developed to allow *in vivo* evaluation of sensors;

- Compare and validate sensor readings from different body compartments (plasma, interstitial fluid) in human subject and animal research;

- Develop objective standards for sensor validation;

- Prioritize outcome-based, multidisciplinary research with emphasis on diseases of major epidemiological importance;

- Related issues concerning sensor applications and remote monitoring need to be addressed including FDA approvals for *in vivo* applications, security and transmittal of sensor information, legal aspects of sensor-mediated management, integration of therapy systems with sensors, and patient variability effects.

Advanced Technologies for Biological and Biomedical Research

Moderator

Tuan Vo-Dinh, Oak Ridge National Laboratory

Panelists

Christopher. H. Contag, Stanford University School of Medicine
Anthony Guidseppi-Elie, Virginia Commonwealth University
Shuichi Takayama, University of Michigan, Ann Arbor
Weihong Tan, University of Florida, Gainesville

Broad Statement

The application of emerging technologies (e.g., sensors, biochips, microarrays, microfluidics, molecular probes, and contrast agents) will be important to develop novel technologies for biological and biomedical research. These advanced technologies include sensing systems that can operate autonomously (e.g., implants) and noninasively; transducers that measure biologically relevant information (e.g., flow, pressure, analyte type, and concentration); and probes that are integrated with surgical tools such as those intended for minimally invasive procedures.

Vision

A new generation of sensors will operate in complex systems (including the human body), will measure multiple analytes and/or physiological phenomena, will incorporate a variety of technologies (i.e. multimodality sensing), will store and integrate a range of data for rapid disease assessment, and will be easy to use.

Objectives

The panel, which was attended by over 200 participants, dealt with the research needs for future sensor technologies in order to address key challenges in medical diagnostics from molecular probes at the single-cell level to whole body systems. Two key points that impacted on nearly every discussion topic were the need to integrate sensor technologies (i.e., multimodality sensing) and incorporation of user-friendly sensor technologies into complex systems including the living body. The development of such advanced medical technologies requires the cross-fertilization of various research areas and collaboration between investigators from different disciplines, thus multidisciplinary approaches must be supported. The blending of “hypothesis-based” and “design-based” approaches should be encouraged in Requests For Applications (RFAs) that pertain to research programs on biomedical sensing technologies and medical instrumentation.

Recommendations, Obstacles and Challenges

The following topics were discussed and considered as key issues for future research and are listed in order of scale—smallest to largest.

<u>Materials</u>

- Biocompatibility is still an important issue for implanted sensors that has yet to be overcome. There needs to be a shift from benign surfaces toward the development of bioactive surfaces to achieve biocompatibility in implantable sensors;

- Bio-smart materials should be developed that combine molecular recognition, signal transduction and controlled drug delivery.

Biomolecular Analyses

- Microarrays;
    - Dedicated disease-directed DNA arrays are needed for diagnostic and prognostic applications;

    - New rapid strategies for tumor typing will require identification of surface markers as targets for therapy, and intracellular markers of disease progression; and

    - Advanced standardization methods and algorithms are needed to improve comparison of data sets.

- Next-generation sensors with high-density data collection are needed that can take advantage of new genomics information, and new bioinformatics technology for data management; and

- Near-patient, multi-element array sensors will be targeted to a given disease.

Intracellular Assays

Needed are:

- Ultra-small and ultra-fast sensors for intracellular monitoring;

- Novel sensors: recognition, manipulation and function;

- Molecular motors for drug delivery, therapy and disease diagnosis;

- mRNA monitoring using molecular beacon DNA probes that bridge biochemical assays and *in vivo* analyses;
    - novel delivery approaches optimized for diagnosis and imaging; and

    - incorporation of multispectral markers for multiplexing the analyses;

- Molecular beacon aptamer probes for intracellular protein monitoring with improved delivery methods;

- Molecular engineering of peptide probes for use as biosensors;

- Need for *in vivo* sensors for analysis at the level of single cells or small numbers of cells at superficial as well as deep tissue sites; and

- Sub-cellular sensors for the in-situ measurement and monitoring of sub-cellular activity of various and transient proteins, mRNA, etc.

Tissues

Links between molecular and cellular assays (e.g. microarrays and cellular fluorescent assays) and whole body studies are needed. Such linked assays will accelerate the development of novel *in vivo* sensor technologies and serve as an intermediate step.

Whole body assays

Needed are:

- Development of *in vivo* gene discovery strategies to complement array technologies—i.e., integrative functional genomics in complex environments;

- Assays for endogenous enzyme activity and the ability to multiplex these assays to achieve multiparameter tumor typing for customized therapies and disease staging;

- Multimodality approaches to *in vivo* sensing in order to provide links between structure and function. We need to combine high resolution structural imaging with accurate and sensitive functional assays. It will be imperative to make these links seamless and efficient for rapid diagnosis and typing. The development of appropriate algorithms to combine information from functional analyses and images is essential;

- Development of probes with dual or multimodality signatures that can be detected by several methods—a means of coregistration of these images; and

- Biocompatible materials and sensors.

System Integration

Needed are:

- Strategies that link *in vivo* and *ex vivo* assays are needed to bridge the gap between high throughput biochemical and cellular assays and *in vivo* analyses (imaging);

- Non-invasive sensors for early diagnostics and typing in deep tissues *in vivo;*

- Integration of various technologies (laser, microchip, sensor, microfluidics) into a user-friendly system. Very often, the individual components are miniaturized, but the overall system is still large and complicated;

- Novel sensing modalities with remote data collection capability;

- Multi-modality sensors with “orthogonal” data collection; and

- The development of signal processing and informatics tools for use in clinical medicine-“accommodating biological complexity in medical metrology.”

Diagnostic Technologies

Moderator

Mark Arnold, University of Iowa, Iowa City

Panelists

Clifton Barry, National Institute of Allergy and infectious Diseases, NIH
Mark Meyerhoff, University of Michigan, Ann Arbor
Paul Yager, University of Washington, Seattle

Broad Statement

Though the biological sciences have improved their ability to detect the signs of many developing diseases, diagnostic medicine remains very much a work in progress. New and more powerful diagnostic tools are greatly needed to equip clinicians to detect the earliest molecular warning signals of human diseases. Given the tremendous progress that has been made in recent years throughout the life and material sciences, an historic opportunity now exists to begin to integrate and expand this knowledge to advance public health. It is expected that the collaboration of the different sciences could lead to an exciting new generation of reliable, sensitive and cost effective diagnostic tools that will allow continuous, noninvasive, real-time monitoring of disease processes in patients. Technologies can be envisioned to permit home monitoring of numerous disease states, assess biological responses to therapeutic treatments, and establish important biochemical differences between healthy and diseased patients. Furthermore, the development of sensors that are rapid, accurate, and inexpensive will have substantial applications in real-time sensing of single-cell function(s) in health and disease. Advances in screening technology are also envisioned where large populations can be rapidly and effectively screened for selected diseases in order to permit early intervention, thereby enhancing quality of life and reducing overall healthcare costs.

Clearly, advances in the different fields of science and technology (e.g., biology, engineering, chemistry, nanotechnology, electronics, optics, and computer sciences) will contribute directly to the development of innovative systems for clinical diagnostics. Examples include real-time, implantable sensors that can not only monitor patients who are at risk, but can also be designed to dispense a drug or other therapeutic agent that would directly attack the detected aberration. Alternatively, advances in automation technology and immunoassay procedures may increase measurement throughput for large-scale testing or may make possible the development of routine home clinical testing kits for self-monitoring purposes.

Vision

The goal of improved diagnosis of disease in the clinic, in large populations, and in third world environments presents diverse challenges and opportunities. New technologies will provide simple, one-step diagnostic tests that can be used in the clinic or the field, and improvements in *in vivo* and continuous monitoring of disease markers. Nano-sensors and molecular probes will facilitate both goals. Most importantly, dependable diagnosis rests on the integration of medical information, and therefore on improvements in bioinformatics systems.

In the following four major areas, special emphasis should be placed on the reliability of the measurements. It is important that the putative test provides useful clinical information. All

techniques need to be rigorously evaluated and validated in appropriate populations prior to widespread use. Evaluating biologic plausibility, technical feasibility, clinical efficacy, patient satisfaction, and cost effectiveness are imperative. Measurement technologies must be validated with "accepted" gold standards.

Simple, One-Step Diagnostic Tests.

Objectives

This technology will be suitable for point-of-care testing, physician office measurements, and home testing by individuals. Such methods must be robust, insensitive to the applied solution volume, and cost effective for screening of mass populations of people. The basic idea is that the user applies a sufficient volume of sample (unmeasured volume) and the entire analytical process is handled internally. A simple readout device provides the desired concentration information. One motivating force is the need for rapid and inexpensive diagnostic tests for infectious and other diseases, to be used for screening large populations in developing nations, and for providing immediate clinical information regarding impoverished populations within the United States. This technology should be based primarily on immunoassays and DNA/RNA probes. Advances will be linked to microfluidics, reagent design, and detection instrumentation.

Recommendations, Obstacles and Challenges

Encourage the development of basic point-of-care (POC) technologies for the detection of infectious diseases in developing countries and in impoverished populations within the United States. Some members of the panel felt that such sensing technology already exists for these types of clinical assays based on known immunoassay technologies and procedures. The principal problem seems to be a lack of a perceived economic incentive by large United States corporations to develop such tests because of low market value. Technological advances and proof of concept studies in areas related to diseases of vulnerable populations will not likely be undertaken by industry and therefore should be emphasized in future public sector initiatives.

An economic analysis of this market is recommended along with financial incentives to encourage small business to participate in the production and distribution of such clinical tests. This economic analysis should extend to the cost-benefit analysis for society as a whole, rather than just for the patients, providers and insurers. Questions were raised concerning who will pay for such technology and who will benefit.

Diagnostic tests are recommended for alternative fluids, such as saliva, urine, interstitial fluid, tear fluids, hair, nails, and sweat. This technology will have impact in point-of-care testing where blood sampling is not ideal. It is recognized by the panel that immunoassays with much lower limits of detection will be required to measure natural concentrations of clinically relevant analytes. Relationships between analyte concentration and clinical disease are clear in some cases but require further verification in some areas. Efforts to correlate such markers with disease status should occur in parallel with efforts to develop the diagnostic capability of monitoring such analytes.

Feasibility must be established for clinical diagnostics on the basis of breath analysis. Examples of potential analytes are keto-acids for diabetes and carbon dioxide following ingestion of labeled urea for the detection of helicobacteral ulcers. It is expected that elevated levels in exhaled breath will generally indicate an extreme imbalance in the blood chemistry, which may render this approach suitable only for initial diagnosis as opposed to monitoring. As noted above, a major effort is required to demonstrate functional correlation between potential biomarkers and disease. In addition, putative biomarkers must be rationally explained on the

basis of known biochemical pathways. Finally, the potential of rapid gas chromatographic techniques should be evaluated as a means to provide reliable and accurate clinical information. Miniaturized gas chromatography hardware should provide an interesting system for this type of analysis and will be capable of identifying and quantifying the major chemical components of the sample. This microfabricated chromatographic approach is an attractive alternative to the existing approach based on non-selective binding arrays (i.e., electronic nose).

Diagnostic systems were suggested for the detection and identification of microbial infection. It was recognized that improved amplification techniques are needed to enhance both detection and identification. One complicating factor is the inaccessibility of contaminating microbial cells, which can be located within tissue as opposed to blood. Therefore, the sequestration of pathogens in tissue will continue to make identification of pathogen-based disease difficult. The idea was proposed to base the analytical measurement on protein mapping as opposed to the conventional DNA analysis, which demands PCR amplification.

There is a need for optical detection of pre-cancerous and cancerous lesions. Real-time assessment could provide point-of-care diagnosis and treatment or imaging to identify invasive corners upon tissue removal. Such devices are 4-5 years from implementation for screening and detection of cervical and other epithelial cancers.

In Vivo and Continuous Monitoring

Objectives

This technology will be suitable for real-time monitoring with applications in intensive care units, less demanding point-of-care hospital situations, feedback control of active implants, and smart therapeutic drug delivery systems. This technology is based on electrochemical and optical sensing technologies, and includes both invasive and noninvasive measurement strategies. In addition, non-chemical transducers fall into this category and can provide information on pressure, temperature, impedance, etc. Advances are linked to the areas of biocompatible materials and a firm understanding of the chemical, biological, and physical characteristics of the *in vivo* environment.

Recommendations, Obstacles and Challenges

Biofouling and sensor stability are major areas of concern for sensors placed within the *in vivo* environment. These issues are critical for devices placed within both subcutaneous and intravascular spaces. Research must continue to explore the fundamental chemistry and biochemistry associated with the biological response to implanted devices. Methods to account for non-specific binding are critical and materials that minimize the impact of *in vivo* biologic effects must be developed. Suggestions were made to enhance biocompatibility efforts by mimicking natural, biological approaches. Another suggestion is to fully encapsulate the analytical measurement within a sealed and isolated implant and carry a sample to this measurement region through a selective biocompatible membrane.

Development of noninvasive and reagentless sensing schemes is recognized as an exciting direction for future research. Current efforts to develop noninvasive sensing technology for blood glucose sensing are recognized as one example of this approach that has received significant attention in recent years. It is recommended that this approach be extended to address other clinical situations, such as urea for hemodialysis optimization, lactate to monitor conditions of stress and shock, localized tissue pH variations, and variations in blood chemistry.

A major impediment to progress is the lack of high performance optics in the key spectral ranges that can pass through the human body and still contain molecular information. In addition, the development of noninvasive tissue imaging techniques that provide chemical information is recognized as a valuable direction for future research.

Nano-sensors and Molecular Probes

Objectives

These sensing devices are suitable for transducing chemical information from molecular regions within and surrounding individual cells. They offer the capability of monitoring *in situ* biochemical processes and therapeutic action of pharmaceutical agents within single cells. They can also be used as tools to explore fundamental intracellular and extracellular biochemistry, such as mechanisms of cell-to-cell communication and may be capable of 1) mediating drug entry into selected cells for targeted therapeutics or 2) identifying and destroying pre-cancerous cells. Advances are linked to nano-structures and materials, control of cellular and membrane biochemical systems, and reagent design.

Recommendations, Obstacles and Challenges

The general development of nano-technology should be encouraged, particularly in the context of developing novel reagents for clinical diagnostics. The inherent stability of nano-particles is recognized as a primary feature of this technology. In addition, the small size permits localization and distribution within single cells.

The idea was proposed to develop labeled reagents that can be injected into the body and then be chemically attracted to specific diagnostic targets. Although this basic idea is not new, the development of novel nano-probes and molecular probes should advance this concept. Efforts targeted toward cancer detection were cited as one example, but other targets should be considered.

Several members of the audience suggested that novel nano-technology systems could be used to enhance the rate of development of diagnostic systems. This should reduce the time required to advance a technology to clinical evaluation.

Bioinformatic Systems

Objectives

Systems are needed to acquire store, and analyze dissimilar sets of data. This initiative recognizes that new diagnostic systems will generate more data of different types. In fact, many of the session participants indicated that the present state-of-the-art diagnostic technology generates an overwhelming amount of clinical data. Systems must be developed to handle all these data and to make these data available to the research community, physicians, and patients for various purposes. Algorithms are needed to extract novel types of diagnostic information from this array of dissimilar data, such as identifying patients of high risk for specific diseases. Algorithms of this type may be used to diagnose disease states. Advances are linked to data mining strategies and the establishment of a central computer database of compiled diagnostic information.

Recommendations, Obstacles and Challenges

The idea of one large database with all available clinical data generated considerable discussion with concerns pertaining to individual privacy, ownership of the data, accessibility of the data, how the data would be used, and problems associated with acquiring accurate data. Nevertheless, the concept was generally supported and recognized as a potentially valuable national resource. It is recommended that NIH determine the feasibility of such a resource and define database content and format standards.

One approach to the above recommendation is to generate a mechanism in which individuals maintain a personal medical record, which includes diagnostic information. This information can be shared only with the person's permission.

General Recommendations

- There is a general need for new technologies for selective analyte binding. Suggested technologies include engineered proteins, signaling aptamers, ionophores, isolated ion-channels, and bio-designed polymers. Any new technology must be robust in function and stable for long-term applications;

- It is important to distinguish between monitoring technology and diagnostic instrumentation. They are subject to different regulation by FDA, and therefore have very different costs for development;

- Clinicians working on diagnosis of disease differ in their opinion of how much information is needed—some want any information they can possibly get about the health of a patient at the time of diagnosis of a disease, whereas others are content to get one piece of reliable information on which they can base a diagnosis;

- The fusion of multi-mode analytical information may be critical in diagnosis. NIH should consider chemical, physical and biopotential information as part of the total diagnostic/monitoring information. Miniaturization is a complementary feature that will permit multiple analyte detection and increase the information content of the measurement. It is important, however, to link treatment options to this multiplicity of analytical information. The need for technical assessment of new technology was mentioned in order to ensure that the information content of a putative device is in fact necessary and clinically valuable. A second complementary technology is on-line computations that will provide rapid and real-time diagnostic processing of information;

- Sample preparation remains a major problem for implementation of many (and perhaps all) sensor technologies, including extraction of nucleic acids from whole cells;

- Funding is necessary for faculty working on technology assessment and validation studies.

Informatics, Validation, and Computational Applications

Moderator

Denise Wilson Ph.D., University of Washington

Panelists

Greg Bearman, Jet Propulsion Laboratory
Karl Booksh, Arizona State University
Vijay Jain, National Science Foundation
Joseph Wang, Catholic University of America

Broad Statement

Signal processing that attends to sensors in the construction of effective sensing systems can be classified into the following three broad categories:

- Validation: verification that each sensor in the system is operating within the limits of a predefined calibration standard;
- Computing: conditioning of sensor signals followed by feature extraction in preparation for system-level decision making;
- Informatics: pattern recognition and interpretation of features and signals extracted by computing.

Vision

A wholistic approach to sensor development and validation in conjunction with appropriate computing and informatics is essential to the design of sensing systems that will effectively meet end-user specifications. Consideration of signal processing, calibration, and validation approaches should occur concurrently with sensor technology development. Concurrent engineering optimizes the interaction of system components in meeting targeted accuracy rates for recognition of analytes and events of interest in the complex and variable environments that are typical of sensing applications.

Objectives

The goal of validation, computing, and informatics is to optimize the performance of a single or multiple sensor system in transducing information from the sensing environment into a usable electronic signal for system level interpretation and decision making. Realizing this goal will require consideration of validation, computing, and informatics when designing sensing technologies for specific applications.

Consideration of sensor validation, both before and during operation in the targeted sensing environment, affects the choice of sensors and the computing architecture at the system level. Since global validation for a particular sensing technology, across all applications and all time is impractical, the development of validation data sets for groups of sensing technologies must focus on representative applications. Appropriate validation data sets will enable valid comparison of sensing technologies across different research and development efforts as well as establish the suitability of a sensing technology for a particular class of application. These data sets must be available to the sensor research community in a timely manner and should be chosen in such a way that they are usable across a variety of sensor technologies for a significant period of time (at least five years). During operation, computing of sensor signals

should validate the sensor against its calibration set by comparing sensor output to redundant sensors (based on the same ('like') and different ('orthogonal') technologies) in the sensing environment. Like sensors enable the detection of single sensor failures while orthogonal sensors enable the detection of systemic failure of a sensing technology as a result of extreme operating conditions. In combination, calibration and *in situ* stage validation of sensor behavior enables optimal performance in the decision-making model that performs the informatics function in the sensing system.

The architecture and signal processing ability of the computing component of a sensing system should optimize the information provided to the informatics (back-end) signal processing. Optimization of data transferred to informatics algorithms maximizes decision-making accuracy (e.g.; recognition of targeted biological components or events of interest) for a given set of informatics algorithms. Computing effort is divided between algorithm development and implementation considerations. Computing algorithms address a wide variety of signal processing needs including:

- the calibration of sensors at the front-end of the system;
- the conditioning of signals (e.g., normalization, noise reduction, scaling, common-mode rejection);
- transformations into an alternative domain (e.g., frequency or wavelet domains);
- extraction of signal features (e.g., mean, spatial and temporal gradients);
- compression of data;
- conversion of data to an appropriate protocol for communication;
- construction of spatially meaningful images (one, two, and three dimensions).

At the end of a successful computing stage, a suite of raw sensor signals is transformed into a meaningful set of low-noise features that is well matched to interpretation by the informatics in the system. Implementation of the computing algorithm is affected by the complexity of the algorithm as well as by power, size, weight, speed and overhead restrictions in the system. Implementation decisions include the choice of hardware over software and the choice of analog, digital or hybrid hardware. The adaptation of the system for the clinician or end-user is also a consideration in the design of the basic sensing system, as is the reconfigurability of the system to adjust to changing conditions of operation. Due to the complexities of the sensing environment and events/targets to be sensed in biological systems, layered implementation architectures are often preferred in sensing systems over single layer architectures.

The primary objective of the informatics is to calculate or recognize the biological elements or events of interest using the features extracted and signals conditioned by the computing stage in the signal processing flow. A wide variety of informatics approaches are available: parameteric and non-parametric, statistical or syntactic, linear and non-linear, established and novel. Intelligent algorithms must be developed and applied to separate composite molecular signatures extracted from sensors in a manner that accommodates blind source/signal separation, the absence of complete ground truth, and little prior information about pattern dependence.

Obstacles and Challenges

Explicit validation of sensors at the individual sensor level by standardized data sets and testing protocols is almost impossible because standardized applications for sensors do not exist. No valid standardized data sets can be generated that span the range of potentially viable applications and working environments. A validation data set or testing protocol is only appropriate across the range of experiences contained in the data set. In order to be useful, sensor validation sets must be applied locally to a class of applications. Sensor validation is a three-step process. First, the validation data set must be defined and constructed. Second, the sensor must be shown to function appropriately within the bounds of the data set or testing protocols. Third, every future sample analyzed by the sensor must be shown to lie within the bounds of the validation set and testing protocols. If a future sample lies beyond the validation set, the sensor has not been validated for that set of circumstances. The inherent challenge in the use of localized data sets is to ensure that any conclusions or benchmarks made for a sensing system are based on the a proper validation set matched to the application of the sensing system.

In order to validate the performance of individual sensors in real time, the capability to assess sensor response in the context of the environment and in the context of the other sensors must be present. For example, validation of the operation of a thermometer requires an external context such as another thermometer. An array of like thermometers would enable detection of a single thermometer failure but would not guard against systemic failure within a particular type of thermometer. In other words, using redundant (like) sensors provides validation of individual sensors but not of the sensing technology (systematic failure). The challenge is to identify a minimal set of redundant like and orthogonal sensors in a sensing system that enable validation of each sensor that is providing information to the decision-making process.

A final challenge in the validation of sensors is the need to understand how a sensor fails in order to address the challenge of effective failure detection. The onset of sensor failure might be detected in time dependent (high or low frequency) signal drift, slow response to finite impulses, or other symptoms. Failure can originate at the sensor/sample interface (i.e., fouling), in a transduction mechanism (enzyme decay), or in the electronics (RF interference or a short). Regardless of the origin of failure, a set of internal standards and controls that can detect and if possible, correct operation errors due to sensor failure must be part of the computational stage of signal processing.

From an informatics perspective, observations from sensors (when using multiprobe, multikinetics, or multispectrum measurement) are often composite signals of mixed hidden sources (e.g., mixed cells of different phenotypes, mixed binding of different receptors, mixed kinetics of different flows). Neither the true individual sources nor the mixing parameters are known, making it difficult to separate the information of interest from less relevant information in the decision making process. Issues of blind source/signal separation, and incomplete understanding of sensor behavior and experiment control further complicate the selection, training, testing, calibration, and validation of the best informatics algorithms. Optimization of the algorithm, once chosen, may be complicated in cases where there is little prior knowledge about the relationship between output patterns and input parameters (pattern dependence) exists.

Technical Recommendations

- Define categories of sensors in such a way as to facilitate standards and validation set definition within the defined classes;

- Develop infrastructure required to provide timely validation data sets for major categories of sensors applied to popular classes of biomedical applications;

- When applicable, develop experimental validation protocols that are compatible between in-vitro and in-vivo situations;

- Validation protocols must be hierarchical and technology/application specific, and must provide a means for standardized input including experimental protocol that is consistent with state-of-the-art sensing technology needs;

- Development of validation protocols should include input from the end user;

- Part of the validation hierarchy needs to support both system level testing (where user outputs such as false negative and false positive rates are the only indicators of system performance) and sensor level testing (where individual sensor behavior, such as noise and drift, are indicators of performance);

- Validation protocols should include the processing of peripheral influences, including sensor drift, in maintaining the calibration validation of sensors during operation;

- The timeline for validation is a major barrier to translation of technology to the community.

Funding and Organizational Recommendations

- Support needs to be committed to the development and verification of sensing systems including validation protocols, computing, and informatics;

- Organized, multidisciplinary efforts need to be dedicated to the development of standardized methodologies for extracting and validating information from biological sensors;

- Workshops, such as those provided by NIST in reference materials for biomedical applications, should be organized to support development of standards and validation protocols for sensor technologies;

- Separation of inputs into those of interest and those of non-interest is complex in sensing systems and does not lend itself well to standardization. For this reason, standardization of validation techniques, methodologies, and protocols will require a significant investment of effort;

- The development of wholistic systems (including sampling, sensing, computation, informatics, standardization, and validation) must be emphasized. An expanded wholistic focus will facilitate technology transfer of effort to usable, marketable sensing systems. Wholistic systems design needs to be structured so that the integration of parts into a coherent and useful whole is as central to the progress of the project as component development.

Recommendations for Standardization

- A significant need in the sensors community exists to define a universal standard for communicating sensor signals through signal processing flow (similar to that defined for

automotive applications). Definition of these types of standards should be driven by the professional societies or appropriate non-profit organizations (IEEE, AIME, ISO, etc.);

- Since standards for other technologies, such as those associated with imager-based systems, have not necessarily been driven by the government, it is quite feasible to negotiate definition of these standards via non-government sources;

- To involve industry in definition of computational standards, better communication of the benefits of these standards should be done by federal sources. The panel recommended looking to industry consortia rather than individual companies for such leadership roles;

- From a clinical trial approach, a serious need exists to standardize interpretation of the user interface for sensing systems. Standardization of the user interface needs to include a hierarchy in the ease and complexity of use (from a simple pushbutton-level interface to a more complex, information-rich interface).

Technologies for Predisposition

Moderator

Michael Heller, Ph.D., University of California, San Diego

Panelists

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Peter O'Connell, Ph.D., Baylor College of Medicine
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Broad Statement

Technologies for predisposition are important for screening large populations to (l) identify an individual's potential (i.e., genes, proteins, etc.) for disease and (2) identify new markers that can be associated with particular diseases. Recently developed technologies, including those based on gene chips and microfluidics, offer new opportunities for rapid analysis of genetic and phenotypic markers.

Vision

Once a method for preventing disease is known, a key element becomes identifying those individuals who are at risk for that disease because of genetics, environment or behavior. Genetic, physiological, anatomical, or chemical disease markers must be identified that can be detected by a sensor. Such sensors, when used in population screening to identify these people, must meet very high standards of speed, low cost, ease-of-use, robustness and reliability, and accuracy. The ideal sensor would accept a sample for analysis and yield an immediate “answer”: information regarding the disease of interest that is meaningful for the health care worker and the individual being screened. This ability to identify people prior to clinical onset of disease will provide savings in lives and money by preventing disease rather than waiting until expensive treatments are required.

Objectives

In many cases, a single parameter, even if measured accurately and reliably, may be insufficient to detect predisposition for complex diseases. This may be due to the large variation between individuals for any given marker. Ideal sensor systems would make multiple measurements – dozens to hundreds if a “fingerprinting” approach is employed - and contain software with sophisticated algorithms capable of integrating this information into a single output regarding the likelihood of risk to the individual for a particular disease or set of diseases. Therefore, it is important first to determine what the right multiple parameters to measure are. This research could be facilitated through the use of novel sensors. For instance, we need faster, more accurate tools, such as comprehensive gene sets on gene expression arrays, to identify the genes that contribute to disease. We also need tools to identify and assay multiple disease marker proteins and small molecules.

There is a need to establish reliable ‘gold standards’ for assessing new technologies for use in sensors, especially for those destined for the clinic or for screening populations. This is

imperative in order to judge current technology and hasten public acceptance of new more reliable technologies.

Obstacles and Challenges

Disease prevention will require a business model different from the current one in which pharmaceutical industries develop, produce and market pharmaceuticals to treat common diseases. These models require a large number of patients to buy the drugs because of the huge R&D, evaluation and promotional expenses. Prevention may more often rely on behavior modification and improved eating habits, which do not support large profits. A related obstacle is a lack of motivation that the public may have to participate in screening for predisposition to a disease. It may be difficult for people to understand the screening results given their statistical nature—a probability of coming down with a disease in the future. It will be necessary to make sure that the benefits provided by screening are apparent so that people will agree to be tested, and to follow up with the appropriate medical personnel should further steps be indicated by the test results. It is also important that the sensors employed are very accurate and reliable to minimize the number of missed high-risk individuals and eliminate false positives. The importance of high sensitivity and specificity, when trying to assess a probability of future disease in an individual, cannot be overemphasized.

Many ethical, legal and social issues surround questions of population screening. For instance, the inclusion in a group may be useful as a surrogate for the eventual determination of each individual's personal genetic variation. Statistical information on groups can be used today to identify people who would be good candidates for diagnostic tests, but society is uncomfortable with using physical characteristics associated with race and ethnicity to identify people for medical purposes. Another question concerns the placebo effect: can the knowledge of a high statistical likelihood of predisposition for disease lead to a real or apparent increased incidence of that disease?

The legal problems associated with deployment of imperfect yet useful technology need to be addressed in a country where people expect perfection from medical products on the market, and have a tendency toward litigation immediately upon discovery of an error. Although technology should be improved until errors are minimized, a parallel effort is needed to alter regulations to permit deployment of useful technology that isn't yet perfect. Along with the ability to test comes the need for policies to determine who should be tested, and for what diseases. Currently, liability mitigates against collecting more information than is necessary for any given purpose, but the most effective predisposition testing would test for the broadest possible array of diseases and conditions and individuals could be continuously monitored for infection and disease emergence to maximally reduce damage due to disease progression.

Devices for clinical use present a problem for manufacturers, in that fewer units are made than for consumer electronics, yet the quality must be higher, they must be more reliable, and relatively inexpensive. As technologies are developed, it must be kept in mind that they will have to be manufactured for relatively little money and without dedicated plants (which are likely to be too expensive and too inflexible). Furthermore, many effective sensor technologies exist that are not patented or patentable, and therefore won't receive much attention from the private sector; these technologies will only be developed into useful sensors under Federal support. Federal leadership will also be necessary to begin to resolve ethical, legal and social issues, because these sensors could do much to improve the health of the nation's citizens and those of the rest of the world.

Recommendations

- Many current technologies could be used immediately for population screening to substantial benefit, if the appropriate populations (e.g., at-risk) were to be identified;

- NIH can help to focus resources on diseases or conditions for which treatments are available, and that have highest prevalence and that cause greatest harm (in suffering and treatment cost);

- Tools are needed to identify biomarkers for disease and to form the basis for new sensors, such as comprehensive gene sets on gene expression arrays;

- There must be a new focus for sensor researchers on reducing false positives, and on making sensors robust and easy to use;

- Many effective sensor technologies will only be developed into useful sensors under Federal support. In addition, Federal leadership is needed to resolve the ethical, legal and social issues surrounding deployment of new sensor technologies.

Biointerfaces and Biomaterials

Moderator

David W. Grainger, Colorado State University

Panelists

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Anne Plant, Ph.D., NIST
Stuart K. Williams, Ph.D., University of Arizona
Kerstin Rebrin, M.D., Ph.D., Medtronic MiniMed

Broad Statement

Once a soft-tissue wound is created, virtually all materials placed into that wound and allowed to remain during the attempted healing process suffer a similar fate long term *in vivo*. While this reaction is dependent to some extent on implant site, all materials extracted from that wound site after several weeks are surrounded by a thin fibrous membrane produced by the host in response to the implanted foreign body. This highly collagenous capsule typifies biocompatibility for a large materials set, regardless of chemistry, modulus, texture or geometry. For the implanted sensor, the encapsulating reaction currently defines the performance limit. The fibrous tissue capsule, poorly vascularized with compromised transport properties, limits both diffusion of analytes and co-reactants (e.g., oxygen) between tissue and sensor, as well as products of sensor reaction chemistry away from the sensor surface. Mechanisms for this fibrosis are rooted in the poorly understood foreign body reaction and its cytokine signaling cascades at the implant site that recruit several key cell types. Establishment of the contiguous fibrous sheath *in vivo* around a sensing element signifies the chronic phase of this response and also of certain limits to analyte movement to and from the sensor. But must this also signify the beginning of the end for sensor performance?

What are the limits in implanted sensor performance when capsule formation appears ubiquitous? Can sensor design accommodate short-term performance in tissue prior to capsule formation, and function in a different performance mode *in vivo* after capsule formation? Can a sensor design dynamically respond to wound site healing histology to accommodate calibration requirements, analyte sensitivity and reliability? Can biomaterials and drug delivery approaches modulate healing enough to eliminate the capsule barrier problems?

The invasion of a sensor into tissue creates a wound. Clearly, the wound healing response in the presence of a biomaterial has certain abnormal traits compared to a sham wound healing response lacking an implant. Sensor biocompatibility is defined by this response, beginning with the first encounter of the sensor surface with host proteins, subsequent encounters with arriving immune cells, inflammatory cytokine cascades, recruitment of fibroblasts, and their fibrotic response locally around the implant. This abrogated healing response is a continuum of dynamic histological events that must locally influence the sensing environment just as dynamically and as continuously. The sensing surface constitutes one component of the sensing environment in this wound site. The surrounding tissue, responding also to the local trauma and inflammatory sequelae, constitutes the other half of this sensing environment. Mutual physiological influences, biochemical crosstalk, and signaling between the two halves are poorly characterized, poorly controlled, and poorly understood in most, if not all, sensor designs to date. Nonetheless, it is this complex environment in which implantable sensor performance and reliability is demanded.

In-dwelling sensors must not necessarily avoid the encapsulation response to be successful. Various capsule reactions are tolerable to permit sensing function; some can be temporally modulated, perhaps eliminated. Yet, reliable, predictable capsule response yielding reliable, predictable sensor response is required. And, importantly, site-to-site implant response, and species-species variability must also be considered very seriously. Add the influences of certain performance enhancing biomaterials features (porous texture, surface shielding from adsorbed proteins, drug delivery, low inflammatory potential) and advanced electronic signal processing algorithms to accommodate complex *in situ* sensing dynamics (and signal:noise limits) and, perhaps, a novel design with requisite new, improved sensing properties in an implantable context is feasible.

Vision

To solve the long-standing challenges preventing practical realization of a long-term implantable sensor, new innovative technical and scientific approaches must exploit recent advances and yet-undiscovered principles governing the behavior of materials implanted into wound sites. The sensor implant site must be considered as a wound bed with compromised, abnormal healing cascades that resolve acutely but persist chronically. These healing dynamics and their physiological consequences and impact on *in vivo* sensing must be appreciated, and to the extent possible, controlled.

The next phase of research in this area must directly resolve the problem of encapsulation by either processing sensor signals in spite of it, or adapting methods to alter it. The sensor and the tissue it affects can be separated into two intimately related problems. A team-based approach must be utilized that capitalizes upon talents from sensor engineers, pathologists and histologists, cell biologists, immunologists, and biomaterials scientists. Contributions from molecular and cellular biology, implant pathology, immunology will help advance knowledge of the tissue reaction to foreign bodies *in vivo*. Materials science, engineering design changes, pharmaceutical adaptations, and novel transduction mechanisms are needed to simultaneously improve the sensor performance component *in vivo*.

Objectives

- New knowledge of the foreign body response mechanisms, their kinetics, dynamics and details, including pathways leading to both foreign body giant cell formation and fibroblast recruitment in wound sites;

- Methods to evaluate the influence of the inflammatory reaction and cytokine presence in a wound site on sensor response;

- Design features for new sensors that enable accurate calibration throughout the wound healing process. This is likely to require dynamic and complex signal processing algorithms to adapt signals logically and reliably over time;

- Improved sensor designs that can anticipate the wound site dynamics and interface with tissue appropriately with multiple behaviors depending on the environment;

- Understanding of site-to-site variability in sensing response and its relationship to wound site-to-site changes;

- Understanding of species differences (histology, physiology, accuracy, weaknesses) in animal models used to evaluate sensors intended for human application;

- Standardization of experimental routines and regimens for *in vivo* evaluation of sensors as a function of site, species and analyte.

Obstacles

Current perceived obstacles are substantial and include:

- The device field is maturing from a materials perspective, but retains serious misconceptions of why devices fail *in vivo*. This includes a long history of disconnects between *in vitro* model test performance with actual performance *in vivo*;

- Lack of appreciation by many sensor engineers and clinicians for molecular details of implant pathology and device biocompatibility that involve wound healing mechanisms, cell signals and the foreign body response;

- Lack of correlation of protein adsorption profiles *in vitro* with biofouling consequences *in vivo*;

- Lack of ability to create and retain non-fouling capabilities on implant surfaces;

- Lack of understanding of what “non-fouling” might mean practically for implanted devices;

- Lack of strategies to control the cytokine cascades that regulate the entrance and reactivity of the many cell types into wound sites;

- Lack of ability to modulate or understand the dynamics of inflammation (cytokine profiles) that accompany foreign body implantation;

- Lack of ability to understand and control the formation of foreign body giant cells at the interface of sensors with tissues;

- Lack of ability to control the recruitment of fibroblasts to implanted sensor sites and their subsequent deposition of fibrous capsule around implanted sensors;

- Little understanding of the relationships between material properties and the inflammatory response;

- Lack of explanation for different sensor responses for identical experiments in different animal species;

- Lack of data for animal-human equivalence for sensor performance in identical formats;

- Lack of methods for generating rapid and retaining continuous perfusion and transport to sensor surfaces *in vivo*.;

- Lack of understanding of how neovascularization might be influenced by sensor implant designs and how to control it locally for optimal transport of analytes to and from sensor surfaces;

- Lack of adequate data on comparative animal models appropriate for sensing various analytes using various tissue compartments;

- Lack of comparative data for animal versus equivalent human performance for sensors as a function of analyte and tissue site.

Challenges

Several unresolved issues must be addressed. These include the capabilities to:

- Develop non-mammalian and animal model experimental systems where *in vitro* sensor performance mirrors or is accurately predictive of performance in human hosts *in vivo*. This includes protein adsorption behavior, fibrosis and inflammatory reaction assays universal to all materials and device biocompatibility problems;

- Produce a device surface *in vivo* that encourages vascular in-growth or integration;

- Produce a non-fouling surface that extends *in vivo* sensor longevity and performance;

- Address the issues of site variability in analyte monitoring, and standardize site selection;

- Address differences in different species models for sensing that plague consistency and interpretation of sensing performance within the field;

- Address the current confusion regarding calibration of sensors *in vivo* and its impact;

- Define in-dwelling sensor duty time scales where encapsulation reactions are not important (< 10 days?) and where such reactions are frequently deleterious to sensing function (>10 days?);

- Standardize sensor biocompatibility characterization protocols so that direct comparisons are made to inflammatory and biocompatibility assessments;

- Further characterize the extent (if any) that angiogenesis induction in proximity to the sensor surface might help both with analyte transport and biocompatibility limitations;

Recommendations

<u>Broad</u>

- Continue the emphasis via BRG and BRP mechanisms on a focused interdisciplinary, team-based approach to problem-solving. Sensor proposals should have clearly defined end-points that exploit sensing capabilities or solve performance issues with in-dwelling sensors. Teams might best include a broad talent base, including a sensor designer (e.g., analytical chemist or bioengineer), an animal specialist, pathologist, chemometrics or signal processing expert, or other appropriate talent that would accommodate a well-defined, focused and comprehensive experimental approach with statistically valid end-points;

- Propose a Request for Applications (RFA) aimed at in-dwelling sensor technical challenges in the short-term acute phase of implantation (< 7 days) versus chronic (long-term, > 7 days) implantation time domains. These sensing periods *in vivo* have substantially different challenges. Clearly defined performance enhancements expected should be defined;

- Propose an RFA focused on comparative animal models (e.g., canine vs. porcine, lapin vs. murine) for sensor performance assessments in defined tissue implantation sites (e.g., subcutaneous, intravenous, intracranial) for specific analytes of clinical interest for both acute and chronic implantation;

- Propose an RFA focused on comparing sensor performance in analyte-specific human vs. appropriate animal models for sensor performance assessments in defined tissue sites (e.g., subcutaneous, intravenous, intracranial) for specific analytes of clinical interest;

- Propose an RFA focused on comparative tissue site selection in human-implanted sensors to evaluate sensor performance in defined tissue sites (e.g., subcutaneous, intravenous, intracranial) for specific analytes of clinical interest;

- Emphasize relevant non-clinical, non-therapeutic sensor applications. Examples include the assessment of implanted sensors as *in situ* probes of biochemical and histological aspects of wound healing around foreign bodies, or dynamic assessments of foreign body response *in situ*, or as sensors for *in vitro* biochemical, cell culture or pharmacological assays.

<u>Specific</u>

- Standardize characterization protocols for assessing implantable sensor performance *in vitro* and *in vivo*. Include tissue site-specific, species-specific and sensor-specific (e.g., amperometric, optical, microdialysis) recommendations for characterization and evaluation;

- Attempt to eliminate use of *in vitro* assays that bear no relevance or exhibit no proven correlation to *in vivo* sensor performance. Emphasize direct experimental links between *in vitro* experiments and *in vivo* sensing end points;

- Establish validity of sensor calibration procedures and how incorrect or different calibration routines impact sensor performance comparisons;

- Emphasize the utility of well-defined angiogenesis designs and assays and performance influences on *in vivo* sensor performance. Additionally, emphasize the use of tissue perfusion assays using sensors to assess sensor-site neovascularization efficacy. A cocktail drug (e.g., VEGF) releasing approach should address issues of pleiotropic wound-site complications, and the ramifications of using only one selected drug or factor;

- Examine sensor implant site differences for different sensor modalities (e.g., potential versus current-based sensing, peroxide evolution, oxygen local consumption) and resulting changes in local tissue properties from different sensing modes using identical device configurations;

- Recognize the utility of developing effective acute and chronic sensors designed for analytes other than glucose;

- Evaluate the influence of different fibrotic capsule properties (thin, thick, vascularization vs. impermeable) on solute transport and sensor performance;

- Evaluate the effect of sensor surface textural influences (topology, porosity) on tissue response and integration using specific histology and biocompatibility assays. Systematize textural definitions for sensor materials surfaces;

- Emphasize an experimental description of sensing dynamics in the wound site in model systems. Evaluate sensor responses to various inflammatory influences and response elements (cytokine presence) *in vivo* as a function of defined foreign body response conditions and wound implant sites and times; and

- Use sensors as probes of wound healing physiology in appropriate animal wound models to profile foreign body responses.

Biomedical Microsystems, Nanosystems, and Integrated Devices

Moderator

David Beebe, Ph. D., University of Wisconsin-Madison

Panelists

Bruno Frazier, Ph. D., Georgia Institute of Technology
Abe Lee, Ph. D., University of California at Irvine
Lydia Sohn, Ph. D., Princeton University

Broad Statement

Miniaturization technology continues to advance with tremendous potential, but the application of micro/nano technologies to medicine and biology presents unique challenges.

Vision

Advances in microfabrication and nanotechnology have enabled the size reduction of individual sensors and actuators for biomedical and biotechnological applications. These advances have also begun to merge functions associated with computation, communication, and power together with sensing, actuation, and control to thus provide new opportunities for facilitating clinical medicine and biological research. Integration provides the basis for large arrays of devices, multiplexed functional systems, and platforms to interact with complex biological systems. The promise of miniaturization including components that probe at single molecule sensitivity combined in systems to monitor and manipulate many parallel events in real-time will provide powerful tools for molecular and cell biology research and medical treatment.

Objectives

Realization of this vision of miniaturized systems will require identification of both fundamental and practical objectives. Key objectives include improved sensing capabilities, complete integration across multiple scales, and improved understanding of clinical environment for miniaturized systems.

Obstacles and Challenges

Specific research problems in this area include:

<u>Improved Sensing</u>

Computation power and information flow have increased greatly over the past several decades. However, sensor technology has lagged far behind. That is, we now have incredible ability to handle and process large amounts of information, but we don't have adequate sensors for the chronic and accurate measurement of many important physiological and biomolecular signals. Detection technologies for sensor systems use a wide variety of sensing mechanisms from mechanical transduction to biochemical reactions. Existing approaches have found some success in acute sensing for well-prepared samples. Areas of sensing needs include sensing of analytes in complex mixtures, and continuous *in vivo* sensing.

The following points further illustrate the sensing challenge.

- Can multiple detection technologies be used to increase selectivity and provide complementary information in other areas of bioanalysis (e.g., cell-based analysis, ionic analysis, proteomics)?

- How does one balance the need to sense analytes in complex mixtures vs. requirement to purify/enrich before sensing? One requires a more complex sensor/strategy, other requires more complex front end to process the sample;

- What is the potential for making dynamic continuous *in vivo* measurements? One of the difficulties of dynamic measurements is that many sensing methods require destruction of the biological material. Reducing the amount of material needed is one partial answer, if one can obtain "identical" material for the next time point.

System Integration

One square inch of skin contains 9 feet of blood vessels, 600 pain sensors, 300 sweat glands, 13 yards of nerves, 9000 nerve endings, 36 heat sensors, 75 pressure sensors and more. Clearly, engineers have a long way to go to achieve a similar level of system integration and functionality. Currently, system components (sensors, actuators, vessels, etc.) are made using a wide variety of methods and materials making integration inherently difficult. Integration of complex function in a cost effective way is one of the challenges in developing biomedical micro/nano systems. Natural systems have evolved very simple and elegant approaches to achieving complex functionality far beyond the capabilities of man-made synthetic systems.

The following points further illustrate the system integration challenge.

- There has been progress in detecting and imaging molecular events for applications ranging from early detection, to sensors, to molecular medicine. However, most of the techniques and assays developed are not easily multiplexed and integrated together. One approach is to create "active" micro/nano scale platforms that have the potential to be programmable for complex biomolecular analyses;

- Simple closed loop feedback control is still beyond the realm of most microsystems, yet this remains the promise of microsystems. Future technology platforms must integrate many more components and functionality onto single chips. Biomedical instruments and devices that co-locate microsensors and microactuators will allow the physician to make therapeutic decisions at the point-of-care;

- Rather than relying on the measurement of a specific analyte, sensor fusion and/or combinatorial approaches that detect a variety of signals may be optimal. It is important to keep in mind that different signals may require very different sensor solutions, which in turn presents a larger challenge in sensor integration onto a single platform.

Clinical environment

A final challenge is to leverage the knowledge gained (e.g., technologies developed, systems demonstrated) through research and development efforts in micro/nano scale technology to maximize the impact in the clinical environment of the future. What are the clinical applications for which microanalysis systems are enabling, or offer a tremendous time savings, or offer other significant advantages? Will more sophisticated miniaturized diagnostics systems become available for over-the-counter or prescription sales? What effects will this have on the clinical environment?

Recommendations

The following recommendations are suggested to address the broad challenges outlined above.

Sensing

Several specific recommendations emerged with respect to sensing issues. First, there was a general feeling that often very simple sensors can provide quite useful information and that NIH should encourage review panels to take this into account when comparing sensing proposals. Second, sensor fusion (i.e. the use of multiple sensing mechanisms/modalities) is important and should be encouraged. Third, sensors that predict device failure may provide great benefit.

Additionally, approaches for accurate sensing in complex environments (chronically implanted sensors, raw blood/saliva samples) require investment. Many analytes of interest are one component of a complex raw sample. This is especially true with respect to samples obtained from animals. Therefore, it is important that sensing systems have a means of obtaining specificity of analysis. Sensing in complex environments is subject to the same performance issues as other sensing systems. How do you keep selectivity and specificity at maximum levels? How does the microsystem, detection, and sample react with the surrounding environment? Can we create systems that actively respond to the changing physiological state of the patient? These are very challenging issues that will require a sustained investment to solve.

Leveraging Existing Platforms/Approaches

Encourage approaches that leverage existing technology platforms. This is particularly important for clinical and *in vivo* applications where regulatory issues often drive and limit the development of technology. By using existing (already approved) technology platforms, the time to clinical use can be shortened. Specifically, this would be facilitated by improved mechanisms for academics to work with industry as commercial companies typically have the approved technology platform, but business goals often prevent exploration of alternative uses.

Programmatic Recommendations

While NIH has made significant progress in incorporating bioengineering themes, ideas and expertise into its mission, further efforts are warranted. As evidenced by the discussions in our session, good progress has been made in bridging the engineering – biology gap, but the engineering – clinical gap is still wide. Thus, we feel it is important for NIH to take a lead role in bringing these communities together. Specific mechanisms might include: (1) Programs to provide incentives for clinicians to spend time with engineers (and vice versa) (e.g. mini sabbaticals), (2) Tutorials on technology at clinically oriented conferences, (3) Create a focused bioengineering conference grant program, (4) Encourage training programs at both the undergraduate and graduate level that provide engineering students with exposure to the clinical environment. More generally, NIH should continue to stimulate interdisciplinary training and research (e.g. BRG and BRPs are good examples) with a specific focus on engineering/clinical interactions.

Two other specific suggestions emerged. First, create a database/clearinghouse for building research teams with relevant skills/knowledge (examples exist within DARPA). Second, create dedicated instrument development programs with review process that ensures that the end users' needs are being met (but without a bias towards purely hypothesis driven research).

Science

Research in basic science areas relevant to micro/nano systems for medicine and biology should be encouraged. Basic research in sensing and actuation mechanisms (in the context of adaptation or merging with synthetic systems) should be fostered. Nature's sensing ability is exquisite and we would be wise to mimic it and use it whenever possible. The interaction of living systems with micro/nano environments will also be critical as the field advances. Microenvironments and surfaces have been shown to interact with living systems in interesting ways. The ability to engineer environments that more closely resemble natural environments has clear potential. For example, there is a need for systems that enable *in vitro* tests that provide more useful and relevant information to minimize the need for animal and human testing and also to expedient development times of new therapies.

Integration

Almost every use of micro/nano systems will require the integration of multiple functions to achieve performance and cost advantages. One can argue that if we want to sense complex systems, we will need complex systems to do so. However, an investment in research towards practical system integration has been limited. Many design concepts are possible: programmable vs. dedicated devices, disposable vs. re-usable, organic vs. inorganic, electronic vs. ionic. Research is needed to explore the many possible paths to system integration with a focus on those with the most promise to meet the demanding needs of *in vivo* applications where true micro/nano scale integration will have the largest impact but also where integration is most challenging to the additional layer of issues: biofouling, calibration, reagents, recording/reporting of results, insertion/retrieval. What are appropriate niches in research, clinical applications where integrated systems can have the most impact?

Cell-Based Sensing

Moderator

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Panelists

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Broad Statement

Some sensor strategies are based on incorporating mammalian cells with electrical or optical transduction schemes. The use of cells as a sensing component results in high specificity and efficient amplification in the detection of biosensitive targets. This session will explore challenges associated with cell-based sensors and opportunities for future research.

Vision

Cell-based sensing involves the processes of detection, amplification and reporting of molecular level information in cellular systems. Advances in molecular sensing will require interdisciplinary approaches and will have broad applications in the development of novel materials and basic research in cell biology.

Objectives

The further development of cell-based sensors would hasten their use in medicine, chemical-threat identification, drug discovery, disease-marker identification, physiological measurements and numerous other uses. Solving use-specific issues such as physiological relevance, standardization, manufacture, sensitivity and cost issues will enable this technology to become mainstreamed.

Obstacles and Challenges

- The single most important advantage of using cell-based biosensors over other detection systems is that the information they provide should be more physiologically relevant than other detection systems, because of the use of tissue specific cell types on the sensor. An obstacle associated with their further development is the determination of appropriate cell types and cell densities to use for a specific endpoint;

- An enormous problem in this field is that by nature, cells are differentially sensitive to environmental stimuli, such as temperature, G-forces, culture medium, and barometric pressure. Complicating the mainstream use of cell-based biosensors is the absence of defined methods for the manufacture and transport of these sensors considering the condition of the cells attached to the sensor at its final place of use;

- The practical use of cell-based biosensors depends on the measurement of endpoints that appear in a timely manner. The development of timely acquired endpoints in these sensors is necessary for their general acceptance;

- Currently, cell-based sensors are not widely used due to the costs related to their development. Until a cost benefit can be demonstrated with cell-based biosensors over sensors of other types, the mainstream use of this technology will not be achieved.

Recommendations

- Support research to develop cell-based sensing applications for disease specific biomarkers or molecules that will preliminarily focus on the development and standardization of cell-based biosensor;

- Encourage research focusing on the construction, production and shipping of sensors to people interested in working with sensors. The sensor community, like others, believes that the NIH does not provide research monies for research that is not entirely hypothesis driven, and realize that the funding of this would be largely development, but would provide many benefits in the future;

- Support research to develop markers that will hasten the identification of endpoints. As with any technology the faster a procedure or device works, the sooner that it will gain acceptance by a user community. Research of this kind will identify molecules and/or biomarkers that occur earlier than any of the known endpoints following cell exposure;

- Support a single site to act as a cell-based sensing core facility to function as not only a core facility but also to address development, standards and reliability issues for a single biomarker or molecule. The startup costs for this technology are presently quite expensive and a core facility could enable this technology to be used by more investigators;

- Convene small meetings between engineers and biologists interested in cell-based biosensors to exchange ideas and develop collaborative efforts. The meeting can be designed as a general meeting for all those interested in cell-based biosensors or all whom are working on developing specific cell-based sensors for neurology, immunology, microbiology or other scientific areas.

Emerging Transduction Technology

Moderator

Timothy M. Swager, Ph.D., *MIT*

Panelists

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Jack Judy, Ph.D., University of California, Los Angeles
Gabriel Lopez, Ph.D. University of New Mexico
Susan Trolier-McKinstry, Ph.D., Pennsylvania State University

Broad Statement

Chemical and biological sensors must generally combine selective recognition of a desired target with a transduction process that translates the binding event into a signal. Incorporation of innovative nanostructures, electronic materials, molecules, and instrumentation that use biological functions or structures into transduction processes offer opportunities for designing sensors with significantly improved sensitivity and response time. This session will address emerging transduction strategies and possible applications for sensor development.

Vision

Advances in identifying new biomarkers, quantification, sensitivity, continuous monitoring, cost and manufacturing will lead to a greater ability to detect, diagnose and treat diseases using sensors.

Objectives

Discussion revolved around a number of interconnected issues associated with needs/limitations/utility of arrayed and multiplexed sensors, lack of quantification in many technologies, specificity, and high sensitivity. The details of these issues varied depending upon the context in which a sensor is used (e.g. discovery vs. clinical or single use vs. continuous monitoring). For example, single use sensors need only be inexpensive and accurate, however sensor elements that need to make multiple measurements or work in a continuous mode need to be both accurate and reliable. This report will therefore discuss the relevant findings in terms of the context in which a sensor will be used.

Challenges and Recommendations

<u>Identifying New Biomarkers</u>

New biomarkers are necessary to best diagnose many diseases. Whereas NIH has active programs directed toward this end, new sensor methods could be developed that would dramatically enhance this critical need. Therefore , NIH should initiate a program designed at developing sensors for biomarker discovery and partner sensor researchers and their technologies with ongoing or new biomarker identification efforts.

Diseases that can be diagnosed on a singular biomarker are the exceptions, and to best find the multiple biomarkers associated with most complex diseases, a multiplexed measurement that can simultaneously monitor many potential biomarkers should be used. Multiplexing can take on many forms. Arrays of sensors with cross-reactivity inherently give a multiplexed response, separation methods can spread analytes out spatially, or coupling to other innovative collection

and capture mechanisms can deliver analytes sequentially to a sensor. The multiplexed data need not be from a single sensor type and multiple orthogonal sensory methods can be used. These multiplexed measurements are critical in understanding Systems Biology and establishing relationships. Multiple classes of molecules (i.e. proteins, cells, metabolites) need to be rapidly measured, thereby requiring considerable diversity in the sensor methods. The complexity of the data generated in these schemes makes for challenges in analysis and the development of database architectures and data mining are critical to the success of such an approach. To best ferret out obscure and/or unknown biomarkers, the multiplexed data should examine broad chemical and biochemical spectrum. Once the character of a biomarker is known sensors with more specific responses and even conventional analytical methods (i.e. chromatography, mass spectrometry) can be used to establish robust correlations. It was the consensus that broad spectrum cross reactive-arrays would potentially be an ideal tool for biomarker discovery but would be of little use in clinical settings. The commercial sector would be most interested in sensors that accurately measure a small number of key robust indicators.

The potential for new biomarkers to impact healthcare is self-evident. To illustrate the potential for a novel biomarker discovery program the example of finding new remote biomarkers was given as an important problem. It was stated that numerous efforts to date had failed to produce remote biomarkers for brain cancers. It was strongly suggested that there is likely a set of biomarkers in readily accessible bodily fluids that had defied identification and that a sufficiently good multiplexed analysis may reveal these factors.

A number of the other sensor issues to be discussed below are critical to the sensor requirements needed to build a superior biomarker discovery effort.

Quantification

A number of sensor schemes have severe limitations due to the inability to produce quantitative analysis. Chip-based schemes and any method wherein the transduction events require immobilized recognition events at an interface (e.g. ELISA) cannot be relied upon for determining concentrations. Cross-reactive arrays are another example of systems that lack the ability to give accurate concentration data. Future efforts in biomarker discovery and disease diagnosis must utilize concentration variations of proteins and small molecules. Indeed in many cases concentration differences may be the only biomarkers.

The potential of chip-based systems for generating a large array of multiplexed data at a low cost underscores the need for new approaches to produce quantitative data from these devices. The hard work of perfecting a sensor to provide quantitative data may not be considered glamorous when compared to detecting an analyte at lower concentrations or with a novel scheme. However, this is one of the most significant limitations to assays/sensors that involve immobilized recognition and/or transduction events at interfaces. It was also pointed out that there are other methods that also have limited quantification, for example mass spectrometry suffers from similar limitations due to the difficulty in volatizing molecules. For the cross-reactive array technologies the analytical limitations are a result of the fact that the responses of multiple chemicals from a non-specific array is impossible to completely deconvolute. Better computer algorithms and greater discrimination (orthogonally) between sensory elements of the array can limit the overlap of the response space of particular analytes and thereby allow for better quantification.

Greater emphasis on quantification must be encouraged by NIH. In fact it may be advisable to encourage proposals that are principally directed at achieving this goal. An interim solution could involve hybrid sensor schemes using established quantifiable methods with chip-based

sensors and cross-reactive arrays. There was also the suggestion that systematic modular approaches would be a superior and more methodical approach to improving chip-based sensors. Innovations that may be applied broadly to existing schemes would have a particularly high pay-off.

Continuous Monitoring Coupled to Drug Delivery

Sensors that can function continuously in clinical or home health care environments present some of the biggest technological and scientific challenges. For a sensor to deliver reliable and reproducible data, it must be very robust and immune from background interference. It was suggested that in some cases the obstacles might be insurmountable and that a more pragmatic approach would be to make frequent measurements with low cost single shot sensor elements. Nevertheless, it was argued that in some situations continuous monitoring is absolutely necessary. Examples of needs that are served by continuous sensors are: (1) implantable sensors for automated monitoring and medication, (2) the observation of correlated changes in multiple biomarkers as they relate to a disease. Implantable glucose sensors for the treatment of diabetes have long been desired as critical elements in automatic sensor/drug delivery systems. There are numerous other diseases that could benefit from implanted sensors, such as rheumatoid arthritis, cancer, and schizophrenia.

The scientific and technological challenges for continuous monitoring are considerable. Biofouling of implantable sensors is one of the best-known problems encountered. The problem is much harder than those encountered in other implants (i.e., hip replacements, pacemakers) due to the fact that the biological fluid-sensor interface needs to be capable of accurately transducing the presence of the analytes. The use of electrically or magnetically active materials to either shake the surface at the biointerface, or to provide local electrical discharges to prevent the onset of protein binding are a potential solutions. A further challenge is the fact that a sensor would optimally require a recognition event that doesn't require chemical or mechanical resetting. Enzyme coupled sensors are desirable because they can be highly specific and the analyte is consumed by the enzymatic action. However, enzyme based schemes have limited application and more general methods are needed. High specificity in a tight binding non-enzymatic receptors leads to slow dissociation of the receptor-analyte complex, thereby complicating continuous monitoring. Extending continuous monitoring technologies will require new receptor designs that allow for highly selective and chemically reversible recognition events. For example, methods are needed for triggering dissociation of analytes from antibodies. Other general approaches for the development of reversible highly selective synthetic or semi-synthetic receptors would also be valuable. Synthetic approaches offer design flexibility for optimizing transduction events and quantification. Alternatively, engineering solutions for refreshing (resetting) sensors or for temporal addressing of sensor arrays could also be developed that allow for continuous sensory monitoring.

Improved Sensitivity

Greater sensitivity is a common need in sensors. High selectivity must accompany gain mechanisms to make them effective. Otherwise, noise and signal are both amplified with no increase of the signal-to-noise ratio. Novel ways to produce high sensitivity are needed. Sensitivity is one of the limiting factors in cross-reactive array technologies due to the fact that these methods must expose the sample to many sensory elements. Ion channels are a key biomimetic method that could be used to provide ultrahigh sensitivity. Natural ion channel systems are too fragile to be practical and synthetic systems should be developed. Ideas such as the use of a synthetic or semi-synthetic ion channel into liposomes and devising ingenious ways to read ion channel activity may be more practical sensory schemes.

Conducting/semiconducting polymers, high performance piezoelectrics, conjugated polymers and magnetic materials may provide additional gain. New materials advances should be exploited in sensor technologies if they can be made to have sufficient selectivity. New highly responsive materials may also have applications other than being the transduction agent. An example offered was the use of sensory elements connected to a set of different superparamagnetic particles. Depending of the size of the particles, families of sensory elements may be made collected at an interface sequentially by applying different field strengths. Such approaches allow for new multiplexing schemes.

The use of nanotube and semiconductor nanowire sensory devices may provide high sensitivity but the Department of Defense (principally DARPA) is making sufficient investments in these technologies. There is little confidence that these methods could move beyond one-of-a-kind devices and that no published device has ever been subjected to proper control experiments such as "real" conditions with a multitude of proteins, small molecules, and ions. While sensational results have been reported in the literature over the last few years, these methods need to be properly tested before NIH expends valuable resources on them.

Cost and Manufacturing

One-shot (disposable) sensor elements have a number of advantages and potentially pose less daunting scientific and technological hurdles. However, they do have the strict requirement that they cost very little. Hence, the ability to manufacture these materials efficiently from inexpensive materials is critical. The biomolecular recognition elements are often very costly, however the fact that the amounts of this can be vanishingly small doesn't preclude expensive materials in miniaturizable devices. Miniature devices often have surface-immobilized transduction elements and hence quantification issues need to be addressed.

Schemes that are amenable to low cost production of single use sensors need to be encouraged. Roll-to-roll continuous processing was offered as a means to reduce production costs. Indeed the success of the photographic industry suggests that complex chemistries and biomolecular units can be assembled easily in roll-to-roll processes. Such methods have yet to be widely applied to sensor production beyond the simple litmus paper type of test. Many of the electronic elements are compatible with this technology and hence there are no obvious "show stoppers".

Underserved Markets

Our national interests require attention toward solving problems of the third world and of creating technology to diagnose and treat diseases that do not directly afflict our homeland, and are not of interest to US commercial enterprises. Examples, of diseases that are underserved include tuberculosis and malaria. It was mentioned that global warming could make malaria a direct concern to the US public. By investing in technologies for these diseases, NIH has the ability to create an artificial market much like the DoD has for technologies relevant to national security. The volume for many detectors related to national security is sufficiently low that an economic incentive for privately funded development programs to develop these technologies doesn't exist. The NIH could coordinate strategic planning with agencies/organizations that are interested in procuring systems.

Enabling Concepts and Materials for Future Biomedical Sensor Technology

Moderator

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Panelists

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Broad Statement

The development of new sensing platforms and new functional materials can have important implications for biological and medical sensors. Specific areas with substantial promise for future improvements in micro-scale integration of sensors and improvements in sensitivity include micro- and nano-technology, functional materials (e.g., dendrimers and switchable polymers) and advanced material concepts (e.g., single molecule techniques).

Vision

While substantial advances have already been realized in the development of biomedical sensors, the fields of nanotechnology, biomaterials, chemistry, and computer science offer opportunities for substantial advances and improvements in the future. Specific areas that show promise for advances in the near term include molecular probe development, novel and improved sensing modalities, nanoscale sensors and actuators, and computational modeling.

Basic questions that need to be addressed include (1) what specific applications of sensors to biomedical research and clinical use are important with regard to future research directions and areas of support, (2) what are the important issues to be addressed with regard to future sensor research and development, (3) what technologies show special promise for advancing future sensor development, and (4) what materials show special promise for advancing sensor development and application?

Obstacles and Challenges

Specific research areas and related issues concerning future sensor development include:

- A key aspect of biomedical sensing is the development of new and improved molecular signaling probes – particularly those that can provide information on both the cellular and tissue levels. Preferably these new agents will facilitate measurement of multiple signatures, can be delivered to the target tissue, and will exhibit specificity. New probes need to be developed for numerous applications including labeling of DNA and RNA, live cell indicators, gene expression, membrane potential, vesicle tracking, etc. This research will require multi-disciplinary, integrative, and collaborative research;

- Probe development for *in vivo* settings is essential for medical applications and long-term monitoring and therapy. Issues that need to be addressed include biocompatibility of materials, biodegradability, long-term implantation, and tissue interactions with inorganic materials. In addition to the technical issues, other concerns that need to be addressed include cost, ease of production, chemical performance and characteristics, and self-assembly;

- Novel sensing modalities (noninvasive and invasive) need to be developed that are applicable to clinical and *in vivo* environments. Optical modalities such as near-infrared excitable fluorescence agents and sensing with molecularly-targeted probes offer promise but need to be developed for general application;

- Suitable *in vitro* and animal models must be developed, standardized, and distributed to provide mechanisms to validate and test novel sensor systems;

- Research on integrated sensor systems (detector/transducer/actuator) is necessary to develop systems capable of therapeutic action and remote monitoring. These systems will require substantial computational and telehealth components. The application of computer science and technology to sensor development and application needs to be promoted.

Recommendations

- Multi-disciplinary, integrative, and collaborative research is necessary to address challenges associated with future sensor development. NIH must develop mechanisms and programs to encourage and support this type of research;

- Research and development specifically aimed at sensing modalities needs to be supported. Specific modalities showing substantial promise include optical systems (near-infrared excitable fluorescent agents) and high-field magnetic resonance (noninvasive imaging);

- Nanoscale research should be supported, especially that aimed at probe development, bioNEMS applied to actuators, integrated sensor systems, and the development of nano-composites that act in concert with sensors (e.g., internal light sources);

- The development of good animal models of human disease is necessary to validate and test sensor systems;

- Funding levels associated with innovative programs (e.g., R21) should be evaluated to ensure that levels are adequate to support multi-disciplinary research;

- Computer and mathematical applications for sensor research must be supported including algorithm development, modeling biological processes using nanosensors, and modeling integrated system performance;

- Related aspects of sensor applications and research must be considered in addition to technical issues. These include FDA approvals for *in vivo* use, legal aspects of remote monitoring and patient record transmittal, and cost/availability issues for research materials.

Invited Participants

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