SOFTWARE BASED BIOASSAY QUANTIZATION USING STANDARD OPTICAL DISC DRIVES

by

Manu Pallapa
Bachelor of Engineering, U.V.C.E. Bangalore, INDIA, 2006

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

In the
School of Engineering Science

SIMON FRASER UNIVERSITY

Fall 2010

APPROVAL

Name: **Manu Pallapa**

Degree: **Masters of Applied Science**

Title of Thesis: **Software based bioassay quantization using standard optical disc drives.**

Examining Committee:

Chair: **Dr. Bonnie Gray**
Associate Professor

School of Engineering Science, Simon Fraser University

_____

Dr. Ash Parameswaran
Senior Supervisor
Professional Engineer

School of Engineering Science, Simon Fraser University

_____

Dr. Hua-Zhong Yu
Supervisor
Professor
Department of Chemistry, Simon Fraser University

_____

Dr. Carlo Menon
Internal Examiner
Assistant Professor
School of Engineering Science, Simon Fraser University

Date Defended/Approved: _____

ABSTRACT

This thesis describes the application of the compact disc technology as a rapid, low-cost, high capacity-screening platform for bioassays. Although the compact disc has been used by modifying the hardware of the detection mechanism (CD-drive) to achieve a ubiquitous and low-cost detection of biomolecules, minimal or no modification is ideal. This thesis investigates the possibility of an inexpensive, software based bioassay detection on CDs with, standard computer drives. Using a CD data analysis software called *IsoBuster,* biotin-streptavidin binding assays prepared on compact discs are read detected and quantized using error based detection mechanism with a high spatial accuracy. The research shows a sensitivity of detection of 270 µg/mL of streptavidin in solution with biotin-modified disc

Keywords: Compact disc, biomarker detection, error detection, *IsoBuster*

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Dr. Ash Parameswaran for his unfailing support and guidance over the years. Without him, neither this work nor my knowledge would have developed. I would also like to thank Ian Foulds, Robert Johnstone, Dan Sameoto, See-Ho Tsang and all my lab members for their assistance and support. My sincere thanks to Dr.Hogan Yu for his accommodating nature and support to help me with the chemistry aspect of the thesis. Many thanks to Yunchao Li and Lily Ou, who assisted me in the critical step of biomolecule deposition. Thanks go out to Ajit Khosla who has shown his support and friendship in hard times. Finally, I am grateful to my parents and Shwetha, who have been a great source of support and confidence in me.

DEDICATION

I would like to dedicate my thesis to my parents.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1: INTRODUCTION

An optical drive and optical storage medium (compact disc) can serve as a high-density biomarker detection tool without any modification of the hardware of the system. *H. Kido et al,* proposed to combine high-density microarrays applied via a piezo-electric inkjet applicator with circular indexing on a compact disc using a commercially available fluorescence scanner for detection (Storm fluorescent scanner, Molecular Dynamics, Sunnyvale, CA). The systems tested were, the inhibition immunoassays for hydroxyatrazine, carbaryl and molinate. The deposition of the inhibition immunoassays employed a slide stepper motor of a print head with three reservoirs. A CD was printed with immunoassays using an inkjet printer. The technique achieved a detection over a linear range from 0.1nM to 1mM (7 orders of magnitude). In comparison, using a 96 well microtiter plate a detection range of 1mM to 0.1µM can be achieved [1]. A colorimetric method to detect multi-parametric DNA and numeric information on a double-sided CD was developed by *Alexandre et al* [2]. The technique chosen was colorimetric because polycarbonate and CD resins auto-fluoresce. This detection technique had a double-sided reader, which was developed for simultaneous analysis of both genomic and numerical data. The fem A gene of various Staphylococci species was the target of detection. This was amplified by a consensus set of primers and then detected on CD microarrays bearing capture probes specific for the fem A of the different Staphylococci species. Instead of using the normal

optical pick-up unit used to read a CD a double-sided reader, which has another optical pick-up head positioned to read the top (label side) of a CD. An arrayer was used to make an arrayed spotting onto the top of the CD, which consisted of an additional aluminium reflection layer. Detection resolution of this process achieved was the successful detection of spot of 300 µm diameter. The concept of acquiring electronic signatures of microspheres deposited onto the read side of a BioCompact Disc (BCD) was reported by Barathur *et al* [3]. Multiplex-amplified DNA from three species of Brucella arrays of 30 - 70 µm diameter spots (150 to 1500spots/mm^2 were detected by their electronic signature waveform. Silver staining detection for increased reflectivity and concentration determination of C- reactive protein (CRP) using a CD reader head mounted on the stage of an optical microscope was done by *Lange et al* [4]. The dynamic range of resolution of four orders of magnitude was achieved with a limit of detection of 1 pM. The work used silver precipitation catalyzed by colloidal-gold-labelled antibodies in a sandwich immunoassay format to increase the reflection and obtain an adequate signal for measurement. Specific digital data based biomolecular detection was shown in *Jones's* work which employed cryptographic hash functions to achieve a CD-based colour recognition. This technique was used for the rapid detection of Gram staining of *Saccharomyces cerevisia* [5]. In an analog signal acquisition, using quantitative biochemical sensing on sensor films *Radislav A et* al [19] achieved a detection resolution of 5 ppm of Ca^{2+}. More recently, different forms of digital data have been used for the detection procedure. *Li et al* [6] used a protocol of reading error levels (Block Error Rate) of pre-recorded audio files to

serve as a quantitative measure of biochemical interaction which shows an order of magnitude more sensitivity than fluorescence labelling and scanning.

1.1 Objective

The main objective of this work is to achieve a low-cost detection mechanism based on CD drives using a CD as the medium. Initially hardware modification of the drive, to acquire an electronic signature of the biomolecule was undertaken. However, this resulted in a signal with high noise even after complementing the hardware with a noise reduction circuit. Following which, a software technique for detection was decided. This reduces cost, and satisfies the condition of having no hardware modification of the CD drive.

In this thesis, the biotin-streptavidin binding assays were deposited on a CD medium, which is read using commercial a CD drive. The quantization of the assays performed using a CD data analysis software called *IsoBuster*.

1.2 Chapter Outline

Chapter 2 introduces the physical structure of optical storage media and the existing information storage hierarchy. Chapter 3 discusses the error detection and correction mechanisms available in optical storage systems. Chapter 4 discusses the simulated error detection and colour detection by hardware modification of CD and DVD drives. Chapter 5 outlines the surface chemistry and deposition techniques of the biotin-streptavidin binding assays on CDs and DVDs. Chapter 6 explains the use of a software-based approach of biomarker detection with DTI RetroBurner software as an example. Chapter 7

details the features and advantages of IsoBuster software in comparison to the DTI RetroBurner software for error detection. Chapter 8 discusses the results obtained from the experiments and explains the findings. Finally, Chapter 9 concludes this work and states the future work.

2: PHYSICAL STRUCTURE AND INFORMATION STORAGE ON CD AND DVD MEDIA

The compact disc (CD) and the digital versatile disc (DVD) are two of the most popular optical storage media. Visually a CD and a DVD are similar in dimensions. The thickness and diameter and centre hole of both the CD and the DVD are the same. Thickness being, 1.2 mm, the diameter being, 120 mm and the centre hole being, 15 mm [7]. The improvement of the DVD over a CD is the combination of improved logical channel coding, smaller physical bit sizes, larger data owing to reduced dimension of the pit lengths and the track pitch.

2.1 CD dimensions

The information on the disc is physically stored in the form of holes called pits and flat surfaces between the pits known as lands. These pits and lands are laid out in a spiral track with a pitch of 1.6 µm.

The disc is manufactured from a clear plastic called polycarbonate with a refractive index of 1.55. The data is read from the disc by focusing a laser beam of 780 nm through the polycarbonate on the track. The laser light that strikes a land is reflected into a photodetector while the light striking a pit is scattered and absorbed. This duality is converted into binary information. The laser beam is about 800 µm in diameter as it strikes the polycarbonate substrate. This optical spot is focused down to a 1µm diameter. Minor imperfections on the reading

surface such as fingerprints scratches and dust are reduced by the shadows cast by them. These imperfections are ignored by the reading system [8].

The detailed physical characteristics of both the CD and DVD are listed in Appendix A. Figure 2-1illustrates the disc diameter, hole diameter, lead-in and lead-out in a compact disc.

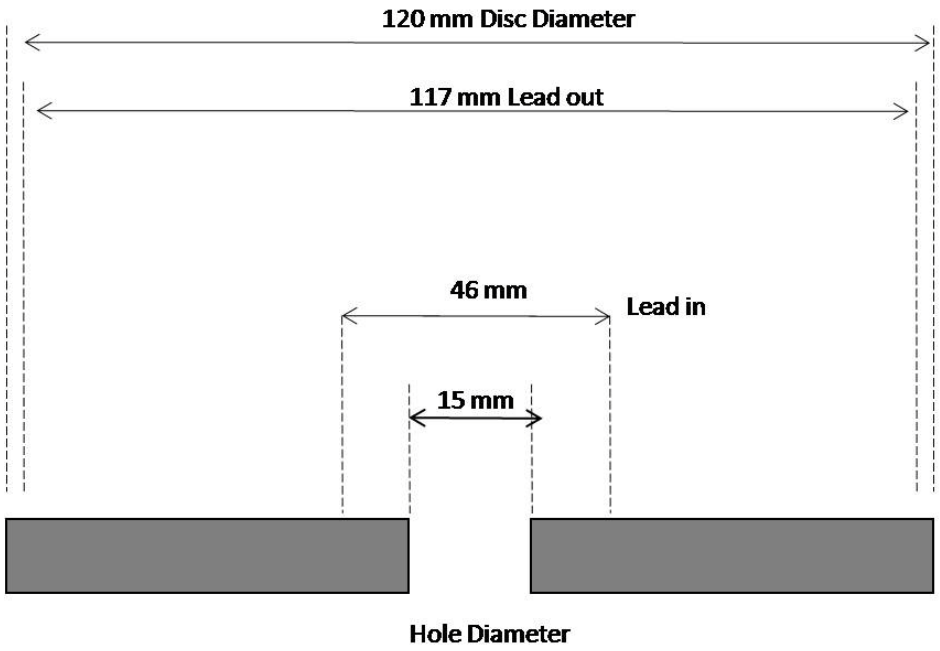


Figure 2-1: Physical layout of a compact disc: Dimensional details

2.2 CD and DVD layers

A transparent plastic substrate forms most of a disc's 1.2 mm thickness. Data is physically contained in pits, which are impressed along its top surface and are covered with a very thin metal layer (50 to 100 µm aluminium, silver, or

gold) layer. Another thin (10 to 30 µm) plastic layer protects the metalized pit surface, on top of which the identifying label (5 µm) is printed. The enlarged cross-sectional view of a CD including its printing ink, lacquer coating, metallised pits and polycarbonate substrate is shown in figure 2-2.

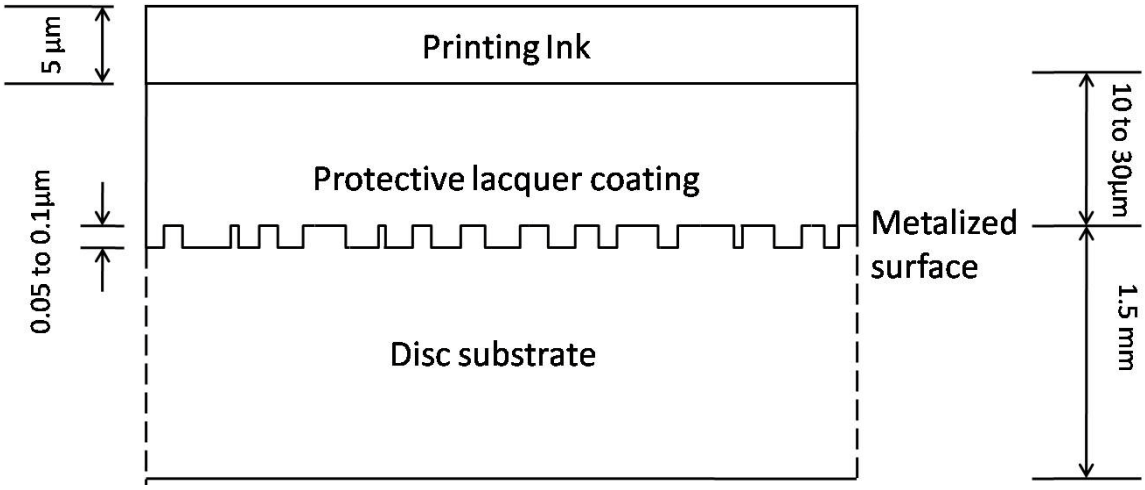


Figure 2-2: Enlarged cross-sectional view showing the layers of a compact disc

A laser beam is used to read the data. It is applied from below and passes through the transparent substrate and back again. The beam is focused on the metalized data surface embedded inside the disc. Since data on a disc is read by a light beam, playing a CD does not cause any wear on the metalized information. Physical format of the DVD is similar to the CD except for the fact that two substrates need to be bonded together [9].

.

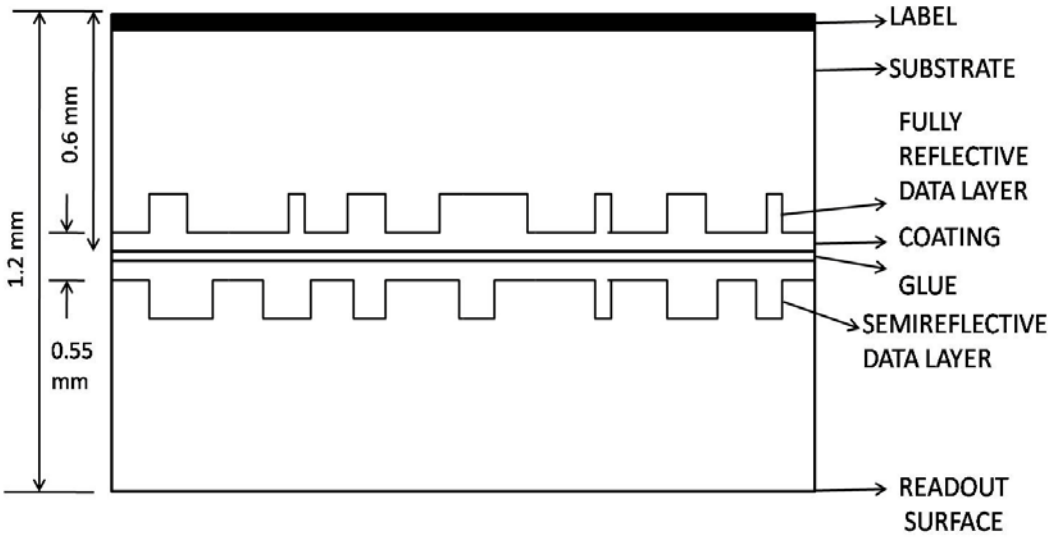


Figure 2-3: Enlarged cross-sectional view showing the layers of a digital versatile disc

2.3 CD read out and optical pickup unit

The optical pickup head that reads the information on the optical media contains a semiconductor laser diode, a focusing system and a photodetector to read the laser beam reflected from the disc. The photodetector contains several photodiodes to convert the laser beam into an encoded signal and determine the focus of the laser spot on the disc track [10]. The schematic of the optical head is shown in figure 2-4.

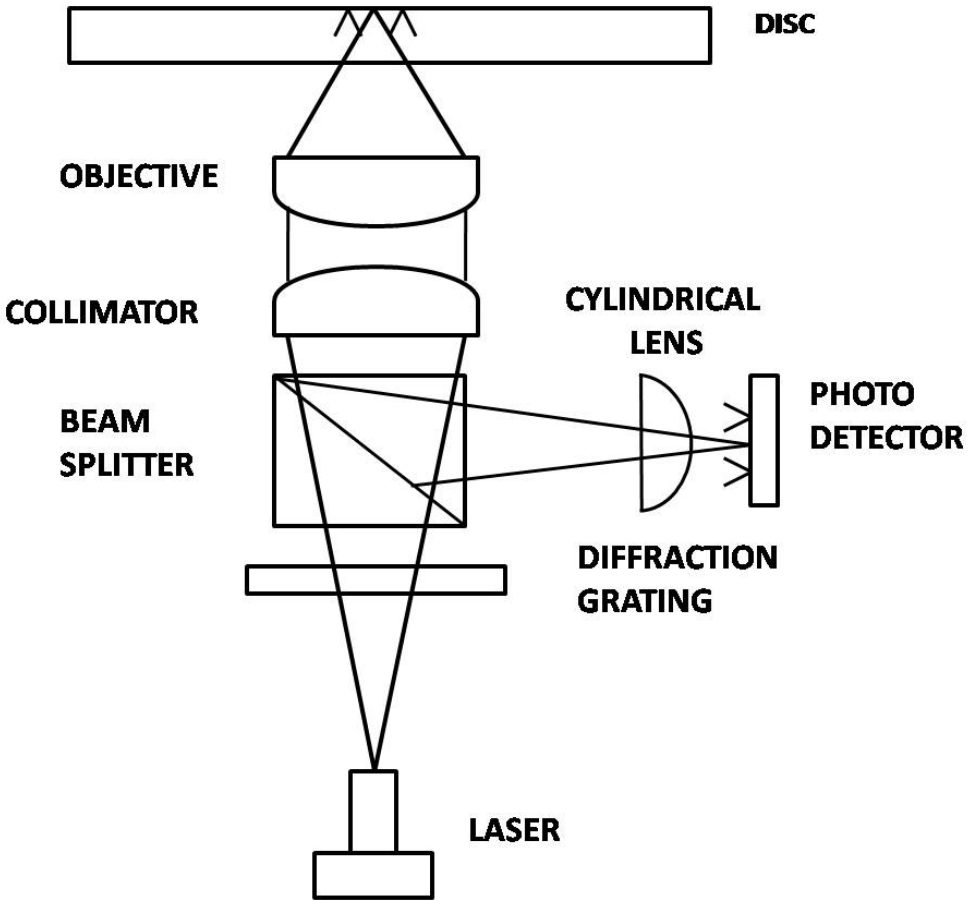


Figure 2-4: Optical reading pickup head schematic

In an event of the spot being out of focus or off track, control voltages indicating the direction and amount of correction required are sent to the control elements holding the objective lens. Due to the combined factors of the refractive index, the thickness of the disc and the numerical aperture (0.45) of the laser's lens, the size of the laser beam on the disc surface is approximately 800 µm but is focused to approximately 1.0 µm at the pit surface. The laser beam is thus focused to a point about twice as large as the pit width. Moreover, the effects of any dust or scratches on the substrate's outer surface are minimized because

their size (and importance) at the data surface are effectively reduced along with the laser beam. A physical obstruction on the reading surface less than 0.5 mm becomes insignificant and causes no error in the readout. The height of the bumps is thus approximately one-quarter of the laser's wavelength in the substrate. Light striking the land thus travels at a distance of one-half the wavelength (one-quarter plus one-quarter) further than light striking a bump. This creates a phase difference of one-half wavelength between the part of the beam reflected from the bump and the part reflected from the surrounding land, as shown in figure 2-5.

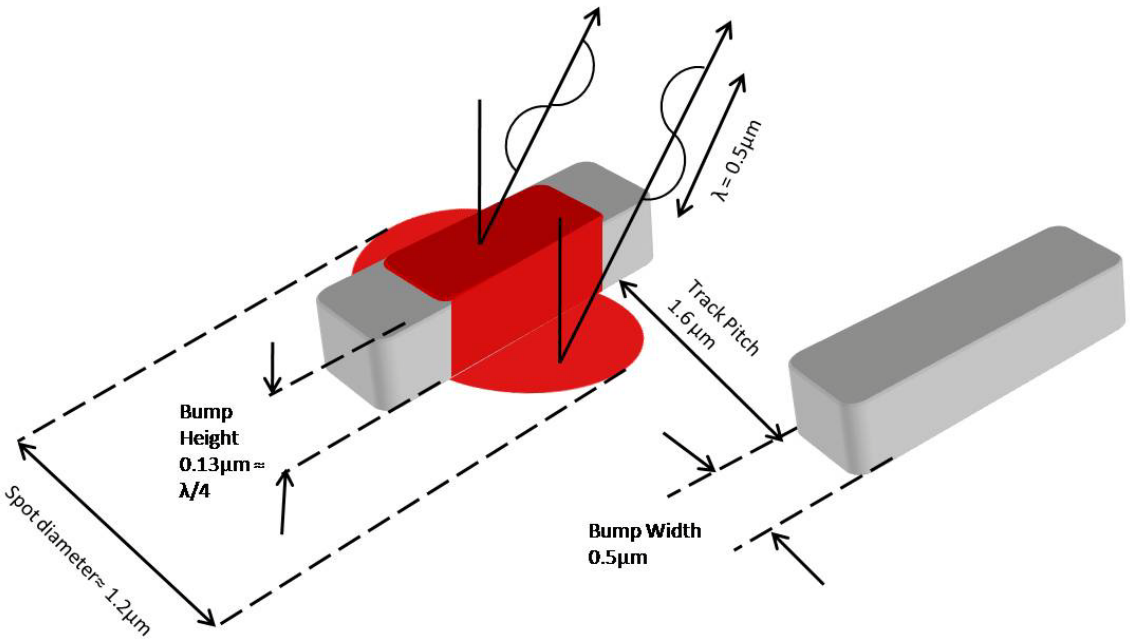


Figure 2-5: Dimensional details of the bump. One-half wavelength path difference relative to surrounding land of the bump creates the phase difference [33]

The CD uses a constant linear velocity during read-out implying that the rotational speed varies depending of the location of the information being read.

The tracks closer to the inner spiral rotate faster when compared to the outer spiral of the disc. The rotational speed of a compact disc varies from 500 rpm on the inner spiral to 200 rpm on the outer spiral, which is between 1.2 to 1.4 m/sec. This allows the track to pass the pickup head. The layout is beneficial as it makes efficient use of disc space but causes a problem of random data access [11] at a constant rate adjustment in rotational speed.

The phase difference causes the two parts of the beam to destructively interfere with and cancel each other, forming a diffraction pattern. In short, a bump disperses light, reducing the intensity of the reflected light. When the beam strikes an area between pits, virtually all of the light is reflected, and when it strikes a pit, virtually all of its light is cancelled. In practice, the laser spot is larger than is required for complete cancellation between pit and land reflections, and pits are made slightly shallower than the theoretical figure of one-quarter wavelength; this yields a better tracking signal. About 25 percent of the power of the incident light is reflected from a long bump. The presence of pits and land is thus read by the laser beam; specifically, the disc surface modulates the intensity of the light beam. Thus, the data physically encoded on the disc can be recovered by the laser and later converted to an electrical signal.

2.4 CD information storage

Although the diameter of a CD is 120 mm as mentioned previously, the recording area is between 46 mm and 117 mm and the signal area is between 50

mm to 116 mm. The minimum pit length is between 0.833 µm for the innermost area, to 0.972 µm which being the outermost area spins at 1.4 m/sec. Similarly, the maximum pit length is 3.05 µm to 3.56 µm. The approximate pit depth and pit width is 0.11 µm and 0.5 µm respectively. The innermost part of the disc does not hold data. Its function is to provide a clamping area for the player to hold the disc firmly to the spindle motor shaft. [12]

The basic information unit of the compact disc is known as a frame. Each frame contains 24 eight-bit characters. Data other than audio data is stored as a frame of eight 24 bytes and audio samples are stored as six 16-bit audio samples each for the right and left stereo channels. Both user data and audio data are encoded as an eight to fourteen modulation (EFM) code on the disc where an 8-bit data is read into an encoder that uses a lookup table to convert it into the CD's 14-channel bit code.

The data on a compact disc is encoded as a frame structure. The audio or user data goes through a number of processes before being written onto a compact disc. The frame serves as a medium to distinguish between audio and user data types and their parity, synchronization word, and subcode. Before modulation is done, a CD frame consists of a 27-bit synchronization word, 8-bit subcode, 182 data bits, and 64 parity bits.

After grouping the data, an error correction encoding called the Cross Interleave Reed-Solomon code (CIRC) takes place. This uses a combination of two techniques called interleaving and parity to make the data more resistant to storage-related errors (Explained in detail in Chapter 3). The CIRC encoding is

followed by adding an 8-bit subcode symbol to each frame. The user data, parity and subcode data are put together and modulated using eight-to-fourteen modulation (EFM). Eight data bits are translated into blocks of 14 bits, to be known as channel bits. A synchronization pattern is added to each frame for recognition.

The total number of channel bits per frame after encoding is 588. This encompasses 24 synchronization bits, 336 data bits, 112 error correction bits, 14 subcode bits, and 102 merging bits. As a frame is too short for numerical applications and there is no provision for addressing, ninety-eight 24-byte frames are summed, thus the effective size for the data area becomes 2352. This 98-frame area is called a *sector*, and it forms the basic CD-ROM data unit. In the case of data other than audio, system data occupies 2048 bytes.

The remaining 304 bytes are used for synchronization, headers, mode selection and extended error detection and correction. A 2352-byte block is divided into a 12-byte synchronization word, and a 4-byte header field used for time and address flags. The header contains three address bytes and a mode byte. The address bytes store the location as time in minutes, seconds and frames (75 blocks per second). In this work, we used the CD-R format, which encompasses both Compact disc – Digital Audio (CD-DA) and Compact disc – Read Only Memory CD-ROM applications. [13].The aforementioned error correction techniques and codes involved in digital data storage is detailed in the following chapter.

3: ERROR DETECTION AND CORRECTION IN OPTICAL STORAGE SYSTEMS

As mentioned in the previous chapter, the laser light passes through the polycarbonate coating well out of focus over a large area. This facilitates the light to pass around dust particles and then comes to a focus within the thickness of the coating. If the dust particles are large enough or spread apart, errors are inevitable.

3.1 Error detection

There are two major methods of error detection systems. They are code error detection using parity check and code error detection using cyclic redundancy check code (CRCC). Code error detection using parity check is carried out during data reproduction. This method has an almost 100% rate of detection for one bit code errors in each block. However, a continuous code error at the same bit position occurs in each word results in the performance of the code error detection to decrease by a large scale. This problem is tackled by either altering the bit order by interleaving or by using bit sequential even and odd parity as shown in the next section. In cyclic redundancy check codes the transmitted data contains the information bits as well as the detection bits. This method does not have a degeneration of the detection method with the occurrence of error patterns.

3.2 Correction

Unlike analog media (magnetic tape, LPs), digital storage media has the advantage of implementing error correction, to correct errors resulting from scratches, dust and data corruption. A physically scratched LP record results in an audible click when the damaged part of the groove passes underneath the phonograph needle. However, when a CD is scratched, the format of the data on the disc and the reading mechanism offers a chance of correction.

Simple redundancy, which is a technique where the data is recorded several times, is the first line of defence for data protection. Though the error is not prevented, the data is protected against the error's effect. This method of incorporating redundant data is not an efficient solution considering the density of data on a CD or DVD.

A second approach known as data redundancy is a technique that involves adding extra information derived from the original data. Existing data redundancy methods in correction codes add a parity bit to the data group, which is the data word plus a parity bit. In the following example, an even parity of 0 or an odd parity of 1 is added at the end of the data word. This parity bit assigned such that the data group is either even or odd.

Data word = 1101100110 Even Parity = 1101100110

Odd Parity = 1101100111

Performance of the error correction code employing data redundancy demands more parity bits and a suitable algorithm to select the parity bits. In

some codes, the data is divided into blocks, and parity values are added to each block.

Figure 3-1a illustrates an original data block as an example. The algorithm used for the parity bits is summation of the data against each row and each column as shown in Figure 3-1b. In addition to the sixteen data value, nine extra parity values are created and appended to the original data block. If the data block received is as shown in Figure 3-1c, the error location is detected and the corresponding error correction can be made using the parity bits [14].

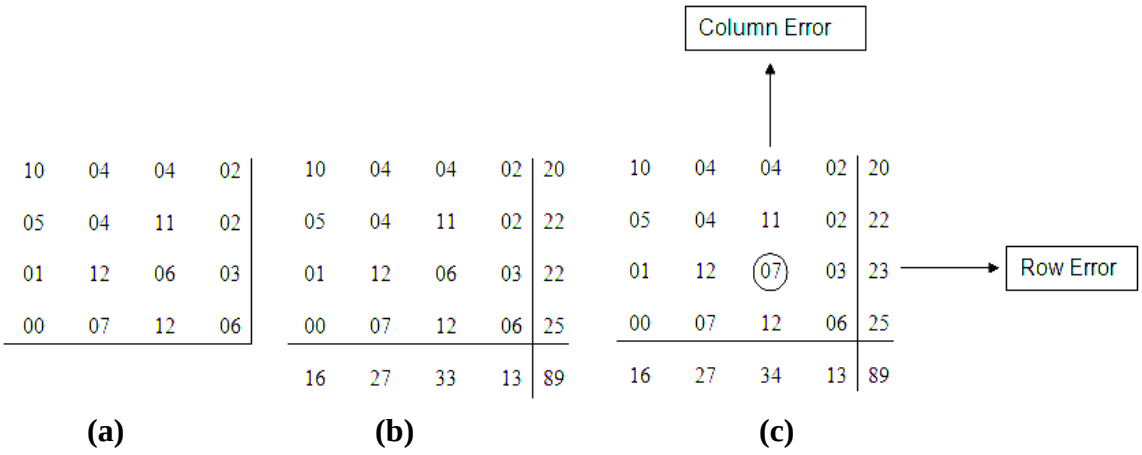


Figure 3-1: Example of parity used to correct the error (a) Data received (b) Data with row column parity (c) Error detected using parity bits

In addition to data redundancy with parity bits, numerous error correction codes have been devised. For example, Hamming codes [15] derive multiple parity bits from combinations of the data bits. Simple Hamming codes can be constructed which will detect two errors and correct one error. Similarly, another

robust error correction code known as the Reed-Solomon [16] code can detect and correct large numbers of errors. The compact disc uses Reed-Solomon error correction code as part of its defence against errors. Using codes such as Reed-Solomon, a digital audio system can detect and correct errors and supply information completely error free

The performance of the error correction system depends on the nature of the error. If a large error eliminates both the data and its parity, there would be nothing left to reconstruct the original data. The error correction systems depend on nature of the errors and device protection to fit their nature.

Errors can occur in large groups, called burst errors, or in isolated instances, called random errors. In a compact disc, a badly formed pit during the manufacturing process could cause a random error. Random error is more common in CD and DVDs and occurs at an average of once in 10^2 or 10^4 bits, whereas a dust particle could cause a burst error. The average occurrence of burst errors is once in 10^3 or 10^6 bits. Errors caused by the variations of the time axis during reproduction of the digital signal are known as jitter. Jitter is predominantly caused by lack of uniformity in the rotational system of the optical drive. A good error correction code uses parity in addition to other processing, such as interleaving.

The requirements of an efficient error correction system include the ability of high random error correctability, a long burst error correctability and in case the burst error is exceeded, the system should enforce graceful data degradation. The error decoder strategy should be simple with a reasonably

sized external random access memory and finally the redundancy of the correction system should be as low as possible to preserve data density.

3.3 Interleaving

Interleaving or scrambling is a process of altering the data order in a fixed pattern. This interleaved data is recorded and subsequently de-interleaved during reproduction. The advantage of using such a process is to achieve effective error compensation by altering the nature of the code errors generated rendering burst errors into random errors. Bit interleave and block (word) interleave are the two types of interleaving techniques. Bit interleave allows the conversion of burst errors into errors of a random nature assisting in the usage of random error correction codes or short burst error correction codes instead of encountering long burst errors. Block interleave applies the same principle of bit interleave to blocks or words. Blocks of uniform length are altered in position according to a fixed pattern. In an event of a burst error, the correct blocks alternate with the error words at a fixed distance. These two techniques are widely used in code error concealment.

Cross interleaving carries the idea further. Data is interleaved numerous times, over both short and long time intervals. This provides correctability for larger errors. The CD system uses the Cross Interleave Reed-Solomon code (CIRC) for error protection. It employs parity checking to correct random errors and cross interleaving to permit parity to correct burst errors.

3.4 Concealment

Although correction of extensive errors is possible, it is impractical to implement. In real-life digital audio systems, when massive error occur these errors are flagged by the correction circuits and passed on to error concealment circuits.

Without concealment, existing erroneous data results in an audible click. Error concealment systems employ interpolation and muting circuits following the CIRC decoder and are independent of the decoder. Using error flags from the CIRC decoder, the player's concealment circuits determine whether to output the data directly, to interpolate it, or to mute the output [18]. Interpolation is the technique of using appropriate data around an error to replace the erroneous data. These values are used to calculate a new value to substitute for the error. Due to the high correlation between music samples, an uncorrected error is virtually inaudible by synthesizing new data from surrounding data. Although clicks are avoided, a momentary increase in distortion is produced.

Numerous interpolation schemes are used, with different performance levels, in its simplest form; zero-order interpolation holds the previous value and repeats it to cover the missing or incorrect word. In first-order interpolation, the erroneous word is replaced with a word derived from the mean value of the previous and subsequent words.

In worst-case scenarios, where the error is so massive that even interpolation would fail, the audio signal is muted. By attenuating the signal before and after the mute, very large errors are made inaudible.

However, the digital audio signal cannot be muted by switching the bit stream to zero; this could result in an audible click. Muting methods vary depending on the player. For example, the signal may be faded down by multiplying the sample by descending coefficients, usually taken from a half cycle of a cosine waveform. The fade-out must begin prior to the bad data; this is accommodated by feeding the signal through a delay before the muting circuit. The mute signal thus arrives before the bad data. Following the bad data, a fade-in is similarly accomplished.

3.5 Cross Interleave Reed-Solomon Code (CIRC)

An efficient error-correction system known as CIRC is used in the CD and DVD systems. This correction system has a range of 4-frame correction to 16-frame correction. The code corrects for most of the errors occurring on the disk. In a case that some error patterns are not correctable, the error is detected and the decoder assists in reconstructing the sample value by interpolation. The performance of CIRC handles 1000 samples per minute and interpolates 10^{-3} Bit Error Ratio (BER) compared to the typical BER of 10^{-4}. Although the probability of an uncorrectable error being detected is nonzero leading to an audible click, the detection capability of the CIRC is designed to ensure less than one click per month at a BER of 10^{-3}. A roughly handled disk with scratches results in long burst error. The CIRC has the ability to correct burst error up to 4000 bits (2.5 mm physical displacement on a CD surface. The complexity of the CIRC is reduced by splitting the decoder into a special-purpose decoder and standard 2000 words of 8 bits. With an efficiency of 75%, the signal format of the CIRC is

designed such that for audio media a four-channel information stream is possible without any changes in the decoder [17].

3.5.1 CIRC encoder and decoder

The CIRC error correction system consists of a C1 (32, 28) and C2 (28, 24) Reed Solomon code. As illustrated in Figure 3.2, the horizontal blocks between C1 and C2 represent 8-bit wide delay lines of unequal lengths to assist in interleaving. Before the C2 encoder, a delay of one symbol is inserted in the even words for concealments. After the C1 encored a delay of one symbol (8 bits) is inserted in the even symbols for data scrambling.

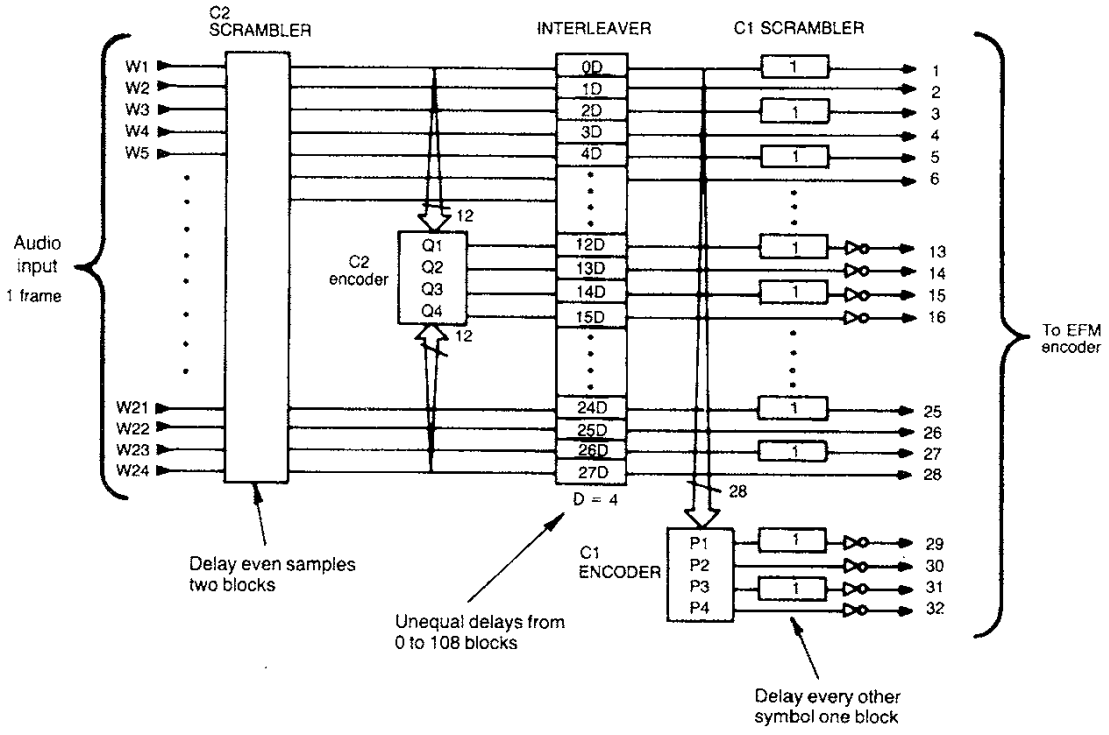


Figure 3-2: Encoder setup of the CIRC system *(Source: Digital Audio Technology (1983)., H. Nakama et al)*

The decoder setup is illustrated in Figure 3-3. The C1 decoder accepts 32 symbols of 8 bits each from which four parity symbols are used for C1 decoding. The parity is generated according to the rules of Reed Solomon coding helping C1 decoder to correct erroneous symbols in every word of 32 symbols. If more than one erroneous symbol is encountered, then regardless of the number of errors, the C1 decoder detects that it has received an uncorrectable word. In this case an erasure flag is set for each symbol to mark that all symbols from C1 are unreliable for the moment.

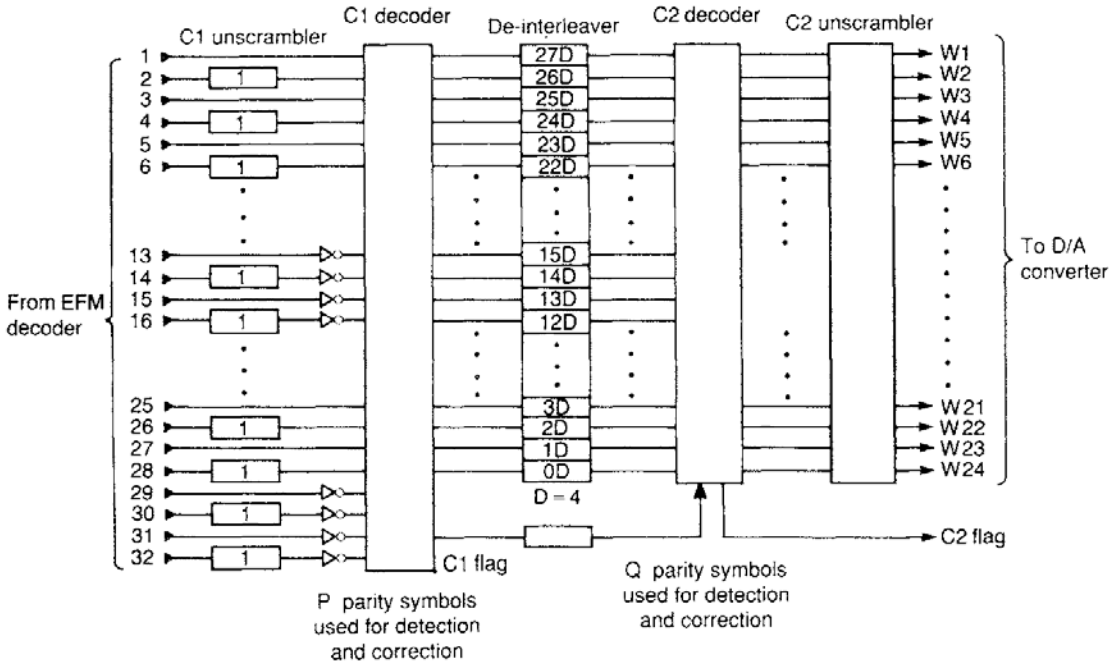


Figure 3-3: Decoder setup of the CIRC system *(Source: Digital Audio Technology (1983)., H. Nakama et al)*

Owing to the fact that the delay lines between the C1 and C2 decoders are of unequal lengths, the symbols marked with an erasure flag arrive at

different delay times at the C2 decoder input. This results in the C2 decoder possessing the information of whether every symbol is error ridden. If a symbol does not have an erasure flag it is error free. The C2 decoder can correct a maximum of 16 frames provided no more than four symbols have an erasure flag.

In the special case that even the C2 decoder cannot correct the error, the decoder lets the 24 data symbols to pass thorough uncorrected. However, these data symbols are marked with erasure flags originally marked by the C1 decoder.

4: DETECTION OF A SIMULATED BIOMARKER: OPTICALLY SHADED CD

4.1 Objective

The objective of this set of experiments was to introduce an error on a CD and obtain the corresponding electronic signal waveform. The error introduced on the CD was in the form of shaded sectors of varying angles on the polycarbonate reading side of the CD. Shading of the sectors was accomplished using a *Staedtler Lumocolor* permanent marker of red, blue and green. The electronic signature of the error, frequency of error appearance, and obtaining different signatures for different colours shaded were of interest in this experiment.

These experiments were based on the technique reported by *Radislav et al* [19], which involved the deposition of colorimetric calcium-sensitive sensor films onto a DVD, exposed to water with different concentrations of Ca^{2+}. The detection and quantization was performed in a modified DVD drive. The modifications included, the extraction of an analog signal from the photodiode of the DVD drive, before the signal is digitized. The extracted analog signal was fed into a LabVIEW based data acquisition program. Secondly, the optical disc drive was further controlled through the enhanced integrated disk electronics (EIDE) interface. The controlled parameters of the optical disk drive include positioning of the laser pickup head at any specified radial position, scanning the laser

pickup head over a range of desired radii with a controlled spatial resolution and the linear rotation velocity of the optical disk.

4.2 Materials and methods

We chose a Lite-On compact disc drive. Upon observation, it was found that the optical pick-up of the drive was connected via a 17-pin sound-cable connector to the printed circuit board of the drive. In order to reproduce the results, the RF-AC and RF-DC pins (as mentioned in the paper) on board were located. In the on board CD drive signal processing circuit the outputs of the RF-AC and RF-DC pins are fed into an RF matrix amplifier circuit that performs the RF signal processing [20]. The RF signal obtained from the optical pickup is fed to an equalizer circuit, to equalize and arithmetically amplify the signal. This output signal is known as RF-AC signal. The RF-DC signal is the output of the A, B, C, and D focusing signals, summed and amplified from the four-quadrant photodiode [21].

The RF-AC and RF-DC pins located were tapped via multi-stranded wires. A TEKTRONIX oscilloscope (TDS 210) accomplished signal monitoring. All the pins emerging from the optical pickup were traced on the circuit board and routed via multi-stranded wires. Figure 4-1 shows the wires routed from the board of the CD drive with the red circle indicating the ENBL pin.

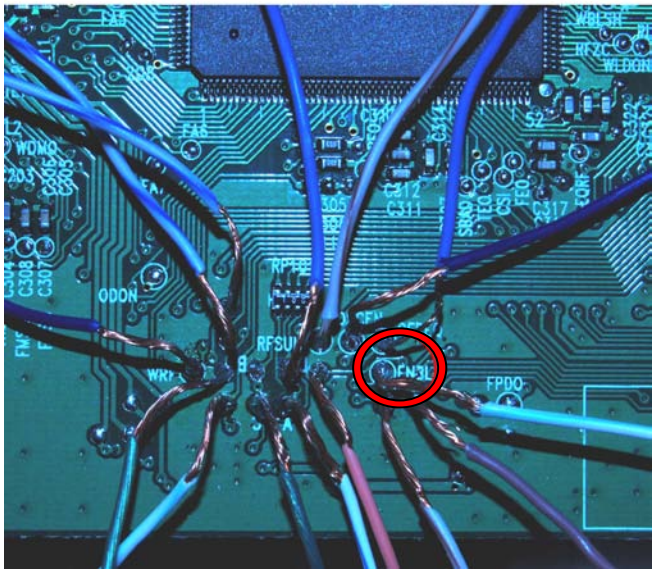


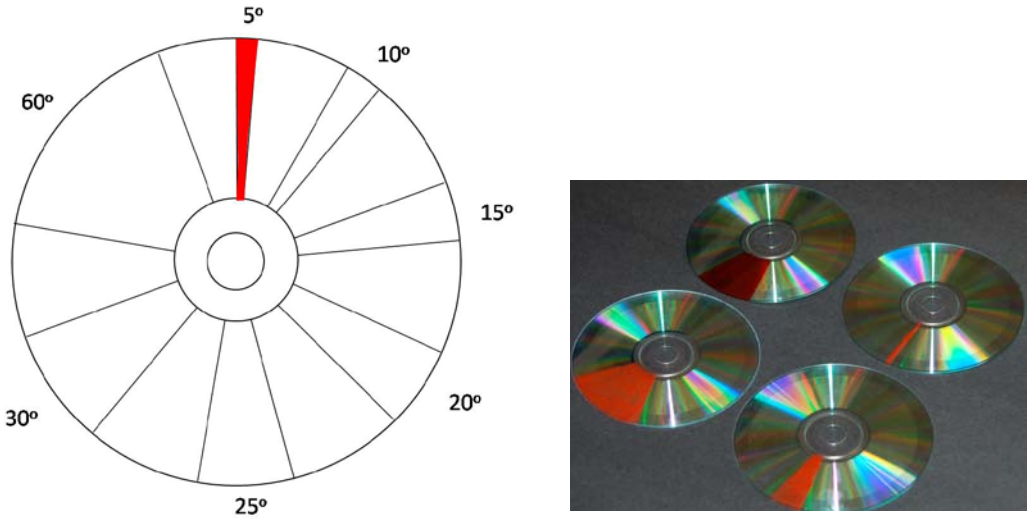
Figure 4-1: Wires routed from the CD drive (the red circle shows the ENBL pin)

Two compact discs (Princo – 700MB, 80min, once writable), were written with 43 tracks of various lengths of audio data. The tracks consisted of single tone frequencies varying from 20 Hz to 20 kHz. As aforementioned in the previous chapter, a burst error on an audio disc is accompanied by an audible click when the media is played. The particular selection of the frequencies was employed to aide audible human verification of the experimental data to observe a correlation between the frequency and the output signal of the compact disc drive. The length and type specification of the 43 tracks is illustrated in Table. 1. This scheme of audio track size and type helps to ascertain whether a particular frequency of the audio signal recorded would have a unique signature on the oscilloscope.

Table 4-1: Information schematic of Audio Tracks

Track Number	Audio type	Length of Track (minutes)
1 to 10	20Hz to 2KHz	0.5
11 to 20	20Hz to 2KHz	1.0
21 to 40	20Hz to 2KHz	2.0
41	White Noise	2.0
42	Pink Noise	2.0
43	Silence	2.0

On the polycarbonate reading side of the compact discs, sectors of different angles were marked using a paper stencil. This was followed by manually shading the sectors using a *Staedtler Lumocolor* permanent marker. One compact disc was uniformly shaded with sectors of red coloured permanent marker and the other with sectors of blue permanent marker. Figure 4-2a shows the stencil used to shade the sectors on the CD with angles subtended to the center of the circle and Figure 4-2b shows the CDs shaded with red permanent marker



(a) (b)

Figure 4-2: a) Stencil used to shade CD surface, b) Shaded CDs of sectors 5^0, 20^0, 45^0, 60^0

With the oscilloscope connected to the inputs RF-AC and RF-DC, the audio CD was read using a Lite-On disc drive. The CD drive was connected to a computer using an external USB adapter.

4.3 Observations

The output obtained, however did not match the electronic waveform presented in the paper. This could have been because the optical drive used was not the same in both experiments. Although, we did perform the same experiment on various other CD and DVD drives, the same waveform was acquired irrespective of the drive used. In continuation, a pin that had shown a change in signal as the CD was played was identified. This on board pin (ENBL)

rendered an electrical waveform with high correlation to the audio track when the media was played.

Using the CDs with shaded sectors, the signal intensity change was visible on the oscilloscope whenever the optical head passed over the shaded area. This visible change was in perfect synchronization to the audible click head as the track was being played. Momentary stopping (track change) of the audio track did not result in any change in the signal. When the track that was being played was completely stopped or paused, the audible hum of the CD drive stopped and this stopped the signal waveform too. The intensity change caused by the shaded region on the CD was observed in the oscilloscope as a visible valley with respect to the reading signal. An increase in the area of the shaded region also registered an increase in the area and amplitude of the valley. The result also showed that with increase in the track number of the audio CD the width of the valley progressively increased. The results are tabulated in Table 2. This experiment was conducted on four CDs having shaded sectors of 5^{o}, 20^{o}, 45^{o} and 60^{o}. Track 5 from the aforementioned audio CD was played and the amplitude, frequency, peak-to-peak amplitude of the electronic signal waveform was recorded. Plotting the amplitude of the ENBL pin signal waveform versus the subtended angle shaded shows a proportional variation.

Table 4-2: Signal Measurements for Track 5

Marked Area (angle subtended) in degrees	Dip width (ms)	Dip to dip width (ms)	Dip depth (mV)	Normal depth (mV)
5	1	17.3	100	44
20	1.2	8.6	112	52
45	3	17	138	42
60	3.6	17.2	158	54

Figure 4-3 shows the increase in peak amplitude of the signal waveform to the degree increase of shading and Figure 4-4 shows the increase in period of the signal waveform to the degree increase in shadings (5^{o}, 20^{o}, 45^{o} and 60^{o})

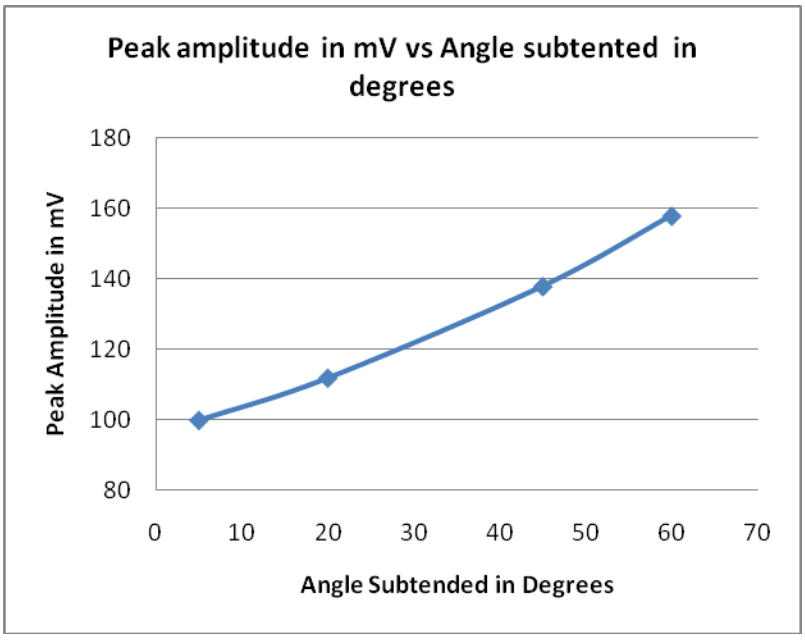


Figure 4-3: Peak amplitude obtained for angle shaded on the CD measured for Track 5

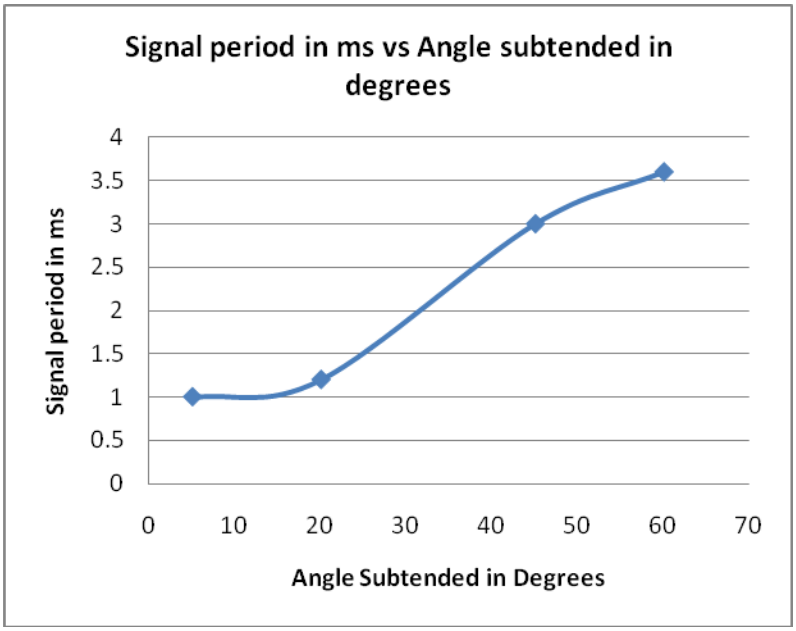


Figure 4-4: Signal period obtained for angle shaded on the CD measured for Track 5

As the tracks are radially written on a CD, an increasing track number suggests that the data position of the track would be radially outward. It was also observed that the output signal obtained was accompanied by a huge amount of noise. The detailed oscilloscope plots of the signals and the shaded angles are illustrated in Appendix B.

4.4 Analysis of the simulated biomarker using a DVD drive

The previous results shown used a CD-ROM drive to achieve error intensity detection by signal waveform on compact discs. In this section, DVD drives are used with both CD and DVD media in reference to the work reported by Radislav et al.

An LG Super Multi DVD Rewriter: GSA-H55N [22] was used. The DVD drive had RFP and RFN (RF positive and RF negative) pins on the printed circuit board. The RFP and RFN pins were traced to the optical head output and wire were soldered from these points. In addition to the RFP and RFN, the following pins – TLT+ and TLT- (tracking signals); F+ and F- (focusing signals); W1SET; WSET, W3SET AND RSET are routed to compare the output signal intensity.

On the polycarbonate reading side of the CD, sectored shadings of a 5^0 red stripe followed by a spacing of 180^0 and a blue stripe of 5^0, were shaded. The 43 audio tracks mentioned previously were written. A DVD with the same red and blue stripe configuration was also made to compare signal signatures between the CD and DVD

4.5 Signal amplification and noise control

The previous results of intensity detection resulted in a signal waveform accompanied by noise. The mitigation of noise can be achieved by passing the output signal through a low pass RC circuit to create an envelope of low frequency signals. A noise reduction circuit with an AD620 differential amplifier to amplify the generated signal and a filter circuit was designed. The filter circuit was designed with a cut-off frequency of 10 kHz. The gain, calculated using Equation 4-1 for the differential amplifier was set to 988, giving a resistance of 50Ω.

$$R_G = \frac{49.4\ k\Omega}{G-1}$$

Equation 4-1

Figure 4-5 shows the filter circuit used to reduce the noise of the output. TL074 [23] is a low noise amplifier. The 1N4148 [24], high speed diode having a forward voltage of 1V at a continuous forward current of 10mA is used.

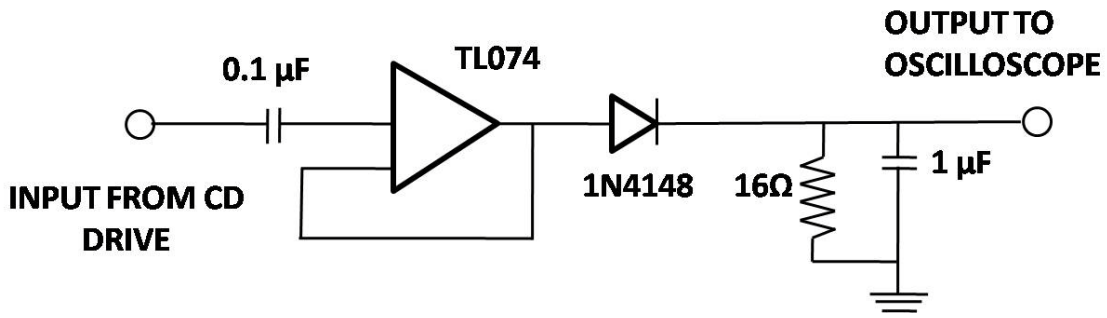


Figure 4-5: Schematic of noise reduction filter circuit

The signal waveform is compared between the circuit using only the differential amplifier and another while using the differential amplifier and filter circuit . In addition, the measurements made in the previous section with the CD were also performed using a DVD. The input signal used were the focusing signals F+ and F-, the tracking signals TLT+ and TLT-, and the RFP and RFN signals.

First, a CD was used and the initial signal using the RFN signal triggered by the focusing signal: F-, was observed in the oscilloscope. The figure 4-6 shows a lot of noise in the signal.

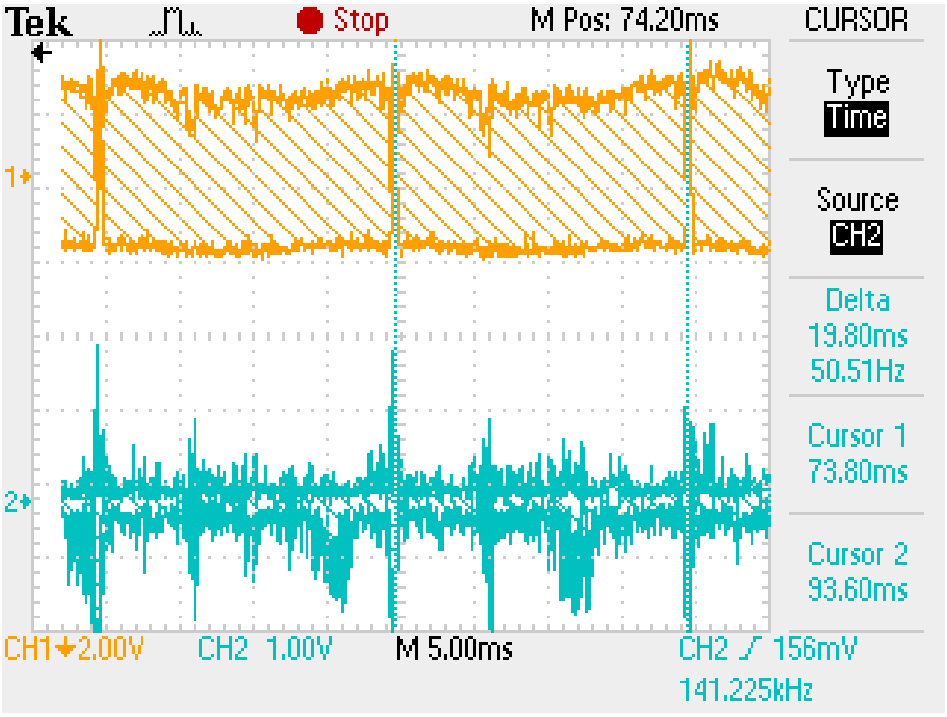


Figure 4-6: Oscilloscope plot showing signal noise (bottom signal) from pins RFN triggered by the focusing signal F-

The frequency of the shaded stripe was measured to be 50Hz for a CD and 100Hz for a DVD. The spin speed of a compact disc is between 500prm (near the center), to 200 rpm (near the circumference). Back calculating the frequency of rotation results in a frequency of 8.33Hz near the circumference and 3.33Hz near the center. For a DVD the spin speed is between 630rpm to 1530rpm, which give a calculated range of frequencies from 10.5Hz to 25.5Hz. this suggests that the signal is being modified after coming out of the optical head

The inputs were connected to the differential amplifier. With inputs as RFN and RFP, the output showed 5 vertical lines on the first channel of the

oscilloscope (output of the differential amplifier). The array of lines showed up when the optical head passed over the shaded region. Using input signals, RFN and F- the voltage measurement at the shaded region, denoted by the spike, was measured to be 79.2mV. Using input signals as RFP and F-, the voltage measurement was 77.6mV. Compared to the previous results, it was observed that the spikes for the shaded region are flipped about the X axis.

With a DVD as the detection media, the initial output was obtained with input signal RFN being triggered by the focusing signal. The voltage measurement for the spikes was 2.6V. With input signals, RFN and F- fed to the differential amplifier the output voltage measurement for the spikes was 77.6mV. Inputs signals set to RFP and F-, to the differential amplifier the output waveform showed a voltage measurement of 77.6mV for the spikes.

With a compact disc used as the media the following CD colour analysis results were obtained. With RFN and RFP signals given as inputs to the differential amplifier circuit the output of the differential amplifier was obtained. This output corresponds to the CD with a red stripe and a blue stripe of 5^0. The voltage measurement of the red spike was 4.96V and for the blue stripe, it was 4.27V. Input signals used RFN, F- gave an output voltage measurement for the red spike of 6.44V, and the blue spike showed a voltage of 6.1V. Input signal as RFP and F- had an output voltage measurement for the red strip of 4.96V and the blue strip, a voltage of 4.27V.

4.6 Observation and discussion

Table 4-3: Results of optical storage disc colour analysis

Input pins to differential amplifier	Output voltage(V) of differential amplifier using CD	Output voltage of differential amplifier using DVD
RFN and RFP	Red (5^0) = 4.96	Red (5^0) = 1.76
	Blue (5^0) = 4.27	Blue (5^0) = 1.52
RFN and F-	Red (5^0) = 6.44	Red (5^0) = 3.57
	Blue (5^0) = 6.10	Blue (5^0) = 0.44
RFP and F-	Red (5^0) = 4.96	Red (5^0) = 4.44
	Blue (5^0) = 4.27	Blue (5^0) = 2.76

With a digital versatile disc used as the media the following DVD colour analysis results were obtained. Input signal as RFN being triggered by the focusing signal showed an output waveform with voltage measurement for the spikes of 2.6V. With the input signals, RFN and F- fed to the differential amplifier, the voltage measurement for the red spike was 1.76V and for the blue stripe was 1.52V. Input signals being RFP and RFN to the differential amplifier the output waveform voltage measurement for the red spike was 3.57V and for the blue spike, it was 440mV.

Input signals, RFP and F- fed to the differential amplifier showed an output voltage for the red spike of 4.44V and for the blue spike of 2.76V. The experiments done using both the differential amplifier circuit and the filter circuit for the signal processing resulted in a lot of noise with a very low voltage range, which was not as observable as the results using only the differential amplifier. Below are the tabulated results for the various inputs used for the amplifier and their respective frequencies and voltages for both the red and blue spikes. All the plots acquired from the oscilloscope for the DVD drive optical media analysis and the DVD drive optical media colour analysis can be found in Appendix C.

Though the marked region the both CD and DVDs showed a significant change with respect to the output of the reading signal, the output analog signal was characterized by a huge amount of noise even after using a noise reduction circuit. Both CD and DVD media as well as drives were tried in this set of experiments. None of the performed configurations offered a coherent signal to perform signal analysis. Further the experiments noted a distinguishable level between red and blue marked regions. Owing to these limitations, the approach of using analog signatures to achieve biomarker detection was shifted to a software based approach. This software based detection would not have any hardware modification of the optical drive. The software based approach is detailed in the next two chapters.

5: PREPARATION AND DEPOSITION OF BIOTIN-STREPTAVIDIN BINDING ASSAYS ON A COMPACT DISC

5.1 Biotin and streptavidin

Biotin is a water soluble B-complex vitamin with a strong affinity for streptavidin [25, 26], a protein found in the bacteria *Streptomyces avidinii*. Being a tetramer, streptavidin forms a β-barrel and has four biotin binding sites in the interior of the barrel. The association between biotin and streptavidin is one of the strongest non-covalent biological interactions and has an association constant (K_a) of approximately 10^{15} M^{-1} [27]. Due to the strong affinity of biotin for streptavidin, this type of interaction is often used as a diagnostic tool in biochemical assays.

5.2 Surface chemistry procedure for streptavidin-biotin binding assay deposition on a CD

5.2.1 Reagents

1-Ethyl-3-(3'-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide (NHS), 2-(N morpholino) ethanesulfonic acid, bovine albumin, 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 MΩ·cm) from a Barnstead EasyPure UV/UF system (Dubuque, IA) was used to prepare the sample solutions.

The first step involves the UV/Ozone surface activation treatment of the CD that includes an 18-minute irradiation process followed by a 20-minute post-treatment. 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 assisting in facilitating the attachment of amino-modified probe molecules such as aminated biotin to the CD surface via amide coupling [28]. Due to the mildness of the immobilization there is no surface deformation and the CD can be read by a conventional optical drive. This is followed by the incubation step wherein the CD in is immersed in the following buffer solution for about 5 hours with intermittent agitation. The buffer solution consists of 0.1M 2-(n-morpholino) ethanesulfonic acid (MES), PH = 5.8 (0.1 × 213.25 × 0.2L = 4.26g/200mL, MES monohydrate), 80 nM EDC (0.08 × 192 × 0.012 = 0.184g) + 18mM NHS (0.024g) in 12mL MES. The CD is washed with this buffer and blow dried by nitrogen.

The deposition of the biomolecule onto the CD surface performed using microfluidic channels that create an array of binding sites for biological interactions with the assistance of two polydimethylsiloxane (PDMS) templates as shown in the figure 5-1. The PDMS templates were prepared using Sylgard 184 Silicone Elastomer Kit procured from Dow Corning Corporation. The

elastomer base and curing agent were mixed in a 6:1 ratio by weight and the mixture was then poured into a silicon mold.

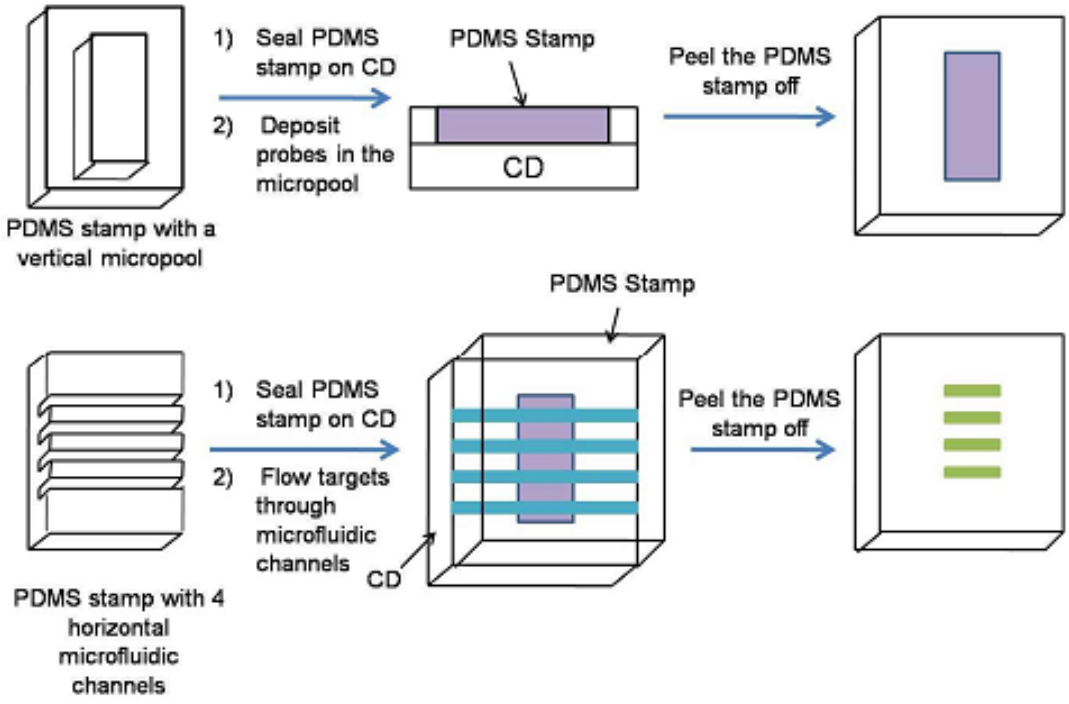


Figure 5-1: Schematic of surface patterning and activation using PDMS microfluidic channel

An aminated biotin molecule can be covalently attached to the surface of the CD. After patterns of biotin probes are generated on the activated surface employing one of the PDMS templates, the streptavidin-nanogold conjugates are introduced using the second PDMS template with microfluidic channels in a perpendicular orientation to the initial template. This allows for the binding of the streptavidin with the immobilized biotin molecules to occur only at the overlapping regions.

Following the surface activation, the NH_2-PEO-biotin probe is immobilized on the CD surface through PDMS plate. The procedure is conducted overnight spanning more than 10 hours. 20µM NH_2-PEO is pipetted into the PDMS channels and is incubated overnight in a humid box. The reaction region is "blocked" with glycogen first and then washed/immersed with 20nM phosphate buffered saline (PBS) containing 0.1% gelatine and 0.1% Tween-20 for 5 minutes. The linking of streptavidin-nanogold conjugate/streptavidin to the CD surface through streptavidin-biotin interaction achieves a streptavidin-nanogold conjugate. The CD is incubated with streptavidin-nanogold conjugate diluted to 1:100 or 1:200 in PBS containing 0.8% BSA for 60 minutes (1µL/99µL or 1µL/199µL). This is then washed with PBS buffer containing 0.1% gelatine and 0.1% Tween-20 for 5 minutes. Following the linking of streptavidin-nanogold conjugate onto the CD, the CD is washed with PBS buffer containing 0.1% gelatine and 0.1% Tween-20 for 5 minutes (blocking), then is repeatedly washed with DI water for at least 10 minutes to remove all of the ions especially chloride ions Cl^-. Enhancement of the reading signal was accomplished by promoting the deposition of silver particles of up to a few hundred nm size [29]. The silver staining is brought about by using LI Silver. As the LI Silver is stored at low temperatures, it is allowed to come to room temperature before using it. An equal amount of solutions A and B of LI Silver is mixed in the dark and the disc is immersed in this solution for 15 to 45 minutes. The development is stopped by rinsing in DI water or 2.5% sodium thiosulfate.

Figure 5-2 illustrates the completed CD with biotin-streptavidin binding assay deposition of five different concentrations (from 0.32 to 1.60 µg/mL) on each disc together with a negative control in which no biotin was deposited.

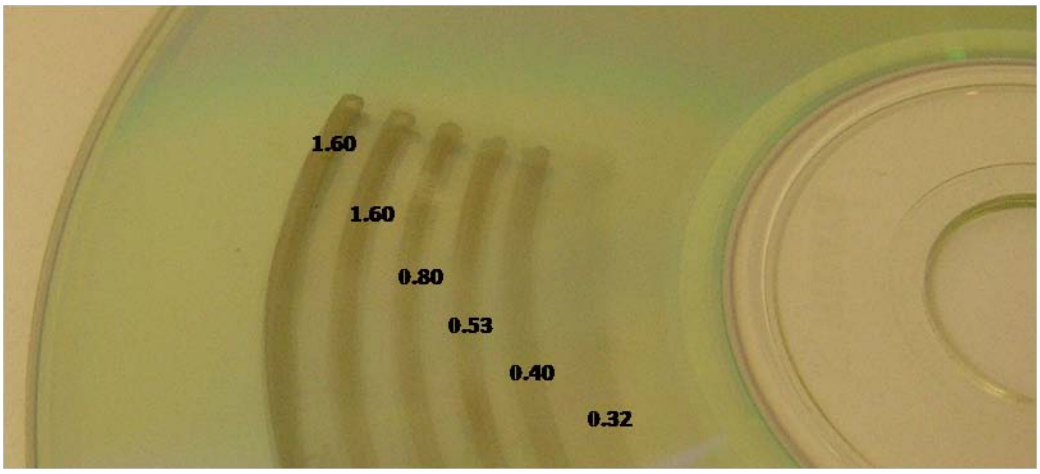


Figure 5-2: The biotin-streptavidin binding "strips" prepared on a CD; the concentrations in µg/mL

6: DISC BASED BIOASSAY READING USING ERROR CORRECTION SOFTWARE

The motivation to explore this methodology was to eliminate any hardware modification contrary to the method employed in Chapter 4, to detect the biomarker. A software-based approach provides information about the spatial location of the deposited biomarker can be determined using a software-based approach.

6.1 Error Detection using *DTI RetroBurner*

The DTI RetroBurner [30] is a program designed to help retrieve lost files from CDs and DVDs that the operating system cannot locate. The program has a feature to extract data for a specific data range of the required portions of files. This assists in the identification of the spatial location of specific sector on which the biomarker is deposited. If the error generated on the optical media by the biomarker is interpreted as an error bigger than a burst error, the software has a feature to scan the CD to recover lost files. The software also extracts data directly from an audio or data CD and converts it into hexadecimal format. A deposition of any biomarker on the reading face of the CD results in a change in the hexadecimal value of the data that is originally stored.

An audio CD was the media used to exploit the data storage schematic for pin pointing a specific location on the CD as there are no synchronization or positioning headers in the audio data. A CD of either audio or data format has

2352-byte sectors and is not a random access format like a data CD that uses 304 bytes in each sector for header, synchronization and error correction. Audio CD uses all 2352 bytes for data [34].

The audio CD written consisted of 43 tracks as mentioned in the previous chapter. The total space occupied by the tracks was 360 MB. The CD was not completely written to serve as visual demarcation between the written and unwritten parts. The demarcation also serves as the binding assay deposition location making it more convenient to observe a change in hexadecimal data only on the last track. The visual demarcation is shown in the figure 6-1.

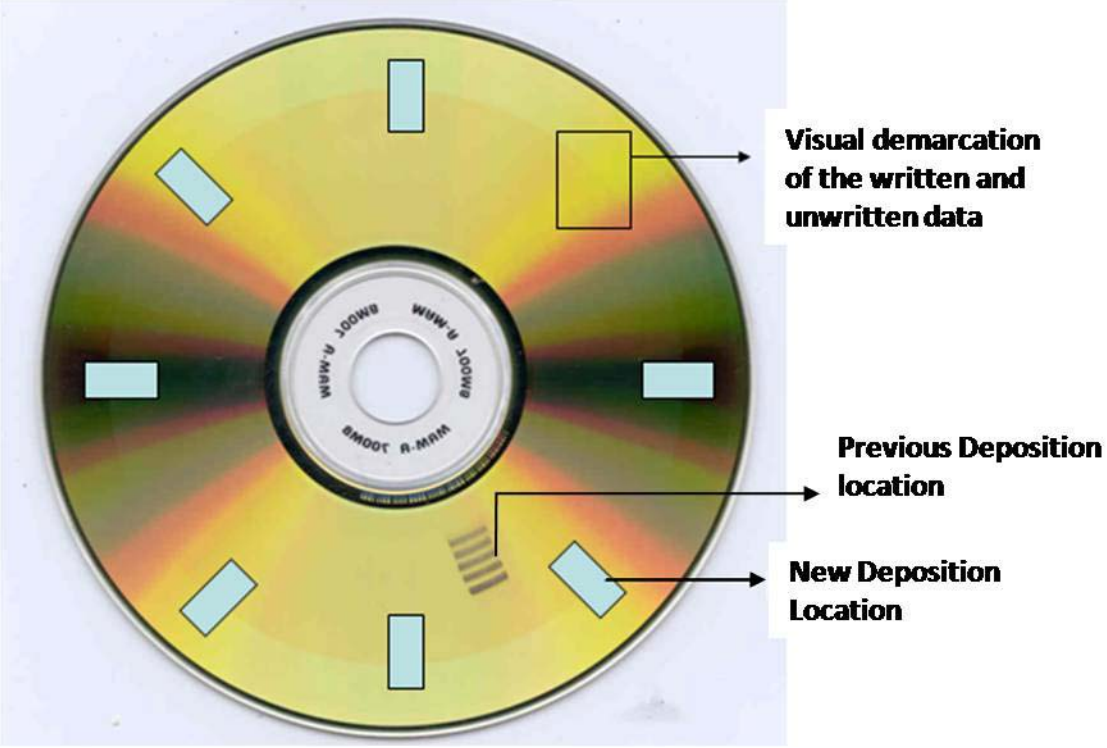


Figure 6-1: Preferred location of deposition

The snapshot of the software is shown in the figure 6-2. The software also has the ability to extract data either as RAW data format or without RAW data format. RAW data is 16-bit PCM data.

In reference to an audio CD, the software can read the hexadecimal data sector by sector. The figure 6-2 shows the hexadecimal data of the 11^{th} sector of track 1 of the audio CD. A CD with information stored in data format as opposed to audio format was also written. The hexadecimal information of the data format with respect to the RAW data and without RAW data was compared.

Figure 6-3 shows the comparison where Retro_207_noraw is the sector information without raw extraction and Retro_207_raw is the raw sector information. In both Retro_207_noraw (04F0 to 07F0) and Retro_207_raw (0630 to 0920) the column with the four digit hexadecimal numbers is the hexadecimal count of the logical block address. The block addresses, 07F0 of Retro_207_noraw and Retro_207_raw are compared. It is seen that the hexadecimal data on block 07F0 of Retro_207_noraw is found on the block 0800 of Retro_207_raw suggesting that extra information of one block is present when there is no raw extraction of data. This extra information is in the form of header, synchronization, and error correction bits.

Figure 6-2: DTI RetroBurner with an audio CD showing sector length and size

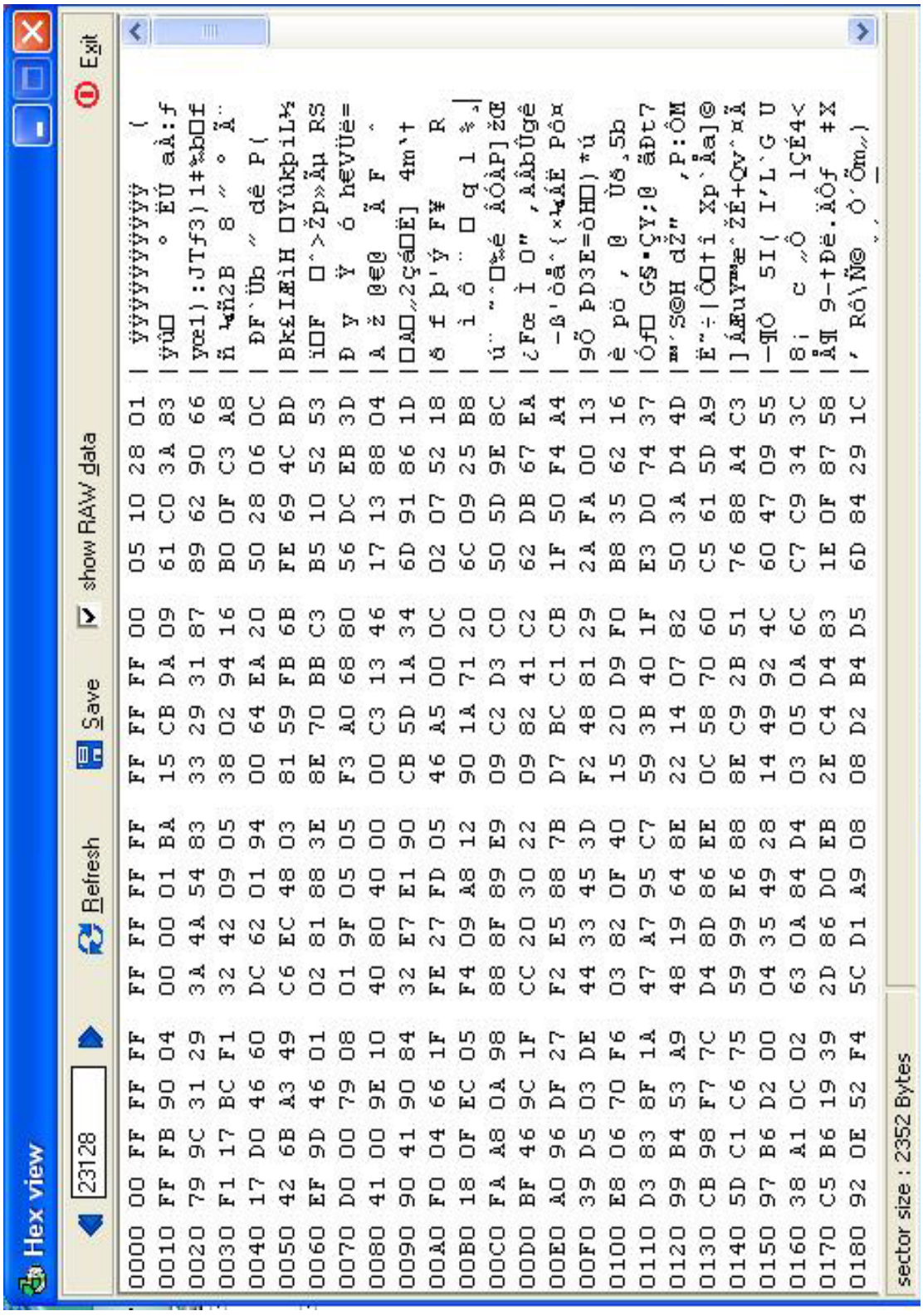
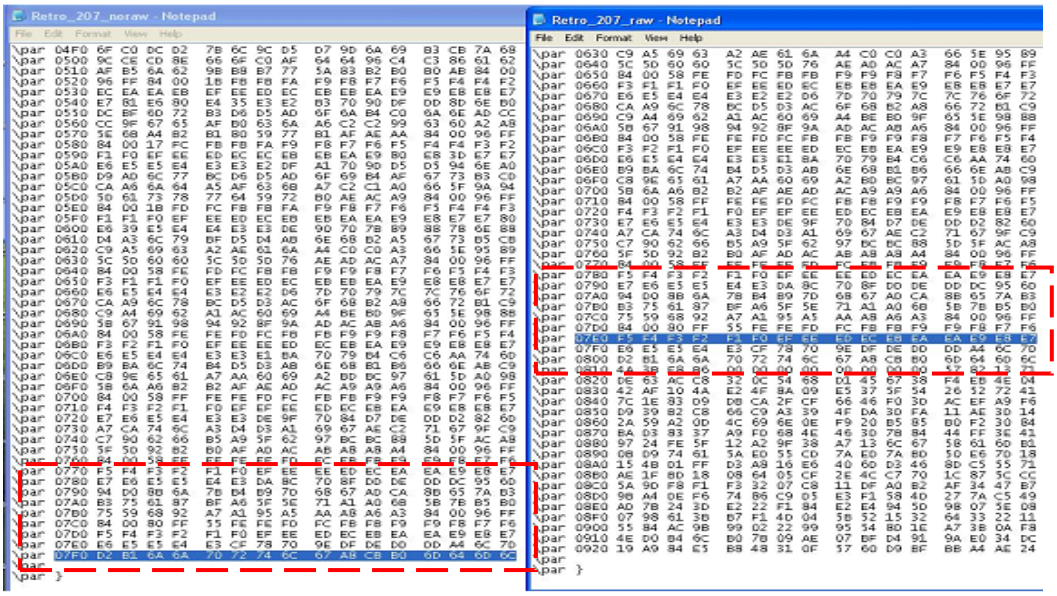
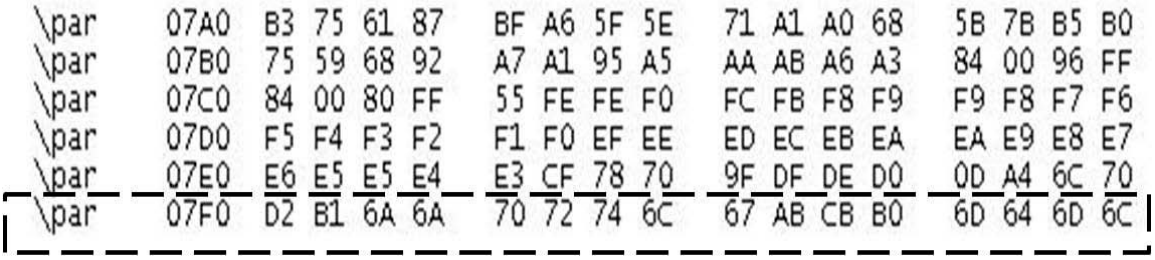


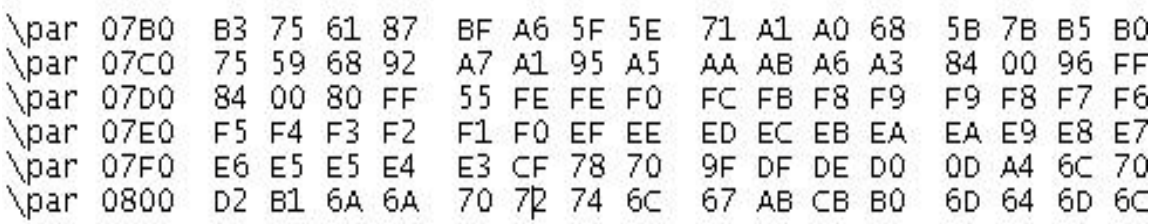
Figure 6-3: Hexadecimal data extracted from sector 21328 with RAW data using DTI RetroBurner



(a)



(b)



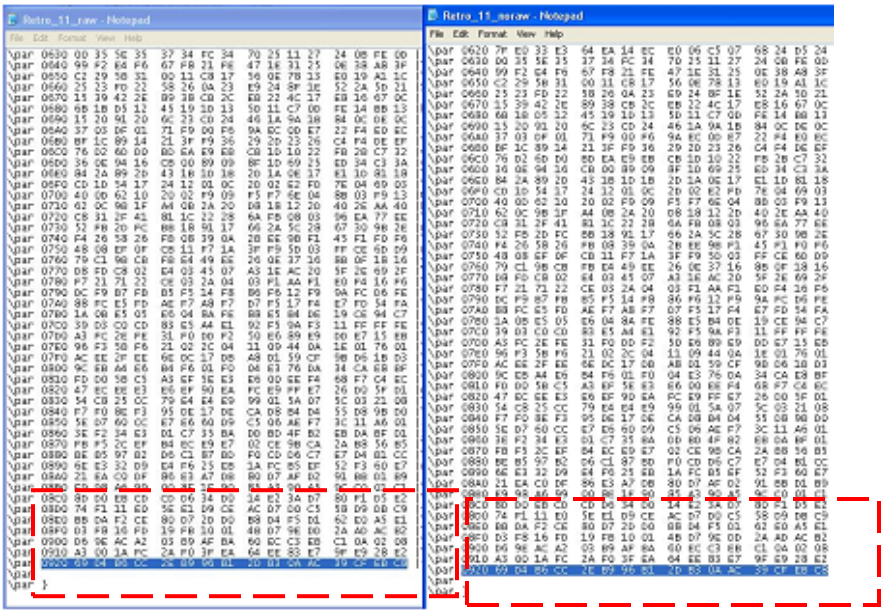
(c)

Figure 6-4: Comparison of RAW and without RAW hex data (b) Enlarged view of RAW hex data (c) Enlarged view of hex extracted without RAW data

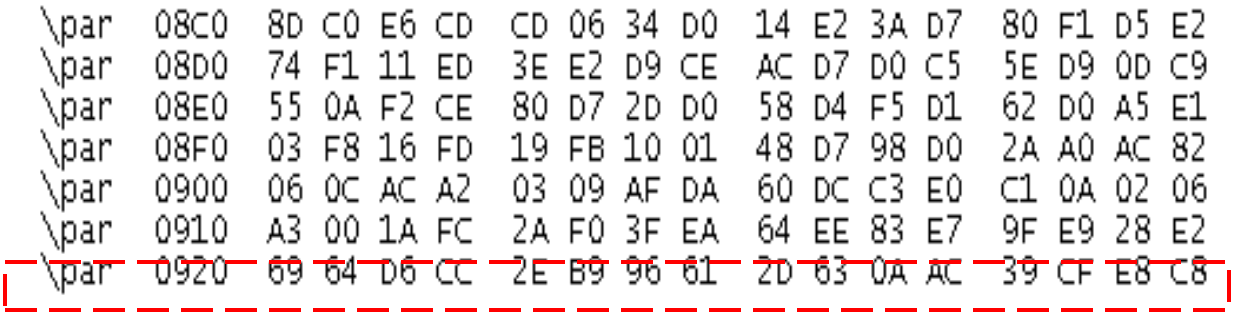
Next, an audio CD was read and the data with respect to the RAW data and without the RAW data was extracted, and compared as shown in figure 6-4. Following the same procedure as before, the hexadecimal data for sector 11 of the audio CD was compared. Here it is evident that the raw extraction data as well as the data without raw extraction are the same. This accounts for the absence of any header, synchronization and error correction bits.

The procedure to compare the hex data is to stream the data acquired from the *DTI RetroBurner* into the *Hex Comparison 2.0* [32] software. The Hex Comparison is a binary file comparison and hexadecimal editor. This is shown in the figure 6-5. The figure shows two files whose hexadecimal values are compared simultaneously. The numbers highlighted represent the difference in the hexadecimal bits.

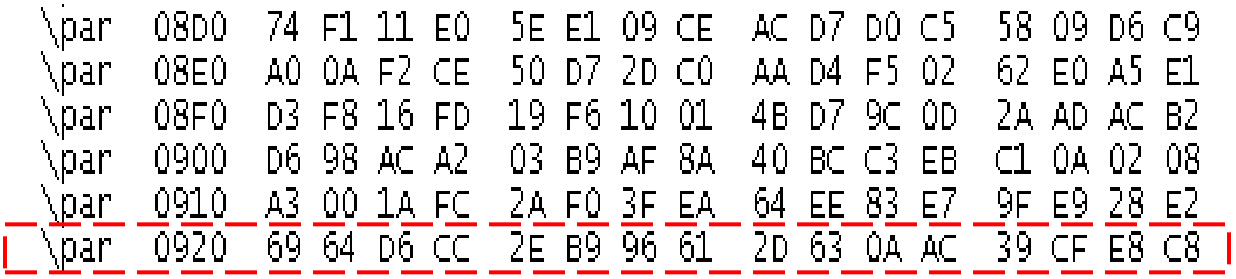
In brief, the procedure to be followed would be to extract the RAW hexadecimal data from a track of the audio CD with no deposition. Extract RAW hexadecimal data from the same track of the audio CD with deposition. Compare the output data of the two hexadecimal files to determine the location of the exact sector that contains the deposition. Finally, tabulating the difference in data of varying concentrations of biomarker to acquire a quantitative analysis of biomarker concentration.



(a)



(b)



(c)

Figure 6-5: (a) Comparison of RAW and without RAW hex data (b) Enlarged view of RAW hex data (c) Enlarged view of hex extracted without RAW data

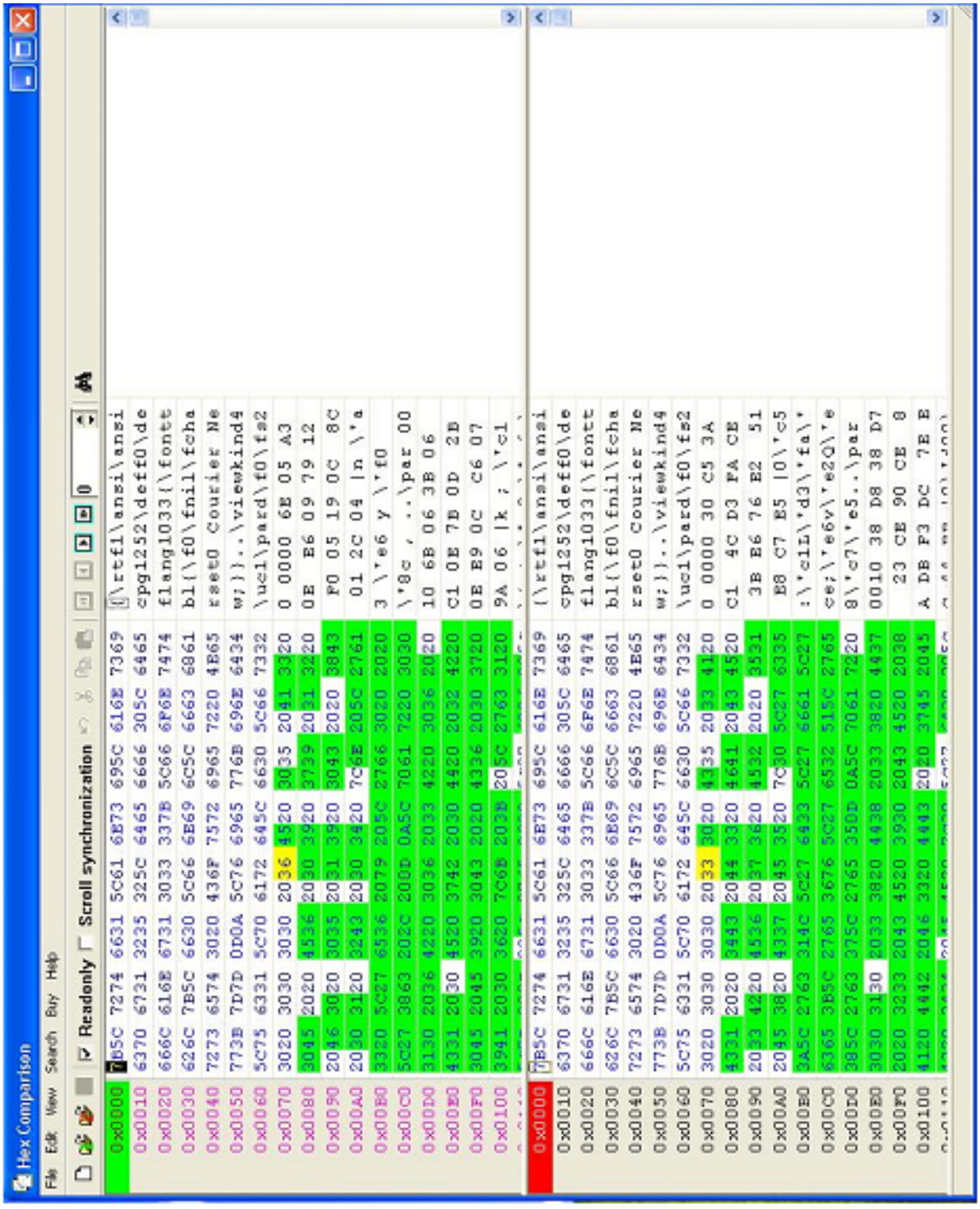


Figure 6-6: Comparison of hexadecimal data from the deposited CD to reference CD using Hex Comparison 2.0

6.1.1 Limitations

The audio CDs were deposited with biomarker: biotin-streptavidin and detected using the aforementioned procedure. Examining the tabulated difference in hexadecimal data did not reveal any definite trend. The difference was random and could not be mapped against concentration. This lead to the need for a program that can detect the errors caused by the deposition; locate the errors in terms of sector addresses and quantize the number of errors to result in a mapping of errors versus concentration. A program known as IsoBuster was identified to perform these actions. The details, working and procedure of the experiments using IsoBuster is described in the next chapter.

7: ERROR DETECTION USING *ISOBUSTER*

7.1.1 IsoBuster

The *IsoBuster* [33] software is a CD/DVD/BD/HD DVD data recovery tool that is compatible with multi-file systems, multi-hard media (CD/DVD/BD/HD DVD) and multi-soft media (CD and DVD data formats used). It has the ability to perform surface scans on any type of hard medium (CD/DVD/BD/HD DVD) to check for physical reading errors. The data written on a disc is distinguished into either Audio Tracks or Data Tracks. It identifies and saves lists of all the files that contain physical read errors. This utility is further exploited by the *Single Sector Extraction* utility that enables viewing of the exact sector and its erroneous data. The *Sector Viewer* gives the logical block address to locate the erroneous sector. It also displays the hexadecimal data of the sector, allowing a comparison of the erroneous sector data with the reference data. IsoBuster, provides the advantage of acquiring the positional and numeric quantization of the errors resulting from the binding “strips”, as well as the freedom to use data formats other than audio data.

7.1.2 Error detection

IsoBuster includes an option to *create a list of all erroneous sectors* of a file, track session or entire medium. Though this option displays the list of erroneous sectors, the error will be detected only if the sector was truly read. The

comprehensive procedure that we employed included performing a *full surface scan* followed by *extraction of the raw data* from an erroneous file.

7.1.3 Error analysis

The first line of analysis involves performing a surface scan on the CD-R upon binding the biomolecules. This flags the tracks that are error affected and the total number of errors occurring on the entire disc. To ascertain the exact number of sectors affected and their location, the error-affected tracks are subjected to an extraction function. This function scans through the affected track and creates a list of erroneous sectors, and gives an option of extracting the file with RAW data or without RAW data. When the function encounters an erroneous sector it provides the following options:

1. Omit the error affected sector with no substituting data written for erroneous sectors
2. Replace the entire sector with zeros while keeping the sector size intact
3. Replace the error ridden sector with user data all zeroes which implies the raw data (sync bytes, header, sub headers, etc.) are intact and the actual user data contains all zeroes
4. Replace the data without raw data and recreate errors

To ensure that the error shown is genuine, the sector view showing the hexadecimal data of the erroneous sector was compared with the hexadecimal

data of the same sector present on the reference CD. Comparison between unaffected sectors and the sectors with the same logical block address (LBA) form the reference CD showed no change in hexadecimal data.

The detection of biomolecules would be done by analyzing the change in the hexadecimal data before and after deposition. This procedure would be followed with different depositions with varying concentrations and the resulting hexadecimal data will be analyzed.

7.2 Media used

A 700 MB compact disc (*RiDATA, Silver-Silver CD-R)* was written with audio data using *Nero 7 Ultra Edition* optical data burning software. This data consisted of a single audio file of size 5 MB repeated 60 times (total memory occupied: 300MB). Three copies of the CD-Rs were made. Two of the CDs were used for detection and the third was used to serve as the reference to compare the hexadecimal data of the erroneous sectors with the clean sectors. The two sample CDs were tested with five different concentrations each of streptavidin.

8: RESULTS AND DISCUSSION

8.1 Biotin-streptavidin Biomarker deposited compact discs

Two compact discs were used to prepare biotin-streptavidin binding assays. Each compact disc had six arcs radially outward with concentrations varying from low to high. The original concentration was 80 microgram per mL. Five concentrations of streptavidin were prepared through dilution. CD-1 and CD-2 had the outer two strips with the same concentration.

For CD-1, the prepared original biomarker was diluted into 5 samples in steps of 50 times the original concentration (50X to 250X). This results in a concentration range of 1.6 µg/mL to 0.32 µg/mL. For CD-2, the prepared original biomarker was diluted into 5 samples in steps of 60 times the original concentration (60X to 300X). This results in a concentration range of 1.33 µg/mL to 0.27 µg/mL.

Table 8-1: Biotin-streptavidin concentration schematic for deposition

	CD 1		CD 2	
	Dilution of conjugates	Concentration in µg/mL	Dilution	Concentration in µg/mL
1	250x	0.32	300x	0.27
2	200x	0.40	240x	0.33
3	150x	0.53	180x	0.44
4	100x	0.80	120x	0.67
5	50x	1.60	60x	1.33
6	50x	1.60	60x	1.33

8.2 Detection and quantization of the biotin-streptavin biomarker

These two biomarker deposited compact discs were thoroughly analyzed using IsoBuster. A physical readability scan was first performed on the compact discs. This gives the total number of errors encountered on the disc as shown in Figure 8-4. On the first CD 1508 erroneous sectors, as shown in the figure were detected. *IsoBuster* gives the option of scanning the error affected tracks to procure the exact number of erroneous sectors.

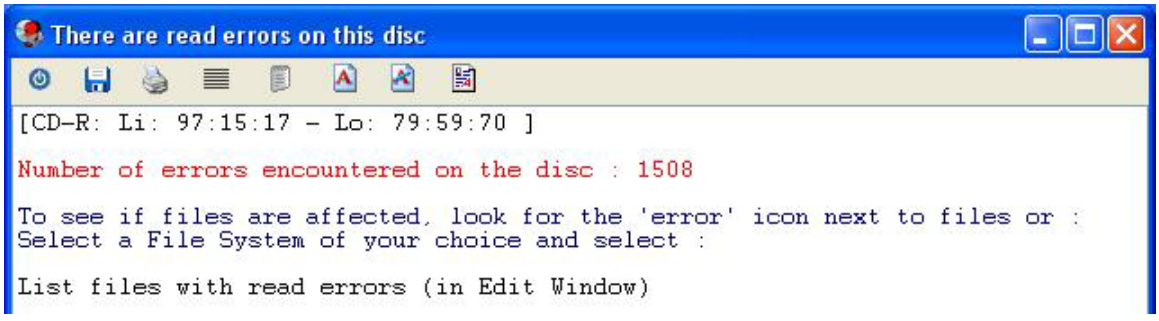
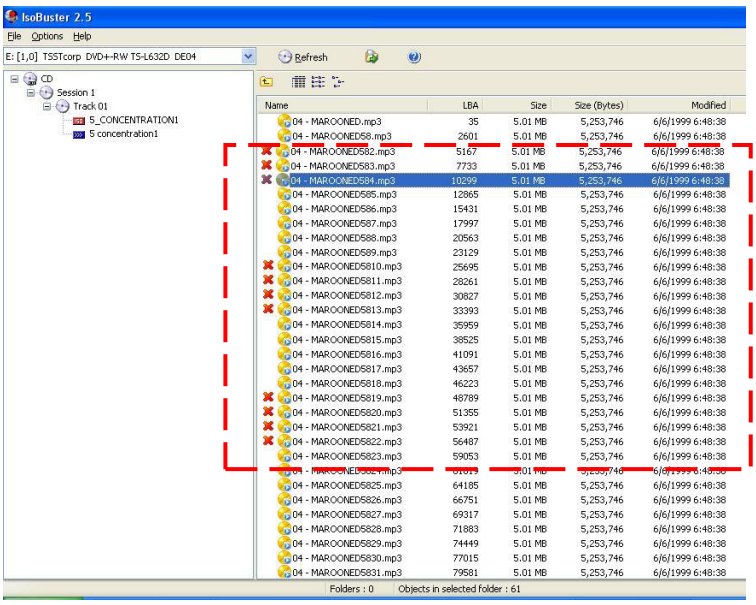
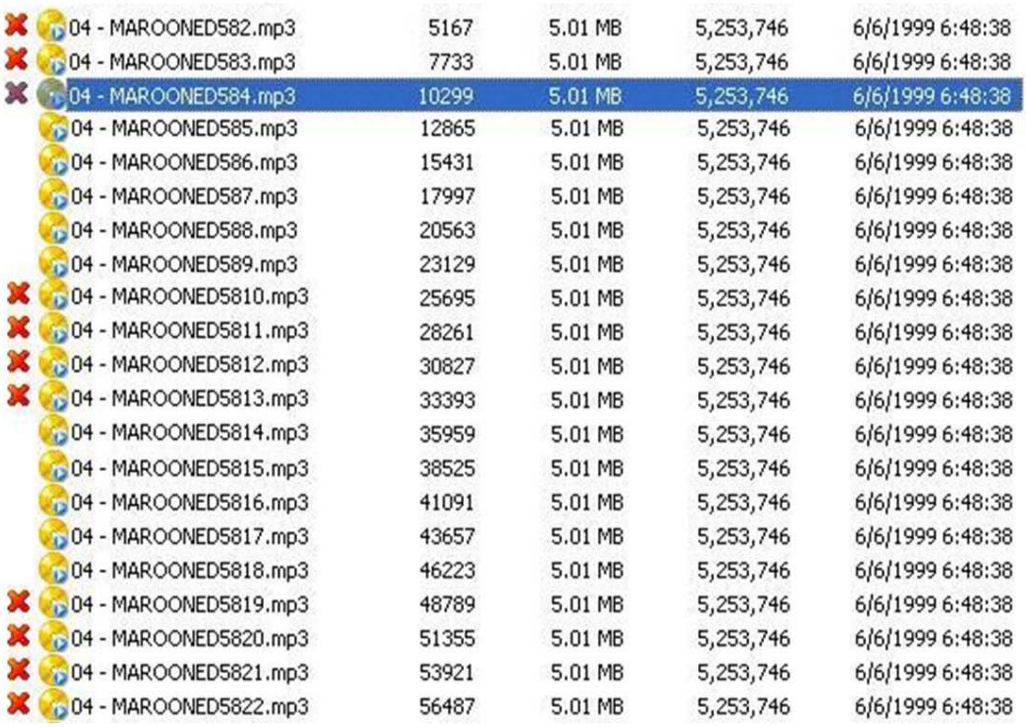


Figure 8-1: Error report showing the number of errors using IsoBuster

The program also shows a list of the tracks, which contained erroneous sectors. Figure 8-2 shows the tracks affected by the concentrations 1.6, 0.8 and 0.53 µg/mL. To acquire the exact number of errors and the sector location of the errors in each track, the error-flagged tracks were further scanned using the extract file option of the program. Extracting each track was performed with the "replace with erroneous sectors" option selected to view the errors present due to the deposited biomarker as illustrated in figure 8-3.



(a)



(b)

Figure 8-2: (a) Grouped errors after error detection using IsoBuster (b) Englarged view showing the errors due to the last 3 depositions

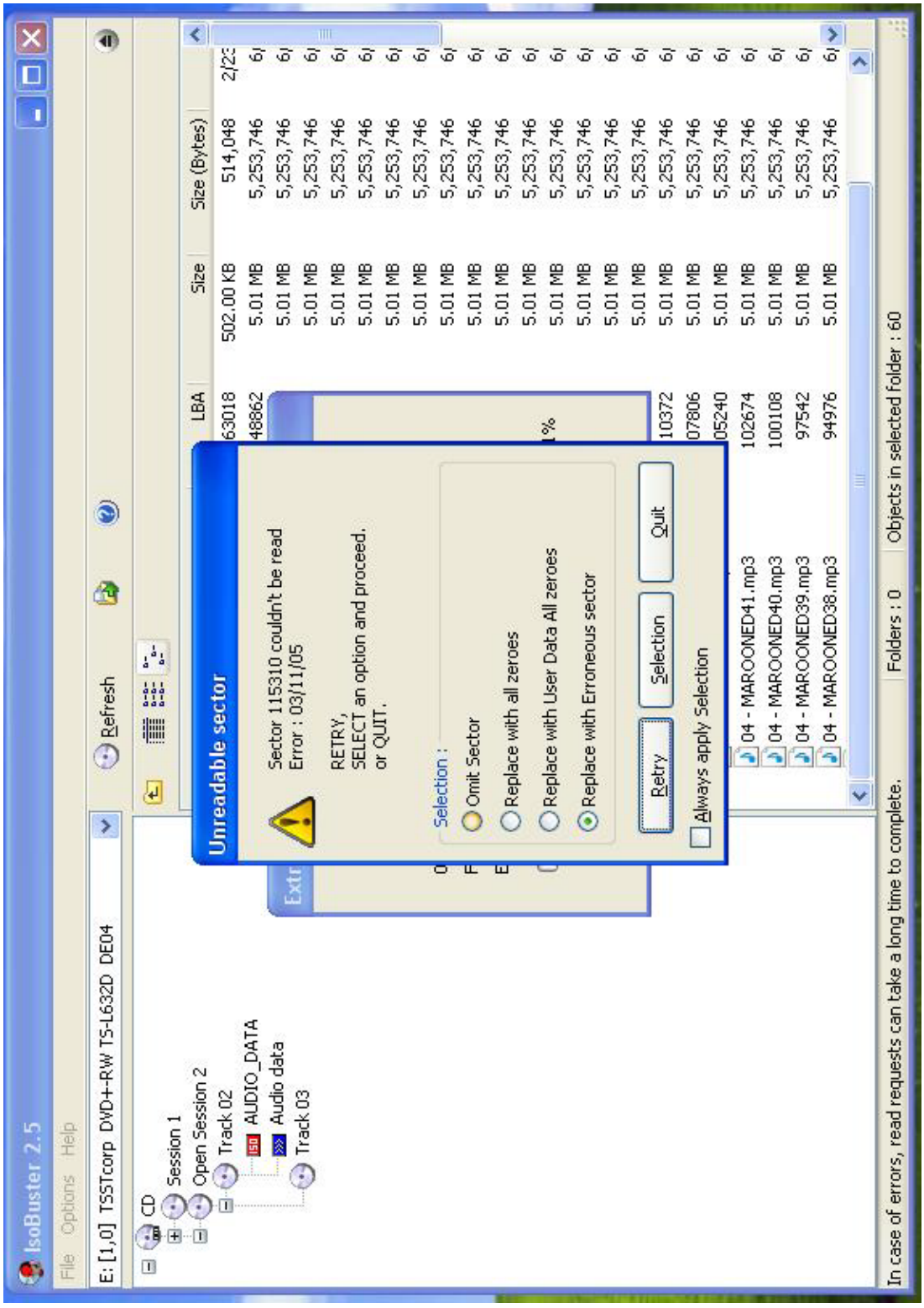


Figure 8-3: IsoBuster showing options to read an unreadable sector affected by errors.

The number of errors detected for each track, with their sector numbers for CD-1 were tabulated. The data of the errors detected versus the concentration of the biomarker is plotted in figure 8-4. Similarly, the error detected versus the biomarker concentration in µg/mL was plotted. A trend similar to the results of CD-2 was observed which is shown in figure 8-5.

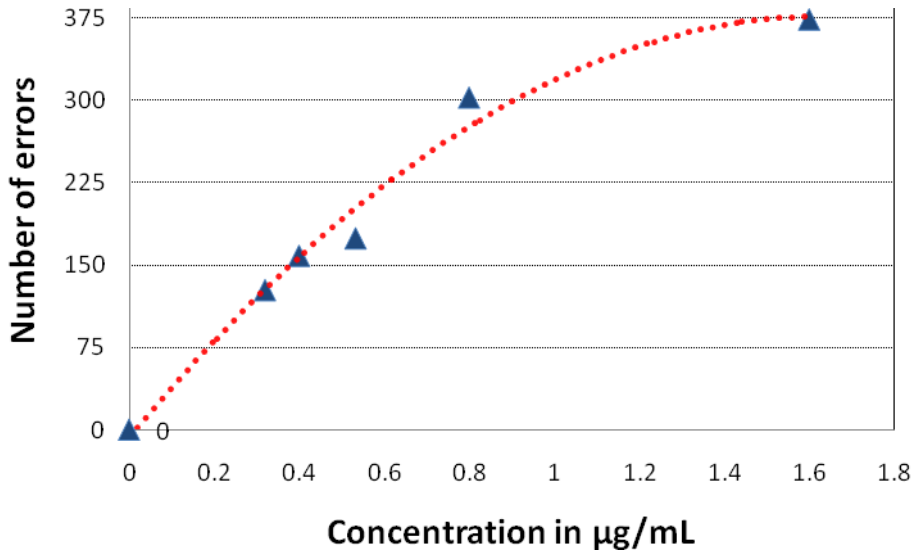
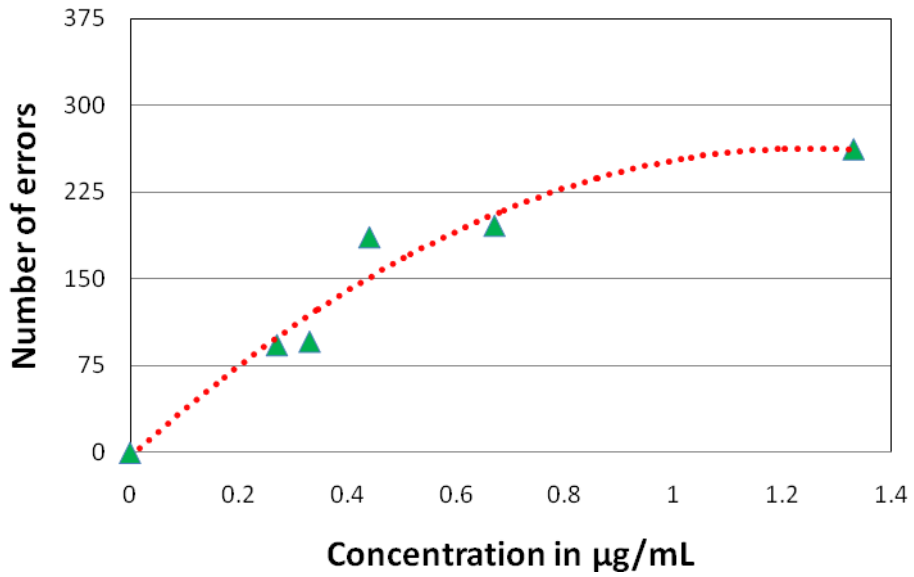


Figure 8-4: Error versus concentration after surface scan of CD-1 with *IsoBuster*



.

Figure 8-5: Errors versus concentration after surface scan of CD-2 with *IsoBuster*

The error-affected sectors were further extracted for hexadecimal data. Error-affected sector numbers from both CD-1 and CD-2 were compared with the reference CD that had no deposition on it. It was found that the sector hexadecimal data between CD-1, CD-2 and the reference CD was not equal. Further, an unaffected or error free sector from CD-1 and CD-2 was selected and compared with the reference CD. It was observed that the hexadecimal sector data was the same in all the three sectors.

Following this set of experiments, three independent CDs deposited with streptavidin-gold nanoparticle conjugates were tested for error sectors versus concentration. The concentration range of the three CDs was 0.32 to 1.6 µg/ml. The number of error sectors affected in a group (corresponding to a specific binding “strip”) and the concentration of streptavidin were plotted as shown in

Figure 8-6. The graph shows a clear monotonic increase as the concentration of target streptavidin increases.

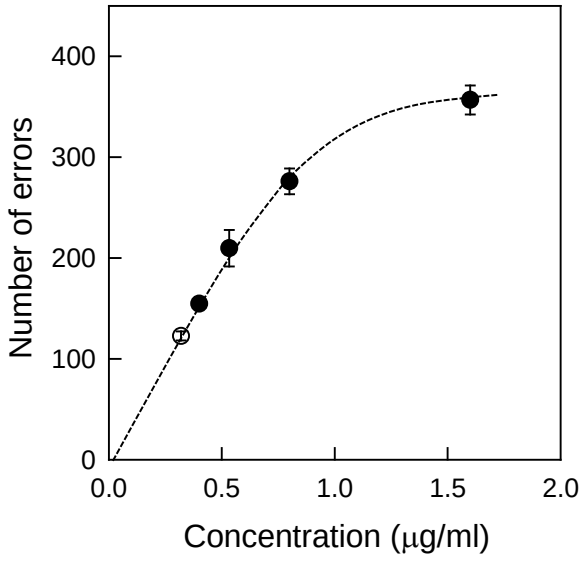


Figure 8-6: Number of error sectors versus concentration of streptavidin-gold nanoparticle conjugates repeated over the course of three independent CD's. The concentration range is 0.32 – 1.6 μg/ml [31]

9: SUMMARY

The work described in this thesis demonstrates the readout and quantization of biotin-streptavidin binding assays deposited on a compact disc. Initially, a hardware-based approach was explored. This method resulted in an electrical signature with lot of noise. Using a filter circuit did not improve the signal to a range that could be used for detection. Further, a software-based approach for error detection was implemented. The process uses a DVD drive with no hardware modifications. The errors detected were quantized using the program, IsoBuster, to obtain a correlation between errors detected versus the concentration of the biomarker. A dynamic range of detection of 0.27µg/mL to 1.60 µg/mL was achieved. 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 specific erroneous bits rather than the total error numbers per frame. Most importantly, the reading can be done with a conventional optical drive without any modification to either the hardware or the software driver, thus augmenting the potential applications in running bioassays with consumer electronic products for biomedical diagnostics.

9.1 Future work

The work describes detection of a concentration range of 0.27 µg/mL to 1.60 µg/mL. However, further we would like to find the limit of detection of the biomarker biotin-streptavidin using our procedure. Exploring other biomarkers and biomolecules for detection can be achieved. By using a double sided DVD a large microarray of a number of biomarkers can be deposited and detected, thus achieving a high-density, low biological and chemical volume detection media. This leads to a low cost; fast turnaround lab on a chip protocol.

9.2 Conclusion

Ten different concentrations of streptavidin ranging from 0.32 µg/mL to 1.60 µg/mL were deposited on two Silver-Silver CD-R discs and the deposited CDs were detected using *IsoBuster*. The exact erroneous sectors caused by six strips of different biotin-streptavidin concentrations were logged. Comparison of the raw hexadecimal data of the erroneous sectors with the clean sectors of the same logical block address showed a change in data suggesting the presence of errors. In contrast, data from the reference disc showed no changes. This technique exhibits the ability to detect the exact location of the deposited conjugates and quantize the errors in relation to their concentration. The graph of erroneous sectors versus concentration showed a curvilinear increase of errors with concentration. Further experiments are being performed to set up a high-density microarray for biomolecule or pathogen detection for screening

APPENDICES

Appendix A: Oscilloscope plots showing the signal obtained from simulated biomarker detection using a CD as the media

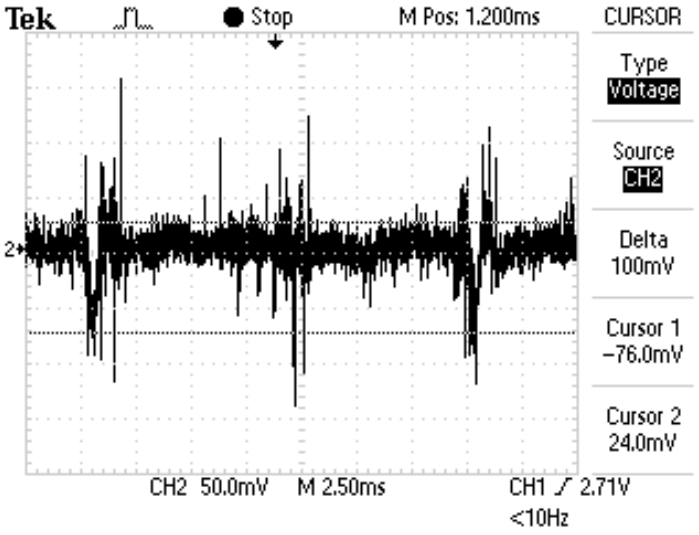


Figure 9-1: 5^0 sector with peak amplitude of 100mV

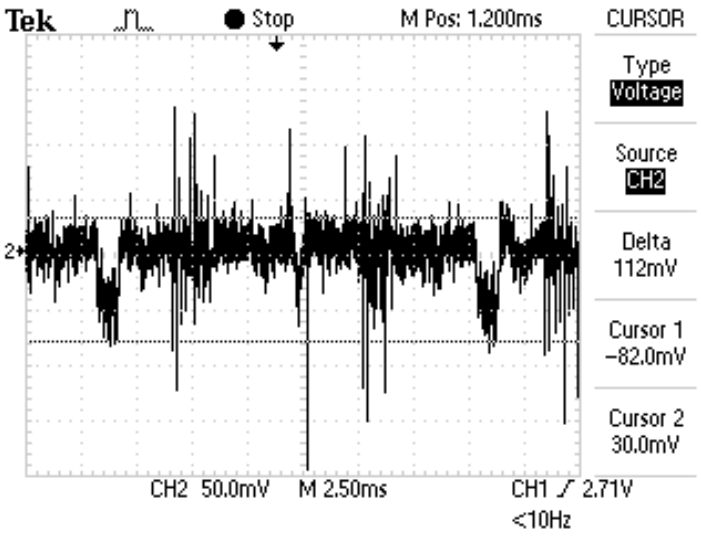


Figure 9-2: 20^0 sector with peak amplitude of 112mV

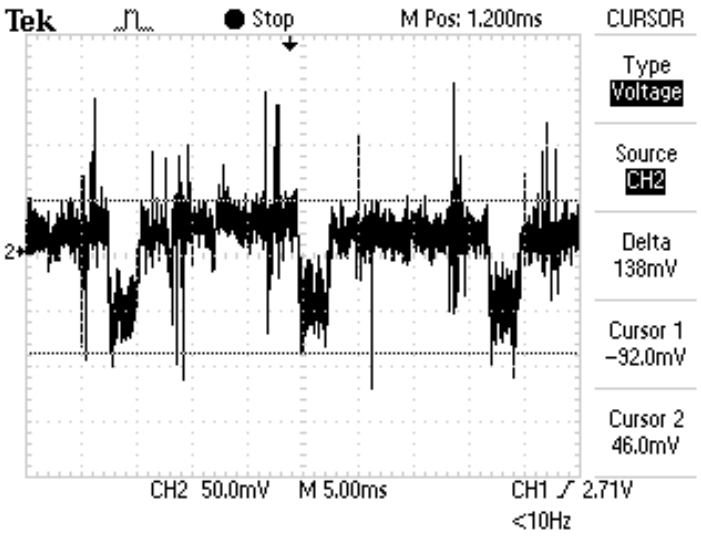


Figure 9-3: 45^0 sector with peak amplitude of 138mV

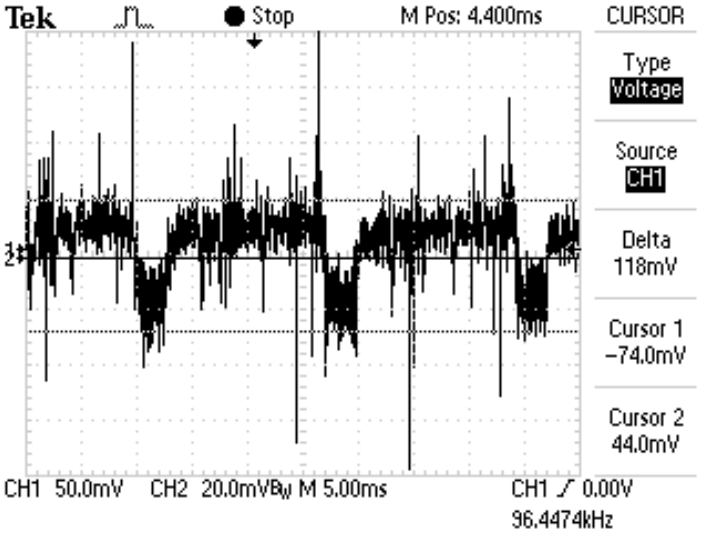


Figure 9-4: 60^0 sector with peak amplitude of 158mV

10: APPENDIX B:

Oscilloscope plots showing the signal obtained from simulated biomarker detection using a DVD as the media Oscilloscope plots

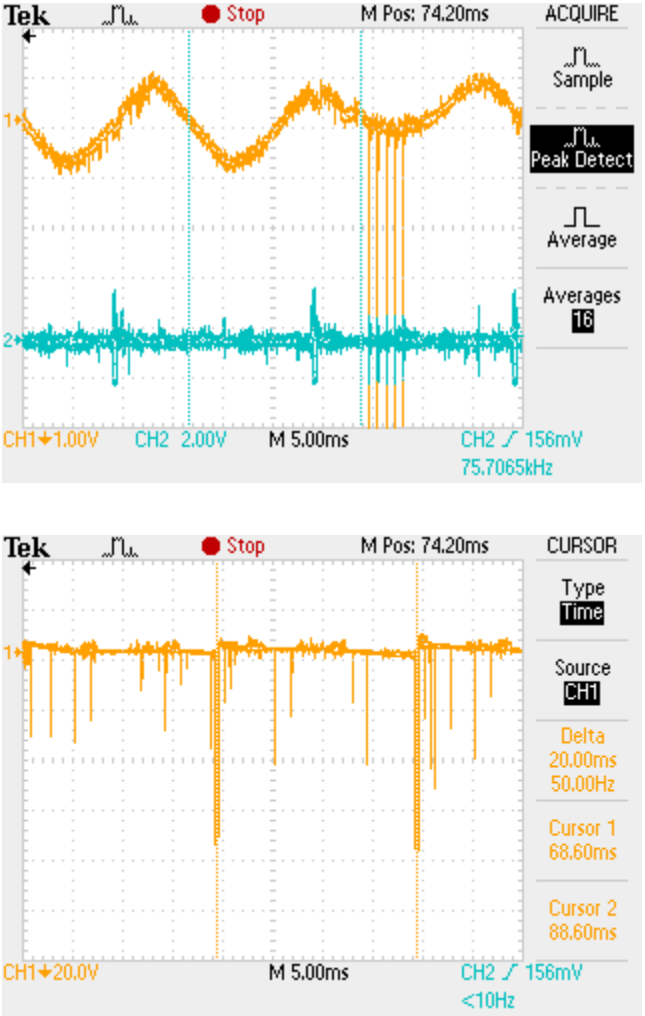


Figure 10-1: DVD media being used with RFN and F- pins as inputs to oscilloscope

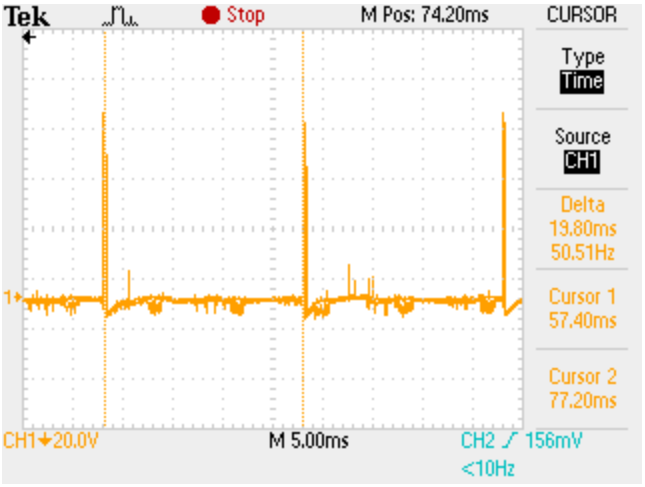


Figure 10-2: RFP and F- as inputs: 50Hz frequency shown by each shading

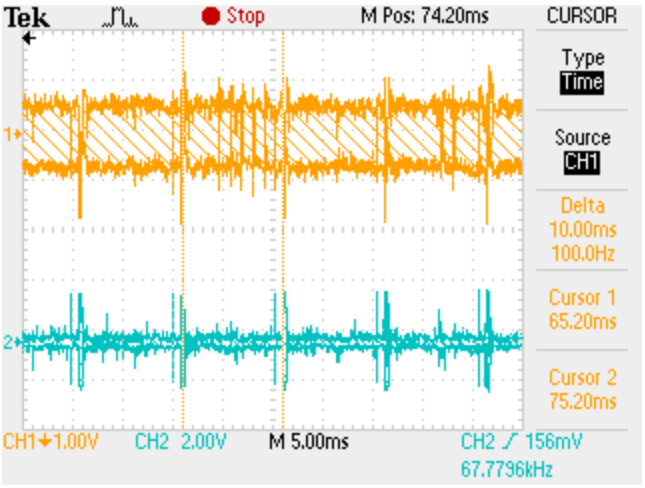
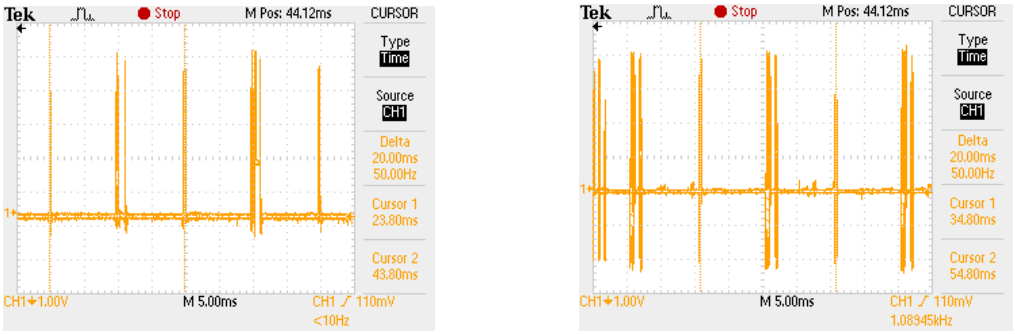
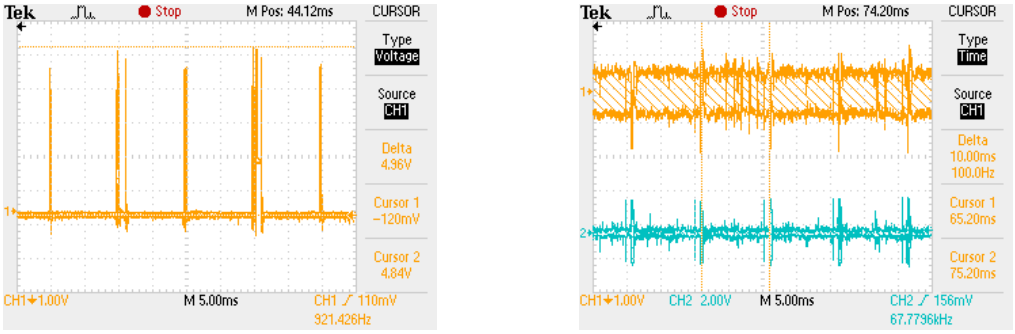


Figure 10-3: RFN triggered by F- as inputs: Channel 1: Blue shading Channel 2: Red shading



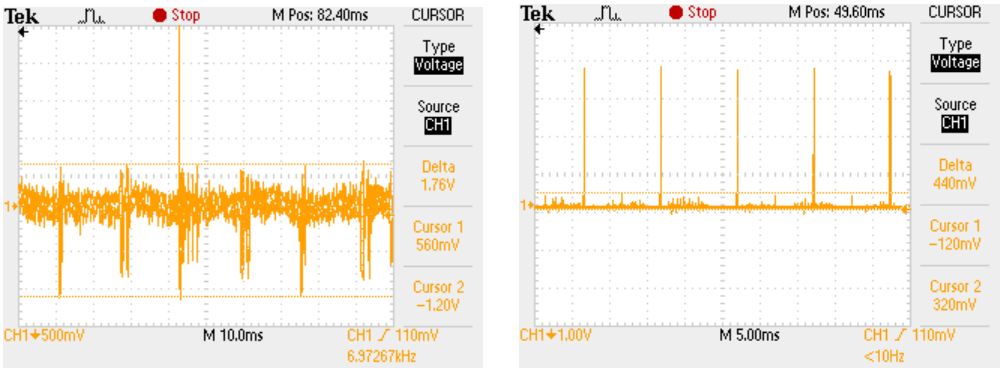
(a) (b)

Figure 10-4: RFN and RFP as inputs: (a) Red spike with 4.96V, Blue spike 4.27V (b) Red spike with 6.44V, Blue spike with 6.1 V



(a) (b)

Figure 10-5: RFP and F- as inputs as inputs: (a) Red spike with 4.96V, Blue spike 4.27V (b) with DVD media 1.2V



(a) (b)

Figure 10-6: RFN and F- as inputs as inputs using DVD: (a) Red spike: 1.76V Blue spike: 1.52V (b) RFN and F- as inputs: Red spike: 3.57V Blue spike: 440mV

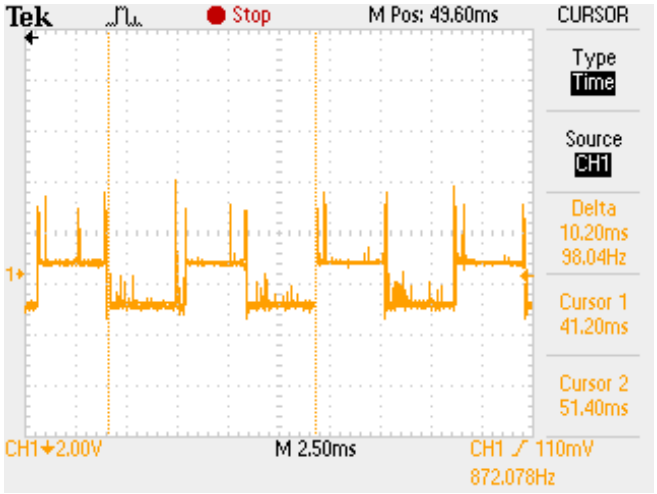


Figure 10-7: RFP and F- fed to differential amplifier: Red spike: 4.44V Blue spike 2.76V

11: APPENDIX C

Physical characteristics of a Digital Versatile Disc [35]

	DVD	**CD**
Thickness	1.2 mm	1.2 mm
Mass	13 to 20 g	14 to 33g
Diameter	120 or 80 mm	120 or 80 mm
Spindle hole diameter	15 mm	15 mm
Lead-in diameter	45.2 to 48 mm	46 to 50 mm
Data diameter (12cm)	48 to 116 mm	50 to 116 mm
Data diameter (8cm)	48 to 76 mm	50 to 76 mm
Lead-out diameter	70 to 117 mm	76 to 117 mm
Outer guardband diameter (12cm)	117 to 120 mm	117 to 120 mm
Outer guardband diameter (8cm)	77 to 80 mm	77 to 80 mm

Reflectivity (full)	45 to 85 percent	70 percent minimum
Readout wavelength	650 or 635 nm	780 nm
Numerical aperture	0.60	0.38 to 0.45
Focus depth	0.47	1
Track pitch	0.74 µm	1.6 µm
Pit length	0.400 to 1.866 µm (SL) 0.440 to 2.054 µm (DL)	0.833 to 3.054 µm (1.2 m/s) 0.972 to 3.560 µm (1.4 m/s)
Data bit length	0.2667 µm (SL) 0.2934 µm (DL)	0.6 µm (1.2 m/s) 0.7 µm (1.4 m/s)
Channel bit length	0.1333 µm (SL) 0.1467 µm (DL)	0.3 µm
Modulation	8/16	8/14 (8/17 with merge bits)
Error correction	RS-PC	CIRC
Error correction overhead	13 percent	23/34 percent

Correctable error (1 layer)	6 mm (SL), 6.5 (DL)	25 mm
Speed (rotational)	570 to 1600 RPM	200 to 500 RPM
Speed (scanning)	3.49 m/s (SL) 3.84 m/s (DL)	1.2 to 1.4 m/s
Channel data rate	26.16 Mbps	4.3218 Mbps
User data rate	11.08 Mbps	1.41/1.23 Mbps
User data: channel data	2048:4836	2352:7203/ 2048:7203
Format overhead	136 percent	206/252 percent
Capacity	1.4 to 8.0 GB per side	0.783/0.635 GB

REFERENCE LIST

[1] H. Kido, A. Maquieira and B. D. Hammock, “Disc-based immunoassay microarrays”, *Analytica Chimica Acta 411*, pp 1–11, 2000.

[2] I. Alexandre, Y. Houbion, J. Collet, S. Hamels, J. Demarteau, J. –L. Gala and J. Remacle **“**Compact disc with both numeric and genomic information as DNA microarray platform”, *BioTechniques 33*: vol. 33, no. 2, pp 435-439, 2002.

[3] R. Barathur, J. Bookout, S. Sreevatsan, J. Gordon, M. Werner, G. Thor and M. Worthington, “New disc-based technologies for diagnostic and research applications” *Psychiatric Genetics*, vol. 12 no 4, pp 193–206, 2002.

[4] S. A. Lange, G. Roth, S Wittemann, T. Lacoste, A. Vetter, J. Grassle, S. Kopta, M. Kolleck, B. Breitinger, M. Wick, J.K. Heinrich Horber, S. Dubel, A. Bernard, “Measuring biomolecular binding events with a compact disc player device”. *Angew. Chem. Int*. Ed. 45, pp 270 –273, 2006

[5] C. L. Jones, “Cryptographic has functions and CD-based optical biosensors”, *Problems of Nonlinear Analysis in Engineering Systems*. v.11, no 2(23), pp.17-36, 2005.

[6] Y. Li, M. L. L. Ou, H. Yu, “Digitized molecular diagnostics: Reading disk-based bioassays with standard computer drives”, *Analytical Chemistry*, vol. 80, pp. 8216-8223, 2008.

[7] K. C. Pohlmann, *The compact disc handbook,* Madison, WI : A-R Editions, 2nd edition pp 48- 49, 1992.

[8] L. Buddine and E. Young, *The Brady guide to CD-ROM*, New York, N.Y.: Prentice Hall Press, pp 59-60, c1987.

[9] J. Taylor, *DVD demystified*. New York; London: McGraw-Hill, pp 123, c1998.

[10] K. C. Pohlmann, *The compact disc handbook,* Madison, WI: A-R Editions, 2nd edition, pp 107, 1992.

[11] L. Buddine and E. Young, *The Brady guide to CD-ROM*, New York, N.Y: Prentice Hall Press, pp 16, c1987.

[12] P. M. Lane; R. Van Dommelen; M. Cada, "Compact disc players in the laboratory: experiments in optical storage, error correction, and optical fiber communication," *IEEE Transactions on Education*, vol.44, no.1, pp.47-60, 2001.

[13] K. C. Pohlmann, *The compact disc handbook,* Madison, WI: A-R Editions, 2nd edition, pp 82-83, 1992.

[14] K. C. Pohlmann, *The compact disc handbook,* Madison, WI: A-R Editions, 2nd edition, pp 62-63, 1992.

[15] G. Salvatore, *Introduction to error control codes*, New York : Oxford University Press, pp 22 – 28, 2001.

[16] H-C. Chang; C. B. Shung, C-Y. Lee, "A Reed-Solomon product-code (RS-PC) decoder chip for DVD applications," *IEEE Journal of Solid-State Circuits*, vol. 36, no.2, pp 229-238, 2001.

[17] H.Nakama, T. Doi, J. Fukuda, A. Iga, *Digital Audio Technology,* USA, 1st Edition, pp 114 – 190, 1983.

[18] H. Hoeve, “Error correction and concealment in the Compact Disc system”. *Philips Technical Review, v*ol. 40 (6), pp: 166-72, 1982.

[19] R. A. Potyrailo, W.G. Morris, A. M. Leach, T.M. Sivavec, M.B. Wisnudel, S. Boyette, “Analog signal acquisition from computer optical disk drives for quantitative chemical sensing”. *Analytical Chemistry*, vol. 78, no 16, pp 5893-5899, 2006.

[20] O. Matsuda, “Optoelectronics in compact disc system”, *Journal of the Institute of Electronics, Information and Communication Engineers,* vol. 76(3), pp 237-41, 1993.

[21] J. Posiello, “The electronics of the compact disc (CD). III”, *Revista Espanola de Electronica,* vol. 33(383), pp 72-75, 1986.

[22] *LG GSA-H55 Internal Super-Multi 20x DVD Rewriter with SecurDisc™ Technology*

http://www.asipartner.com/Microsite/LG/Products/GSAH55N_Spec%20Sheet.pdf (Last Accessed: August 2009).

[24] *Low-noise JFET-input operational amplifier* http://focus.ti.com/lit/ds/symlink/tl074.pdf (Last Accessed: August 2009).

[25] *High-speed diodes* *http://www.nxp.com/documents/data_sheet/1N4148_1N4448.pdf* (Last Accessed: August 2009).

[26] T. Meier, F. Fahrenholz, Basel. *A laboratory guide to biotin-labeling in biomolecule analysis* Boston: Birkhäuser Verlag, c1996.

[27] Christian Heise, Frank F. Bier. *Topics in Current Chemistry*, Springer Berlin / Heidelberg, vol. 261, pp 11, 2005.

[28] Y. Li, Z. Wang, L. M. L. Ou, H.-Z. Yu, DNA detection on plastic: Surface activation protocol to convert polycarbonate substrates to biochip platforms, *Analytical Chemistry*, vol. 79, pp. 426-433, 2007.

[29] M. González, L. A. Bagatolli, I. Echabe, J. L. R. Arrondo, C. E. Argaraña, C. R. Cantor, G. D. Fidelio, Interaction of Biotin with Streptavidin*, Journal of Biological Chemistry*, vol. 272, no 17, pp 11288-11294, 1997.

[30] *DTI RetroBurner* http://www.dtidata.com/cd_dvd_recovery.htm (Last Accessed: January 2009).

[31] M. Pallapa, L.M.L. Ou, M. (Ash) Parameswaran, H-Z. Yu, Software-based quantitation of bioassays on CD, Sensors and Actuators B: Chemical, In Press, Accepted Manuscript.

[32] *Hex Comparison 2.0*

http://exeicon.com/hex-comparison/ (Last Accessed: January 2009).

[33] *IsoBuster*

http://www.isobuster.com/ (Last Accessed: September 2009).

[34] J. Watkinson, *The art of digital audio*, Oxford; Boston: Focal Press, 3rd edition. pp:242 243, 2001.

[35] L. Buddine and E. Young, *The Brady guide to CD-ROM*, New York, N.Y.: Prentice Hall Press, pp 35, c1987.