TOWARDS DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) USING MICROCANTILEVER SENSORS

ABDULLAH ALODHAYB

TOWARDS DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) USING MICROCANTILEVER SENSORS

By

© Abdullah Alodhayb

A thesis submitted to the
Department of Physics and Physical Oceanography
in partial fulfillment of the
requirements for the degree of
Master of Science

Department of Physics and Physical Oceanography
Memorial University of Newfoundland

September 14, 2011

St. John's Newfoundland

Abstract

In this work, attempts have been made to develop an early HIV virus detector through DNA hybridization. The reason for detecting the genetic materials of the HIV rather than the virus is because HIV is asymptomatic during the first weeks (sometimes months) after an infection. Microcantilever sensors were used in this work to detect the hybridization process. The active microcantilever was functionalized with thiol modified single stranded DNA with the sequence (5'-/ThioMC6-D/ TCT GTA TGT CAT TGA CAG TCC AGC T-3)'. The reference microcantilever was exposed to TE buffer solution. Samples containing complementary sequences were introduced into the microcantilever sensor cell in a constant flow. The microcantilever sensors were able to detect concentrations as low as 0.2 nM. Experiments were also conducted by varying the chain length of the target DNA. A sample consisting of 1497 bases produced from actual HIV RNA was successfully detected at a concentration of 0.2 nM. Based on the deflection signal obtained, it should be possible to detect a sample concentration as low as 0.1 nM without having to modify the current system.

Acknowledgements

I would first like to express my appreciation and gratitude to my supervisor Dr. Luc Beaulieu for giving me the opportunity to work on this interesting project. His patience, support, enthusiasm, and understanding have contributed to the completion of this thesis. This work would not have been completed without his intellectual and insightful guidance.

I also would like to thank a number of people who have helped me throughout this work:

- Nicole Brown for preparing DNA solutions and for her great job in clarifying the chemical aspect of this work.
- Victor Hyden for helpful discussions.
- Sarah Ayoub for coating the microcantilevers with gold.
- The machinist Gordon Whelan for his great work in developing the experimental apparatus.

A very special thanks to my parents, my wife Norah, my friends Majed Sager, Hamad Alkhureiji, Saad Alanazi, Zead Alfayz, Abdulaziz Alanazi, Adel Hammas and others who have constantly encouraged and inspired me during this work.

Table of Contents

List of Symbols and Acronyms

- $\Delta\sigma$: Change in surface stress.
- R : The radius of the curvature of the microcantilever.
- E : Young's modulus.
- t : Microcantilever thickness.
- υ : Poisson's ratio.
- μm: Micrometers = 10^{-6} meters.
- PSD: position sensitive detector (PSD).
- HIV: Human immunodeficiency virus.
- AIDS: acquired immunodeficiency syndrome.
- DNA: Deoxyribonucleic acid.
- ssDNA: Single stranded DNA.
- dsDNA: Double stranded DNA.
- HS-ssDNA: Thiol-modified single stranded DNA.
- RNA: Ribonucleic acid.
- RT-PCR: Reverse transcription polymerase chain reaction.
- nm: Nanometers = 10^{-9} meters.

- SAM: Self assembled monolayer.
- bDNA: Branched DNA.
- MCH: Post- treatment 6- mercaptor-1- hexanol
- D: The distance between the cantilever base and the incident laser on the cantilever.
- L: The distance between the incident laser on the cantilever and PSD.
- CL: The cantilever length.
- θ: The angle of incident beam.
- ζ: The PSD angle.
- φ: The azimuthal angle.
- S : The PSD signal.
- δ : Deflection of the microcantilever.

List of Figures

List of Tables

Chapter 1

Introduction

The need for devices capable of detecting a large number of physical and chemical phenomena is currently in demand. Detecting such phenomena as the presence of molecules requires devices that are small in size, reliable, highly sensitive, have fast response time, and are inexpensive. To date, a significant amount of research has been concentrated on making devices that meet such requirements.

A biosensor is an analytical device capable of detecting the presence of biomolecules. A biosensor consists of two main elements. The first element is the recognition layer which is a layer of biomolecules that interacts with the target molecules to be detected. The second element is the transducer which detects the interaction between the recognition layer and the target molecules and then converts the resulting event into a measurable electronic signal.

Biosensors have attracted substantial interest and have been under continuous investigation in the last decade due to their wide range of application in medical diagnostics, and environmental screening [1]. Microcantilever sensors are a type of biosensors which have been employed to detect a wide range of physical and chemical phenomena such as a change in temperature, surface stress, antigen-antibody interaction, proteins, and DNA hybridization [2-5]. Moreover, microcantilever sensors have successfully met the aforementioned requirements making them available to be studied and utilized in many applications.

1.1 Microcantilever Biosensors

A microcantilever is a free standing beam fixed at one end and free at the other. Microcantilevers are typically formed into two different shapes; rectangular or v-shaped. Microcantilevers are normally fabricated from silicon (Si) or silicon nitride (Si_3N_4) using micromachining techniques. The longest microcantilever E (shown in figure 1.1), which was used in our lab due to its high sensitivity, was 350 μm long, 35 μm wide, and 1 μm thick.

Although microcantilever sensors are in micrometer dimensions, they may also be called nanomechanical sensors due to their nanometer deflection which occurs in response to changes either on their surfaces or their surrounding environment.

a) b)

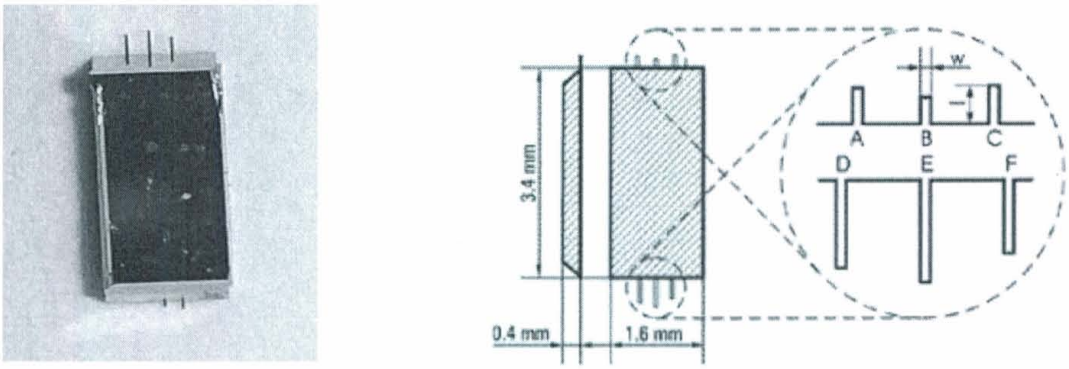


Figure 1.1: a) A photograph of the microcantilevers used in this work. b) A schematic of MikroMasch CSC12/Tipless microcantilever (Image courtesy of MikroMasch company) .

1.1.1 Functionalizing the Microcantilever Surface

Functionalizing the microcantilever surface with the proper receptive layers is fundamental in order to employ it as effective and active transducer. Microcantilever sensors are made active by coating them on one side with a thin gold film.

It has been found that a thin layer of gold is best for the microcantilever surface for several reasons. Unlike other metals, gold attracts a high number of receptor molecules that bond strongly to its surface. A second reason is that gold does not oxidize which prevents receptor molecules from deadsorbing from the microcantilever surface. On the other hand, the deposition of gold may induce a surface stress on the microcantilever sometimes leading to undesired measurements [6]. Having a microcantilever coated with a uniform, flat gold layer is significant in order to achieve accurate and reproducible measurements.

On the gold-coated microcantilever, the receptor molecules, which have been attached to the gold surface, will react with the target molecules. This reaction induces a surface stress on the microcantilever surface, thus resulting in the deflection of the microcantilever as shown in figure 1.2. This deflection can be detected using the optical beam deflection system.

a) b)

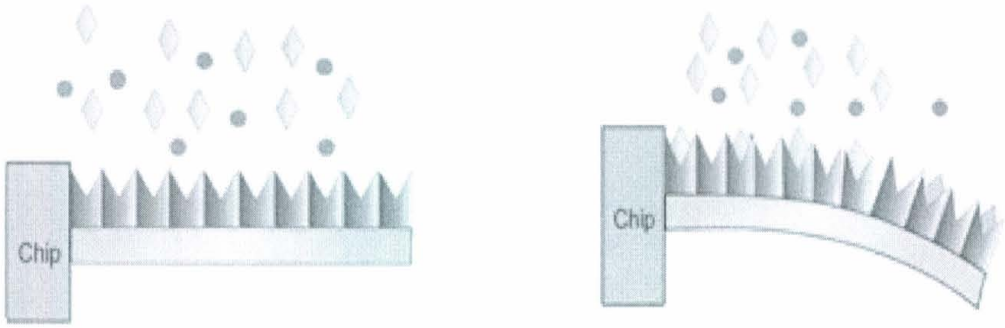


Figure 1.2: Schematic representation of a microcantilever sensor operating in static mode. a) The cantilever before the interaction between probe and target molecules. b) The cantilever bends due to the interactions on its surface.

Two types of surface stress may be formed on the microcantilever surface as molecule adsorption and interactions take place. The first is where the microcantilever bends downward and it is called compressive stress as shown in figure 1.3a. This stress is caused by the repulsive interactions between the atoms over the microcantilever surface. The second type is where the microcantilever bends upwards and it is called tensile stress as shown in figure 1.3b. This stress is caused by attractive interactions between the atoms over the microcantilever surface.

Moreover, the lower side of the microcantilever surface should either be passivated or left uncoated. This process of only coating one side and leaving the other uncoated or passivated would contribute to prevent complex situations from occurring.

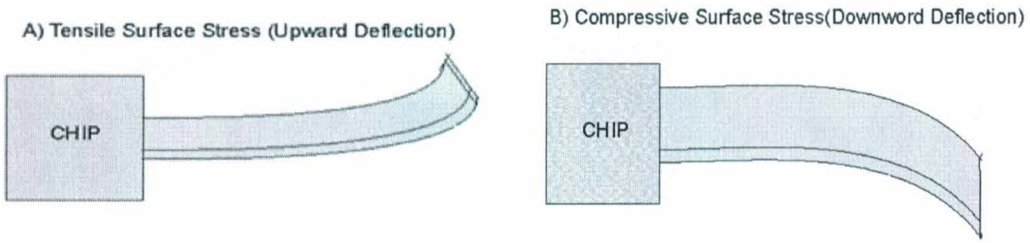


Figure 1.3: Types of surface stress that may be formed on the microcantilever sensor either during deposition or experiments. A) tensile surface stress , B) compressive surface stress.

1.1.2 Modes of Operation

Microcantilever sensors operate predominately in two modes: static and dynamic. In static mode, the deflection occurs when the microcantilever experiences a surface stress as a result of the adsorption and/or interaction of the target molecules with the receptive layer on the microcantilever surface (see figure 1.2).

Microcantilever deflections can be measured precisely by using multiple approaches such as the optical beam deflection and using piezoresistive cantilevers. In the former approach, which has been used in our work, a laser beam is focused at the free end of the microcantilever which then reflects into a position sensitive detector (PSD). In the latter approach, when the microcantilever deflects due to a surface stress the piezoresistive cantilever undergoes a change in resistance proportional to the deflection which can then be measured. In the dynamic mode, the resonant frequency at which a microcantilever is vibrating is monitored. Such vibrating can be detected and translated into a useful signal via several methods including piezoresistive and optical readout method.

The change in surface stress on the microcantilever sensor is described by Stoney's equation which is written as [7],

$$\Delta\sigma = \frac{Et^2}{6R(1-\nu)} \tag{1.1}$$

where R is the radius of the curvature of the microcantilever, E is Young's modulus , t is the microcantilever thickness, and υ is Poisson's ratio .

In this work, microcantilever sensors were used to detect the HIV virus in static mode by targeting a specific sequence within the RT portion of the RNA genome of the virus. In order to achieve this aim, the microcantilever surface was first coated with 20 nm inconel followed by 100 nm of gold.

The microcantilever surface was then functionalized with single stranded ssDNA modified with a thiol linker at the 5' end of the strand. The sulfur atom in thiol binds with gold resulting in the formation of a self-assembled monolayer (SAM). When ssDNA of a complementary sequence was introduced into the microcantilever sensor cell, they hybridize with the ssDNA on the microcantilever. This hybridization caused the microcantilever to deflect which was monitored and detected using an optical beam deflection system (OBDS).

1.2 Motivation

The Human Immunodeficiency Virus (HIV) is a deadly virus which infects many people worldwide. Often, the virus can present inside a subject for many months before being detected. This property of the HIV virus, which is asymptomatic inside the human body for long time, is the main reason for the wide spread of the virus. According to the recent statistics, 33.4 million people worldwide are living with HIV [8]. This number of infected people clarifies the need of increased awareness about this virus and the need to find a proper method capable of detecting HIV earlier in order to prevent the spread of the virus.

The most hazardous effect of the HIV virus is its ability to destroy the immune system of the human body, thus preventing the body's defense against further diseases and illness. When the HIV virus infects the body, it attacks vital cells of the immune system, such as CD4+T cells, macrophages and dendrite cells. CD4 is the main receptor used by HIV to enter into host T cells. Upon entry into host T cells, the HIV virus starts undergoing three stages. The first stage is the primary infection stage (also known as the acute infection stage), which lasts for a few weeks. This stage is often asymptomatic but sometimes some symptoms such as flu, fever, and sore throat can be seen. Such symptoms are often not recognized or considered as signs of HIV infection since they are common symptoms for other diseases. In addition, the body takes several months (1 to 3 months) to produce antibodies that fight the virus, therefore HIV antibody tests during the primary infection stage may yield negative results. The period of time between when a person is first infected and the production of antibodies is termed as the window period, during which the infected person may still transmit the virus [9]. Because antibodies tests are ineffective during the primary infection stage, doctors may order other tests such as Reverse transcriptase Polymerase Chain Reaction (RT-PCR) and p24 antigen test which examine the genetic material of the HIV virus itself rather than the HIV antibodies.

With such tests, it is then possible to detect the presence of the HIV virus even if it is in the window period. In the RT-PCR test, reverse transcriptase is used to convert the viral RNA in the HIV virus into a complementary DNA. This sequence is then amplified by PCR and the resulting DNA is then hybridized to specific probe DNA which has been attached to a solid support [10, 11]. This hybridization is then analyzed to determine if the person is either infected with the HIV virus or not. The secondary stage of HIV infection is known as the latency stage, which usually lasts approximately 10 years, during which the person has no signs or symptoms of illness despite the fact that CD4 count continues to decline. When CD4 count is less than 200 cells per microliter, an infected person is diagnosed with AIDS which is the last stage of the HIV infection. Opportunistic infections such as Pneumocystis carinii pneumonia (PCP), Mycobacterium avium complex (MAC) disease appear during this stage due to the severe damage of the host immune system [12] . Additional details about this virus and tests used to detect and monitor it are presented in Chapter 2.

1.3 Scope of the thesis

In this work, microcantilever sensors were used to detect the HIV virus by targeting the RNA Genome inside the virus in the hope of developing an early detection sensor for HIV. In Chapter 2, a brief introduction to the DNA, RNA and the HIV virus will be presented. A detailed discussion about DNA immobilization as well as DNA hybridization will be given. In Chapter 3, the experimental system and apparatus such as the optical beam deflection system, fluid cell, and sputter deposition will be described. In Chapter 4, chemicals, which were being used to prepare the probe DNA, which acts as receptor molecules on the cantilever surface, and the target DNA, which acts as the target molecules on the cantilever surface, will be described. In addition, the experimental results will be shown followed by a detailed discussion of each result. Finally, in chapter 5, we will summarize the work presented herein and provide proposals on how this work can be improved and continued.

Chapter 2

Human Immunodeficiency Virus (HIV)

Introduction

In this chapter, the chemical and biological aspects of this work will be given. In order to understand the reasons behind the proposed mechanism for detecting HIV using microcantilever sensors, it is worthwhile to first provide a brief Introduction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In section 2.2, the HIV virus is discussed including a brief history of the virus, its function, and some of its effects. In section 2.3, DNA immobilization on the microcantilever surface is presented followed by how to detect the hybridization in the fluid cell presented in section 2.4.

2.1 Deoxyribonucleic Acid (DNA) and Ribonucleic acid (RNA)

Each cell of the human body contains a nucleus where genetic information is stored. The genetic information is stored in the form of DNA. A DNA molecule is a long, double strand helix that resembles a winding staircase, consisting of two separate strands which are bound together by base pairs as shown in figure 2.1.

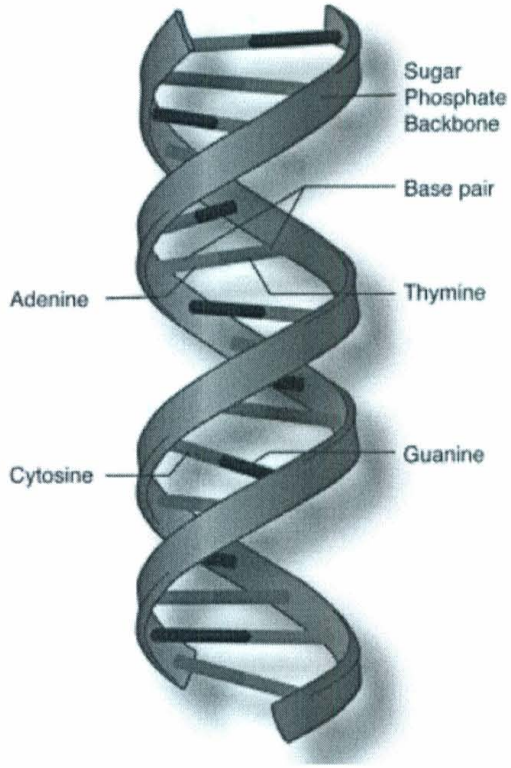


Figure 2.1: A schematic representation of the structure of DNA. Two strands are bounded together by base pairs (A,T,G, and C) through the hydrogen bond. (Image courtesy of National Human Genome Research Institute) [13].

DNA is a polymer of nucleotides which are composed of phosphate, a five-carbon sugar called deoxyribose, and one of four nitrogenous bases which are divided into two groups. The purine bases, adenine (A) and guanine (G), which have two nitrogen ring structures, and the pyrimidine bases, thymine (T) and cytosine (C), which have one nitrogen ring structure. The purine and pyrimidine bases precisely join to form a base pair. Adenine is paired with thymine, and guanine is paired with cytosine as depicted in figure 2.2.

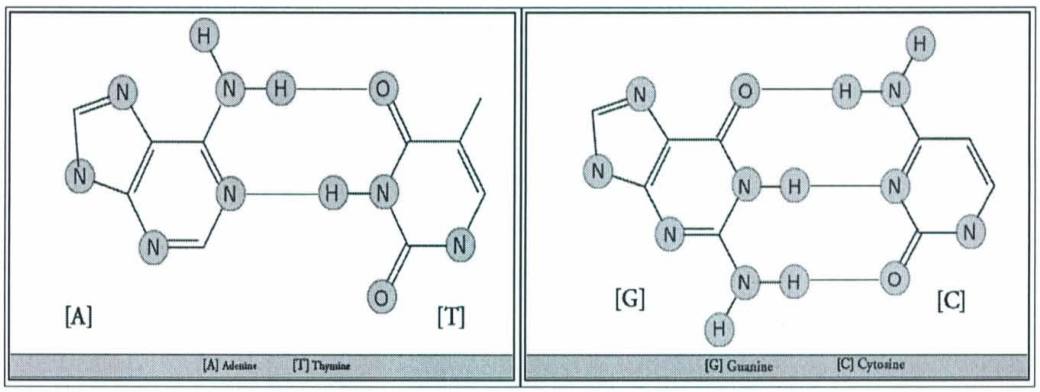


Figure 2.2: A schematic depiction of the DNA base pairs.

Physically, the base pairs project inward from the sides of the DNA molecule which resemble the steps on a spiral staircase. The base pairs are held together by hydrogen bonds which, although weak are extremely stable under normal conditions. Enzymes called DNA helicases are required to separate the two strands so that the genetic information can be exactly duplicated. This information is stored and arranged in units known as genes.

A gene is the unit of heredity passed from generation to generation and is also responsible for the daily functions of the cells in the body. A gene is represented by a number of base pairs ranging from thousands to almost one million base pairs. There are two main processes that are carried out through the use of DNA, transcription and translation, which ensures function and duplication of each cell. Before discussing the functions of these processes, a second type of nucleic acid must be introduced called ribonucleic acid (RNA). Protein synthesis takes place in the cytoplasm of the cell however DNA is located in the nucleus of the cell, therefore RNA is used to direct the proteins which carry out translation [14]. Furthermore, there are three types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). RNA is essential for transcription and translation to take place.

Transcription involves copying of the genetic code from DNA to a complementary strand of mRNA, the genetic code being a successive sequence of four bases (adenine, thymine, guanine and cytosine) with thymine in DNA being replaced by uracil in RNA. The genetic code controls the sequence of amino acids in a protein molecule that is being synthesized in a cell. Once the mRNA has transcribed the genetic code, it detaches from the DNA and is transported into the cytoplasm where it controls the assembly of proteins. Translation is the next process that takes the instructions transcribed from DNA to mRNA and transfers them to the rRNA of ribosomes in the cytoplasm of the cell. When the mRNA comes into contact with the ribosome it binds to the small subunit, the instructions are then communicated to tRNA which delivers the correct amino acid to the proper position on the peptide chain. There are 20 different amino acids and 20 different types of tRNA for each one. Each type of tRNA carries an anticodon which is complementary to the mRNA codon calling for the amino acid carried by the tRNA. The tRNA anticodon recognizes the mRNA codon which ensures the proper sequence of amino acids in a synthesized protein [15].

Understanding DNA is important for realizing how the basic cellular processes in our bodies keep us alive and allow us to reproduce. There is however an example of DNA transmission that impacts negatively on humans, viral DNA.

A virus is a small infectious agent that uses its DNA to replicate inside the living cells of an organism. In general, viruses attach to host cells, inject their DNA, use the host cell's DNA replication cycle to create multiple copies of the viral DNA, and are released from the cell to infect the body.

2.2 Human Immunodeficiency Virus (HIV)

The origin of the HIV virus and how was it introduced to humans have been extensively studied. There are several theories describing the origin of the HIV virus even though the exact origin is still unknown. The most accepted theory states that the origin of the virus was zoonsis, which means that the virus was first transmitted to human via animals [15]. HIV (shown in figure 2.3) is an example of a lentivirus which belongs to the Retroviridae family.

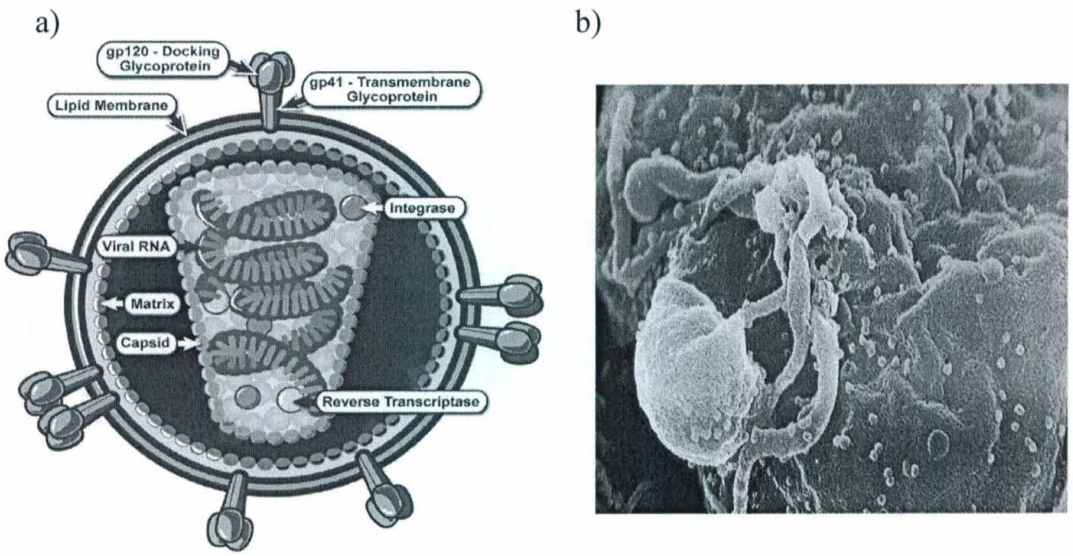


Figure 2.3: a) A schematic presentation of the HIV virus. Used with permission from [16]. b) An SEM image of the HIV virus. (Image courtesy of CDC of http://phil.cdc.gov/Phil/details.asp)

HIV causes the immune system to progressively become weaker leading to life threatening opportunistic infections. These infections are unusual with a healthy immune system but a compromised immune system allows the opportunity for infection [12]. An HIV infection can be transmitted through infected blood, semen, vaginal fluid or breast milk. The HIV infection undergoes three phases: the primary infection phase, the latency phase and the overt AIDS phase [17]. As mentioned in chapter 1, HIV is characterized by a long incubation period which is the time between initial exposure to the virus to when signs and symptoms first appear. Lentiviruses can inject a considerable amount of their genetic information into the DNA of the host cell and can replicate in non-dividing cells, which is why HIV is so devastating and at the moment can only be treated but not cured. Because of such a long incubation period, symptoms do not usually appear until significant damage is already inflicted on the immune system. HIV infects vital cells of the immune system, such as CD4+T cells, macrophages and dendrite cells. CD4 is a primary receptor used by HIV to enter into host T cells. An HIV infection leads to a constant reduction in T cells that possess CD4 receptors.

CD4 levels are used to decide when to begin treatment of infected patients. Normal blood CD4 values in a non-infected person are 500-1200 X 10^6 cell /μL [17]. When the CD4 count reaches 350 cells per microliter, patients usually begin treatment. The reduction in CD4 levels caused by the HIV occurs through three main mechanisms. First, the virus directly attacks and kills infected cells. Second, the rate of apoptosis in infected cells is high. In other words, all normal body cells have a programmed death to ensure new cell formation however HIV infected cells die faster. Third, the virus uses CD8 cytotoxic lymphocytes, which are immune cells that recognize and kill infected cells, to destroy infected CD4 cells. The susceptibility to opportunistic infections in the body is increased as CD4 cell numbers continue to decline. This is known as the latency phase during which the person has no signs or symptoms of illness but CD4 count continues to fall. This stage of infection usually lasts 10 years. The overt AIDS phase occurs when CD4 count is less than 200 cells per microliter [17]. Without treatment during this phase, the infected person can die within 2 to 3 years due to opportunistic infections. These infections can develop rapidly because the immune system is rendered almost incapable of fighting off pathogens at this point, therefore treatment is essential for survival.

Patients with HIV are usually treated with a combination of three drugs which help to suppress HIV replication, increase CD4 cell count, and slow the progression to AIDS to help improve the overall quality of life and survival time. The clinical course of HIV varies from person to person: 60-70% develop overt AIDS 10 to 11 years after initial HIV infection (typical progressors), 10-20% develop overt AIDS in less than 5 years (rapid progressors), and the remaining 5-15% are called slow progressors and do not experience the progression to overt AIDS for more than 15 years [17].

2.2.1 Detection methods of the HIV virus

HIV virus could be detected by several tests such as the HIV antibody test, P24 antigen test and HIV Viral Load test. Despite the HIV antibody test being accurate and inexpensive, it is not very effective during the primary phase of the HIV virus. The reason for this is that an HIV antibody test detects the antibodies produced by the body in response to the virus. Unfortunately, this can take up to 3 months for the body to develop antibodies to combat the virus leading to negative results when conducting this test.

Hence, testing the genetic material of the HIV virus itself rather than the HIV antibodies is needed during the primary phase. HIV Viral Load test is a sensitive, accurate, and efficient test used for the early detection of the HIV virus. Viral load is measured via a blood test which determines the amount of the HIV virus in the blood by RNA copies per milliliter HIV Viral load test can be measured by two main methods: Reverse transcriptase Polymerase Chain Reaction (RT-PCR) and branched-chain DNA (bDNA) [18]. The RT-PCR test encompasses two main procedures: extracting the genetic material of the HIV virus and then applying the PCR technique process. The first step begins by extracting the RNA from the person's blood followed by using reverse transcriptase which converts the extracted RNA into a complementary DNA. PCR technique is then used to amplify the complementary DNA (the target DNA) and the resulting DNA is then hybridize to specific probe DNA which has been attached to a substrate [10,11].

The viral load test is also used along with other test such as CD4 test to monitor the HIV virus during its progression inside the body. As has been previously mentioned, the HIV viral load test measures the amount of the HIV virus in the body by RNA copies per milliliter.

A high viral load between 3000 to 10000 copies/ml indicates that the virus has a high likelihood of progression to the later stages where treatment is more complicated and less successful. A low viral load value between 40 to 800 copies/ml indicates that the virus is at a controllable level where it may be treated and monitored. The main purpose of doing this test is to maintain the viral load amount as low as possible for as long as possible. After conducting this test much information can be obtained. This information includes how active the virus is, what treatment is currently needed, and the future treatment plan [19].

2.3 DNA Immobilization

DNA immobilization is a process where single stranded ssDNA (the probe) is functionalized onto a substrate. In this work, single stranded ssDNA was synthesized to match a portion of a sequence of the RT portion of the RNA in the HIV virus. This is called the probe.

The RNA inside the virus is therefore called the target. Detecting the hybridization of the probe and the target is equivalent to detecting the virus. In order to immobilize the ssDNA on a solid surface, the molecules must first be modified with a functional end group. Most common examples of functional groups are Amino- groups, AcryditeTM-groups, and Thiol-groups.

In this work, covalent bonding was used to immobilize the oligonucleotides to the gold surface by using oligonucleotides modified with a thiol end group. The SH modifier could either be placed at the 5'end or 3'end of the oligo. The sulfur atom in thiol has a high affinity to gold and therefore binds with gold leading to the formation of a self assembled monolayer (SAM). It has been demonstrated that the amount of oligos that can be immobilized on gold surface is 100 times higher than those on silicon or silicon nitrate surfaces [20]. Single stranded DNA modified with a thiol group will be referred to as HS-ssDNA. Immobilization of ssDNA on the microcantilever surface is depicted in figure 2.4. Details about our experimental method for immobilizing probe HS-ssDNA on the microcantilever surface is presented in chapter 4.

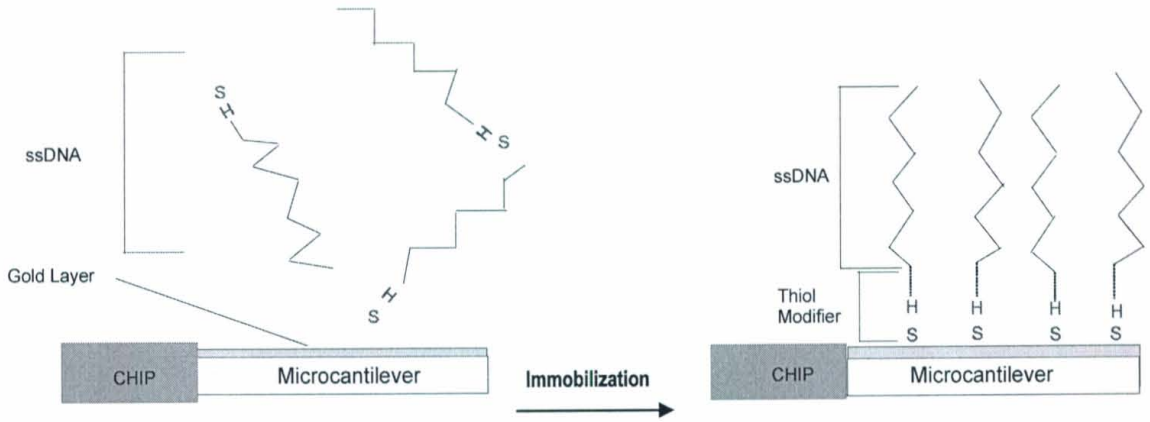


Figure 2.4: A schematic representation of the immobilization process of HS-ssDNA on the microcantilever surface.

2.4 DNA Hybridization

DNA hybridization is the binding of two complementary sequences leading to the formation of a double stranded DNA (dsDNA). DNA hybridization has been studied extensively with an emphasis on understanding the hybridization mechanism. New and developed technologies such as DNA microarray have offered fast, selective, and sensitive detection of DNA hybridization [21,22].

DNA microarray technology (also known as gene chips) have made a significant revolution in genetic analysis leading to a wide range of medical and biological applications such as the detection of infectious diseases, drug discovery, and gene expression analysis [21,22,23].

The concept of DNA microarrays relies first on the immobilization of a single stranded DNA called probe DNA onto a solid surface and then on the hybridization between the immobilized probe and the complementary target DNA. However, several difficulties associated with DNA chips such as sample size, and the preparative time have been reported [23]. Moreover, most DNA hybridization methods including gene chips rely on labeling techniques either with radioactive or nonradioactive labels. Free-labeling techniques have attracted much attention due to the disadvantages associated with labeling techniques. These disadvantages include additional time required to tag the molecules as well as the exposure to radiation [20]. Consequently, alternative technologies such as DNA biosensors are being developed and enhanced. Microcantilever biosensors have successfully been used to detect DNA hybridization due to their high sensitivity and selectivity [24,25].

In this work, a label-free technique based on microcantilever sensors has been used for detecting HIV through DNA hybridization. As will be discussed in greater details, hybridization induces a change in surface stress on the microcantilever surface causing the nanomechanical deflection of the microcantilever. DNA hybridization between the probe and the target molecule forms double stranded DNA (dsDNA) causing the microcantilever deflection as depicted in figure 2.5.

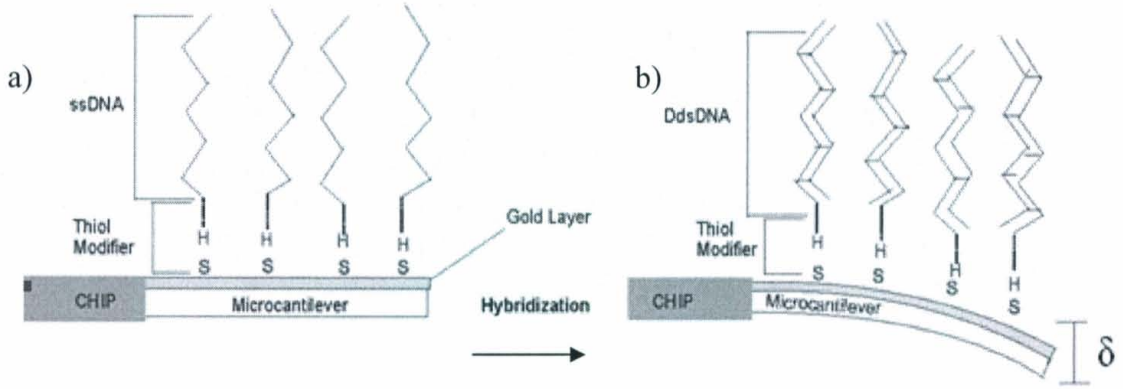


Figure 2.5: A schematic representation of the hybridization process. a) HS-ssDNA is immobilized on a microcantilever surface, b) hybridization of two complementary strands forming a double stranded DNA(dsDNS) which causes the deflection of the microcantilever (δ).

Chapter 3

Experimental Apparatus and Techniques

Introduction:

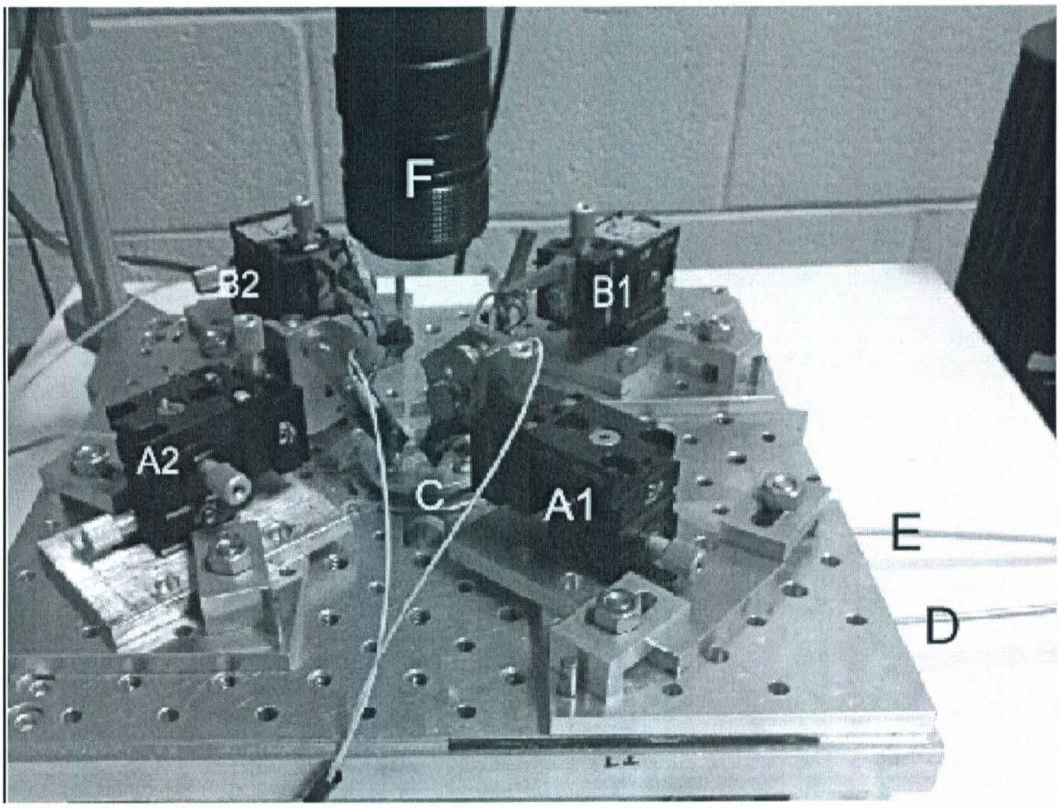
In this chapter, the experimental set-up and techniques used in this work to conduct the microcantilever sensor experiments are presented. An overview of the microcantilever sensor system which is composed of the fluid cell, laser, and photo sensitive detectors (PSDs) will be given. A brief description of the sputter deposition technique by which the microcantilever sensors were coated with a thin gold film will also be provided. Lastly, a detailed discussion on calibrating the instruments as well as the optical beam deflection system used to monitor the mechanical deflection of the microcantilever sensor will follow.

3.1 Overview

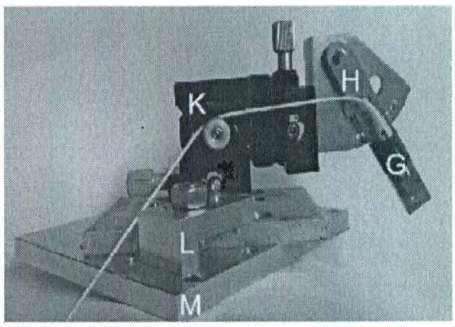
The majority of the apparatus used in this work were contained on the microcantilever sensor set-up including the fluid cell, optical focusers, optical microscope, and PSDs. These are shown in figure 3.1.

Briefly, the microcantilevers were mounted in the fluid cell where they were exposed to the injected fluids. Once the microcantilevers were positioned and the fluid cell sealed with a glass cover, the lasers were focused on the free end of the microcantilevers using the optical focusers. The reflected beams from each microcantilever surface were directed into photo sensitive detectors (PSDs). It was important to ensure that the optical beams were made incident on the microcantilevers at the desired position. This was done by viewing the positioned microcantilever on the fluid cell using an optical microscope placed above the fluid cell as shown in figure 3.1.

a)



b)



c)

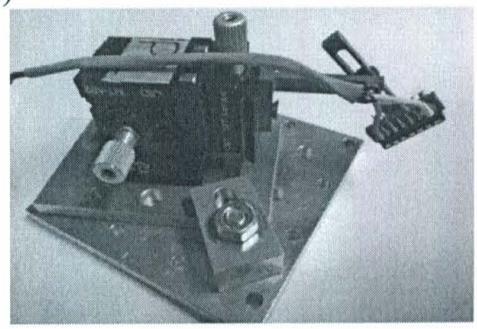


Figure 3.1: a) A photograph of the microcantilever sensor system. A1, A2, B1, B2) Translation stages, C) Fluid cell, D, E) Input and output tube. F) Optical Microscope placed above the fluid cell used to view the position of the optical beam on the microcantilever surface. b) A picture of the focuser assembly. G) The adjustment arm, H) Brass holster with several holes used to hold the focuser arm at a particular angle, K) x-y-z translation stage, L) two clips used to fasten the laser assembly. c) A photograph of the PSD and PSD holding mechanism.

3.2 Fluid cell

As shown in figure 3.1, the fluid cell (c) was placed at the center of the platform. The fluid cell was made of aluminum which is relatively inert and is non-reactive with the reagents used in this work. This ensures a minimal influence of the fluid cell on the experimental results thus increasing the accuracy of the measurements. The microcantilevers were secured and sealed in the fluid cell during the initial set-up. The fluid cell was sealed with a rubber o-ring covered with a glass disk held in place by an aluminum bracket. The glass disk was coated on both sides to prevent reflections of the laser beam from the air/glass and glass/liquid interfaces. The fluid cell is attached to two tubes from below which are used to transport fluid to and from the fluid cell as shown in figure 3.2. These two tubes were attached to a syringe pump which allows the injection of different solutions. The tubes are made of Polyether Ether Ketone (PEEK), which is not known to react with organic compounds.

1) 2)

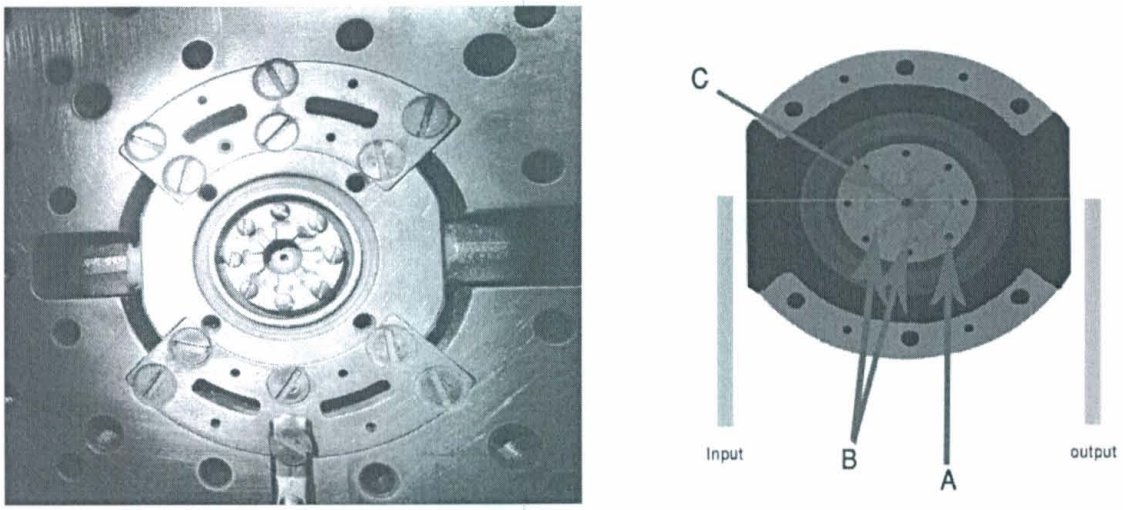


Figure 3.2: 1) A photograph of the fluid cell used in this work to house the microcantilevers. 2) Schematic representation of the fluid cell clarifying its main parts: A) Spring clamps used to secure the microcantilevers in position. B) Slots in which the microcantilevers were held and secured during testing, C) A hole from which a solution entered the fluid cell.

In our experiment it is necessary to inject two different solutions into the cell. In order to accomplish this as efficiency as possible, and without injecting any bubbles into the system, a flow divider was constructed as shown in figure 3.3.

This device allowed for two syringes to be used without the need to disconnect them and hence eliminating the creation of bubbles in the cell. Once the microcantilevers were positioned and the bubbles were removed, the fluid cell was sealed and flushed using de-ionized water to eliminate contaminants.

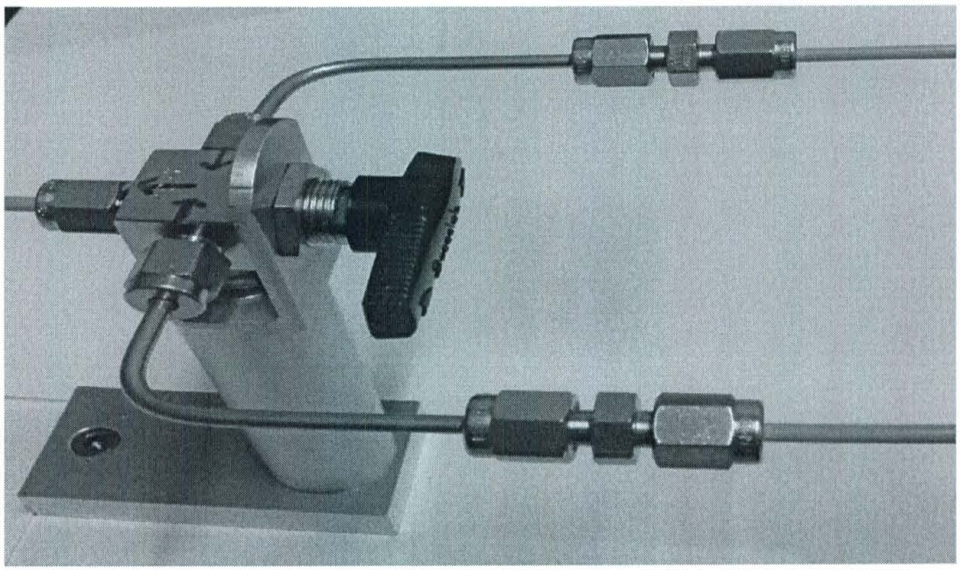


Figure 3.3: A photograph of the flow device used to control which solution was allowed to flow into the fluid cell.

3.3 Lasers and PSD'S

Optical focusers ((LPF-01-635-4/125-S-2.4-15-4.7GR-40-3S-1-2, OZ optics) were used to focus the laser beam from a laser diode ((FMXL112-00, Claire Lasers) onto the free end of the microcantilever. As can be seen from figure 3.1, the optical focusers were attached to translation stages so that the incident beam on the microcantilever could be precisely adjusted to the desired position. The optical focusers were also attached to an adjusting arm through which the angle of the incident beam could be controlled precisely. The laser diode was powered by a precision current source (LDX-3412,ILX Lightwave Corp). In order to keep the beam intensity constant, the diode was mounted on a constant temperature stage controlled by a temperature controller (LDT-5412, ILX Lightwave Corp).

The reflected beam was detected using a position sensitive detector (PSD). The PSD was adjusted so that the laser beam reflecting off the microcantilever surface was incident on the active area of the device. The PSD's were also attached to translation stages allowing them to be moved and aligned with precision (see figure 3.1-2).When the laser beam hit the PSD, a photo current was induced which, in turn, was converted into a voltage signal which was then read by the data acquisition board.

The relationship between the beam position on the PSD and the PSD voltage is linear. Each PSD has an active area 10 mm long as shown in figure 3.4. When the output voltage is 0 V, the beam position is directly in the center of the PSD as depicted in figure 3.4. The +/- 5 mm position on the PSD surface corresponds +/- 10 V correlate. Therefore, +5mm indicates that the beam position is at the top on the active area of the PSD and -5 mm indicates that it is at the bottom. A program written by Meng Xu was used to collect the signal from each PSD and plot them in real time on the display monitor.

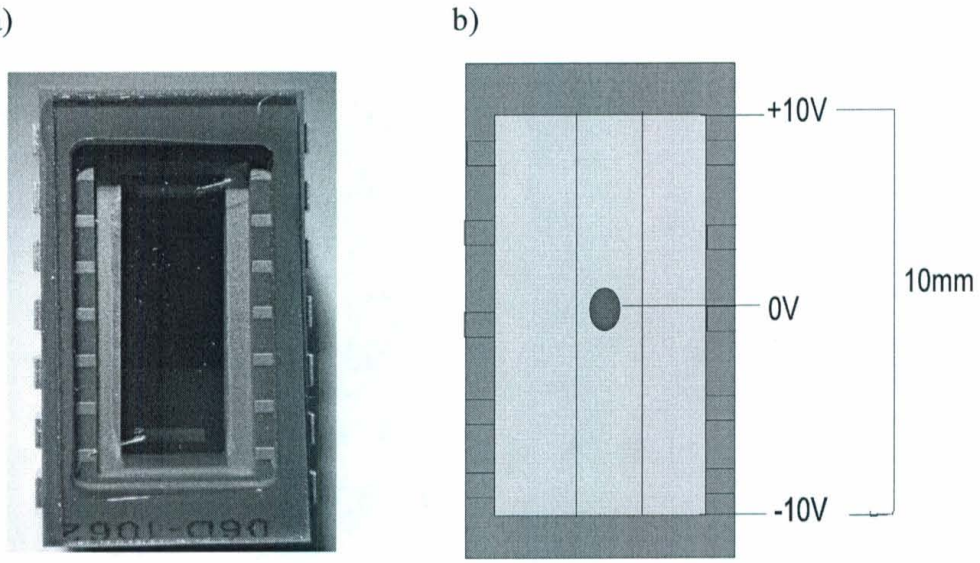


Figure 3.4: a) A photograph of the PSD. The active area is colored black. b) Schematic representation of the PSD active area. The laser spot is in the middle of the active area corresponding to a PSD voltage of 0 V.

3.4 Optical Microscope

An optical microscope with a CCD camera (shown in figure 3.1) was placed above the fluid cell in order to gather images of the microcantilever and view the position of the laser spot on the microcantilever surface. An image of the laser beam focused on the apex of the microcantilever surface captured by the microscope is shown in figure 3.5.

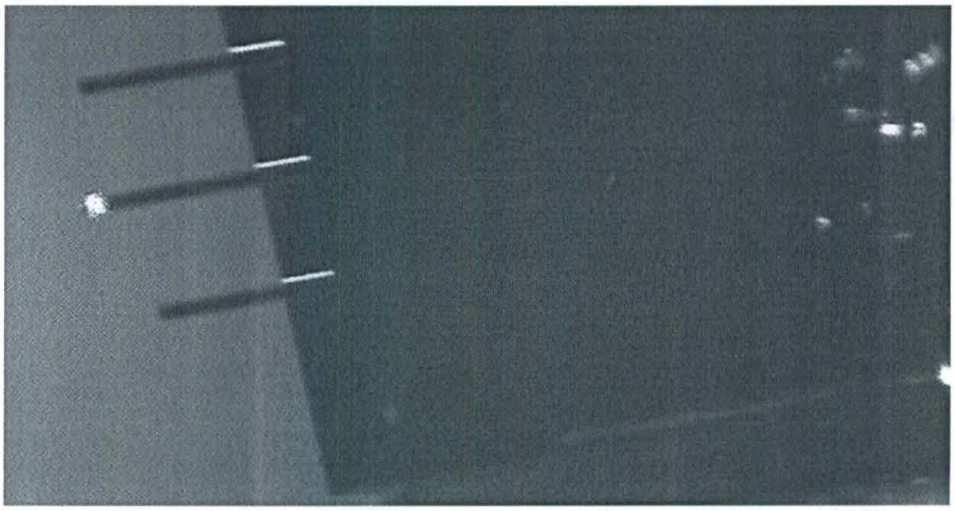


Figure 3.5: An image taken by the optical microscope showing the position of the laser beam on the apex of microcantilevers.

3.5 Sputter Deposition:

In this work, sputter deposition was used to deposit a thin Au film on the microcantilever. Gold is used to attach the organic receptive layer to the microcantilever because it is inert and forms a strong bond with thiol-based molecules.

Sputter deposition is a common technique used to deposit thin films onto a substrate. Sputter deposition is a process whereby target atoms, (gold in this case), are ejected from the target and deposited on the substrate surface. During the sputtering process, argon gas is allowed to flow inside a vacuum chamber which houses both the target and the substrate. A high potential difference is applied between the target and the substrate producing an electric field. The produced electric field causes argon gas to ionize to Ar^{+} which becomes attracted to the target. Collisions between the gold target and ionized argon atoms (Ar^{+}) cause target atoms to be ejected which then deposit on the substrate surface creating a thin film [26].

In this work, 100 nm of gold on 20 nm of (inconel / chromium) were deposited on the microcantilevers at a power of 80 W, with a gas flow rate of 20 SCCM (Standard Cubic Centimeters Per Minute) for 10 mins. Au was deposited at a deposition rate of 0.6 Å/s and power of 20 W for nearly three hours.

3.6: Optical Beam Deflection System (OBDS):

The optical beam deflection system (OBDS) was used in the sensor experiments in order to monitor the cantilever deflection. This system was well characterized by Beaulieu et al [27,28], who optimized the geometry of the optical beam deflection system.

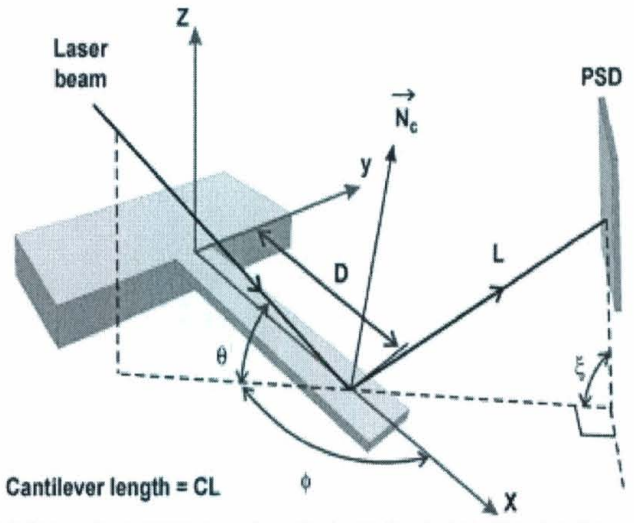


Figure 3.6 : A schematic representation of the optical beam deflection system [27].

In addition, the authors showed that the values of D, L, CL, θ, ξ and φ uniquely characterize the system where D is the distance between the cantilever base and the incident laser on the cantilever, L is the distance between the incident laser on the cantilever and PSD, CL is the cantilever length, θ is the angle of incident beam , ξ is the PSD angle, and φ is the azimuthal angle.

It has also been stated that for small deflection the microcantilever deflection (δ) is linearly proportional to the PSD signal (S).

$$\delta = \gamma S \tag{3.1}$$

In order to gain an accurate conversion from the PSD signal into microcantilever deflection, γ needs to be determined. The value of γ has mathematically described by Beaulieu et al [28] and a program calculating its value was written. In the previous work of Beaulieu et al. did not account for the glass cover used to seal the liquid cell from the environment as used in this work. Also, the optical path was considered from the focuser to the PSD. However, in real experiments it is necessary to work backward from the PSD signal to the microcantilever deflection.

Therefore, an improved program was written by others in our group by modifying the formalism first published by Beaulieu et al. in order to obtain an algorithm that took as input the PSD signal and gave as output the microcantilever deflection.

Chapter 4

Results and Discussion

Introduction

In this chapter, we will discuss the capability of the microcantilever sensor for detecting the HIV virus through DNA hybridization between the probe and the target molecules. In section 4.1, we will show how to functionalize the microcantilever surface by first depositing a thin gold film followed by immobilizing a single chain DNA monolayer. A complete explanation of the chemical and biological methods used to prepare the DNA solutions will also be provided. The experimental results obtained in this work as well as a detailed discussion for each result will be presented in the section 4.2 of this chapter. Lastly, we will examine the influence of a new adjustment added to the fluid cell in section 4.3.

4.1 Microcantilever Surface Functionalization

Functionalizing the microcantilevers involved first depositing thin gold film on the cantilever as a receptive layer. Prior to deposition, the microcantilevers were first cleaned with a Piranha solution (H_2SO_4: H_2O_2 =3:1) for 5 to 10 minutes and then washed twice with ethanol and then de-ionized water to remove any residue and contamination on the surface. After rinsing, the microcantilevers were dried in an oven for 24 hours at 275 C. The cleaned and dried levers were then coated on the top surface with a 20 nm adhesion layer of Inconel/Chromium followed by a 100 nm thin gold film by sputtering deposition. Depositing the gold film allows for the microcantilever to be functionalized with the receptive layer which is presented in section 4.1.1.

4.1.1 DNA Immobilization Procedures

Immobilizing DNA on gold-coated microcantilevers has been investigated by many groups. These investigations have led to exploration of the mechanism behind the DNA immobilization on gold surfaces.

Factors governing the DNA immobilization such as the surface probe density, the total number of DNA strands adsorbed on the microcantilever surface, and the resultant surface stress have all been measured [20, 25, 29 ,31 ,32]. As has been stated in chapter 2, DNA immobilization on gold surface could be accomplished through several methods including adsorption, affinity interaction, and covalent bonding. The latter method was used in this work to immobilize the single stranded DNA or oligonucleotides on the gold surface. Using this method involved modifying the oligonucleotides with a thiol end group (SH modifier). The sulfur atom in the thiol molecules has a high affinity for gold and therefore binds with gold leading to the formation of a self assembled monolayer (SAM) (see figure 2.5). The HS-ssDNA (probe molecule) concentrations were 1 μM throughout all experiments. This specific concentration was chosen due to the fact the surface in which the probe molecules are immobilized can be saturated with probe concentrations between 1 to 10 μM [29].

The DNA probe sequence used in this work was 25 bases long and synthesized with a 5'-thiol linker (5'-/ThioMC6-D/ TCT GTA TGT CAT TGA CAG TCC AGC T-3)'. The single stranded DNA modified with thiol group, abbreviated as, HS-ssDNA, is often delivered with a double thiol end group which creates a disulfide bond. This bond prevents the thiol end group from oxidization and therefore maintains the DNA sequence active and usable.

Prior to immobilization, the extra thiol end group had to be reduced. Such reduction can be accomplished using several methods. One of the most common methods is using a reducing agent such as DDT which must be removed before the immobilization can take place.

In this work, a treatment with solid-phase DDT has been used to reduce the extra thiol group. In this treatment, chemical DDT is available in the form of an acrylamide resin called Reductacryl. The oligos were combined with DDT at a ratio of 1:50 mg to ensure full reduction [30]. The resulting mixture was then resuspended in TE buffer (PH: 7.9) and agitated for 20 minutes at room temperature. The Reductacryl (DDT) was then removed by filtration using a syringe filter. The solution of HS-ssDNA was then used directly to functionalize the microcantilever surface with the probe molecule layer.

The active and reference microcantilevers were placed in a cleaned container in which they were completely covered with HS-ssDNA solution for the active and with TE buffer for the reference. During all experiments, all active and reference microcantilevers were taken from the same deposition batch to ensure precise measurements. The process where the microcantilevers were immersed in a solution is called incubation.

In this work, the incubation time was three to five hours unless otherwise mentioned. The reason for such a short incubation time is that immobilization is known to occur within a few hours even though some researchers prefer longer time to ensure a complete assembly of the probe molecules on the microcantilever surface [31]. However, we noticed that short incubation time gave better results than longer ones. In order to test the effect of the incubation time on the DNA hybridization efficiency, the microcantilever was immersed in HS-ssDNA for 24 h and was compared to the microcantilever incubated for 3 hours. The microcantilever shows higher deflection when incubated for 3 hours whereas longer incubation time gave smaller deflections.

A possible reason for this is that a short incubation time may lead to an SAM coverage of less than 100% leading to a smaller cantilever deflection (pre deflection due to the cantilever functionalization - see figure 2.5) whereas longer exposure time would lead to a high percentage surface coverage leading to a possibly large cantilever deflection. Therefore in an analogous manner that pulling a spring becomes more difficult with the extension length, the cantilever deflection due to the hybridization process may be larger for a cantilever that has an initial smaller deflection. In some experiments, the probe-immobilized microcantilevers were treated with 1 mM treatment 6- mercaptor-1- hexanol (MCH) to minimize non-specific binding of ssDNA as will be described later [33].

Once the microcantilever was functionalized with the probe molecule layer, it was then placed in the cantilever sensor fluid cell which was previously rinsed with ethanol followed by TE buffer.

DNA immobilization experiments were not studied in this work since such experiments have been extensively conducted and roughly similar results obtained [25, 33, 34]. These results clearly show that DNA immobilization leads to a small change in surface stress on one side of the microcantilever surface relative to the other causing a small deflection. This deflection which is smaller than the resultant deflection from DNA hybridization is contributed to the induced surface stress on its surface caused by the covalent binding between the sulfur atom of thiol modifier and the gold atoms on the microcantilever surface [25].

4.1.2 DNA Hybridization Procedures

DNA hybridization is the binding of two complementary strands of DNA forming a double stranded DNA (dsDNA) molecule. In this work, the main purpose of conducting DNA hybridization experiments is to develop an HIV sensor capable of detecting the genetic materials of the virus in a label-free manner using microcantilever sensors.

Label-free detection of DNA hybridization has been reported in several articles [25, 31,34]. However, in some articles, reference microcantilevers were not used. In this work, reference microcantilevers were used throughout all experiments. Reference microcantilevers ensure that the microcantilever deflection is caused by the hybridization between the probe and the target molecules and not to nonspecific interactions. In this work, DNA hybridization took place in the fluid cell.

After immobilizing the probe DNA on the microcantilever surface and placing it in the fluid cell, TE buffer was then injected at a consistent flow rate of 0.1 ml/min until a baseline was obtained as shown in figure 4.2. This baseline indicates that the DNA molecules are stabilized. TE buffer is often used to stabilize the DNA molecules and protects them from degradation [33]. This process also removes physisorbed oligos from the microcantilever surface. Once a baseline has been obtained, a solution containing complementary target DNA was injected at a flow rate of 0.1 ml /min. Although this flow rate was set for most experiments, it was sometimes changed in order to examine the effect of flow rate on the DNA hybridization process which was not found to be influential.

A number of target sequences with different lengths and concentrations were used in this work in order to gain a deep understanding of DNA hybridization. Not only DNA –DNA hybridization was investigated in this work but also DNA- RNA hybridization has been studied. This variation in using different sequences, concentration, as well as sequence lengths would finally lead us to optimize the DNA hybridization conditions thus developing a proper HIV detector. All the DNA sequences, shown in table 4.1, were purchased from IDT (Integrated DNA Technologies) and were consensus sequences in the HIV gene pol coding for RT genome from the HIV sequence database (see figure 4.1). RNA sequence was synthesized from DNA Technology, Denmark.

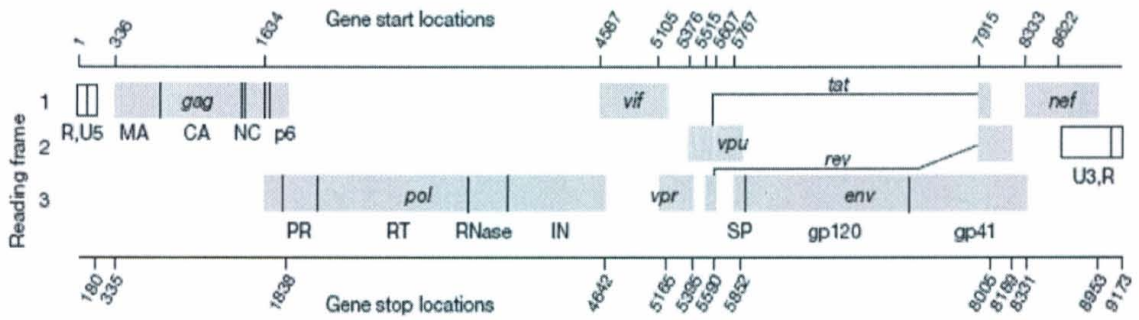


Figure 4.1: A schematic representation of the HIV-1 RNA genome. The RT portion from which our sequences were derived is shown in the Pol polyprotein. Used with permission from [35]

Table 4.1: DNA and RNA sequences used in this work.

Name	Length (bases)	Sequence
Probe	25	5'-/ThioMC6-D/ TCT GTA TGT CAT TGA CAG TCC AGC T-3'
DNA target (1)	25	5'-AGC TGG ACT GTC AAT GAC ATA CAG A-3'
Noncomplementary target DNA	25	5'-TGT TTC CTG TCC TGT CTC TGC TGG G -3'
RNA target	75	5'-GCC UAU AGU GUU GCC AGA AAA AGA CAG CUG GAC UGU CAA UGA CAU ACA GAA GCU AGU GGG AAA AUU GAA UUG GGC-3'
DNA target (2)	200	5'-AAA AAC ATC AGA AAG AAC CTC CAT TCC TTT GGA TGG GTT ATG AAC TCC ATC CTG ATA AAT GGA CAG TAC AGC CTA TAG TGC TGC CAG AAA AAG ACA GCT GGA CTG TCA ATG ACA TAC AGA AGT TAG TGG GAA AAT TGA ATT GGG CAA GTC AGA TTT ACC CAG GGA TTA AAG TAA GGC AAT TAT GTA AAC TCC TTA GAG GA-3'

4.2: Results and Discussion

Once the gold-coated microcantilevers were functionalized with the probe DNA molecules, a solution containing the target DNA was injected into the fluid cell which contained the functionalized and reference microcantilevers. The optical beams were focused on the microcantilevers and the reflected beams were monitored using the PSDs. Figure 4.2 shows the microcantilever response as a function of time after the injection of buffer and a complementary target DNA (DNA target 1 as shown in table 4.1) .

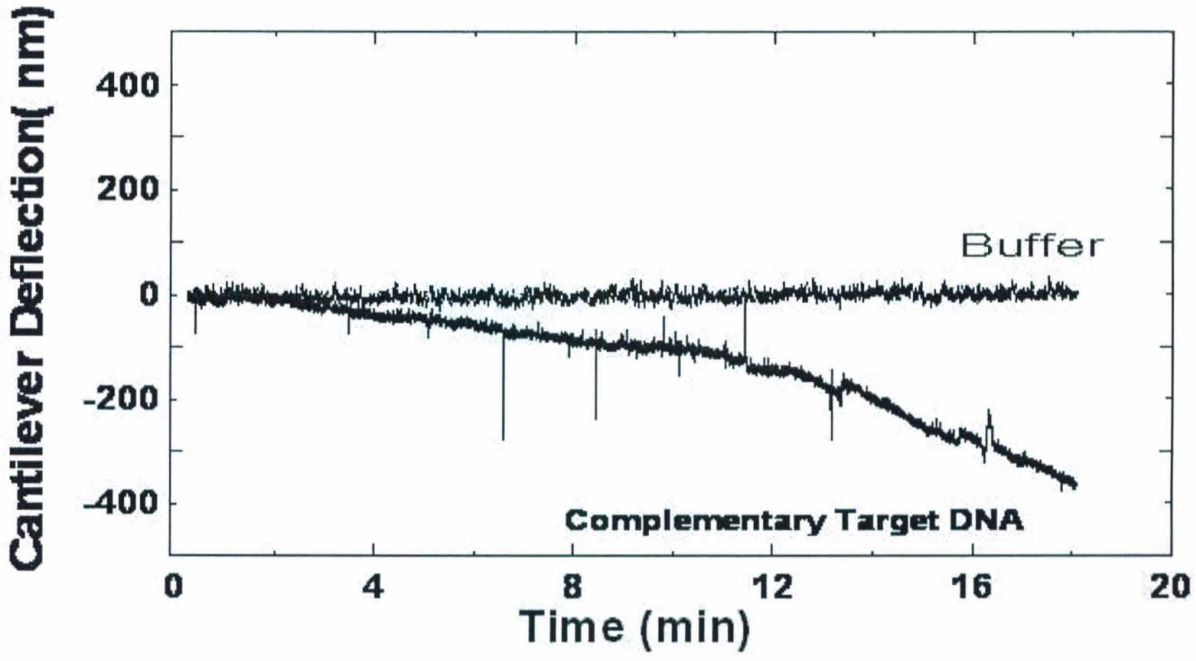


Figure 4.2: Injection of complementary solution where it is clear that the probe-functionalized microcantilever deflects as the target DNA is injected whereas a marginal deflection is seen with the reference microcantilever.

As can be seen in the graph, the active microcantilever deflects as a result of the formation of double strand DNA (dsDNA) which induced a surface stress on the microcantilever surface.

The microcantilever deflection caused by the probe-target hybridization is due to the intermolecular forces which induced surface stress on the probe oligo-fuctionalized microcantilever. On the other hand, the reference microcantilever exposed to buffer solution showed no apparent deflection indicating that the deflection of the active microcantilevers is indeed caused by the DNA hybridization between the probe and the target molecules. In some experiments, we have exposed the HS-ssDNA functionalized microcantilever to the post treatment 6- mercaptor-1- hexanol (MCH). This exposure minimizes nonspecific binding of ssDNA molecules by assuring that ssDNA molecules are only binding to the gold surface through the sulfur atom. Such specific binding is known to greatly increase the hybridization efficiency and therefore the microcantilever deflection [36]. In this work, we have observed such efficiency and higher microcantilever deflection when using MCH.

After immobilizing the HS-ssDNA molecules on the microcantilever surface, the microcantilever was exposed with 1mM post- treatment MCH for 1 hour. As demonstrated in figure 4.3, the microcantilever exposed to MCH treatment deflects faster and greater than the microcantilever without the MCH treatment. Such behavior was expected as a highly organized and packed monolayer may be obtained when using the MCH post treatment.

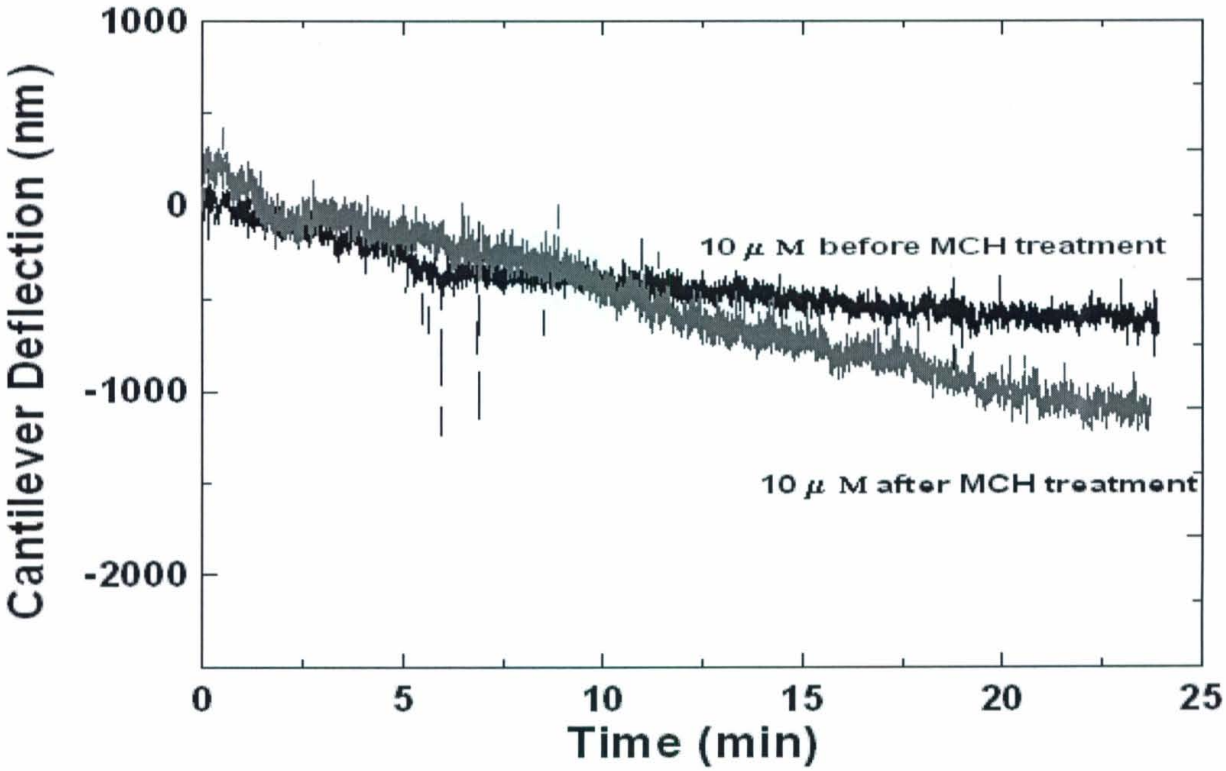


Figure 4.3: The microcantilever shows higher deflection when it is being exposed to the post treatment MCH.

Subsequent experiments included studying the effect of the target DNA concentration on the microcantilever deflection. We have introduced four different concentrations of complementary DNA to the microcantilever functionalized with the probe DNA. These concentrations were 1 μM, 0.1 μM, 2 nM, 0.2 nM respectively. All these different experiments were conducted under the same conditions; i.e. the buffer concentration, incubation time, and the probe concentration. The primary objective of these experiments is to see what the smallest concentration of target DNA can be detected with our system. We have been able to detect a target concentration of 0.2 nM. As clearly demonstrated in the figure 4.4 and 4.5, the microcantilever deflection is a function of the target DNA concentration. Smaller microcantilever deflection is observed for low target DNA concentrations whereas higher deflections are seen for high concentration of target DNA. As can also be seen in the graph, the microcantilevers start bending almost immediately upon injection of the target solutions.

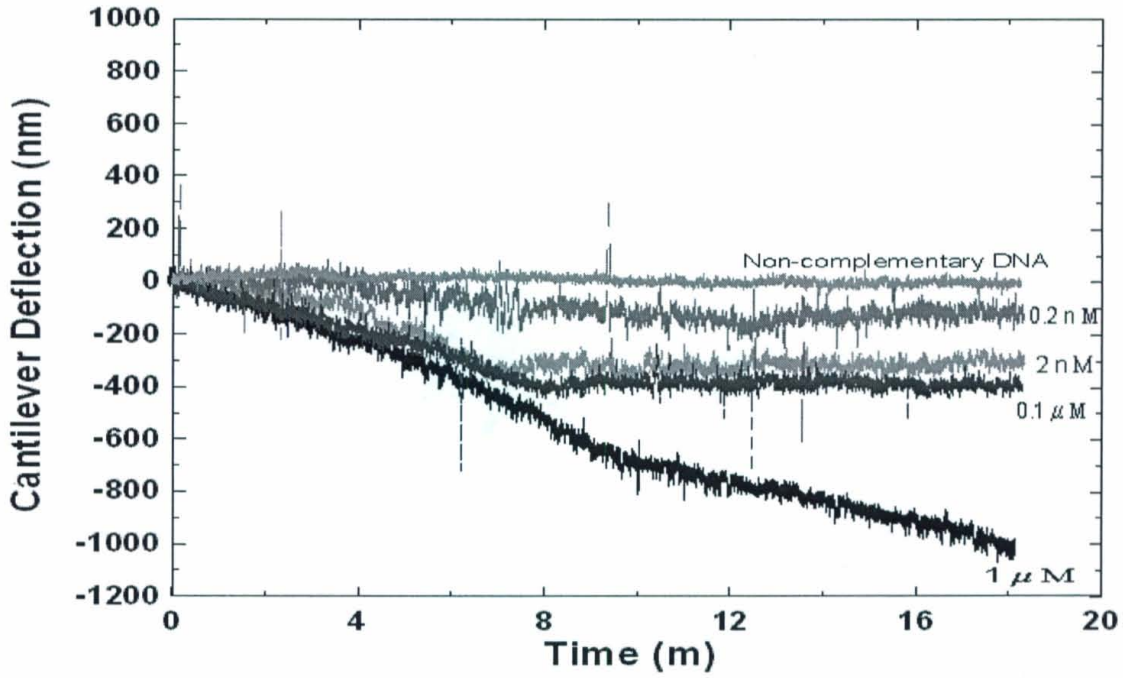


Figure 4.4: Injection of different concentrations of complementary target DNA. Higher concentrations of target DNA increase the microcantilever deflection however lower concentrations give smaller deflections.

Figure 4.4 also shows the microcantilever response due to the introduction of ssDNA with a noncomplementary sequence solution to the probe on the microcantilever. In this case the microcantilever showed a negligible deflection due to nonspecific binding between noncomplementary portions.

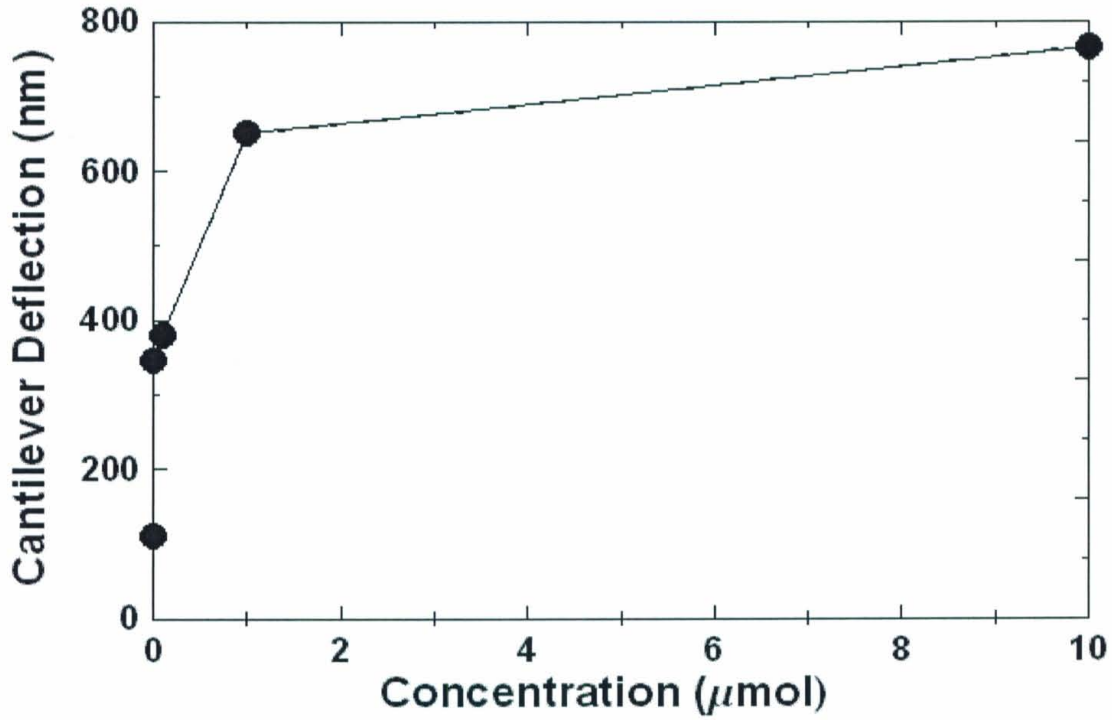


Figure 4:5: Target DNA concentration versus cantilever deflection.

As shown in figure 4.4, the microcantilever deflection reaches a saturation state after a certain amount of time. This state indicates that the all target molecules have hybridized with all the probe molecules which were immobilized on the microcantilever surface. Saturation state is often achieved faster by small concentrations of target DNA rather than high concentrations. We believe that this occurs because the system is out of chemical equilibrium. When the concentration is high more molecules are needed to react with the probe DNA to achieve a chemical equilibrium.

The variation in the microcantilever deflection upon the injection of different target DNA concentrations reflects and exhibits the high sensitivity of the microcantilever. Although the microcantilever deflection is small with lower DNA target concentrations it still demonstrates a sufficient sensitivity to detect these small concentrations. In order to interpret the various deflections of the microcantilever upon the injection of different concentrations, it is first significant to highlight the main causes of the microcantilever deflection after DNA hybridization.

Causes of the microcantilever deflection as a result of DNA hybridization have been investigated and reported in several studies [20, 25, 29, 31, 34, 36]. In most studies regarding DNA hybridization, the microcantilever sensor responds to DNA hybridization by bending to different values. DNA hybridization, as stated before, induces a surface stress on the microcantilever causing a mechanical deflection. Our results show a downward bending of the microcantilever (compressive surface stress) as the DNA hybridization take place however upward bending (tensile surface stress) was also reported [37,38]. The downward bending of the microcantilever is explained as an increase in electrostatic repulsive forces on the microcantilever surface during hybridization [34].

The same groups also attributed the downward bending to the increase in chain packing of the DNA molecules on the microcantilever surface as well as to the interactions between neighbouring DNA molecules [38]. On the other hand, the upward bending of the microcantilever is explained as the decrease in configurational entropy during hybridization [40].

Reports have shown that microcantilever deflection depends strongly on the probe density, the hybridization efficiency, the target sequence length and concentration, as well as salt concentration [31,36,38]. In order to investigate the effect of probe coverage density on the nanomechanical response of the microcantilever, we have varied the incubation time of the DNA immobilization on the microcantilever surface.

Figure 4.6 demonstrates two probe-functionalized microcantilevers exposed to 1 μM target DNA. The microcantilever which was immersed in the probe DNA solution for 3 h shows higher deflection than the microcantilever which was immersed for 24 h. These results are in close agreement with some reports stating that high incubation times lead to a high-density probe coverage which reduces the DNA hybridization efficiency and therefore the microcantilever deflection [37,38].

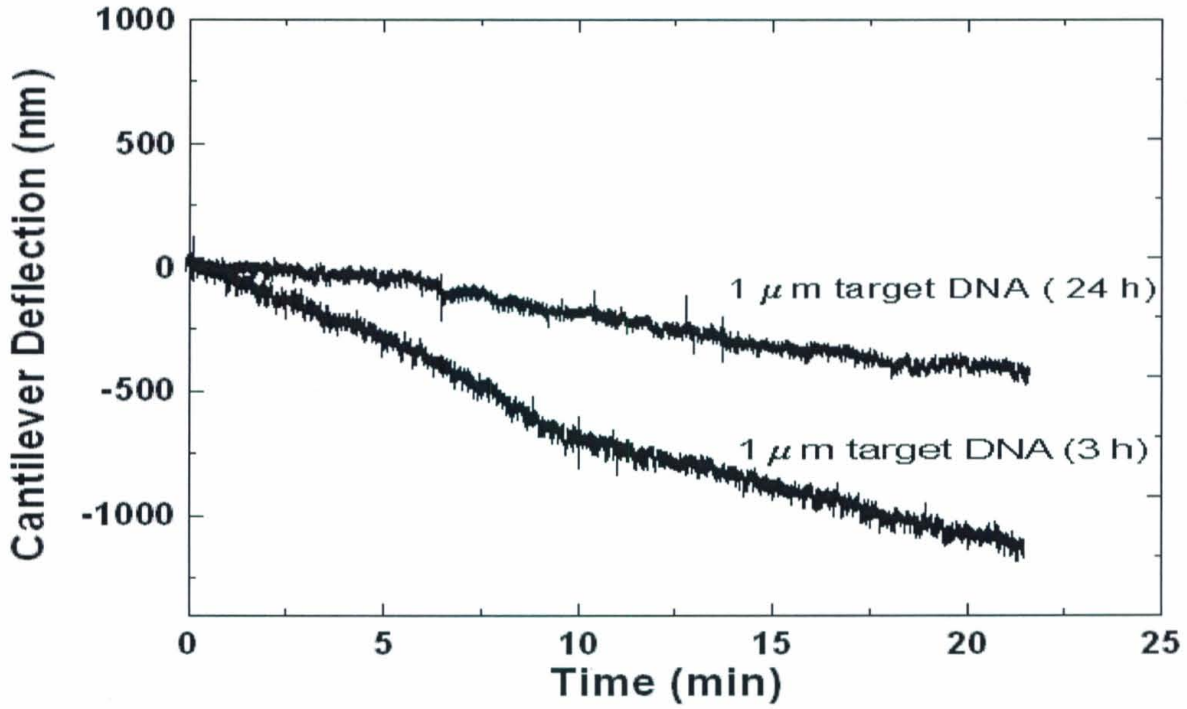


Figure 4.6: Two probe-functionalized microcantilevers were exposed to the same target sequence and concentration show different deflection because of the variation in incubation time.

In this work, we have observed that hybridization efficiency depends, in addition to the probe density, critically on the salt concentration. The dependence of DNA hybridization efficiency on the salt concentration was seen by the injection of target DNA solution with two different salt concentrations (200 mM and 400mM). These concentrations were the same in all immobilization and hybridization process.

These two concentrations were chosen as they are known to be the optimal concentrations within which the hybridization efficiency as well as the microcantilever deflection are the greatest [31]. Studies have also revealed that at salt concentrations greater than 400 mM the microcantilever deflection did not show increased deflection than those using salt concentrations between 200-400 mM [31,38]. Figure 4.7 illustrates the microcantilever deflection as a function of time after the injection of target DNA with different salt concentrations.

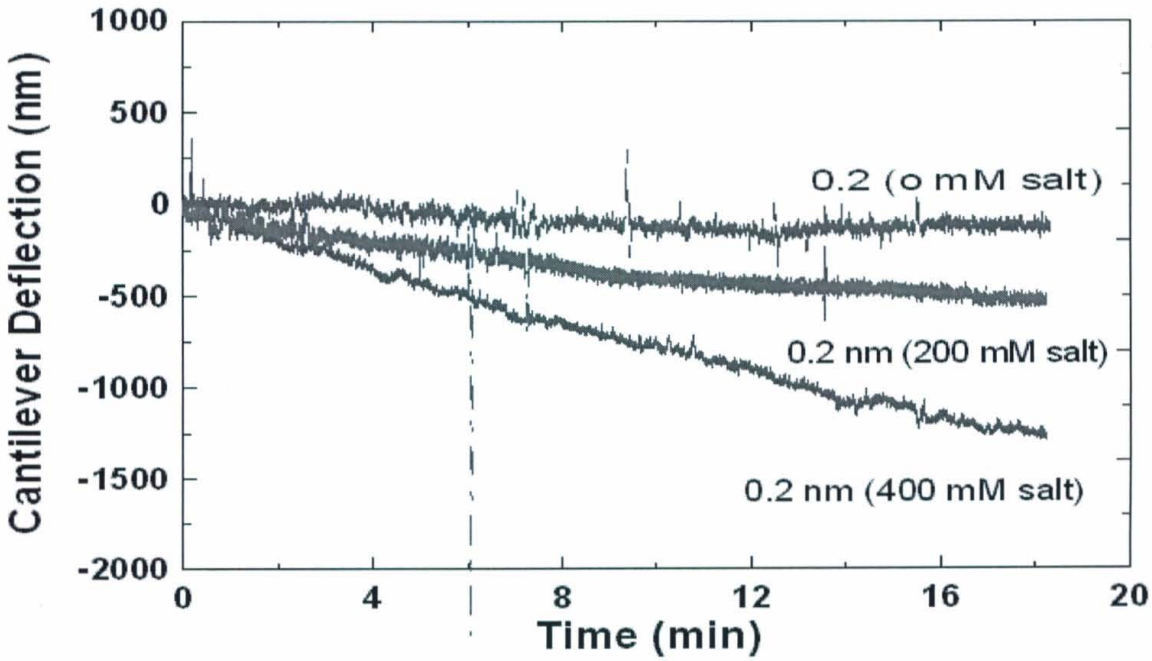


Figure 4.7: The influence of salt concentration is seen to increase the microcantilever deflection.

Figure 4.7 clearly shows that the microcantilever deflection increases with increasing the salt concentration. There are two possible contributions to the increase in the microcantilever deflection when using salt. First, increasing the salt concentration increases the melting temperature (Tm) of dsDNA at which the double stranded DNA separate [39]. This means that at high salt concentration the double stranded DNA would be more stable and hybridized. Secondly, as DNA is negatively charged, the strands repel each other to some degree. Salts contain a positive charge which will congregate around negatively charged DNA allowing them to pair with other ssDNA sequence with less charge repulsion.

After investigating the influence of the target concentration on the microcantilever responses, different lengths of target DNA were investigated. Four different lengths of target DNA and RNA (25 bp, 75 bp, 200 bp, and 1497 bp) were used. The introduction of different target DNA lengths aimed to examine the capability of the microcantilever sensor to detect different length with full and partial complementary templates. Full complementarily between two ssDNA indicates that the two strands have the same length and all target bases are complementary to the probe bases.

Partial complementarily between two ssDNA, however, indicates that the two strands have different length and therefore not all the target bases are complementary to the probe bases. Moreover, the hybridization between the probe DNA and target DNA (target DNA 1 as shown in table 4.1) reflects full degree of complementarity between the probe and target DNA. The hybridization between the probe DNA and RNA target reflects an intermediate degree of complementarity whereas the hybridization between probe DNA and DNA target (DNA target 2 as shown in table 4.1) reflects a low degree of complementarity. We have found the microcantilevers functionalized with probe molecules have been able to discriminate all these sequences with multiple degrees of complementarity at very low concentrations (0.2 nM) as shown in figure 4.8. It is clear from figure 4.8 that the microcantilever deflection is proportional to the sequence length of the target DNA and RNA where the microcantilever deflection increases with increased target length. The expected behaviour during the hybridization between the probe and target with partial complementarily is that complementary portions will hybridize and the interactions between noncomplementary portions are nonspecific. The net deflection of simultaneous complementary and noncomplementary bindings would result in higher deflection than the total deflection of only complementary bindings. In other words, besides the induced surface stress by the hybridization between complementary sequences, additional surface stress induced due to nonspecific bindings is considered to give rise to the microcantilever deflection.

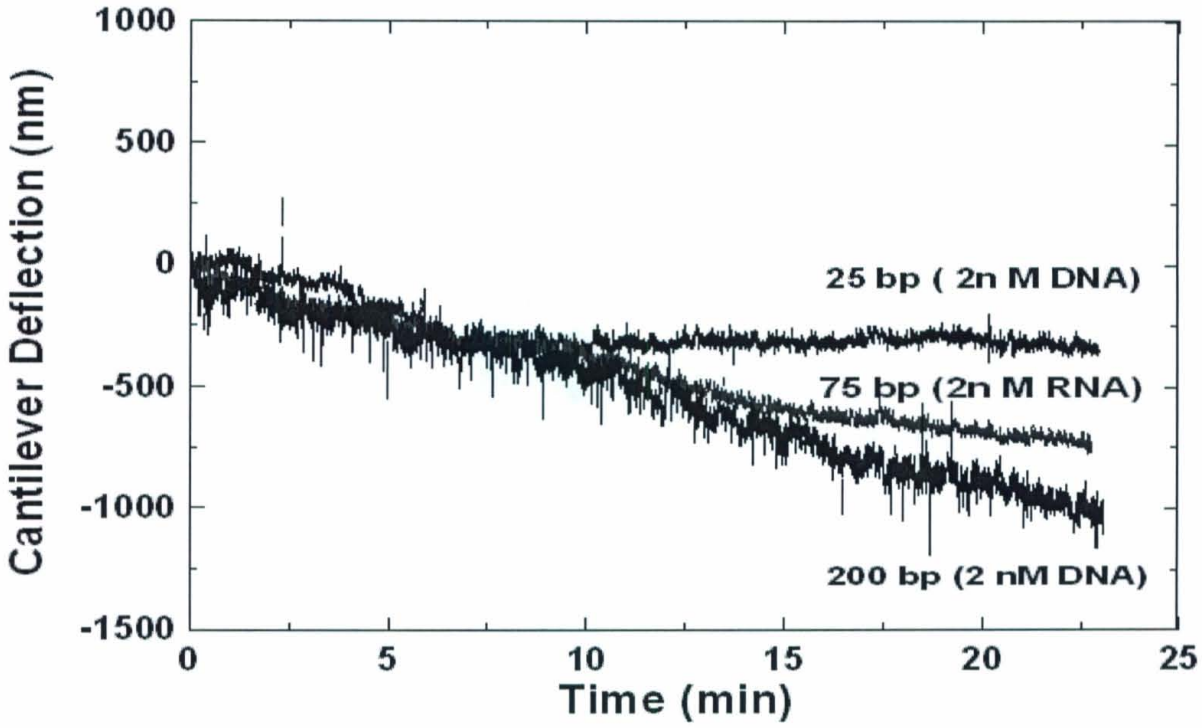


Figure 4.8: Microcantilever deflection as a function of time after the introduction of target DNA and RNA with different lengths.

Furthermore, DNA with a length of 1497 bp was provided to us by the British Columbia Centre for Excellence in HIV/AIDS (BC-CfE). The DNA was created from the RT portion of the HIV genome and amplified to a concentration of 0.2 nM using PCR (Polymerase Chain Reaction).

In this experiment, we have followed the same immobilization and hybridization procedures we used as mentioned elsewhere. As seen in figure 4.9, the active microcantilever deflects after the injection of the target DNA which does not occur with the reference microcantilever. This ensures that the active microcantilever deflection is caused by the hybridization between the probe and target molecules.

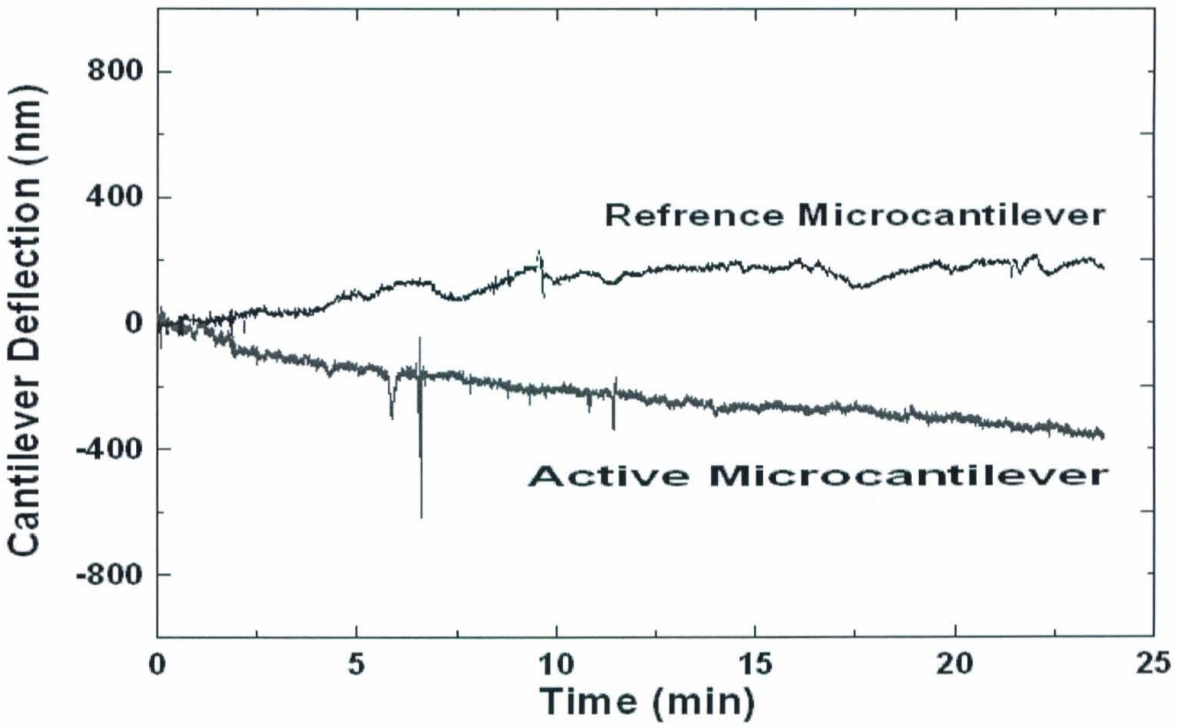


Figure 4.9: The microcantilever deflection after the injection of the 1497 bp target DNA.

This result assures the microcantilever capability of being a powerful and promising technique capable of detecting the hybridization of PCR-amplified DNA strands which is being detected with expensive techniques. Although there have been several methods to detect the amplified target, we here exploit the capability and the sensitivity of the microcantilever sensor to detect the amplified target. Figure 4.10 shows how the 1497 bp DNA fall within other results showing the effect of chain length on the microcantilever deflection.

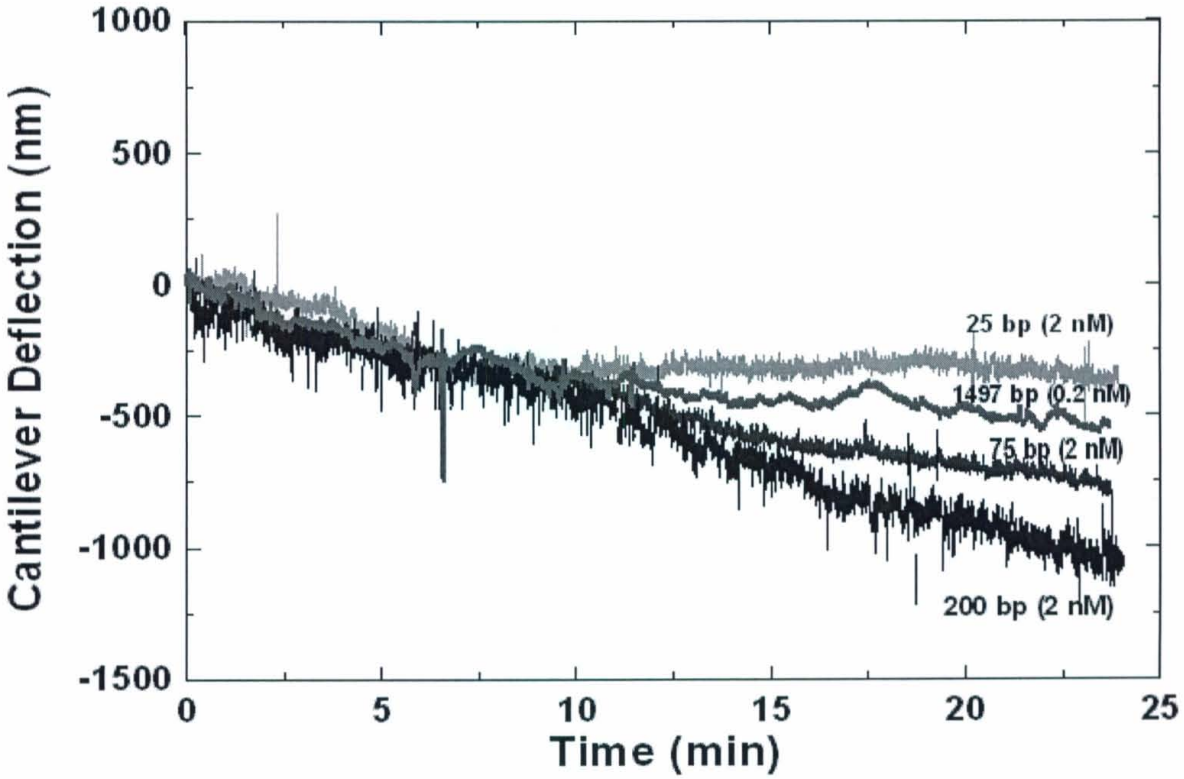


Figure 4.10: Microcantilever deflection as a function of time after the introduction of target DNA with different lengths at the same concentration (2nM) except the HIV target DNA which is 0.2 nM.

Although the HIV DNA is longer than all the other sequences shown in figure 4.10, it had the smallest concentration. However, because of its length, it produces a comparable deflection. We propose that if the HIV DNA was at the same concentration it would produce an even greater deflection than other sequences made.

4.3. New Adjustment to the Set-up

.

To further our attempts to optimize the conditions that govern the nanomechanical response of the microcantilever sensor during DNA hybridization process, a new adjustment was added to the experimental set-up. This new adjustment is a small cylinder piece placed in the fluid cell as shown in figure 4.11.

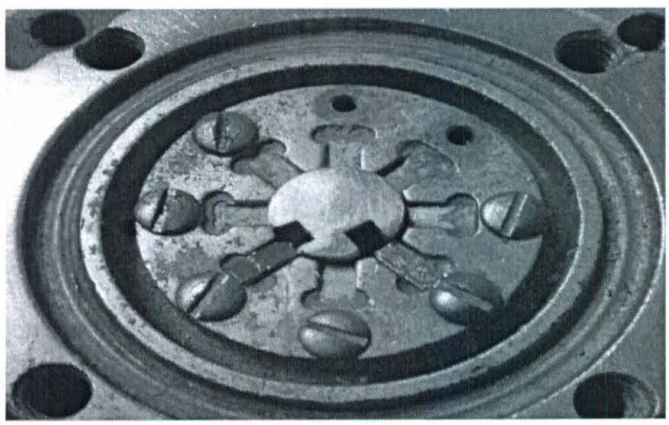


Figure 4.11: The new fixture added to the fluid cell (shown in the middle of the fluid cell) which focuses the injected solution to more directly interact with the microcantilevers .

This fixture focuses the injected solution to more directly interact with the microcantilevers in the fluid cell ensuring a faster and more concentrated diffusion of the solution molecules. As anticipated, this new adjustment produced higher microcantilever deflections of about 250 nm as shown in figure 4.12 than a microcantilever that performed without the fixture.

The injected solution containing complementary target ssDNA interacts with the receptor molecules on the microcantilever surface upon entry into the fluid cell increasing the interactions over the surface, thus raising the microcantilever deflection. This work is in collaboration with other students in our research group.

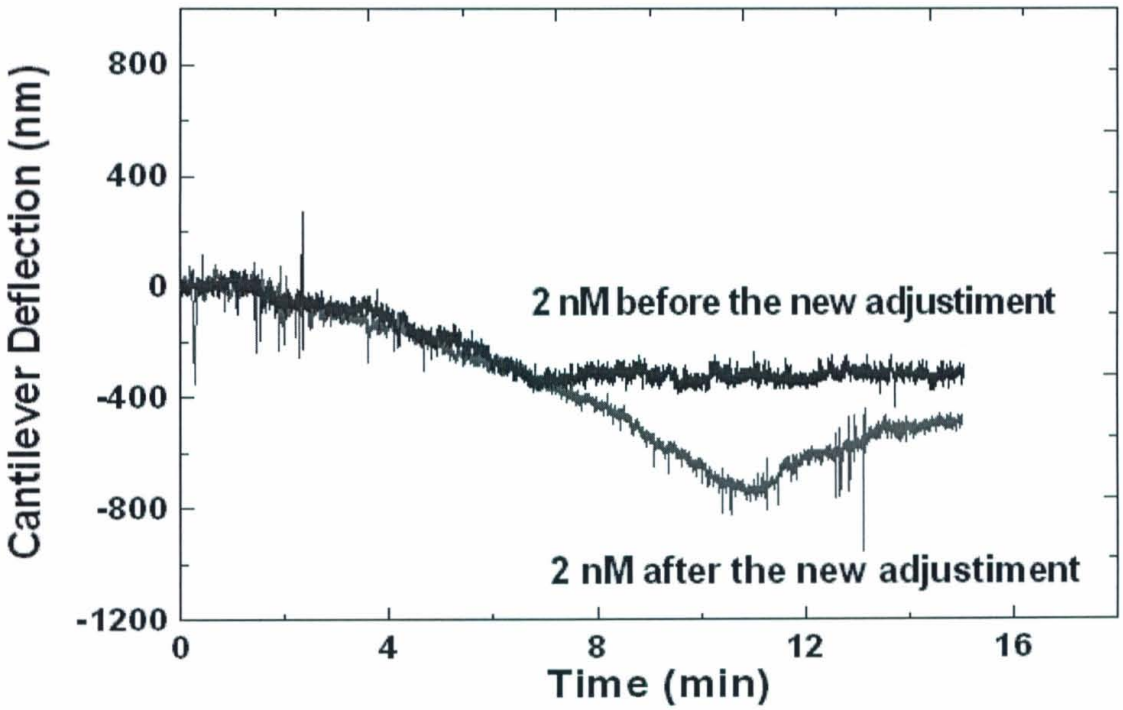


Figure 4.12: Microcantilever deflection upon the injection of target DNA with the same concentration using the new fixture.

Chapter 5

Conclusion and Future Work

5.1 Conclusion

In this work we examined the capability of microcantilever sensors to detect the HIV virus through DNA hybridization in the hopes of developing a cheaper, more sensitive, and more reliable sensor for the early detection of the HIV. Therefore, DNA hybridization experiments were conducted so that a deep insight into the microcantilever response due to DNA hybridization may be gained. Our experimental results showed that the microcantilever responded to the DNA hybridization between the probe and target molecules by deflection. In order to ensure that this deflection was indeed caused by DNA hybridization, reference microcantilevers exposed to either a noncomplementary sequence or buffer were used throughout all experiments.

We have also studied several factors that may affect the nanomechanical response of the microcantilever including the sequence length, concentration, incubation time, and salt concentration.

Variations of the hybridization conditions not only allowed for an understanding of the mechanism behind DNA hybridization but also allowed for an understanding the microcantilever deflection due to the molecular interactions. Furthermore, it has been shown in this work that microcantilever sensors can be employed to offer a label-free, accurate, sensitive, and specific detection of DNA hybridization.

This work also included examining the effect of chain length on the microcantilever response. The microcantilever was able to detect a PCR-amplified target DNA with a length of 1479 bp. This kind of hybridization between a short probe DNA and a long PCR-amplified target DNA has not previously been investigated by other groups. Although we have been able to employ the microcantilever to detect a small concentration of target DNA as 0.2 nM, we are still far from the actual HIV concentration of a blood sample taken from infected patients.

The concentration of HIV RNA in a blood varies drastically over the course of infection. For early infection, this concentration is approximately 8.305 $0x10^{-14}$ nM per ml.

Performing DNA hybridization experiments in this work required the instrument to be well calibrated. Calibration of the instrument involved converting the PSD into an actual microcantilever deflection. Therefore, a program was made which converts the acquired PSD signal into microcantilever deflection.

5.2 Future Work

Future work of this project may include using the microcantilever sensor for developing treatment drugs. HIV entry into the host cell is mediated by binding of the viral gp 120 envelope protein to a cell surface coreceptor (most commonly the CCR5 or CXCR4 chemokine coreceptor), followed by binding to the primary HIV cell-surface receptor CD4. A class of drugs known as HIV coreceptor antagonists act by binding to cellular coreceptor, thereby blocking HIV entry into host cells. Maraviroc, a CCR5 antagonist, is an example of such a drug. Host cell coreceptor CCR5 and CXCR4 can be immobilized on cantilevers to measure their affinity to new small-molecule antagonists which may have potential as novel coreceptor inhibitors.

Future work may also include studying the effect of the cantilever cell temperature in order to better promote DNA hybridization. DNA and RNA hybridization process are more efficient at higher temperatures (such as the body temperature) than the room temperature at which our experiments were conducted. Thus, increasing the cantilever cell temperature to be similar to that of the body temperature would result in more realistic results.

Another important component of this work is to compare the sensitivity of hybridization type sensing platforms to platforms based on the antigen/antibody capture mechanism.

Bibliography

1- Raiteri, R., Grattarola, M., Butt, H., & Skladal, P. (2001). Micromechanical cantilever-based biosensors. Sensors and Actuators B-Chemical, 79(2-3), 115-126.

2- Godin, M., Tabard-Cossa, V., Miyahara, Y., Monga, T., Williams, P. J., Beaulieu, L. Y., et al. (2010). Cantilever-based sensing: The origin of surface stress and optimization strategies. Nanotechnology, 21(7), 075501.

3- Boisen, A., Dohn, S., Keller, S. S., Schmid, S., & Tenje, M. (2011). Cantilever-like micromechanical sensors. Reports on Progress in Physics, 74(3), 036101.

4- Grogan, C., Raiteri, R., O'Connor, G., Glynn, T., Cunningham, V., Kane, M., et al. (2002). Characterisation of an antibody coated microcantilever as a potential immuno-based biosensor. Biosensors & Bioelectronics, 17(3), 201-20.

5- Su, M., Li, S., & Dravid, V. (2003). Microcantilever resonance-based DNA detection with nanoparticle probes RID B-6688-2009. Applied Physics Letters, 82(20), 3562-3564.

6- Meng Xu. (2006). Characterizing the initial state of cantilever sensors. (M.Sc., Dept. of Physics and Physical Oceanography: Memorial University of Newfoundland.).

7- Hwang, K. S., Lee, S., Kim, S. K., Lee, J. H., & Kim, T. S. (2009). Micro- and nanocantilever devices and systems for biomolecule detection. Annual Review of Analytical Chemistry, 2, 77-98.

8- UNAIDS http://data.unaids.org/pub/Outlook/2010/20100713_fs_outlook_epi_en.pdf

9- Luo, R. (2002). Understanding the threat of HIV/AIDS. Jama-Journal of the American Medical Association, 288(13), 1649-1649.

10- Verheyden, B., Thielemans, A., Rombaut, B., & Kronenberger, P. (2003). RNA extraction for quantitative enterovirus RT-PCR: Comparison of three methods. Journal of Pharmaceutical and Biomedical Analysis, 33(4), 819-823.

11- Malhotra, K., Kyger, E., O'Donnell, P., Johnson-baugh, J., Shyu, J., Luk, K., et al. (2003). Development of a highly sensitive and reproducible one-tube real-time quantitative RT-PCR (TaqMan) assay for the detection of HIV type 1 RNA in human plasma. Clinical Chemistry, 49(6), A140-A140.

12- Benson, C., Kaplan, J., Masur, H., Pau, A., & Holmes, K. (2005). Treating opportunistic infections among HIV-infected adults and adolescents: Recommendations from CDC, the national institutes of health, and the HIV medicine association/infectious diseases society of america. Clinical Infectious Diseases, 40, S131-S235.

13- National Institutes of Health. National Human Genome Research Institute http://www.genome.gov/glossary/index.cfm?id=53

14- Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D., Darnell, J., 2000, Molecular Cell Biology, 4th Ed., W.H. Freeman and Company, NY, New York. ISBN 0-7167-3136-3

15- Kalichman, Seth C, (1998)."Understanding AIDS: A_advances in research and treatment . Kalichman, Seth C.". American Psychological Association, 420-421.

16- National Institute of Allergy and Infectious Diseases. http://www.niaid.nih.gov/factsheets/graphics/howhiv.jpg

17- Carol Mattson Porth and Glenn Matfin. (2009). Acquired immunodeficiency syndrome. Porth pathophysiology: Concepts of altered health states (8th Edition ed., pp. 412-428) Lippincott Williams & Wilkins.

18- Constantine, N., & Zink, H. (2005). HIV testing technologies after two decades of evolution. Indian Journal of Medical Research, 121(4), 519-538.

19-Relucio, K.,. (2002). HIV-1 RNA and viral load. Clinics in Laboratory Medicine, 22(3), 593.

20- Vikholm-Lundin, I., Piskonen, R., & Albers, W. M. (2007). Hybridisation of surface-immobilised single-stranded oligonucleotides and polymer monitored by surface plasmon resonance. Biosensors & Bioelectronics, 22(7), 1323-1329 .

21- Bellis, M., & Casellas, P. (1997). DNA chip: A bench multi-reactor. M S-Medecine Sciences, 13(11), 1317-1324.

22- Rockett, J.,. (2000). DNA arrays: Technology, options and toxicological applications. XENOBIOTICA, 30(2), 155-177

23- Vercoutere, W.,. (2002). Biosensors for DNA sequence detection. Current Opinion in Chemical Biology, 6(6), 816-822

24- Fritz, J., Lang, H. P., Rothuizen, H., & Vettiger, P. (2000). Translating biomolecular recognition into nanomechanics. Science, 288(5464), 316-318.

25- Alvarez, M., Carrascosa, L., Moreno, M., Calle, A., Zaballos, A., Lechuga, L., et al. (2004). Nanomechanics of the formation of DNA self-assembled monolayers and hybridization on microcantilevers. Langmuir, 20(22), 9663-9668.

26- Joshua Rideout (2007). Analysis of the E®ect of Sputtering Parameters on the Grain Size of Thin Cu Films on Si(001) Substrates. (Honours thesis, Dept. of Physics and Physical Oceanography: Memorial University of Newfoundland.).

27- Grüter, P., Laroche, O., Tabard-Cossa, V., & Grutter, P. (2006). Calibrating laser beam deflection systems for use in atomic force microscopes and cantilever sensors. Applied Physics Letters, 88(8), 083108

28- Beaulieu, L. Y., Laroche, O., Tabard-Cossa, V., & Grutter, P. (2007). A complete analysis of the laser beam deflection systems used in cantilever-based systems. Ultramicroscopy, 107(4-5), 422-430.

29- Steel, A., Levicky, R., Herne, T., & Tarlov, M. (2000). Immobilization of nucleic acids at solid surfaces: Effect of oligonucleotide length on layer assembly. Biophysical Journal, 79(2), 975-981

30- Integrated DNA Technologies. (2005). Strategies for attaching oligonucleotides to solid supports.

31- Castelino, K., Kannan, B., & Majumdar, A. (2005). Characterization of grafting density and binding efficiency of DNA and proteins on gold surfaces. Langmuir, 21(5), 1956-1961

32- Peterson, A., Heaton, R., & Georgiadis, R. (2001). The effect of surface probe density on DNA hybridization. Nucleic Acids Research, 29(24), 5163-5168.

33- Herne, T., & Tarlov, M. (1997). Characterization of DNA probes immobilized on gold surfaces. Journal of the American Chemical Society, 119(38), 8916-8920. doi:10.1021/ja9719586

34- Levicky, R., Herne, T., Tarlov, M., & Satija, S. (1998). Using self-assembly to control the structure of DNA monolayers on gold: A neutron reflectivity study. Journal of the American Chemical Society, 120(38), 9787-9792.

35- Watts, J. M., Dang, K. K., Gorelick, R. J., Leonard, C. W., Bess, J. W., Jr., Swanstrom, R., et al. (2009). Architecture and secondary structure of an entire HIV-1 RNA genome. Nature, 460(7256), 711-U87.

36- Steel, A., Levicky, R., Herne, T., & Tarlov, M. (1999). Electrochemical quantitation of DNA immobilized on gold. Abstracts of Papers of the American Chemical Society, 218, U173-U173.

37- Hansen, K., Ji, H., Wu, G., Datar, R., Cote, R., Majumdar, A., et al. (2001). Cantilever-based optical deflection assay for discrimination of DNA single-nucleotide mismatches. Analytical Chemistry, 73(7), 1567-1571.

38- Wu, G., Hansen, K., Thundat, T., & Datar, R. (2001). Origin of nanomechanical cantilever motion generated from biomolecular interactions. Proceedings of the National Academy of Sciences of the United States of America, 98(4), 1560-1564

39- Biswal, S. L., Chaiken, A., Birecki, H., & Majumdar, A. (2006). Nanomechanical detection of DNA melting on microcantilever surfaces. Analytical Chemistry, 78(20), 7104-7109.