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Fiber Optic Couplers for Protein Detection

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Numerous applications in areas such as medical diagnostics, environmental monitoring, and food safety require selective protein detection. A variety of optical detection methods have been developed, but many of them require the use of a fluorescent labeling dye. Label-free optical techniques such as surface plasmon resonance (SPR), use of resonant mirrors, and interferometry have also been developed. Here we report the use of a fiber optic coupler as a platform for bioassays/biosensing. The coupler fabrication and design elements crucial to the sensor performance are described, and protein detection is demonstrated.

1. Introduction

1.1 *Refractive index-based detection*

The response of many biosensors and assays currently in use is based on absorbance or fluorescence of the analyte molecule or of the dye used as a tag. Fluorescence-based techniques are much more sensitive than absorbance-based ones and, therefore, provide lower limits of detection. Unfortunately, most common analyte molecules are not naturally fluorescent. Therefore, a fluorescent dye molecule or a tag, such as a quantum dot, must be incorporated in the analysis. Methodologies include linking the dye/tag to the analyte molecule or the receptor, performing a "sandwich" assay with a secondary-labeled molecular recognition element, or displacing fluorescently labeled molecules with the unlabelled analyte molecules in a competitive format. Such techniques complicate detection by adding steps and reagents. In addition, the attachment of fluorescent tags can interfere with the molecular binding event. The typical protein limit of detection for fluorescence-based techniques is 1 ng/mL (pM for 150 KDa protein).[1–3]

Label-free detection methods simplify detection by reducing sample preparation and eliminating reagents. Many state-of-the-art, label-free optical detection methods measure

refractive index changes induced by analyte binding. Such techniques include surface plasmon resonance (SPR), use of resonant mirrors, and interferometry. Table 1 provides a list of many state-of-the-art bio-detection systems, the corresponding limits of detection, and select representative publications.

1.2 *Fiber optic couplers*

A variety of biosensors that utilize optical fibers have been developed.[14,16–19] Here we describe the use of fiber optic couplers as a bioassay/biosensor platform. These couplers are fabricated by simultaneously heating and pulling two single-mode fibers to create a "waist" region where the two fibers are thermally fused and the fiber diameter is reduced from an initial value of 125 μm per fiber to 5–15 μm per fiber. After this fusion, light propagating in a single input fiber is split at the "waist" region and then propagates down the two fibers. In this "waist" region, the fiber cores become too small to contain the propagating mode, and therefore light propagates through the surrounding cladding. Thus, in the coupled region, the light is guided through a "core" composed primarily of the original fiber cladding material, and whatever surrounds the fused region, such as the sample solution, becomes the new cladding. As a result, the division of power between the two fibers becomes a strong function of the index of refraction in the small volume surrounding the fiber surfaces in the coupled or "waist" region. Theoretical approximations of the response of a simplified version of such a coupler have been described previously.[20,21]

The coupler can be used as a biosensor if receptor molecules are attached to the coupler surface. Binding of the analyte molecule to the receptor molecule changes the refractive index and hence the critical angle at the coupler surface, thereby inducing a shift in the coupling ratio. Therefore, the change in the ratio of the output couplers can then be used to detect a binding event. A simplified diagram depicting the coupler response is shown in Fig. 1. The application of fiber optic couplers as an immunoassay detection platform has been suggested previously,[22] and here we report experimental results and design modifications made to improve the reproducibility and sensitivity of the coupler response to analyte binding.

Table 1
A list of many state-of-the-art bio-detection systems, the corresponding limits of detection, and select representative publications.

Sensor Type	Limit of Detection*(analyte = protein)	Select References
Fluorescence	ng/mL	(1–3)
SPR	ng/mL	(4, 5)
Resonance mirror	ng/mL	(5, 6)
Grating couplers	μg/mL	(7)
Bidiffractive grating couplers/ Multilayer gratings	ng/mL	(8, 9)
Hartman interferometer	ng/mL	(10–12)
Mach-Zehnder interferometer	ng/mL	(13, 14)
Optical difference interferometer	μg/mL	(15)

* For a 150 KDa protein, such as IgG, ng/mL = pM = ppb.

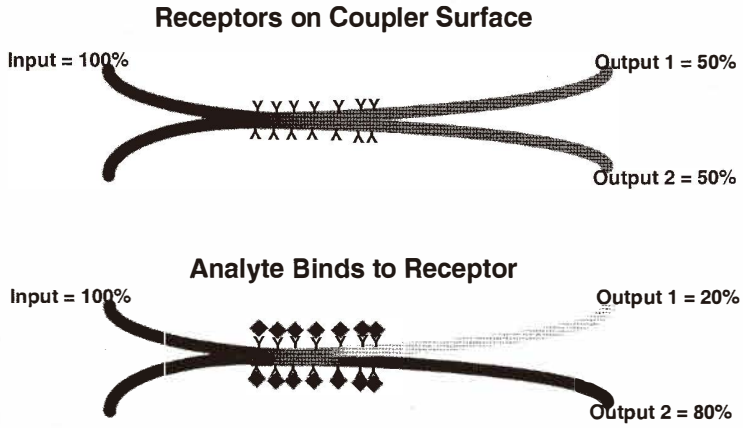

Fig. 1. Schematic depicting the light output change induced in a fiber optic coupler due to analyte binding to a receptor immobilized on the coupler surface. Not to scale.

2. Materials and Methods

Couplers were either purchased (Empirical Technologies Corporation, ETC) or fabricated in-house. The Veridian fabrication involves an automated coupler pulling station.[23] Two single-mode fibers (Corning SMF-28) are held at a fixed position at one end, and the opposite ends are run to a moveable stage through a tensometer. The pull length is measured with encoder motors and the entire pulling process is monitored by computer software. The central region of the fibers is positioned in a 2300°C furnace (ETC) that automatically moves during the drawing process in order to keep the furnace positioned in the critical "waist" region of the fibers. If the furnace is not moved during the pulling process, the couplers produced are skewed in the taper because heat was not being applied consistently to the coupled region. The ETC couplers used in this study were produced using such a system and, thus, had a skewed coupling region.

During the Veridian pulling process, light is launched into one fiber input and the output intensity of the two fibers is monitored. Collecting this data in real time and using the software-controlled pulling station allow a coupler to be drawn to a specific coupling ratio, or length, which can be monitored with the pulling software. The station[23] was designed to pull couplers with only small variations coupler to coupler in order to reduce the number of variables affecting the coupling ratio.[20,21]

A crossover occurs as a coupler is pulled through the 50:50 split in the light output. With the current automated station, couplers have been pulled up to 7 crossovers. Modeling of the single-mode-fiber coupler showed that the sensitivity of the coupler was substantially affected by the number of crossovers during the pulling process.[24] Experiments[25] performed using a series of couplers pulled to the first through the fifth crossover

also indicated that properties such as sensitivity and signal-to-noise ratio were affected by the pull length. Couplers pulled to the third crossover provided optimal performance; therefore Veridian couplers pulled to the third crossover (Fig. 2) were chosen for this study, and were compared to ETC couplers which were pulled to the first crossover. The Verdian couplers tested have a "waist" diameter of approximately 18±1 μm, and the ETC couplers had a waist diameter of approximately 30±2 μm. The waist sizes of both ETC and Veridian couplers were measured using an optical microscope.

The Veridian couplers and the purchased couplers tested were fabricated using a single-mode fiber, in which theoretically there is only a single core mode propagating in the fiber. However, even in a single-mode fiber there can be multiple core modes and, additionally, as light is launched into a fiber through a connector, higher order and cladding modes are often generated that can interfere with the dominant mode.[26] These modes are introduced at the connection from the light source to the coupler and are generated as light is coupled back into the output fiber cores from the waist region. Due to the short length of the fiber optic coupler, these modes will not be attenuated simply by the fiber length, as is common in communications applications. Therefore, standard cladding/higher order mode reduction techniques were used to eliminate or significantly reduce these modes on both the input and output ends of the couplers. The response of the modified versions of both the Veridian couplers and the ETC couplers was compared with that of unmodified couplers. Both the Veridian couplers and the ETC couplers were mounted in a quartz half shell measuring 3.5 inches in length and 0.25 inches in diameter. In order to prepare the coupler for use as a biological sensor, receptor molecules were immobilized on the coupler surface,

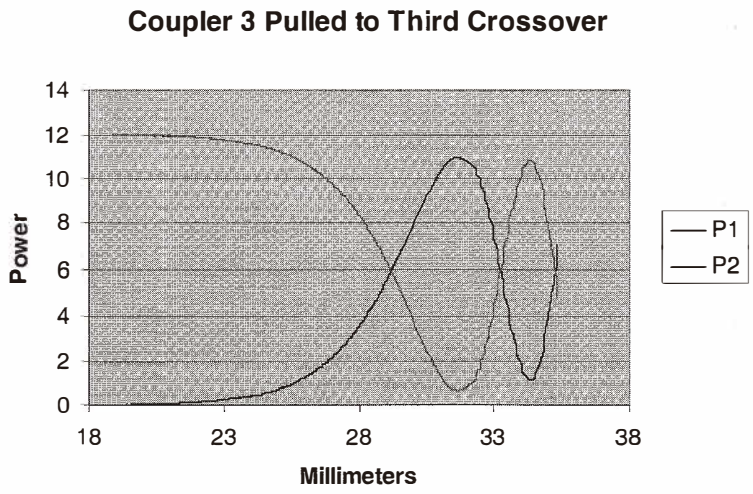

Fig. 2. Real-time monitoring of coupler fabrication. The two coupler output powers (arbitrary units), P1 and P2, are plotted as functions of the coupler pull length (mm). The intersections of the two lines indicate crossovers where the power distribution between the two fibers is 50:50.

as described below. Because both the coupler holder and the coupler are made of quartz, the receptor molecules will be immobilized on both surfaces and, therefore, analyte binding will also occur on both the coupler and the holder. Loss of analyte to the holder surface may reduce the sensitivity of the system because the area of the quartz half is many orders of magnitude larger than the area of the fiber optic sensing region. Therefore, the Veridian coupler holder was passivated to minimize the adsorbance of biological molecules to the coupler frame.

The couplers were treated to immobilize receptor molecules on the fiber surface. The cells were prepared in sets of five couplers that were mounted in a holder and treated simultaneously. The receptor molecule was an antibody, rabbit anti-goat immunoglobulin G (IgG), and the analyte molecule was goat IgG. All couplers sets (Veridian and purchased) were prepared using the following procedure: 1) 30-min soaking in a 1:1 solution of methanol and hydrochloric acid, 2) rinsing in water, 3) 30-min soaking in 50% H_2SO_4 solution, 4) water rinse, 5) rinsing in phosphate buffer saline (PBS), 6) incubation with rabbit anti-goat IgG solution, 7) incubation in a non-protein blocker. Colorimetric and fluorescence techniques were used to verify the immobilization of the rabbit anti-goat IgG on the coupler surface.[27]

3. Results and Discussion

Experiments were run to show the capability of fiber optic couplers to function as bioassays/biosensors and the efficacy of the various design enhancements described above. The receptor-modified couplers were interfaced with an input light source and the output light from each fiber was monitored and collected a rate of 5 points/s. The couplers were tested in a continuous fluid flow system. To measure the response to the analyte, the following solutions were introduced into the continuous flow system: 1) buffer containing the non-protein blocker, 2) 27 μg/mL whole-molecule goat IgG solution, and 3) buffer containing the non-protein blocker. Due to the high binding constant of antibody-antigen interactions, a final buffer rinse (step 3) should not cause a significant change in the coupler output if the analyte (goat IgG) binds to the receptor (rabbit anti-goat IgG).

The results achieved with the Veridian and the ETC couplers that were not modified to reduce higher order/cladding modes were inconsistent (data not shown). Therefore, the mode reduction modification appears crucial to the coupler operation as a biosensor, and both Veridian and the ETC couplers were modified for further testing. Prior to mode reduction modification, the average percent light ratio for the two output fibers was between 50:50 and 25:75 for both the Veridian and the ETC couplers. When the cladding/higher order modes were suppressed in the Veridian couplers, the output light ratios remained similar. However, when these modes were suppressed in the ETC couplers, the light output from one lead was almost completely eliminated and the average percent light ratio for the two output fibers became 95:5 to 99:1, indicating that the light in one output was primarily made up of cladding and higher order modes.

Figure 3 shows typical results achieved using the mode-suppressed ETC couplers and Veridian couplers. All couplers were tested in sets of five, but the response of only one coupler from each set is shown to avoid redundancy. As described above, the couplers

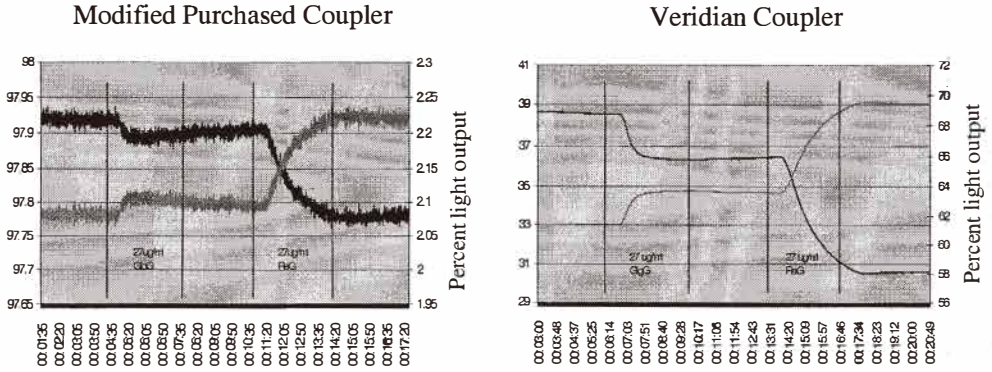

Fig. 3. Typical coupler response to analyte binding. Left - Purchased coupler (ETC) modified to reduce higher order/cladding modes. Right - Veridian coupler pulled to the third crossover and modified to reduce higher order/cladding modes. Fiber output 1 (black) - left axis. Fiber output 2 (grey) - right axis. Data shown are responses to the following solutions: 1) buffer, 2) 27 μg/mL goat IgG, 3) buffer, 4) 227 μg/mL rabbit anti-goat IgG.

were exposed to a buffer, then to a solution of goat IgG and then, again, to a buffer. Both coupler types responded irreversibly to the goat IgG. In order to verify that the goat IgG was bound, a sandwich assay was performed by introducing rabbit anti-goat antibody into the flow system. If the goat IgG was bound to the couplers, it would bind its conjugate, the rabbit anti-goat IgG, and there would be an additional change in the index of refraction surrounding the sensing region of the coupler. As shown in Fig. 3, both coupler types also responded irreversibly to the rabbit anti-goat IgG, as expected. The signal-to-noise ratio achieved is much higher for the Veridian couplers, indicating that the Veridian design modifications used may produce a more sensitive biosensor/bioassay platform.

Experimentation has shown that the fiber optic coupler does provide a viable platform as a biological/chemical sensor. However, the original coupler design[22] must be modified to enhance the signal output, increase the signal-to-noise ratio, and isolate the detection area on the coupler. Veridian Corporation accomplished these goals and showed significant improvement in signal output, and reproducibility by suppressing higher order modes, pulling the couplers beyond the first crossover, and passivating the quartz half.

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