

# Tapered fiber tips for fiber optic biosensors

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**Abstract.** Tapered fiber tips with different geometries are fabricated for developing a fiber optic biosensor. Fluorescence experiments are performed to compare the coupling efficiency of light for different fiber tip configurations. When light is generated in a "thick" layer ( $>1 \mu\text{m}$ ) around a fiber core, the continuously tapered tip with the steepest taper collects light more efficiently than the longer combination tapered tip. To demonstrate the applicability of our results, we have successfully detected weak chemiluminescent signal collected by a bundle of fibers with the short continuously tapered tips using a cooled CCD array detector. The chemiluminescence reaction was catalyzed by alkaline phosphatase immobilized on the fiber tips by a sol-gel technique.

*Subject terms:* fiber optic biosensor; chemiluminescence; sol-gel; fluorescence; alkaline phosphatase.

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## 1 Introduction

Fiber optic biosensors have been studied extensively in the last few years.<sup>1,2</sup> A tapered sensor tip has been used frequently in the development of fiber optic biosensors because of its superior performance in coupling light to a detector and mode conversion capabilities compared to the nontapered ones.<sup>3-6</sup> Because the tip configuration of a fiber optic sensor plays an important role in collecting and transmitting the light signal, both theoretical and experimental investigations have been conducted. Computer simulation by Marcuse<sup>7</sup> pointed out that light generated by a bulk source in a cladding couples more high-order modes into the core than low-order ones in

a positive guiding fiber. Theoretical modeling by Egalon and Rogowski<sup>8</sup> showed that a fiber has a higher coupling efficiency when it has a thin-film source located at the core/cladding interface compared to a source relatively distant from the core. Golden et al.<sup>3,6</sup> and Anderson et al.<sup>4,5</sup> have investigated the effects of different tapering configurations on the fluorescence signals collected by the fiber. For their experiment, which involved binding of fluorescent tags on the side surface of the fiber tip, the combination tapered tip (see Fig. 1) couples more fluorescence signal than the continuously tapered fiber tip.

Recently, a chemiluminescence technique has been investigated<sup>9,10</sup> in our laboratory to detect organophosphorous-based pesticides with high sensitivity (e.g.,  $\sim 50$  ppb for paraoxon in solution). The same detection scheme can be applied in developing a fiber optic chemiluminescence-based biosensor. The enzymes immobilized on the fiber tip catalyze the reaction and the chemiluminescence signal can be collected and transmitted by the same fiber. Because the chem-

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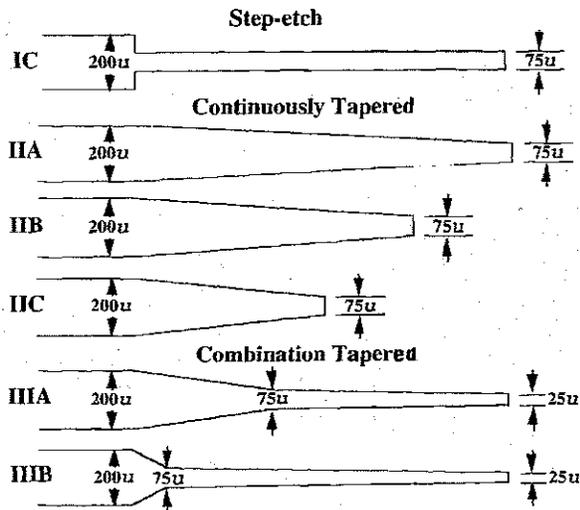


Fig. 1 Configurations of different tapered fiber tips. All tips have lengths of 10 cm except IIB, which is 7.5 cm long, and IIC, which is 5 cm long. Tip IIIA was etched down to 75  $\mu\text{m}$  in 3.5 cm, whereas tip IIIB was etched to 75  $\mu\text{m}$  in only 1 cm.

luminescence signal is weak, efficient coupling of the signal into the fiber is essential. The objective of this study is to investigate the effect of different tapered fiber tip configurations on coupling efficiency of chemiluminescence light. In our fiber optic biosensor, the chemiluminescence signal is not confined to the vicinity of the fiber core and can be generated far away from the core. We want to collect as much of the chemiluminescence light as possible, from both the side surface of the fiber tip and the distal end surface to increase sensitivity.

There are three different cases of decladded fiber being used as a sensor tip: (1) the optical signal generated in a very thin layer ( $< 1 \mu\text{m}$ ) immediately outside the core/cladding interface and the distal end, (2) the optical signal generated in a "thick" layer ( $> 1 \mu\text{m}$ ) around the fiber core, and (3) the light source is uniformly distributed around the fiber tip. In a fluorescence-based fiber sensor, case 1 corresponds to thin film distribution of sources in the cladding, case 2 corresponds to bulk distribution of highly fluorescent sources in the cladding using evanescent wave excitation for the side surface, and case 3 corresponds to bulk distribution of sources in the cladding using side excitation.

To establish the most efficient coupling configuration of the tip for a fiber optic biosensor, we performed experimental studies on the tapering effect of the fiber tips in these three cases.

## 2 Material and Method

### 2.1 Fabrication of Tapered Fiber Tips

Fiber optic biosensors involve immobilization of biological molecules, such as receptors, antigen/antibody, enzymes, or fluorescent-tags at the tip region of the decladded fiber. There are two factors that affect the detected signal for a tapered tip. One is the number of immobilized biomolecules that depends on the surface area of the tip. The other is  $V\#$  mismatch at the boundary between the clad fiber and

the fiber tip resulting from the change of the cladding material.

The  $V\#$  is defined as

$$V\# = \frac{2\pi r_{\text{core}}}{\lambda} [(n_{\text{core}})^2 - (n_{\text{clad}})^2]^{1/2}, \quad (1)$$

where  $r_{\text{core}}$  is the radius of the core,  $n_{\text{core}}$  is the index of refraction of the core, and  $n_{\text{clad}}$  is the index of refraction of the cladding.

In our experiment, pure silica core fiber (from SpecTran Co., Massachusetts) with  $r_{\text{core}} = 100 \mu\text{m}$  and  $n_{\text{core}} = 1.4571$  at  $\lambda = 0.6328 \mu\text{m}$  were used. The fiber has silica cladding with  $n_{\text{clad}} = 1.440$ . The fiber has  $V\# = 221$  at the specified wavelength. However, when the fiber cladding is removed and the core is in direct contact with the aqueous medium, then  $V\# = 584.3$  if we assume the index of refraction of the aqueous medium to be 1.333. The mismatch of  $V\#$  will cause a major loss of signal at the boundary, especially those in higher order modes. The radius of the fiber core must be etched down to  $37.8 \mu\text{m}$  to have matched  $V\#$ . This is called a step-etched tip. The drawback of this tip is that there is a considerable loss of light as it travels across the boundary and experiences an abrupt change of the core radius. For a fluorescence-based sensor, the loss of excitation light is even more than that of the signal light, which is traveling in the opposite direction.<sup>6</sup> A better design is to have the core radius gradually tapered down to match the  $V\#$  at the distal tip. This is called a continuously tapered tip. The combination tapered design goes one step further by sharply reducing the core radius to have the  $V\#$  matched first, then slowly tapering it down to a smaller radius. Figure 1 depicts the configurations of different tapered fiber tips fabricated in our laboratory.

To make tapered fiber tips, approximately 10 cm of the fiber jacket was removed by immersion in a chloroform solution for about 15 min. The fiber cladding was then etched away by immersion in a solution containing 48% by volume hydrofluoric acid (HF) for 8 min. To control the dipping and the lifting speed of the fiber tip vertically into the HF etch bath for tapering, a film lifter from a Langmuir Blodgett trough (Lauda) was used. The etching time and the dipping/lifting speed differ for different types of the tapered tip. After the tapered tips were fabricated, their dimensions were measured by using a calibrated optical microscope.

### 2.2 Fluorescence Experiment

To study the light collection efficiency of the different tapered configurations, fluorescence experiments were conducted in all three cases discussed in Sec. 1. Figure 2 shows the schematic setup of the fluorescence experiment.

An argon ion laser was used as the light source. The laser beam (either  $\lambda = 488 \text{ nm}$  or  $\lambda = 496 \text{ nm}$ ) passes through a dichroic filter (passband between 400 and 500 nm) and a set of collimating lenses before it is reflected by a beamsplitter and focused onto the front end of the fiber by a plano convex lens. This lens ( $f = 12 \text{ mm}$ ,  $D = 14 \text{ mm}$ ) was chosen in such a way that its numerical aperture (NA) is larger than that of the fiber ( $\text{NA} = 0.226$ ), so that all the light signal coming out of the fiber can be collected by the lens. The launching angle of the laser was adjusted to ensure maximum signal count at peak wavelength. Traveling back through the same fiber, the

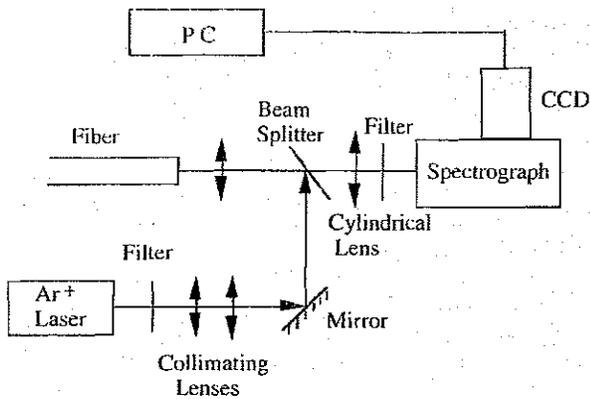
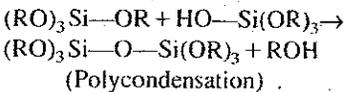
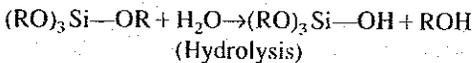


Fig. 2 Block diagram of the fluorescence experiment setup.

fluorescence signal was collected and collimated by the same lens. The signal light then went through the high-transmission beamsplitter and a long-pass filter with a cutoff wavelength at 530 nm. The light was finally focused by a cylindrical lens onto the input slit of a spectrograph with a CCD array detector (Oriel InstaSpec IV). The fluorescence signal detected by the CCD array was processed by a personal computer.

To study the tapering effect of the various fiber tips in case 1, we coated a thin layer of rhodamine 6G dye in a sol-gel matrix on the different tapered tips. The sol-gel process is a technique to produce transparent glass without a high-temperature process. It involves hydrolysis and polycondensation of alkoxides. The following schematic shows a typical sol-gel process to produce silica<sup>11</sup>:



The sol-gel layer containing rhodamine 6G dyes was produced by dipping the whole fiber tip into the sol-gel solution for 1 min, followed by drying the sol-gel layer in air at room temperature for 24 h. Both side surface and distal end of the fiber tip were coated with the sol-gel layer. Optical microscopic studies indicate that the thickness of the sol-gel film is in the submicrometer regime. Observation using the same microscope reveals smooth coating of the sol-gel layer on the fiber surface. The submicrometer sol-gel film containing rhodamine 6G is a good simulation of case 1, because the fluorescence source is a dye-doped thin film at the core/cladding interface.

To study the tapering effect for cases 2 and 3, dilute rhodamine 6G solution was used as a fluorescence source. The tip portion of the fiber was immersed in the rhodamine 6G solution in a glass pipette. The fluorescence signal was coupled into the fiber at the side surface as well as at the distal end of the tip. The main difference in the experimental setup for case 2 and case 3 is the manner in which the laser light was used to excite the rhodamine 6G solution. To simulate case 2, the laser light was coupled into the prox-

imal end. The reason we can simulate case 2 by using this setup is that the fluorescence of the rhodamine 6G around the fiber core extends much farther than the penetration depth of the evanescent wave because of the self-absorption of the dye solution.<sup>10</sup> To simulate case 3, the rhodamine 6G solution was excited directly by the laser beam, not through the fiber. This results in a small change of the setup. In case 3, the beamsplitter in front of the fiber was taken away and the unfocused laser beam was used to excite the dye solution in the glass pipette. The fiber only collects the fluorescence signal from the tip.

### 3 Results and Discussion

Figures 3, 4, and 5 illustrate the results of our fluorescence experiments for cases 1, 2, and 3, respectively. For each configuration of the fiber tip, more than two fibers were used in the experiment. Each experiment was repeated at least two times per fiber. The mean of the fluorescence peak signals was calculated for each configuration and compared in the figures. The result from integrating over the area under each spectrum gives similar trends for each case. Error bars were obtained from different measurements for the same type of fiber. The normalized data with respect to the largest value in each case are shown in the figures. The signal level in all cases is at least 10 times larger than the background level. The SNR is well over 100:1 for all the measurements.

For case 1, the excitation wavelength was 496 nm and the peak fluorescence is about 580 nm. From Fig. 3, we observed that combination tapered tip IIIB has the best coupling efficiency. It collects about 2.5 times more light than the con-

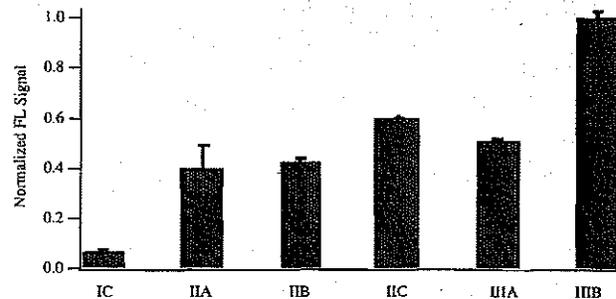


Fig. 3 Case 1: fluorescence signal of rhodamine 6G dye for different tapered fiber tips.

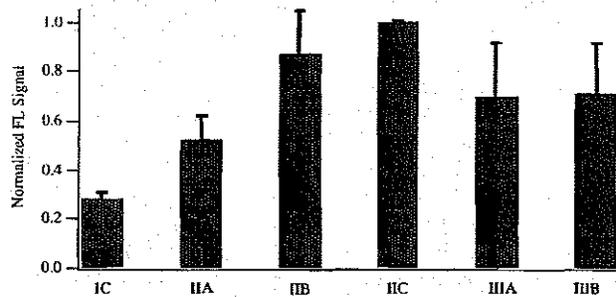


Fig. 4 Case 2: fluorescence signal for different tapered fiber tips in diluted rhodamine 6G solution.

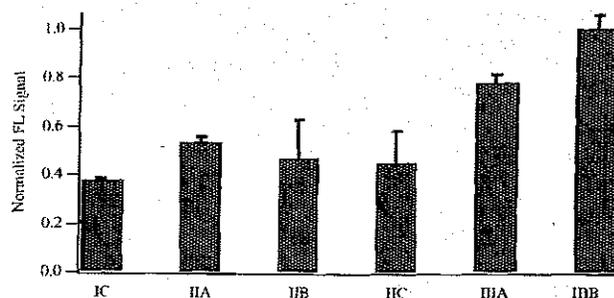


Fig. 5 Case 3: fluorescence signal for different tapered fiber tips in diluted rhodamine 6G solution.

tinuously tapered tip with the same tip length. As we can see from Fig. 1, along most of the combination tapered tip IIB, the  $V\#$  is smaller than the "matched  $V\#$ " because of its smaller radius. The smaller  $V\#$  results in all the light signal collected at the distal end being transmitted to the other end of the fiber, whereas for the continuously tapered tip, the  $V\#$  is matched only at the distal end of the tip. The mismatch of  $V\#$  at the other end of the tip will cause loss of the fluorescence signal as it couples into the clad fiber. Among continuously tapered tips with different tip length, the shortest length fiber tip IIC (see Fig. 1), which has the largest tapering angle, collects more light than the longer ones. This is because the larger tapering angle makes it possible for more light outside the tip to satisfy total internal reflection (TIR) condition. These photons become bound modes as they are coupled into the fiber tip.

For case 2, the excitation wavelength was 496 nm and the peak fluorescence is around 563 nm. Our results indicated that the shortest continuously tapered tip IIC has about 1.4 times higher coupling efficiency than the longer combination tapered tip IIB (Fig. 4). This probably results from the fact that the fluorescence signals were collected both at the side surface of the tapered tip and at the distal end surface of the tip. Those fluorescence signals collected by the distal end are far larger than those collected by the side surface of the tip. However, if the fiber tip is untapered, most of the light collected by the distal end will get lost as it travels along the tip.<sup>7</sup> The collection efficiency can be increased drastically with the mode conversion capability of the tapered tips.<sup>3-6</sup> According to Marcuse,<sup>7</sup> for a bulk distribution of light source in the cladding, fluorescence signals coupled into a fiber are mostly carried as higher order modes. With larger tapering angle, the tip converts more high-order modes into low-order ones. Once the light signals are in lower order modes, they can easily become bound modes. Comparing continuously tapered tip with combination tapered tip that has the same tip length, the combination tapered tips collect light more efficiently for the same reason ( $V\#$  matching) discussed in case 1.

In case 3, 488-nm laser light was used as the excitation beam and the emission peak is around 595 nm. The measured fluorescence signals were much weaker compared to the first two cases. The data shown in Fig. 5 were normalized with respect to the largest signal in case 3 only. Combination tapered tips coupled much more light into fiber than continuously tapered tips. The step-etched tip IC has about 38% of

coupling efficiency compared to that of the combination tapered tip IIB, which is the highest for tip IC among all cases.

Comparing with tapered tips, our experiments indicated that untapered step-etched geometry is the least efficient configuration for light collection in all three cases discussed, provided that no light is collected in the "etched-in" ring area of the core where the etched tip starts. However, if the tip is immersed deeper into the dye solution, in which case light is also collected in the "etched-in" ring area, then the collection efficiency for tip IC increases approximately by 12% for case 3 in our experiment.

Note that there is no difference for case 3 between fluorescence application and chemiluminescence application, because the fiber tip only collects light and its geometry does not affect the excitation of the solution around the tip, whereas in cases 1 and 2, different tapered geometries do affect the excitation by effectively changing the penetration depth of the evanescent wave.<sup>6</sup> The optimum conditions for fluorescence collection may not be the same as those for chemiluminescence.

#### 4 Detection of Chemiluminescence Signal by Tapered Fiber Tips

To check the applicability of our results in the development of our chemiluminescence (CL)-based biosensor, we made use of various fiber tips with different geometries, already described, to collect weak CL signal. In our fiber optic CL biosensor, even though enzymes are immobilized in the sol-gel layer on the surface of the fiber tip and they catalyze the substrate solution around the tip, it is the substrate solution that is responsible for emitting CL signal. Although most of the CL light is in proximity to the tapered fiber core, it extends beyond the sol-gel layer or penetration depth. This situation is similar to case 2 described earlier. Among all the tips tried in our experiment, the short continuously tapered tip IIC gave the best performance for CL light collection. The experiment was developed for detection of organophosphorous-based pesticide. The CL signal is generated from the dephosphorylation of CSPD<sup>®</sup> substrate (from Tropix, Inc., Massachusetts) by alkaline phosphatase, as illustrated in Fig. 6. Inhibition of the enzyme-substrate reaction by the pesticide results in a decreased CL signal. This decrease is directly proportional to the pesticide concentration in a certain range. By monitoring the change of the chemiluminescent signal in

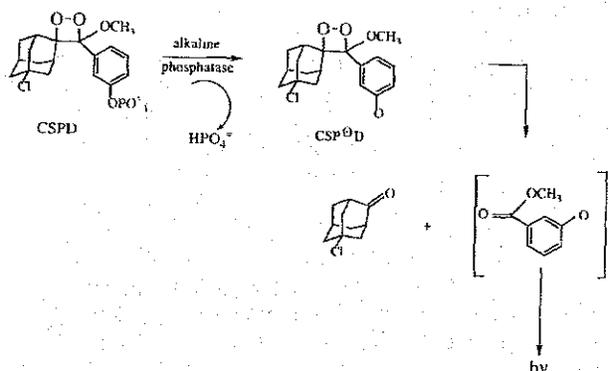


Fig. 6 Alkaline phosphatase catalyzed dephosphorylation of CSPD<sup>®</sup> and subsequent chemiluminescence.

the presence of the pesticide, one can determine the concentration of the pesticide.<sup>9</sup>

In our fiber optic biosensor, alkaline phosphatase was immobilized on the surface of the fiber tip by a sol-gel layer as described earlier. The chemiluminescent signal was collected at the tapered fiber tip and transmitted by the same fiber to a CCD array detector cooled to 0°C. Four fibers were used in a bundle to increase the detection sensitivity. The proximal ends of the fibers were lined up directly in front of the CCD array detector and the continuously tapered tip portions (sensor part) of the fibers were placed in a capillary that contained the CSPD® substrate solution. Figure 7 is a schematic diagram of our CL experimental setup.

Typical chemiluminescence signal for the CSPD® dephosphorylation reaction by alkaline phosphatase immobilized on the tapered fibers is given in Fig. 8. Each point in the graph represents the accumulated CL signals detected by the CCD array over 3 s.

The described experiments demonstrated that weak CL signals can be detected by choosing the tip geometry of the optical fiber that has the highest coupling efficiency. In a fluorescence experiment, one can control the signal intensity by adjusting the excitation light. In a CL experiment, however, one has very limited control over the signal intensity, which is determined by the chemical reaction. To increase the SNR, it is critical to use the most efficient tapered fiber tip. The results of coupling efficiency resulting from the tip geometry also provide guidance for other fiber optic sensor systems that utilize tapered fiber tips as the light collector.

### 5 Conclusion

The most efficient taper geometry of fiber optic biosensor has been determined for detection of light signal in our study. In our experiments, the continuously tapered fiber tip with the largest tapering angle (IIC) was the best configuration to collect light when the light source extends beyond the penetration depth of the evanescent wave. However, the combination tapered tip (IIIB) proved to be the most efficient configuration when the light source is in the penetration depth of an evanescent wave or is uniformly distributed around the fiber tip. Continuously tapered tips with the shortest length

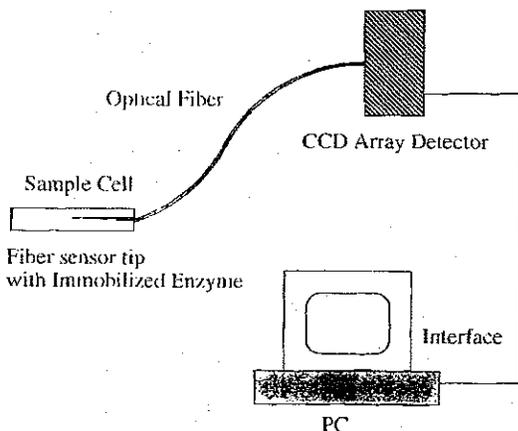


Fig. 7 Schematic of fiber optic chemiluminescence experimental setup

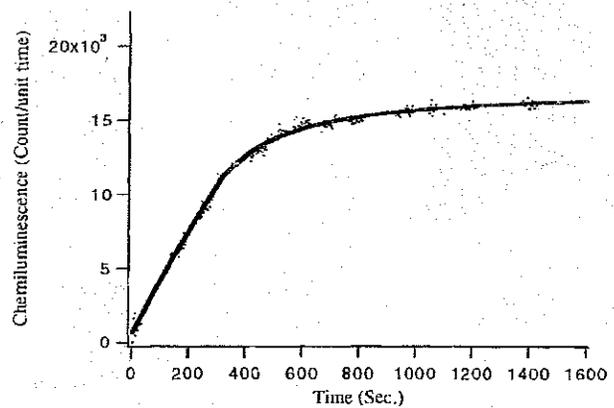


Fig. 8 Chemiluminescence signal of CSPD® substrate catalyzed by alkaline phosphatase immobilized on the tapered fiber tips. Concentration of CSPD®: 1.6mM. Dotted symbols are experimental data and solid line is a fitted curve.

(IIC) were used successfully to detect the chemiluminescent signal from dephosphorylation of CSPD® substrate by alkaline phosphatase. Even though the results of our tapered tip study were demonstrated only in detection of the chemiluminescent signal, it is clear that they can be applied to other fiber optic sensors that make use of a tapered tip to collect light.

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