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Digitized Molecular Detection on Off-the-shelf Blu-ray Discs: Upgraded Resolution and Enhanced Sensitivity

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Abstract

Beyond the intrinsic capability of storing and archiving high-definition movies and games, off-the-shelf Blu-ray discs have been adopted for the preparation of molecular binding assays, which are subsequently read and quantitated with a standard computer drive in conjunction with the disc-quality check program. The performance of this digitized molecular detection system has been examined first with an artificial "ink assay" (an array of microsize ink dots) to define the quantitation capability and the lateral resolution; the conventional biotin-streptavidin binding assay was then tested with the Blu-ray detection platform, and the results compared with that obtained on a DVD. The upgraded lateral resolution (<100 μm) and enhanced assay performance (linear response up to 0.4 μg/mL and LOD estimated to be < 0.1 μg/mL for the trial biotin-streptavidin system augments its potential to be adapted as a cost-effective and quantitative diagnostic tool for on-site analysis and point-of-care medical diagnosis at trace amounts.

Key words: Blu-ray disc, Optical drive, Error correction, Molecular detection, Bioassay

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1. INTRODUCTION

With the goal of adapting computer drives as precise signal-reading devices or modifying disc media as substrate materials for biochip fabrication, optical disc technology-based molecular detection systems promise to be a new generation of analytical tools for biomedical diagnosis and environmental monitoring [1-2]. In the past two decades, one of the key advances in this field was the development of integrated optical detection/scanning system from conventional computer drives by either retrieving analog signal directly from the circuit [3], or incorporating an additional detector to the existing laser pick-up unit [4-6]. In both cases, the data writing/reading function of the drive was disabled or altered, and the data storage capability of the disc was not utilized. We have developed a software approach for molecular detection on off-the-shelf discs with unmodified computer drives [7], and demonstrated its applications for ultrasensitive heavy metal detection in water [8]. In the meantime, Maquieira and co-workers have compared the software approach with their hardware modification method [9]; Imaad *et al.* have pioneered the integration of digital reading with microfluidic compact discs for cell counting [10]. Recently, we upgraded the methodology to work with digital versatile discs (DVDs) [11], and carefully compared the performance of different diagnostic software programs for reading DVD-based bioassays [12].

With the ever increasing data storage requirements (particularly high-definition movies and gams), optical disc technology has evolved rapidly from DVD (4.7 GB) to Blu-ray disc (BD, 25 GB per layer). BD technology utilizes a laser of shorter wavelength (405 nm) and an objective lens with larger numerical aperture (NA = 0.85) in comparison with DVD (λ = 650 nm, NA = 0.6), which essentially decreases the diameter of the focused laser spot from 1.32 μm (DVD) to 0.58 μm (BD) [13], and enables the recording of high density pits on the disc (320 nm track pitch with a minimum of 150 nm pit length). The nanostructured BD media have recently found unique applications beyond their intrinsic capability of storing digital data; Huang and co-workers “repurposed” Blu-ray movie discs as quasi-random nanoimprinting templates to prepare a patterned active layer on the metal electrode of polymer solar cells, leading to high absorption and power conversion efficiencies [14]. Nieuwoudt *et al.* sputtered thin gold films onto Blu-ray disc substrates to create cost effective

sensors for surface enhanced Raman spectroscopy [15]. Maquieira and co-workers have prepared high density microarrays on Blu-ray discs for massive DNA/protein screening; in their work the attenuated analog signals were directly acquired from the photodiode in a Blu-ray drive and processed with custom Biodisk software [16]. Hansen and co-workers reported the first implementation of a Blu-ray optical pick up unit (OPU) for the high-performance, low-cost readout of a homogeneous assay in a multi-chamber microfluidic disc [17]. Independently, Tian *et al.* have reported Blu-ray optomagnetic measurements of a competitive immunoassay for Salmonella [18].

Conceptually different from the pioneering studies of adapting modified Blu-ray drives as powerful analytical devices described above [16-18], we present herein our software approach to adopt off-the-shelf Blu-ray discs (BDs) to prepare bioassays for reading with standard computer drives or disc players. By testing both ink-dot arrays and a standard biotin-streptavidin binding assay, we have examined the performance of this digitized molecular detection platform, including different modes of error detection, lateral resolution, and the assay. The direct comparison with our established DVD assay system reported previously [11-12] will also be presented.

2. EXPERIMENTAL

2.1. Materials and Reagents

Blank BD-Rs (Verbatim Co.) and DVD-Rs (Sony Inc.) were obtained locally. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino)ethanesulfonic acid (MES), Tween 20 and gelatin were purchased from Sigma-Aldrich. Sodium chloride, sodium phosphate dibasic, sodium dihydrogen phosphate and bovine serum albumin (BSA) were purchased from Aladdin; sodium azide from Solarbio, and EZ-link@Amine-PEG2-biotin from Thermo Scientific. The nanogold–streptavidin conjugate and LI silver enhancement kit were purchased from Nanoprobes, Inc. Deionized water (>18.2 MΩ·cm) to prepare buffers and other solutions were produced from a Barnstead UF/UV compact water system (Thermo Fisher Scientific Inc.).

2.2. Preparation of Ink-Dot arrays on disc

High-definition video files were recorded on a blank BD-R using Power2GO software (V6, Cyberlink). The large size ink-dot arrays (0.2 ~ 1.5 mm in diameter) were prepared using a Pilot metallic marker (extra-fine tip, silver). The micro-size ink dots on BD/DVD were prepared by using a BioJet Ultra dispenser (AD2030, BioDot Inc.).

2.3. BD surface activation and biotin-streptavidin assay preparation.

A specially designed polydimethylsiloxane (PDMS) plate with three sets of embedded microchannels (0.5 mm × 12 mm × 50 μm, arc shaped) was used for sample delivery and channel washing. The BD-R was first treated with 0.1 M NaOH at 55 ± 1 °C for 90 min, rinsed with deionized water and dried in a stream of N_2. The disc was subsequently activated with EDC (100 mM) and NHS (25 mM) in a MES buffer solution (pH 5.8) for 4 h. Biotin solution in the MES buffer was then delivered to the reaction zone by using the PDMS plate. After incubation for 8 h (in a humidity chamber) at room temperature it was blocked with 20 mM phosphate buffer at pH 7.4 (containing 150 mM NaCl, 0.8% BSA, 0.1% gelatin, 0.05% Tween 20, and 0.05%NaN_3) for 2 h, followed by adding different concentrations of nanogold–streptavidin conjugate solutions in 20 mM phosphate buffer (pH 7.4: 50 mM NaCl, 0.1% BSA). After the binding reaction, the disc was incubated at room temperature for one hour. Finally it was subjected to silver staining treatment for 5 min with a freshly made solution (containing 48 mM silver nitrate and 182 mM hydroquinone).

2.4. Digitized assay reading and data analysis

A standard Plextor external Blu-ray drive (PX-LB950UE) in conjunction with the error correction software program PlexUtilities (v 1.3.3, downloaded from http://plextoramericas.com) was used to read the assay discs. For error tests, the program controls the Blu-ray drive to run at the 8× speed. The raw data was exported to either Microsoft Excel or SigmaPlot for further analysis (to integrate the error numbers and determine the error densities).

Optical images of the assay discs were taken with a Nikon D5300 DSLR camera equipped with a macro lens and then analyzed with Adobe Photoshop to determine the grayscale intensities (luminosity). The exact sizes of the ink-dots were determined from the 40× enlarged images

captured with an optical microscope (BA310Met, Motic China Group Co.). A FEI NanoNova 430 scanning electron microscope was employed to obtain high resolution images of the binding assays on BD-R/DVD-R.

3. RESULTS AND DISCUSSION

3.1. Blu-ray technology-based digital assay reading protocol: quantitation and lateral resolution

The data stored on a BD are organized by frames: each frame contains 2052 bytes of data in total, consisting of 2048 bytes of user data and 4 bytes of error detection code. As shown in Fig. 1, a logical Error-Correction Block (ECC Block) in a standard BD has a total of 64 kilobytes of user data (432 rows×152 columns; 32 data frames) with 64 rows of parity symbols and 4 equally spaced picket columns [13]. Specifically the user data are protected by the Long Distance Code (LDC) that has 304 code words with 216 bytes of data and 32 bytes of parity symbols. These code words are interleaved 2×2 in the vertical direction such that a block of 152 bytes×496 bytes is formed. The left-most picket column is formed by the sync patterns at the start of each row. The other three pickets are protected by the Burst-Indicating Subcode (BIS) that has 24 code words with 30 bytes of address information and 32 bytes of parity symbols. The BIS code words are interleaved into three columns of 496 bytes each. For standard BD drives, a logical ECC block is the basic unit to test the disc quality by counting the number of parity errors on LDC code words (LDC error) or the BIS code words (BIS error). In comparison, the DVD data system does not contain picket columns [11-12], for which the error correction capability is not as powerful as the Blu-ray technology.

Based on the error detection principle discussed above [13,19], we carried out initial tests with the two error detection modes by using an “artificial” array, i.e., a set of 10 ink dots of different sizes deposited on a BD-R along the radial direction (Fig. 2a). In both LDC and BIS modes, we observed 10 distinct peaks in the error histograms exported from the PlexUtilities program. These peaks are of different sizes and heights, which are apparently in good correlation with the dimensions of the ink dots (*vide infra*). In both plots, the background noise is low, but the error counts in LDC mode are about 50 times higher than that in BIS, which can be explained in terms of the differences in the data

sets protected by them. In Fig. 2(c) slightly higher background signals were observed, indicating that BIS is more sensitive to the errors created on the disc from writing and storing. As shown in the two error histograms (Fig. 2b, c), the x-axis was plotted as the logical position (GB); this is corresponding to the data location of pre-recorded high-definition video file. However, for the multiple assay tests, the physical position of the binding site is more important, as it can be correlated with the sample identity easily. For a commercial 25 GB BD-R, the actual capacity is 23.31 GB and the data zone is between 24 mm and 58 mm in terms of the radius. Based on the direct proportion between recordable capacity and recording area, we can establish a relationship between the logical position and radial distance (from the center of the disc) of each ink dot, which is described by the following equation:

$$r = \sqrt{\frac{x}{23.31} \times (58^2 - 24^2) + 24^2} \qquad (1)$$

where x is the logical position (GB), and r is the radial distance (mm). In Fig. 3a, the solid line is plotted according to eqn.1, and the solid circles represent measured values. It is clear that eqn. (1) indeed provides a perfect fit to the experimental data, confirming the direct correlation between physical location and logical position of an assay site. The other aspect of the ink assay we have examined is the dependence of the error numbers on the size of the ink dots. As mentioned above, these ink dots are of different sizes, and their areas were determined accurately from enlarged photos captured with an optical microscope. We have shown that both BIS and LDC counts are proportional to the area of the ink dot (Fig. 3b,c); the R^2 values for the best linear fits are 0.991 and 0.994, respectively. Slight deviations have been observed for the larger dots, which may be due to the uneven coating of the ink when the dots become big (> 0.5 mm).

High throughput is one of the preferred aspects for assay design, as it means that more samples/standards can be tested at once. The smallest dot we have tested was around 200 μm in Fig. 2a, which provided a distinct peak in both error plots. In order to test the lateral resolution of the BD assay method, we have prepared a set of microsize dots (60 to 80 μm in diameter) with a BioJet Ultra dispenser on a BD; for comparison we also prepared a DVD-R with a set of larger dots (from 70 to 120 μm). As shown in Fig. 4a, five peaks were detected by the BD system; no obvious error peaks appeared except for random background errors for the DVD system (Fig. 4b). The measured

full width at half maximum (FWHM) of the highlighted peak is 74 ± 5 μm (right inset of Fig. 4a), which matches the dimension determined using an optical microscope (76 ± 4 μm). The DVD system was not able to resolve the "peak" corresponding to the largest ink dot (114 ± 4 μm), although scattering error signals are present (right inset of Fig. 4b). The lateral resolution of the error reading is not simply determined by the diameter of the focused laser spot, rather it is dictated the laser spot size on the disc surface. Because the dye recording layer is 0.1 mm away from the surface (0.6 mm for DVD), the laser spot size on the surface is as small as 138 μm in diameter [13]. On a DVD surface, the laser spot is substantially bigger (520 μm), for which small ink dots (~100 μm) cannot be resolved.

3.2. Quantitative analysis of biotin-streptavidin binding assay

With the success of reading and "quantifying" artificial assays on a BD (ink dot arrays), we went ahead to explore the feasibility of reading a trial bioassay (biotin-streptavidin binding) created on off-the-shelf BD-Rs. Because the association between biotin and streptavidin is one of the strongest non-covalent interactions (K_d = ~10^{-14} M), it is often used as a model system in testing biochemical assays [7, 20]. As shown in Fig. 5a, we have prepared a biotin-streptavidin binding assay on BD with 7 different concentrations of the analyte (three replicates each) with the aid of a PDMS microfluidic channel plate. In both LDC and BIS mode, the error histogram showed unequivocal responses when the concentration of the analyte (streptavidin) increased (i.e., the higher the concentration, the stronger the observed error peaks). Two observations should be noted: (1) for the three independent repeats in each concentration the signals are fairly close to each other; (2) for high concentrations (0.6-0.8 μg/mL) the LDC signals are saturated. Although the latter can be considered as an indication of higher sensitivity of using LDC errors for quantitation, it is not practical in the present system. Subsequently we have plotted the BIS error density, i.e., the number of BIS errors per unit assay area, as a function of the analyte concentration (Fig. 6). It is clear that the assay signal (BIS density) rises proportionally at low concentrations (< 0.4 μg/mL), but no longer shows any increases above 0.6 μg/mL. For a direct comparison we also plotted the assay results obtained on a DVD [11]; beyond the different error reading principle (BIS vs. PIF, Parity

Inner Failure) the signal rises much slower and the signal does not reach a plateau until 1.0 μg/mL of the analyte being added. In the DVD system, an PIF is reported when more than five bytes are in error in a row within an ECC block [11]. For an accurate determination of the limit of detection (LOD), we would need to test more samples at the low concentrations; nevertheless, it can be estimated based on the 3σ rule from Fig. 6 that the LOD for the BD system is certainly below 0.1μg/mL (as the experimental uncertainties are fairly small); this is significantly better than that of the DVD system (0.2 to 0.4 μg/mL).

The main factor contributing to the improved detection sensitivity is the upgraded error detection and correction principle as discussed above. The data density on a BD is about five times higher than that on a DVD; the adoption of Picket code significantly improved the error correction ability in a BD system. Particularly the redundant check data are placed at the column (i.e., vertical) direction, and the BIS code assures the address and control information can be accurately read [19]. We should also consider the differences in the surface activation and coupling steps; BDs adopt a new type of hard coating (e.g., a (meth) acrylate based lactone material) instead of polycarbonate used for both CDs and DVDs [21]. It was discovered that a base-hydrolysis would make the surface more hydrophilic, and that the coupling of amine-PEG2-biotin upon EDC/NHS activation is straightforward [7,11].

To further understand the different assay performance and to validate the Blu-ray digital reading system we also analyzed the optical property and microscopic morphology changes in the assay strips (Fig. 5a). Because of the dark background of BD-Rs, the standard ODR (optical darkness ratio,) analysis [7-8,22] is no longer applicable; instead we have obtained the luminosity values of the binding strips and plotted the average against the analyte concentration (Fig. 7a). It is intriguing that direct reading of the luminosity (the brightness in a grayscale picture, with a value from 0 to 255) does provide a quantitative measure of the assay results. As of relatively large experimental uncertainties (shown as bigger error bars) and the high background signal, it is not as accurate as to obtain the BIS densities for quantitation. Nevertheless, the correlation of optical reading signal (luminosity) and BIS density showed good linearity for concentrations above 0.1 μg/mL. The scanning electron microscope (SEM) images showed significant variations for the

biotin-streptavidin binding assays (upon silver enhancement) prepared on a BD vs. a DVD (Fig. 8). For the same analyte concentration, the surface coverage of the silver particles is much higher; at a concentration of 0.8 μg/mL, the BD surface is mostly covered with a film of silver particles, while for the DVD assay, bare areas are still evident. The other clear difference is the shape and size of the individual silver particles; on a DVD surface they are much bigger than on a BD surface, which may be an indication of the different densities of nucleation sites. This is, eventually, dictated by the surface aviation step (i.e., density and distribution of reactive groups generated on the surface). Further investigation of the BD surface chemistry including potential interference of non-specific adsorption for the trial biotin-streptavidin assay as well as other practical bioassays is currently underway in our laboratory, and is beyond the scope of the present work.

4. CONCLUSIONS

The Blu-ray technology-based molecular detection system showed unpreceded lateral resolution and sensitivity with respect to previously developed methodologies (the DVD system in particular). With the help of ink dot arrays at the microscale, the ultimate resolution of the Blu-ray system was confirmed to be smaller than the laser spot on the disc surface (138 μm), i.e., ink dots in the range of 50-70 μm can be easily resolved. The trial biotin-streptavidin binding assay tested at limited concentrations on a BD showed much improved performance (with a linear response up to 0.4 μg/mL and the estimated LOD below 0.1 μg/mL) in comparison with those on a DVD (LOD: 0.2 μg/mL); this is due to the upgraded error detection and correction mechanism, and the newly developed surface chemistry for making high-density assay sites. Extended studies of the improvement of reading time and the test of real-world samples are certainly warranted.

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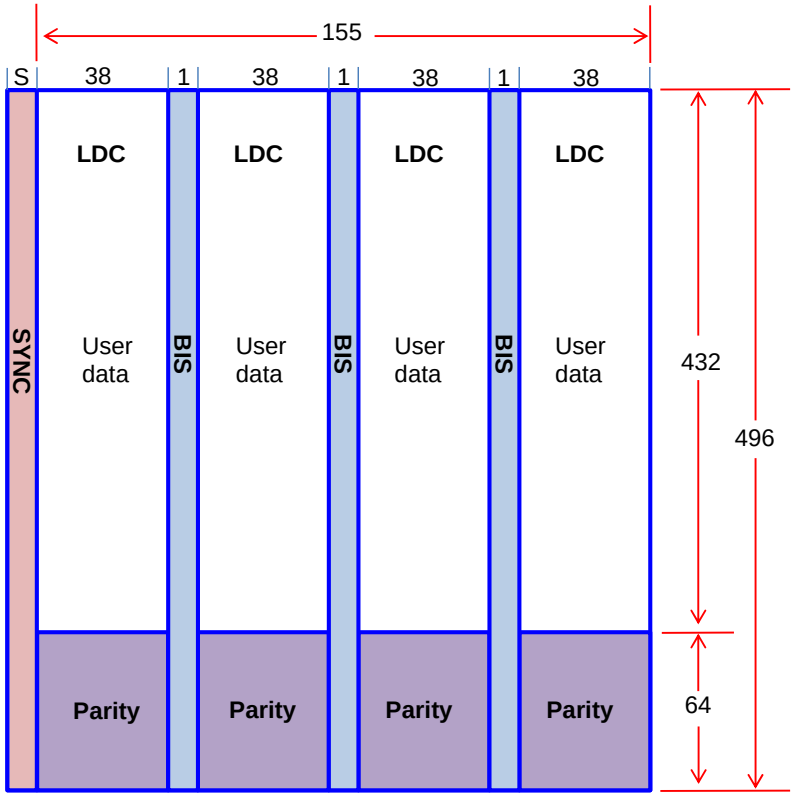

Fig. 1. Schematic representation of the error correction code (ECC) block in a Blu-ray disc.

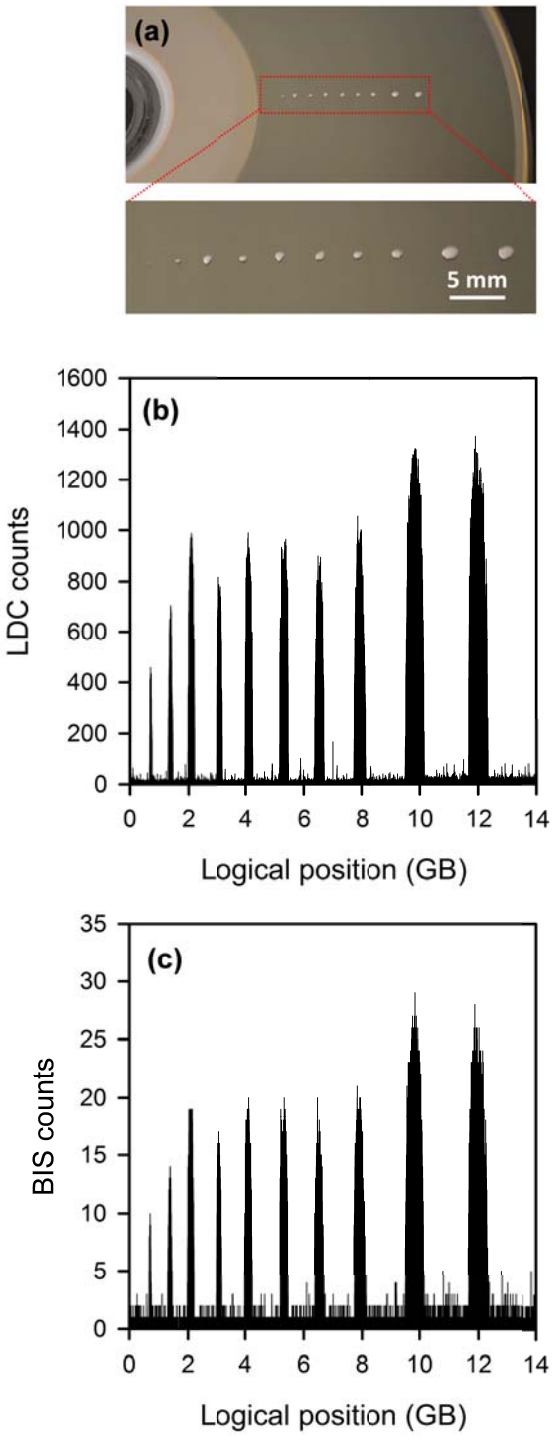

Fig. 2. An ink-dot array prepared on a Blu-ray disc (a) and the corresponding error histograms produced in Long Distance Code, LDC (b) and Burst indicator Subcode, BIS (c) mode, respectively.

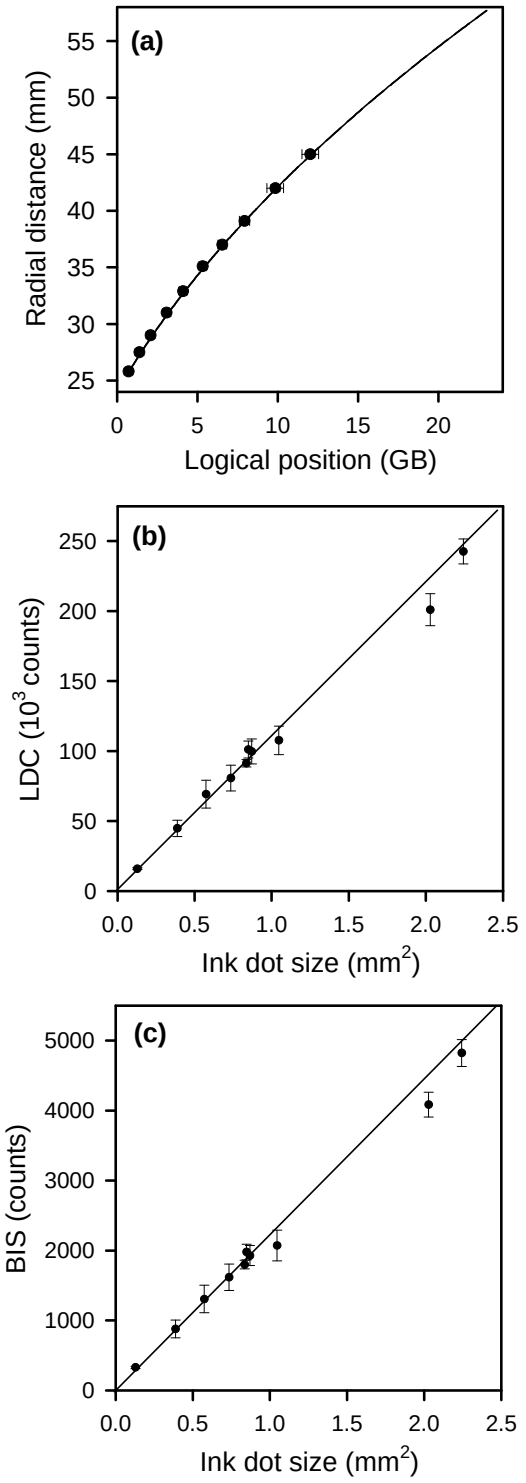

Fig. 3. (a) Relationship between the radial distance and logical position of the ink dots on a BD-R. (b) LDC counts and (c) BIS counts plotted as function of the size of the ink dots. The data were derived from error histograms shown in Fig. 2b,c, respectively.

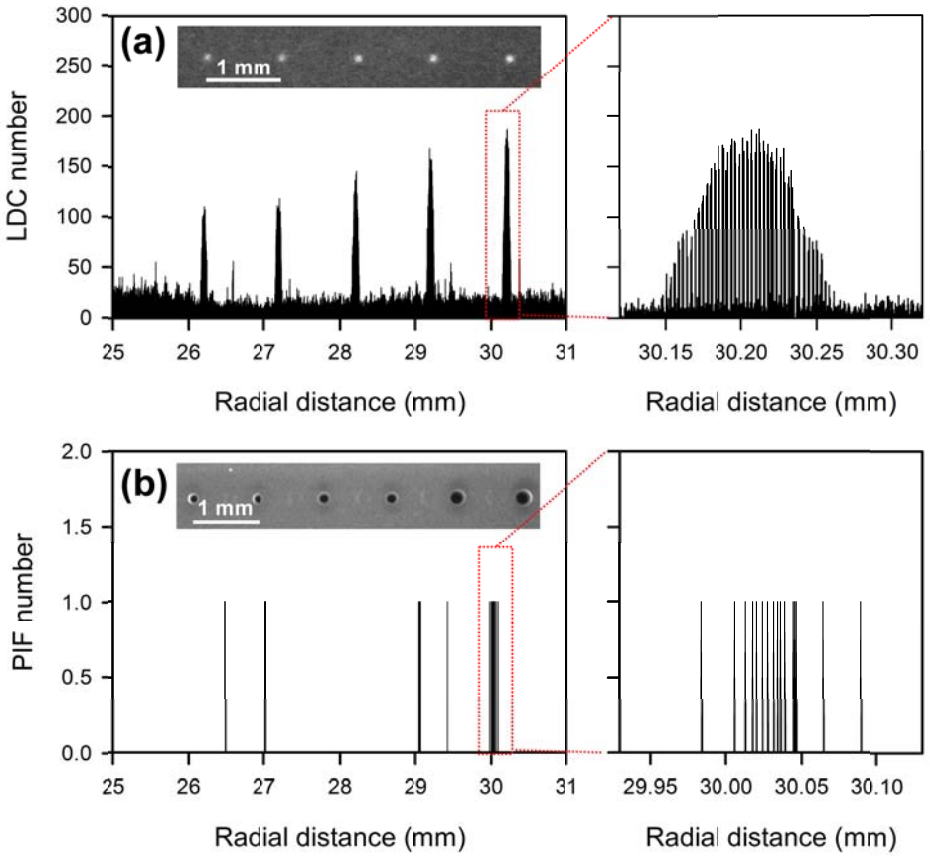

Fig. 4. (a) LDC histogram of the BD-R with deposited ink spots with diameters of 56 to 76 μm; (b) PIF histogram of the DVD-R with deposited ink spots with diameters from 76 μm to 114 μm. The insets on the right are the zoom-ins of the highlighted peaks.

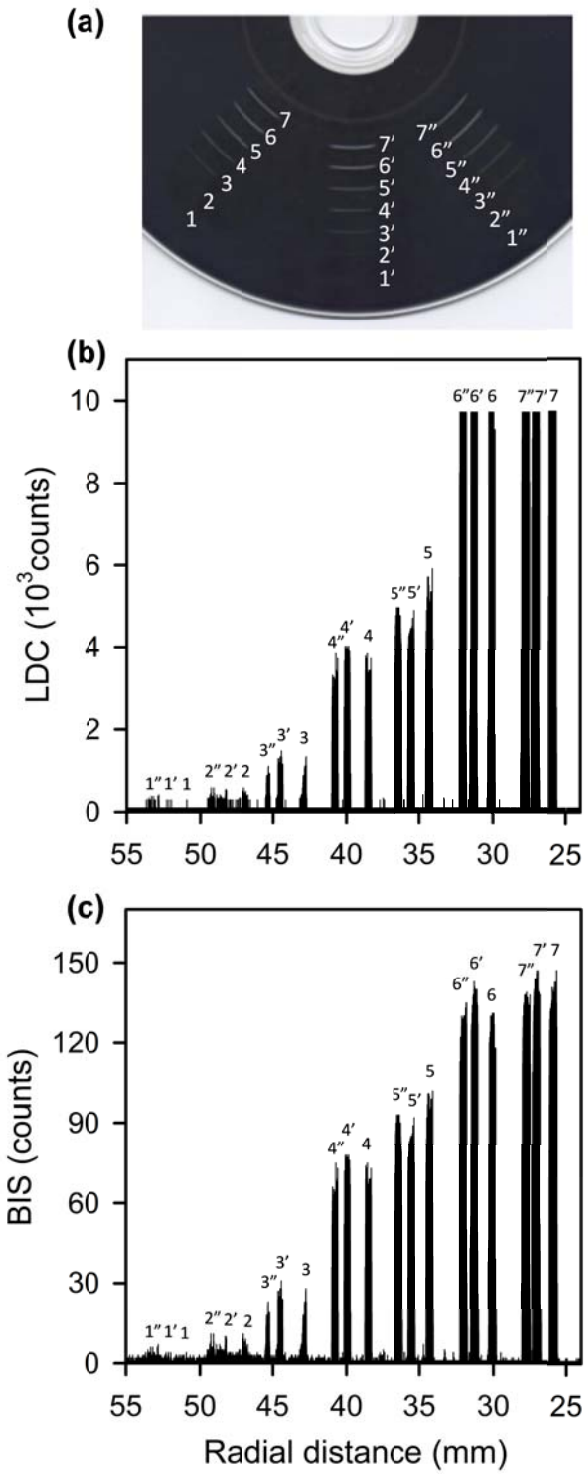

Fig. 5. (a) Optical image of the biotin-streptavidin binding assays on a BD-R with 7 different concentrations of the nanogold-streptavidin conjugate (three replicates each); the concentrations for samples 1 to 7 are 0, 0.01, 0.07, 0.10, 0.20, 0.40, and 0.80 μg/mL. The corresponding reading error histograms are shown in (b) LDC and (c) BIS.

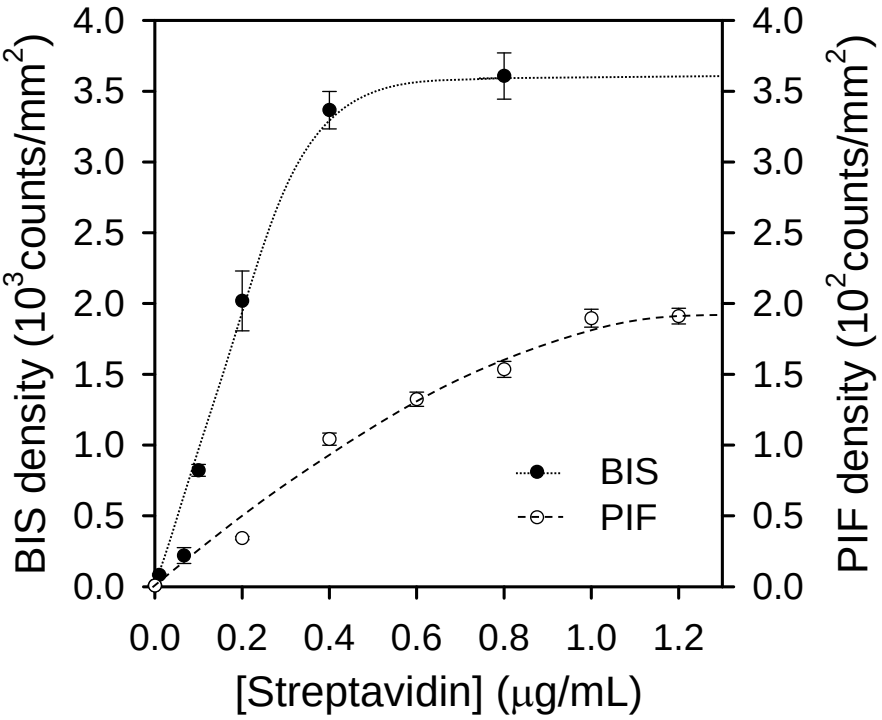

Fig. 6. BIS density as a function of the analyte concentration in the BD-based biotin-streptavidin assay (shown in Fig. 5); the data of a DVD-based assay was included for comparison [11].

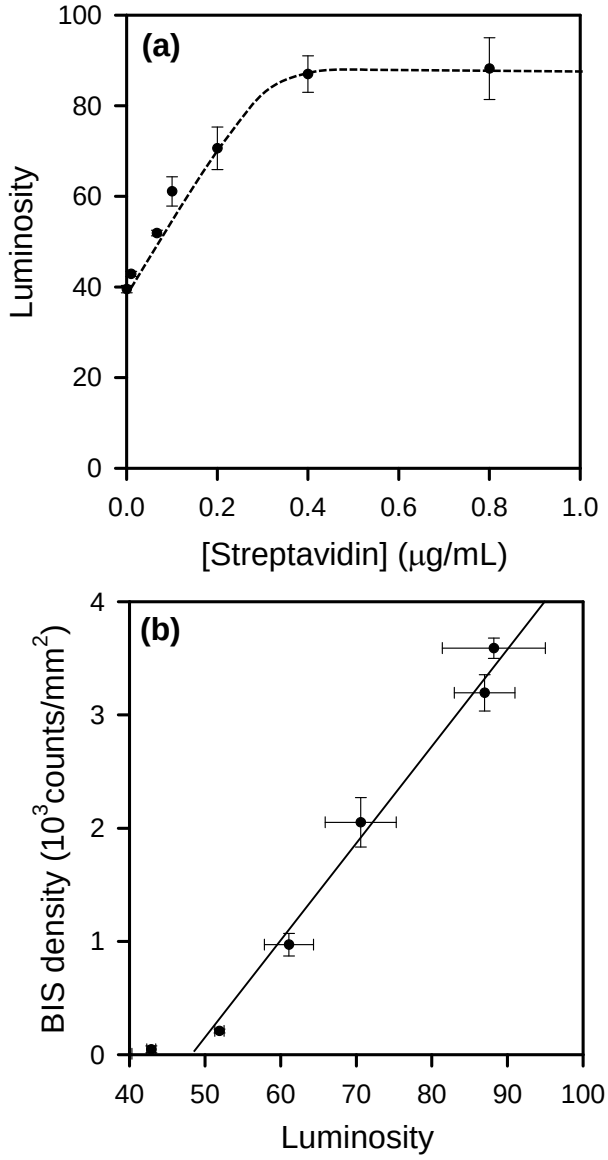

Fig. 7. (a) Optical luminosity of the binding strips as a function of the analyte concentration of BD-based biotin-streptavidin assay; (b) Correlation of the determined BIS density and the optical luminosity.

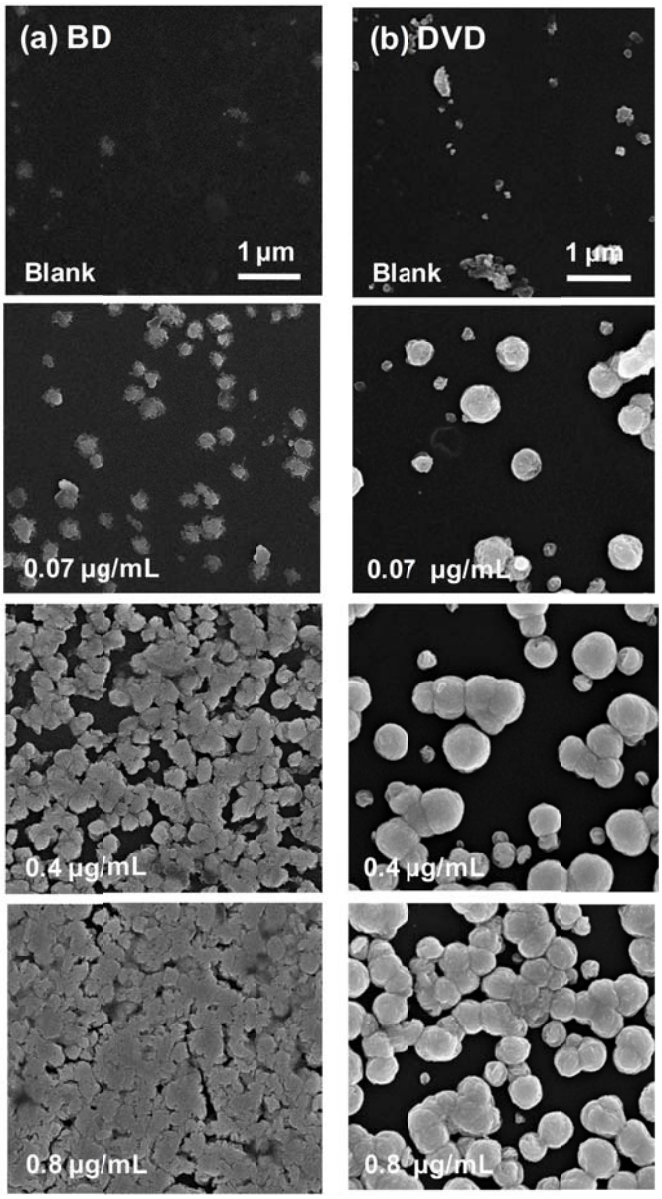

Fig. 8. SEM images of the binding strips to show the size and density of silver particles on the surface of (a) BD-R and (b) DVD-R.