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Reading Disc-Based Bioassays with Standard Computer Drives

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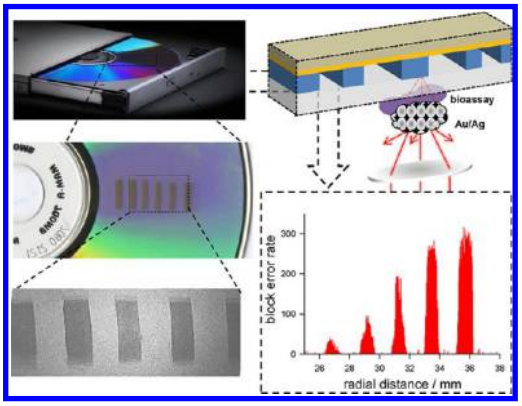
CONSPECTUS

Traditional methods of disease diagnosis are both time-consuming and labor-intensive, and many tests require expensive instrumentation and trained professionals, which restricts their use to biomedical laboratories. Because patients can wait several days (even weeks) for the results, the consequences of delayed treatment could be disastrous. Therefore, affordable and simple point-of-care (POC) biosensor devices could fill a diagnostic niche in the clinic or even at home, as personal glucose meters do for diabetics. These devices would allow patients to check their own health conditions and enable physicians to make prompt treatment decisions, which could improve the chances for rapid recovery and cure.



Compact discs (CDs) provide inexpensive substrate materials for the preparation of microarray biochips, and conventional computer drives/disc players can be adapted as precise optical reading devices for signal processing. Researchers can employ the polycarbonate (PC) base of a CD as an alternative substrate to glass slides or silicon wafers for the preparation of microanalytical devices. Using the characteristic optical phenomena occurring on the metal layer of a CD, researchers can develop biosensors based on advanced spectroscopic readout (interferometry or surface plasmon resonance). If researchers integrate microfluidic functions with CD mechanics, they can control fluid transfer through the spinning motion of the disc, leading to "lab-on-a-CD" devices.

Over the last decade, our laboratory has focused on the construction of POC biosensor devices from off-the-shelf CDs or DVDs and standard computer drives. Besides the initial studies of the suitability of CDs for surface and materials chemistry research (fabrication of self-assembled monolayers and oxide nanostructures), we have demonstrated that an ordinary optical drive, without modification of either the hardware or the software driver, can function as the signal transducing element for reading disc-based bioassays quantitatively.

In this Account, we first provide a brief introduction to CD-related materials chemistry and microfluidics research. Then we describe the mild chemistry developed in our laboratory for the preparation of computer-readable biomolecular screening assays: photochemical activation of the polycarbonate (PC) disc surface and immobilization and delivery of probe and target biomolecules. We thoroughly discuss the analysis of the molecular recognition events: researchers can "read" these devices quantitatively with an unmodified optical drive of any personal computer. Finally, and critically, we illustrate our digitized molecular diagnosis approach with three trial systems: DNA hybridization, antibody–antigen binding, and ultrasensitive lead detection with a DNAzyme assay. These examples demonstrate the broad potential of this new analytical/diagnostic tool for medical screening, on-site food/water safety testing, and remote environmental monitoring.

Introduction

Conventional chemical analysis and medical diagnosis are typically time-consuming, labor-intensive, and limited to well-equipped laboratories because they require special instrumentation and highly trained personnel. To cope with those limitations, "point-of-care" (POC) methods and tools have been developed, which not only allow molecular analysis and biomedical diagnosis to be carried out on-site by nonprofessionals but also provide test results promptly. Thus treatment can be started on-time and subsequent monitoring can be improved, leading to better outcome.[1] The personal glucose meter is the best example of

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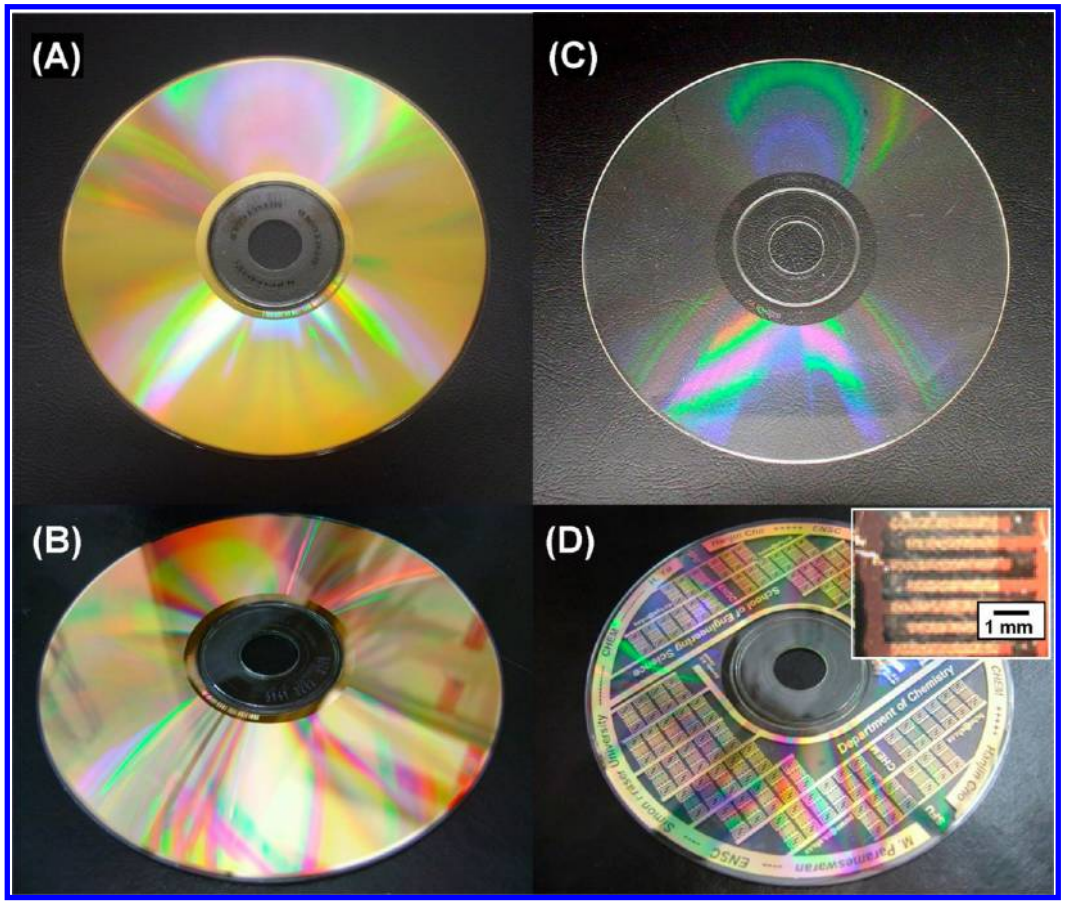

FIGURE 1. Photos of a gold CD-R before (A) and after (B) removal of the protective polymer layer,[7] (C) the polycarbonate substrate of a CD upon removal of all other layers,[13] and (D)a multiplex electrical sensor constructed on a gold CD-R (for the detection of iodine vapor).[18] The inset shows a patch of phospholipids (yellow) inkjet-printed on one set of the interdigitated sensing electrodes.

established POC devices;[2] it is as compact as a cell phone and able to determine the blood sugar level within a few seconds.

Compact disc (CD) and digital versatile disc (DVD) technology is a promising resource for the development of inexpensive materials and tools for POC molecular screening; the discs offer versatile fabrication materials, and conventional computer drives can be adapted as quantitative optical reading devices for microarray biochips. A major research theme of the last two decades has been the development of micro-total-analysis systems (μTAS)[3,4] on CD-like platforms, which have already attracted widespread interest in commerce.[5] These "lab-on-a-CD" devices integrate several laboratory functions on circular plastic or glass substrates (without data storage/processing functions); they require only a simple motor to provide the centrifugal forces needed for fluid manipulation. As the disc spins, centrifugal forces induced in sample fluids drive liquids radially outward from the center toward the edge of the disc.[5a,b] By embedding electrochemical or optical measurements on the same disc, one can obtain test-specific information. Several companies, including HSG-IMIT, GenePOC, and Tecan, have been working on the commercialization of this method; the Swedish firm Gyros has produced special CDs to run nanoliter-scale immunoassays.[5c]

Our early endeavors on the application of CD technology started with the utilization of the reflective metal layer in a CD for chemistry research.[6] For some brands of high-quality CD-Rs (Kodak/Mitsui), this layer is made of pure gold due to its high stability and ideal optical performance (others are typically aluminum and silver). After immersion of a regular gold CD-R in concentrated HNO_3 for 3−5 min, the protective polymer coating can be peeled off. Figure 1A,B shows the difference before and after the protective film is removed; that is, we readily convert an ordinary "old" CD to a "shining" gold substrate that is suitable for the preparation of alkanethiolate self-assembled monolayers.[7] For electrochemistry studies, Angnes et al. have pioneered the preparation of gold electrodes of various sizes and shapes from regular CDs.[8,9] Subsequently, gold substrates from CDs have been

adapted to various spectroscopic sensing devices[10] and microchip-based electroanalytical systems.[11,12]

We and others have also explored the application of CD substrates as templates for the fabrication of material nanostructures.[13−19] For an example, inexpensive CD/DVD polycarbonate bases (Figure 1C, after removal of all other layers) have been adapted as microcontact printing stamp to pattern gold nanostructures.[13,14] We have constructed disc-based multiplex conductivimetric chemical sensors for detecting volatile analytes.[18] The key is to prepare a miniaturized electrode array (Figure 1D) via inkjet printing of a patterned alkanethiolate monolayer on a gold CD-R and subsequent wet chemical etching.[18,19]

Surface Activation of CD Polycarbonate Base

The aforementioned application of CDs or DVDs involves the destruction of the disc structure to expose either the gold reflective film[7−12,17−19] or the polycarbonate (PC) base;[13−16] those discs are no longer readable by a conventional computer drive. Activation of the transparent side for the covalent attachment of probe molecules (without destruction of the CD structure) is essentially the first task in the fabrication of computer-readable bioassay discs. We have established a UV/ozone treatment protocol to convert polycarbonate CD substrates to biochip platforms.[20] It is safe and mild because the UV power is low (1.5 mW/cm^2) and ozone is generated from the ambient oxygen near the reaction site. As shown in Figure 2A, the water contact angle dropped from 88° ± 2° to 20° ± 2° after less than 10 min as a result of the generation of hydrophilic reactive end groups (e.g., −COOH). UV irradiation alone takes much longer (from 30 min to 10 h), depending on the wavelength, power, and distance of the light source.[21−23] We also confirmed that the surface morphology did not change significantly (Figure 2B), which ensures the disc readability after activation.[20]

The next task is the patterned immobilization/delivery of probe/target molecules on the activated disc; a microfluidic network method[24,25] was explored in our research to create disc-based DNA microarrays.[20,26] As illustrated in Figure 3, a PDMS (polydimethylsiloxane) channel plate is first placed on the activated PC disc for the immobilization of probe DNA strands (to form a line array). After its removal the target samples are delivered in perpendicular channels with another PDMS plate. The hybridization event happens at the intersection of the microchannels on the second plate and the preformed probe line array. To be adapted to computer drive reading, the channels were designed to follow the direction of the spiral track of a CD, and the second plate was a simple rectangular mask (*vide infra*).

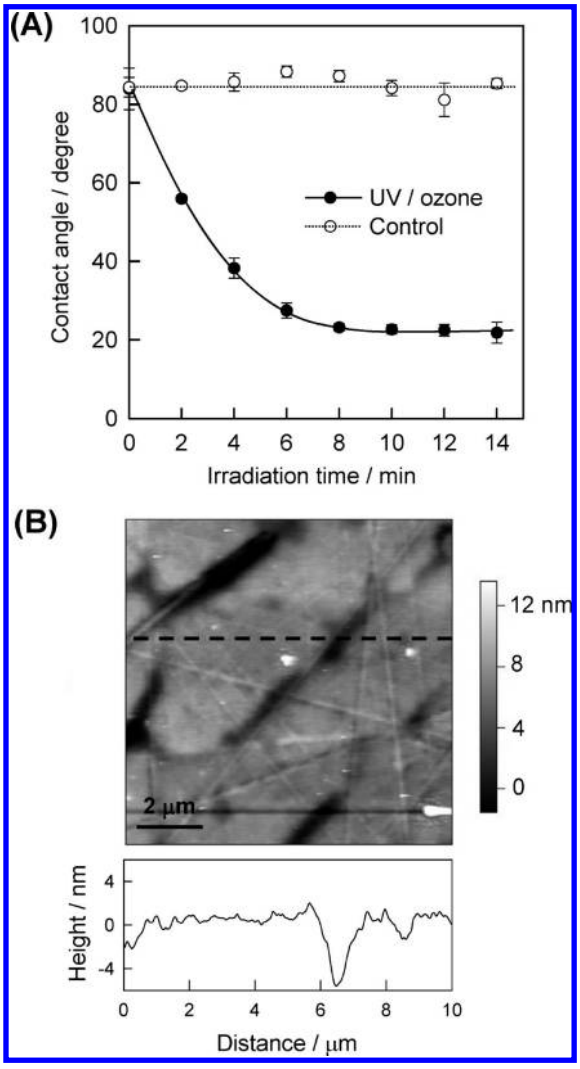

FIGURE 2. (A) Dependence of water contact angle at the PC surface on UV/ozone activation time. (B) Atomic force microscope (AFM) image and corresponding cross-section of the PC surface of a CD after UV/ozone treatment for 10 min.

Figure 4 shows a DNA hybridization assay performed with the same DNA target strand and three different probe strands. The hybridization of Cy5-labeled target DNA with probe strands containing a single-base mismatch (probe II) resulted in low fluorescence signals at the intersections with lines 1−3. In comparison, no signal was discernible in the fluorescence image when the target and probe DNA strands (probe III) were noncomplementary (expected at the intersections with line 4), and the fluorescence intensities at the intersections of target sample with lines 5−7 (complementary probe/target) were high.

It should be noted that such a nondestructive surface activation method, along with protocols recently developed

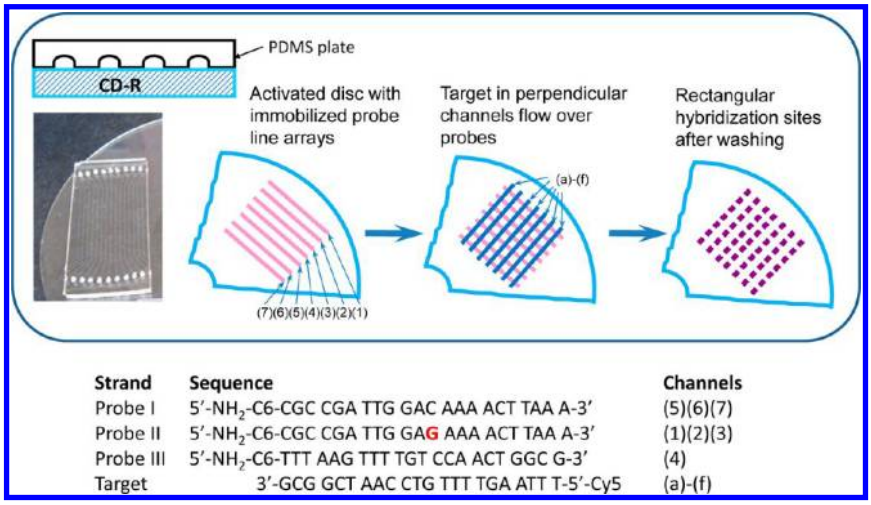

FIGURE 3. Preparation of DNA hybridization assay on a disc using microfluidic network approach; the schematic diagram at the top shows the formation of hybridization patches at the intersections between seven sample channels and seven probe lines. The DNA sequences listed at the bottom were used to generate the results shown in Figures 4 and 8.

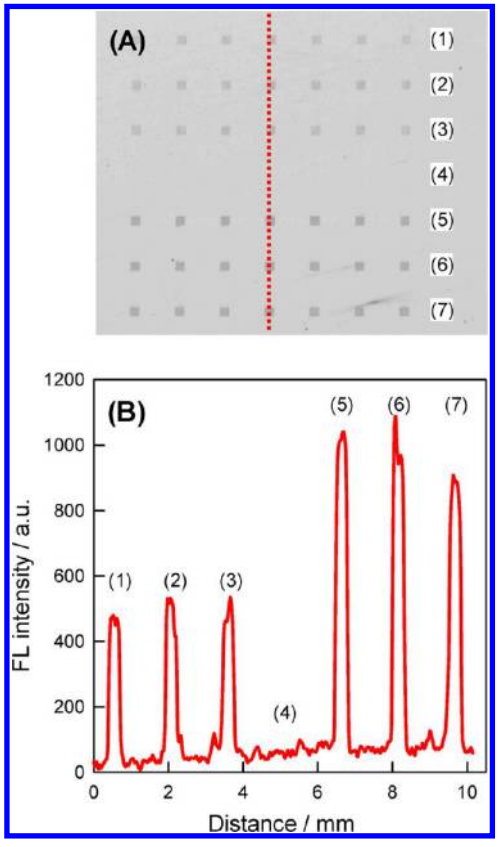

FIGURE 4. Hybridization experiments using microfluidic channels to deliver the same target sample (1.0 μM) on a preformed DNA probe line array at 40 °C for 5 min. (A) Fluorescence images with the probe numbers listed; (B) relative fluorescence intensities vs distance along the projection shown as dotted line in panel A. The oligonucleotide sequences are shown in Figure 3: lines 5−7 were obtained with complementary probes (probe I), line 4 with noncomplementary probes (probe III), and lines 1−3 with strands containing a single base-pair mismatch (probe II).

by other teams,[21−23,27−29] is applicable not only for making computer-readable bioassay discs (as described below) but also for fabricating other types of polymer-based microanalytical devices.[21−23,26]

Assay Signal Amplification and Digital Readout

Recent research to adapt computer optical drives as signal readout devices for disc-based bioassays has been focused on innovative hardware modifications, either by altering the optics of the laser pickup unit[30−33] or by acquiring analog signals from a photodiode detector.[34] Since Kido et al. proposed the concept in 2000,[35] software approaches have also been explored to analyze the digital signals obtained from a CD using optical drives directly.[36,37] Of particular interest, Jones employed CD drives as photonic signal processing devices (functioning as optical microscopes) to image stained bacterial cells physically adsorbed on a disc.[37] With the typical size of cells ranging from a few to tens of micrometers ($\gg\lambda/4$, λ = 780 nm for a CD drive), this is sufficient to disrupt the laser reading process.

The key to replace complex and expensive test equipment with ordinary computers for on-site chemical analysis or POC medical diagnosis is reading of biorecognition reactions on disc with standard (unmodified) optical drives.[38] We have therefore developed a digital readout protocol for the multiplex biomolecular screening based on the reading-error correction function embedded in standard audio CDs. To prepare the computer-readable bioassay discs, we integrated the UV/ozone surface treatment/amide coupling

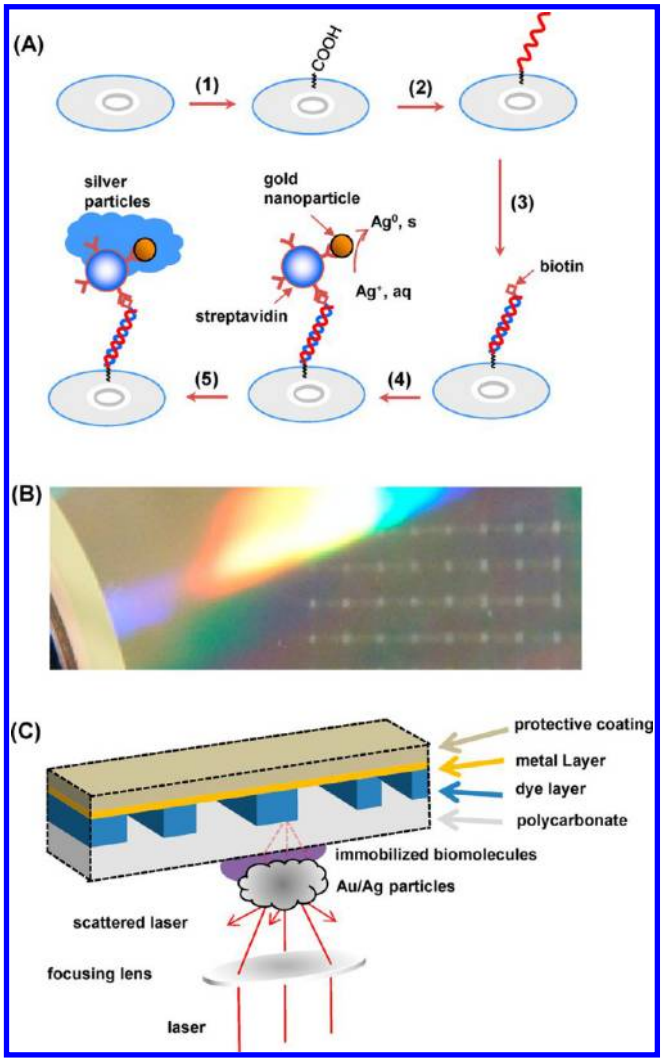

FIGURE 5. (A) Preparation of disc-based bioassay and signal amplification via gold/silver staining. (1) UV/ozone activation to generate carboxylic acid groups on CD; (2) immobilization of amino-tethered DNA probe strands via amide coupling; (3) hybridization with biotinylated DNA target strands; (4) binding of nanogold–streptavidin conjugates; (5) reductive precipitation of silver particles for signal enhancement. (B) Optical image of DNA microarray formed on a regular CD-R. (C) Digital reading of bioassay with CD drive: the biomolecule/Au/Ag particle conjugates block the reading laser and generate digital errors.

protocol (described above) and the gold nanoparticle-based autometallography method developed previously.[32,39–41]

As mentioned above, objects of ~200 nm size ($> \lambda/4$) are needed to induce significant disruption of the laser light reflection (to create "readable" digital signals) in a standard optical drive.[35,37] Biological macromolecules (typically a few nanometers) are too small to be detected; therefore the molecular binding signals must be amplified. Steps 3–5 of Figure 5A illustrate the procedure for the case of reading a DNA hybridization assay. Biotin-labeled target DNA is hybridized with the probe DNA immobilized on the disc (step 3) and subsequently treated with gold nanoparticle–streptavidin conjugate (step 4). Silver is then deposited on the gold "seed" (step 5) to increase the particle size from a few to several hundred nanometers. At saturated concentrations, the binding spots can be observed with the naked eye (Figure 5B).

Conceptually different from the colorimetric method for analyzing combinatorial DNA arrays,[42] we expect that the large silver particles associated with binding events would block the laser and cause significant scattering,[35,37] thereby producing reading errors detectable by the optical drive (Figure 5C). An optical drive is able to distinguish and correct these errors using a data-protection strategy (cross interleave Reed–Solomon code) in conjunction with the parity bits check protocol.[43] We have identified several free CD-quality diagnostic programs that have the ability to extract the error-statistic information in a CD/DVD drive and to generate a plot displaying the reading error level (the block error rate) as function of playtime.[44,45] Because the playtime of a digital audio file corresponds to a specific physical position on the CD, we can locate where the binding event occurred if it causes a significant disruption of the laser reading according to eq 1:

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

where r (mm) is the radius of the location (radial distance from the disc center) and t is the playtime (min). As shown in Figure 6A, the radius of the programmable area of a typical 700-MB CD-R that contains 79.7 min of audio data is 58 mm, and 25 mm is the radius of the center section.

The performance (lateral resolution and sensitivity) of this digital readout protocol was first evaluated by applying a series of color stains at different locations on the CD surface.[44] As shown in Figure 6B, these stains generate significant errors when read by a standard optical drive, in contrast to the minimal error level shown in the bottom panel. The same error distribution trends can be obtained from different CD-quality diagnostic programs and different optical drives, which confirms the reproducibility of the error reading method.[44,45]

With this digital readout approach, we initially examined the biotin–streptavidin binding assay; one of the most frequently studied biological interactions. Five binding strips were formed on the PC surface of an audio CD-R ; they were designed to test the binding of surface-bound biotin (prepared

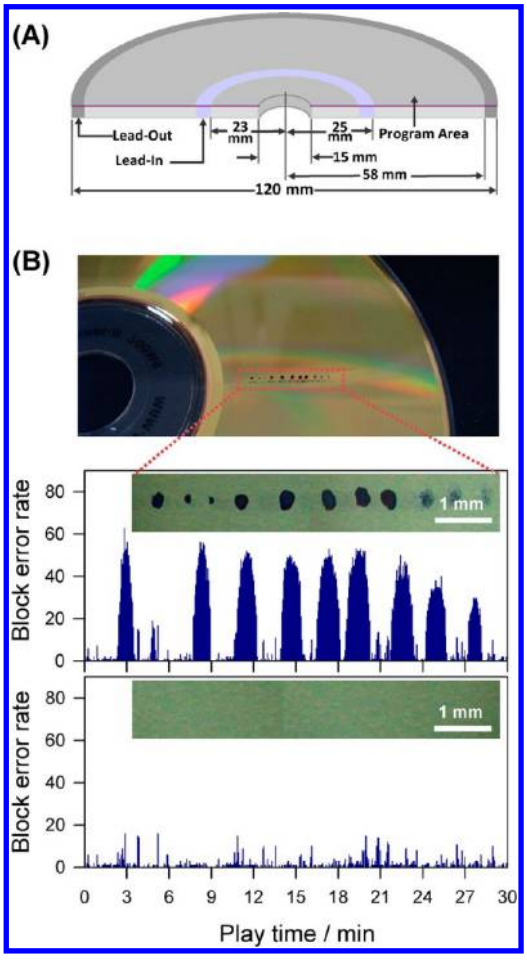

FIGURE 6. (A) Layout of a typical CD. (B) Optical image of a disc with color stains and the corresponding reading error (block error rates) vs play time (min). The bottom panel shows the background error rate distribution without color stains.

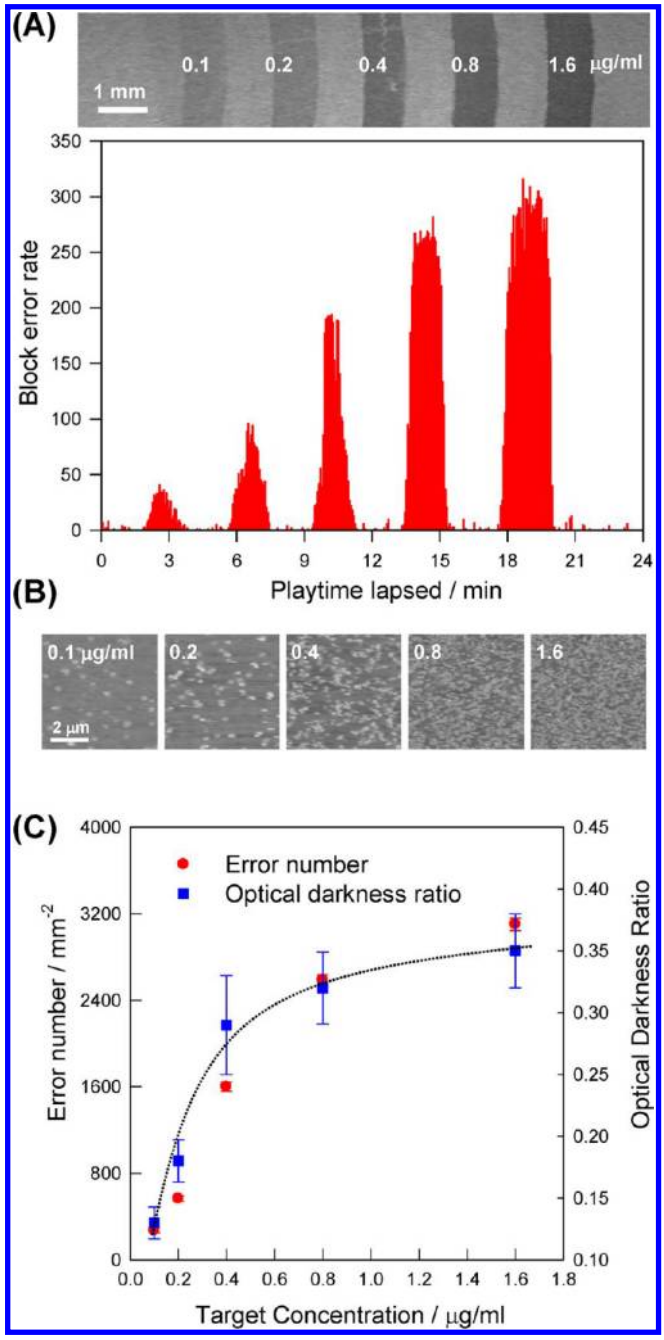

FIGURE 7. (A) Distribution of block error rates on a CD modified with biotin/streptavidin binding assay strips; the top inset is an optical image of the binding strips. (B) AFM images of the binding strips to show the size and density of silver particles on the surface. (C) Relative error level intensity and optical darkness ratio as function of the target concentration; the dashed line is to direct the eyes only.

by coupling biotinyl-3,6,9-trioxaundecanediamine to −COOH groups on an activated CD surface, Figure 7A) to five different concentrations of gold nanoparticle−streptavidin conjugates. 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 7A). Different from playing the audio tracks, the error correction reading takes less than 92 s for a 52× optical drive, during which no heating effects are observed (i.e., the disc or drive does not get hot). Atomic force microscopy (AFM) images (Figure 7B) revealed that they were composed of large-sized nanoparticles (90−300 nm) with different particle densities (increasing gradually with increasing streptavidin concentration).

The data plotted in Figure 7C 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 ODR value 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 is the value for the binding site, which is a function of

particle size and density.[41] 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. The consistency between the two reading results confirms the validity of our disc-based assay preparation and digital reading protocol.

Applications and Recent Developments

The successful quantitation of the biotin−streptavidin biorecognition reactions on a disc using a standard computer drive (with free CD quality control software) laid the foundation for studying more complex biorecognition reactions. To illustrate the application potential, herein we describe the results of analyzing DNA hybridization, antibody−antigen recognition,[44−46] and a DNAzyme assay for ppb-level Pb^{2+} detection.[47]

DNA Hybridization Assays. To test DNA hybridization (the nucleotide sequences are the same as those used in Figure 4), we prepared a line array on the PC surface by hybridizing probe I with increasing concentrations of biotinylated DNA (target) and subsequent treatment with a gold−streptavidin conjugate solution (Figure 6A). As shown in Figure 8A, the error level increased rapidly with the target concentration in the low concentration range and reached saturation levels at higher concentrations of the cDNA target strands. Without further optimization, we could detect the target sample at a concentration as low as 25 nM. Considering the small sample volume (2.0 μL), this is equivalent to 50 fmol of DNA molecules.

More importantly, we were able to detect single-nucleotide polymorphism (SNP), that is, a GG mismatch in the middle of a 22-mer oligonucleotide. Upon hybridization of the same target with complementary (PM), single-base-pair mismatched, and noncomplementary (NP) probe strands on the same array, the three types of strips showed marked differences in their ODR values and, upon playing, in their error levels. As shown in Figure 8B, strong error signals were produced only for the hybridization of complementary strands (lines 4 and 5, probe I + target); two weaker error peaks due to the DNA duplexes containing SNP (lines 1 and 2, probe II + target) were visible, while no hybridization with noncomplementary probe strands (line 3, probe III + target) was detected.

"ELISA" on a Disc. To further demonstrate the versatility of the CD-based biomolecular screening protocol, we

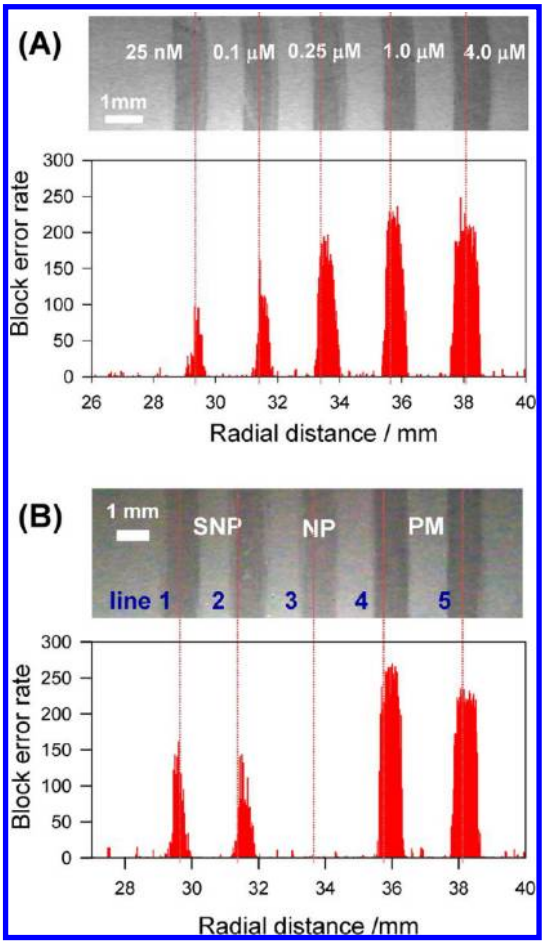

FIGURE 8. (A) Block error rate distribution of the DNA hybridization line array of complementary sequences (probe I and target I) and optical image of the binding strips. (B) Block error rate distribution of a CD modified with a DNA hybridization array prepared from three types of probes: single base-pair mismatched (SNP), noncomplementary (NP), and complementary strands (PM). The sequences of the DNA strands are listed in Figure 3.

examined the anti-human IgG/human IgG binding assay, a model system of medical immunoassays.[48] Conventional immunoassays (e.g., ELISA) are performed in a sandwich-format (lowering the background signal due to specific antigen recognition), particularly for rather large protein biomarkers.[48] As shown in Figure 9A, anti-IgG was first immobilized on the disc surface. After introducing IgG (the trial analyte), biotin-labeled anti-IgG was added to the system. The subsequent binding of nanogold−streptavidin conjugates and silver enhancement provided sufficient signal amplification to disrupt laser reading in an unmodified optical drive (Figure 9B). The detection limit is 25 ng/mL, while the responding range is over 2 orders of magnitude (i.e., up to 2.5 μg/mL).[46]

Morais et al. have recently developed multiplexed microimmunoassays on DVDs in an indirect, competitive

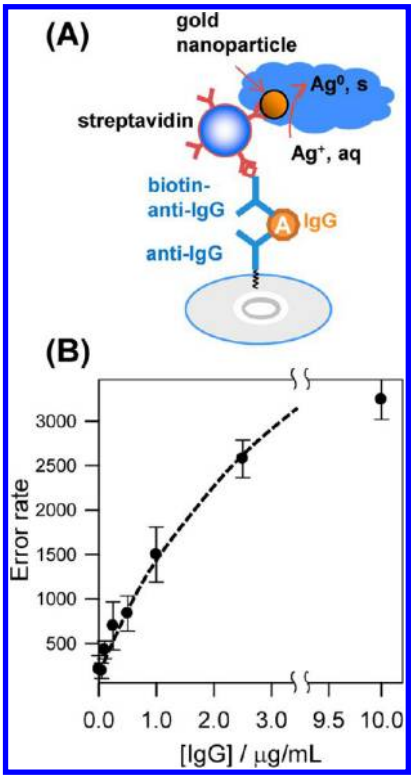

FIGURE 9. Quantitation of a sandwich-format human IgG binding assay on disc. (A) The construction of an anti-IgG/IgG/biotin−anti-IgG sandwich assay and the signal enhancement; (B) block error rate as function of the IgG concentration.

format:[49] five small molecular analytes of different chemical nature (pesticides and antibiotics) were quantified without sample treatment or preconcentration in less than 30 min with similar sensitivity and selectivity to the ELISA kits. Notably, they also compared the performance of acquiring analog signals[34] vs analyzing reading errors[44] and concluded that both are valid for multiplex assay detection with satisfactory limits of quantitation.

Lead Detection at Part-per-Billion Level. This disc-based detection method can also be applied to many other aspects of analytical science beyond medical diagnosis.[47,50−52] For example, Maquieria and co-workers have developed microsensor arrays to determine biotoxins (microcystins) in river water and a DNA microarray on disc to simultaneously detect traces of multiple food allergens.[50,51] We have recently designed a disc-based DNAzyme assay for the detection of Pb^{2+} at the part-per-billion (ppb) level.[47]

DNAzymes are specific DNA sequences that catalyze activities such as DNA/RNA ligation, cleavage, porphyrin metalation, thymine dimer photoreversion, and nucleopeptide formation. During the *in vitro* selection process,[53] a metal ion is typically used as cofactor for a specific DNAzyme sequence, and this feature can be utilized to design a sensor

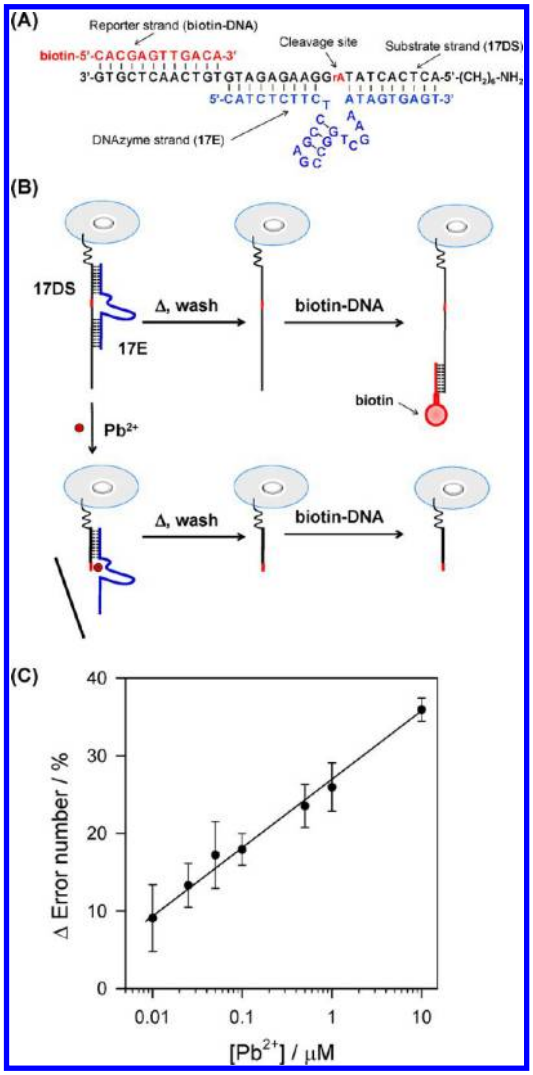

FIGURE 10. (A) Design of the Pb^{2+}-specific DNAzyme sensing construct and DNA strand sequences. (B) Surface immobilization and reaction of DNAzyme sensors in the presence/absence of Pb^{2+}; the binding of nanogold streptavidin and subsequent silver enhancement are shown in Figure 6A. (C) Dependence of the reading error level on the concentration of Pb^{2+}: semilog calibration curve in the range of 10 nM to 10 μM; the solid line is a linear fit, $\Delta E_r = 2.67 + 8.54 \log [Pb^{2+}]$, with $R^2 = 0.99$.

for that ion.[54] After the Pb^{2+}-specific DNAzyme sensing construct[54] (Figure 10A) has been immobilized on a CD-R surface, the Pb^{2+} concentration can be determined by the error diagnostic program (Figure 10B). The reading error increases linearly over 3 orders of magnitude of Pb^{2+} concentrations (from 10 nM to 10 μM, Figure 10C); the detection limit is 10 nM (equivalent to 2 ppb), which is adequate for environmental monitoring and food/water safety tests. The selectivity was confirmed by testing several other divalent cations (Zn^{2+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, and Hg^{2+}).[47] More importantly, this detection method offers a platform

technology for the fabrication of DNAzyme sensors to analyze other metal ions because DNAzymes specific for Hg^{2+}, Cu^{2+}, UO^{2+}, and Zn^{2+} have also been obtained by *in vitro* selection.[55]

Conclusions and Outlook

We have developed a digital readout methodology for screening biomolecular binding reactions on a disc using a standard computer drive. Different types of bioassays can be prepared in a line array format with a microfluidic approach on a photoactivated disc. 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.

As a breakthrough in advancing on-site chemical analysis and POC medical diagnosis,[56] this 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-correction function inherent in the standard digital data of audio files (recorded on the same disc) to detect the reading errors, which is different from the method proposed by La Clair et al. (in which such function was disabled and replaced by a unique data structure).[36] In principle, to read biomedical assays with an ordinary computer is now accessible to every user, and it is feasible to extend the reading mechanism to stand-alone CD/DVD players. (2) The surface chemistry developed herein involves simple and mild reactions that can be carried out by nonspecialists. (3) Because the measured error levels and the optical darkness ratios are consistent with each other over the entire dynamic range, they can be used for the construction of calibration curves to quantitate the analyte concentrations.

As mentioned above, the signal enhancement treatment by incorporating gold/silver nanoparticles enables a clear contrast between binding sites and background area which can be directly visualized (Figure 5B). Based on the optical darkness ratio analysis, we can obtain both qualitative and quantitative results by examining the optical image (Figure 7B); this means that any digital camera or scanners could accomplish data interrogation with simple object identification software. Camera phones have been recently adapted as integrated rapid-diagnostic-test readers for various immunoassay biochips;[57] it should be possible to read the bioDisc assays in the same manner. Besides the better sensitivity achievable with the error correction protocol, the chemistry to prepare robust and selective assays on plastic substrates and the flexibility of solution handling with disc spinning system are clear advantages of the CD-based detection methodology.[56]

In the past few years, we have witnessed significant progress in this exciting field, including the adaptation of DVDs for making multiplex microimmunoassays.[50,51] DVDs and Blu-ray discs are gradually replacing the conventional CD/CD-Rs on today's market. DVDs use a laser of 650 nm, and Blu-ray discs use a laser of 405 nm wavelength for retrieving data stored on the disc. The digital reading protocol is based on the basic error identification and correction algorithm for audio files;[44] the rapid upgrades of the recording media and optical drives do not affect the quantitative measurements but provide new opportunities for investigating multiwavelength operation for multiplex molecular analysis.[58] Particularly with shorter wavelengths, the laser beam is focused on the polycarbonate surface with a smaller size; thus the minimum particle size on the disc creating a reading error can be considerably reduced. Consequently, the error detection method would be more sensitive with a better lateral resolution, and its multiplex throughput is therefore increased as well. Nevertheless, the 405 nm excitation wavelength may lead to a higher probability of photodegradation when running bioDisc assays. The other advancement would be the handling of liquid samples; in conjunction with microfluidic technology, Imaad et al. have recently made significant progress in the construction of digital microfluidic CDs for counting polystyrene microparticles and living cells.[52]

We are unable to provide more details on the relevant research of many other colleagues because of space limitations; we hope all those who have contributed to making "bioDisc" exciting will accept a collective acknowledgment. This research has been financially supported by the Natural Science and Engineering Research Council (Canada), the Simon Fraser University Innovation Office, and the National Science Foundation of China. We thank Dr. Eberhard Kiehlmann for proofreading the manuscript.

BIOGRAPHICAL INFORMATION

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FOOTNOTES

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REFERENCES

1 (a) Soper, S. A.; Brown, K.; Ellington, A.; Frazier, B.; Garcia-Manero, G.; Gau, V.; Gutman, S. I.; Hayes, D. F.; Korte, B.; Landers, J. L.; Larson, D.; Ligler, F.; Majumdar, A.; Mascini, M.; Nolte, D.; Rosenzweig, Z.; Wang, J.; Wilson, D. Point-of-care biosensor systems for cancer diagnostics/prognostics. *Biosens. Bioelectron.* **2006**, *21*, 1932–1942. (b) Gubala, V.; Harris, L. F.; Ricco, A. J.; Tan, M. X.; Williams, D. E. Point of care diagnostics: Status and future. *Anal. Chem.* **2012**, *84*, 487–515.

2 Heller, A; Feldman, B. Electrochemical glucose sensors and their applications in diabetes management. *Chem. Rev.* **2008**, *108*, 2482–2505.

3 Manz, A.; Graber, N.; Widmer, H. M. Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sens. Actuators B* **1990**, *1*, 244–248.

4 (a) Telleman, P. In *Microsystem Engineering of Lab-on-a-chip Devices*, 2nd ed.; Geschke, O., Klank, H., Telleman, P., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008; Chapter 1, pp 1−7. (b) Kim, J. T.; Manz, A.; Arora, A.; Simone, S.; Saliebe-Beugelaar, S. B. Latest developments in micro total analysis systems. *Anal. Chem.* **2010**, *82*, 4830–4847.

5 (a) Gorkin, R.; Park, J.; Siegrist, J.; Amasia, M.; Lee, B. S.; Park, J.-M.; Kim, J.; Kim, H.; Madou, M.; Cho, Y.-K. Centrifugal microfluidics for biomedical applications. *Lab Chip* **2010**, *10*, 1758–1773. (b) Nolte, D. D. Review of centrifugal microfluidic and bio-optical disks. *Rev. Sci. Instrum.* **2009**, *80*, No. 101101. (c) http://www.gyros.com/, accessed on July 16, 2012.

6 (a) Yu, H.-Z. New chemistry on old CDs. *Chem. Commun.* **2004**, 2633–2636. (b) Ho, C.; Soolaman, D. M.; Yu, H.-Z. Analytical/materials chemistry on old CDs: Beyond self-assembly. *Can. J. Chem.* **2005**, *83*, 403–412.

7 Yu, H.-Z. Self-assembly on "recordable CDs". *Anal. Chem.* **2001**, *73*, 4743–4747.

8 (a) Angnes, L.; Richter, E. M.; Augelli, M. A.; Kume, G. H. Gold electrodes from recordable CDs. *Anal. Chem.* **2000**, *72*, 5503–5506. (b) Daniel, D.; Gutz, I. G. R. Quick production of gold electrode sets or arrays and of microfluidic flow cells based on heat transfer of laser printed toner masks onto compact discs. *Electrochem. Commun.* **2003**, *5*, 782–786.

9 Westbroek, P.; De Strycker, J.; Dubruel, P.; Temmerman, E.; Schacht, E. H. Flow-through cell for on-line amperometric determination of Ce(IV) during polymerization reactions. *Anal. Chem.* **2002**, *74*, 915–920.

10 (a) Singh, B.; Hillier, A. C. Surface plasmon resonance imaging of biomolecular interactions on a grating-based sensor array. *Anal. Chem.* **2006**, *78*, 2009–2018. (b) Gopinath, S. C. B.; Awazu, K.; Fons, P.; Tominaga, J.; Kumar, P. K. R. A sensitive multilayered structure suitable for biosensing on the BioDVD platform. *Anal. Chem.* **2009**, *81*, 4963–4970.

11 Tang, C. K.; Vaze, A.; Rusling, J. F. Fabrication of immunosensor microwell arrays from gold compact discs for detection of cancer biomarker proteins. *Lab Chip* **2012**, *12*, 281–286.

12 Kirpatrick, D. C.; Antwi, C.; Martin, R. S. Use of recordable compact discs to fabricate electrodes for microchip based analysis systems. *Anal. Methods* **2010**, *2*, 811–816.

13 (a) Helt, J. M.; Drain, C. M.; Batteas, J. D. A benchtop method for the fabrication and patterning of nanoscale structures on polymers. *J. Am. Chem. Soc.* **2004**, *126*, 628–634. (b) Helt, J. M.; Drain, C. M.; Bazzan, G. Stamping patterns of insulated gold nanowires with self-organized ultrathin polymer films. *J. Am. Chem. Soc.* **2006**, *128*, 9371–9377.

14 Meenakshi, V.; Babayan, Y.; Odom, T. W. Benchtop nanoscale patterning using soft lithography. *J. Chem. Educ.* **2007**, *84*, 1795–1798.

15 Hazarika, P.; Chowdhury, D.; Chattopadhyay, A. Fabrication of submicron scale patterned plastic thin film fluidic devices with controllable thickness. *Lab Chip* **2003**, *3*, 128–131.

16 Tabuchi, M.; Kobayashi, K.; Fujimoto, M.; Baba, Y. Bio-sensing on a chip with compact discs and nanofibers. *Lab Chip* **2005**, *5*, 1412–1415.

17 Yu, H.-Z.; Rowe, A. W.; Waugh, D. M. Templated electrochemical deposition of zirconia thin films on "Recordable CDs". *Anal. Chem.* **2002**, *74*, 5742–5747.

18 Cho, H.; Parameswaran, M.; Yu, H. Z. Fabrication of microsensors using unmodified office inkjet printers. *Sens. Actuators B* **2007**, *123*, 749–756.

19 Li, Y.; Li, P. C. H.; Parameswaran, M.; Yu, H.-Z. Inkjet printed electrode arrays for potential modulation of DNA self-assembled monolayers on gold. *Anal. Chem.* **2008**, *80*, 8814–8821.

20 Li, Y.; Wang, Z.; Ou, L. M. L.; Yu, H.-Z. DNA detection on plastic: Surface activation protocol to convert polycarbonate substrates to biochip platforms. *Anal. Chem.* **2007**, *79*, 426–433.

21 McCarley, R. L.; Vaidya, B.; Wei, S.; Smith, A. F.; Patel, A. B.; Feng, J.; Murphy, M. C.; Soper, S. A. Resist-free patterning of surface architectures in polymer-based microanalytical devices. *J. Am. Chem. Soc.* **2005**, *127*, 842–843.

22 Liu, Y. J.; Ganser, D.; Schneider, A.; Liu, R.; Grodzinski, P.; Kroutchinina, N. Microfabricated polycarbonate CE devices for DNA analysis. *Anal. Chem.* **2001**, *73*, 4196–4201.

23 Welle, A.; Gottwald, E. UV-based patterning of polymeric substrates for cell culture applications. *Biomed. Microdevices* **2002**, *4*, 33–41.

24 Delamarche, E.; Bernard, A.; Schmid, H.; Michel, B.; Biebuyck, H. Patterned delivery of immunoglobulins to surfaces using microfluidic networks. *Science* **1997**, *276*, 779–781.

25 Situma, C.; Wang, Y.; Hupert, M.; Barany, F.; McCarley, R. L.; Soper, S. A. Fabrication of DNA microarrays onto poly(methyl methacrylate) with ultraviolet patterning and microfluidics for the detection of low-abundant point mutations. *Anal. Biochem.* **2005**, *340*, 123–135.

26 Peng, X. Y.; Li, P. C. H.; Yu, H.-Z.; Parameswaran, M.; Chou, W. L. Spiral microchannels on a CD for DNA hybridizations. *Sens. Actuators B* **2007**, *128*, 64–69.

27 Banuls, M. J.; Gonzalez-Pedro, V.; Puchades, R.; Maquieira, A. PMMA isocyanate-modified digital discs as a support for oligonucleotide-based asssays. *Bioconjugate Chem.* **2007**, *18*, 1408–1414.

28 Tamarit-Lopez, J.; Morais, S.; Banuls, M.-J.; Puchades, R.; Maquieira, A. Development of hapten-linked microimmunoassays on polycarbonate discs. *Anal. Chem.* **2010**, *82*, 1954–1963.

29 Tamarit-Lopez, J.; Morais, S.; Uchades, R.; Maquieira, A. Oxygen plasma treated interactive polycarbonate DNA microarraying platform. *Bioconjugate Chem.* **2011**, *22*, 2573–2580.

30 Alexandre, I.; Houbion, Y.; Collet, J.; Hamels, S.; Demarteau, J.; Gala, J.-L.; Remacle, J. Compact disc with both numeric and genomic information as DNA microarray platform. *BioTechniques* **2002**, *33*, 435–439.

31 Barathur, R.; Bookout, J.; Sreevatsan, S.; Gordon, J.; Werner, M.; Thor, G.; Worthington, M. New disc-based technologies for diagnostic and research applications. *Psychiatr. Genet.* **2002**, *12*, 193–206.

32 Lange, S. A.; Roth, G.; Wittemann, S.; Lacoste, T.; Vetter, A.; Grässle, J.; Kopta, S.; Kolleck, M.; Breitinger, B.; Wick, M.; Hörber, J. K. H.; Dübel, S.; Bernard, A. Measuring biomolecular binding events with a compact disc player device. *Angew. Chem., Int. Ed.* **2006**, *45*, 270–273.

33 Morais, S.; Carrascosa, J.; Mira, D.; Puchades, R.; Maquieira, A. Microimmunoanalysis on standard compact discs to determine low abundant compounds. *Anal. Chem.* **2007**, *79*, 7628–7635.

34 Potyrailo, R. A.; Morris, W. G.; Leach, A. M.; Sivavec, T. M.; Wisnudel, M. B.; Boyette, S. Analog signal acquisition from computer optical disk drives for quantitative chemical sensing. *Anal. Chem.* **2006**, *78*, 5893–5899.

35 Kido, H.; Maquieira, A.; Hammock, B. D. Disc-based immunoassay microarrays. *Anal. Chim. Acta* **2000**, *411*, 1–11.

36 La Clair, J. J.; Burkart, M. D. Molecular screening on a compact disc. *Org. Biomol. Chem.* **2003**, *1*, 3244–3249.

37 Jones, C. L. Cryptographic hash functions and CD-based optical biosensors. *Problem. Nonlinear Anal. Eng. Syst.* **2005**, *11*, 17–36.

38 Ligler, F. S.; Erikson, J. S. Diagnosis on disc. *Nature* **2006**, *440*, 159–160.

39 Alexandre, I.; Hamels, S.; Dufour, D.; Collet, J.; Zammatteo, N.; De Longueville, F.; Gala, J.-L.; Remacle, J. Colorimetric silver detection of DNA microarrays. *Anal. Biochem.* **2001**, *295*, 1–8.

40 Liang, R. Q.; Tan, C. Y.; Ruan, K. C. Colorimetric detection of protein microarrays based on nanogold probe coupled with silver enhancement. *J. Immunol. Methods* **2004**, *285*, 157–163.

41 Gupta, S.; Huda, S.; Kipatrick, P. K.; Velev, O. D. Characterization and optimization of gold nanoparticle-based silver-enhanced immunoassays. *Anal. Chem.* **2007**, *79*, 3810–3820.

42 Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. Scanometric DNA array detection with nanoparticle probes. *Science* **2000**, *289*, 1757–1760.

43 Lane, P. M.; Dommelen, R. V.; Cada, M. Compact disc players in the laboratory: Experiments in optical storage, error correction, and optical fiber communication. *IEEE Trans. Educ.* **2001**, *44*, 47–60.

44 Li, Y.; Ou, L. M. L.; Yu, H.-Z. Digitized molecular diagnostics: Reading disc-based bioassays with standard computer drives. *Anal. Chem.* **2008**, *80*, 8216–8223.

45 Pallapa, M.; Ou, L. M. L.; Parameswaran, M.; Yu, H.-Z. Software-based quantitation of bioassays-on-CD. *Sens. Actuators B* **2010**, *148*, 620–623.

46 Ou, L. M. L., Reading disc-based bioassays for protein biomarkers with standard computer drives, M.Sc. thesis, Simon Fraser University, Burnaby, BC, Canada, 2011.

47 Wang, H.; Ou, L. M. L.; Suo, Y.; Yu, H.-Z. Computer-readable DNAzyme assay on disc for ppb-level lead detection. *Anal. Chem.* **2011**, *83*, 1557–1563.

48 Lequin, R. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin. Chem.* **2005**, *51*, 2415–2418 and references cited therein.

49 Morais, S.; Tortajada-Genaro, L. A.; Arnandis-Chover, T.; Puchades, R.; Maquieira, A. Multiplexed microimmunoassays on a digital versatile disk. *Anal. Chem.* **2009**, *81*, 5646–5654.

50 Tortajada-Genaro, L. A.; Santiago-Felipe, S.; Morais, S.; Gabaldon, J. A.; Puchades, R.; Maquieira, A. Multiplex DNA detection of food allergens on a digital versatile disk. *J. Agric. Food Chem.* **2012**, *60*, 36–43.

51 Morais, S.; Tamarit-Lopez, J.; Puchades, R.; Maquieira, A. Determination of microcystins in river water using microsensor arrays on disk. *Environ. Sci. Technol.* **2010**, *44*, 9024–9029.

52 Imaad, S. M.; Lord, N.; Kulsharova, G.; Liu, G. L. Microparticle and cell counting with digital microfluidic compact disc using standard CD drive. *Lab Chip* **2011**, *11*, 1448–1456.

53 (a) Breaker, R. R. In vitro selection of catalytic polynucleotides. *Chem. Rev.* **1997**, *97*, 371–390. (b) Lorsch, J. R.; Szostak, J. W. Chance and necessity in the selection of nucleic acid catalysts. *Acc. Chem. Res.* **1996**, *29*, 103–110.

54 Liu, J.; Lu, Y. A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643.

55 (a) Liu, J.; Cao, Z. H.; Lu, Y. Functional nucleic acid sensors. *Chem. Rev.* **2009**, *109*, 1948–1998. (b) Lu, Y.; Liu, J.; Li, J.; Bruesehoff, P. J.; Pavot, C. M. B.; Brown, A. K. New highly sensitive and selective catalytic DNA biosensors for metal ions. *Biosens. Bioelectron.* **2003**, *18*, 529–540.

56 Erickson, J. S.; Ligler, F. S. Home diagnostics to music. *Nature* **2008**, *456*, 178–179.

57 (a) Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas, S. W., III; Sindi, H.; Whitesides, G. M. Simple telemedicine for Developing Regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem.* **2008**, *80*, 3699–3707. (b) Mudanyali, O.; Dimitrov, S.; Sikora, U.; Padmanabhan, S.; Navruza, I.; Ozcan, A. Integrated rapid-diagnostic-test reader platform on a cellphone. *Lab Chip* **2012**, *12*, 2678–2686.

58 Potyrailo, R. A.; Morrisa, W. G.; Wroczynskia, R.; Hassiba, R.; Millera, P.; Dworkena, B.; Leacha, A. M.; Boyetteb, S.; Xiao, C. Multi-wavelength operation of optical disk drives for chemical and biological analysis. *Sens. Actuators B* **2009**, *136*, 203–208.