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DVD diagnostic software for reading disc-based bioassays, a comparative study

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ABSTRACT

Compact disc (CD) technology-based bioassays have been developed as novel point-of-care (POC) tools for various applications in chemical analysis and biomedical diagnosis. Herein we investigate the accuracy of screening disc-based bioassays with standard optical drives of ordinary desktop/notebook computers with free DVD diagnostic software. In particular, we have compared the performance of three free online DVD diagnostic software programs: PlexUtilities, Kprobe and Optical Drive Control (ODC), first with arrays of mm-size ink dots and then with biotin–streptavidin binding assays. We have confirmed that the PIF (parity inner failure) number is related to the size of the ink spots, while the PIF density (integrated PIF counts per unit area) is proportional to the optical darkness ratio (ODR) of the spots. The reading results with a biotin–streptavidin binding assay prepared on a standard DVD-R further prove the accuracy of quantitation with all three types of disc diagnostic software programs.

1. Introduction

Compared with the conventional medical diagnosis (traditional blood tests or biomedical imaging), point-of-care (POC) methods and tools have the advantages of easy operation and rapid detection which are vital for patients to improve the chances for fast recovery and cure. Tremendous efforts have been focused on the development of such POC methods and devices for applications in biomedical diagnosis (identification and quantitation of medically relevant molecular analytes) in the past two decades [1–3]. We and others have independently developed compact disc (CD) technology-based bioassays as novel POC tools for a variety of practical applications in both chemical analysis and medical diagnosis [4,5]. In the past, the adaptation of computer optical drives as signal readout devices for running disc-based bioassays focused on the modification of the drive optics [6–9]. Potyrailo et al. explored a unique method of acquiring analog signals from the photodiode detector of an optical drive for quantitative chemical sensing [10]. These groundbreaking studies demonstrated that molecular screening can be realized by either modifying the optics of computer drives or altering the signal output protocol [6–10]. However, disc-based bioassays could not be directly read with commercial optical drives. We have explored software approaches to cope with this limitation, i.e., to realize the quantitative analysis of a CD-based bioassay by using an unmodified optical drive and free CD diagnostic software programs (PlexTools Professional, LE V3.12) [5,11]. Our error-reading protocol for digitally reading disc-based bioassays is different from that reported previously by La Clair et al. [12] who have implemented a specially designed data structure and error correction algorithm. In a subsequent publication [13] we described another protocol to read and quantitate biotin–streptavidin binding assays with CD-data analysis software (IsoBuster) which identifies erroneous sectors by locating the exact error position bit-by-bit and allows various data formats to be used.

While we demonstrated the detection of Pb^{2+} at the ppb level with our digital reading protocol for disc-based DNAzyme assays [14], others have explored its application for reading multiplex micro-immunoassays [15], and in the construction of digital microfluidic CDs for counting polystyrene microparticles and living cells [16]. Notably, Morais et al. compared the acquisition of analog signals with the analysis of reading errors, and concluded that both are valid for multiplex assay detection with satisfactory limits of quantitation [15]. Parallel to these developments [14–16], Digital Versatile Disc (DVD) technology was adapted to the preparation of microimmunoassays on the new disc media

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based on the attenuated analog signal reading strategy [17,18] and to the construction of multiple nanostructured sensing layers on DVD (read by a special reflectance-based optical reader) [19,20]. Ramachandraiah et al. recently developed a new HIV diagnostics device by modifying a commercial DVD drive into a laser-scanning microscope to identify and count the number of CD^{4+} cells in blood samples [21]. It must be pointed out that these bioassays with DVD as substrate materials cannot be read by unmodified, standard optical drives.

The motivation to develop DVD-based bioassays is to catch up the technology advances in the optical discs: today DVD technology plays a central role in data storage, replacing the CD due to its larger storage capacity and stronger error correction performance. In addition, compared with the CD drive, a DVD drive adopts shorter wavelength lasers and larger numerical aperture objective lenses which decrease the focus diameter of the reading laser from 3.2 μm to 1.48 μm. The smaller focus laser size permits higher resolution and more sensitive error detection [5]. Therefore, we were encouraged to switch the CD detection platform to the more advanced DVD technology. It is known that the substrate of DVD media is also made of polycarbonate; therefore the surface activation and assay signal amplification procedure for preparing DVD-based bioassays is the same as that for CD [11,14]. However, in DVD technology different data coding and error correction principles have been adapted [22,23]. In this work we have investigated the accuracy of screening disc-based bioassays with standard optical drives of ordinary desktop/notebook computers using free DVD diagnostic software. In particular we have compared the performance of the three most popular free on-line DVD diagnostic software programs PlexUtilities, Kprobe and Optical Drive Control (ODC), first with arrays of mm-size ink dots and then with biotin–streptavidin binding assays. Such fundamental studies are of great importance for promoting potential applications of DVD technology-based bioassay in a range of new areas such as environmental monitoring and biomedical diagnosis.

2. Experimental

2.1. Reagents

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, phosphates 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 (Yaphank, New York).

2.2. Fabrication of printed spot arrays on DVD

Video data were recorded on blank DVD-R (Sony Inc.) with Power2GO software (V6, Cyberlink). The DVD-Rs were activated in a UV/ozone cleaner (PSD-UV, Novascan Technologies Inc.) photochemically. To verify the surface activation efficiency, water contact angles of the DVD surface were measured with a contact angle goniometer (VCA optima S, AST Products Inc.). After 15-min of UV/ozone treatment, we noticed that the water contact angle reduced from $89 \pm 2^\circ$ to $14 \pm 5^\circ$, indicating that high-density hydrophilic groups (e.g., —COOH) were generated on the polycarbonate (PC) base of DVD [24]. A commercial inkjet printer (Epson R270) with a front-printing tray was employed to print ink-spot arrays on DVD PC substrate [25]. A free software program (PrintCD) was used to design the pattern of the spot arrays and control the printing process. We designed three types of ink-spot arrays: spots with identical diameter and darkness, spots with the same darkness but different diameters, and spots with identical diameters but different darkness values. The variation of darkness was realized by changing the number of printing times. The printed discs were dried in a drying cabinet at room temperature for 20 min.

2.3. Preparation of biotin/streptavidin binding assays on DVD

To mimic the size and location of the ink-spot arrays, six binding spots were formed on the PC surface of a DVD-R by using polydimethylsiloxane (PDMS) plates with six pinholes of 1.4 mm diameter. The DVD-R disc was first treated in a UV/ozone cleaner (PSD-UV) for 15 min and incubated for 20 min. Then it was activated with EDC (100 mM) and NHS (25 mM) prepared in 0.1 M MES buffer solution (pH 5.8) for 3 h. Biotin solution in the MES buffer was then delivered into the reaction zone. After incubation for 4 h (in a humidity chamber) at room temperature it was blocked with a 20 mM phosphate buffer at pH 7.4 (containing 150 mM NaCl, 0.8% BSA, 0.1% gelatin, 0.05% Tween20, and 0.05% NaN_3) for 15 min, followed by addition of different concentrations of streptavidin–nanogold conjugate solutions in 20 mM phosphate buffer at pH 7.4 (containing 50 mM NaCl, 0.1% BSA, and 0.05% NaN_3). After the binding reaction, the DVD was incubated at room temperature for 1 h. Finally it was subjected to the silver staining treatment wherein freshly prepared silver enhancement solutions (silver acetate) and reducing agent (hydroquinone) were used as directed [5,11].

2.4. Software-based digital reading protocol

We have identified three most commonly used DVD diagnostic software programs: Kprobe (v 2.5.2, downloaded from http://www.k-probe.com), ODC (version 1.7, downloaded from http://www.optidrivecontrol.com/), and PlexUtilities (v 1.3, provided by Plextor Corp, downloaded from http://www.plextoramericas.com) for testing ink-spot arrays and biotin–streptavidin binding assays prepared on a DVD-R. Kprobe and ODC were originally designed for LITE-ON DH-12B2SH-12 Blu-ray drive (Lite-on It Co.), and PlexUtilities designed for PX-LB950UE Blu-ray drive (Plextor Co.).

3. Results

3.1. Quantitative analysis of ink-spot arrays on DVD-R

Before testing the ink-spot arrays prepared on a DVD disc, we should understand the data structure and basic DVD error correction algorithm. The data on a DVD are organized by sectors: each sector contains 2064 bytes (12 rows × 172 columns) in total, consisting of 2048 bytes of user data and 16 bytes of logic and error detection code. As shown in Fig. 1, a logical error correction code (ECC) block in a standard DVD has a total of 32,768 user bytes made up of 16 data sectors (192 rows × 172 columns), 16 rows of outer parity (PO) codes, and 10 bytes of inner parity (PI) data in each row of the block. For standard DVD drives, a logical DVD ECC Block is the basic unit to test the disc quality by counting the number of either parity inner errors (PIE) or parity inner failures (PIF). When a row in an ECC block contains at least one byte of error, it will generate a PIE; a PIF is reported when more than five bytes in a row within an ECC block are in error [23].

Based on the error detection principle described above, the screening capability of the DVD error reading protocol was first evaluated by marking a DVD-R with a series of ink-spots of the same size and darkness (Fig. 2, top inset). All three DVD diagnostic software programs identified above can generate a standard error plot (Fig. 2a), showing the number of PIF as function of the logical