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A Sensitive Multilayered Structure Suitable for Biosensing on the BioDVD Platform

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Several technologies are currently available for the analysis of biomolecular interactions with high sensitivity and efficiency. However, these instruments are invariably expensive and, thus, are not suitable for bedside analyses. To circumvent this issue, we have previously reported a BioDVD platform that allowed us to use a DVD mechanism to monitor various biomolecular interactions [Gopinath et al., 2008, *ACS Nano* 2, 1885–1895]. In the present study, to improve the sensitivity of the BioDVD platform for various analyses, we have performed computer simulations to optimize the ZnS–SiO₂ layer thicknesses and determined an optimized optical interferometric response after adjusting the ZnS–SiO₂ layer thickness to 65 and 60 nm for the inner and outer layer thicknesses, respectively. Biomolecular interaction analyses performed with the optimized BioDVD disks revealed a 3-fold improvement in the sensitivity, compared to our previously reported multilayered structure. In this study, we have also shown that the BioDVD platform is suitable not only for analyzing nucleic acid hybridization and interactions between RNA–small ligands and RNA–proteins, but also for antigen–antibody interactions. Furthermore, our evaluations revealed that each sample required no more than 10 tracks of data to analyze the biomolecular interactions on the BioDVD platform, which permits a greater number of spots per BioDVD disk and also reduces the time needed to measure the biomolecular interactions.

The post-genome era began after the sequencing of the genomes of various organisms was completed, and research is currently focusing on deciphering the functions of different biomolecules. The biological processes in all living organisms are driven by biomolecular interactions between diverse macromolecules, such as proteins, nucleic acids, lipids, carbohydrates, and other natural products. To analyze their interactions, several methodologies are currently available to detect biomolecules,

either without labeling (in the native condition) or after labeling. While the latter methodologies offer high sensitivity in establishing readout formats, the labeling of molecules is sometimes problematic, because the labeling molecule may occupy an important binding site or cause steric hindrance, resulting in false information regarding the interactions. Some of these limitations stimulated interest in the development of novel technologies to detect the biomolecules in their native form, without labeling. The prominent methodologies adopted for the label-free analysis of biomolecules include surface plasmon resonance (SPR) and interferometric approaches.^{1–7} However, these methodologies involve static structures, which prevent repetitive scanning, and, thus, they lack high-speed capabilities.

In view of this, an optical biosensor based on spinning disk interferometry has recently been reported, which explored the well-established technology of the optical compact disk (CD) to develop ultrahigh-throughput immunological assays, and is referred to as the BioCD.^{8–10} As an alternative to the BioCD, we have recently described a spinning-disk biosensor that utilizes the optical interference of reflected light from a multilayered structure, consisting of dielectric, metal, and optical phase-change thin films, to monitor different interactions on its surface. We refer to this platform as the BioDVD, because it utilizes the optical system of a digital versatile disk (DVD) to measure changes in reflected light intensity. The basic operating principle of the BioDVD with an integral optical phase-change film relies on optical interferom-

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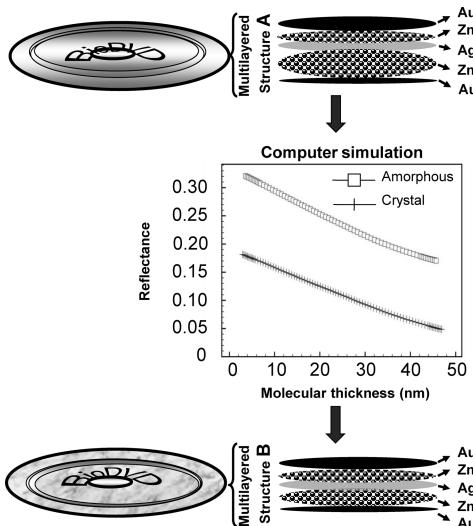


Figure 1. Multilayered structures used in the BioDVD system before (structure A) and after (structure B) adjusting the thicknesses, and reflection curves for simulations in which molecules are immobilized on the new multilayered structure. The BioDVD structures were fabricated by radio frequency (rf) magnetron sputtering on pregrooved polycarbonate disk substrates with a diameter of 12 cm and a thickness of 0.6 mm.

etry: two gold films (one thick and one thin) generate a standing wave between them, and the sandwiched dielectric film, as well as the phase-change film thickness, are designed to optimize the optical multi-interferometric response to be highly sensitive to a tiny refractive index change in the top thin gold film. Once a molecule binds to the surface, the optical standing waves generated in the structure are suppressed, leading to a change in reflectivity. Using this system, we have demonstrated that biotin–streptavidin interactions and nucleic acid hybridization, as well as RNA–protein and RNA–small-ligand interactions, can be measured efficiently and with high sensitivity.^{11–13} We also found that the BioDVD fabricated with a phase-change layer offers the possibility for highly accurate and reversible masking of the sensitivity of regions on the platform, using currently available DVD technology. In addition, the presence of the phase-change layer can be used to label different detection regions on the sensor surface, allowing flexible implementation of the biomolecular interactions used on a given disk structure in the field. In our previous studies, we suggested that manipulating the ZnS–SiO₂ layer thickness may further improve the sensitivity, because these manipulations may lead to a more ideal optical interferometric response, because of optimization of the phase contrast of the aggregate structure. In the present studies, we performed computer simulations and found a more optimized structure in which the interferometric response was large by adjusting the ZnS–SiO₂ layer thicknesses to 65 and 60 nm for the inner and outer layers, respectively. Using this optimized multilayered structure disk, we found that the sensitivity of the BioDVD platform was improved over 3-fold,

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Table 1. Summary of Matrix Parameters

multilayer	refractive index	thickness (nm)
layer 1 (top molecule)	1.45 + 0.00	5
layer 2 (thin Au film)	0.13 + 3.16	5
layer 3 (ZnS–SiO ₂ film)	2.20 + 0.00	65
layer 4 (phase-change, amorphous)	4.50 + 2.30	15
layer 5 (ZnS–SiO ₂ film)	2.20 + 0.00	60
layer 6 (bottom-Au film)	0.13 + 3.16	50
layer 4 (phase-change, crystal)	3.30 + 2.90	15

as compared to our previous multilayered disk structure. In addition, we have determined that the BioDVD platform is suitable for antigen–antibody analysis. Taken together, our previous and current studies show that the BioDVD platform technology is suitable for analyzing different biomolecular interactions, including nucleic acid hybridization, and RNA–protein, RNA–small-ligand, and antigen and antibody interactions.

MATERIALS AND METHODS

Oligonucleotides and Protein. 5'-thiolated DNA was prepared chemically with 20 deoxythiamine residues (dT₂₀) with protected thiolated groups. To deprotect the thiolated groups, the oligos were treated with dithiothreitol (DTT, 60 mM) and Tris HCl (250 mM, pH 8.0) for 16 h at room temperature. After the reaction, the SH-poly dT₂₀ oligo was subjected to purification by passage through ODS-120T column [TOSOH, Japan; TSK-gel size 7.8 mm (inner diameter) × 30.0 cm (long)] in a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) run with 2.5 mM triethylammonium acetate (TEAA) under 5.0 MPa of pressure and eluted with a linear concentration using TEAA and acetonitrile at a ratio of 60:40 and a flow rate of 2 mL/min. All fractions were monitored at OD 254 nm. The solution from the peak area was collected and dialyzed twice for 2 h each against double-distilled water. The dialyzed sample was concentrated to dryness in a speed vacuum, and the concentration of DNA was measured. RNA molecules were enzymatically synthesized, as described previously.^{12,14–16} Human Factor IX was purchased from American Diagnostica (Stamford, CT). The Haemagglutinin (HA) protein of the influenza A and B viruses, which had been purified as described previously,^{15,17} was kindly provided by Dr. Kawasaki at the National Institute of Advanced Industrial Science and Technology, Japan.

Fabrication of the Multilayered BioDVD. Two BioDVD disks with different multilayered structures were fabricated by radio frequency (rf) magnetron sputtering on a pregrooved polycarbonate disk substrate with a diameter of 12 cm and a thickness of 0.6 mm. The surface contained a prefabricated, spiral groove from an inner radius of 22.3 mm to an outer radius of 59.8 mm, with a 1.2-μm track pitch and a depth of 60 nm. One of the multilayered structures used here (designated as A) consisted of

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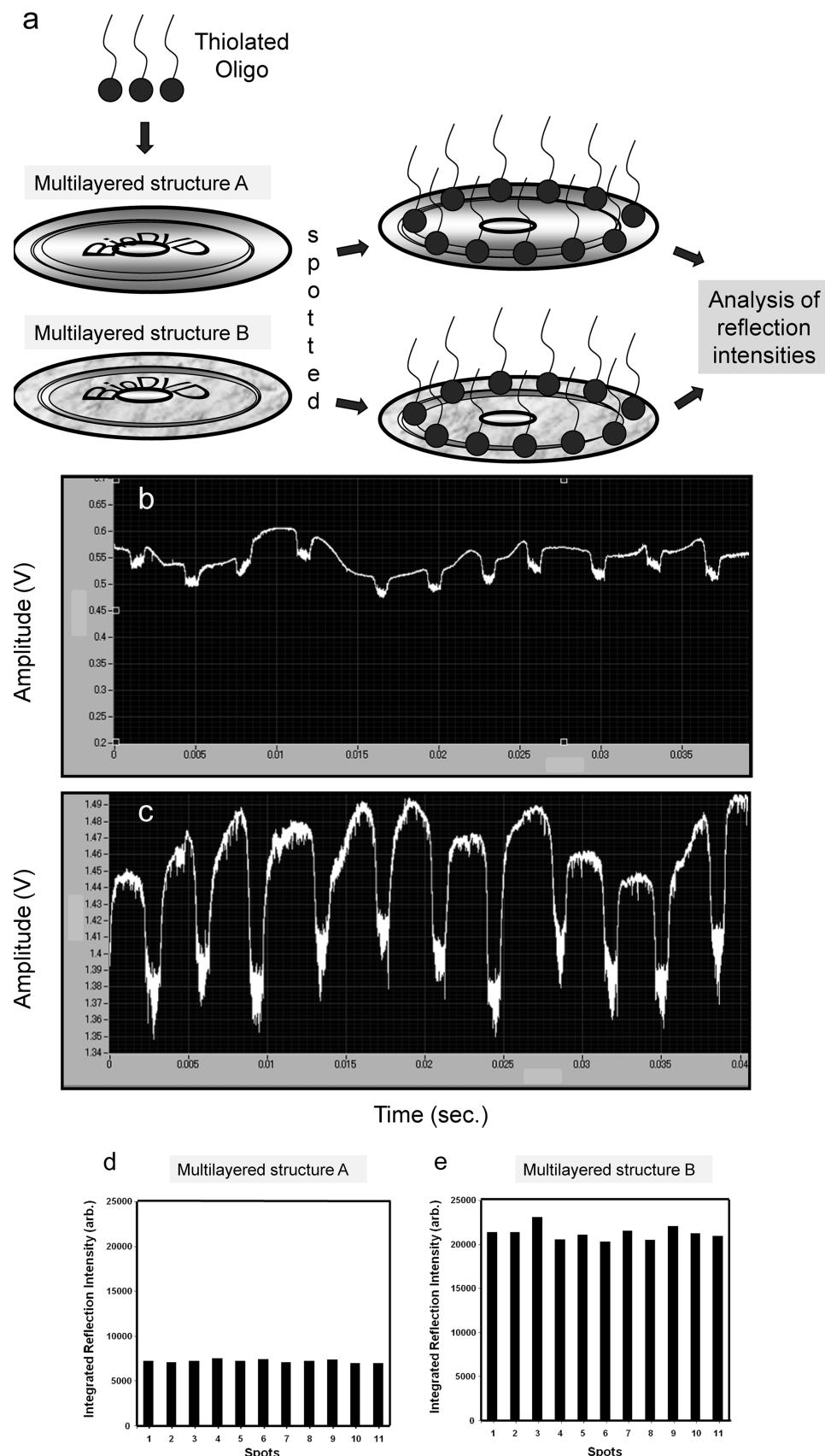


Figure 2. (a) Scheme to analyze the single-stranded thiolated oligo on multilayer BioDVD structures. (b) Observed reflected light intensity obtained from thiolated oligo spots on structure A. (c) Observed reflected light intensity obtained from thiolated oligo spots on structure B. (d) Quantitative analysis of thiolated oligo spots on structure A. (e) Quantitative analysis of thiolated oligo spots on structure B. Thiolated DNA molecules were spotted at a concentration of $1 \mu\text{M}$. Each spot was $\sim 1.0 \text{ mm}$ in diameter, and the spots were placed on a concentric circle with a radius of $\sim 45 \text{ mm}$.

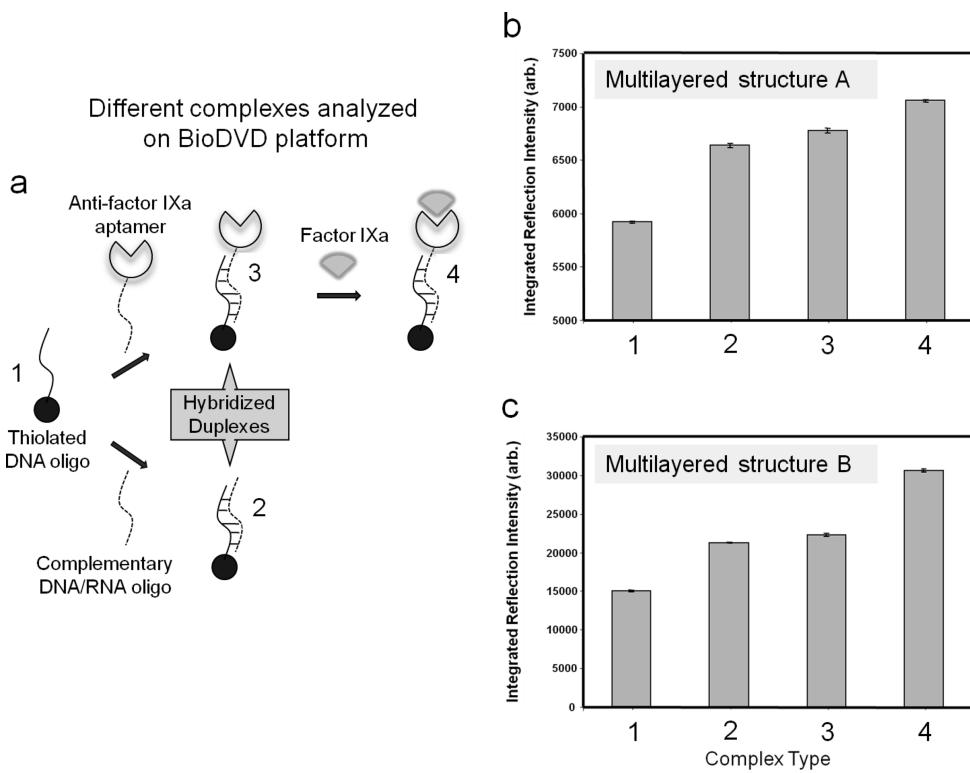


Figure 3. (a) Scheme to analyze different biomolecular complexes on BioDVD structures A and B. (b) Quantitative analyses of single-stranded thiolated oligo (**1**), DNA–DNA (**2**), DNA–RNA (**3**), and DNA–RNA–protein (**4**) complexes on structure A. (c) Quantitative analyses of single-stranded thiolated oligo, DNA–DNA, DNA–RNA, DNA–RNA–protein complexes on structure B.

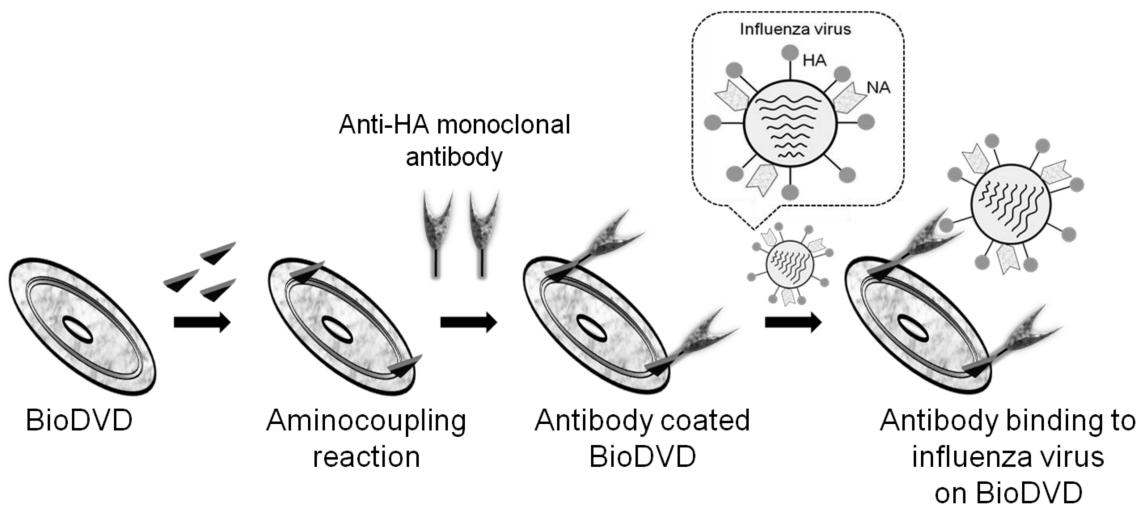
five layers of the structure: Au (5 nm)/(ZnS)₈₅(SiO₂)₁₅ (85 nm)/Ag_{6.0}In_{4.4}Sb_{61.0}Te_{28.6} (AIST, 15 nm)/(ZnS)₈₅(SiO₂)₁₅ (45 nm)/Au (50 nm), and the another five-layer structure (designated as B) was comprised of Au (5 nm)/(ZnS)₈₅(SiO₂)₁₅ (65 nm)/Ag_{6.0}In_{4.4}Sb_{61.0}Te_{28.6} (15 nm)/(ZnS)₈₅(SiO₂)₁₅ (60 nm)/Au (50 nm), respectively. These structures are shown in Figure 1. The optical interference between the thin and thick Au layers is the most important element in the BioDVD to enhance the optical signal of a ligand molecule immobilized on the thin Au surface. In both BioDVD structures, the optical interference was slightly detuned from the condition in which destructive interference was generated at the thin Au layer position. Disk A contained an asymmetric structure about the phase-change layer, while disk B utilized an almost-symmetric structure about the phase-change layer. These structures were designed by computer simulations of the phase shifts arising from the aggregate structure. Before immobilizing the ligands, we tested and confirmed the calculated reflectivity by depositing different thicknesses of SiO₂ films ($n = 1.45$), which represented the biomolecules to be analyzed. We also measured the index of refraction (n) of different biomolecules using optical ellipsometry (MIZOJIRI, Model DHA-OLX/S4M, $\lambda = 632.8$ nm) and found n values of 1.24, 1.34, and 1.43 for the complexes of DNA–DNA, DNA–RNA, and DNA–RNA–protein, respectively, demonstrating consistency with our approximation of SiO₂ as being representative of the bilayer. Spotting and further processing details were performed as described previously.¹²

Signal Readout. Before attaching the recognition molecules onto the BioDVD sensor surface, the desired track was marked by the formation of a crystalline region (0.2 mm from the desired track). All measurements were obtained from a head position

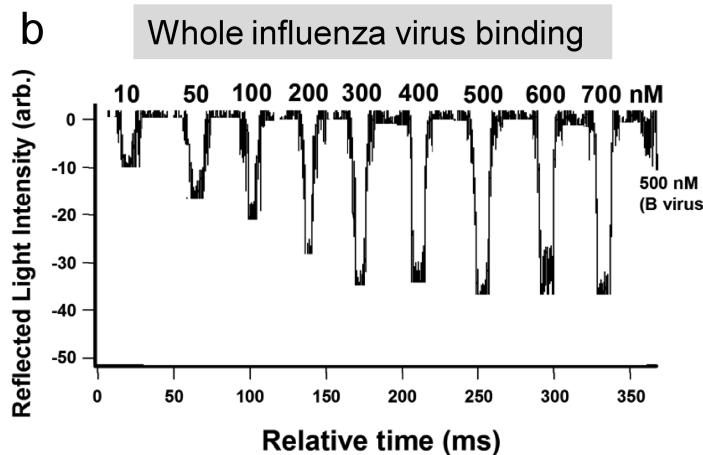
manually referenced from the center of the DVD, and the sensor surface faced the laser beam. The intensity of the reflected light was measured from the sensor surface by an optical disk drive tester (DDU-1000, Pulstec Industrial Co., Ltd.) that was equipped with a 635-nm diode laser and a pickup lens with a numerical aperture (NA) of 0.6. The laser beam was focused on the sensor surface of the BioDVD by inserting a dummy plate with a thickness of 0.6 mm between the BioDVD and the pickup lens. The readout laser power (P_r) was fixed at 1.0 mW, and the BioDVD was rotated at a scanning speed of 6.0 m/s during the measurements. The measurements were performed using the autofocus and autotracking modes available on the DDU unit. The intensity of the reflected light was measured via the output voltage from a photo detector located in the pickup unit, using a sampling rate of 1 MHz, and was smoothed using boxcar averaging.

BioDVD Reader and Measurements of Biomolecular Interactions. The DDU-1000 is a standard optical disk tester used to evaluate optical disks such as DVDs and CDs. Unlike a conventional commercial DVD unit, which only allows manipulation of read/write parameters within ranges specified by the DVD forum, all parameters relevant to the recording and readout process, such as laser tilt, beam focusing offset, and laser power, as well as tracking, can be individual manipulated at will. The DDU-1000 apparatus is an optical disk testing unit that is equipped with a 635-nm wavelength semiconductor laser and an NA = 0.60 lens. The linear constant velocity of the disk substrate could be varied over a range of 0.4–19 m/s, allowing a wide range of recording/readout speeds to be investigated. The maximum laser power was 15 mW, and typically 1.0 mW was used for BioDVD

a



b



c

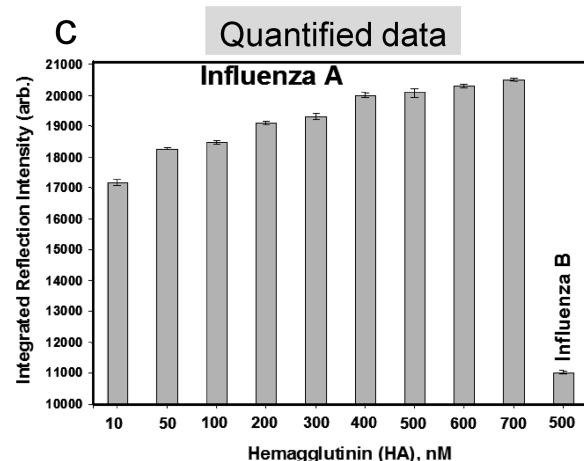


Figure 4. (a) Scheme to analyze antibody–antigen complexes on BioDVD structure B. (b) Observed reflected light intensity for antibody–antigen complexes at different concentrations (10–700 nM). (c) Quantitative analyses of antibody–antigen complexes at different concentrations. As a negative control, 500 nM of influenza B virus was spotted. The observed reflection intensity signal was normalized with the IGOR Pro Version 6.0 software (WaveMetrics, Inc., Lake Oswego, OR).

signal readout. Both the DDU and commercial DVD read/write units use identical autofocusing and tracking mechanisms.

Our measurement of the BioDVD was done within the DVD specifications, except for the insertion of a dummy plate between the BioDVD disk and the optical pickup unit. The dummy plate was made of transparent plastic with a refractive index of 1.56. The BioDVD platform is compatible with use in commercial DVD drive with only minor circuit changes. Because of these minor modifications, the cost of a BioDVD unit will be slightly more expensive than a mass production conventional DVD drive, but still would be significantly less expensive than a laser scanning or SPR setup.

Analysis of Antibody–Antigen Interactions on the BioDVD. 16-Mercaptohexadecanoic acid was obtained from Sigma-Aldrich, USA. For the experiment, a 10- μ L aliquot of 5 mM 16-mercaptopropionic acid was combined with 100 μ L of a mixture of 50 mM *N*-hydroxysuccinimide (NHS) and 200 mM *N*-ethyl-*N'*-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and incubated at room temperature for 10 min. The antibody for Haemagglutinin (HA) was diluted to a final concentration of 100 nM and incubated for 30 min at room temperature. To remove the unbound small ligands, these complexes were passed through a Microcon centrifugal filter device (Amicon) with

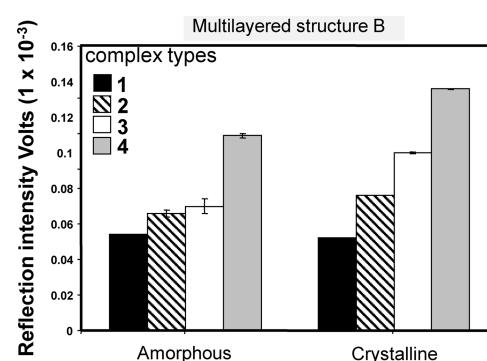


Figure 5. Quantitative analyses of single-stranded thiolated oligo (**1**), DNA–DNA (**2**), DNA–RNA (**3**), and DNA–RNA–protein (**4**) complexes on structure B in the amorphous and crystalline states of AIST. Changes between the two states were achieved by adjusting the laser power.

a 30 kDa cutoff membrane. The complexes retained on the membrane were recovered with buffer solution. The samples were prepared by a buffer with pH 4.0. The samples were spotted on the BioDVD surface and incubated for 1 h at room temperature. To determine the antibody–antigen interactions, after thorough washing, different concentrations of the whole virus (Influenza

A) were spotted on the antibody spots. The disk was incubated for 15 min, and then measurements were obtained after washing the disk. (See the figure given in the Supporting Information for more-detailed information regarding this process.)

RESULTS AND DISCUSSION

The operating details of our BioDVD system have been described recently.¹² Briefly, it is a five-layer structure that consists of phase-shifting layers to allow sensitive optical interferometric measurement of biomolecular interactions. The disk surface has an Au layer to attach thiol-linked biomolecules. A spiral groove from an inner radius of 22.3 mm to an outer radius of 59.8 mm was prefabricated on the disk surface; thus, areas between 22.5 and 58.5 mm were used for spotting biomolecules. A 5- μ L sample was spotted to cover an ~1-mm-diameter area and was analyzed with a disk drive unit (DDU-1000) at different positions. A single track on the BioDVD has both a 0.6- μ m-wide groove and a 0.6- μ m-wide land, and the sample covered tracks are averaged to obtain a noise-free signal. The resulting reflectivity signals of the biomolecular interactions were averaged using LabVIEW 7.1 (National Instruments Corporation, Austin, TX). The remaining specifications are almost identical to those of a DVD-RW disk. Therefore, the signals obtained from the BioDVD can be retrieved using a conventional DVD drive. The only difference between a conventional drive and the BioDVD system is the presence of the multilayered structure in the BioDVD disk.

Simulation and Comparative Analyses of Nucleic Acid Interactions on Two Different Multilayer Structures. To further improve the sensitivity of our established platform, in this study, we performed computer simulations of the optical response of the BioDVD structure for different thicknesses of ZnS–SiO₂ to optimize the changes induced in the reflectance that are due to the presence of biomolecules (see Figure 1). We simulated the reflectivity from optical multilayers using the “matrix” method.¹⁸ In this technique, the reflectivity of an optical multilayer can be simulated using a matrix product of matrices that represent individual layers. Each of the six layers in the stack is represented by an individual matrix of the form

$$\begin{bmatrix} \cos(\delta) & \frac{i \sin(\delta)}{n} \\ i n \sin(\delta) & \cos(\delta) \end{bmatrix}$$

where δ represents the phase shift due to the propagation of the electromagnetic field through a layer of thickness d and complex index of refraction n ($\delta = 2\pi n d / \lambda$). For the current experiment, the wavelength was fixed at $\lambda = 635$ nm, which is the standard wavelength used in DVD players. The index of refraction used for the construction of each matrix is summarized in Table 1. To optimize the sensitivity of the optical multilayer to changes in the thickness of the biomolecular sensing layer, we utilized an iterative procedure in which a 5-nm-thick biomolecular top layer was assumed to be present. The thickness of each of the different optical layers (layers 2–5) was then varied and the maximum in the derivative of the total reflectivity of the optical multilayer, as a function of the biomolecular sensing layer thickness (dR/dt_{bio}), was determined. The thickness of each layer was iteratively

varied until no further increase in dR/dt_{bio} was observed, yielding an optical multilayer structure that is sensitive to small variations in immobilized biomolecule thickness.

The optical phase differences at the thin Au layer between structures A and B are almost opposite. Our new optimized structure B exhibits an 8-fold increase in reflectivity difference over structure A in biosensing. The large optical contrast is more suitable for the detection of biological molecules on the BioDVD platform with low error rates. The simulations showed that, as the surface biomolecular layer thickness was increased by 1 nm, the reflectivity decreased by 0.4% for structure B, while the same calculations for structure A only showed a reflectivity change of 0.1%–0.2%. To confirm these computer models, we have prepared BioDVD disks using both structures A and B and compared their biosensing performance.

In the comparison experiments, we measured the change in reflectivity after attaching single-stranded thiolated DNA to both types of structures. A total of 11 spots that consisted of single-stranded thiolated DNA were attached to different regions on the disk to immobilize the oligo on the Au surface of the BioDVD (see Figure 2a). These molecules were attached through a thiol linker, as reported previously,¹² and the resulting reflectivity was measured. Each spot was determined to occupy at least 100 tracks, based on the spot volume. Using typical DDU settings, which allow the continuous acquisition of different tracks in a concentric manner, we collected data from 50 tracks of the BioDVD for each sample spot and averaged the data along these tracks. We used at least three different DVDs for each sample analysis. The reflectivity of all 11 spots was similar for each type of disk, which suggests good reproducibility of the BioDVD platform. As shown in Figures 2b–e, we observed a clear difference between the two different disk structures. The reflectivity from structure B was determined to show a greater than 3-fold improvement in reflectivity over structure A, which is consistent with predictions. The baseline was also found to improve significantly, as a consequence of the improved sensitivity (see Figures 2b and 2c). The signal-to-noise ratio was also observed to improve in going from structure A to structure B with values of 10:1 and 20:1, respectively.

To compare the sensitivity of structures A and B directly for the analysis of different biomolecular interactions, such as DNA–DNA and DNA–RNA duplexes and DNA–RNA–protein complexes, we measured the thiolated DNA oligo (**1**), its cDNA oligo (to analyze duplex formation, **2**), the aptamer RNA that binds to the thiolated DNA oligo with an extra sequence at its 3' end (which is complementary to the thiolated DNA oligo, **3**), and the complex of **3** with a coagulation factor (specifically recognized by the antifactor IXa aptamer, **4**). (See Figure 3a.) We could efficiently monitor all of the aforementioned interactions (complexes **1–4**) on structure A, as previously reported¹² (see Figure 3b). Similarly, when these interactions were analyzed on structure B, the reflectivity was determined to be more than 3 times larger than that observed on structure A (see Figure 3c).

Analysis of Antibody–Antigen Interactions on Structure B. The ability of the same structure to detect antibody–antigen interactions on structure B was also investigated. For this purpose, we initially immobilized a monoclonal antibody against the hemagglutinin of Influenza A, using an amino-coupling reaction on the Au layer of structure B, by spotting (10 spots) radially the

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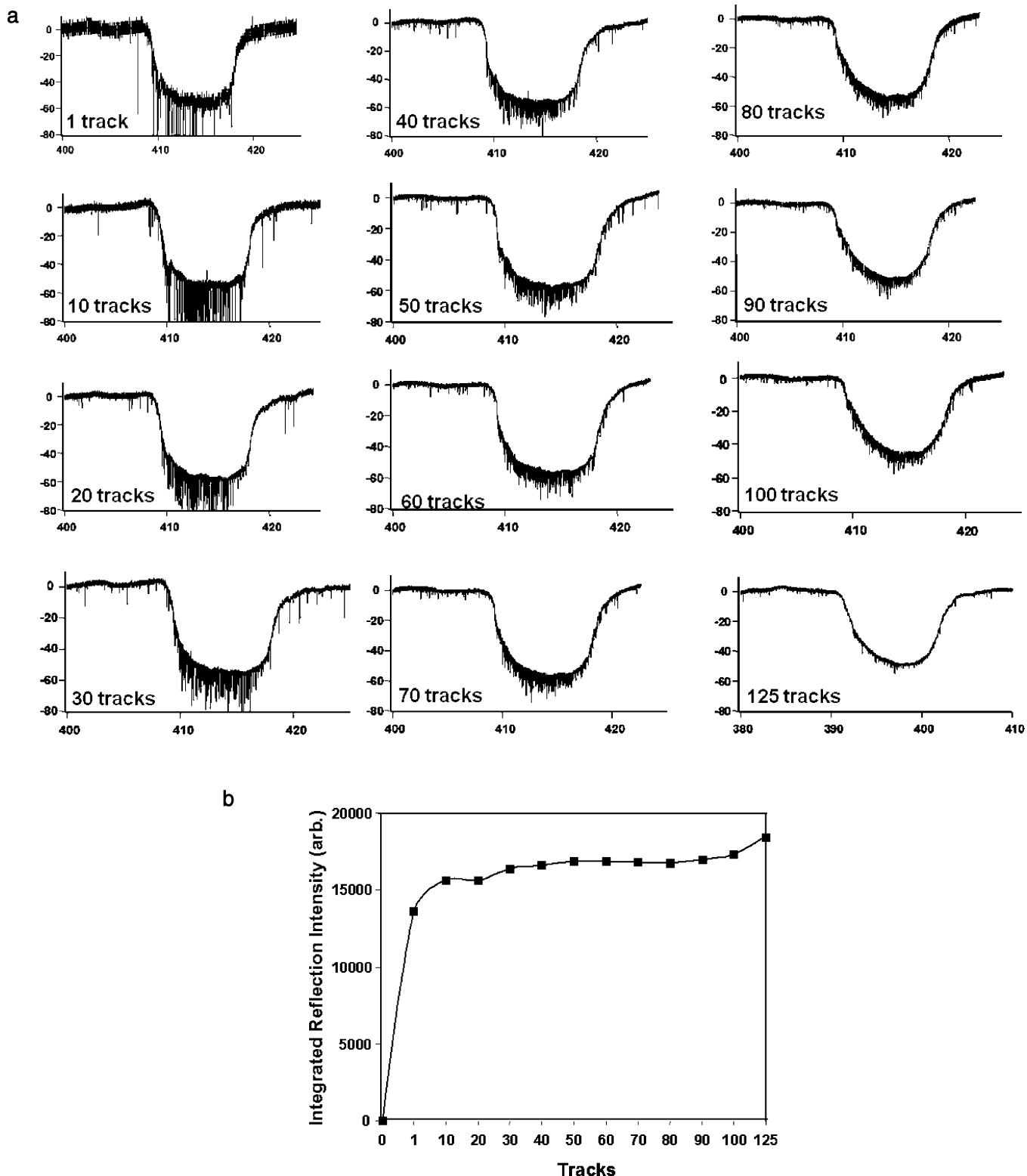


Figure 6. (a) Determination of the number of tracks needed to obtain an optimum signal with thiolated DNA and the aptamer complex at a concentration of $1 \mu\text{M}$. (b) Averages of the observed intensity of reflected light for each track are plotted. The signal reflectivities from different tracks were averaged using LabVIEW 7.1 software (National Instruments Corporation, Austin, TX), and were normalized using IGOR Pro Version 6.0 software (WaveMetrics, Inc., Lake Oswego, OR).

antibody on the disk (see Figure 4a). To these immobilized antibody spots, we added the whole Influenza A virus at different concentrations (ranging from 10–700 nM, calculated based on the total amount of protein). As a negative control, we also added the Influenza B virus (at 500 nM) to the 10th spot and then

measured the change in reflectivity for all 10 spots (Figure 4b) and quantitated them (Figure 4c). As shown in Figure 4b, the reflectivity was determined to increase with increasing concentration and the data were fitted using a nonlinear regression curve fitting algorithm to calculate the equilibrium dissociation constant

(K_d), which was 12 nM. For the same complex, we reported a K_d value of 2 nM using surface plasmon resonance instrument (Biacore),¹⁵ which suggests that the BioDVD has ~6-times-lower sensitivity than a Biacore-based analysis, which is presently the gold standard for the analysis of biomolecular interactions. Taken together, the intensities observed with the thiolated oligo and other complexes on structure B clearly suggest that our optimized structure B has better sensitivity, compared to structure A.

Manipulating the State of the Phase-Change (AIST) Layer Enhances the Biosensing Response. To tune the sensitivity of the multi-interferometric system to local changes in the refractive index of the sensor surface, the inclusion of a phase-change layer offers a strong advantage. We previously observed that the response of the system underwent a large change when the phase-change layer was switched from the amorphous state to the crystalline state, which is a process that can be reversibly achieved by simple changes in the readout laser power.¹² We determined that, when the phase-change layer in structure A was in the amorphous state, the sensitivity for biomolecular interactions was higher than that of the crystalline state. This allows for regions of the BioDVD disk to be selectively and reversibly masked by a simple transformation of the phase-change layer by the application of two different laser powers.¹² In the present studies, we performed a similar biomolecular interaction analysis for structure B. As observed in the aforementioned studies, structure B yielded over a 3-fold improvement in signal intensity for all of the complexes (**1–4** in Figure 3a), compared to structure A in the amorphous state. Interestingly, in contrast to our previous study,¹² when the multilayered structure B was converted to the crystalline state from the amorphous state by an irradiation process with the laser power (7 mW, six times), we observed an enhancement of the signals for all of the biomolecular interactions that were analyzed, including nucleic acid hybridization and RNA/protein interactions (complexes **2–4** in Figure 3a). However, the signal intensity for the thiolated oligo did not increase significantly on structure B in the crystalline state. These results suggest that manipulating the ZnS–SiO₂ layer thickness seems to enhance the phase contrast of the aggregate structure, because of its large refractive index. This significantly affects the intensity of the reflected light and thereby increases the sensitivity for analysis of biomolecular interactions (see Figure 5).

Track Averaging and Noise Reduction. Biomolecular interaction data can be acquired rapidly using the BioDVD platform with higher reproducibility at higher speeds (6.0 m/s); however, we determined that the data acquisition from 50 tracks, the track averaging, and the noise reduction time are bottlenecks in the overall analysis. To reduce the time required for all of these processes, we will next address how many tracks are required to

obtain data with an adequate signal-to-noise ratio. In our previous studies, we collected data from 50 tracks of the BioDVD and averaged along these tracks to quantify the biomolecular interactions. In this study, we immobilized a thiolated DNA and an aptamer (antifactor IX) complex on the Au layer of the BioDVD using a 7.5- μ L sample, which occupies ~150 tracks. We collected reflectivity data (of the same spot) that originated from 1–125 tracks and integrated their peak area (see Figure 6a). The quantitated data were plotted for all different tracks after averaging, except for the single-track data. As seen in Figure 6b, the intensity of the reflected light originating from a single track clearly is sufficient to measure biomolecular interactions. However, to obtain an acceptable standard deviation between tracks, it seems that 10 tracks could be sufficient, instead of the 50 tracks used previously. Once the number of tracks used for the data acquisition was reduced, the spot volume could be reduced to 50 nL, which occupies ~10 tracks. By decreasing the spot size, the total number of spots possible on a single disk could be further increased to a total of ~400 000, based on the total available area of the disk.

CONCLUSION

In the present study, we have optimized the disk structure and improved its sensitivity to detect biomolecular interactions, which allowed expansion of its applications beyond nucleic acid–based interactions. Interestingly, in this study, we determined that optimizing the ZnS–SiO₂ layer thickness on either side in the multilayered structure of BioDVD allowed us to improve the sensitivity by more than a factor of 3, compared to our previously reported structure for analyzing different biomolecular interactions. Moreover, we have determined that only 10 tracks of data are required for analyzing biomolecular interactions on the BioDVD platform.

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SUPPORTING INFORMATION AVAILABLE

Figure describing the amino-coupling reaction between 16-mercaptophexadecanoic acid and anti-Haemagglutinin (HA) antibody (upper panel) and the attachment of the 16-mercaptophexadecanoic acid linked anti-HA antibody to the BioDVD disk and further interaction with the HA of the Influenza A virus (lower panel). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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