

# A plastic colorimetric resonant optical biosensor for multiparallel detection of label-free biochemical interactions

Brian Cunningham<sup>\*</sup>, Bo Lin, Jean Qiu, Peter Li, Jane Pepper, Brenda Hugh

SRU Biosystems 14A Gill Street, Woburn, MA 01801, USA

## Abstract

A high sensitivity plastic biosensor based on detection of changes in optical density on the surface of a narrow bandwidth guided mode resonant filter is demonstrated. Using sub-micron microreplication of a master sensor surface structure on continuous sheets of plastic film, the sensor can be produced inexpensively over large surface areas. In this work, the sensor structure is incorporated into standard 96-well microtiter plates and used to perform a protein–protein affinity assay. A surface receptor immobilization protocol demonstrating low nonspecific binding is used to detect an antibody with 8.3 nM sensitivity. By measuring the kinetic interaction of a protein–protein binding pair simultaneously at several concentrations, the affinity binding constant can be quickly determined. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Optical biosensor; Microreplication; Resonant filter; Genomics; Proteomics; Diagnostics

## 1. Introduction

For the majority of assays currently performed for genomics, proteomics, pharmaceutical compound screening, and clinical diagnostic applications, fluorescent or colorimetric chemical labels are commonly attached to the molecules under study so they may be readily visualized [1–3]. Because attachment of a label substantially increases assay complexity and possibly alters the functionality of molecules through conformational modification or epitope blocking, various label-free biosensor technologies have emerged. Label-free detection phenomenologies include measuring changes in mass [4], microwave transmission line characteristics [5], microcantilever deflection [6], or optical density [7,8] upon a surface that is activated with a receptor molecule with high affinity for a detected molecule. The widespread commercial acceptance of label-free biosensor technologies has been limited by their ability to provide high detection sensitivity and high detection parallelism in a format that is inexpensive to manufacture and package. For example, biosensors fabricated upon semiconductor or glass wafers in batch photolithography/etch/deposition processes are costly to produce and package if the sensor area is to be large enough to contain large numbers of parallel assays. Similarly, the requirement of making electrical connections to individual biosensors in an array poses difficult challenges

in terms of package cost and compatibility with exposure of the sensor to fluids.

In previous work, we have described a novel technology based upon a narrow bandwidth guided mode resonant filter structure that has been optimized to perform as a biosensor [9]. The sensor utilizes a sub-wavelength grating waveguide structure to provide a surface that, when illuminated with white light at normal incidence, reflects only a very narrow (resonant) band of wavelengths. The resonantly reflected wavelength is modified by the attachment of biomolecules to the waveguide, so that small changes in surface optical density can be quantified without attachment of a label to the detected biomolecule. Unlike optical detection approaches that rely upon interaction of detected molecules with an evanescent wave, the detection phenomenon in this work actually occurs *within* the waveguide, and thus, provides for a strong interaction between surface binding events and the transduced signal. Further advantages of the sensor approach are that the resonant reflected signal is measurable with the sensor either dry or immersed in liquid, and the simplicity of the non-contact excitation/detection instrumentation. The sensor structure, when fabricated on a glass substrate, demonstrated surface binding sensitivity of  $4.2 \times 10^{-13}$  g/mm<sup>2</sup>. Assays were performed that demonstrated the ability to perform a protein–protein binding assay with a sensitivity of 0.0167 nM for streptavidin, and the ability to measure attachment/detachment of molecules with molecular weight of 130 Da was demonstrated. Because sub-micron sensor feature definition and processing was

<sup>\*</sup> Corresponding author. Tel.: +1-787-933-7255.

E-mail address: bcunningham@srubiosystems.com (B. Cunningham).

performed on glass wafers in small batches, the sensor structure was limited in its use for low-cost disposable applications, such as microtiter plates or microarray slides.

In this work, we report on the design, fabrication, and evaluation of an equivalent sensor structure that is fabricated into sheets of plastic film. Rather than perform sub-micron definition of grating features using photolithography on the sensor itself, a master wafer is created in silicon that is used as a template for producing the sensor structure on plastic by a high-definition microreplication process. The use of a continuous plastic roll for the sensor substrate enables other processes, such as dielectric thin film coating and surface activation, to be performed in a substantially more efficient manner. Most importantly, the ability to produce a high-sensitivity biosensor in plastic over large surface areas enables incorporation of the sensor into large area disposable assay formats such as microtiter plates and microarray slides. In this work, we demonstrate incorporation of the plastic sensor into the bottoms of bottomless 96-well microtiter plates, and the ability to use the sensor plate to perform multiple protein–protein binding assays in parallel. The detection sensitivity of the plastic-substrate sensor is found to be equivalent to previously reported glass-substrate sensors.

## 2. Materials and methods

### 2.1. Sensor design and fabrication

The sensor structure requires a grating with a period lower than the wavelength of the resonantly reflected light [10,11]. As shown in Fig. 1, the grating structure is fabricated from a low refractive index material that is overcoated with a thin film of higher refractive index material. The grating structure was microreplicated within a layer of cured epoxy, as described below.

First, an 8 in. diameter silicon master wafer was produced. The 550 nm period linear grating structure was defined in photoresist using deep-UV photolithography by stepping and repeating the exposure of a 9 mm diameter circular grating reticle over the surface of a photoresist-coated silicon wafer. The exposure step/repeat procedure produced patterns for two standard format 96-well microtiter plates with eight rows and 12 columns each. The exposed photoresist was developed, and the grating structure was permanently transferred to the silicon wafer using a reactive ion etch with a depth of  $\sim 200$  nm. After etching, the photoresist was removed.

The grating structure was replicated onto a 0.005 in. thick sheet of polycarbonate by distributing a thin layer of epoxy between the silicon master wafer and a section of the polycarbonate sheet. The liquid epoxy conforms to the shape of the master grating, and is subsequently cured by exposure to ultraviolet light. The cured epoxy preferentially adheres to the polycarbonate sheet, and is peeled away from the silicon wafer. Sensor fabrication was completed by sputter

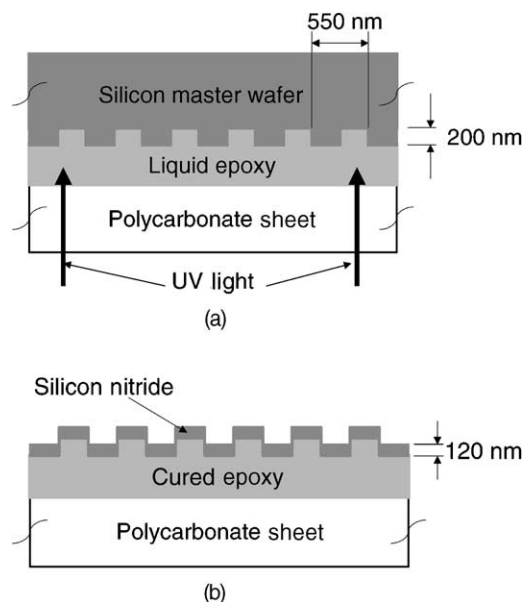


Fig. 1. Fabrication process used to produce the sensor. First (a) the silicon master wafer is used to replicate the sensor structure into a thin film of epoxy between the silicon and a sheet of plastic film. After the epoxy is cured, the plastic sheet is peeled away. To complete sensor fabrication (b), a thin film of silicon nitride is deposited over the structure.

deposition of 120 nm silicon nitride on the cured epoxy grating surface. Following silicon nitride deposition, 3 in.  $\times$  5 in. microtiter plate sections were cut from the sensor sheet, activated with amine functional groups (using the protocol defined further) and attached to the bottoms of bottomless 96-well microtiter plates (Costar and Greiner) with epoxy.

### 2.2. Surface activation and attachment of receptor molecule

After silicon nitride deposition, the sensors are activated with amine functional groups to enable various bifunctional linker molecules to be attached to the surface in a known orientation. Amine activation was performed by immersion of the sensor in 10% 3-aminopropyltriethoxysilane (Pierce) solution in ethanol (Aldrich) for 1 min, followed by a brief ethanol rinse. Activated sensors were then dried at 70 °C for 10 min.

A simple, colorimetric method using a modified protocol from Pierce was used to determine the density of amine groups on the surface [12]. The amine-activated sensor was immersed in 0.1 mM of sulfo-succinimidyl-4-O-(4,4'-dimethoxytrityl)-butyrate (*s*-SDTB, Pierce), solution made in 50 mM sodium bicarbonate (pH 8.5), and shaken vigorously for 30 min. The sensor was then washed with deionized water and subsequently treated with 30% perchloric acid (Sigma). The solution turned orange when the sensor was amine-activated or remained colorless otherwise. This method indicated that the surface density of the amine groups is  $\sim 2 \times 10^{14}$  groups/cm<sup>2</sup>.

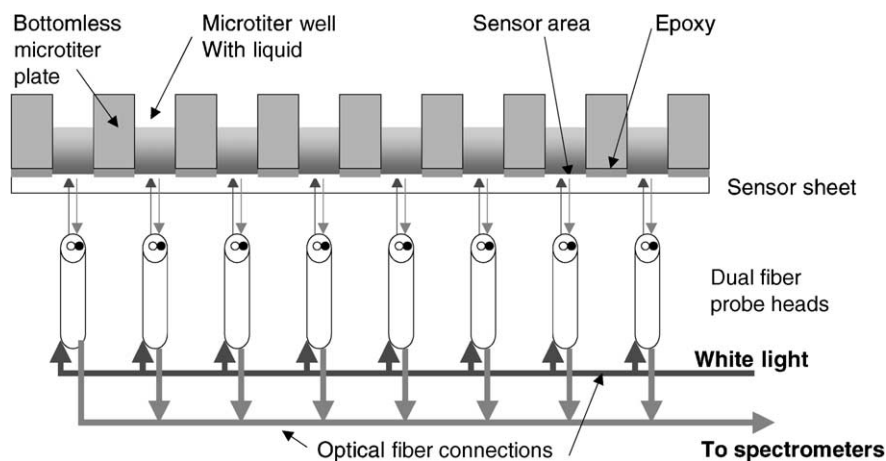


Fig. 2. Instrumentation used to illuminate and read the output of the sensor structure. Eight separate probe heads are arranged to read an entire column of a microtiter plate at once. Each probe head contains two optical fibers. The first fiber is connected to a white light source to cast a small spot of collimated light on the sensor surface. The second fiber collects reflected light for analysis by a spectrometer.

The purpose of this work is to demonstrate the use of the plastic biosensor for the detection of a well-characterized protein–protein binding interaction. The protein–protein system selected for this study was detection of anti-biotin IgG antibody using biotin immobilized on the sensor surface as a receptor molecule. Therefore, a protocol for immobilization of biotin on the sensor surface was developed that utilizes a bifunctional polyethyleneglycol-*N*-hydrosuccinimide (NHS–PEG) linker molecule (Shearwater Polymers Inc.) to act as an intermediary between the amine surface group and the biotin. The NHS–PEG molecule is designed specifically to enable NHS to preferentially bind to the amine-activated surface, leaving the PEG portion of the molecule oriented away from the surface. The NHS–PEG linker molecule serves to separate the biotin molecule from the sensor surface by a short distance so it may retain its conformation, and thus its affinity for other molecules. The PEG also serves to prevent nonspecific binding of proteins to the sensor.

After attachment of amine-activated sensor sheets into the bottom of microtiter plates, individual microtiter wells were prepared with three different surface functional groups in order to provide sufficient experimental controls for the detection of anti-biotin IgG. First, amine-activated surfaces are studied without additional modification. The amine-activated surface is expected to bind proteins nonspecifically, but not with high affinity. Second, microtiter wells with the NHS–PEG bifunctional linker molecule were prepared. The NHS–PEG molecule is expected to provide a surface that does not bind protein. Third, microtiter wells with an NHS–PEG–biotin linker molecule were prepared. The NHS–PEG–biotin molecule is expected to bind strongly to anti-biotin IgG.

To activate an amine-coated sensor with biotin, 2 ml of NHS–PEG–biotin (Shearwater) solution in TPBS (a reference buffer solution of 0.01% Tween 20 in phosphate buffer solution, pH 8) at 1.0 mg/ml concentration was added to the

sensor surface, and incubated at 37 °C for 1 h. An identical procedure was used for attachment of the NHS–PEG (Shearwater) molecule without biotin. All purchased reagents were used as packaged.

### 2.3. 96-Well plate scanner instrument

A schematic diagram of the system used to illuminate the sensor and to detect the reflected signal is shown in Fig. 2. In order to detect the reflected resonance, a white light source illuminates a ~1 mm diameter region of the grating surface through a 100 μm diameter fiber optic and a collimating lens at nominally normal incidence through the bottom of the microtiter plate. A detection fiber is bundled with the illumination fiber for gathering reflected light for analysis with a spectrometer (Ocean Optics). A series of eight illumination/detection heads are arranged in a linear fashion, so that reflection spectra are gathered from all eight wells in a microtiter plate column at once. The microtiter plate sits upon a motion stage so that each column can be addressed in sequence. The instrument is currently capable of measuring all 96 wells in ~15 s, limited by the rate of the motion stage.

## 3. Results

### 3.1. Reflected resonance signal and response uniformity

With water in the microtiter plate well, the *p*-polarized reflected resonance spectrum is shown in Fig. 3. The measured peak wavelength value (PWV) is 857 nm, and the full-width at half-maximum (FWHM) of the resonant peak is 1.8 nm. The ability of the sensor to measure shifts in optical density on its surface may be calibrated by measuring the sensor PWV when two solutions with known refractive index values are added to the microtiter plate wells, and by calculating the PWV shift ( $\Delta$ PWV) between the two

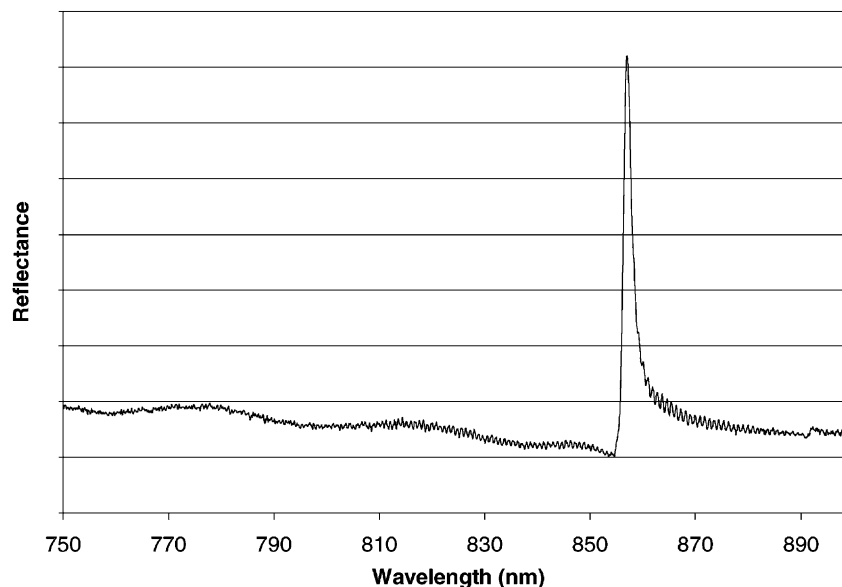


Fig. 3. Reflected intensity as a function of wavelength for the sensor structure within a microtiter plate well filled with water.

solutions. To measure sensor response uniformity across a plate, the PWV of all 96 wells were measured in water ( $n = 1.333$ ) and subsequently in glycerol ( $n = 1.472$ ). We define the shift coefficient,  $\sigma = \Delta\text{PWV}/\Delta n$ , to be a figure of merit for comparing the response of sensors with different designs or fabrication processes. The average shift in PWV from water to glycerol across all wells was 15.57 nm, providing a shift coefficient of  $\sigma = 112$  nm. This value is higher than the shift coefficient of glass-substrate sensors ( $\sigma = 88$  nm) reported previously, indicating improved sensitivity to optical density changes on the sensor surface. The standard deviation of  $\sigma$  across 96 sensor wells was 1.07 nm, indicating a very high degree of sensor uniformity across a large surface area.

### 3.2. Protein–protein binding assay

A protein–antibody affinity assay was performed to demonstrate operation of the plastic biosensor. A matrix of three separate sensor surface states ( $\text{NH}_2$ , NHS–PEG, NHS–PEG–biotin) were prepared and exposed to seven concentrations of goat anti-biotin IgG (Sigma). Each matrix location was measured within a separate microtiter plate well, for a total of 21 wells measured simultaneously. Because the NHS–PEG wells are not expected to bind protein, they provide a reference for canceling common mode effects such as the effect of the refractive index of the test sample and environmental temperature variation during the course of an assay. Data are reported here without the use of any mathematical correction.

Fig. 4 plots the PWV shift—referenced to a sensor with no chemical functional groups immobilized, recorded due to attachment of  $\text{NH}_2$ ,  $\text{NH}_2 + (\text{NHS-PEG})$ , and  $\text{NH}_2 + (\text{NHS-PEG-biotin})$  molecules to the sensor surface. The error bars indicate the standard deviation of the recorded

PWV shift over seven microtiter plate wells. The data indicates that the sensor can differentiate between a clean surface, and one with immobilized  $\text{NH}_2$ , as well as clearly detecting the addition of the NHS–PEG (MW = 2000 Da) molecule. The difference between surface immobilized NHS–PEG and NHS–PEG–biotin (MW = 3400 Da) is also measurable.

Fig. 5 shows the PWV shift response as a function of time for the sensor wells when exposed to various concentrations of anti-biotin IgG (0–80  $\mu\text{g}/\text{ml}$ ) and allowed to incubate for 20 min. The NHS–PEG surface (Fig. 5b) provides the lowest response, while the amine-activated surface (Fig. 5a) demonstrates a low level of nonspecific interaction with the anti-biotin IgG at high concentrations. The NHS–PEG–biotin surface (Fig. 5c) clearly demonstrates strong specific interaction with the anti-biotin IgG—providing strong PWV shifts in proportion to the concentration of exposed anti-biotin IgG.

The PWV shift magnitudes after 20 min from Fig. 6c are plotted as a function of anti-biotin IgG concentration in Fig. 6. A roughly linear correlation between the IgG concentration and the measured PWV shift is observed, and the lowest concentration IgG solution (1.25  $\mu\text{g}/\text{ml}$ , 8.33 nM) is clearly measurable over the negative control PSB solution.

### 3.3. Characterization of sensitivity using polymer multilayers

In order to study the sensitivity apart from the context of a biomolecular assay—which is subject to the effects of surface capacity, binding conditions, and molecular orientation—an experiment was performed in which a polymer multilayer of defined thickness and refractive index is built on the surface. A procedure for deposition of a sequence of positively and negatively charged polyelectrolyte films has

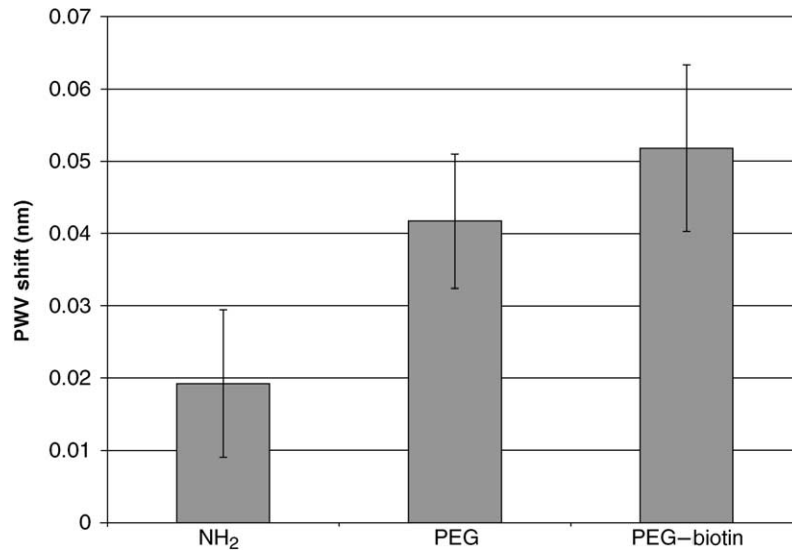


Fig. 4. Peak wavelength shift relative to a clean sensor surface for three sensor surface activation states. The error bars indicate the standard deviation of the shift over seven separate sensor wells.

been demonstrated as a means for reliably calibrating and comparing the response of various optical biosensors [13]. The polyelectrolyte multilayers behave as homogeneous and isotropic monolayers, while multilayers with proteins are expected to have more complex refractive index profiles.

The polyelectrolytes used in this study were anionic poly(sodium 4-styrenesulfonate) (PSS, MW = 60,000), cationic poly(allylamine hydrochloride) (PAH, MW = 70,000) and cationic poly(ethyleneimine) (PEI, MW = 60,000). The polymers were purchased from Aldrich and

used without further purification. The polyelectrolytes were dissolved in 0.9 M NaCl in HPLC water (filtered before use) at a concentration of 5 mg/ml. The 0.9 M NaCl solution is also used as buffer/rinse solution for the experiment. The buildup of the polyelectrolyte multiplayer film is then realized by the following sequence of steps, during which the PWV of the sensor is monitored at 10 s intervals:

- (i) Hundred microlitres of 0.9 M NaCl buffer is pipetted into a BIND microtiter well, and allowed to incubate

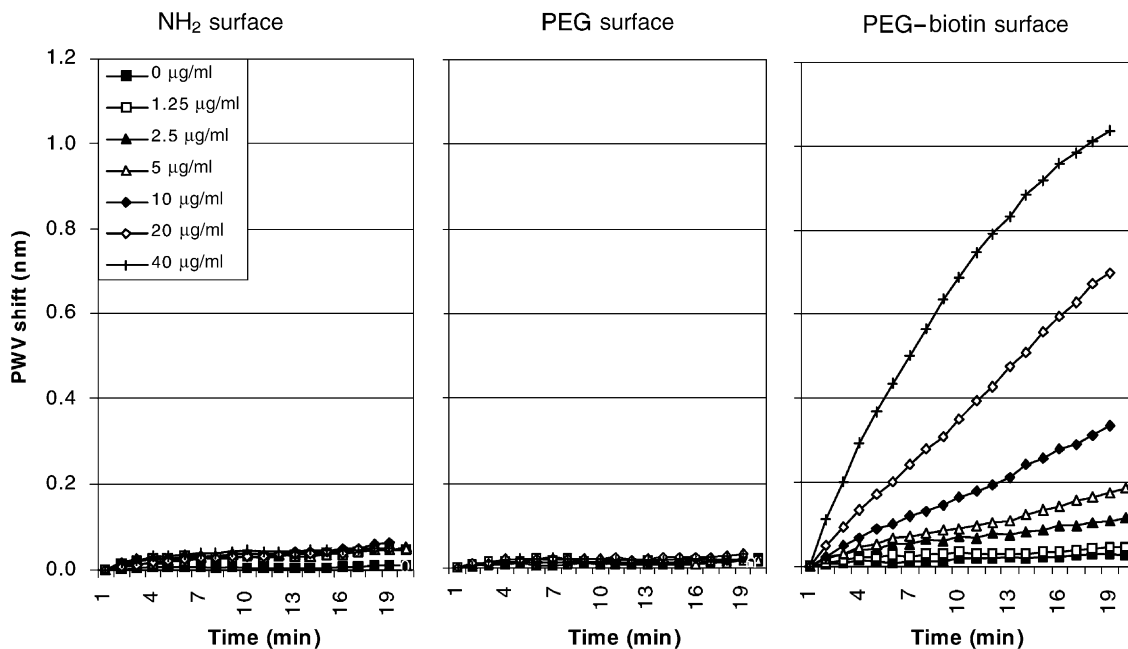


Fig. 5. Exposure of NH<sub>2</sub>, PEG, and PEG-biotin activated surfaces to seven concentrations of anti-biotin IgG. The NH<sub>2</sub> surface displays low levels of nonspecific protein binding at high protein exposure concentrations, while the PEG surface displays low levels of nonspecific binding. The PEG-biotin surface has a strong binding interaction with the anti-biotin IgG.

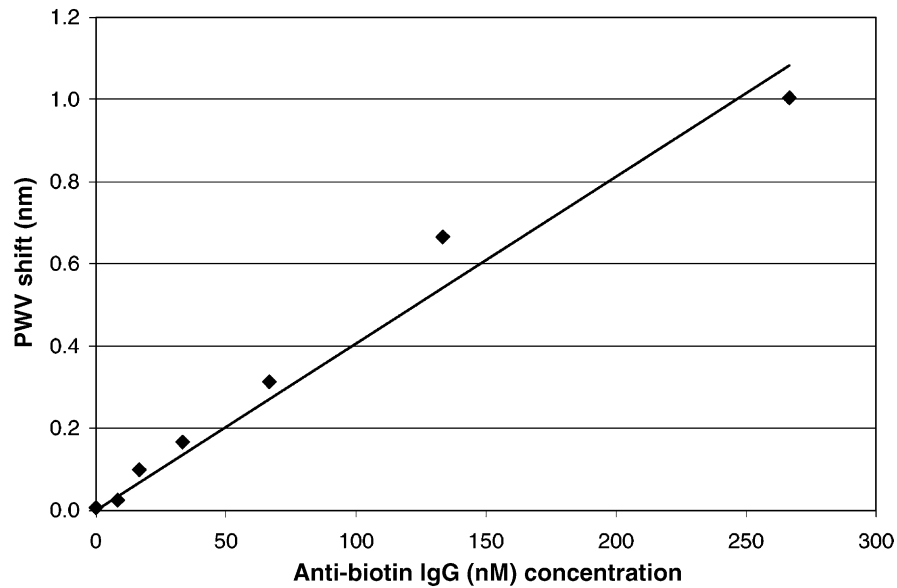


Fig. 6. Peak wavelength value shift as a function of anti-biotin IgG concentration for the PEG–biotin activated wells after a 20 min incubation. The plotted line indicates a least-squared fit linear function.

on the sensor surface for 5 min to establish a stability baseline.

- (ii) The buffer is removed, and 100  $\mu$ l of PEI solution is added to the microtiter well, and allowed to incubate for 5 min.
- (iii) The PEI solution is removed, and the well is rinsed with buffer. The sensor is monitored with buffer for 5 min.
- (iv) PSS and PAH are alternately adsorbed on the PEI surface using the same procedure as described for PEI. A buffer rinse is used after every PSS and PAH incubation to re-establish a baseline for comparing net

PWV shifts without bulk solution effects. Progressively, a PEI–(PSS–PAH)<sub>n</sub> surface is deposited. A total of 10 polymer layers were deposited in sequence.

The buildup of the PEI–(PSS–PAH)<sub>n</sub> multilayer, as monitored by the sensor, is shown in Fig. 7. The data correspond very well to results reported from other optical biosensors, which also measure a linear relationship between polymer coating thickness, and sensor response [13]. Because the growth of each layer in the multilayer stack is self-limiting, previous research has determined that the thickness of each layer is approximately 50  $\text{\AA}$ , and that the refractive index of

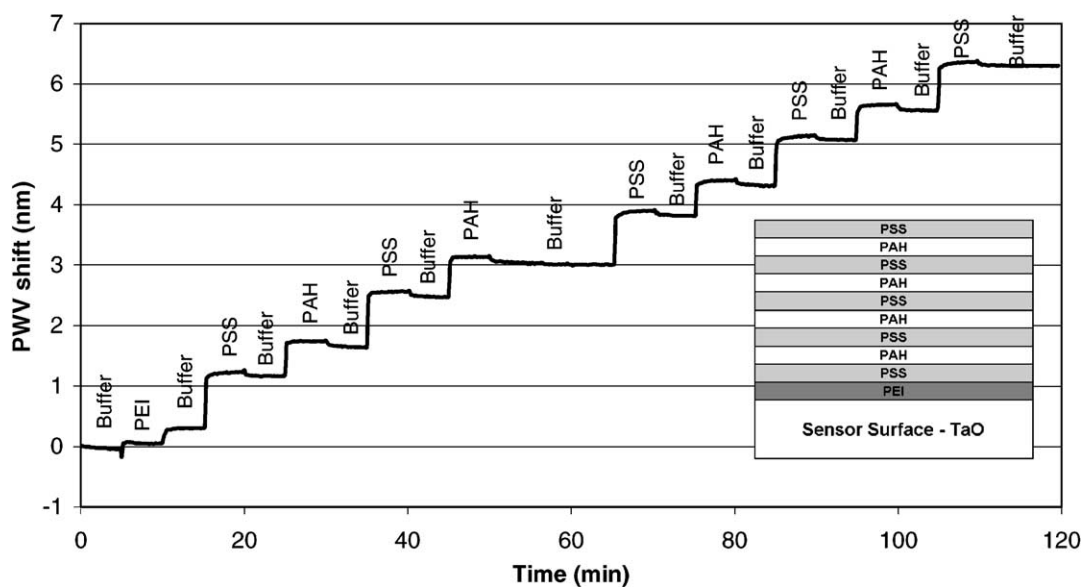


Fig. 7. PWV shift signal obtained during the in-situ buildup of a polyelectrolyte PEI–(PSS–PAH)<sub>n</sub> multilayer. The thickness of each alternating positively charged and negatively charged polymer is self-limiting to a thickness of  $\sim 50 \text{ \AA}$ / layer.

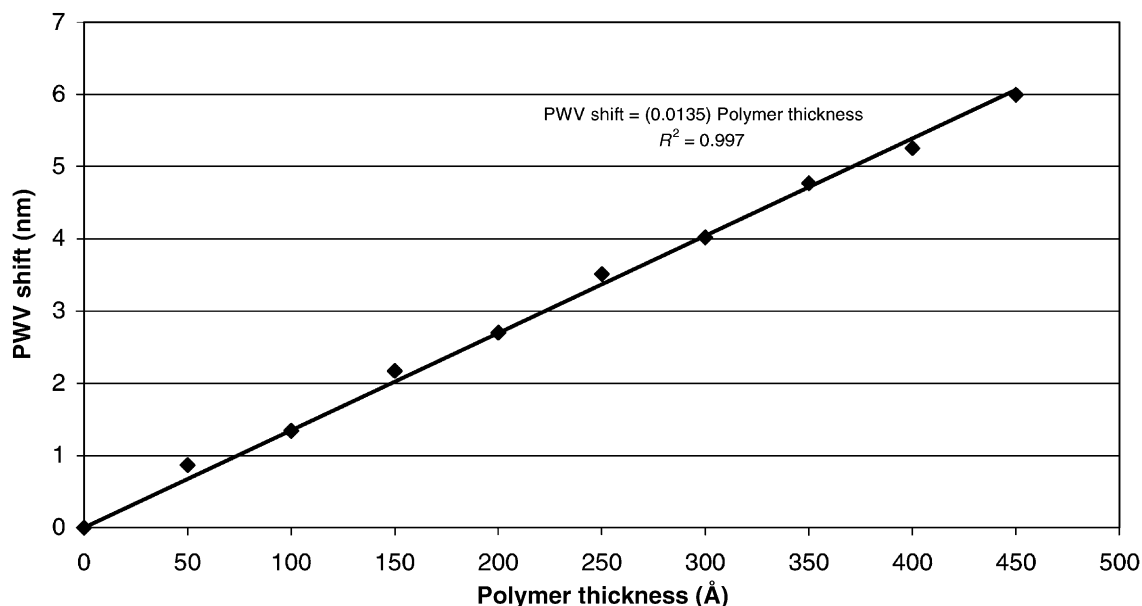


Fig. 8. Evolution of the polyelectrolyte film thickness during the different steps of the PEI-(PSS-PAH)<sub>n</sub> film buildup.

the film is approximately  $n = 1.49$ . Using the buffer measurement after PEI injection as a reference baseline, the PWV shift for each subsequent buffer measurement can be plotted as a function of total multilayer thickness, as shown in Fig. 8. A linear relationship is obtained for the first  $\sim 500$  Å of deposited material, as predicted by earlier computer models. Using current sensors, readout instrumentation, and signal processing algorithms, PWV shifts of as little as 0.003 nm can be reliably determined [9]. Using the relationship plotted in Fig. 8, a PWV shift of 0.003 nm would correspond to a polymer thickness shift of 0.22 Å.

#### 4. Discussion

By fabricating a high sensitivity biosensor into large continuous sheets of plastic, a manufacturing approach is demonstrated that will enable sensors to be inexpensively incorporated into disposable laboratory items such as micro-titer plates and microarray slides. Assay formats such as these are readily compatible with the liquid dispensing and robotic handling infrastructure that is most commonly used in biological research, and potentially provide a cost-per-assay this is compatible with the goals of laboratories that wish to screen for large numbers of molecular interactions.

The experiments described in this report exemplify how the plastic biosensor structure can be used to rapidly study a protein-protein binding interaction. In this case, the availability of multiple parallel sensors enables incorporation of multiple positive and negative controls to measure a binding interaction with statistical surety with compensation for nonspecific protein binding and environmental factors. The parallelism afforded by this approach will also enable

many different protein-protein interactions to be studied simultaneously.

It is anticipated that the ability to monitor large numbers of biochemical interactions in parallel without the use of fluorescent tags will be fundamentally important to several research fields. For such measurement capability to gain widespread acceptance, the cost of manufacturing sensors must be low enough that they can become single use disposable laboratory items. The sensor fabrication approach described utilizes a silicon master wafer that contains the sub-micron sensor surface structure. While great care must be taken in the fabrication of the master structure, and microfabrication techniques such as deep-UV photolithography are required to produce it, the cost of the master wafer is spread over many thousands of sensors that can be produced from a single tool. While the current work utilizes the silicon master as a tool for microreplication, it is also possible to use the silicon master to produce daughter tools by methods such as electroplating of nickel replicas. Such techniques are commonly employed in the manufacture of compact disks. The microreplication technique reported in this work may be carried out by sequentially producing structures within a continuous sheet of plastic film that is advanced past the tool. Likewise, deposition of dielectric thin film coatings may also be inexpensively performed on continuous sheets of plastic within specially outfitted sputter deposition chambers. Using a continuous film approach to sensor fabrication, a single 1000 ft roll of material can contain several thousand sensors, yielding tremendous cost advantages compared to batch fabrication approaches most commonly used in wafer microfabrication.

One of the most significant advantages of the colorimetric resonant biosensor is the method by which sensors are

transduced. Sensor excitation requires illumination with collimated white light provided through the tip of a fiber optic probe located up to several centimeters away from the sensor surface. Sensor readout is performed by collecting light through a second optical fiber that is bundled with the illumination probe. This transduction scheme, therefore, requires no electrical connection and associated cost of electrical wire bonding, packaging, and interface to external circuitry. Likewise, no coupling prisms with associated index matching fluids are required to contact the sensor in any way. The ability to excite and read the sensor in this way enables simplified incorporation of the structure into common assay formats such as microtiter plates, while enabling integration of instrumentation with microtiter plate readers. Adaptation of the sensor structure into other formats such as microarray slides and microfluid flow channels is anticipated.

The protein–protein binding assay presented was mainly selected to demonstrate the use of multiple wells, the use of PEG surfaces to control nonspecific binding, and the ability to attach linker chemistry with correct orientation. The standard deviation of the PWV measurement is currently 0.001 nm, therefore, a minimum detected signal can be defined as an event resulting in a shift of 0.003 nm. Based upon a 0.003 nm minimum signal, a sensitivity limit of 0.61 nM is calculated for the protein–protein binding assay using the defined protocol. The sensitivity limit can be further enhanced through use of higher binding capacity surface chemistry (such as hydrogels and polymers) and longer assay incubation time.

## 5. Conclusion

A high sensitivity plastic biosensor based on detection of changes in optical density on the surface of a narrow bandwidth guided mode resonant filter is demonstrated. Using sub-micron microreplication of a master sensor surface structure on continuous sheets of plastic film, the sensor can be produced inexpensively over large surface areas. The sensor structure was incorporated into standard 96-well microtiter plates and used to perform a protein–protein affinity assay. A surface receptor immobilization protocol

demonstrating low nonspecific binding is used to detect an antibody with 8.3 nM sensitivity. It is anticipated that the sensor will be incorporated into disposable laboratory assay formats to enable label-free detection of molecular and cellular binding interactions in the fields of genomics, proteomics, and molecular diagnostics.

## References

- [1] A.P. Pandey, M. Mann, Proteomics to study genes and genomes, *Nature* 405 (2000) 837–846.
- [2] N. Leigh Anderson, A.D. Matheson, S. Steiner, Proteomics: applications in basic and applied biology, *Curr. Opin. Biotechnol.* 11 (2000) 408–412.
- [3] G. MacBeath, S.L. Schreiber, Printing proteins as microarrays for high-throughput function determination, *Science* 289 (2000) 1760–1763.
- [4] F. Caruso, E. Rodda, D.N. Furlong, K. Nijkura, Y. Okahata, Quartz crystal microbalance study of dna immobilization and hybridization for nucleic acid sensor development, *Anal. Chem.* 69 (1997) 2043–2049.
- [5] J. Hefli, A. Pan, A. Kumar, Sensitive detection method of dielectric dispersions in aqueous-based surface-bound macromolecular structures using microwave spectroscopy, *Appl. Phys. Lett.* 75 (1999) 1802.
- [6] G. Wu, R.H. Datar, K.M. Hansen, T. Thundat, R.J. Cote, A. Majumdar, Bioassay of prostate-specific antigen (PSA) using microcantilevers, *Nat. Biotechnol.* 19 (2001) 856–860.
- [7] W. Huber, R. Barner, C. Fattinger, J. Hubscher, H. Koller, F. Muller, D. Schlatter, W. Lukosz, Direct optical immunosensing (sensitivity and selectivity), *Sens. Actuators B* 6 (1992) 122–126.
- [8] A. Brecht, G. Gauglitz, Optical probes and transducers, *Biosens. Bioelectron.* 10 (1995) 923–936.
- [9] B.T. Cunningham, P. Li, B. Lin, J. Pepper, Colorimetric resonant reflection as a direct biochemical assay technique, *Sens. Actuators B* 81 (2002) 316–328.
- [10] R. Magnusson, S.S. Wang, New principle for optical filters, *Appl. Phys. Lett.* 61 (9) (1992) 1022.
- [11] S. Peng, G.M. Morris, Resonant scattering from two-dimensional gratings, *J. Opt. Soc. Am. A* 13 (5) (1996) 993.
- [12] G. MacBeath, A. Koehler, S.L. Schreiber, <http://www-schreiber.chem.harvard.edu/home/protocols/SMP.html>.
- [13] C. Picart, G. Ladam, B. Senger, J.-C. Voegel, P. Schaaf, F.J.G. Cuisinier, C. Gergely, Determination of structural parameters characterizing thin films by optical methods: a comparison between scanning angle reflectometry and optical waveguide lightmode spectroscopy, *J. Chem. Phys.* 115 (2) (2001) 1086–1094.