

BIOSENSORS, NANOSENSORS AND BIOCHIPS: FRONTIERS IN ENVIRONMENTAL AND MEDICAL DIAGNOSTICS

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ABSTRACT

This presentation describes three areas of research related to the development of biosensors, nanosensors and biochips for chemical, biological and medical analysis: (1) nanostructured plasmonics-based probes for surface-enhanced Raman scattering (SERS) biochemical analysis, and (2) nanosensors for *in vivo* analysis of a single cell and (3) multi-functional biochips for medical diagnostics.

INTRODUCTION

The field of photonics has recently experienced an explosive growth due to the non-invasive or minimally invasive nature and the cost-effectiveness of biophotonic modalities in environmental sensing, medical diagnostics and therapy [1]. This lecture discusses the development and application of advanced biomedical photonics, molecular spectroscopy, biosensors and biochips for environmental and biomedical diagnostics.

PLASMONIC-BASED NONOPROBES

The first research area involves the development of plasmonic nanoprobess having enhanced electromagnetic properties of metallic nanostructures. The term plasmonics is derived from "plasmons", which are the quanta associated with longitudinal waves propagating in matter through the collective motion of large numbers of electrons. Incident light irradiating these surfaces excites conduction electrons in the metal, and induces excitation of surface plasmons leading to enormous electromagnetic enhancement for ultrasensitive detection of spectral signatures: surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF).

Raman spectroscopy has proven to be an effective technique as an analytical tool. This is partly due to its non-destructive nature and structural fingerprinting capability with very narrow and highly resolved bands (0.1 nm). In addition, the spectral measurement is rapid and requires little sample preparation, which gives it the potential for on-line analysis and field applications. However, conventional Raman

spectroscopy suffers from low sensitivity and often requires powerful and expensive lasers for excitation. In the mid-to-late 1970s, it was discovered that when molecules were adsorbed onto specific solid substrates, an enhanced Raman signal of the adsorbate was obtained with intensity enhancements of 10^6 - 10^{15} . This effect has since become known as surface-enhanced Raman scattering (SERS) spectroscopy [2]. The SERS enhancement is thought to be the result of a combination of intense localized fields arising from surface plasmon resonance in metallic nano-structures and chemical effects. The exact nature of the SERS phenomenon is still under intense investigation [6-8]. The intensity of the normally weak Raman scattering process is increased by factors as large as 10^6 - 10^{11} for compounds adsorbed onto a SERS substrate, allowing for trace-level detection. Fig. 1 shows a scanning electron micrograph (SEM) of a SERS-active nanospheres (300-nm diameter coated with a 100-nm layer of silver). Our nanoparticle-based SERS technology that has enabled sensitive detection of a variety of compounds of environmental and medical interest. The SERS nanoprobe technology has also been incorporated in several fiberoptic probe designs for remote analysis. The development of a SERS gene probe technology based on the solid surface-based technology has also been reported. In one study, the selective detection of HIV DNA and cancer gene was demonstrated [3].

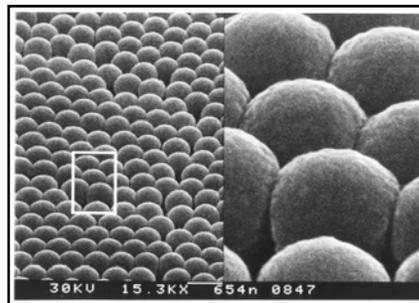


Figure 1: SEM photograph of Silver-Coated Nanosphere-based SERS substrates

During the last decade, our laboratory has been interested in the development of optical techniques for genomics analysis due to the strong interest in non-radioactive DNA probes for use in biomedical diagnostics, pathogen detection, gene identification, gene mapping and DNA sequencing. We have focused on not only the development of efficient SERS-active solid substrates for trace organic analysis in biological and environmental applications [4] but also the development of SERS-active solid substrates for biomedical diagnostics. We have developed SERS gene probes to detect the human *BLC2* gene, which is an important representative of a family of cancer genes.

Due to their non-radioactive nature, there is strong interest in the development of optical techniques for biomedical diagnostics, pathogen detection, gene identification, gene mapping and DNA sequencing. The hybridization of a nucleic acid to its complementary target is one of the most definite and well-known molecular recognition events. Therefore, the hybridization of a nucleic acid probe to its DNA target can provide a very high degree of accuracy for identifying complementary DNA sequences [2-4].

OPTICAL NANOSENSORS

Biology has entered a new era with the recent

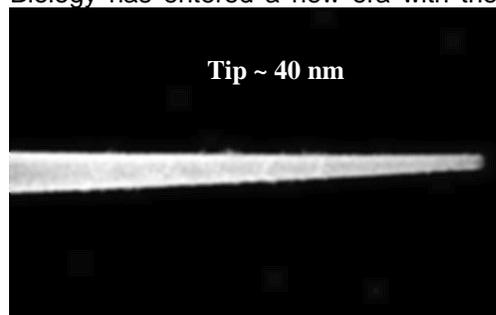


Figure 2 Photograph of an Antibody-based Nanoprobe. (The small size of the probe (200-nm diameter) allows manipulation of the nanoprobe at specific locations within single cells).

advances in nanotechnology, which have recently led the development of biosensor devices having nanoscale dimensions that are capable to probe the innerspace of single living cells. Nanosensors provide new and powerful tools for monitoring *in vivo* processes within living cells, leading to new information on the inner workings of the entire cell [5, 6]. Such a systems biology approach could greatly improve our understanding of cellular function, thereby revolutionizing cell biology. Fiberoptic sensors provide useful tools for remote *in situ* monitoring. Fiberoptic sensors could be fabricated to have extremely small sizes, which make them suitable for sensing intracellular/intercellular physiological and biological parameters in microenvironments. A wide variety of fiberoptic chemical sensors and biosensors have been developed in our laboratory for environmental and biochemical monitoring [1].

We have developed nanosensors for *in situ* intracellular measurements of single cells using antibody-based nanoprobes. In this work, we describe the development of nano-biosensors for *in situ* monitoring of single cells using biosensors having antibody-based nanoprobes having 40-nm diameter (Fig. 2).



Fig. 3 shows a photograph of an antibody-based nanoprobe used to measure the presence of BPT inside a single cell. The small size of the nanoprobe allowed it to be manipulated to specific locations within the Clone 9 cells.

The small size of the nanoprobe allowed it to be manipulated to specific locations within the Clone 9 cells. This study illustrates the use of antibody-based nanoprobe for measurements of chemicals inside a single cell. These nanodevices could also be used to develop advanced biosensing systems in order to study in situ intracellular signaling processes and to investigate gene expression inside individual living cells. Programmed cell death called apoptosis is an important process for multi cellular organisms, from which they benefit during development and homeostasis. The nanosensor was used to detect caspase-9 activation following apoptosis.

Dynamic information of signaling processes inside living cells is important to fundamental biological understanding of cellular processes. Many traditional microscopy techniques involve incubation of cells with fluorescent dyes or nanoparticles and examining the interaction of these dyes with compounds of interest. However, when a dye or nanoparticle is incubated into a cell, it is transported to certain intracellular sites that may or may not be where it is most likely to stay and not to areas where the investigator would like to monitor. The fluorescence signals which are supposed to reflect the interaction of the dyes with chemicals of interest, is generally directly related to the dye concentration as opposed to the analyte concentration. Only with optical nanosensors can excitation light be delivered to specific locations inside cells. An important feature of nanosensors is the minimal invasiveness of the monitoring process. A cell survival study was previously performed whereby an investigation was performed to determine whether penetration of the cell by the nanosensor resulted in intracellular or membrane damage of such a nature as to compromise cellular viability. It was determined that the process of mitosis continued normally and that nanosensor insertion and withdrawal did not affect the life cycle of the cell. Nanosensors are an important technology that can be used to measure biotargets in a living cell and that does not significantly affect cell viability. Combined with the exquisite molecular recognition of antibody probes, nanosensors could serve as powerful tools capable for exploring biomolecular processes in sub-compartments of living cells. They have a great potential to provide the necessary tools to investigate multi-protein molecular machines of

complex living systems and the complex network that control the assembly and operation of these machines in a living cell. Future developments would lead to the development of nanosensors equipped with nanotool sets that enable tracking, assembly and disassembly of multi-protein molecular machines and their individual components. These nanosensors would have multifunctional probes (antibody as well as DNA probes) that could measure structure of biological components in single cells. Until now, scientists have been limited to investigate the workings of individual genes and proteins by breaking the cell apart and study its individual components *in vitro*. The advent of nanosensors will hopefully permit research on entire networks of genes and proteins in an entire living cell *in vivo* [5-7]

THE MULTI-FUNCTIONAL BIOCHIP (MFB)

The MFB is an integrated multi-array biochip, which is designed by combining integrated circuit elements, an electro-optics excitation/detection system, and bioreceptor probes into a self-contained and integrated microdevice [8-14]. Fig. 4 depicts a schematic diagram of the MFB device. The MFB system includes the following elements: 1) an excitation light source, 2) multiple bioprobes having different types of bioreceptors, 3) a sampling platform, 4) sensing elements, and 5) a signal amplification and treatment system. The development of the multichannel sampling platform involves immobilization of bioprobes on multiarray (4 x 4 or 10 x 10 channels for current biochips) substrates, which can be performed on a transducer detection surface to ensure optimal contact and maximum detection.

The integrated electro-optic microchip system developed for this work involved integrated electrooptic sensing photodetectors for the biosensor microchips. Such an integrated microchip system with on-board integrated circuit (IC) electronics is not currently available commercially. Therefore, we have designed IC electrooptic systems for the microchip detection elements at Oak Ridge National Laboratory. Highly integrated biosensors are made possible partly through the capability of fabricating multiple optical sensing elements and microelectronics on a single integrated circuit [10, 11]. Such an integrated microchip system is not currently available commercially.

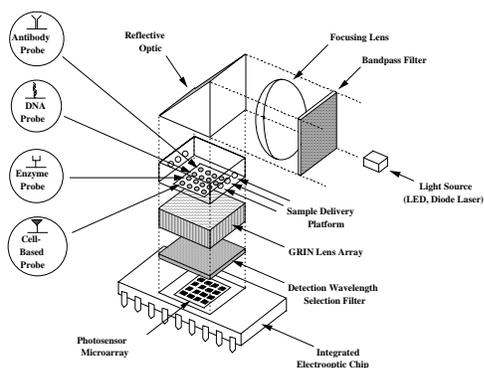


Figure 4. Schematic Diagram of the Multi-Functional Biochip (MFB) optical system.

To develop a biochip system with optimized performance, we have developed and evaluated several biochip IC systems based on photodiode circuitry, one system having 16 channels (4 x 4 array), and another having 64 channels (8 x 8 array) having four types of electronic circuits on a single platform. The biochips include a large-area, n-well integrated amplifier-photodiode array that has been designed as a single, custom IC, fabricated for the biochip. This IC device is coupled to the multiarray sampling platform and is designed for monitoring very low light levels. The individual photodiodes have 900- μm square sizes and are arrayed on a 1-mm spacing grid. The photodiodes and the accompanying electronic circuitry were fabricated using a standard n-well CMOS process. The use of this type of standard process allows the production of photodiodes and phototransistors as well as other numerous types of analog and digital circuitry in a single IC chip. This feature is the main advantage of the CMOS technology in comparison to other detector technologies such as charge-coupled devices or charge-injection devices. The photodiodes themselves are produced using the n-well structure that is generally used to make resistors or as the body material for transistors. Since the anode of the diode is the p-type substrate material, which is common to every circuit on the IC chip, only the cathode is available for monitoring the photocurrent and the photodiode is constrained to operate with a reverse bias.

We designed an analog multiplexer that allows any of the elements in the array to be connected to an amplifier. In the final device, each photodiode could be supplied with its own amplifier. The multiplexer is made from 16 cells for the 4 x 4 array device. Each cell has a CMOS switch controlled by the output of the address decoder cell. The switch is open when connecting the addressed diode to an amplifier. This arrangement allows connecting a 4 x 4 (or 10x10) array of light sources (different fluorescent probes, for example) to the photodiode array and reading out the signal levels sequentially. With some modification, a parallel reading system can be designed. Using a single photodiode detector would require mechanical motion to scan the source array. The additional switches and amplifier serve to correctly bias and capture the charge generated by the other photodiodes. The additional amplifier and switches allow the IC to be used as a single, large area (nearly 4 mm square) photodetector.

Fig. 5 shows a photograph of the 4x4 IC microchip. With the CMOS technology, highly integrated biosensors are made possible partly through the capability of fabricating multiple optical sensing elements and microelectronics on a single IC. A two-dimensional array of optical detector-amplifiers was integrated on a single IC chip. To evaluate and select an improved IC system for the biochip, we have also designed and fabricated a chip with an 8x8 CMOS sensor array. This microchip contains 4 quadrants, each having a different electronic design, which was evaluated for optimal performance.

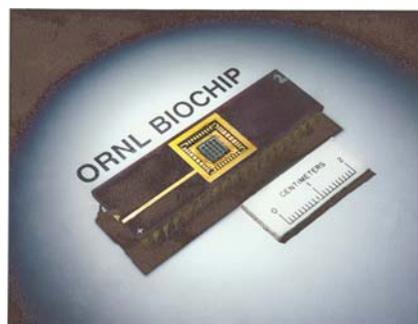


Figure 5. Photograph of the 4x4 integrated circuit microchip.

In general biosensors and biochips employ only one type of bioreceptor as probes, i.e., either nucleic acid or antibody probes. Biochips with DNA probes are often called gene chips, and biochips with antibody probes are often called protein chips. An integrated DNA biochip that uses multiple bioreceptors with different functionalities on the same biochip, allowing simultaneous detection of several types of biotargets on a single platform has been developed. This device is referred to as the multi-functional biochip (MFB). The unique feature of the MFB device is the capability to perform different types of bioassay on a single platform using DNA and protein-based probes such as antibody probes simultaneously.

Hybridization of a nucleic acid probe to DNA biotargets (e.g., gene sequences, bacteria, viral DNA) offers a very high degree of accuracy for identifying DNA sequences complementary to that of the probe. In addition to DNA probes, the MFB uses also another type of bioreceptor, i.e., antibody probes that take advantage of the specificity of the immunological recognition. The results of this study using antibody against *E coli* and DNA probes for *B. anthracis* demonstrate the feasibility of the multi-functional biochip for the detection of multiple biotargets of different functionality (DNA, proteins, etc.) using a single biochip platform. *Mycobacterium tuberculosis* bacterium or the *p53* cancer protein have also been detected on the same biochip. Fig. 6 illustrates typical signals detected on the biochip.

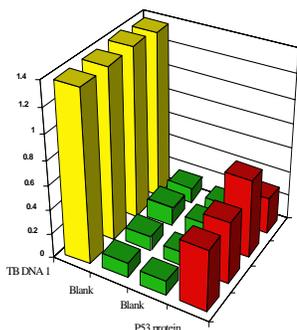


Figure 6. Detection of the *Mycobacterium tuberculosis* gene fragment and the *p53* cancer protein using the multifunctional biochip system.

The left row of the biochip corresponds to the detection of TB gene segment using DNA probes, while last row used to the detection of the p53 protein using antibody probes. The two middle rows correspond to the blank.

Infectious diseases are moving across borders, becoming a global treat to our lifestyle and well-being. Over half of TB cases in some wealthy countries are among foreign-born populations. With increasing travel, increasing cases of malaria were reported among travelers. Infectious diseases are becoming a matter of national security for many developing countries. Sustainable development is feasible if countries can tame the infectious diseases that disempower their people. If these diseases remain unchecked, they could damage the social fabric, diminish agricultural output, affect industrial production, undermine political, social and economic stability, and ultimately contribute to regional and global insecurity.

For the above reasons, there is an urgent need to develop rapid, simple, cost-effective medical devices for screening multiple medical diseases simultaneously and to monitor infectious pathogens for early medical diagnosis. Such a system will also be useful in physician offices or for personal use at home. Other important applications involve monitoring pathogens for diseases by relatively unskilled personnel in the field far remote from clinical laboratories. The MFB, which is a truly integrated biochip system that comprises probes, samplers, detector as well as amplifier, and logic circuitry on board, could enable a rapid and inexpensive test for multiple diseases and for a wide variety of applications. With its multi-functional capability, the MFB technology is a system that allows simultaneous detection of multiple biotargets simultaneously. Such a device could provide information on both gene mutation (with DNA probes) and gene expression (with antibody probes against proteins) simultaneously. It is expected that advances in miniaturization and mass production technology will significantly reduce the cost of fabrication of biochip systems for widespread use worldwide to address the urging need for improved health care at a reduced cost.

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