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Technical Notes

Integrating Waveguide Biosensor

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A capillary biosensor is demonstrated which uses the waveguiding properties of the capillary to integrate the signal over an increased surface area without simultaneously increasing the background noise from the detector. This biosensor achieves limits of detection of 30−50 pg/mL in immunoassays using a diode laser for excitation and a PMT for detection. This is ∼2 orders of magnitude greater sensitivity than was achieved using the same immunoassay reagents in a fiber optic biosensor or a planar array biosensor. Two different approaches to using the capillaries as immunosensors are described, either of which could be adapted for multianalyte sensing.

The concept of combining optical analysis with a capillary device has appeal, because the capillary can provide two functions simultaneously: it can guide fluid flowing through its lumen and guide light in its wall.[1] Capillaries have been used successfully as supports for proteins in several types of optical biosensors.[2−8] In these biosensors, the excitation and emission are handled using a variety of approaches. In the flow immunosensor and the enzyme-based glutamate biosensor, the assay occurs upstream from a detection region; the detection region functions as a cuvette with excitation and collection of emitted light normal to the long axis of the capillary (Figure 1.1).[3,5,8] In the capillary fill device as described using a capillary,[2] the excitation light is introduced at the end of the capillary and propagates on the inner surface as an evanescent wave. When the evanescent excitation generates a signal from an antigen−antibody-fluorophore complex, the emitted light is coupled into and propagates along the capillary. At the distal end, a grating is used to couple the light out of the waveguide and into a detector (Figure 1.2). This geometry is analogous to an evanescent fiber optic biosensor,[9−11] except that the sample solution is inside instead of outside the waveguide. In the third geometry,[4] the excitation is evanescent, but the emitted light is collected normal to the capillary axis using a CCD camera (Figure 1.3). This sensor is analogous to an array biosensor based on a planar waveguide.[12−16]

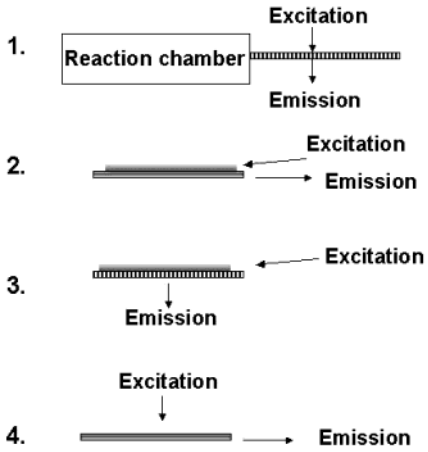

Figure 1. Capillary and optical path geometries. Excitation light can be used to illuminate the capillaries from a 90° angle (1,4) or via waveguiding and evanescent illumination (2,3). The fluorescent signal is collected at a 90° angle from the capillary (1,3) or after being coupled into the waveguide and transported to the end of the capillary (2,4).

The fourth geometry is less obvious in terms of effective collection of emitted light than the previous three arrangements

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of excitation and emission. Furthermore, an analogous biosensor with a comparable optical configuration is not obvious. In this configuration, the excitation is produced by a light source normal to the axis of the capillary, and the emitted light is coupled into the waveguide and collected at one end (Figure 1.4).[6−7] Misiakos and Kakabakos used this geometry as a way to demonstrate detection of multiple spots with a single photodiode or photomultiplier (PMT). They coupled biotinylated bovine serum albumen to the surface in bands ∼5 mm wide and measured binding of Europium-streptavidin to the biotin. By pulsing the capillary with 340 nm light, each band could be roughly discriminated by correlating the signal with the capillary length (60 mm). The detection limit for the avidin was ∼1 ng/mL. In the first set of experiments in this paper, we use a photoactive cross-linker to immobilize antibodies in spots on the inside of a smaller glass capillary and demonstrate antigen−antibody binding using a diode laser to scan the capillary.

However, although the capillary used with a scanning diode laser and PMT in this way provides a simple and inexpensive biosensor with potential for multianalyte sensing, the real power of this excitation/emission configuration for ultrasensitive detection is not utilized. When the active surface area is increased inside the capillaries, the fluorescence signal increases, but the electronic (PMT) background noise remains constant. When the entire internal surface of the capillary is active, the fluorescence signal accumulates, or integrates, over its length, while the background noise essentially remains constant. In the integrating capillary biosensor we describe in this paper, a single antibody is coated on the entire inner surface of the capillary. After the immunoassay procedure, the entire coating is illuminated using a diode laser with a line generator to produce excitation light normal to the capillary and along most of its length. The capillary performs the signal integration as the fluorescence emitted from the film couples into the waveguide and passes to the ends, where a PMT or photodiode can collect the integrated fluorescence. Thus, in addition to the advantage of using simple, low-cost optical components, this biosensor can detect significantly lower concentrations of analyte. Multianalyte detection can be attained by passing the sample through multiple capillaries, each coated with a different antibody, either sequentially or in parallel, depending on the amount of sample available.

EXPERIMENTAL SECTION

Materials. Fused-silica capillary pieces 38 mm long (0.70-mm i.d., 0.85-mm o.d.) with optically polished ends and coated on the outer surface with PTFE were obtained from Polymicro Technologies (Phoenix, Az). Mouse immunoglobin G (IgG), biotin-SP-conjugated goat anti-mouse IgG (H+L), and Cy5-conjugated anti-mouse IgG were obtained from Jackson ImmunoResearch Laboratories, Inc. (dye:protein ratio = 2.6; West Grove, PA). Purified staphylococcal enterotoxin B (SEB) and affinity-purified sheep anti-SEB were purchased from Toxin Technologies (Sarasota, FL). The SEB detection antibodies were fluorescently labeled using cyanine Cy5 Bisfunctional Reactive Dye Kit (λ_{ex} = 649 nm, λ_{em} = 670 nm; Amersham Pharmacia Biotech, Pittsburgh, PA). After reconstitution of the freeze-dried fraction in deionized water, 1.0 mg of sheep anti-SEB was incubated in Cy5 reactive dye for 30 min. The free dye was separated from the labeled protein over a BioGel P-10 gel permeation column (Bio-Rad Laboratories, Hercules, CA). All procedures were in accordance with the dye kit manufacturer's instructions**.** The dye:protein ratio for this reagent was found to be 6.8 (Complete dye labeling and characterization procedure is detailed in Amersham Pharmacia Product Specification #PA-25000). All reagents were purchased as freeze-dried, sealed samples, which were reconstituted per manufacturer recommendations. Anti-SEB antibody was biotinylated using a 5-fold excess of biotin-LC−NHS ester (Pierce, Rockford, IL) according to previously published protocols.[14] Unincorporated biotin was separated from biotinylated antibody using gel filtration of BioGel P-10.

The following chemicals were used as obtained from the respective suppliers as indicated: ethanol (Warner-Graham, Cockeysville, MD); hydrochloric acid, sulfuric acid, methanol, anhydrous toluene, sodium hydrogen phosphate heptahydrate, and Tween-20 (Aldrich, Milwaukee, WI); sodium phosphate, monobasic (Mallinckrodt, Hazelwood, MO); 3-mercaptopropyl trimethoxysilane, *N*-(maleimidobutyryloxy)-succinimide ester (Fluka, Milwaukee, WI); dimethyl sulfoxide (DMSO), and bovine serum albumin from Sigma (Milwaukee, WI).

Patterned Capillaries. The photochemically active silane, 2-nitro-5-[11-(trimethoxysilyl)undecyl]oxybenzyl methoxy poly(ethylene glycol) propanoate (NMPEG-silane), developed by Conrad,[17,18] was employed to immobilize antibodies in discrete bands inside the capillaries. Briefly, NMPEG-silane is a unique molecule that provides a protein-"rejecting" poly(ethylene)glycol surface prior to ultraviolet light irradiation and a site-specific protein-immobilization surface after irradiation. Photolysis of the NMPEG-silane cleaves the carbamate, removing the hydrophilic PEG moiety and yields a surface-bound *o*-nitrosobenzaldehyde self-assembled monolayer. The capillaries were coated with the NMPEG-silane under nitrogen at 1 mg/mL in pH 4 acetate buffer, 60 °C for 24 h. The capillaries were then removed from the reaction mixture, rinsed with copious amounts of water, and dried in a desiccator for 2 days.[17] For use, the NMPEG-silane film was rehydrated with double-distilled water for 30 min and rinsed twice, and the capillary was filled with water. A mask was placed over the capillary, and photolysis was performed using a pulsed UV-curing system (model RC-250B, Xenon Corporation) equipped with a 340 nm ± 10 band-pass filter. The 4-s exposure delivered ∼400 mJ/cm^2 to the sample surface. After illumination, the capillaries were rinsed twice with water, refilled, sonicated for 5 s, and rinsed twice more. The capture antibody solution was prepared by adding 40 μg/mL antibody in PBS to an equal volume of 1 mg/mL in $NaCNBH_3$ in 0.1 M acetate/acetic acid buffer pH 4.8.[17] This solution, with a final antibody concentration of 20 μg/mL antibody, was introduced into the capillary and reacted on a rocker at room temperature for 30 min. The reaction between the aldehyde and the available primary amines on the antibody yields a Schiff base. Thus, the antibodies were bound to the surface in a stable, site-specific, and covalent manner. After two rinses with PBS, the capillaries were ready for an assay.

Integrating Waveguide Capillaries. The acid cleaning methodology used in these experiments has been demonstrated to be effective in preparing glass surfaces for antibody attachment.[19]

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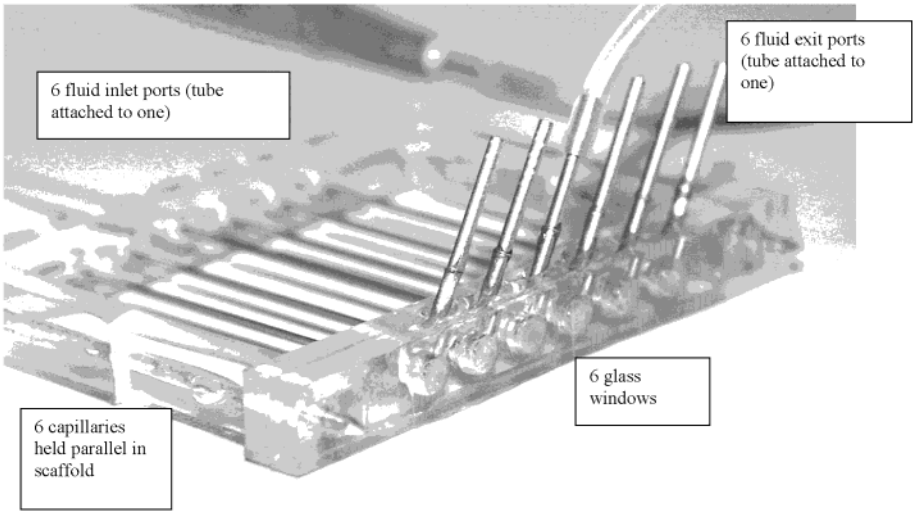

Figure 2. Scaffold holding capillaries. Fluid is pulled through the capillaries and out the metal tubes using a peristaltic pump. Emitted fluorescence is directed by the capillary through the glass window at the end of the scaffold through the lens to the PMT.

Capillaries were first cleaned with 1:1 methanol:hydrochloric acid solution for 30 min. This was followed by liberal rinsing with deionized water and subsequent incubation for 30 min in concentrated sulfuric acid. Great care must be taken in loading and unloading the sulfuric acid into the capillaries, because the solution is viscous, and heat is generated during initial application. Following the sulfuric acid treatment, the capillaries were rinsed exhaustively in deionized water until no schlieren lines were evident. The Tygon tubing segments between capillaries, when more than one were prepared simultaneously, were changed, and the capillaries were dried by purging the interiors with a gentle flow of purified nitrogen gas.

To facilitate the application of solution to only the interiors of the capillaries during the preparation of the integrating waveguides, the capillaries were attached to the tips of 1 cm^3 Luer tip syringes using 1.0 cm lengths of Tygon tubing affixed to tubing-barb-to-Luer adapters. Simultaneous treatment of as many as 20 capillaries was accomplished by attaching capillaries in series using 1.0-cm lengths of Tygon tubing. The capillary ends were inserted into the tubing and held by the friction between the capillary and the tubing. A 2.0-cm length of the Tygon tubing was attached to the end of the capillary distal to the syringe, allowing the end of the assembly to be dipped into treatment solutions without contacting the outer cladding of the capillary. Using this series assembly, solutions could be flushed through the capillaries with ease by drawing on the syringe plunger. Drawing solution in and clamping the end tubing with a metal pinch clamp enabled the incubation of macromolecules within the capillaries. Care was taken to exclude air pockets within the capillaries.

For safety, care must be taken to avoid subjecting the capillaries to high positive pressures such as those induced when solutions are ejected from the capillaries by depressing the syringe plunger. This can cause the tubing-to-capillary junctions to fail and pressurized streams of solution to be expelled unexpectedly. To avoid this, solutions should be drawn through the capillaries using the syringe to generate a negative pressure. The syringe may then be detached from the capillaries and the solution discharged directly into a waste container. This is especially crucial to safe performance of the acid cleaning steps, since the solutions are quite viscous.

For the integrating waveguides, avidin was covalently attached to the internal surface of the capillary, essentially using the silane chemistry described by Bhatia et al.[20] In preparation for the silanization, the capillary interiors were rinsed with 10 mL of anhydrous toluene. A solution of 2% 3-mercaptopropyl trimethoxysilane in anhydrous toluene was drawn into the capillaries using a glass syringe. After rinsing the solution through once, the silane was allowed to incubate within the capillaries for 1 h in a nitrogen-filled glovebag. The capillaries were then rinsed three times with anhydrous toluene and allowed to dry under nitrogen for 30 min. The silanized capillaries were then treated for 30 min with 1 mM *N*-(maleimidobutyryloxy)-succinimide ester dissolved in 100 μL of DMSO and diluted in anhydrous ethanol. The capillaries were removed from the glovebag and rinsed thoroughly with double-distilled water. A freshly prepared solution of 30 μg/mL NeutrAvidin biotin-binding protein (Pierce, Rockford, IL) in 10 mM phosphate-buffered saline (PBS, pH = 7.4) was incubated in the capillaries overnight at 4 °C. The avidin-coated capillaries were rinsed with 10 mL of PBS to remove unbound NeutrAvidin and stored at 4 °C filled with PBS containing 0.03% sodium azide until use.

In preparation for each assay, the avidin-coated capillaries were treated with appropriate biotin-conjugated capture antibodies. Capillaries for mouse IgG assays were treated with a 10 μg/mL solution of biotinylated goat anti-mouse IgG prepared in PBS with 0.05% Tween-20 (PBST) for 1 h. The capillaries for SEB assays were prepared identically, except that 10 μg/mL solutions of biotinylated sheep anti-SEB in PBST were utilized for the incubation. The antibody incubation was followed by a rinse with PBST containing 1.0 mg/mL bovine serum albumin (PBSTB).

Optics and Signal Electronics. The optical bench for the capillary immunosensor consisted of an excitation source, the capillary holder, system optics, and the photomultiplier tube. The capillary was securely held within a custom-fabricated scaffold assembly (Figure 2). This component, milled in-house of poly(methyl methacrylate) (PMMA), held the capillary perpendicular to the laser excitation beam. A window at one end of the capillary scaffold allowed optical access to that end of the capillary while

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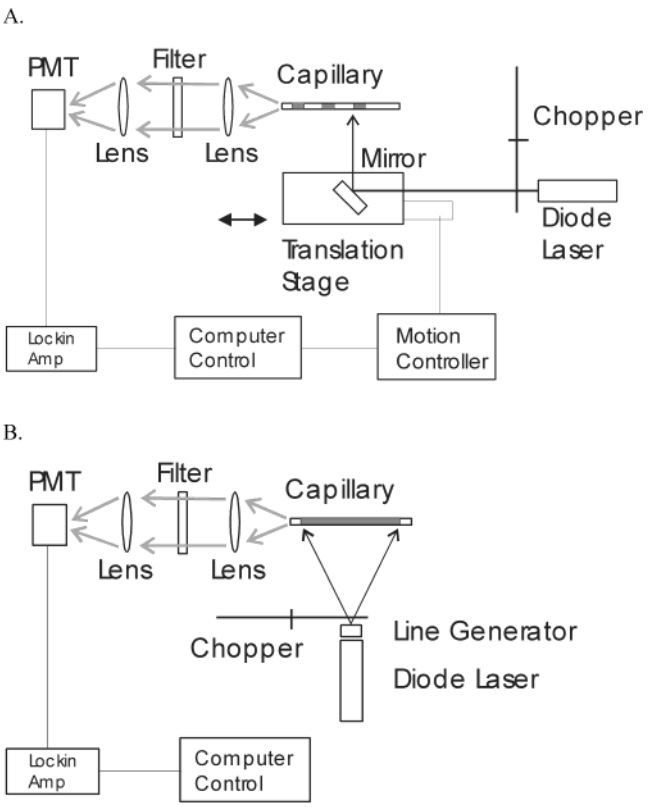

Figure 3. Schematic representation of optics for capillary biosensor: A, components used to measure fluorescence from immunoassays in patterned capillaries; B, simplified version of sensor used to measure fluorescence from immunoassays in single analyte capillaries.

facilitating fluid flow-through. It was through this window that the emission was directed by the capillary to the detector. The detector was a Hamamatsu HC-120−05 photosensor (PMT) module (185−900 nm, 20 kHz, 15 V dc). The detector received the emission from the end of the capillary after passing through a biconvex collimating lens, a 670-nm band-pass filter, a 665-nm-long pass filter, and a focusing lens. The PMT output was connected to the input of a SR-510 lock-in amplifier (Stanford Research Systems, Sunnyvale, CA). External reference input was provided from the optical chopper trigger output. As Figure 3 indicates, the laser beam was oriented 90° relative to the emission optical path. The laser excitation source employed here was a 635-nm, 12 mW diode laser (LaserMax, Bridgewater, NJ). An optical chopper (315 Hz, Stanford Research Systems) was placed in front of the laser, as shown in Figure 3A.B. For the patterned capillary experiments, a mirror was mounted on a stepper translation stage so that the excitation beam could be moved automatically along the capillary length (Figure 3A). Data acquisition software written in LabWindows (CVI, National Instruments) controlled the translation stage and collected the data from the lock-in amplifier at 5-s intervals. For the integrating waveguide experiments, the laser was equipped with a 45° line generator (Figure 3B). The line generator was aligned to provide a beam parallel to the bench surface and extending the entire length of the capillary. The beam was focused on the capillary by a biconvex lens (20-cm focal length, Newport Corp, Irvine, CA). Masking was used to match the length of the emitted line with the exposed length of the capillary only in order to reduce the scattered stray light.

Fluidics. Tygon tubing was utilized for all solution delivery and waste lines except where noted. The solutions introduced into the capillary immunosensor were drawn from their respective reservoirs using a Pharmacia P-1 peristaltic pump placed in circuit following the capillary holder. Switching between buffer, air, antigen, and detection antibody reagents was effected using manually activated plastic stopcocks. The valve configuration allowed for the introduction of air pockets between the solutions applied. This prevented diffusion of reagents into the buffer and cross contamination of the reservoirs. The experiments were performed at a volume flow rate of 0.12 mL/min, and open-circuit, unidirectional flows were used.

For the preliminary patterned capillary experiments, the capillaries were exposed to sample and fluorescent antibody, dried, and mounted in front of the laser for analysis. The patterning experiments were performed before the fluidics scaffolding was constructed, necessitating the open-ended tubes to be scanned dry. For the integrating waveguide measurements, the assays were run while the capillary was in the instrument, and measurements were made while the capillary contained PBSTB.

Sandwich Immunoassays. Samples of mouse IgG and purified staphylococcal enterotoxin B (SEB) were prepared in PBSTB with concentrations ranging from 10 pg/mL to 1000 pg/mL. At least three replicate assays were performed for each solution. New capillaries were used for each assay. Tracer antibodies with Cy-5 fluorescent label were used at a concentration of 10 μg/mL in PBSTB.

A sandwich assay procedure was utilized to detect mouse IgG or SEB. The assay protocol was adapted from those worked out for other sandwich immunoassays on glass surfaces.[10,14,21,22] The flow of reagents and buffer through the fluidics was maintained at 0.12 mL/min for an average linear velocity of 0.52 cm/s through the capillary. Capillaries were initially incubated in flowing PBSTB for 2 min. Following this, an air bubble was introduced into the fluidics, followed immediately by the sample. The sample was exposed for 15 min under flow, after which the capillary was washed with PBSTB for 5 min. (Again, an air bubble was inserted at the solution interface to reduce mixing of solutions.) The capillary was then exposed to the Cy-5 labeled tracer antibody for 5 min under flow and flushed with buffer for 5 min. The PMT output was monitored during the course of each experiment.

Each series of assays was accompanied by blanks incubated in flowing PBSTB for the 15 min that the standard samples were incubated in an antigen solution. Any response measured in these blanks could be attributed to the nonspecific binding of the Cy-5 modified tracer antibody to the surface. Experimental controls were also performed to confirm the biological specificity of the reaction. These included assays for mouse IgG with immobilized anti-SEB capture antibody using Cy5-labeled anti-mouse IgG as the tracer antibody and with immobilized anti-mouse IgG capture antibody using Cy5 anti-SEB as the tracer antibody.

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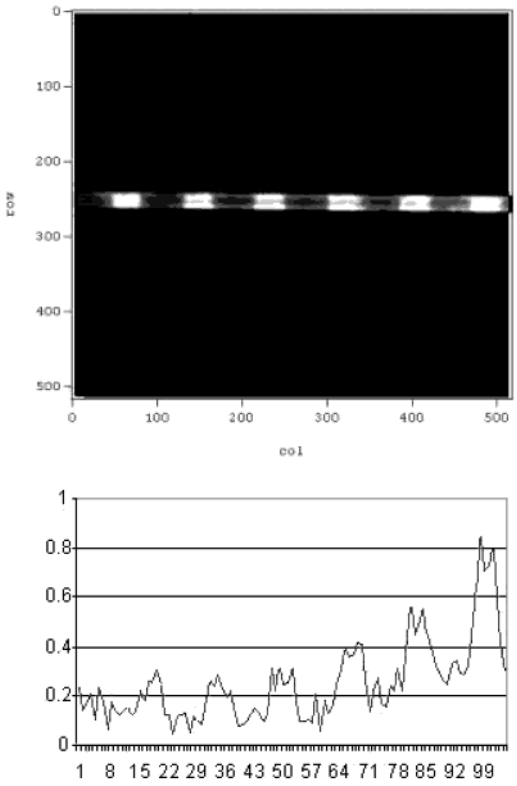

Figure 4. CCD image of patterned capillary after SEB immunoassay. A sample containing 500 ng/mL SEB was assayed using a capillary patterned with anti-SEB. To visualize the pattern, the capillary was illuminated from the end using a 635-nm diode laser, and the resulting fluorescence was imaged using a CCD camera. The image is (2.5 cm)^2. The graph shows the fluorescence signal that was obtained as the laser scanned the capillary from 90° and the fluorescence waveguided to one end was collected by the PMT. The background increases toward the right as the laser light approaches the end of the capillary closest to the PMT.

RESULTS AND DISCUSSION

Patterned Capillaries. Anti-SEB was patterned on the inside surface of a capillary in bands of ~2 mm width using a photoactive silane. Because of the PEG remaining on the silane between the bands, there is minimal immobilization of the capture antibody to the surface of the capillary between the bands and minimal nonspecific adsorption of antigen or labeled antibody between the bands (Figure 4). The increase in the baseline at the end of the capillary nearest to the PMT is due to scattered excitation light that increases as the laser gets closer to the PMT during the scan of the capillary. Despite the increasing baseline, the signal from each peak is evident after samples as low as 10 ng/mL SEB have been assayed (Figure 5). The limit of detection in this format, as measured using the mean + 3 SD of the baseline values, was 10 ng/mL; however, because of the variation that can be seen in Figure 5, we are skeptical of the reliability of this limit of detection. Much of the deviation in the 6 values may be due to the more efficient recovery of fluorescence from the bands nearest to the PMT, as can be seen in Figure 4.

In comparison to the experiments of Misiakos and Kakabakos, the limit of detection of our patterned capillary system was 10-fold higher (10 ng/mL compared to 1 ng/mL). However, Misiakos and Kakabakos were measuring biotin binding to avidin, which has a higher association constant than the antigen−antibody

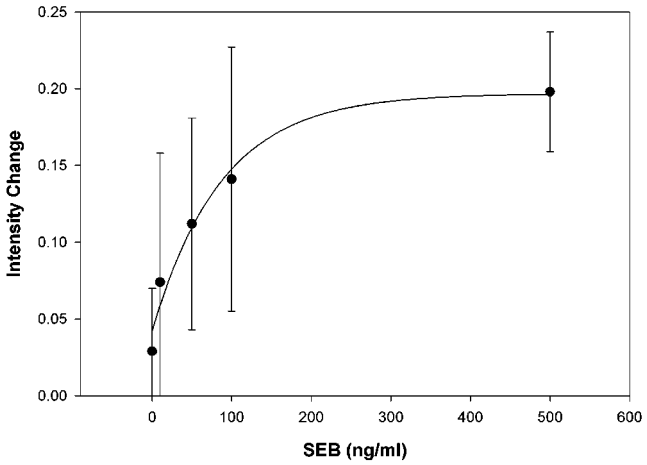

Figure 5. Dose−response curve for SEB assay in patterned capillary. Each concentration of SEB was exposed to six bands of antibody in a single capillary. Values represent the mean + SD (n = 6) in microvolts.

binding measured in these experiments (10^{15} M^{-1}, as compared to 6×10^5 M^{-1} for this particular IgG−anti-IgG pair[23] and 10^6−10^7 M^{-1} for the SEB−anti-SEB pair, unpublished results). Furthermore, they used a more powerful light source and time-resolved fluorescence, which eliminated the background excitation signal. Last, the surface area covered by the biotin-coated bands was 3.6 times greater than that of the antibody-coated bands used here (length, 5 mm vs 2 mm; i.d., 1 mm vs 0.7 mm). Considering the greater simplicity, smaller component size, and lower cost of the system used here, the results compare admirably. In addition, the photochemistry used for immobilization produced a much tighter control over the location of the bands of immobilized proteins than did the nonspecific adsorption method used by Misiakos and Kakabakos.

Integrating Waveguide Capillaries. For several reasons, the antibodies were immobilized via an avidin−biotin bridge rather than by using a covalent attachment to the surface. First, the avidin seems to passivate the surface against nonspecific binding, which not only prevents an increase in background signal due to adsorption of the tracer antibody, but also prevents denaturation of the antibody via adsorption to the glass over long association times after covalent attachment. Second, the number of biotins attached to the capture antibody can be limited so that the number of attachment sites varies from 1 to 3; direct covalent attachment of the antibody to the silane can result in a much higher number of attachment sites and reduced activity. Ligler et al.[21] showed an increased activity in capture antibodies bound to fiber optic waveguides when attachment to the silanized surface was through the avidin instead of through covalent cross-linkers to the silane.

To determine if there was, in fact, some particular step in the capillary preparation that impacted the background intensity, the intensities of a series of 12 capillaries were measured during the preparation and assay procedures. Without a capillary in the holding scaffold, a signal of 60 μV was consistently recorded at the lock-in amplifier. The capillaries were placed in the optical system, buffer flowed through at 0.12 mL/min, and the intensity

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was recorded. The initial intensity of the capillaries averaged 124 μV. This may indicate that there is a significant contribution to the background from reflection within the capillary or at the end of the capillary proximal to the PMT. Starting from the initial intensity of an acid-cleaned capillary filled with PBSTB, there was an 8% drop in the intensity following the silanization and avidin immobilization. There was then a slight (<1%) increase following immobilization of the capture antibody and a 3% increase following incubation with 200 pg/mL mouse IgG for 15 min (assay conditions). From these measurements, it appears that the preparation steps contribute very little to the generation of the background, indicating that most of the background signal prior to the addition of the fluorophore is probably due to scattering or reflection within the optical path. Steps were taken to mitigate optical reflection within the detector optics, although the intensity changes are negligible compared to the 46% increase in intensity for a capillary assay of a 10 pg/mL solution of mouse IgG.

The *y* axis is labeled as intensity change. The change in intensity is defined as the intensity of the capillary signal following incubation with Cy-5 and subsequent wash with buffer minus the intensity following antigen exposure. Therefore, the reported intensity is only that intensity gained by bound Cy-5. Upon exposure of the capillary used for the antigen blank to Cy-5 (and subsequent buffer wash) there is a small, but consistent *increase* in the intensity. One would expect there to be no increase due to binding of the tracer antibody because there is no antigen; but there is a limited amount of nonspecific binding. So the offsets of the blank and the curves shown in the figures are indicative of the extent of nonspecific tracer antibody binding.

The mouse IgG dose−response curve is based on multiple sandwich immunoassays with fresh capillaries used each time. Ten to twelve capillaries were prepared simultaneously and incubated in a goat anti-mouse IgG solution and assayed individually. The triplicate data show good precision, as indicated by the error bars in Figure 6. The lowest concentration discernible above the mean + 3 SD of the blank was 40 pg/mL. The range of quantitation for the curve is between 40 and 300 pg/mL. Beyond 300 pg/mL, the curve levels off asymptotically. The signal-to-noise ratio for the 40 pg/mL standard concentration was 26. The signal-to-noise ratio at the upper limit of quantitation was 41.

A dose−response study was also conducted using the protein toxin SEB and antigen concentrations ranging from 10 pg/mL to 1 ng/mL (Figure 7). The lowest concentration discernible above the mean of the blank + 3 SD was 30 pg/mL. The range of quantitation for the curve was between 30 and 400 pg/mL. Beyond 400 pg/mL, the curve levels off asymptotically. The signal-to-noise ratio for the 30 pg/mL concentration was 53. The signal-to-noise ratio at the upper limit of quantitation was 137.

The dose−response curves in this study had a narrow dynamic range. It was the intention of this work to determine the limit of detection of this methodology while still maintaining somewhat reasonable incubation times. To do so, the capillaries were exposed to antigen for 15 min. With the limited surface area inside the capillary interior and the flow rate of the antigen solutions during this time, the binding sites were saturated, causing the leveling effect seen at the higher concentrations. Running the assays for shorter incubation times would increase the dynamic range but might elevate the limit of detection.

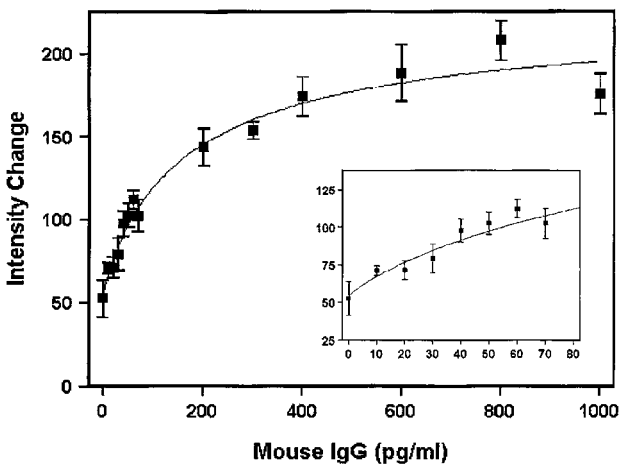

Figure 6. Dose−response curve for mouse IgG assay in single-analyte capillaries. Mouse IgG concentrations ranging from 10 to 1 ng/mL were analyzed in triplicate. The capillaries were prepared with capture antibody and exposed to mouse IgG for 15 min under a 0.12 mL/min flow rate. The detection antibody was introduced for 5 min at a flow rate of 0.12 mL/min. The intensity changes represent the increase in signal in microvolts after exposure to the mouse IgG and Cy5-antibody, as compared to the value for the capillary prior to incubation with analyte. The inset shows an expanded view of the responses for 10−80 pg/mL mouse IgG. The data fits an asymmetrical sigmoid model with the equation $y = 219/(1 + (x/150)^{-0.89})$ with a good correlation ($R^2 = 0.97$).

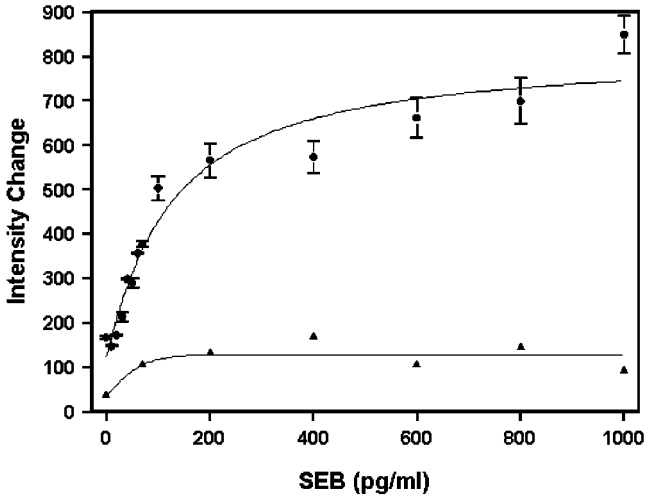

Figure 7. Dose−response curve for SEB in single analyte capillaries. Circles are triplicate analyses of SEB for concentrations ranging from 10 to 1000 pg/mL in buffer. Capillaries treated with anti-SEB are exposed to SEB solutions for 15 min and to the detector antibody for 5 min. Triplicate data points (in microvolts) were averaged and fit to an asymmetrical sigmoid model ($y = 808/(1 + (x/122)^{-1.075})$) with a good fit ($R^2 = 0.97$). Triangles depict a control experiment in which capillaries coated with anti-SEB capture antibody are exposed to mouse IgG and Cy-5 anti-mouse IgG tracer antibody.

Control experiments confirmed the specificity of the assays. Capillaries prepared with biotinylated anti-SEB were deliberately exposed to concentrations of mouse IgG from 70 pg/mL to 1 ng/mL. The capillaries were then exposed to Cy-5 anti-mouse IgG. The Cy-5 anti-mouse IgG tracer antibody should bind to any mouse IgG bound by or adsorbed to the anti-SEB and the capillary surface. Although there was a slight increase in the intensity compared to the blanks, the intensities level off at ~200 pg/mL,

and the values were not statistically different from values for the blanks. This control, shown in Figure 6, demonstrates the relative insensitivity of the immunosensor to an inappropriate antigen. The specificity of the binding of the detection antibody to the antigen was also demonstrated in a control experiment in which capillaries treated with biotinylated anti-mouse IgG were incubated in mouse IgG solutions, and detection was with Cy-5 labeled anti-SEB. These capillaries showed no significant increase in fluorescent intensity over the corresponding blank.

CONCLUSIONS

An important feature of the capillary biosensor system is that the waveguide and surface upon which the immobilization takes place also function as integral components of the fluidics system. This serves to simplify the design of instrumentation; however, a more important feature of the capillary biosensor is demonstrated here: With the illumination of a significant fraction of the capillary or the entire capillary, the signal can be integrated over the length of the capillary via the waveguiding capability of the capillary while the electronic background remains constant. This feature significantly enhances the sensitivity of the assay. The assays performed with the integrating waveguide capillaries (Figure 7) have a limit of detection ~2 orders of magnitude lower than assays performed with the same SEB reagents in patterned capillaries (Figure 5), a fiber optic biosensor,[11] or a planar waveguide-based array biosensor.[22] This factor is particularly impressive because the optic components (capillary, diode laser, and PMT) are so small and simple.

Another reason that the results are so surprising is that the fluorophores were excited at 90°, and only the fluorescence coupling into the waveguide was measured. According to theories modeling the emission from a single dipole at a surface, this process should not be very efficient.[24] Misiakos and Kakabakos[6] assumed that the light excites photons that are emitted isotropically from the fluorophore and that, on the basis of the critical angle and refractive index of the capillary, 14% of the emitted fluorescence is coupled into the capillary and guided to one end for detection. MacCraith and colleagues[25] have a different theory, however, which better explains the relatively high level of signal detected using the capillary biosensor. They developed an electromagnetic theory of fluorescence radiation based on evaluating radiation from a layer of dipoles rather than a single dipole. When this layer of radiating dipoles is placed at the interface between two dielectric materials with different refractive indices, the radiation is distributed preferentially into the material with the higher refractive index (in this case, the fused silica of the capillary). Furthermore, when the dipole layer is placed on a waveguide, the angles of the emitted fluorescence correspond to the angles of guided TE and TM modes propagating within the waveguide system, that is, the radiation of the dipoles is efficiently coupled into the guided modes of the waveguide.

Not only do the integrating waveguide capillaries provide an efficient means for measuring a fluorescent signal, but multiple capillaries can also operate in parallel for multianalyte sensing. Figure 2 shows the fluid exit end of the manifold used for the experiments described here and demonstrates the ability to hold six capillaries. All of the capillaries could be flooded with light simultaneously for excitation. The figure also shows capillaries clad with a colored polyimide. If white light is used, the cladding could be selectively colored and, thus, serve as an excitation filter. The fluorescence is emitted from the end of the capillary through the glass window and could be collected by a linear array of detectors instead of a single detector. The geometry is also amenable to fabrication in an integrated format with waveguides photolithographically polymerized on the inside of each channel.[26] Thus, one could make small, integrated optical components using this approach.

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