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Optical Fiber Microarrays for Chemical and Biological Measurements.

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ABSTRACT

We have used coherent imaging fiber arrays as a platform for preparing chemical sensors and biosensors. Sensors can be made with spatially-discrete sensing sites for multi-analyte determinations. Micrometer sized sensors have been fabricated by etching the cores of an optical imaging fiber to create microwells and loading them with microspheres. These arrays possess both high sensitivity and reproducibility and can be used for making thousands of measurements simultaneously such as for genetic analysis or for the analysis of complex biological fluids. Both optical and optoelectrochemical arrays have been used for multiplexed sensing. In another scheme, the arrays can be used for single molecule detection. In this format, individual molecules, such as enzymes, can be trapped in the microwells by sealing each microwell with a silicone gasket. The enzyme molecules catalyze the formation of a fluorescent product that can be detected readily. The kinetic properties of hundreds to thousands of single enzyme molecules can be monitored simultaneously using this format. By observing the stochastic nature of the single molecule responses, new mechanistic insights into the fundamental nature of the enzymes can be obtained.

INTRODUCTION

Optical fibers have been used for chemical sensing for nearly three decades. A variety of sensing formats have been employed. Optical fibers can carry light signals due to the refractive index difference between the clad and core materials comprising the fiber. By attaching chemically-sensitive species to the distal end of the fiber, one can interrogate these species by illuminating the proximal fiber end. Our laboratory has used a fluorescence transduction mechanism because fluorescence enables a simple optical setup. In our implementation, excitation light is carried down the fiber core via total internal reflection and the isotropically-emitted fluorescence signal can be collected by the same fiber core. In this way, the fiber provides the ability to interrogate a sample remotely.

Chemical sensing often requires the measurement of either multiple analytes or of the spatial distribution of a single analyte. To address these sensing needs, we have developed optical fiber array sensors [1]. In this approach, a unitary coherent fiber bundle comprising thousands of single micron-sized individual fibers is used (Figure 1-left). The most common implementation of this format involves etching one end of the fiber array. The composition of the fiber cores is slightly different from the clad, resulting in a preferential etch when the array is placed in an acid solution [2]. Etching results in an array of user-defined wells at the end of the fiber where each well is optically "wired" by the fiber core that defines each well's bottom

(Figure 1-center). In the following section, we describe a number of ways in which these fiber arrays can be used for sensing and other analytical applications.

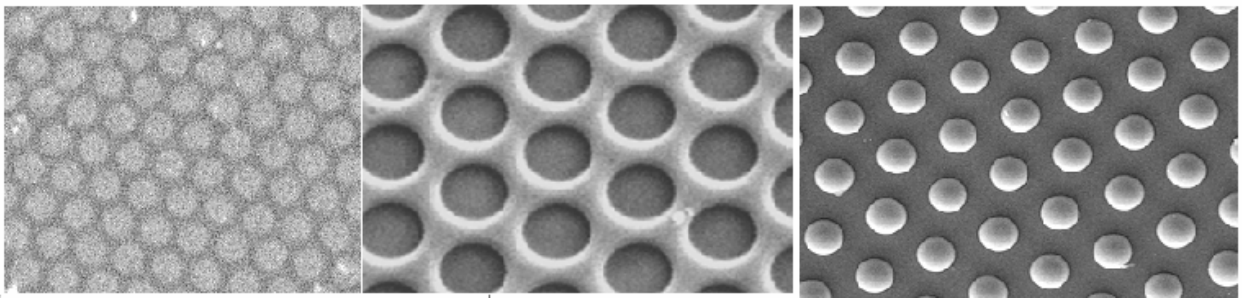

Figure 1. Optical fiber arrays. (Left) Lighter gray circles are the fiber cores while darker gray is the surrounding clad. (Center) Cores are preferentially etched to give wells. Bottom of each well (dark gray) is the end of an optical fiber. (Right) Microspheres assemble into the etched wells. All the fiber cores in each image are approximately 3 microns in diameter.

RESULTS AND DISCUSSION

Microsphere-based sensing

Optical sensor arrays have been produced by etching the cores (~50,000) of the distal face of imaging fiber bundles. Microsphere sensors have been created by covalently coupling micrometer sized polymer beads to biological receptors such as oligonucleotide capture probes. The microspheres randomly distribute via self-assembly and are held in the wells via capillary and electrostatic forces. Each sensor type is optically encoded with one or more fluorescent dyes at specific concentrations [3]. Alternatively, we can tag the beads with oligonucleotide labels that are decoded via sequential hybridization. Target nucleic acids are labeled through amplification techniques such as PCR or RCA or via signal probes in a sandwich assay format. Using this platform, we have detected DNA, RNA, and rRNA isolated from a variety of bacterial and viral pathogens.

Analyses of complex biological fluids and environmental matrices such as saliva, seawater, and animal fecal suspensions are currently underway. Chronic obstructive pulmonary diseases are a significant source of morbidity and place a high burden on the health care system. Exacerbations are often caused by viral or bacterial infections of the upper respiratory tract, secretions of which are in constant natural exchange with saliva. We have designed microarrays with hundreds of sequences specific to tens of pathogens of interest. These arrays are being screened with PCR and RT-PCR amplicons produced from nucleic acids isolated from whole or centrifuged saliva. Using analogous methods, we are also analyzing samples of groundwater and fecal suspensions spiked with a variety of bacteria and viruses intimately associated with the safety of food and water supplies. The high feature redundancy and sensitivity of these methods enable massively parallel measurements of many targets of interest with low occurrence of false positive or negative results.

Electrogenerated Chemiluminescence (ECL)

The bead-based platform used for fluorescence assays has also been adapted to use electrogenerated chemiluminescence (ECL) for signal transduction. ECL occurs when light is emitted following an electrochemical redox reaction of a label molecule, such as ruthenium tris(bipyridine), $Ru(bpy)_3^{+2}$, and a coreactant, tripropylamine (TPA). Since the stimulus is electrical and the signal is optical, the background signal is very low. We have performed ECL-based sandwich immunoassays, where the ECL from individual sensing beads is imaged. As with the fluorescence platform, the ability to image single beads enables highly multiplexed assays to be performed. The system uses a gold electrode that contains an array of wells to hold the sensing beads. A schematic of the sandwich immunoassay is shown in Figure 2. Briefly, the experiments were performed by depositing 3 μm polystyrene beads, which had antibodies covalently attached, into the wells on the electrode. The electrode was first placed in a sample containing antigen, and then in another solution containing biotinylated detection antibodies. Finally, streptavidin modified with $Ru(bpy)_3^{+2}$ was used to attach the ECL label. Readout was performed by imaging the electrode on a microscope using an electron multiplying CCD camera. Figure 2 shows the detection of the antigen IL-8 at a concentration of 2 μg/mL using ECL.

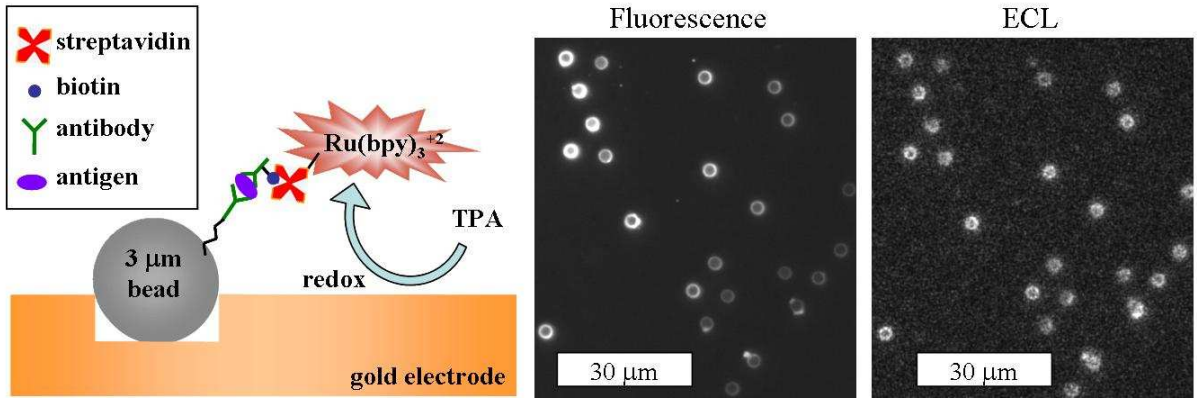

Figure 2. (Left) Schematic of a sandwich immunoassay where the presence of an antigen results in the immobilization of $Ru(bpy)_3^{+2}$ to a solid support. The ruthenium complex produces an optical signal from chemiluminescence following an electrochemical reduction of tripropylamine. The fluorescence micrograph indicates the location of the beads, and the micrograph of the ECL indicates the successful detection of IL-8 at a concentration of 2 μg/mL.

Array of Optical Traps

Work is also ongoing into ways to expand the utility of microsphere-based arrays by removing the physical fiber support. This goal will be accomplished by creating an array of three-dimensional optical traps (tweezers). Activating and deactivating the traps will allow the sensor array to be rapidly reconfigured by using different populations of beads. With individually addressable traps, ordered array formation will be possible. Adding bead types sequentially and observing or controlling their locations in the array will obviate the need for encoding of bead types. This approach will make arrays with a larger variety of bead types feasible.

One implementation of the array format is to use the beaded wells as an array of lenses. Each bead acts as a micro-ball lens and can focus at a point with an intensity sufficient to trap a

particle such as a bead or a cell. An alternative is to use a gradient index (GRIN) fiber array to trap particles [4]. By turning the traps on and off, different populations can be trapped, observed, and released as shown in Figure 3. A proof-of-principle design using a digital micromirror device has enabled actuation of individual traps [5]. Each micromirror directs the laser light to a fiber or set of fibers and can be independently actuated.

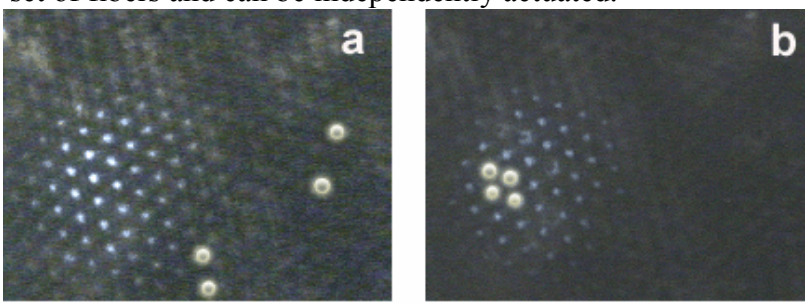

Figure 3: An array of individual optical traps created by an optical fiber array. The small white spots are the optical traps formed at the end of each fiber while the large white circles are the beads. Each bead is trapped at a focal point. The figure shows four beads being trapped as they flow from the right side of the field of view into the trap array.

Single Enzyme Molecule Detection

Single molecule measurements provide unique information about heterogeneous molecular behaviors that are hidden using bulk methods. We separated and enclosed single β-galactosidase molecules with the fluorogenic substrate resorufin- β-galactopyranoside in an array of 50,000 femtoliter-sized chambers on the distal end of an etched optical fiber bundle. According to Poisson statistics an appropriate low enzyme concentration (one enzyme molecule in 20 microchambers) ensures that the microchambers contain a maximum of only a single enzyme molecule while the rest are empty [6]. The catalytic activity of each enzyme molecule, which resulted in the production of fluorescent resorufin, was detected individually by epifluorescence microscopy (Figure 4).

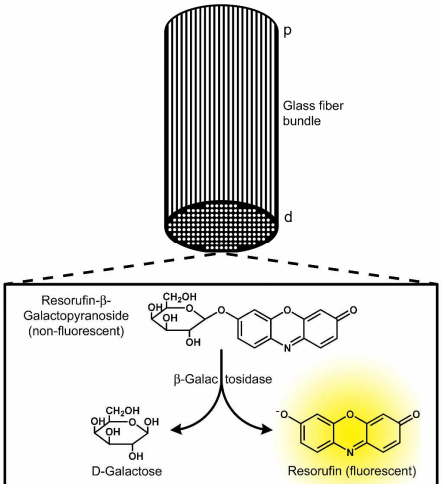

Figure 4: Substrate turnover of single enzyme molecule a fiber bundle array. Some of the wells at the end of the fiber contain a single enzyme molecule which converts a non-fluorescent substrate to fluorescent resorufin. The substrate turnover can be reversibly inhibited.

With our novel detection platform for single enzyme molecules we demonstrated that individual β-galactosidase molecules exhibit a distinct catalytic activity which is broadly distributed ("static heterogeneity") [7]. In each experiment hundreds of individual enzyme molecules in the fiber bundle array were observed simultaneously – enough data for a thorough statistical analysis of single enzyme molecule kinetics.

When we added the slow-binding inhibitor D-galactal to the enzyme/substrate solution in the microchamber array (Figure 5), we were able to observe enzyme inhibition kinetics at the single molecule level for the first time [8]. Inhibited and active states of β-galactosidase could be clearly distinguished and the large array size provided very good statistics. With a pre-steady-state experiment, we demonstrated the stochastic character of inhibitor release, which obeys first-order kinetics. Under steady-state conditions, the quantitative detection of substrate turnover changes over long time periods revealed repeated inhibitor binding and release events, which are accompanied by conformational changes of the enzyme's catalytic site. We proved that the rate constants of inhibitor release and binding derived from stochastic changes in the substrate turnover are consistent with bulk reaction kinetics.

Single DNA Molecule Detection

Recently, we demonstrated the further application of this femtoliter sized reaction vessel array technology for highly sensitive DNA analysis with an experimental limit of 1 fM [9]. To validate the utility of this technology in our study, we employed a fiber optic array to create thousands of femtoliter-sized reaction wells, each specifically functionalized with oligonucleotide probes capable of capturing biotinylated target DNA. After hybridization, the fiber was incubated with streptavidin-labeled enzyme solution (streptavidin-β-galactosidase conjugate). The bound single enzyme molecules were confined to individual reaction vessels containing excess fluorogenic substrate. The enzyme molecules catalyzed the production of a sufficient number of fluorescent product molecules to generate a detectable signal (Figure 5). At low target DNA concentrations with relatively short incubation times, only a small percentage of the capture sites bind target DNA, enabling a binary readout of target concentration from the high-density fiber array. This simple binary readout-based scheme is easy to perform and exhibits a high signal to noise ratio in the presence of trace amounts of DNA target. Furthermore, it also should be possible to extend this technology to protein detection by modifying the reaction wells with specific capture antibodies. We expect this assay to be useful in a number of biomedical applications where accurate and highly sensitive target analysis is critical.

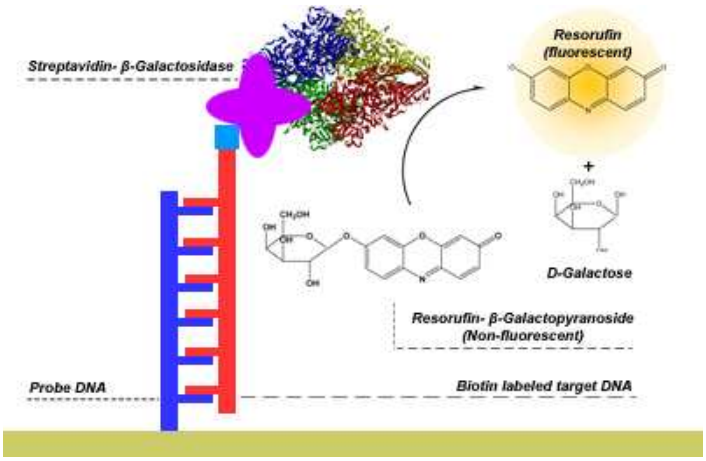

Figure 5. Schematic illustration of DNA sensing technology performed in femtoliter-sized reaction wells of a fiber array employing enzymatic amplification for signal generation.

CONCLUSIONS

Optical fiber arrays are a flexible platform and can be used for a variety of applications. We have demonstrated their utility for multiplexed analysis as well as for optical trapping and single molecule detection. The ability to interrogate samples remotely using the optical fiber arrays as light conduits, simplifies the optics and provides an expedient way to make high density measurements.

ACKNOWLEDGMENTS

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