Digitized Molecular Diagnostics: Reading Disk-Based Bioassays with Standard Computer Drives

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Digitized Molecular Diagnostics: Reading Disk-Based Bioassays with Standard Computer Drives

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We report herein a digital signal readout protocol for screening disk-based bioassays with standard optical drives of ordinary desktop/notebook computers. Three different types of biochemical recognition reactions (biotin−streptavidin binding, DNA hybridization, and protein−protein interaction) were performed directly on a compact disk in a line array format with the help of microfluidic channel plates. Being well-correlated with the optical darkness of the binding sites (after signal enhancement by gold nanoparticle-promoted autometallography), the reading error levels of prerecorded audio files can serve as a quantitative measure of biochemical interaction. This novel readout protocol is about 1 order of magnitude more sensitive than fluorescence labeling/scanning and has the capability of examining multiplex microassays on the same disk. Because no modification to either hardware or software is needed, it promises a platform technology for rapid, low-cost, and high-throughput point-of-care biomedical diagnostics.

Microarray technology is a powerful tool for the high-throughput analysis of specific interactions between biological macromolecules (DNA, proteins, and carbohydrates). However, its applications (e.g., gene profiling, clinical diagnosis, immunoassays, and drug discovery) are currently limited to well-funded biomedical laboratories or hospital settings due to the requirement for expensive equipment (such as robotic spotters and laser fluorescence scanners).[1−4] Therefore, the development of inexpensive materials and tools for rapid biomolecular screening is important to the improvement of public health care, particularly for early-stage, on-site biomedical diagnostics. Compact disk (CD) technology is a promising candidate,[5,6] as it may offer versatile fabrication materials and convenient optical reading devices for microarray biochips. It has been shown recently that polycarbonate (PC) plates can be employed as alternative substrates to glass slides/silicon wafers for the preparation of microanalytical devices.[7−9] In addition, the characteristic optical phenomena occurring on the metal layer of a CD can be utilized to develop biosensors based on interferometry[10] or surface plasmon resonance.[11] If microfluidic functions are integrated with CDs, particularly the control of fluid transfer by disk spinning, laboratory-on-a-disk devices can be fabricated,[12,13] which have already attracted widespread interest in commerce.[14]

Recent research to adapt computer drives as optical readout devices for biochips has been focused on innovative hardware modifications. With a second laser-based detector attached on the upper part of a commercial CD drive, Alexandre et al. fabricated a double-sided reader to examine both numeric and genomic information on "bioCDs".[15] Barathur et al. reported that a modified optical drive can be used to read special disks with embedded microfluidic channels.[16] Recently, Lange et al. converted the pickup unit inside a CD drive to a laser scanning microscope to monitor the light reflection from gold nanoparticle-stained immunoassays microcontact-printed on CD surfaces.[17] Potyrailo et al. were able to acquire analog signals directly from the photodiode of a DVD/CD drive for the quantitation of metal cations adsorbed on the disk surface.[18] Maquieira and co-workers detected DNA hybridization spots and immunoassays located on the top side of the CD by installing an additional photodiode to monitor the light transmitted through the disk.[19,20]

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Meanwhile, other scientists have explored software approaches to analyze the digital signals obtained from a CD. The protocol pioneered by La Clair et al. for screening ligand−protein interactions on CDs was based on an error determination routine, for which a specially designed software was used to create unique data structures and to detect the reading errors by comparing the original with the retrieved data byte by byte.[21,22] Nevertheless, the multistep phosphorylation reactions for attaching ligand molecules to the polycarbonate surface reported therein need to be carefully handled,[21] as polycarbonate is not compatible with harsh organic solvents (e.g., prolonged exposure to acetonitrile or acetone will ruin the optical properties of CD substrates).[23] The detection sensitivity may also be limited because unlabeled biomolecules (usually smaller than 10 nm) are not large enough to either effectively block/diffract the laser beam (780 nm) in a typical CD player[6,17] or to create significant interferometric signals.[10] Jones employed CD drives as photonic signal processing devices (optical microscopes) to image stained bacterial cells physically adsorbed on disk;[24] with the typical size of a cell ranging from a few to tens of micrometers, this is sufficient to disrupt the laser reading process. Since the entire CD was used for one type of stained cells in his experiments,[24] the potential of high-throughput analysis and the detection of biorecognition reactions at the molecular level deserves further investigations.

The key question to replace complex and expensive test equipments with ordinary computers for on-site biomedical diagnosis is this: can we really "read" biorecognition reactions on disk with standard optical drives?[25] Herein we give an affirmative answer by describing a novel readout protocol for the multiplex screening of biomolecular binding events, which takes advantage of the reading-error correction function embedded in standard audio CDs. After "enhancement" of the assay signals by autometallography,[26−28] a free CD-quality analysis program is employed to detect and quantitate the binding events. Because this technique does not require any changes to the computer hardware or software, it is readily accessible and suitable as platform technology for rapid, high-throughput, and point-of-care biomedical diagnostics. In addition, the surface chemistry we have demonstrated herein, i.e., to prepare robust, multiplex bioassays (for biotin−streptavidin binding, DNA hybridization, and antibody−antigen interaction) on polycarbonate surfaces via covalent bonding, is potentially useful in the fabrication of other types of polymer-based microanalytical devices.[7−9]

RESULTS

Two prerequisites must be met before commercial CD drives can be adapted for screening and quantitation of disk-based biomedical binding assays: immobilization of probe molecules onto the disk without surface destruction, and signal enhancement to make the reaction "readable" by the laser system of an unmodified computer drive.

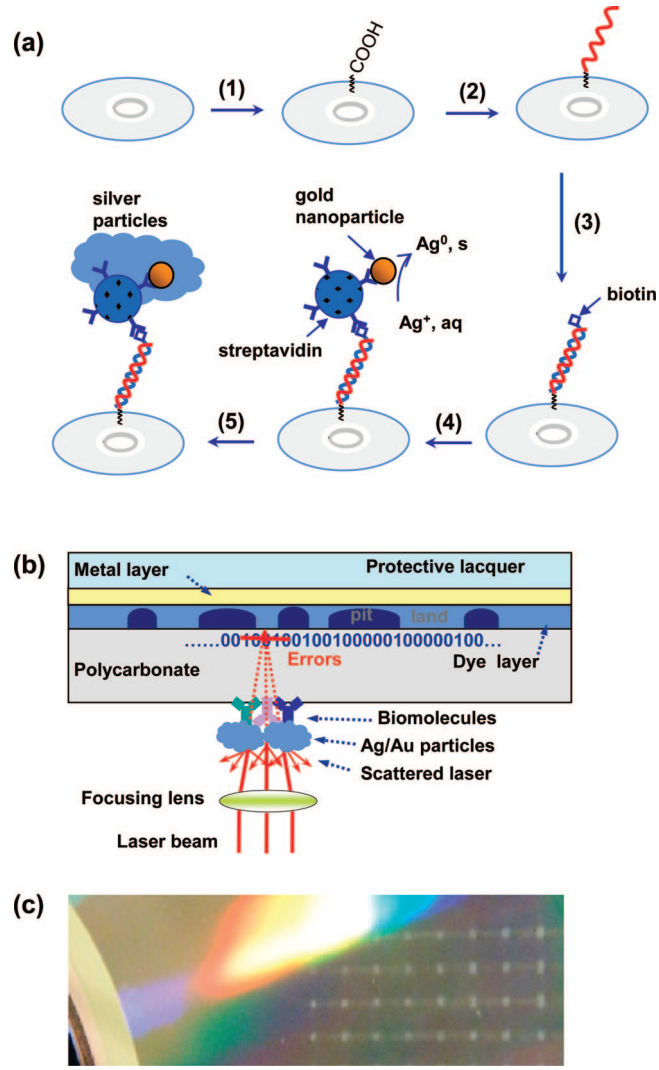

Figure 1. (a) Preparation of disk-based bioassay and signal amplification via gold/silver staining. (1) UV/ozone activation to generate carboxylic acid groups on CD; (2) immobilization of amino-tethered DNA probe strands via amide coupling; (3) hybridization with biotinylated DNA target strands; (4) binding of gold nanoparticle−streptavidin conjugates; (5) reductive precipitation of silver particles for signal enhancement. (b) Digital reading of bioassay with CD drive: the biomolecule/nanoparticle conjugates block the reading laser and generate significant errors. (c) An optical image of DNA microarray formed on a regular CD-R according to the above surface reaction and signal amplification procedure.

Probe Immobilization and Signal Amplification. We integrated the UV/ozone surface treatment/amide coupling protocol and the gold nanoparticle-based autometallography method to accomplish the above-mentioned tasks. Figure 1a illustrates the surface reaction procedures for a DNA hybridization assay. The PC face of a CD/CD-R was first irradiated with UV light in the presence of ozone to produce a hydrophilic surface with a high density of carboxylic acid groups (step 1). Then the probe molecules (biotin, DNA, or human plasma IgG) were covalently attached to the PC surface via amide coupling (step 2). By using microfluidic channel plates made of poly(dimethylsiloxane) (PDMS)

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Table 1. Comparison of the Detection Performance of the Fluorescent Method and the CD-Based Detection Method

	detection limit		dynamic detection range	
	FL	CD	FL	CD[a]
biotin /streptavidin binding	1.25 μg/mL	0.1 μg/mL	1.25−10 μg/mL	0.1−1.6 μg/mL
DNA hybridization	0.1 μM	0.025 μM	0.1−1.0 μM	0.025−1.0 μM
IgG/anti-IgG interaction	0.2 μg/mL	0.025 μg/mL	0.2−10 μg/mL	0.025−1.0 μg/mL

[a] In CD-based detection, the dynamic range for each biorecognition reaction can be manipulated by the variation of staining time.

as on-disk sample delivery and positioning tools,[9,29] the immobilization and binding reactions can be carried out in a flexible arraying format and at any desired location. We monitored the biochemical binding reactions on the disk surface initially with the conventional fluorescence (FL) method (see Supporting Information). In this case, the target molecules were labeled with fluorescent tags. The results confirmed that the immobilization of the probes on the PC surface and the subsequent recognition of target molecules are efficient and sensitive. The detection limits were found to be in the submicromolar range for all three classical binding assays (Table 1) tested herein. It should be noted that the surface activation procedure for PC substrate works well for the attachment of small ligands and proteins (besides DNA strands), which had not been demonstrated in our previous publication.[9]

Objects of ~200 nm size (~1/4 λ) are needed to induce significant disruption of the laser light reflection (to create "readable" signals) of a standard optical drive.[6,17] Since most biological macromolecules are too small to be detected by the reading system of a standard computer drive, we amplified the binding signals by autometallography.[26−28] Steps 3−5 of Figure 1a illustrate the procedure for the case of reading DNA hybridization assay. Biotin-labeled target DNA was hybridized with the probe DNA immobilized on the disk (step 3) and subsequently treated with gold nanoparticle−streptavidin conjugate (step 4). Silver was then deposited on the gold "seed" (step 5) to increase the particle size from a few to several hundreds nanometers. As shown in Figure 1b, we expected that the large silver particles would block the laser beam hitting them and cause significant scattering,[17] thereby producing reading errors detectable by the optical drive. In fact, at saturated concentrations, the binding sites could even be observed with the naked eye or with a standard flatbed scanner (Figure 1c). It should be noted that a colorimetric method for analyzing combinatorial arrays using DNA-modified gold nanoparticle probes and a conventional flatbed scanner has been previously demonstrated by Taton et al.;[30] nevertheless, the digital reading protocol described herein is conceptually different.

Digital Readout Protocol. Our bioassay screening protocol is based on the analysis of reading errors produced on prerecorded audio files by molecular binding events; i.e., the position and level of the resulting reading error correspond to the physical location and the intensity of the bioassay signal, respectively. In fact, every optical drive is "smart" enough to distinguish and correct the errors occurring in the reading process. To do this, it employs an algorithm called Cross Interleave Reed-Solomon Code (CIRC).[31] This coding system uses a data-interleaving strategy to record and distribute the original data (including potential errors) and employs a parity checking function (redundant data) to protect them.[31,32] Upon playing, the CD drive only needs to decode the data in a reverse sequence and extract the parity bits (redundant data) to check their accuracy. Any disagreement during the parity check indicates an error. Upon playback, after demodulation of each frame, each base unit (including 24 bytes of audio and 8 bytes of parity checking data) of the audio file is sent to the CIRC decoder for deinterleaving, error detection, and correction. Because every block of audio data passing through the CIRC decoder has an address corresponding to a specific physical position on the CD surface, the computer drive can detect every reading error and track its original location simultaneously.

We have identified several free CD-quality diagnostic programs, such as PlexTools Professional, Kprobe, and CD-DVD Speed, which can be used to access the error-statistic information in a CD/DVD drive and to generate a plot displaying the variation of the block error rate as function of playtime. Because the playtime of a digital audio file corresponds to a specific physical position on the CD surface, we can identify where the binding event occurred if it causes a significant disruption of the laser reading. For example, a typical 700-MB CD-R contains 79.7 min of audio data: if an error peak occurs at the playtime around the 15-min mark in the error distribution plot, we can tell that the biomolecular binding event happened at an approximate orbicular location with a radius of 33.77 mm, as calculated from the following equation,

$$r = \sqrt{\frac{t}{79.7}(58^2 - 25^2) + 25^2} \qquad (1)$$

where t is the playtime (min) and r is the radius of the location (radial distance). As shown in Figure 2a, the radius of the program area of a typical CD is 58 mm, and 25 mm is the radius of the blank center part.

Based on the detection principle described above, the performance (lateral resolution and sensitivity) of this digital readout protocol was first evaluated by applying a series of microsize color stains at different locations on the CD surface. As shown in Figure 2b, these stains generated significant errors when read by a standard optical drive, in contrast to the minimal errors shown in the bottom plot. It is evident that an object with a diameter larger than 260 μm can be recognized by an optical drive and that the block error rates depend on the darkness of each stain spot. We

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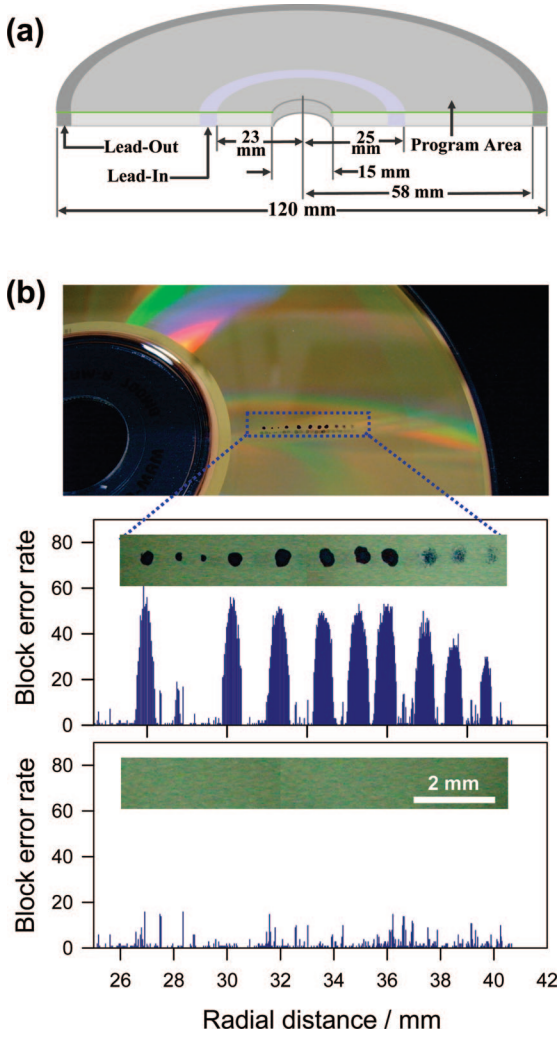

Figure 2. (a) Layout of a typical compact disk. The physical location of a binding site is correlated with the playtime (audio CD only). (b) Optical image of a disk with color stains and the corresponding reading error (block error rates) vs radial distance (mm). The bottom plot shows the background error rate distribution before staining.

note that the detection range of this reading program is between 10 and 300 errors/s (block error rate); below the lower limit, the signal is difficult to distinguish from the background, while over the upper limit, the detected signal will be saturated. The time needed to obtain the test results shown in Figure 2 is much shorter than the play time of the audio tracks; for example, less than 92 s is required to examine an entire CD if a 52 × optical drive is used for the detection.

Quantitation of Biomolecular Binding Assays. The successful detection of microsize features on the CD surface gave us the confidence to screen classical biomolecular interactions with this digital readout system. First we examined biotin−streptavidin binding, one of the most frequently studied biological interactions. Five binding strips were formed on the PC surface of an audio CD-R with the assistance of PDMS plates. They were designed to test the binding of surface-bound biotin (prepared by coupling biotinyl-3,6,9-trioxaundecanediamine to carboxylic acid groups on an activated CD surface, Figure 1a) to five different concentrations of gold nanoparticle−streptavidin conjugate. To exclude potential contaminations from the multistep surface reaction, we run the diagnostic program to check the error distribution of the CD after

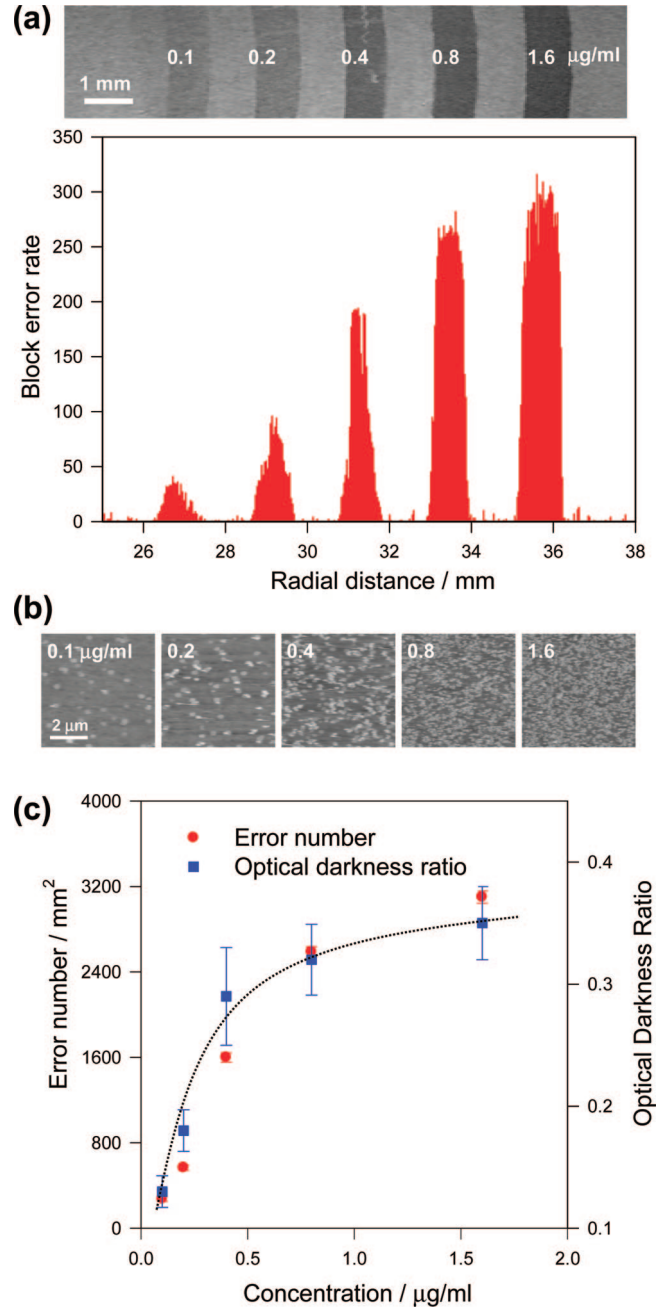

Figure 3. (a) Quantitation of biotin/streptavidin binding assays; the distribution of block error rate on a CD modified with five strips of biotin/streptavidin binding lines. The top inset is an optical image of the binding strips. (b) AFM images of the binding strips to show the size and density of silver particles on the surface. (c) Relative error level intensity and optical darkness ratio as function of the target concentration; the dashed line is to direct the eyes only.

each treatment. Upon the completion of the silver staining reaction, the CD exhibited a characteristic error distribution with five distinct peaks whose digital positions (playtime) perfectly matched the corresponding physical locations of the binding strips (Figure 3a). Atomic force microscopy (AFM) images (Figure 3b) revealed that they were composed of large-sized nanoparticles (~90−300 nm) with different particle densities (increasing gradually with increasing streptavidin concentration). The particle size variations are due to differences in the number of gold nanoparticle "seeds" and the effects of competitive growth. The data plotted

Table 2. Oligonucleotide Sequences of Probe and Target DNA Samples

DNA strand	sequence
probe I	H_2N-$(CH_2)_6$-5′-CGC CGA TTG GAC AAA ACT TAA A-3′
probe II	H_2N-$(CH_2)_6$-5′-CGC CGA TTG GA*G* AAA ACT TAA A-3′
probe III	H_2N-$(CH_2)_6$-5′-TTT AAG TTT TGT CCA ACT GGC G-3′
target I	3′-GCG GCT AAC CTG TTT TGA ATT T-5′-biotin
target II	3′-GCG GCT AAC CTG TTT TGA ATT T-5′-Cy5

in Figure 3c demonstrate that both the error rates and the optical darkness ratios (ODR) of the binding signals (determined with an optical microscope) depend on the target concentrations. The optical darkness ratio (ODR) is defined by eq 2

$$\mathrm{ODR} = (I_b - I_s)/I_b \qquad (2)$$

where I_b is the average luminosity of the background and I_s the value for the binding site, which is a function of particle size and density.[28] In the low target concentration range, error level and ODR are approximately proportional to the concentration of the target molecules (streptavidin); at higher concentrations, both reach a plateau due to signal saturation. This result not only validated our disk-based assay preparation and digital reading protocol but also laid the foundation for studying more complex biorecognition reactions.

To test the DNA hybridization illustrated in Figure 1a (the nucleotide sequences are listed in Table 2), we prepared a line array on the PC surface by hybridizing probe I with increasing concentrations of biotinylated DNA (target I) and subsequent treatment with a gold−streptavidin conjugate solution. As shown in Figure 4a, after silver enhancement, the hybridization array became optically visible and also detectable by a CD drive. Both the error level and ODR value increased rapidly with the target concentration in the low concentration range and reached saturation levels at higher concentrations of cDNA strands (Figure 4b). Without optimization, we could easily detect the target sample at a concentration as low as 25 nM. Considering the small solution volume (2.0 μL) needed, this is equivalent to 50 fmol of DNA molecules, i.e., the sensitivity is about 1 order of magnitude higher than that of the conventional fluorescence labeling/scanning method.[9]

In addition, we were able to detect single-nucleotide polymorphism (SNP). Upon hybridization of the same target (target I) with complementary (PM) (probe I), single-base-pair mismatched (probe II), and noncomplementary (NP) probe strands (probe III) on the same array, the three types of strips showed marked differences in their optical darkness and, upon playing, in their error levels. As shown in Figure 5, strong error signals were produced only for the hybridization of complementary strands (lines 4 and 5) after silver staining for a shorter time (e.g., <60 min). After 80 min of staining, two weaker error peaks due to the DNA duplexes containing SNP (lines 1 and 2) became visible, while no hybridization with noncomplementary probe strands (line 3) was detected.

To further demonstrate the versatility of the CD-based biomolecular screening protocol, we also examined antihuman IgG/human IgG binding, a model system of biomedically relevant

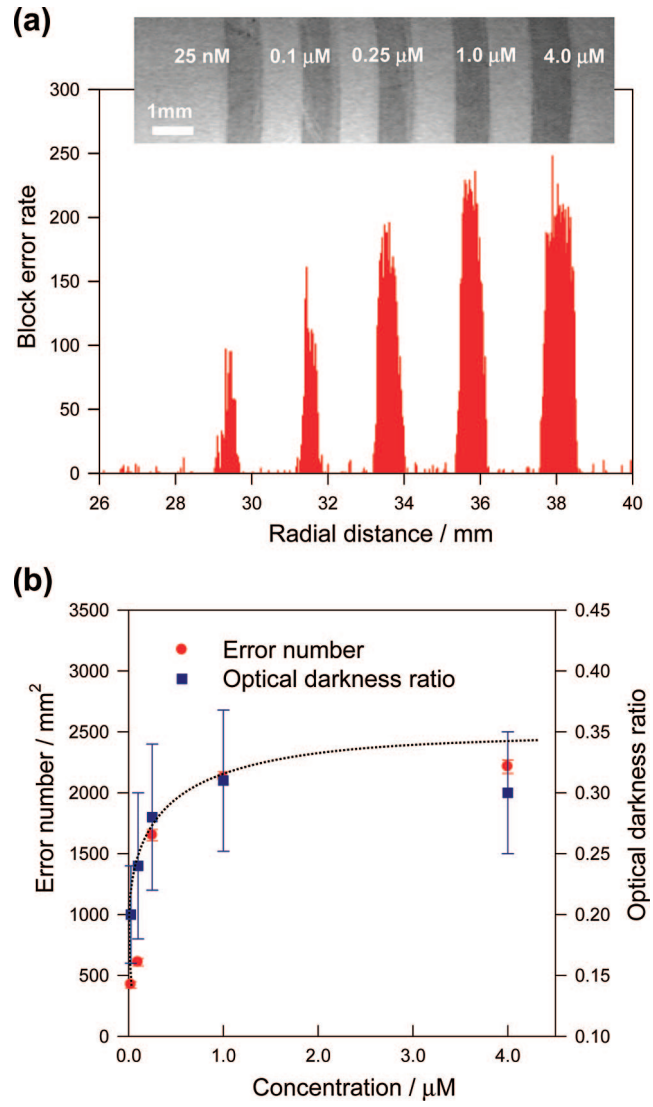

Figure 4. Quantitation of DNA hybridization assays based on error levels read with a standard computer drive: (a) block error rate distribution of the DNA hybridization line array; inset, an optical image of the binding strips; (b) dependence of error levels and optical darkness ratios on target concentrations.

immunoassays. In general, the immobilization of proteins is more challenging because they easily lose activity. We have obtained promising results (Figure 6) as indicated by the observed higher detection sensitivity in comparison to the DNA hybridization assays. The optical image and error distribution plots show that not only can a readable signal be obtained with a concentration as low as 25 ng/mL anti-IgG but also the saturation level is reached at a lower concentration (250 ng/mL). The silver staining step required only 30 min to obtain sufficient contrast, which confirms the high efficiency of IgG immobilization and of anti-IgG binding. It should be noted that conventional immunoassays (e.g., ELISA) are typically performed in a sandwich format (lowering the background signal due to nonspecific antigen adsorption), for which we would expect even better results.[33]

DISCUSSION

The use of standard computing devices for analysis of disk-based bioassays has been a technical challenge and would

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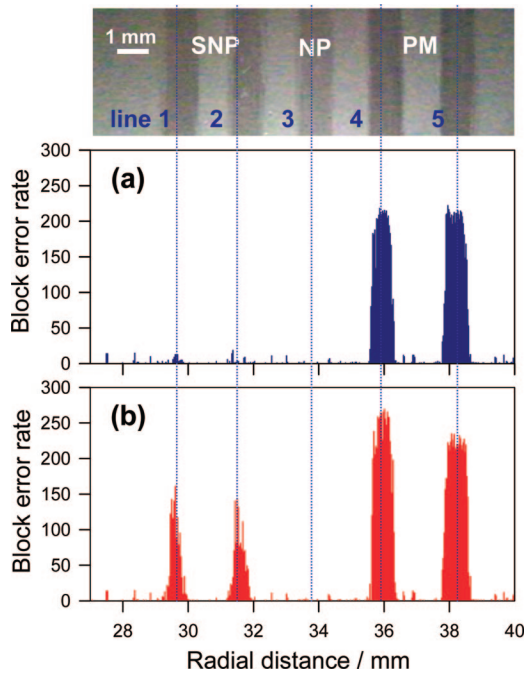

Figure 5. Block error rate distribution of a CD modified with a DNA hybridization array prepared from three types of probes: single-base-pair mismatched (SNP), noncomplementary (NP), and complementary strands (PM): (a) scanned after 60 min of silver enhancement, (b) scanned after 80 min of silver enhancement. The top inset is an optical image of the five hybridization strips.

constitute a breakthrough in advancing point-of-care biomedical diagnosis.[25] "Prior art" has relied on the fabrication of special disks with embedded microchannels or on complete alteration of the read/write mechanism of optical drives.[15−24] Our approach has the following major advantages: (1) No modifications to either the optical drive or the operation software are needed. We took advantage of the error-indentifying/correcting function inherent in the standard digital data of audio files (recorded on conventional CD/CD-Rs) to detect the reading errors, which is different from the method reported by La Clair et al. (in which such function was disabled and replaced by a unique data structure).[21] It is true that a dedicated software may do better in reading particular data structures and serving specific needs of particular users, which may not be available with a free software. Nonetheless, to read biomedical assays with an ordinary computer now becomes feasible (i.e., accessible to other users), and it is possible to extend the reading mechanism to stand-alone CD/DVD players. (2) The surface chemistry proposed and tested herein involves simple and mild reactions that can be carried out by nonspecialists as easily and safely as ELISA assays.[33] (3) As the measured error levels and the optical darkness ratios are consistent with each other over the entire testing range, they can be used for the construction of calibration curves to quantitate the analyte concentrations. Conventional colorimetric diagnostic kits, in contrast, are useful only for qualitative measurements (positive or negative response).

In terms of the effect of hardware variations on the readout reproducibility, we did observe slightly different error levels when reading the same disk with different types of optical drives. However, because the same digital readout principle as for audio-CDs (the simplest format) was followed,[31,32] the trend in change

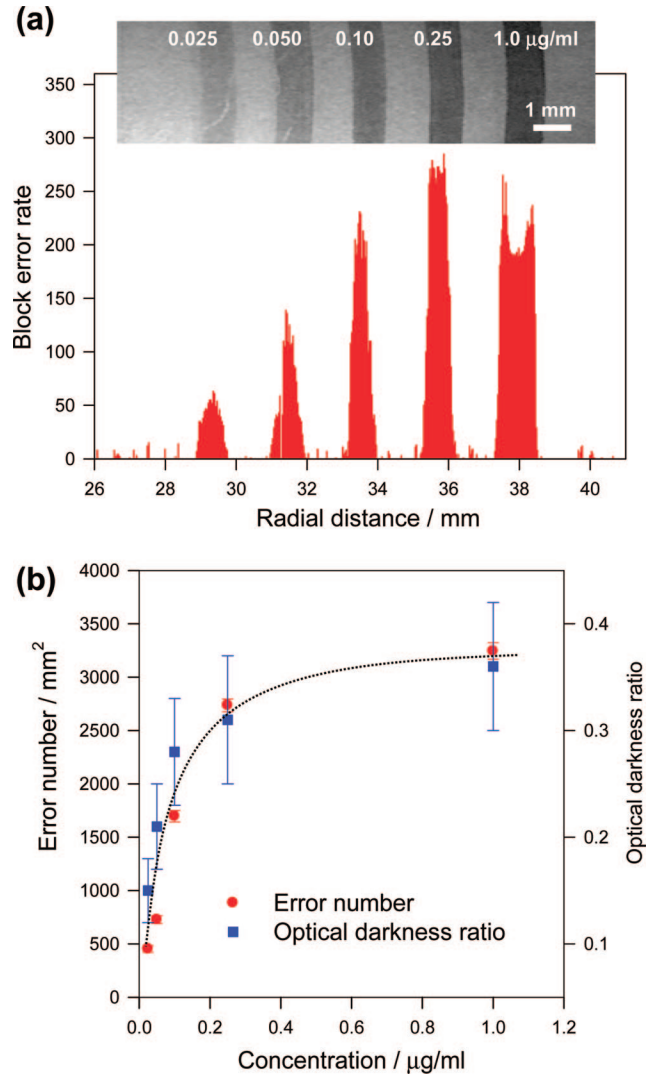

Figure 6. Quantitation of human IgG/antihuman IgG binding assays on disk. (a) Block error rate distribution plot with an optical image at the top; (b) dependence of error levels and optical darkness ratios on the target concentrations.

of error levels was found to be the same in all tests, which is essential for quantitative analysis (see Supporting Information).

The results obtained for DNA hybridization and IgG/anti-IgG interaction show that the CD-based readout system is more sensitive than traditional fluorescence labeling/scanning (Table 1). In addition, the dynamic range for each biorecognition reaction can be manipulated: if the signal upon incubation in the target solution is strong, the silver staining time can be shortened, and vice versa. Thus, the target concentration detection range is actually much wider than our data indicate. It should be pointed out that prolonged silver staining reaction will eventually saturate the reading errors and also generate high background interferences.

As far as the signal throughput is concerned, theoretically each byte of data stored on the CD (700 MB) can be utilized. However, because the detection resolution on the PC surface depends on the minimum width of each reaction "strip" (260 μm as shown in Figure 2b), the screening capacity of a CD is ~100 reactions/disk at the current stage. We expect that a DVD system (higher digital capacity and smaller detection laser spot size) would have an even higher capacity.

CONCLUSION

We have developed a digital readout methodology for screening biomolecular binding reactions, which is based on the reading-error determination principle of audio CDs. Using a standard computer drive without any modification to the hardware or control software, this novel signal-readout system "recognizes" staining spots larger than 260 μm on a CD surface. Different types of bioassays (DNA hybridization and antibody/antigen binding) can be prepared in simple array format with microfluidic equipment/tools. When read by a conventional optical drive, the error rate of prerecorded audio files is a quantitative measure of the concentration of molecular analytes in the testing solution. In all cases, the readout error levels were found to be well-correlated with their ODR values. The CD-based readout system has a much higher sensitivity than the conventional fluorescence method, and it has the potential to screen a large number of binding assays on the same disk simultaneously.

EXPERIMENTAL SECTION

Surface Reactions on CD-Rs. Before reaction, audio information (such as WAV files) was burned into a blank CD-R (Mitsui Inc.). After recording, the PC surface was cleaned with ethanol and then activated in a UV/ozone cleaner (model PSD-UV, Novascan Technologies, Inc.) for 15 min. The disk was subsequently immersed in a 0.1 M phosphate buffer at pH 6.0 (also containing 5 mM 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide and 0.33 mM *N*-hydroxysuccinimide (NHS)) for 5 h. Then amine-PEO_3-biotin (biotinyl-3,6,9-trioxaundecanediamine, Pierce Biotechnology Inc.), amino-modified DNA strands (Sigma-Genosys, sequences listed in Table 2), and human plasma IgG (Athens Research & Technology Inc.), respectively, were immobilized onto the activated PC surface, followed by on-disk binding with their corresponding target molecules: gold-conjugated streptavidin (1.4-nm diameter, Nanoprobes Inc.), biotinylated DNA target (Sigma-Genosys), and biotinylated goat antihuman IgG (biotin-SP-conjugated goat anti-human IgG (H+L), Jackson ImmunoResearch Inc.).

(I) Biotin−Streptavidin Binding. After the NHS activation step, 10 μL of a 30 μM solution of amine-PEO_3-biotin in 0.1 M phosphate buffer at pH 7.0 was delivered onto the PC surface through a mask (made from a PDMS plate), and the disk was kept in a humid box (a sealed plastic container with its bottom filled with water) for 5 h. After the PDMS mask was peeled off, the reaction zone was passivated by treatment with a 20 mM phosphate blocking buffer at pH 7.4 (containing 150 mM NaCl, 0.8% bovine serum albumin (BSA), 0.1% gelatin, 0.05% Tween 20, and 0.05% NaN_3) for 15 min to reduce nonspecific adsorption. Then a second PDMS plate with six microchannels oriented perpendicularly was placed on top of the disk. Five different concentrations of gold-conjugated streptavidin solutions (0.1, 0.2, 0.4, 0.8, and 1.6 μg/mL) in 20 mM phosphate buffer (pH 7.4, 150 mM NaCl, 0.1% BSA, and 0.05% NaN_3) were injected into the channel reservoirs on one side and passed through the channels by suction from the other ends. The solutions were allowed to stay in the channels for 60 min at room temperature. After the PDMS plate was peeled off, the chip was washed with the buffer, dried under N_2, and subjected to the silver staining treatment.

(II) DNA Hybridization. (See Figure 1.) After DNA probe immobilization, the PC surface was passivated by treatment with 1 mg/mL solution of BSA for 5 min. Five concentrations of DNA target solution (0.025, 0.1, 0.25, 1.0, and 4.0 μM target I) in 1× saline−sodium citrate (SSC) buffer (pH 7.0, 150 mM NaCl, 15 mM sodium citrate, 0.05% sodium dodecylsulfonate) were hybridized in a humid box at 40 °C for 30 min with three different DNA probe sequences (probes I, II, and III; see Table 2). After hybridization, the disk was washed with SSC buffer, treated with phosphate buffer for 20 min, and immersed in 0.4 μg/mL gold−conjugated streptavidin solution for 60 min. Then the disk was washed and subjected to the staining treatment.

(III) Antigen−antibody Interaction. Human IgG (250 μg/mL) in 20 mM PBS-BSA buffer was allowed to react with the NHS-activated PC surface for 2 h at room temperature. The disk surface was then washed with phosphate buffer for 20 min, five concentrations of antihuman IgG solution (0.025, 0.05, 0.10, 0.25 and 1.0 μg/mL) were delivered, and the disk was incubated at room temperature for 90 min.

For the silver staining treatment, the biomolecule-modified CD-Rs were thoroughly washed with distilled water to remove anions (especially chloride). After washing, they were immersed in freshly made silver enhancement solution for different periods of time. A reagent kit for silver enhancement reaction (LI Silver, Nanoprobes Inc.), which consists of two solutions, silver salt (silver acetate) and reducing agent (hydroquinone) respectively, was used as directed.

Digital Readout Protocol. Three different systems (drive plus corresponding software), PX-755UF CD/DVD writer (Plextor Corp.) + PlexTools Professional (LE V3.12, downloaded from http://www.plextools.com), Plextor PX-760A CD/DVD writer (Plextor Corp.) + PlexTools Professional, and SHW-160P6S CD/DVD writer (Liteon It Corp., OEM)+ Kprobe (V2.50, downloaded from http://www.k-probe.com), were tested for biomolecular screening. The free Plextor Professional program runs on PC-Windows; it was used in this work to generate the error plots presented in Results. For error tests, this program controls the CD drive to run at a 8× speed, so that it typically takes 10 min to screen the entire CD and less than 2 min to screen a specified zone. After reading, it will export an error distribution plot and provide a statistical result on error numbers and types.

Optical/AFM Imaging and Data Analysis. Optical images of all samples (the binding strips formed on CDs) were captured by a Motic Digital Microscope (DM143, Micro-Optic Industrial Group Co.) and analyzed by measurement of position and size (area) of each binding strip upon silver enhancement. The ODR of each strip was determined by measuring its average intensity (I_s), using the luminosity histogram tool of a Photoshop software, and compared to the value for the background (I_b).[28]

The surface topographies of the binding assays were examined with an MFP-3D-SA atomic force microscope (Asylum Research, Inc.) in tapping mode using a rotated monolithic silicon tip (Innovative Solutions Bulgaria Ltd., resonance frequency 13 kHz, force constant 0.2 N/m). The number, size, and morphology of the particles after silver enhancement were analyzed with IGOR Pro 4 software.

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SUPPORTING INFORMATION AVAILABLE

Additional information and experimental details including the data structure of audio CDs, the error detection and correction mechanism, and the fluorescence detection of disk-based bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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