

WTEC Biosensing Study, U.S. R&D Overview Workshop
Draft Agenda

National Institutes of Health
Natcher Auditorium
Balcony C

December 3, 2002

<u>Time</u>	<u>Presentation</u>	<u>Presenter</u>
9:00 AM	Welcome	Christine Kelley, NIH/NIBIB
9:05 AM	Introduction to the study	Jerry Schultz, Univ. Pittsburgh (WTEC panel chair)
9:10 AM	Opening Remarks	Milan Mrksich, Univ. of Chicago (WTEC panel vice chair)
9:15 AM	Introduction to bio/cell-based sensors	Sangeeta Bhatia, UCSD (WTEC panel)
9:20 AM	Living cells as sensitive sensors	Robert S. Burlage, Ph.D., Department of Health Sciences, University of Wisconsin-Milwaukee
9:55 AM	Controlling the behavior of cells at interfaces	Chris Chen, Johns Hopkins Univ.
10:30 AM	break	
10:45 AM	Mammalian cell sourcing for use as sensors	Ron Faris, Brown Univ.
11:20 AM	Introduction to electro-chemically based sensors	Milan Mrksich, Univ. of Chicago (WTEC panel vice chair)
11:25 AM	Electrochemiluminescence Based Micro-Array Systems for Biochemical Assays and Detection of Biological Agents	James L. Wilbur, Ph.D., Meso Scale Diagnostics, LLC.
12:00 PM	lunch (Natcher cafeteria is recommended)	
1:00 PM	Bio/Chemical Sensing using Thin Film Recognition Elements	Dick Crooks, Texas A&M University
1:35 PM	Introduction to informatics and system integration	Imants Lauks, Epocal (WTEC panel)
2:10 PM	break	
2:25 PM	Rapid, Reliable, Confident PCR for Bio-Detection	Kurt Petersen, Cepheid
3:10 PM	Sensors/systems fusion overview	David Brady, Duke Univ. (WTEC panel)
3:50 PM	Network integration	TBD
4:25 PM	Introduction to optical methods	David Walt, Tufts University (WTEC panel)
4:30 PM	Chemical and Biological Sensors based on Optical Properties of Materials: luminescence, reflectivity, and plasmon resonant effects	Michael Sailor, UCSD
5:05 PM	Adjourn for the day	

December 4, 2002

<u>Time</u>	<u>Presentation</u>	<u>Presenter</u>
9:00 AM	Welcome	Geoff Holdridge, WTEC
9:05 AM	Optical Methods (recap)	David Walt, Tufts University (WTEC panel)
9:10 AM	Optical Arrays for Biosensing	Frances S. Ligler, Center for Bio/Molecular Science & Engineering Naval Research Laboratory
9:45 AM	Surface Methods for Optical Biosensing	Richard Van Duyne, Northwestern Univ.
10:20 AM	break	
10:40 AM	Mass. Spectrometric Methods.	Charles Wilkins, Univ. of Arkansas (WTEC panel)
11:15 AM	Introduction to mass sensors, MEMS, and microfluidics	Tony Ricco, Aclara (WTEC panel)
11:20 AM	MEMS for biosensing applications	Michael Roukes, Caltech
11:55 AM	Lunch (Natcher cafeteria is available)	
12:55 PM	Mass-sensitive devices for biosensing applications	Amit Lal, Cornell Univ.
1:30 PM	Micro- and Nanofabricated Fluidic Devices for Biosensing	J. Michael Ramsey, Oak Ridge National Lab.
2:05 PM	Review of U.S. Government Agencies' Interests and Activities (introductions by Geoff Holdridge, WTEC)	
2:05 PM	DOE	Dean Cole (BES), NNSA representative (invited)
2:20 PM	NIH/NIBIB	Christine Kelley
2:30 PM	NIH/NIA	Winnie Rossi
2:40 PM	NIH/NIAID	Gregory Milman
2:50 PM	Air Force	John Brewer
3:05 PM	NRL	Richard Colton
3:20 PM	DARPA	Millie Donlon
3:35 PM	USDA	Dan Schmoldt
3:50 PM	NSF	Fred Heineken, Bruce Hamilton
4:05 PM	NIST	John J. Kasianowicz
4:20 PM	Other invited agencies (NASA, ARO, EPA, CDC, etc.)	TBD
4:45 PM	Discussion	
5:00 PM	Conclude	

WTEC Study on

BIOSENSING RESEARCH AND DEVELOPMENT

PROCEEDINGS OF THE DECEMBER 2ND – 3RD 2002 U.S. REVIEW WORKSHOP

Final Proceedings

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PREFACE

The National Science Foundation, the National Institutes of Health, and other agencies of the U.S. Government have asked the World Technology Evaluation Center, Inc. to perform an assessment of status and trends in biosensing research and development around the world in comparison to that in the United States. The purpose of this study is to assess the U.S. biosensing R&D effort in comparison to activities abroad, provide the scientific/engineering community with a critical view of the field and identify the most promising areas for future research and industrial development, stimulate the development of an interdisciplinary and international community of biosensing researchers, and identify opportunities for international collaboration in the field. WTEC has recruited a panel of U.S. experts in the various related fields to perform this assessment (see inside cover). The panel is charged with analyzing and comparing research in the United States with that being pursued abroad. This panel has will visit relevant R&D facilities in Japan and Western Europe during the winter and spring of 2003. Prior to these visits the panel first needed to develop an understanding of the state-of-the art in these technologies in the United States.

Towards this end, WTEC invited leading U.S. biosensing researchers to a workshop held at NIH in Bethesda, MD on December 3 and 4, 2002. This volume is a collection of papers presented at the workshop. Paper authors were asked to provide a broad description of all related U.S. work in their respective fields (i.e., not necessarily just the activities in their own laboratories). Authors were chosen to be representative of cutting edge U.S. research in each of the topic areas. This edition also includes overviews of each session prepared by the respective WTEC panelists, as well as some revised contributions.

Thanks to all the participants for their contributions to this volume and the workshop, and to our sponsors for supporting this activity.

Sincerely,

Geoff Holdridge, WTEC Vice-President

World Technology Evaluation Center (WTEC), Inc.

R. D. Shelton, President

(Staff working on this study)

Geoffrey M. Holdridge, Vice-President

Bobby A. Williams, Financial Officer

Roan E. Horning, Project Manager

Hassan Ali, Consultant. Japan Advance Contractor

Nick Clemens, Consultant., Europe Advance Contractor

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EXECUTIVE SUMMARY

Workshop to Review U.S. Science and Technology in Biosensors

The WTEC study panel hosted a two day meeting in Washington DC to survey the state-of-the-art and current trends in biosensor technologies within the United States. Several sessions, with each addressing a technical theme in biosensors, provided an overview of recent successes in technology development and of the barriers for translating these technologies to the marketplace. The workshop identified several general needs that pertain to the broad field of biosensors, and specific needs for each technical theme.

GENERAL NEEDS AND APPLICATIONS

- ∄ Rapid, inexpensive, and broad based tests must be developed for detection and identification of toxic materials and organisms.
- ∄ Standards must be developed for validation and comparison of technologies.
- ∄ Methods are required that can be fielded as sentinels in the environment to monitor food, water, soil and air quality.
- ∄ Improved sampling and preprocessing techniques are needed.
- ∄ System automation for unskilled operators needs to be addressed

OPTICAL METHODS

Growth in optical methods stems largely from the developing infrastructure in the telecommunications (optical components such as new light sources, detectors, and optical fibers) and computer sectors (ability to read decreasing feature sizes in optical storage media, such as DVDs). These components enable smaller, low-powered, integrated devices to be prepared and used for a variety of fieldable biosensing applications. Because multiple frequencies can be employed without interference, optical methods are well suited for multi-channel assays. This aspect is important since many applications require the measurement of a panel of analytes.

The most well established optical detection principles for biosensing at surfaces include ellipsometry, interferometry, waveguiding, and the evanescent electromagnetic field methods such as propagating surface plasmon resonance (SPR) spectroscopy. Electrochemiluminescence techniques show promise of very high specificity. Recently, nanoparticle-based methods for optical biosensing have been explored. In addition, Fourier transform infrared reflection absorption spectroscopy (FT-IRRAS) and surface-enhanced Raman spectroscopy (SERS) have also been applied to biosensing. Research on highly sensitive labeling techniques such as quantum dots, upconverting phosphors, and magnetic bead technologies is being actively pursued.

A primary research issue concerns the development of novel materials that integrate analyte recognition with optical signal transduction in an array format. Arrays also offer the ability to make replicate measurements to minimize false responses and to employ less selective sensing schemes coupled with intelligent processing to remove the requirement for absolute specificity.

Some of the technical challenges are the need for more sensitive CMOS chips, VIXELs, organic light emitting diodes (OLEDs), and organic photodiode arrays (OPAs). Advances in battery technology remain critical to stand-alone operation of sensors.

BIOLOGICAL RECOGNITION ELEMENTS

Biological materials, such as cells and proteins, are intrinsically fragile, and therefore require the development of methods for engineering robustness into sensor design. Efforts that are developing novel recognition units, including aptamers (polynucleic acids) and imprinted polymers, may provide alternatives to protein-based systems.

There are three primary failure modes when biologics are used within thin-film-based biosensors: denaturation and loss of viability (depending on the specific biologic), desorption, and contamination. Fluid phospholipid membranes can be immobilized onto planar supports and patterned into discreet arrays providing a means for using membrane proteins, which account for about half of all proteins known, as biosensing elements. Pore-forming proteins such as hemolysin can be placed in bilayers to allow the detection of transport events rather than a binding between a receptor and target, which is the phenomenon upon which nearly all existing thin film biosensors are based. This in turn provides an opportunity for implementing stochastic sensing, in which analytes are detected one at a time. For applications aimed at detecting slight differences (size, charge, etc.) between otherwise similar molecules, stochastic sensing may be more useful than methods that provide average information about an ensemble of targets.

Several inorganic thin films have been found to be effective platforms for biosensing. For example, a porous silicon-based optical interferometric biosensor has been reported. The sensor operates by measurement of the Fabry-Perot fringes in the white light reflection spectrum from the porous silicon layer. Molecular binding is detected as a shift in wavelength of these fringes.

MASS SPECTROMETRY

For biosensor applications, present laboratory-based mass spectrometry provides superior performance that can provide an excellent “gold standard” to compare various other methods. Key developments in mass spectrometer sources for biological applications were the development of matrix assisted laser desorption/ionization and electrospray ionization.

Among the most intriguing possible applications for mass spectrometry as a biosensor tool, is the identification of biomarker signals that are expressed by viruses, bacteria, and spores, with the interpretation aided by comparison with genomic information for each organism. The successful approach will most likely involve identification of the biomarkers with high performance laboratory instruments, with subsequent routine analysis and detection by lower performance inexpensive instruments. Another possible application is the use of mass spectrometry as a tool for clinical diagnostics (e.g. protein biomarkers for cancer).

A primary goal of present research is to produce an instrument for so-called “field-portable” applications. Small magnetic sector analyzers, linear quadrupole and quadrupole ion trap, Fourier transform, and time-of-flight mass spectrometers are being developed and evaluated, many for biosensor applications. As is seen in the publications that have resulted so far, most of the miniaturization efforts have necessarily resulted in mass spectral performance compromises, to a greater or lesser degree, depending on the type of mass analyzer involved.

It is quite clear that development of miniaturized low power vacuum systems should be an area of the highest priority, if the promise of compact and analytically effective mass spectrometers is to be fully realized for truly portable (or perhaps personal) mass spectrometers.

MASS DETECTION

Mass detection does not, however, remove the need for specific interfacial biochemical recognition: analyte molecules must be selectively recognized and anchored in preference to all other species. Herein lies a key limitation (and a key area for improvement) of label-free detection: nonspecific adsorption. Suitable reference devices and clever surface chemistries have the potential to prevent false positive signals from arising due to simple physical adsorption of a component of the sample matrix.

MICROFLUIDIC SYSTEMS

Developments in MEMS have increased the range of materials of construction beyond those of the semiconductor industry, with increasing use of polymers offering the promise of less expensive devices for some applications, and integration of diverse materials enhancing functionality for others. Discrete devices are now giving way to integrated subsystems that include input/output capabilities, data processing, closed-loop sensing and actuation, and multiparameter measurements from a single microsystem.

MEMS devices have expanded to handle many thousands of biochemical measurands, often requiring a unique, tailored interfacial sensing material for each and every analyte, i.e. a different antibody for each protein, a different strand of nucleic acid for each gene. The range of materials that must be used in device manufacture is much vaster than for physical or even chemical sensors, and many pose unique challenges for deposition, characterization, and maintenance of long-term viability. The potential for high-impact technological advances is significant.

Foremost among the goals is the so-called “sample-to-answer” device that accepts a raw biological sample, performs a complex series of biochemical manipulations—everything from filtration to “amplification” (replication) to purification—and then detects multiple target analytes with high sensitivity, high selectivity, and wide dynamic range.

The complexity of biological samples was addressed by the implementation of a range of laboratory processes in integrated chip format to both reduce the complexity of the sample and to make it more readily detectable. The role of interfacial chemistry is central to biosensing with such systems, and there is a key enabling role and opportunity for structured as well as molecularly defined materials.

CELL BASED SENSORS

Cell- and tissue-based sensors offer several potentially unique functionalities:

- ∄ Ability to detect and/or classify unanticipated threats (e.g. novel pathogens)
- ∄ Relate sensor data to human physiology/pathology (e.g. toxicity)
- ∄ Ability to integrate numerous input stimuli into a nonlinear cellular response
- ∄ Self-replicating, biodegradable sensors
- ∄ Adaptive dynamic range
- ∄ Ability to leverage emergent phenomena being elucidated by microscale control of cells and tissues

The most well developed strategy is based on interfacing excitable cells (neurons, hippocampal slices, cardiomyocytes) with microelectrode arrays. Both spontaneous and induced action potentials can be detected extracellularly, offering a platform for detection of cytotoxic agents and receptor antagonists

Research activity to address several key challenges is in its early stages:

- ∄ Integration of cells and tissues with synthetic materials. Strategies to improve genotypic and phenotypic stability of cells, increase longevity, preserve physiologic input/output responses, and localize and confine cells via micropatterning are being investigated.
- ∄ Cell sourcing. The need to produce uniform populations of cells that retain physiologic responses have led to several avenues of investigation: tumor-derived cell lines that are robust but suffer from genotypic drift and perturbed physiology, stem cells, from both adult and embryonic sources, tissue slices, primary cells, and immortalized cells (e.g. SV40 T antigen). In the US, adult stem cells, adult progenitor cells (more committed than stem cells), and immortalized cells appear to be the most promising cell sources to date.
- ∄ Sensitivity and specificity. The need for sensitive and specific sensors has led to both genetic approaches (e.g. use of knock out cells as control populations) and computational approaches for pattern

recognition. The incorporation of detection technologies, e.g. green fluorescent proteins, into the genome of the cells is an emerging area of research.

- ∄ Data mining and informatics. Methods to interpret and classify data, and strategies to link data to physiologic responses are being explored.
- ∄ Delivery. Issues related to storage, automation, and portability have prompted efforts in miniaturization, fluidic and temperature control, sample handling and preparation, cell preservation, maintenance of pH and aseptic environment, portable power, user interface, and modular design.
- ∄ Maintenance of cell viability and development of strains that can exist anaerobically must be a high priority..

DATA MANAGEMENT

Genomics and combinatorial chemistry have demonstrated the value of measuring thousands of reactions at the same time and in a semi-selective manner. Such reactions, which are often slow, technically complex, and require complicated bioinformatics approaches for data interpretation, nonetheless emphasize our ability to measure and interpret highly complex and interrelated molecular processes.

The manufacture of biosensing systems requires the integration and feedback of performance information of the biosensor in the field to the manufacturing process. Thus field test data results needs to be included in the design of the biosensing devices.

More attention needs to be given to the structure of sensing systems to handle information from wildly divergent data sources and types. For example some sensing systems such as mass spectrometry provide discrete data while cell based systems can provide continuous data streams. Data sampling, reduction and integration for knowledge generation and decision management is a critically underfunded area.