



Short communication

## Software-based quantitation of bioassays on CD

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## ABSTRACT

Compact discs (CDs) can be used as rapid, low-cost, high-capacity screening platforms for running bioassays with no modification of the detection hardware (conventional standard optical drive). We describe herein a new protocol to read and quantitate biotin–streptavidin binding assays with a standard optical drive by using a 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. The numbers of erroneous sectors increase as a function of the streptavidin concentration in the tested solutions. High spatial accuracy and detection sensitivity (0.3 µg/mL) were achieved.

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## 1. Introduction

The compact disc (CD) has been explored as an assaying platform for extremely low-volume biological and chemical analyses; an optical drive can be adapted to serve as a signal readout device for biomarker detection. In 2000, Kido et al. reported the preparation of disc-based high-density immunoassay microarrays via piezoelectric inkjet printing, for which the signal readout relied on a conventional fluorescence scanner [1]. They also proposed the use of an unmodified optical drive to examine reflective immunobead-based sandwich assays on CD [1]. Alexandre et al. [2] developed a colorimetric method for the detection of multiparametric DNA on a specially prepared “bioCD” (“storing” DNA hybridization assays on the external and numeric information on the inner portion of the same disc). The signals were detected with a custom-made double-sided CD reader for simultaneous analysis of both genomic and numerical data [2]: the spatial resolution was 300 µm. Barathur et al. from Burstein Technologies Inc. (BTI) developed their version of “BioCompact Disc (BCD)”, an enclosed disc format read by a modified CD drive [3]. The key feature of their system is the sample analysis in the liquid phase within an enclosed chamber on the disc, which is performed concurrently with optical tracking of information features on the disc.

Recently, Lange et al. introduced a silver staining method (with antibody-labelled gold nanoparticles promoting the precipitation of silver particles) to increase the reflectivity of C-reactive protein (CRP) immunoassays on disc, which can be imaged with a CD reading (pickup) head mounted on an optical microscope stage [4]. With an analog signal acquisition approach, i.e., making electrical connections to obtain a differential signal from the optical photodiode component, Potyailo et al. have shown the application of conventional CD/DVD drives for quantitative chemical sensing of metal cations [5].

Other researchers have explored software-based approaches to analyze the digital signals obtained from a CD. La Clair et al. reported an error determination routine for screening ligand–protein interactions on CDs with a specially designed software; they created unique data structures to detect reading errors by comparing the original with the retrieved data byte by byte [6]. Jones employed CD drives as photonic signal processing devices (optical microscopes) to image stained bacterial cells physically adsorbed on disc [7]. We have also developed a method for reading error numbers per frame of pre-recorded audio files on a CD-R [8]. Herein we examine the feasibility of using a commercial CD-data analysis software (e.g., IsoBuster) to read and quantitate biochemical binding assays prepared on a disc. The versatility of IsoBuster provides the advantage of using any data format/media. IsoBuster also allows the detection to be more specific; it identifies the erroneous bits rather than the total error numbers per frame. This provides the most “direct” approach to use consumer electronic products for biomedical diagnostics.

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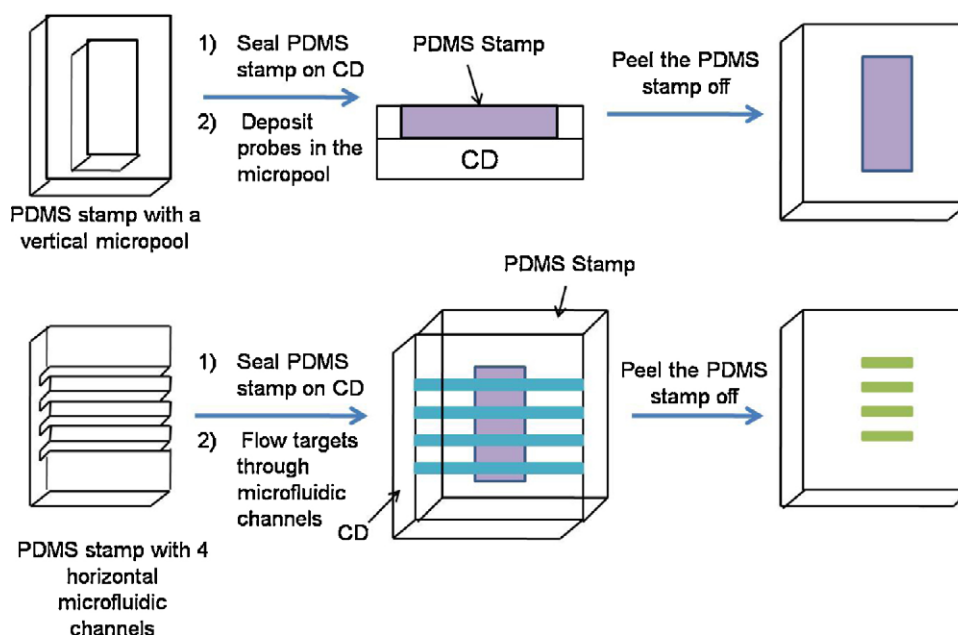


Fig. 1. Surface patterning and assay preparation using PDMS microfluidic channel plates. The figure is not to scale.

## 2. Experimental

### 2.1. Reagents

1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino)ethanesulfonic acid (MES), bovine serum albumin (BSA), Tween 20 and gelatine were purchased from Sigma–Aldrich. Sodium chloride and phosphates were from Caledon Laboratories Ltd. Sodium azide was purchased from BioShop Canada Inc. EZ-link amine-PEG2-biotin was purchased from Thermo Scientific. The nanogold–streptavidin conjugate (1.4 nm in diameter) and LI silver enhancement kit were purchased from Nanoprobes. Deionized water ( $>18.3\text{ M}\Omega\text{ cm}$ ) from a Barnstead EasyPure UV/UF system (Dubuque, IA) was used to prepare the sample solutions.

### 2.2. Preparation of biotin–streptavidin binding assays on CD

For the surface activation the polycarbonate side of the written CD was irradiated with UV light in the presence of ozone for 20 min. The UV/ozone cleaner (Model PSD-UV) was from Novascan Technologies, Inc. This UV/ozone treatment generates a high density of carboxylic acid groups on the CD surface which facilitates the attachment of amino-modified probe molecules such as aminated biotin to the CD surface via amide coupling [9]. Due to the mildness of the immobilization, the CD can still be read by a conventional optical drive. Before the immobilization of biotin molecules, the CD was treated with EDC (100 mM) and NHS (25 mM) prepared in a MES solution (0.1 M, pH 5.8) for 4 h. Surface patterning and bioassay preparation were carried out by microfluidic delivery of the amino-terminated biotin molecules and the target streptavidin samples (Fig. 1) via two polydimethylsiloxane (PDMS) channel plates (prepared using the Sylgard 184 Silicone Elastomer Kit). The elastomer base and curing agent were mixed in a 6:1 ratio by weight and the mixture was then poured into a silicon moulding master. After introducing  $60\ \mu\text{L}$  of the MES solution containing biotin molecules (4 mM) into the opening ( $24\text{ mm} \times 8\text{ mm}$ ), the CD was incubated at room temperature overnight. Another PDMS stamp with microchannels ( $1\text{ mm} \times 28\text{ mm}$ ) was then used for the delivery of  $10\ \mu\text{L}$  of the streptavidin–nanogold conjugate solution of various

concentrations in each channel. The testing solution was made in a phosphate buffer (20 mM) containing 0.8% BSA. After the binding reaction, the CD was incubated at room temperature for 1 h. The enhancement of the reading signal was accomplished by promoting the deposition of silver particles of up to a few hundred nm size [8].

### 2.3. Software-based detection procedure

Being stored in the form of pits and lands in a CD, digital information is read spirally from the centre outward by a laser in an optical drive. These pits and lands form the basis of binary codes referred to as bits. Eight bits constitute a byte, which is another commonly used unit for digital information. A frame, the basic unit of information, is comprised of 24 bytes of audio data, 8 bytes of parity bits, 3 bytes of sync data, and 1 byte of sub-code bits in an audio CD. A sector is a group of 98 frames [8].

Audio data was recorded on RiDATA Silver–Silver 700 MB CD-Rs with Nero 7 (Ultra Edition). A single audio file of 5 MB size repeated 60 times was written on each of two CDs for a set of experiments, one for the detection and the other as a reference to compare the hexadecimal data of the erroneous sectors with clean sectors. In each case, at least five different concentrations of streptavidin–gold nanoconjugate were tested.

IsoBuster was used as the software to detect and quantitate the bioassays on CDs. This is a data recovery tool compatible with multi-file systems, multi-hard media (CD/DVD/BD/HD DVD), and multi-soft media (various CD and DVD data formats), which provides the freedom to use any data format/media to conduct biomolecule detection [10]. Though other CD scanning softwares have the ability to acquire the block error rate from media, they only count error frames, but not to the byte-by-byte details (as can be done with the single sector extraction utility of IsoBuster). It performs surface scans on hard media to check for physical reading errors which are identified and saved as a list of erroneous files. This utility is further exploited by the single sector extraction function which enables viewing of the exact sector and its erroneous data. The *sector viewer* gives the comprehensive logical block address to pinpoint the erroneous sector to the resolution of individual bits. This feature displays the hexadecimal data of a particular

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