Thursday Morning, November 6, 2003

Homeland Security Topical Conference
Room 309 - Session HS-ThM

Detection of Biological Agents and Self-Cleaning of Contaminated Surfaces

Moderators: L.J. Buckley, Defense Sciences Office, DARPA, D.W. Grainger, Colorado State University

8:20am **HS-ThM1 Direct Electrical Detection of Specific Protein Binding at Surfaces**, ***T.L. Lasseter***, *W. Cai, W. Yang, R.J. Hamers,* University of Wisconsin, Madison

Most biological analyses ultimately rely on optical methods of detection. Here, we show that the binding of proteins to surfaces can be detected electrically in a completely label-free manner using electrochemical impedance spectroscopy. Experiments have been conducted on several different substrates including gold, glassy carbon, and silicon. In each case, we covalently linked biotin to the surface, and then investigated the changes in electrical impedance when the surface was exposed to avidin over the frequency range from 5 mHz to 1 MHz. The changes in impedance are most apparent at low frequencies, < 1 Hz. By using fluorescently-labeled avidin, we also correlated the magnitude of the observed electrical changes with the intensity of the observed fluorescence from the surface. Measurements of the impedance changes as a function of avidin concentration show that the detection limit from these electrical measurements is comparable to or even better than the detection limit observed from fluorescence spectroscopy. Finally, circuit modeling of the interface is being use to relate the electrical changes observed to the physical structure of the interface.

9:00am **HS-ThM3 Autonomous DNA Testing for Bio-Detection**, ***K. Petersen***, Cepheid **INVITED**

After 18 months of extensive testing and evaluation, the US Postal Service has made a decision to deploy automatic bio-threat detection systems in mail sorting facilities throughout the United States. A team, including Northrop Grumman, Cepheid, Smiths Detection, and Sceptor has developed this system called BDS (Bio Detection System). The BDS system will be the first and only automated, nationwide bio-threat detection system approved through rigorous testing of stringent systems requirements. The highest possible detection sensitivity is achieved by using the polymerase chain reaction (PCR), which is increasingly becoming the "gold standard" for biological detection and identification; oftentimes better than culturing. The lowest possible false positivity rate is achieved by extensive internal controls and assay validation procedures. Some aspects of the performance of the BDS system actually exceeds that of the US blood banking system for pathogen detection. Yet, all of this sophisticated, state-of-the-art biological processing is performed completely automatically, in a dusty, industrial environment in about 30 minutes. This presentation will describe the advanced fluidic, biological, chemical, and engineering aspects of this revolutionary technology. We will also discuss how a key sub-system of the BDS, the GeneXpert, is being applied to many other biomedical applications such as the detection of infectious diseases and cancer.

9:40am **HS-ThM5 Magnetically Based Microarray Platform for Rapid Handheld Bioassays**, ***M.C. Tondra***, NVE Corp.

Biochemically functionalized magnetic beads are being used as labels in the development of rugged, handheld bioassays. These magnetic labels can be adapted for use in both protein and DNA assays. A palm-sized magnetic excitation module has been fabricated and demonstrated with integrated Giant Magnetoresistive (GMR) detector arrays. The arrays have 20 sites, each of which is a GMR magnetic sensor underneath a 200 micron diameter functionalizable assay surface. The sites are functionalized by attaching a reference protein or oligonucleotide to the surface. The assay is typically a sandwich-type assay, though other techniques are possible. The sensitivity of the assay is quite good, with reports of better than 1 femtoMolar detection limits. The integrated detectors can detect a single immobilized magnetic label, and has a dynamic range of better than 10^3. The ultimate performance of a given assay is dependent on microfluidics and sample handling. The magnetic detection scheme has several advantages over common optically based techniques. The excitation device and detector array are both solid state devices and are inherently rugged. Both are amenable to low cost mass- manufacturing techniques. And it is possible, with sophisticated sample handling, that PCR amplification may be unnecessary due the ability to detect a single label. Magnetic forces may also be used to enhance reaction rates and improve specificity. This presentation will address issues related to fabrication and surface modification of the GMR sensor array chip. The most common assay surfaces include silicon nitride, silicon dioxide, aluminum dioxide, and gold. In all cases, the surface quality must be very high to achieve predictable and desirable assay results. With continued development, this magnetic bioassay platform should prove to be ideal for applications where high speed multiplexed sandwich-type assays are done on a disposable platform.

10:00am **HS-ThM6 Microfluidic Approaches to Improving Biosensor Performance**, ***J.C. Rife***, *P.E. Sheehan, L.J. Whitman,* Naval Research Laboratory

The sensitivity of a microarray biosensor depends on many factors other than the sensor performance, including sensor area, analyte diffusion, and non-specific binding of target and/or label. However, when the detection system is sensitive enough to detect single labels, the performance will be limited by these factors. We are confronting such limitations in the development of two systems for biowarfare pathogen detection. The Bead ARray Counter (BARC) and the Force Discrimination Biosensor (FDB) systems use biomolecular recognition to bind magnetic microbeads to either a solid or porous substrate. At our current detection limits, we are labeling one analyte molecule per detectable microbead. Under these conditions, the BARC system can detect DNA concentrations as low as 1 fM (10@super 5@ molecules/ml) and FDB can sense protein toxins at concentrations <0.5 pg/ml (10@super 6@ molecules/ml). Further gains in system performance will depend on careful design of the fluidics systems. We will present analytical and finite element calculations aimed at understanding and optimizing the microfluidic delivery of assay reagents to the sensor surfaces. The role of flow profile, fluidic forces, and sensor geometry in maximizing the assay performance will be discussed.

10:20am **HS-ThM7 Comparison of Bioassay Surface Chemistries on Gold and Alumina Films**, ***S.P. Mulvaney***, *C.L. Cole, J.C. Rife, K.A. Wahowski, L.J. Whitman,* Naval Research Laboratory

The surface bioaffinity coating is arguably the most critical component of any biosensor based on ligand-receptor capture on a solid substrate. The characteristics of this interface have profound effects on the overall performance of the sensor, affecting the assay sensitivity and selectivity (including background due to non-specific binding), and signal transduction. The physical properties of the sensor surface must be compatible with the detection method employed and chemically suitable for functionalizing with receptor biomolecules such as antibodies or oligonucleotides. The utility of gold films for electrochemical and optical detection schemes has made it one of the most commonly used sensor surfaces. One common approach to functionalizing gold is to first conjugate the desired biomolecules to a mercaptan functional group which can then immobilized on the surface via a Au-S bond. Alternatively, thiolated self-assembled monolayers (SAMs) can be used as a base for subsequent conjugation with biomolecules. SAM-based films, while effective, often lack the reproducibility required for reliable, quantitative assays. Therefore, we are exploring alternate surfaces and surface chemistries. We have developed and characterized multilayer, biocompatible polymer films on alumina surfaces and found them to be more reproducible than similar films on gold. The effects on assay performance for various chemistries on top of gold and alumina films will be compared and contrasted as used in the Bead Array Counter (BARC),@footnote 1@ a biosensor system that uses paramagnetic beads. CL Cole and KA Wahowski are employees of Nova Research, Inc., Alexandria, VA. @FootnoteText@ @footnote 1@Edelstein et. al., Biosens. Bioelectron. 2000, 14, 805-813.

10:40am **HS-ThM8 Biocatalytic Nanocomposites as Self-Cleaning Surface Coatings**, ***J.S. Dordick***, Rensselaer Polytechnic Institute **INVITED**

The interface of biology and materials science has led to the development of new materials, with unique structural and functional properties, and new process technologies complete with the ability to produce, from "bottoms up", a wide range of biomimetic structures. These materials and their designs have broad application as catalysts, sensors, and devices for use in synthesis, cell and tissue engineering, bioanalysis and screening, and nanoelectronics. We have focused on the generation of nanostructures that are functionalized with and in some cases constructed from biological molecules, complete with tailored selectivities and biocatalytic activities at the molecular and nanoscales.@footnote 1,2@ In one example, we have incorporated enzymes attached to carbon nanotubes and further embedded into polymeric films, coatings, and paints to form biocatalytically active surfaces. These materials are capable of degrading

proteins, fats, polysaccharides, and other organic and biological molecules. Furthermore, in some cases, these materials can prevent the microbial fouling often associated with surface coatings, and this may have significant impact in medical devices and in battlefield and homeland defense. This presentation will be focused on the preparation, characterization, and use of different enzyme-nanomaterial-polymer composites and potential broad-based applications arising from this technology. @FootnoteText@ @footnote 1@K. Rege, N.R. Raravikar, D.-Y. Kim, L. S. Schadler, P.M. Ajayan, and J.S. Dordick (2003), "Enzyme-Polymer-Single Walled Carbon Nanotube Composites as Biocatalytic Films", Nano Letters (submitted).@footnote 2@ P. Wang, M. V. Sergeeva, L. Lim, and J. S. Dordick (1997), "Biocatalytic Plastics as Active and Stable Materials for Biotransformations", Nature: Biotechnology 15, 789-793.

11:20am **HS-ThM10 Novel Surface-Segregating Materials for Chem-Bio Applications***, **J.A. Orlicki**, M.S. Bratcher, R.E. Jensen, C.A. Winston, S.H. McKnight,* Army Research Laboratory, AMSRL-WM-MA

Macromolecular surfactants based upon hyperbranched polymer scaffolds have been employed to deliver functional groups to the surface of a substrate. Careful selection of end group chemistry allows the control of thermodynamic phase segregation, leading to an increased loading of additive at a substrate surface relative to the bulk concentration. The high number of end groups of a hyperbranched polymer permits the covalent attachment of additional moieties for added functionality (e.g. solubility control, reactive groups, ligand binding sites). Employing these molecules as additive to bulk polymer systems will provide a method to impart chemical or biological resistance upon delicate materials (electronics packaging, clothing) at minimal cost. We have developed polymers capable of chelating metal centers (polyoxometalates), and have shown their ability to transport the metals to a substrate surface. Contact angle and XPS analysis indicated the segregation of metals to the surface only when interacting with the macromolecular surfactant. These results will be discussed, along with the accompanying synthetic techniques to spur the development of novel active groups for surface functionalization.

11:40am **HS-ThM11 Opportunities in Materials Chemistry at DARPA***, **L.J. Buckley**,* Defense Sciences Office, DARPA

Materials chemistry at the surface impacts many areas of science and engineering. As one approaches nanometer dimensions, the surface properties will dominate the material behavior. The Defense Advanced Research Projects Agency (DARPA) has many opportunities for the application of materials chemistry for the general modification of surface behavior. A summary of these opportunities will be presented.

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