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Self-referenced assay method for photonic crystal biosensors: Application to small molecule analytes

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Abstract

Using an image-based method for label-free detection of biochemical binding density distribution on the surface of a photonic crystal biosensor embedded within standard format multiwell microplates, a new method for automatic referencing of assay errors due to variability in the bulk refractive index of the analyte test sample is demonstrated. The new method involves application of the immobilized ligand upon the biosensor surface as a small spot, so that uncoated regions of a microplate well may serve as an accurate reference for the "active" regions containing the ligand. Due to the high spatial resolution of the image-based detection approach, each well in the microplate may be represented by hundreds of independent measurements of biochemical binding density, as measured by the shift in reflected wavelength from the photonic crystal biosensor. A linear plot of detected analyte density as a function of immobilized ligand density is constructed, in which the slope represents the ligand/analyte affinity and the intercept represents common-mode error effects. Streptavidin–biotin is used as a ligand–analyte model system to demonstrate the ability of this method to separate intentionally introduced bulk refractive index errors from the detection of a small molecule analyte. This referencing approach will be important in the context of small molecule drug compound library screening, where drug compounds within a large library are often suspended in solutions of inconsistent bulk refractive index.
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Keywords: Optical biosensor; Label-free detection; Photonic crystal; Small molecule

1. Introduction

One of the major concerns for many types of optical biosensors is the detection of signals that are not due to the quantity of interest, but rather due to a common-mode error such as temperature, nonspecific binding, and bulk refractive index of the test sample. For label-free detection of small molecules, the detected signals often have a magnitude that is similar or lower than the noise introduced by the experiment [1], thus it is necessary to accurately separate the error signals from the biochemical binding signals in order to produce meaningful data. For small molecule screening assays, small molecules often are suspended in solutions that are partly comprised of glycerol, dimethyl sulphoxide (DMSO) [2], or ethanol to prevent precipitation. These solvents usually have high refractive index values that introduce error signals for optical biosensor measurements. Such errors are typically corrected through the use of a "reference" sensor that is monitored in parallel with the "active" sensor. In the context of biosensors embedded within 96-, 384-, or 1536-well multiwell microplates, individual wells may be designated as references, and the position and number of reference wells are selected based upon the accuracy required for a particular assay and the expected variability in common-mode error effects.

A novel biosensor technology based upon photonic crystals was described previously [3]. The biosensor utilizes a subwavelength periodic surface structure that, when illuminated with white light at normal incidence, reflects only a very narrow resonant band of wavelengths. The resonant peak wavelength value (PWV) changes when biomolecules are attached to the sensor surface, so that small changes in surface dielectric permittivity can be quantified without attachment of labels to the detected biomolecule. The sensor surface structure is mass-manufactured from continuous sheets of plastic film and is

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incorporated into disposable 96-, 384-, or 1536-well microplates for high throughput assay applications in pharmaceutical discovery and life science research [4]. Using an appropriate imaging instrument described previously [5], the density of biochemical binding on the sensor surface can be measured in a high spatial resolution imaging mode.

Since the photonic crystal optical biosensor measures changes in dielectric permittivity on the sensor surface, when biomolecules are added to the sensor, the change in refractive index between the different liquid media in contact with the sensor will induce a signal that is indistinguishable from an actual biochemical binding event [6]. Especially when using high refractive index solvents such as DMSO, glycerol, and ethanol, the variability of the solvent content from one sample to another within a chemical compound library results in a measurement variability for optical biosensors that is due solely to the refractive index of the test sample.

In this work, we demonstrate that when small molecules are suspended in buffer with variable bulk refractive index values, the bulk variability can be eliminated through the use of a self-referencing method by integration of active and reference regions of the photonic crystal biosensor within each biosensor microplate well. The method requires the application of the immobilized ligand to only a portion of the biosensor well, so the active and reference regions of the biosensor surface can be exposed to the same analyte test sample. Using the high spatial resolution imaging capabilities of the biosensor detection instrument, hundreds of independent biosensor measurements are gathered simultaneously for each well to rapidly construct a plot of detected analyte concentration as a function of immobilized ligand density, where reference regions of the sensor have an immobilized ligand density of zero. The slope of this plot is used to quantify the detection of analyte, while the *y*-intercept contains information about error effects that occur to the "active" and "reference" regions in common. This approach is insensitive to the exact location, size, uniformity, and shape of the immobilized ligand region, and reduces the complex image analysis to a single number (slope of plot) for each biosensor microplate well.

This work represents the first time that this method has been applied to the detection of small molecule analytes. The streptavidin–biotin system is used as a model system demonstrate the effectiveness of this assay/data analysis approach for measuring samples with bulk refractive index variability. For screening of small molecule compound libraries against an immobilized protein target, highly automated and robust detection and referencing techniques are required to clearly separate "hits" with a single measured parameter.

2. Materials and methods

2.1. Sensor and instrument

The design and fabrication of the photonic crystal sensors used in this work have been published previously [6,7]. The sensors used in the work presented here were incorporated into standard 96-well SBS (Society for Biomolecular Screening) format microplates. The biosensor imaging instrument has been described previously [5,8]. The instrument measures the biosensor resonant reflected peak wavelength value (PWV) as a function of position on the biosensor surface in order to generate a PWV image of the entire sensor surface in a single scan (Fig. 1). A pixel resolution of 89.2 μm × 89.2 μm was used for this work, and the time required to scan a microplate is ∼600 s.

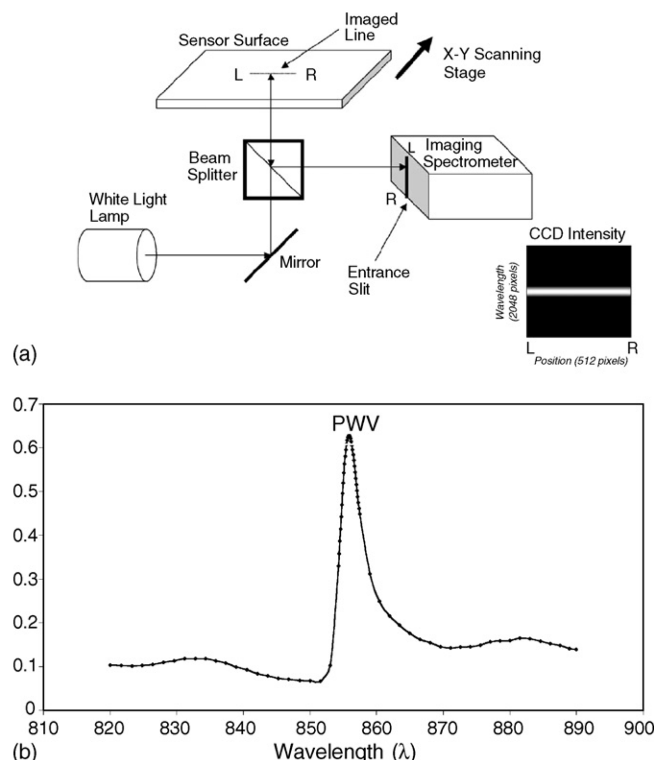


Fig. 1. (a) Schematic diagram of the imaging system used in this work. A single line from the biosensor surface is imaged into the entrance slit of an imaging spectrometer, which gathers a reflectance spectrum for each pixel along the length of the imaged line. Each pixel is represented by a reflected resonant spectrum, as shown in (b) from which the peak wavelength value (PWV) is mathematically determined. A PWV image is constructed by translating the sensor perpendicular to the image line direction by several micrometers and gathering reflected spectra for each line.

Typically, a biosensor experiment involves measuring *shifts* in PWV, so the sensor surface is scanned twice: once before and once after biomolecular binding. The images are aligned and subtracted to determine the difference in PWV as detected by the sensor. This scanning method does not require the PWV of the imaged surface to be completely uniform, either across the surface or within a set of probe locations, or tuning of the sensor angle to a resonance condition as with SPR-imaging [9].

2.2. Streptavidin–biotin binding experiment

A protein/small molecule interaction bioassay was performed that measures the binding affinity of streptavidin to biotin in the presence of intentionally introduced DMSO bulk refractive index errors. Streptavidin (Prozyme, MW = 55,000 Da) was prepared with 0.005 M PBS and filtered with a 0.22 μm syringe filter (Nalgene) to a concentration of 2.5 mg/ml. Biotin was pur-

chased from Sigma–Aldrich (MW = 244 Da), and was prepared with 0.005 M PBS to a concentration of 0.5 mg/ml. Six DMSO concentrations, 0.0, 0.4, 0.8, 1.2, 1.6, 2.0%, were selected to introduce into the 0.005 M PBS biotin buffer solution.

The 96 wells on the microplate were pre-coated with a layer of active aldehyde groups on the sensor surface, and then incubated with 100 μl of 0.005 M PBS for 45 min on a rotator (LAB-LINE®) and the top of the microplate was sealed with a thermal seal (Fisher Scientific) to prevent evaporation of buffer solution. After the surface of the biosensors were stabilized, the microplate was scanned (SCAN A) and then the PBS was removed from each well and the surface was completely dried with pressurized N_2. The microplate was allowed to dry for another 30 min.

The 72 wells were spotted with two 10 nl spots of streptavidin, one overlapping the other, by a multi-spot dispenser (Piezoarray, Perkin Elmer, Inc.) in the center of the well and allowed to incubate in dried surface condition for ∼4 h in a 4 °C environment. The microplate was then washed with deionized water, incubated with 100 μl of PBS in each well, and then placed under 4 °C for another 18 h of incubation. After 18 h, the microplate was stabilized at room temperature for 45 min for the second scan (SCAN B). During the spotting session, an ionizer and a chiller were used to keep the microplate stage temperature 2° above the dew point in order to prevent fast evaporation of the spots.

The biotin sample was allowed to stabilize at room temperature for 45 min prior to injection into the wells. The PBS solution was replaced with 90 μl of 0.0, 0.4, 0.8, 1.2, 1.6, 2.0% DMSO solution in columns 1–2, 3–4, 5–6, 7–8, 9–10, and 11–12, respectively. The DMSO solutions were allowed to incubate for 30 min before the addition of 10 μl of the 0.5 mg/ml biotin solutions into 72 microplate wells. The microplate was immediately scanned for the third time after the addition of the biotin solution (SCAN C). The sequence of steps is summarized in Fig. 2.

2.3. Self-referencing data analysis

The first scan (scan “A” in Fig. 2a) establishes the pre-assay baseline PWV image for the chemically functionalized plate immediately before the protein ligand is applied. The second scan (scan “B” in Fig. 2a) measures the PWV image after the protein ligand spot is applied to the surface, and after any unbound protein has been washed away. By mathematically subtracting the PWV image of scan “A” from the PWV image of scan “B”, we obtain a PWV *shift* image that displays the immobilized protein density as a function of position in each well of the microplate. The final scan (Scan “C” in Fig. 2a) measures the PWV image of the microplate after exposure to the small molecule analyte. By mathematically subtracting the PWV image of scan “B” from the PWV image of scan “C”, we obtain a PWV *shift* image that displays the detected analyte density as a function of position in each well of the microplate.

The data gathered from the three scans are used to generate a plot of detected analyte density as a function of immobilized protein density (Fig. 2c), where each individual pixel from the PWV shift image represents one point (pixel) from the protein density continuum generated by the immobilized protein spot.

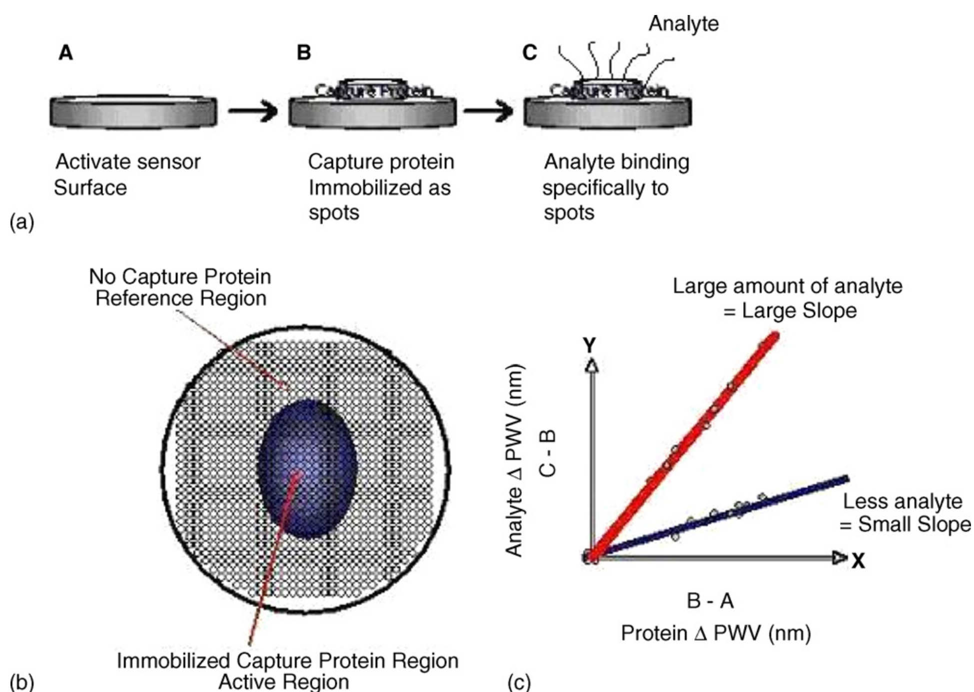


Fig. 2. Schematic representation of the slope method for self-referenced label-free binding analysis. (a) Sequence of three scan required to determine the spatial density of immobilized protein (Scan B–Scan A) and the density of detected analyte (Scan C–Scan B). (b) Representation of a single biosensor microplate well, subdivided into a grid of independent PWV determinations from an array of pixels. Pixels without captured protein function as reference measurements for correction of common-mode errors for pixels within the active regions with immobilized protein. (c) A plot of immobilized protein density (*x*-axis) vs. detected analyte density (*y*-axis) yields a linear plot, whose slope indicates the relative affinity between analyte and ligand.

The range of PWV was selected to include every "reference" pixel and every "active" pixel in the generated plot. Because detected analyte density is linearly dependent upon the availability of immobilized protein on the sensor surface, a linear trend is measured. The slope of this linear trend serves as a means of quantifying the strength of the interaction between the immobilized protein and the analyte: greater slope indicates greater interaction strength than lesser slope, and zero slope indicates no interaction. An important consequence of this data analysis method is that *the bulk refractive index of the analyte solution has no effect on the slope of the plot*, although it can change the *y*-intercept of the curve because the bulk refractive index of the buffer solution will change the PWVs collected for each pixel, thus moving all of the data points along the *y*-axis. In this way, the regions of the microplates that do not contain immobilized protein serve as a reference for correcting the effect of bulk refractive index in the analyte sample. Although the data analysis method is based upon high-resolution images of biochemical binding density, the image analysis is reduced to the reporting of a single number, the slope of the immobilized ligand density/analyte density curve that can be utilized as a means for screening higher affinity binders from lower affinity binders. This method has been used recently to rank the binding affinity of a panel of antibodies against an immobilized protein target [10,11]. In this work, we show for the first time the effectiveness of this approach for detecting a small molecule analyte with an immobilized large protein target.

Also reported for the first time is the use of an annulus function is to automatically group the reference and active pixels. In Fig. 3, the outermost ring represents the diameter of a single well within the microplate, and the diameter of the inner-most ring is selected to include the entire protein spot. The annulus between the two middle rings defines the reference region, which contains zero immobilized protein density. The pixels in the gray regions are removed from the data set used in the plot of detected analyte density as a function of immobilized protein density. The average PWVs in Region 2 (the annulus: bulk refractive index + nonspecific binding background error) is mathematically subtracted from the PWVs in Region 1 (protein + analyte + bulk refractive index + nonspecific binding background error), thus removing the background noise. Note that the annulus represents the overall reference for an entire single microplate well.

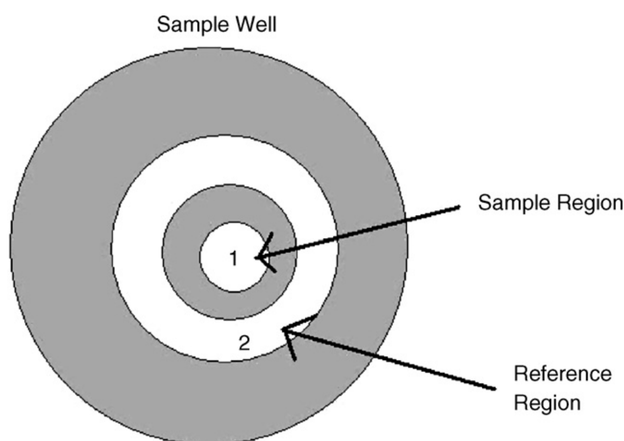


Fig. 3. A sample grid analysis of a single microplate well, where the pixels in the gray regions are eliminated. Region 1 represents the sample region that contains the immobilized protein pixels, which also includes bulk refractive index error signals. Region 2 (annulus) represents the region without protein binding that only contains the pixels of background bulk refractive index and nonspecific binding noise. The annulus functions as the overall reference for both "reference" and "active" pixels because by subtracting the average PWV shift in Region 2 from Region 1, the background bulk refractive index and nonspecific binding noise introduced into both "reference" and "active" pixels is eliminated from the protein binding signal.

3. Results

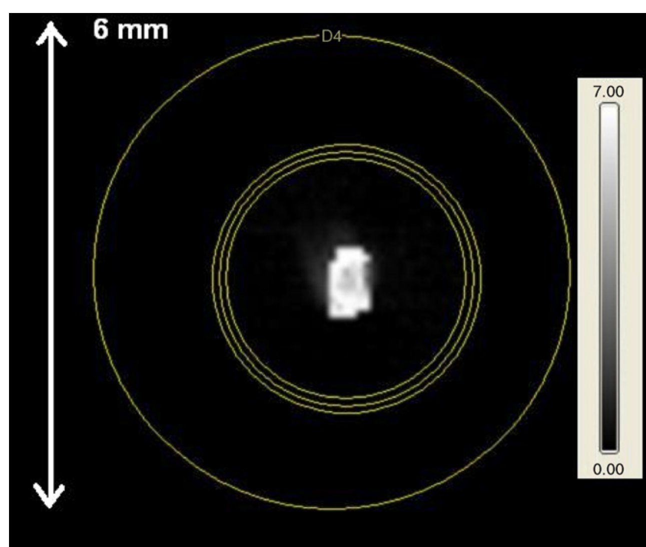
3.1. Streptavidin–biotin affinity with DMSO solution error

The measured PWV shift of streptavidin was approximately 8.5 ± 1.3 nm. Fig. 4 shows the streptavidin–biotin PWV shift images for a single microplate well with a streptavidin spot size of $900\,\mu m \times 580\,\mu m$ at a dispensed volume of 20 nl. Fig. 4a shows the PWV shift image of the immobilized streptavidin (SCAN B–SCAN A) and Fig. 4b shows the PWV shift of the biotin binding to the streptavidin spot (SCAN C–SCAN B) with a PWV shift approximately 0.12 ± 0.05 nm. Fig. 5 shows a single well (D4) of the plot generated from the PWVs collected of the detected biotin (*y*-axis) as a function of the immobilized streptavidin (*x*-axis), where each point in the scatter plot represents a single pixel of the PWV shift image.

Best-fit lines were generated with the pixels obtained from each of the 72 microplate wells by a least-square algorithm, where the slope and *y*-intercept can be determined. The scatter plots with the best-fit lines for each microplate well are shown in Fig. 6, where greater slope values represent greater binding of biotin to the immobilized streptavidin. Fig. 7 shows the measured average slope value at each DMSO concentration, which had a low correlation coefficient of 0.26. The *y*-intercept values are shown in Fig. 8, where the value increases as the DMSO concentration increases as expected.

4. Discussion and conclusion

With the incorporation of chiller and ionizer for humidity control in the spot dispensing instrument, the streptavidin spots were able to stay in liquid phase for ~5 min, which allowed enough time for the biomolecules to interact with the aldehyde groups on to the sensor surface. Slow evaporation of streptavidin prevented crust formation on the outer region of the spot. The images of the streptavidin spots showed binding to the sensor surface with little spreading effects even after washing with deionized water (Fig. 4a), which might be due to allowing the microplate to dry for a long period of time. For some binding experiments it is critical to use a blocking agent to prevent nonspecific binding of the analyte, but it was unnecessary to apply a blocking step in this experiment because biotin has low affinity for the aldehyde groups on the sensor surface (Fig. 4b). However, there was still a small amount of nonspecific binding observed in Fig. 4b, where some positive PWV shift pixels are located in regions without the streptavidin spot, which were eliminated



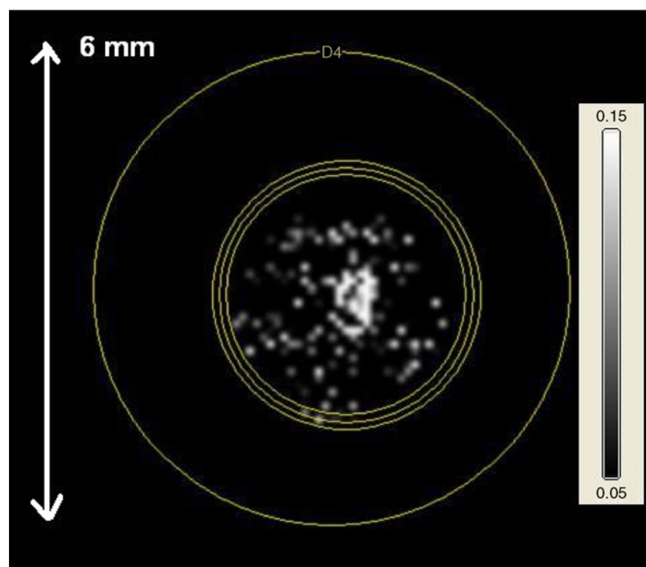


Fig. 4. PWV shift images for a single microplate well scanned at a pixel resolution of 89.2 μm × 89.2 μm. (a) PWV shift image (Scan B–Scan A) of two overlapping immobilized streptavidin spots dispensed at 10 nl per spot. (b) PWV shift image (Scan C–Scan B) for small molecule biotin binding to the immobilized streptavidin spots.

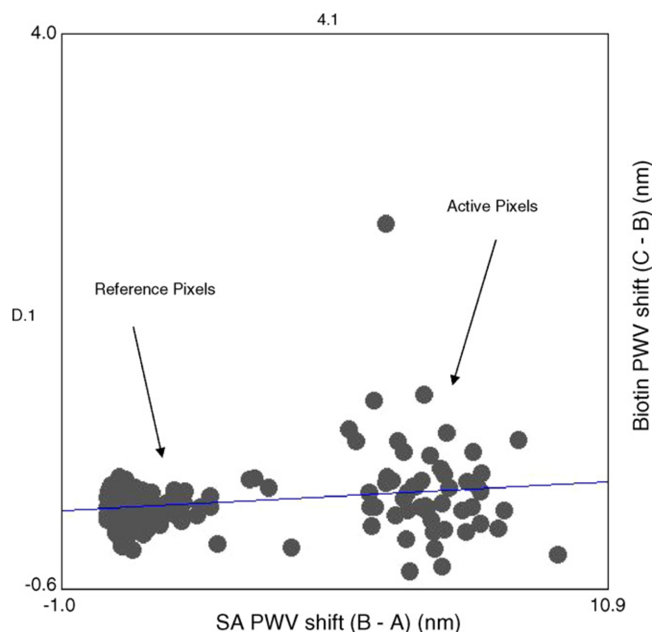


Fig. 5. A sample scatter plot of the biotin (MW = 244 Da) binding density as a function of immobilized streptavidin (MW = 55,000 Da) density for a single well (D4) of the biosensor microplate. Each point in the plot represents a single pixel from the PWV image. Therefore, points near the origin represent pixels with no immobilized streptavidin (reference), while points away from the origin represent pixels with immobilized streptavidin (active).

by the use of the annulus function in data analysis. The scatter plots for each microplate wells shows two clusters of pixels. The cluster near the origin represents the reference pixels, while the active pixels are in the cluster away from the origin shown in Fig. 5. Note that the active cluster is very close to the reference cluster in the vertical direction because of the small molecule low binding signals. The intercept of the plots represents the bulk PWV shift due to the addition of different DMSO concentrations to the buffer solution in the microplate wells. Fig. 7 shows that, for small molecules suspended in DMSO buffer, where the concentration varies from 0 to 2%, the variability has little impact upon the actual binding signal as measured by the slope. Because the actual DMSO variability is expected to be no more than 10% in a small molecule affinity screening campaign, determination of binding affinity by this self-referenced slope method is effective for elimination of bulk refractive index errors. Fig. 8 plots the bulk PWV shift for each concentration of DMSO. The intercept values increase as the DMSO concentration increases because every pixel in the (Scan C–Scan B) image is equally affected by the bulk refractive index shift. The 0.0% DMSO condition resulted in a slightly negative *y*-intercept, which is presumed to be the result of a small negative PWV drift between Scan B and Scan C. Such drift, if occurring on the entire biosensor microplate in common, is factored out of the slope analysis.

The difference in molecular weight between streptavidin (55,000 Da) and biotin (244 Da) is very large, and four potentially active binding sites for biotin are available on each immobilized streptavidin molecule. Using this information, we may compute a theoretically achievable biotin binding PWV shift based upon the measured streptavidin PWV shift, the ratio of streptavidin molecular weight to biotin molecular weight, and the number of available binding sites. For a measured immobilized streptavidin PWV shift of 8.50 nm, the theoretically achievable biotin shift would be ∼0.17 nm. In our assay, we measured a biotin PWV shift magnitude of ∼0.178 nm, which indicates that ∼100% of the immobilized streptavidin binding sites are functional. The assay method described in this work requires only a few nanoliters of immobilized streptavidin solution per well, resulting in use of only ∼3.6 μg of protein per assay. This is particularly important for assays requiring expensive purified protein that is available in small quantity, but which

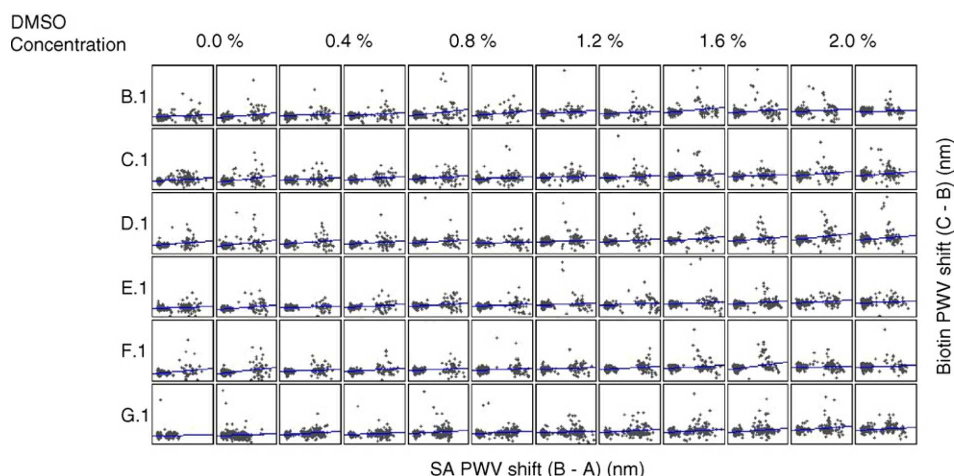


Fig. 6. Scatter plots with best-fit lines for 72 biosensor microplate wells. Each well contains a single immobilized streptavidin spot, and is exposed to biotin at 0.05 mg/ml concentration in the presence of variable concentrations of DMSO. For each scatter plot, the Protein A binding density (Scan B–Scan A) is plotted on the *x*-axis and the ligand binding density (Scan C–Scan B) is plotted on the *y*-axis.

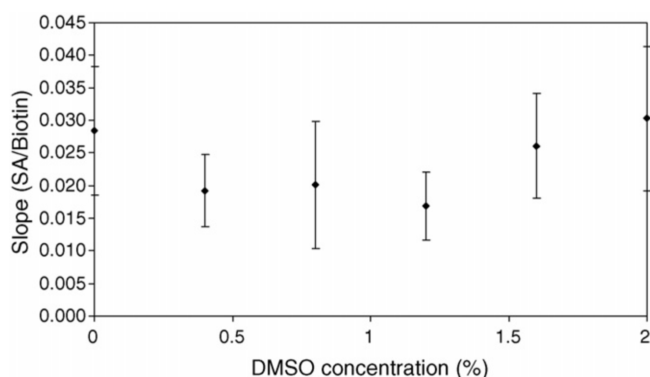


Fig. 7. The slope value as a function of DMSO concentration for biotin. The determined slope varies for each DMSO concentration with a correlation coefficient of 0.26, which shows it is not dependent upon the DMSO concentration.

must be used in a small molecule screening campaign involving potentially millions of wells.

The self-referencing assay protocol presented here is enabled by the ability to gather high-resolution spatial images of label-free biomolecular binding on the biosensor surface. The lack of pixel crosstalk and high sensitivity for detecting reflected wavelength shift originates from the photonic crystal biosensor structure, which prevents lateral propagation of light. Furthermore, the image analysis method does not require the immobilized protein spot to be deposited in any particular location within the biosensor microplate well, and does not require the spot to be deposited with any particular shape. In fact, the slope analysis method does not require absolutely uniform immobilized protein density, provided that the activity of the protein is uniform. Although three sequential scans of the microplate are required, each scan takes ∼10 min. The image alignment, subtraction, and slope determination functions are automated by the imaging instrument software. Future work on the detection of small molecule interaction by the use of self-referencing slope method will involve experimenting with a panel of drug compounds screened against multiple enzyme targets, as they would be measured in affinity screening campaign.

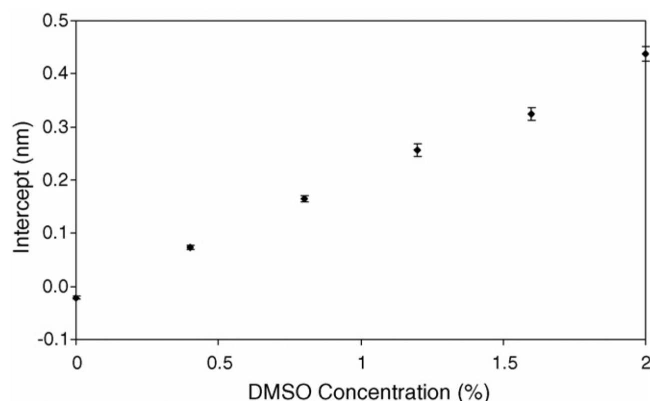


Fig. 8. The *y*-intercept values as a function of DMSO concentration for biotin. The relationship between DMSO concentration and *y*-intercept values contain information on assay artifacts that affect both the reference and active regions of the well.

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References

[1] B. Lin, J. Pepper, P. Li, H. Pien, B.T. Cunningham, A label-free high throughput optical technique for detecting small molecule interactions, Biosens. Bioelectron. 17 (2002) 827–834.

[2] H.J. Lee, G. Shan, T. Watanabe, D.W. Stoutamire, S.J. Gee, B.D. Hammock, Enzyme-linked immunosorbent assay for the pyrethroid deltamethrin, J. Agric. Food Chem. 50 (2002) 5526–5532.

[3] 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.
[4] B.T. Cunningham, P. Li, S. Schulz, B. Lin, C. Baird, J. Gerstenmaier, C. Genick, F. Wang, E. Fine, L. Laing, Label-free assays on the BIND system, J. Biomol. Screening 9 (2004) 481–490.
[5] P. Li, B. Lin, J. Gerstenmaier, B.T. Cunningham, A new method for label-free imaging of biomolecular interactions, Sens. Actuators B 99 (2004) 6–13.
[6] B.T. Cunningham, J. Qiu, P. Li, C. Baird, Enhancing the surface sensitivity of colorimetric resonant optical biosensors, Sens. Actuators B 87 (2002) 365–370.
[7] B.T. Cunningham, J. Qiu, P. Li, J. Pepper, B. Hugh, A plastic colorimetric resonant optical biosensor for multiparallel detection of label-free biochemical interactions, Sens. Actuators B 85 (2002) 219–226.
[8] B. Lin, P. Li, B.T. Cunningham, A label-free biosensor-based cell attachment assay for characterization of cell surface molecules, Sens. Actuators B (2005) (in press).
[9] E.A. Smith, W.D. Thomas, L.L. Kiessling, R.M. Corn, Surface plasmon resonance imaging studies of protein-carbohydrate interactions, J. Am. Chem. Soc. 125 (2003) 6140–6148.
[10] B.T. Cunningham, Biosensor Multiwell Microplate with Self-Reference Capability, in: IEEE Sensors, Vienna, Austria, 2004.
[11] B.T. Cunningham. L.L. Chan, P.Y. Li, D. Puff, A self-referencing method for microplate label-free photonic crystal biosensor, IEEE Sens. J. (2006) (Submitted for publication).

Biographies

Leo Chan is a graduate research assistant at the University of Illinois at Urbana-Champaign in the Nano Sensors Group directed by Dr. Brian T. Cunningham. His research focuses on the characterization of photonic crystal optical biosensors and the optimization of small molecule biodetection using this platform. Before joining Dr. Cunningham's group, Leo Chan served as an undergraduate research at Keck Graduate Institute: Claremont, California, where he worked on the application of free solution electrophoresis to DNA finger printing. He earned his BS and MS in Electrical and Computer Engineering with a minor in Biomedical Engineering from the University of Illinois at Urbana-Champaign, where he is currently pursuing a PhD.

Brian Cunningham is an Associate Professor of Electrical and Computer Engineering at the University of Illinois at Urbana-Champaign, where he is the director of the Nano Sensors Group. His group focuses on the development of photonic crystal-based transducers, plastic-based fabrication methods, and novel instrumentation approaches for label-free biodetection. Prof. Cunningham is a founder and the Chief Technical Officer of SRU Biosystems (Woburn, MA), a life science tools company that provides high sensitivity plastic-based optical biosensors, instrumentation, and software to the pharmaceutical, academic research, genomics, and proteomics communities.

Prior to founding SRU Biosystems in June, 2000, Dr. Cunningham was the Manager of Biomedical Technology at Draper Laboratory (Cambridge, MA), where he directed R&D projects aimed at utilizing defense-related technical capabilities for medical applications. In addition, Dr. Cunningham served as Group Leader for MEMS Sensors at Draper Laboratory, where he directed a group performing applied research on microfabricated inertial sensors, acoustic sensors, optical switches, microfluidics, tissue engineering, and biosensors. Concurrently, he was an Associate Director of the Center for Innovative Minimally Invasive Therapy (CIMIT), a Boston-area medical technology consortium, where he led the Advanced Technology Team on Microsensors. Before working at Draper Laboratory, Dr. Cunningham spent 5 years at the Raytheon Electronic Systems Division developing advanced infrared imaging array technology for defense and commercial applications. Dr. Cunningham earned his BS, MS, and PhD degrees in Electrical and Computer Engineering at the University of Illinois. His thesis research was in the field of optoelectronics and compound semiconductor material science, where he contributed to the development of crystal growth techniques that are now widely used for manufacturing solid state lasers, and high frequency amplifiers for wireless communication.

Derek Puff is the Principal Software Engineer at SRU Biosystems, Inc., a Woburn, MA biotechnology firm developing high-sensitivity biosensors for label-free molecular interaction detection. Dr. Puff earned his PhD in Biomedical Engineering from the University of North Carolina-Chapel in 1995, where he specialized in medical image processing and analysis. He has worked as a software developer in the medical imaging and biotechnology industry for the past 10 years. Dr. Puff leads the development of software applications for the SRU Biosystems instrumentation product line.