ELECTRICAL CHARACTERIZATION OF THREE DIMENSIONAL DNA-BASED BIONANOELECTRONICS PLATFORMS

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DEDICATION

I would like to dedicate this master’s thesis to my husband, Shannon Tyler Winchester, who assisted me a great deal throughout my entire master’s program and has been a great source of motivation and inspiration.

Also, this thesis is dedicated to my family and who have supported me all the way since the beginning of my studies.

Finally, this thesis is dedicated to all those who believe in the richness of learning.
Obstacles are things a person sees when he takes his eyes off his goal.

- E. Joseph Cossman
ABSTRACT OF THE THESIS

The electrical conductivity of DNA remains to be controversial with various studies which report DNA properties varying from insulator to extreme conductivity (superconductive).

This study investigates experimentally the electrical behavior and performance of a double-stranded Lambda DNA wire on two and three dimensional electrodes by suspending the DNA thereby eliminating the effect of substrate that is thought to be the culprit of inconsistent results. The two dimensional DNA platform design developed in this study helped to obtain an optimized three dimensional platform. The 3-D chip architecture is made of layers of a negative photoresist (SU8) and gold layered on silicon dioxide substrate. The DNA attachments on 3-D and 2-D electrodes were demonstrated based on the following methods: Oligo-DNA self assembly, electrostatic and, electrical field attractions. Electrical results based on I-V and R-V curves showed measurable and significant conductivity through the DNA wire that we believe could establish it as a semi-conductor. A mathematical model based on I-V data as well as an electrical circuit model for lambda DNA are also developed in this study. An equivalent electrical circuit was created in PSpice where DNA is modeled as a voltage-controlled current source. This is important because having models of DNA molecules in the form of equivalent electronic circuits would be useful in the design of nanoelectronic circuits and devices.

The research presented here is characterized by a significant departure from previous studies and made unique contributions by (i) DNA assembly on three dimensional structures which showed lower resistance and higher conductivity in comparison to 2-D or flat electrodes. (ii) 3-D DNA platform structure demonstrated better stability than 2-D structure. (iii) Additionally, these high aspect-ratio 3-D electrodes prevented the suspended DNA from contacting the substrate. This helps to collect more accurate resistance measurements.(iv) design of narrow and tapered electrode tips helped to guide and attract DNA electrostatically between two gold posts.(v) Furthermore, design of four electrodes in this study had an advantage that almost no current flowed in the sense wires, thus the voltage drop was extremely low. This allowed more precise measurements than traditional two sensing probes.
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CHAPTER 1
INTRODUCTION

In the past several years, the discovery that DNA perhaps can conduct electrical current has made it an interesting candidate for roles that nature did not intend for this molecule [1]. On the surface DNA and electronics seem to be worlds apart; however, a series of recent research have highlighted the unusual ability of DNA to form electronic components [2]. DNA could be useful in nanotechnology for the design of electric circuits [3], which could help to overcome the limitations that classical silicon-based electronics is facing in the coming years. Moreover, DNA-the blueprint of life- has taken centre stage in biological research during the past few decades. The field of biotechnology is revolutionizing by the elucidation of the DNA molecule’s structure and its electrical characters. With study of the electrical properties of DNA it is possible to detect mismatches in double stranded DNA because such a mismatch would hamper conductivity. This promises further advances in understanding DNA-repair processes in damaged DNAs\(^1\), DNA correlation with disease, and the process of ageing [4]. Further advances in this field, could allow the use of DNA-based electronic sensors to diagnose DNA-based disease [5].

\(^1\) DNA damage is induced by ultraviolet radiation or oxidative stress, causing oxidation of guanine, and electron holes then migrate along the DNA molecule.
In general, advancement in DNA structural knowledge has the ability to create new industries and various tools in electronic and biology fields.

1.1 BACKGROUND

The structure of DNA was discovered by Watson and Crick who received a Nobel Prize for their work in 1962 [6]. The question of whether the molecule could be used as an electrical conductor or insulator was ripe even at that time, but the technology was not. The idea of using organic molecules for building electronic components dates back to 1974 [7]. Fink & Schonenberger (1999) were the first to measure current flow through DNA using a modified low-energy electron point-source microscope. More recently, Porath et al. (2000) have shown that a 10.4 nm long (30-base-pair) poly(G)–poly(C) sequence has electrical characteristics similar to that of a semiconducting diode that allows current to flow in one direction only.

Gordon Moore, from the Intel Corporation, formulated a law in 1965, now known as Moore’s Law, stating that the number of transistors on a chip would double every 18 months, but that this trend drastically changed from 2010 and the doubling rate dropped to every 4–5 years. DNA-based electronics has the potential to extend beyond Moore’s Law, proclaiming the end of conventional microelectronics. However, building a computer based on DNA molecules is still a long way off.

1.2 DNA STRUCTURE

DNA, deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). DNA bases pair up with each other, A with T and C with G, to form units called base pairs (Figure 1.1). Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide (Figure 1.2). Nucleotides are arranged in two long strands that form a spiral called a double helix (Figure 1.3, Figure 1.4). The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder’s rungs. The sugar and
phosphate molecules are forming the vertical sidepieces of the ladder. The DNA double helix is stabilized by hydrogen bonds between the bases attached to the two strands [8].

Figure 1: DNA is a double helix formed by base pairs attached to a sugar-phosphate backbone. 1-A&T, C&G bases pairs, 2-Two Single strand DNAs, 3- Double strand DNA, 4- DNA helix. Photo Source: Wikipidia
The most important property of DNA is its ability to self assemble, which makes it possible to produce nanostructures with a precision that is not achievable with classical silicon-based technologies [9]. It also can adopt various states, conformations, and it is able to self-replicate economically from a process standpoint [10].

1.3 MOTIVATION FOR THIS STUDY

DNA is very complex molecular wire and has demonstrated a wide variety of electronic properties in various experiments including insulator, semiconductor and, superconductor behavior[11]. This diversity in experimental results is based on different factors such as environmental effects (temperature, PH and, humidity), differences in the base-pair sequence of the DNA, length, molecule orientation, number of DNA molecules in a sample, DNA molecule internal vibrations, metal molecule contact, and the quality of the DNA-electrodes interface (Figure 2). All these conditions can affect the conductive properties of DNA.

![Figure 2: Conditions that effect on conductive properties of DNA wire assembled between two electrodes, Photo source: nano.tu-dresden.de](image-url)
One of the experimental parameters that affects accuracy of DNA electrical characterization experiments, is the effect of the substrate. Eliminating this effect is the main motivation of this work.

This study has some advantages over the previous related research efforts. DNA attachment on three dimensional platforms has been done for the first time in this research which helps to easily suspend DNA between two metal posts. In addition, this new DNA platform design helps to concentrate electrostatic force on the electrode tips which can absorb the negatively charged DNA to selectively be located between the electrodes. Moreover, the electrodes with four bump pads give us the opportunity to use four point probes to measure electrical impedance technique that uses separate pairs of voltage-carrying and current-sensing electrodes to make more accurate measurements than traditional two-terminal sensors.

1.4 Organization of This Thesis

In this study, Chapter 1 gives Introduction. Chapter 2 covers the investigation of electrical characters of DNA in related literatures. In chapter 3, a fabrication process for positive lithography to achieve a 2-Dimensional chip and negative lithography to achieve a 3-Dimensional platform are explained. In chapter 4, the process of attachment of DNA on these two platforms is discussed which contains two methods for DNA assembly by use of oligos and electrostatic force. Chapter 5 consists the electrical characterizations of the suspended DNA between two gold electrodes on 2-D and 3-D platforms in which resistivity and I-V properties of DNA wire will be discussed. A mathematical and an electrical model for DNA wire are also introduced. Finally, chapter 6 discusses and concludes DNA wire experiment and purposes future research and development (Figure 3).
Figure 3: Overview of the flow of this study; (a) chip fabrication, (b) DNA attachment, (c) DNA electrical measurements
CHAPTER 2
LITERATURE SURVEY

This chapter summarizes recent research efforts of electrical properties of DNA nanowires.

2.1 DNA-BASED BIO-NANOELECTRONICS

Although several experiments proposed DNA as an insulator (Dunlap et al.) [12], Conductor (Cai et al. 2000) [13] or, superconductor (Kasumov et al. 2001) [14], more recent research demonstrates semiconductor-like behavior for DNA (Porath et al. 2000) [15]. At 2008, Felice and Porath reviewed the current level of understanding of the behavior of DNA polymers as conducting wires, based on experimental and theoretical investigations of the electronic properties, determined by the $\pi - \pi$ superposition along the helical stack [16]. Contrary to earlier beliefs that the conductivity of double stranded DNA molecules is negligible, they carried out clear-cut measurements of high currents under controlled experimental conditions that rely on avoiding nonspecific molecule-substrate interactions and realizing electrode-molecule covalent binding to indicate that dsDNA could have considerable conductivity. Their work outlined the importance of immobilizing molecules onto inorganic substrates in view of technological applications and noted selected observations by suitable imaging techniques. As a parallel effort, there is some research work in tracing the route toward the exploration of tailored DNA derivatives that may exhibit enhanced conductivity [17].

To begin characterizing DNA wires, we need to select a species of DNA that possesses conductive qualities need to be selected. Cohen et al, compared electrical transport through a single stranded DNA (ssDNA) monolayer and double stranded DNA (dsDNA) monolayers with and without upper thiol end-groups. They found that the ssDNA monolayer is unable to transport current. In contrast, the dsDNA monolayer with thiols on both ends can transport significant current [18].
These results reconfirm the ability of dsDNA to transport electrical current under the appropriate conditions, demonstrate the efficiency of an ssDNA monolayer as an insulating layer, and emphasize the crucial role of an efficient charge injection through covalent bonding for electrical transport in single dsDNA molecules [19].

The fabrication of DNA-conjugated nanoparticles based on self-assembly seems to have received the most attention in recent years. Along these lines, Mirkin et al. and Alivisatos et al. [20] were the first to describe self-assembly of gold nanoclusters into periodic structures using DNA. Mirkin et al. [21] described a method of assembling colloidal gold nanoparticles into macroscopic aggregates using DNA as linking elements. The method involved attaching non-complementary DNA oligonucleotides to the surfaces of two batches of gold particles capped with thiol groups, which naturally bind to gold. When another oligonucleotide duplex with ends which are complementary to the grafted sequence is introduced, the nanoparticles self-assemble into aggregates. Mucic et al. [22] have also described the construction of binary nanoparticle networks composed of 9 nm particles and 31 nm particles, both composed of citrate-stabilized colloidal gold. The same concept has recently been extended to metallic nanowires/rods [23, 24, 25]. Here, the basic idea is to fabricate gold and/or platinum metal wires, functionalize these wires with ssDNA, and assemble them on substrates which have the complementary ssDNA molecules attached at specific sites. Thus, self-assembly of interconnects and wires can be made possible. In one approach, metallic wires are formed by electroplating in porous alumina membranes with pores sizes of about 200 nm. Metallic rods, ranging from 1–6 µm in length were produced, depending on the electroplating conditions.

In a series of publications, Braun and his colleagues demonstrated the use of DNA as a template for the fabrication of nanowires [26, 27, 28, 29]. Braun et al. formed a DNA bridge between two gold electrodes, again using thiol attachment. Once a DNA bridge is formed between the 12–16 µm spacing of the electrodes, a chemical deposition process is used to vectorially deposit silver ions along the DNA through AgC/NaC ion exchange and formation of complexes between the gold and the DNA bases. The result is a silver nanowire which is formed using the DNA as a template or skeleton. Current–voltage characteristics were measured to demonstrate the possible use of these nanowires. The authors also reported the formation of luminescent self-assembled poly (p-phenylene vinylene) wires for possible
optical applications [30]. The work has a lot of potential and much room for further research to control wire width, contact resistances between the gold electrode and the silver wires, and use of other metals and materials. DNA self assembly method of this paper used as a guideline for our scheme to hybridize the DNA molecule with surface bound oligonucleotides and stretch it between two gold electrodes [31].

Braun’s group has also recently extended its work in the use of DNA molecules as templates for self-assembled nanometer-scale conductive metallic wires - this time using gold. Standard molecular biology techniques allow addresses, at nanometer-scale resolution, on the underlying DNA sequences, for the precise localization of electronic materials (e.g. gold particles), converting the insulating biological molecules into functionalized electronic components. Moreover, recombinant DNA, the natural mechanism of gene mixing in cells, can be employed for the formation of molecularly accurate DNA junctions that can serve as templates for more complex electronic devices [31]. They used a two-step self-assembly approach, where the inherent molecular recognition capabilities of DNA molecules are first utilized to construct a network that serves as a template for the subsequent assembly of electronic materials into a functionalized circuit. Specifically, RecA protein that is responsible for recombination in E. Coli bacteria was utilized to construct accurate DNA junctions. These junctions can serve as templates for electronic switching devices that are localized there.
Ground breaking research by Seeman and colleagues [32, 33, 34] has laid a foundation for the construction of structures using DNA as scaffolds, which may ultimately serve as frameworks for the construction of nanoelectronic devices. It is now well recognized that the application of biological self-assembly systems for electronic applications, particularly for features below 100 nm range carries huge potential in advancing technological progress in nanoelectronics. In such an approach, DNA acts as a general template for metal deposition, and the specific molecular recognition of base sequences can be exploited to address electronic components. So far, a growing number of largely independent approaches to nanowire fabrication have been proposed; but it is believed that these methods still require extensive development and optimization before functional nanoelectronic applications could be realized.

In general, the application of biomolecules in nanostructure generation is an interesting alternative to techniques in physical nanotechnology. Nucleic acids, particularly DNA, provide suitable chemical and physical properties to become a feasible object for molecular construction. The core principle of such DNA-based complexes is the self-organization of the DNA molecules. Synthetic short single-stranded DNA can be coupled to substrate surfaces, and the resulting thin films represent functional monolayers. Further, long DNA molecular chains provide the framework for nano-constructions. The connections between these molecules and biologically functionalized planar or nanoparticulate substrates is realized by self organization guided by the predefined complementarity and affinity of the utilized DNA. This coupling reaction can be controlled by parameters such as temperature, pH, and ionic concentrations.

In the past several years, a significant amount of interest has focused on developing concepts and approaches for self-assembled systems. It has also been suggested that complex network of potentially useful particles for a variety of optical, electronic, and sensing applications could be manufactured exploiting the exquisite molecular recognition of various natural biological materials [35].

Washizu’s (2003) method of DNA stretching based on electrostatic effects was inspired us to try stretching and attachment of DNA between two gold electrodes electrostatically.
DNA in water solution can be stretched to a straight shape, and immobilized with its terminus onto the electrode edge. When the electrode gap is made equal to the length of DNA, DNA can be immobilized at both ends bridging over the electrodes [36].

Nogues et al, reported electrical transport measurements through dsDNA molecules that are embedded in a self-assembled monolayer of single-stranded (ss) DNA and attached to a metal substrate and to a gold nanoparticle on opposite ends. The measured current flowing through the dsDNA amounts to 220 nA at 2V (Figure 2) [37]. This result is comparable with I-V curves received from λDNA in our research.

In another study, Hodzic et al have developed a PSpice model of the electrical behavior of DNA molecules for use in nanoelectronic circuit design base on the I-V curve of dsDNA. The equivalent electrical circuit of DNA showed wide-band-gap semiconductor behavior (Figure 5). It was modeled as a voltage-controlled current source described by the mathematical model that includes temperature dependence GPOLY(T).

The mathematical model is described by the "fitted fifth-order polynomial" with temperature dependent coefficient of the linear term.

\[ Y=A_1+A_2X+A_3X^2+\ldots \]

Where, \( Y = \) Current, \( X = \) Voltage (V), \( A = \) Temperature dependent coefficient

This data helped us to build an electrical model based on our I-V curve. Having models of DNA molecules in the form of equivalent electronic circuits would be useful in the design of nanoelectronic circuits and devices [38].
CHAPTER 3

DESIGN AND MICROFABRICATION OF 2-D AND 3-D DNA WIRE PLATFORMS WITH (30-0.5) µM GAP

With electrical characterization in mind where the effect of substrate is eliminated, we purpose to build a 3-dimentional (3-D) platform containing of 3-dimentional electrodes (covered with gold) from witch ds DNA is suspended. To enable that, we start with the design and fabrication of a 2 dimensional chip (2-D) to achieve the best design. Then, we moved to 3-D chip to optimize our DNA wire chip fabrication.

In this chapter, a complete discussion of the design and microfabrication of both 2-D and 3-D DNA wire platforms is given. The difference between 2-D and 3-D structures is based on two different lithography techniques, positive lithography and negative lithography. The end-goal of these fabrication methods is to create a platform consisting of two micro-electrode bridges which will provide attachment points for double stranded DNA (dsDNA). In positive lithography, a positive photoresist is coated on top of a substrate and when exposed to UV light, the underlying material is removed wherever the positive photoresist exists. The reason for this phenomenon is UV light changes the chemical structure of the photoresist so that it becomes more soluble in developer solution (Figure 6).
In negative lithography, the negative photoresist behaves in the opposite manner; the negative photoresist becomes polymerized\(^2\) and difficult to dissolve in the developer wherever it is exposed to UV light (Figure 7).

![Figure 6: +Positive Resist](image)

![Figure 7: - Negative Resist](image)

Typically, positive and negative lithography have some common and some discrete steps. Some of the common steps are: wafer dicing, chip cleaning, dehydration, photoresist coating, pre-backing, UV exposure, gold sputtering, and stripping. The individual steps for positive lithography include developing the chip before gold sputtering and stripping the gold layer after sputtering. This method provides a gold feature in direct contact with its substrate. This top-down approach provides a flat feature which appears two dimensional (2-D) while individual steps for negative lithography such as adding post-backing and omitting the chemical photo developer provide a chip consisting of two layers of gold and negative resist (on top of the substrate). The final feature with this bottom up approach creates a three dimensional (3-D) structure.

In this chapter, speed of spin-coating in rpm (revolutions per minute), UV intensity rates with specific time durations for coating, exposure, development, gold-sputtering and, stripping will be presented. These factors are required to develop new protocols to fabricate 2-D and 3-D DNA wire platforms with gap sizes of 30\(\mu\)m to 0.5 \(\mu\)m between electrodes. There are some advantages to utilizing each of these methods. For instance, 3-D (negative lithography) offers high aspect ratio features with smaller gaps than 2-D (positive

\(\text{\textsuperscript{2}}\) Polymerization is a process of reacting monomer molecules together in a chemical reaction to form three-dimensional networks or polymer chains
lithography). Additionally, negative lithography features exhibit better adhesion to both substrate and upper gold layers in comparison to positive lithography features. Moreover, negative photoresist demonstrates higher temperature resistance than positive photoresist. However, in the case of removing photoresist with chemical or mechanical forces, positive photoresist was more easily removed than negative photoresist [39]. During this process, a total of 44 DNA wire platforms were fabricated of which 32 were positively lithographed and consisted of 2-D features and 12 were negatively lithographed with 3-D features (Figure 8).

Figure 8: Number of positively and negatively fabricated chips
3.1 2-DIMENSIONAL DNA WIRE PLATFORM USING POSITIVE LITHOGRAPHY

There are several steps in fabricating a 2-D DNA wire platform. Initially, designs are generated with proper features consisting of two electrodes with a gap size equal or less than the length of λDNA. The best 2-D design would be the one that experimentally demonstrates better DNA attachment where the attachment can easily be observed under a microscope. After designing various features, those features are converted to a mask through a commercial vendor. Later, this mask will be used along with positive lithography to fabricate the DNA wire platform that we will subsequently call Generation I. In this section, we will discuss mask design, mask layout, and microfabrication processes for DNA wire chips on 2-D platform.

3.1.1 2-Dimensional DNA Wire Feature Design

Four basic designs of electrodes are drawn with various dimensions and gap sizes from 5, 10, 15, 20, 25 to, 30 µm as shown below (Table 1). CoventorWare® 2009 software was used to layout the features (Figure 9-Figure 12) [40]. The reason for obtaining various designs and gap sizes is to test each design separately to find out which one is the most suitable feature for this study. The best feature will be the one that provides the cleanest and sharpest gap, focuses electrostatic forces through the electrodes (which direct and locate DNA strands between two electrodes) and demonstrate materials stability during chemical DNA attachment process.

The naming for the various chip designs was inspired by Persian and Indian mythology³. Figure 9-12 summarize the designs.

---

³Ara: A town in the Province of Armenia.
Mithras: The ancient Persian god of light.
Zarathustra: Persian prophet who founded Zoroastrianism.
Vayu: is a primary Hindu deity, the Lord of the winds.
Figure 9: Design 1 (Ara: A1)

Figure 10: Design 2 (Mithras: M1, M2)

Figure 11: Design 3 (Zarathustra: Z1, Z2)
Figure 12: Design 4 (Vayu: V1, V2)

<table>
<thead>
<tr>
<th>DIE SERIAL</th>
<th>DIE SIZE (cm)</th>
<th>GAP SPACINGS (μm)</th>
<th>COVENTOR CELL NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1x1</td>
<td>5,10,15,20,25,30</td>
<td>ara_1cmx1cm_allgaps</td>
</tr>
<tr>
<td>M1</td>
<td>1x1</td>
<td>5,10,15,20,25</td>
<td>mithras_1cmx1cm_allgaps</td>
</tr>
<tr>
<td>M2</td>
<td>1x1</td>
<td>15</td>
<td>mithras_1cmx1cm_LARGE_15um_gap</td>
</tr>
<tr>
<td>Z1</td>
<td>1x1</td>
<td>15, 5-25 Gradient</td>
<td>zarathustra_1cmx1cm_15_5_25_gaps</td>
</tr>
<tr>
<td>Z2</td>
<td>1x1</td>
<td>15</td>
<td>zarathustra_1cmx1cm_LARGE_15um_gap</td>
</tr>
<tr>
<td>V1</td>
<td>1x1</td>
<td>5,10,15,20,25</td>
<td>vayu_1cmx1cm_allgaps</td>
</tr>
<tr>
<td>V2</td>
<td>1x1</td>
<td>15</td>
<td>vayu_1cmx1cm_LARGE_15um_gap</td>
</tr>
</tbody>
</table>

Table 1: Die sizes and gap spacings of DNA wire electrode features
3.1.2 Mask Design for 2-Dimensional DNA Wire Chip

Using the layout editing CoventorWare, seven 1cm×1cm microarray designs including A1 (Ara), M1 (Mithras), M2 (Mithras’ arrays), Z1 (Zarathustra), Z2 (Zarathustras’ array), V1 (Vayu), V2 (Vayu’s array) are arranged on a 4 inch (100mm) wafer (Figure 13). After chip population, the mask file is built in CoventorWare®, and then sent to the printing company, CAD/Art Services (CAS) to fabricate a transparent film mask that will be used for photolithography (Figure 14).

Figure 13: Schematic diagram of mask population (A1, M1, M2, Z1, Z2, V1, V2)

Figure 14: Generation I Mask
3.1.3 Material and Fabrication Process for 2-Dimensional DNA wire platforms

To be able to build a chip with 2-D electrodes positive photolithography process with Shipley’s 1813 photoresist is used. The complete instruction is explained in the following sections.

3.1.3.1 Wafer

The wafer used in this MEMs fabrication process is a thin slice of semiconductor material, Silicon (Si), covered with silicon oxide (SiO2). Silicon (Si) is a chemical element with atomic number 14. Si is a tetravalent metalloid which has an atomic state with four electrons available for covalent chemical bonding in its valence (outermost electron shell) and with an intermediate property (either a metal or nonmetal) [41].

![Figure 15: Silicon crystallizes in the diamond cubic crystal structure](image1)

![Figure 16: A silicone compound](image2)

To microfabricate a layer of oxide on the surface of the silicon wafer thermal oxidation is used to produce a thin layer of silicon dioxide on the surface of the wafer. The technique forces an oxidizing agent to diffuse into the wafer at high temperature (800 and 1200°C) and react with it [42]. The thermal oxidation reaction may use either water vapor or molecular oxygen as the oxidant agents as follows:

\[
\text{Si} + 2\text{H}_2\text{O} \rightarrow \text{SiO}_2 + 2\text{H}_2\text{(g)} \quad \text{(water vapor)}
\]

\[
\text{Si} + \text{O}_2 \rightarrow \text{SiO}_2 \quad \text{(molecular oxygen)}
\]
Thermal oxide incorporates silicon consumed from the substrate and oxygen supplied from the ambient environment. Thus, it grows both down into the wafer and up out of it. If a bare silicon surface is oxidized, 46% of the oxide thickness will lie below the original surface and 54% above it [43].

3.1.3.2 Dicing, Cleaning and Dehydrating the Substrate

A 1cm×1cm chip is diced from the 100mm thick wafer. To remove contaminants from the wafer the surface is rinsed thoroughly with acetone, Isopropyl alcohol (Figure 17). Then blown dry with compressed N2 (nitrogen). It is better to dehydrate the chip on a preheated oven (65° C) for 1 minute. Then, the chip is removed and left to cool down on a clean surface.

![Figure 17: Cleaning station](image)

3.1.3.3 Photoresist Coating

3.1.3.3a Coating Material:

The Shipley S1813 photoresist coating that has been used in this experiment is a positive resist. The positive photoresist is a type of photoresist in which the portion of the photoresist that is exposed to light becomes soluble to the photoresist developer. The portion of the photoresist that is unexposed remains insoluble to the photoresist developer [44].
Shipley S1813 resist is a standard novolak\textsuperscript{4} based positive photoresist and its resistance depends on the etch process and can vary a lot. This Positive photoresist consists of a base resin (novolac/phenol formaldehyde) (Figure 18), photoactive compound and, volatile solvent (diazonaphthoquinone (DNQ)) (Figure 19). The base resin gives the resist film-making properties. The photoactive compound and volatile solvents are used to make the material liquid for application [45].

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{fig18}
\caption{Chemical structure of Novolacs/phenol-formaldehyde resin, Photo source: Wikipedia}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{fig19}
\caption{Chemical structure of Diazonaphthoquinone (DNQ), Photo resource: Wikipedia}
\end{figure}

3.2.3.3b Coating Method:

The chip mounts on the spinner in the presence of suction via a vacuum pump (Figure 20). Then, 1mL Shipley is dispensed onto the chip all at once and subsequently spin-coated. Figure 21 shows the spin curve for 1813 Shipley resist which has been coated on wafers using different speeds and baked at 115°C for 60s. According to this spin curve, equation and, experimenting at various spin speeds and time, the best results were achieved at a spin rate of 1950 RPM for 45 seconds. At this spin rate the film thickness is 2µm/20000Å\textsuperscript{4} [46].

\textsuperscript{4} Novolacs: are phenol-formaldehyde resins made where the molar ratio of formaldehyde to phenol of less than one. Novolacs are commonly used as photoresists.
3.1.3.4 Soft Bake

The chip is soft baked at 115° C for 1minute after which it is cooled for 4 minutes (Figure 22).
3.1.3.5 UV Exposure

Photolithography can be defined as a method for transferring a desired pattern into a photoresist (photosensitive material), which has been placed on the top of the chip. Under UV light exposure the chemical properties of the photoresist changes. In the case of a positive photoresist the exposed areas becomes less resistant by altering the chemical properties of the photoresist \[47\]. Exposure modifies the photoresist chemically due to destruction of the inhibitor by the light in a suitable wavelength range \[48\]. In Shipley, DNQ inhibits the dissolution of the novolac resin but upon exposure to light the dissolution rate increases even beyond that of pure novolac. The mechanism by which unexposed DNQ inhibits novolac dissolution is believed to be related to hydrogen bonding \[49\]. Photoresist thickness is closely related to exposure dose. In positive resist exposure dose has a direct relationship with photoresist thickness, and reverse relation to remaining photoresist thickness after exposure and development (Figure 23) \[50\].

![Figure 22: Hot plate](image-url)
3.1.3.5a UV Exposure Method

After turning ON the UV source, it needs to warm up for 5 to 10 minutes. Meanwhile, the mask is cleaned with acetone and IPA then rinsed with DI water. Then it is blown dry with compressed N2 (nitrogen). To achieve a 10µm feature for the photoresist layer of 2 µm by running several experiments, the UV intensity determined was 6.06 mW/cm² for an exposure time of 40s. To calculate the exposure dose the following equation has been used:

\[
\text{Exposure dose (mJ/cm}^2\text{)} = \text{Measured intensity (mW/cm}^2\text{)} \times \text{Exposure time (s)}
\]

Exposure dose = 6.06 (mW/cm²) × 40 = 242.4 mJ/cm²

Now we set the correct intensity and exposure time for OAI system (Figure 24). Next, the mask is aligned on the chip in the presence of a vacuum 10⁻⁹ torr⁵. To achieve the best alignment a microscope was used to position the mask. Finally, the chip with a mask on top is slid under a UV source and exposure begins (Figure 24).

---

⁵ 1 torr = 1 mm of Hg = 133.3 Pa
To develop the DNA wire platform we need to provide a specific ratio of developer and DI water (Microposit 351 Developer: Water = 1 : 5). 350mL of this combination of developer and water is poured in a developer container. Several experiments were done to determine the required development time for different sizes of DNA wire platform. The best time for 1cm×1cm DNA wire platform is 15s. To achieve the best feature, the chip needs to be monitored constantly during developing process. Immediately at 15 s the wafer is removed from the ultrasonic bath and rinsed with DI water for 30 s. Finally, the chip is blown dry with an N₂ gun [51].

*Note: Developing time changes base on the freshness of developer, size of chip and, kind of feature.

Figure 24: The OAI UV light source consists of a stand-alone light source, a constant intensity controller, and a shutter timer.
3.1.3.7 Initial Imaging

After developing the chip, it is visualized by an optical microscope or scanning electron microscope (SEM) to confirm achievement of small gap (10µm-30µm) between 2 electrodes before gold sputtering process.

3.1.3.8 Gold Sputtering

After developing the chip with 351developer a coat of gold is deposited on top of the Chip. Gold sputtering is used for 2 reasons, it increases electrical conductivity of a sample and during the attachment process oligos and DNA stick to gold layer much better than other materials. Hummer gold sputter deposits a very thin layer of Gold onto a chip. Sputtering proceeds during Argon glow discharge between the instrument anode and cathode [52]. (See Appendix [1])

![Gold sputtering machine](image)

Figure 25: Gold sputtering machine

3.1.3.8a Sputtering Method

The chip is placed on the center of the sputtering pedestal. Vacuum pump main power is turned on. When the vacuum reaches 70 m torr, the timer is set for 4-8 min. Now, we turn on the high voltage switch and set the voltage to 9V. The current should be less that 10mA, to obtain this current we need to open the Argon leak valve. To have current remained constant at 10mA, the leak valve need to be adjusted slowly. At the end of the time period,
we set the high voltage knob to zero. Then, the system shuts down in this order: High voltage, main power, mechanical pump, Argon tank (not tightly). Now we can remove the chip. Through this method the chip should receive a 0.2µm gold layer on top [53,54]. (See Appendix [2]).

3.1.3.9 Photoresist Stripping

After gold sputtering we pour 400mL of Shipley stripper, 1165, into the Ultrasonic bath. Then the chip is placed into the bath with a pair of tweezers and processed for 5 min (Figure 26). The ultrasonic cleaner bath has a transducer at the bottom that produces a high-powered ultrasonic wave throughout the entire oscillating tank. Constant power and automatic frequency control distribution of ultrasonic energy [55]. It is important to monitor the stripping process to make sure we are not going over time and losing features. When we see nice and clean golden color on top of the chip, it is the time to remove the chip and rinse it with DI water immediately for 30s. Then, the chip is blown dry. Note1 that to achieve the best features, the chip needs to be monitored constantly during the entire developing process. Also, Stripping times depend on the freshness of stripper, size of chip, and kind of feature.

Figure 26: Ultrasonic bath
3.1.3.10 Final Imaging

Now, the chip is ready to receive final visualization. The optical microscope, Hirox microscope, is used to visualize the Micro DNA wire platform features. Two different lenses are used a MX-10 and MX(G)-5040SZ. The MX-10 lens covers a magnification range of 35 to 7000x while, MX(G)-5040SZ has a rotary head adapter that achieves 360°3-D image detection with a magnification of 40x to 800x.

![Hirox Microscope](image)

**Figure 27: Hirox Microscope**

3.1.3.11 Result

Imaging results with repeating the same process, showed 2-D DNA features with gap between (30µm-10µm). However, to obtain this optimum result, we went through different processes which are explained in Index [4]. Some of the related pictures are also included.
Figure 28: Positive Photolithography Steps to Fabricate A 2-D DNA Chip
Table 2: Summary of fabrication of 2-D (Positive Lithography) DNA Wire chip to achieve $10\mu m$ feature gap.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Chip Cleaning</th>
<th>Dehydration Bake</th>
<th>Positive Photosist</th>
<th>Spin Coat Rates</th>
<th>Spin Coat Time</th>
<th>Photoresist Coat Thickness</th>
<th>Soft Bake Temperature and Time</th>
<th>UV intensity</th>
<th>UV Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cm x 1cm chip</td>
<td>Deionized water</td>
<td>15s</td>
<td>AcetoneIPA: N2</td>
<td>Rinse</td>
<td>2000 RPM</td>
<td>45s</td>
<td>$2\mu m$</td>
<td>$115^\circ C, 60s$</td>
<td>6.06 mW/cm²</td>
</tr>
<tr>
<td>Silicon + Silicon dioxide</td>
<td>Microposit 351:1:5</td>
<td>Manual Agitation</td>
<td>DLE20, 30s</td>
<td>4.8 min</td>
<td>Microposit 1165</td>
<td>Ultrasound Bath</td>
<td>5min</td>
<td>DLH20, 10s</td>
<td>Optical microscope, SEM</td>
</tr>
</tbody>
</table>
3.1.4 Visualization of 2-D DNA Wire Platforms with Optical Microscope

To image the positively fabricated chips, an optical microscope (Hirox), located at SUSU MEMs lab, was used with two different lenses. The pictures are shown below.

Figure 29: 2-D (Positive Lithography) Zarathustra (Z1) feature with 15µm gap
Figure 30: 2-D (Positive Lithography) Vayu (V1) with 15µm gap
Figure 31: 2-D (Positive Lithography) Mithras (M1) with 25 & 20 µm gap

Figure 32: 2-D (Positive Lithography) Mithras (M1) with 15 µm gap
Figure 33: 2-D (Positive Lithography) Mithras (M1) 10µm gap
3.2 3-Dimensional DNA Wire Platform Using Negative Lithography

During fabrication of the 2-D DNA wire platform, we found out that the best design was Mithras (M1) due to its well defined features, small and precise gap size and large bump pads for measuring the electrical character of DNA. These four large pump pads connected to the parallel electrodes will be used to measure electrical characters of DNA with 4 point sensing electrodes.

However, the long vertical length of Mithras gap (100µm) can trap many DNA strands which would affect the electrical test results by changing the overall resistance due to adjacent parallel DNA strands (Figure 34). To avoid this problem, the gap width was changed from 100µm to 12 µm to minimize the amount of attached adjacent DNA strands between two electrodes. This change in new design (Figure 35) also would help to concentrate more electrostatic force across the positively charged gold electrodes, in compare to Figure 34 to force negatively charged DNA to attach between the electrodes.

![Figure 34: Bundle of DNA attach between (15µm- 100 µm) gap](image)

![Figure 35: Less DNA strands attach between (8µm-12 µm) gap](image)
After iterating on the original design, an optimal design was selected and converted to a mask. The mask later is used along with negative photolithography to fabricate three dimensional DNA wire platforms.

The fabrication process starts with dicing a silicon wafer into 1cm×1cm chips. These chips were later cleaned and prepared for photoresist coating. Negative photoresist was poured on a silicon oxide layer and spin coated. Following the chip is pre baked and left to cool down at room temperature. Next, a mask is aligned on the chip and placed under UV light. The UV exposure polymerizes the feature wherever the negative resist is exposed. Contrary to positive resist, we need to gradually post bake the negative resist. Finally, a layer of gold is sputtered on top of the SiO2 layer. The stripping process will remove negative resist and the gold layer from the areas which have not received UV exposure. The final product is a 3-D DNA platform, consisting of two layers of negative photoresist and a gold layer, which have been raised from the SiO2 substrate. These steps are discussed in detail as follows.

### 3.2.1 3-D DNA Wire Chip Design

Following the Mithras (M1) model, the resulting design (generation 2) with a smaller gap dimension was designed with CoventorWare® software (Figure 36). Three 1cm×1cm “Generation II” design layouts consisting of the following gap dimensions (length, width) were constructed: (12µm, 12µm), (10µm,12µm) and, (8µm,12µm). Subsequent to concept layout and CAD drafting, the new designs will be ready to convert to a mask. The minimum feature size obtainable through the mask production company (at the time of this study) was 5µm. To fabricate a 5µm gap on the chip, in negative lithography, it is necessary to select a feature size of 8µm. Therefore, by designing the length 8, 10, 12µm on the mask we will obtain gap sizes less than the specified (< 8, 10, 12µm) on the chip. For instance an 8µm gap on the mask will result in a 5µm gap on the chip. Also, fabricating chips with different gap length (< 8, 10, 12 µm) offer opportunities to attach various lengths of DNA between the electrodes.
3.2.2 3-D DNA Chip Population and Mask Design

Generation II mask consisting of six 1cm×1cm regions are created. This mask contained cell quadrants K, L, M, N, O, P which were populated on different parts of a 4inch (100mm) mask file built using CoventorWare® (Figure 37). Cells N, O and P are the new design with 8, 10 and 12 µm length gap. Cells K, L and, M are previous Mithras (M1) design with 8, 10 and 12 µm length gap. After chip population, the mask file is sent out to be printed. The mask comes back as a transparent film which will be used for fabrication of 3-D chips (Figure 38).
Figure 37: Chip Population for generation II design

Figure 38: 1. Generation II mask file, 2. Cells with 8, 10 and 12 µm length gaps
Table 3: Demonstration of the die sizes and gap spacings for gen II mask.

<table>
<thead>
<tr>
<th>LETTER</th>
<th>DIE SIZE (cm)</th>
<th>FEATURE</th>
<th>DIAMETER, WIDTH, GAP (µm)</th>
<th>COLUMN SPACING (µm)</th>
<th>ROW SPACING (µm)</th>
<th>TOTAL NUMBER OF FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1x1</td>
<td></td>
<td>8</td>
<td>NO STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>1x1</td>
<td></td>
<td>10</td>
<td>NO STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>1x1</td>
<td></td>
<td>12</td>
<td>NO STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>1x1</td>
<td></td>
<td>8</td>
<td>STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>1x1</td>
<td></td>
<td>10</td>
<td>STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>1x1</td>
<td></td>
<td>12</td>
<td>STEP</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Q</td>
<td>1x1</td>
<td></td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>109</td>
</tr>
</tbody>
</table>

Figure 39: Gen II transparent mask
3.2.3 Material and Fabrication Processing of 3-D DNA Wire Platforms

The material used in this study to construct 3-D structures on silicon oxide substrate is negative photoresist SU-8. SU-8 originally was invented by IBM in 1989, but is now sold by Microchem and Gersteltec. The SU-8 resin contains eight epoxy groups per molecule which gives the polymer very high functionality (Figure 40). It is a very viscous polymer that can be spread over a thickness ranging from 0.1µm to up to 2mm and still be processed with standard lithography. Its UV maximum absorption is at 365nm wavelength. Solidification of the material happens during UV exposure when SU-8 molecular chains cross-link. SU-8 is highly transparent in the ultraviolet region, allowing for processing of very thick film up to 2mm with nearly vertical side walls. [56, 57].

Figure 40: SU-8 molecule

There are a few advantages to using SU-8 over Shipley in our research. First, it is its ability to pattern high aspect ratio >20 [58]. This provides a 3-D structure with high thickness (e.g. 90µm) for whole feature with a small gap distance (e.g. 5 µm) between two electrodes. This 3-D structure later helps to suspend the DNA between two electrodes

---

6 It may be applied to two characteristic dimensions of a three-dimensional shape, such as the ratio of the longest and shortest axis
without touching the substrate verses 2-D (Shipley). Second, SU-8 tends to demonstrate better adhesion to not only silicon substrates but also to the gold layer on top compared to Shipley positive photoresist [59]. Third, after exposure and development, its highly cross-linked structure gives it high resistance to chemicals and radiation damage. Cured cross-linked SU-8 shows very low levels of out-gassing under vacuum\(^7\) [60]. Additionally, SU-8 exhibits higher temperature resistance over positive resists, Shipley [61]. Negative lithography process is explained in details as follow.

**Negative Lithography Procedure to Fabricate 3-D DNA Wire Chip with SU-8**

3.2.3.1. Preparing the Wafer
The 3-D DNA wire platform is fabricated using the facility at SDSU MEMS Lab clean room. 3-D DNA MEMs chip fabrication starts with a clean (100) silicon wafer covered with Silicon dioxide. The wafer diameter is 4 inch and has 0.5 \(\mu\)m thickness. After dicing 1cm×1cm die chip from the wafer, we need to clean the chip with acetone, Isopropyl alcohol (IPA) and, DI Water and blow dry it with N2 gun. *Note: To obtain a proper adhesion between the photoresist and substrate, make sure the substrate is thoroughly clean and dry.*

3.2.3.2 Dehydration Bake
The chip is dehydrated on oven plate in 65° C for 60s. Then, it is kept out until it cools down to room temperature.

3.2.3.3. Photoresist Coat
The chip is mounted on the spinner. Two different viscosities of SU8, 10 and 100 are used as negative resist coaters. A “quarter sized” puddle of SU-8 is applied on to the chip carefully in a single dispense without causing air bubbles on the coating surface.

---

\(^7\) Outgassing is the release of a gas that was dissolved, trapped, frozen, absorbed or adsorbed in some material
Multiple drops may trap air bubbles which can decrease the quality of the features. Immediately after SU8 was applied; the chip spin coating is started at the speed 2000 rpm and gradually increased to 3000 rpm for 45 seconds under a suction $10^{-9}$ Torr via a vacuum pump. Through, this process by changing the viscosity, speed and, time parameters we can produce the coating thickness of 15 to 90 µm.

*Note: The time and speed rate were determined with respect the following spin curve and running several lab experiments.

![SU-8 Spin Speed Curve](image)

**Figure 41: Spin speed vs. thickness, curve for SU8-100 resist is in blue**

3.2.3.4. Soft bake:

After the resist has been applied to the substrate, it is soft baked at 65°C for 10min then let the chip cools down for 4 min. The chip is put back on the oven and the temperature increased from 75°C to 95°C gradually in 5min. Now, the chip is left on the oven at 95°C for an additional 25min to evaporate the solvent and increase the density of the film. Bake time depends on the solvent evaporation rate, influenced by the rate of heat transfer and ventilation. We have derived the bellow backing time for our chip, referencing Microchem’s recommended soft bake parameters (Table 4) and through various lab tests. When the time has elapsed, the chip is set aside to cool down for 15 minutes.
3.2.3.5. Expose

After cleaning the mask, it is placed and aligned on the chip then the suction is applied through a vacuum pump. Now, we need to set the proper exposure dose and time. The exposure dose depends on film thickness and it is determined from the Curve (Figure 42) and also various tests. The best dosage is ~6mW/cm². If we assume the light intensity of the UV source is ~15mW/second. The exposure time is calculating as follow:

Exposure time = exposure dose / measured intensity

Base on this equation and our lab experiments the best exposure time is 30s. At this point, the mask aligner set up is slid to the UV source and we press expose button.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Thickness (µm)</th>
<th>Pre-bake @ 65°C</th>
<th>Softbake @ 95°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-8 50</td>
<td>40</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>SU-8 100</td>
<td>150</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>30</td>
<td>90</td>
</tr>
</tbody>
</table>

**Table 4: Microchem recommended soft bake parameters**

![SU-8 2000 Exposure vs Film Thickness](image)

**Figure 42: Microchem recommended exposure dose processes**
3.2.3.6. Post Exposure Bake

Following exposure, a post expose bake must be performed to selectively cross-link the exposed portions of the film. The post backing is performed on the hot plate. According to Microchem recommended post back process (Table 5) and our fabrication experiments, the backing temperature starts with 65°C for 5°C, it is increased gradually to 95°C for 20 min, then raise to 120°C for 5 more min. The reason for this gradual ramping is SU-8 readily cross-linked and can result in a highly stressed film. To minimize stress, wafer bowing and resist cracking, a slow ramp process is recommended. When, the time is up the chip is removed and cooled down slowly [62,63].

* Note: Optimum cross-link density is obtained through careful adjustments of the exposure and post exposure baking process.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Thickness (μm)</th>
<th>PEB 1 @65°C</th>
<th>PEB 2 @95°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SU-8 50</td>
<td>50</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>SU-8 100</td>
<td>150</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 5: Microchem recommended post exposure bake parameters
3.2.3.7. Gold sputtering
The chip is placed inside the chamber. Then, the vacuum pump is turned on. The chamber flushes three times till vacuum gauge shows below 70 mTorr. Now, we set the timer for 4-8 min depend on the desired thickness of gold layer and switch on high voltage. To start sputtering, we set the voltage to 9V and current less than 10mA. At the end of adjusted time, we shot down high voltage, main power, argon tank. Then, the sample is removed from the chamber. It should be a layer of gold on SU8 [64].

3.2.3.8. Develop / Stripping
300 ml SU8 developer solution is purred into the ultrasonic bath. The chip is left there for 10 min (Table 6) then removed, rinsed with DI water for 10s and blow dried. At this point we should be able to see the gold 3-D DNA platform on the blue SiO2 substrate with the necked eyes.

* Note: Stripping time plus UV intensity dose play important roles to achieve very fine gaps.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Thickness (μm)</th>
<th>Development (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-8 50</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>SU-8 100</td>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6: Microchem recommended devedevelop processes

3.2.3.9. Hard Bake (cure)
This part is optional. We can bake the chip at <65°C for a few more min to further cross link the material.
3.2.3.10. Imaging:
Finally, the chip is viewed with an optical microscope to visualize the features. [65,66]. The thicknesses of the chips are measured with Keyence LT-9000M laser measurement system with the resolution 0.01[67].

3.2.3.11. Result
The result of the imaging with repeating the above fabrication process helped us to achieve 3-D DNA features with gap length of 8 µm to 3 µm. To obtain this optimum result, we went through different processes which are explained in Index [5]. Some of the related pictures are also included.
Figure 44: Negative photolithography Steps

1cm x 1cm Si+SiO₂ substrate
Negative Photoresist purred on the substrate
Negative Photoresist spin coating
Pre Baking

Mask + UV Exposure
Post Baking
Gold Sputtering
Negative Resist & Gold Stripping

3D DNA Chip
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Chip Cleaning</th>
<th>Dehydration Bake</th>
<th>Negative Photore sist</th>
<th>Spin Coat Rates</th>
<th>Spin Coat Time</th>
<th>Photoresist Coat Thickness</th>
<th>Soft Bake Temperature</th>
<th>Soft Back Time (2 steps)</th>
<th>Stripping time</th>
<th>Stripping Condition</th>
<th>Stripping time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1cm x 1cm chip, Si+SiO2</td>
<td>Acetone $\rightarrow$ IPA $\rightarrow$ N2gun</td>
<td>65°C for 60s</td>
<td>SU-8 (10,100) : 1.5mL</td>
<td>(CO2000-3000) RPM</td>
<td>45s</td>
<td>(15-90) µm</td>
<td>(65-95) °C</td>
<td>65°C, 10min</td>
<td>10min</td>
<td>DI-H20, 10s</td>
<td>6.16 mW/cm²</td>
</tr>
</tbody>
</table>

Table 7: Summary of fabrication of 3-D (Negative Lithography) DNA chip to achieve (8-3)µm feature gap
3.2.4 Visualization of 3-D DNA Wire Platforms with Hirox Microscope

Figure 45: Chip#1, Negatively fabricated with layers of SU8-100+Au, Gap=8.186 μm, Thickness=90 μm
Figure 46: Chip#2, Negatively fabricated with layers of SU8-100+Au, Gap=5µm, Thickness=50 µm
Focus on the gap (2-D view)

Focus on the gap (3-D view)

Figure 47: Chip#3 Negatively fabricated with layers of SU8-100+Au, Gap=3µm, Thickness=50 µm
CHAPTER 4

EXPERIMENTAL RESULTS FOR DNA ATTACHMENT

In this chapter two different procedures for attachment of Lambda DNA (λDNA) (15µm length and 2nm diameter) on 2D and 3D DNA wire chips will be described. The two procedures include λDNA attachment on gold electrodes via oligo primers and electrostatic-covalent bonding. These two procedures are explained in detail below.

4.1 Lambda DNA Attachment Between Two Golden Electrodes With use of Oligonucleotides (Method1)

This process of attachment is based on the Braun et al method and the previous DNA attachment method at SDSU MEMs lab where λDNA was synthesized between two gold electrodes based on the self assembly property of DNA [68]. In its simple form, this process is based on synthesizing molecules of DNA between two gold electrodes by use of two complimentary strands of oligonucleotides. This function is based on the self assembly property of DNA.
Our first attempt was attachment of DNA via oligos on a 2D chip. DNA attachment on the 2D positive lithography chip was found to be unstable because after attachment the sputtered gold flaked off and the feature was destroyed. After experimentation to improve the robustness of the gold layer, we discovered (in this positive structure) that gold does not strongly bond to a silicon substrate. Analysis should that it flaked off by exposure to chemical solutions during hybridization and visualization. Ultimately we chose to use the 3D negative structure as a platform for DNA attachment. A new revised version of the SDSU DNA attachment protocols was also utilized for this study (See Appendix [6]). In 3D negative structures we found that gold bonds to the SU-8 layer stronger than the SiO2 substrate in 2D positively structured chips. As a result the gold layer does not peel off during DNA attachment and visualization.

The purpose of this experiment was to confirm the existence of DNA between two DNA wire platform electrodes. To confirm the presence of DNA a positive control test was utilized. The positive control test is based on biotechnology standards where fluorescence is used to confirm DNA attachment. As previously mentioned, oligos are used to promote DNA bonding to the desired surfaces. In this case the contact between the DNA and the gold electrodes was oligo thiol-mediated8. The gold has a natural affinity for sulphur and 3’-thiolated DNA (Figure 48) which promotes bonding. The attachment process is initiated by dispensing prepared oligo A and B solutions onto the DNA wire platform electrodes. After a designated amount of time oligo-gold bonding is achieved. The chip is rinsed with Milli-Q water to remove any unattached DNA and followed by 50µL of lambda DNA which is pipetted onto both electrodes. This process results in hybridization after which the sample is stained and visualized under a fluorescent microscope.

8 A thiol is an organosulfur compound that contains an carbon-bonded sulphydryl (-C-SH or R-SH) group were R represents alkane or other carbon-containing moiety.
Lambda DNA comes from a virus called Phage Lambda. The phage is isolated from *E. coli*. The DNA is isolated from the purified phage by phenol extraction and dialyzed against 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. This virus is harmless to man and therefore makes an excellent source of DNA for experimentation [69]. λDNA contains 48,502 base pairs (bp) and it is presented in a double-stranded linear. This is the way that λDNA exists in the intact phage. When λDNA enters the host cell it becomes a circle (Figure 49). λDNA consists of a head containing double-stranded linear DNA as its genetic material and a tail at each end. The 5’ strand overhangs the 3’ strand by 12 bases [70, 71]. These single stranded overhangs are complementary to oligos and their sequences are:

5’ GGGCGGCGACCT 3’ and 5’ AGGTCGCCGCCC 3’

**4.1.1 Lambda DNA**

Lambda DNA comes from a virus called Phage Lambda. The phage is isolated from *E. coli*. The DNA is isolated from the purified phage by phenol extraction and dialyzed against 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. This virus is harmless to man and therefore makes an excellent source of DNA for experimentation [69]. λDNA contains 48,502 base pairs (bp) and it is presented in a double-stranded linear. This is the way that λDNA exists in the intact phage. When λDNA enters the host cell it becomes a circle (Figure 49). λDNA consists of a head containing double-stranded linear DNA as its genetic material and a tail at each end. The 5´ strand overhangs the 3´ strand by 12 bases [70, 71]. These single stranded overhangs are complementary to oligos and their sequences are:

5’ GGGCGGCGACCT 3’ and 5’ AGGTCGCCGCCC 3’
\( \lambda \)DNA has a molecular weight of \(31.5 \times 10^6\) daltons and is stored in buffer 10mM Tris-HCl (pH 7.5), 10mM NaCl and, 1mM EDTA at \(-20^\circ\)C. The restriction buffer provides optimal conditions, NaCl provides the correct ionic strength, and Tris-HCl provides the proper pH.[72] (see Appendix [4]).

Figure 49: A: The lambda genome depicted in its linear state (the way the DNA exists in the intact phage). B: The lambda genome presented in its circular state (the way the DNA forms after it enters the host cell). Photo source: National Taitung University, Chung Hua
4.1.2 Oligo

An oligonucleotide is a short nucleic acid polymer, typically with twenty or fewer bases. They can be formed by bond cleavage of longer segments [73].

![Oligo A](image1.png) ![Oligo B](image2.png)

**Figure 50: Oligo A**

22.40 D, 204.2nm, 0.8mg

5’-GGG CGG CGA OCT β Thio MC3-O/-3’

**Figure 51: Oligo B**

21.50D, 202.6nm, 0.78mg

5’-AGGTCGCCGCCC/3 Thio MC3- O/-3’

4.1.3 Hybridization

The process of forming a double stranded nucleic acid from joining two complementary strands of the oligonucleotides correspond to a short DNA single strand of λDNA is hybridization [74].

![Hybridization Diagram](image3.png)

**Figure 52: Hybridization and complementary strands of λDNA, Oligo A and, OligoB**
4.2 Oligos and Lambda DNA Attachment on Gold Electrodes Procedure

4.2.1.1 Oligo Preparation

a) Two oligo sequences from IDT (Integrated DNA Technologies) are obtained: Oligo A (5'-GGG CGG CGA OCT β Thio MC3-O/-3’) and Oligo B (5’-AGGTCGCCGCCC/3 Thio MC3- O/-3’).

b) 100µM solution is formed from both oligo sequences by combining the lyophilized contents (dry, powdery substance) with 2mL of Tris-EDTA (TE) solution9. The contents is vortexed for 5s.

9. TE is commonly used as a stabilizer and diluting buffer solution. It remains viable through multiple thaw cycles.
c) 100:1 dilutions on both solutions were performed to obtain a concentration of 1µM in each container then each tube is microcentrifuged (Figure 54).

![Vortex Mixer](image)

**Figure 54: Vortex Mixer**

d) 18uL of solution from step (a), 1uL of T4 Polynucleotide Kinase (Fermentas) and, 1uL of enclosed buffer solution are mixed. This modifier phosphorylates the oligos in preparation for attachment and hybridization. This step repeats for both oligo solutions.

e) Both oligo solution tubes are placed into a water bath at 37°C for 30 minutes to allow the reaction to take place (Figure 56).

f) The Polynucleotide Kinase (PK) reaction is stopped by chloroform extraction. This is done at the same time as a 10:1 PK dilution to obtain a 100nM concentration of oligos in both vials. 20uL of 100uM solution is added to 25uL of EDTA/Chloroform mixture and 155uL of water. This mixture was prepared under a hood (Figure 55). Final solution is spun in a centrifuge for 1 minute and set aside in ice until needed.
4.2.1.2 REDUCTION OF Oligo

To use the free thiol (–SH) in our application the disulfide linkage must be reduced with dithiothreitol (DTT). To reduce the oligo a 10nM solution of oligos is prepared by mixing 1µL oligo with 9µL reducing agent solution (DDT). The solution incubates at room temperature for 3 hours.

4.2.1.3 Oligo Purification

The solutions are passed through the BioRad Spin-6 column using the following procedure:
Two Biospin 6 Tris Columns (A) and two Spin column wash tubes (B) are selected. The columns are inverted several times to resuspend the settled gel and remove any bubbles. The tip is snapped off and the columns are placed in a 2.0 ml microcentrifuge. Next the cap is removed and the excess packing buffer (A) is allowed to drain to the top of the gel bed (B). The drained buffer is discarded and the column is placed back into a 2 ml tube. The tube is centrifuged for 2 min at 3,300 RPM and the buffer is discarded (Figure 57). The column is placed into a clean 2.0 ml microcentrifuge tube. Next the 50µm Oligo A is dispensed directly to the center of the column.
After loading oligo A, the column is centrifuged (spinner Beckman Accuspin FR) for 4 min at 3,300 RPM\textsuperscript{10}. Following centrifugation the purified sample is placed in Tris /SSC buffer. Molecules smaller than the column’s exclusion limit will be retained. The same method was followed for Oligo B [75]. Collect the flow through by pipette and place in two strip caps after which they are kept on ice for 17 hours.

![Diagram of Oligo Purification](image)

**Figure 57: Oligo Purification**

\textsuperscript{10} Alternatively, to calculate the speed in RPM required to reach a gravitational force of 1,000 x g, use the following equation:

\[
\text{RCF (x g)} = (1.12 \times 10^{-5}) \times (\text{RPM}) \times 2 \times r
\]

where RCF is the relative centrifugal force, r is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column, and RPM is the speed of the rotor.
4.2.1.4 λ DNA PREPARATION

λ DNA (New England Biolabs) is linear when mailed and suspended in a TE solution (10mM Tris-HCl, 1mM EDTA). A new solution is formed by mixing 2μL of λ DNA (~1ug) with 42μL of water, 1μL of Fast-AP\textsuperscript{11} (Fermentas), and 5μL of enclosed buffer solution. This mixture incubates at 37°C for 10 minutes. In order to stop the phosphotase reaction the mixture is heated in a water bath at 75°C for 5 minutes. After heating the product is placed on ice patch until needed.

\textsuperscript{11} Fast-AP is a form of alkaline phosphatase that cleaves the phosphate groups from the λ DNA. This is used in the place of Shrimp Alkaline Phosphatase.
4.2.1.5 DNA ATTACHMENT

Using a small-volume pipette, a 2µL droplet of oligo is deposited onto a section of the chip while viewing the electrodes through an optical microscope (for better control). The chip is soaked for 5 minutes. The sulfur groups on the functional ends of the oligos will selectively attach to the gold substrate\(^\text{12}\). The chip is then rinsed with a droplet of Milli-Q water\(^\text{13}\). The same method as oligo A is used to affix oligo B to the second electrode. After oligos attachment a 50 µL solution of λDNA is dispensed onto the chip covering the region where the oligos are located (Figure 60). This is set aside at room temperature for 20 min. The chip is then placed in a Petri dish with Milli-Q water (Figure 61) and sheet of Parafilm is stretched over the top (Figure 62). The petri dish is then placed in an incubator (VWR scientific Model 2720) at 55°C for 1hour 30min (Figure 63). Finally, the chip is removed from the incubator and rinsed with Milli-Q water and set aside to air dry.

* See Appendix [5] for summery of chemical list used during DNA attachment process.

\(^{12}\) A technique used by research teams investigating self-assembling monolayers

\(^{13}\) Milli-Q is water that has been purified and deionized to a high degree by a water purification systems.
4.2.2 DNA attachment on positive structure (2D Chip) and Gold flaking issue

A DNA attachment method slightly different from the aforementioned method was attempted first on a positive platform at SDSU MEMs lab by the Bionanoelectronics group (see Appendix [6] for Instruction). After attachment of DNA on 2D structure it was found that the gold layer is not stable. To investigate further, three SiO2 covered silicon substrates were gold sputtered and used to run several tests.

Test 1:
Water dropped on the first chip then placed in a oven (Ls-314) at 45°C for 4 hours. Result: the gold layer peeled off.

Test 2:
2µl of SYBR gold\(^\text{14}\) and mounting solution\(^\text{15}\) placed on the gold layer of the second chip for an hour. Result: the gold layer peeled off

Test 3:
The third chip immersed in the water for 38 hours at room temperature. Result: the gold layer was stable.

\(^{14}\) The SYBR® Gold Gel is used to visualize dsDNA.

\(^{15}\) Mount solution is used to stick the bottom of the chip to the microscope slide to prevent dislodging.
The result showed that stability of the gold layer on a positively formed platform made by Shipley1314 depends on temperature, time and, type of solution during hybridization and imaging steps. During these steps the chip is exposed to water, SYBR gold, and mounting solutions. The gold layer flaked off due to the weak bonding between gold and the SiO2 layer (Figure 64, Figure 66 and, Figure 65).

4.2.3 DNA Attachment on Negative Structure (3D Chip) and Chip Visualization Process

To avoid the gold flaking issue we decided to attach DNA on the negatively fabricated chip (3D DNA chip) because it offers strong bonding between gold and SU-8 [76]. The SYBR® Gold Gel Stain is used to visualize the DNA wire chip. Molecular Probes SYBR® Gold nucleic acid gel stain is the most sensitive fluorescent stain available for detecting double- or single-stranded DNA by standard ultraviolet transilluminators\(^\text{16}\). The method to stain double stranded DNA and visualize it under fluorescent microscope is as follows:

\(^{16}\) Ultraviolet transilluminators are commonly used to visualize fluorescent markers.
a) Before opening a SYBR gold vial it should be allowed to warm to room temperature and then centrifuged in a microfuge to deposit the DMSO solution\(^{17}\) at the bottom of the vial. Staining reagent diluted in buffer can be stored protected from light either at 4°C for several weeks or at room temperature for three or four days [77] (see Appendix [7]).

b) After preparing SYBR gold the chip is placed in a Petri dish. 2 µl of SYBR Gold\(^{18}\) is deposited on the electrodes gap. The dish is wrapped with foil (to block light) and the chip is soaked with SYBR gold. SYBR gold is a DNA binding dye that inserts itself into double-stranded DNA (Figure 67). After 10 min, the SYBR gold solution is rinsed from the DNA wire chip with a few drops of Milli-Q water.

![Figure 67: SYBR gold binds to dsDN and illuminate under fluorescent microscope. Photo source: lia02000](image)

4.2.3.c A fluorescent microscope is used to visualize the DNA wire chip. To prepared the chip for visualization we placed it between two glass lab slides (Figure 68) and added a small drop of mounting solution to the four corners of the chip. A drop of immersion

---

17 Dimethyl Sulfoxide facilitates DNA strand separation (in GC rich difficult secondary structures) because it disrupts base pairing and has been shown to improve PCR efficiency

18 2x: (2 µl) SYBR Gold solution from 10000x stock solution which is calculating with following method:  
\[ V1 \times C1 = V2 \times C2, \quad 1000 \times X = 10,000 \mu l \times 2X, \quad V1 = X = 20 \mu l, \quad V2 = 2X = 2 \mu l \]
solution was placed on the top glass and the DNA chip was visualized under a fluorescent microscope (refer to Appendix [5] for list of chemicals used in this chapter).

Figure 68: DNA wire chip placed between 2 glass slides via four mounting drops.
4.2.4 Visualization of λDNA attached on 3D Chip by Fluorescent Microscope

This section consists of images of attached λDNA between two gold electrodes. The pictures were captured with a Fluorescent Microscope at the SDSU life science lab. The following pictures are the results of the "Oligos and λDNA Attachment on Gold Electrodes Procedure (part 4.2)".

Following are the microscope settings used for our visualization:
Resolution: W:1392, H:1040
Final preview resolution: 696×520
Set Area: (0,0) - (1391,1039)
Camera Exposure upper limit: 500ms

In Figure 69 (1-3) the glowing wire demonstrates the successful attachment of λDNA between two gold electrodes (based on the fluorescent property of SYBR gold stained hybridized Oligos and DNA). The area between the electrodes is approximately 20µm×8µm. At the stated fixed resolution, microscope photos of the electrode surface, substrate, and midline were obtained which demonstrates the depth of the electrode features (~20µm). The different levels of focus detail how the DNA is suspended between two gold electrodes and not in contact with the silicon substrate.

Figure 69 (1-3). Reduced illumination in midline plane focus in Figure 69.4 exhibits the highest edge fluorescence as well as between electrodes which are the areas most likely to attract oligos and DNA due to electrostatic charge.

Figure 70, slides 1,2,3,4 highlight other regions of the electrodes. The glowing edge regions seen under fluorescent microscope are most likely the result of attached oligos and folded DNA.
Figure 69: Slides 1, 2 and 3 focused on different depths e.g. electrodes, substrate and their midline. 4. Reduced illumination in midline plan focus is shown in this slide. The highest fluorescence is along the edges and between electrodes which are the areas that most likely attract oligos and DNA due to electrostatic charge.
Figure 70: Slides 1-4 shows some other part of the electrodes. The parts of edges that are shining under florescent microscope can be the result of attached oligos and folded DNA.
4.2.4.1 CONTROL EXPERIMENT

An experiment which uses controls is called a controlled experiment. The control group is practically identical to the treatment group, except for a single variable of interest whose effect is being tested. The control experiment used to verify existence of DNA affixed between the two electrodes is a positive control\textsuperscript{19}. To run a control test first the electrodes were visualized before DNA attachment under a Hirox optical microscope at SDSU MEMs lab (Figure 71). In this chip the gap is 8µm and the thicknesses of the 3D electrodes are 20µm. The same chip is then visualized after attachment of DNA under the fluorescent microscope (Figure 72). In this picture the assembled DNA is observed glowing between two gold electrodes. Lastly mounting solution was dispensed onto the electrodes and a coverslip was placed on top of the chip. The mounting solution is used as a temporary adhesive to adhere the sample to the coverslip. After 15min the coverslip was peeled off and the chip was again observed under a fluorescent microscope. After removal of the coverslip the glowing wire (hybridized DNA) in addition to some unhybridized DNA were removed (Figure 73). Figure 74 exhibits photos from other regions of electrodes after removing the DNA wire. DNA which was hybridized with just one end to their match oligo on each gold electrode was also removed.

\textsuperscript{19} A positive control confirms that the procedure is observing the related effect.
Figure 71: Gold electrodes before DNA hybridization visualized with Hirox microscope.

Figure 72: Gold electrodes visualized after DNA hybridization with fluorescent Microscope.

Figure 73: DNA removal visualized under the fluorescent microscope.
Figure 74: Other regions of the chip after DNA removal.
4.3 **Lambda DNA Attachment on Gold Electrodes by Electrostatic and Covalent Forces**

**(Method 2)**

In this method λDNA attaches to gold covalently and electrostatically (no voltage and current applied from outside). Electrostatic phenomena arise from the forces that electric charges exert on each other (Figure 75) [78]. In Figure 76 the electrostatic field (lines with arrows) of a nearby positive charge (+) causes the mobile charges in conductive objects to separate due to electrostatic induction. Negative charges (blue) are attracted and move to the surface of the object facing the external charge. Positive charges (red) are repelled and move to the surface facing away. Covalent bonding is a form of chemical bonding that is characterized by the sharing of electron pairs between atoms of gold and oligo thiol groups (Figure 76) [79].

**Figure 75: Covalent bond**
*Photo source: Tutornext*

**Figure 76: Electrostatic field**
*Photo source: Wikimedia, Chetvorno*

At the nano scale the volume of objects is less dominant as opposed to the total surface area of the same objects. Hence the types of force acting are different at this scale. For example, gravity and inertia are negligible at the nanoscale. In contrast, the electrostatic and covalent bonding of thiolated DNA which deposited on the gold surface is strong enough to form a stable bond between sulfur and the gold particles.
Table 8 lists different types of forces and their interaction strength. These unique forces provide scientists with a broader toolset for manipulating properties at the nanoscale. However, when designing these systems, a more complex world exists at the nanoscale. By understanding nature’s method of using forces at the nanoscale one can nanoengineer highly functional structures with better electrical properties [80].

<table>
<thead>
<tr>
<th>Type of Force</th>
<th>Strength (kJ/mol)</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalent</td>
<td>&gt;210</td>
<td>C–C bond</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>&gt;190</td>
<td>Li⁺−F⁻</td>
</tr>
<tr>
<td>Dipole–dipole</td>
<td>5–40</td>
<td>H⁺–Cl⁻−H⁺–Cl⁻</td>
</tr>
<tr>
<td>π–π interaction</td>
<td>10–20</td>
<td>CNT–CNT</td>
</tr>
<tr>
<td>Hydrogen bonding</td>
<td>5–40</td>
<td>ssDNA–ssDNA</td>
</tr>
<tr>
<td>Dispersion</td>
<td>&lt;5</td>
<td>H⁺–O⁻–H⁺–Cl⁻–Cl⁻</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>5–40</td>
<td>H₂O–metal</td>
</tr>
<tr>
<td>Dative</td>
<td>20–380</td>
<td>S–Au</td>
</tr>
<tr>
<td>Ionic bonding</td>
<td>20–30</td>
<td>Na⁺Cl⁻–crystal</td>
</tr>
<tr>
<td>van der Waals</td>
<td>0.1–40</td>
<td>H₂O–H₂O</td>
</tr>
</tbody>
</table>

*Carbon nanotube.
*Single-stranded deoxyribonucleic acid.

Table 8: List of forces acting at nanometer scale. Source: Handbook of nanoscience, engineering and technology

In addition to the method of hybridization of Oligos and DNA on the electrodes (4.2), we ran the second experiment in which λDNA attached to the gold electrodes directly by electrostatic charge and covalent bond.

Method: A pipette was used to dispense 0.1 µl of prepared λDNA²⁰ onto the electrode gap (Figure 79). The chips were set aside for 30 min at room temperature (Figure 78). This time is sufficient to attract the negatively charged DNA between electrostatically positive charged gold electrodes. During this attraction thiolated DNA builds covalent bonds between sulfur and the gold layers. The chips were rinsed with Mill-Q water and dried at 80°C for 10 min then visualized via a Hirox optical microscope, Scanning Electron Microscope (SEM), an SEM (Quanta FEG 450) at the SDSU electron microscope facility.

²⁰ See 4.2.1.4 “λDNA Preparation” section
4.3.1 Visualization of λDNA Attachment on Gold Electrodes by Electrostatic and Covalent Forces

After direct attachment of λDNA on to the chip it was visualized with a Hirox optical microscope before and after rinsing with Milli-Q water. In Figure 80 λDNA solution is mixed with Tris-HCl buffer, NaCl and, EDTA. After rinsing the chip with Milli-Q H2O (Figure 81), DNA still appeared stuck to the gold electrodes which indicates the covalent bonding between thiolated DNA and the particles is strong.
4.3.2 Visualization λDNA Attached on Gold Electrodes by Electrostatic and Covalent Forces by Quanta SEM

A SEM (Quanta FEG 450) was used to visualize the electrostatically and covalently attached λDNA (on the same chip as above). Quanta SEM is based on passing voltage and current through the chip to generate an image. If the specimen is conductive less current and voltage is needed to generate an image. Otherwise the system will inject more voltage and current into the sample. Following are the microscope settings used for this visualization:

1.2 kv <V< 5kv, 1.2 kv: low voltage, 5kv: high voltage
Chamber pressure: 20pa
Software: XT microscope control

The following SEM images were taken before rinsing. The trapped DNA appears in a circle, the other substances are dried Tris-HCl buffer, NaCl and, EDTA (Figure 82). Figure 83 shows the spectrum analysis of the chip. The yellow region indicates the different materials on the chip.

Figure 82: Picture of dried DNA solution is taken by Quanta FEG 450SEM.

Figure 83: Spectrum analysis of the chip.
4.3.3 Visualization λDNA Attachment on Gold Electrodes by Electrostatic and Covalent Forces by SEM

The final chip contained a solution of prepared lambda DNA directly attached to the gold electrodes with the same method explained on section 4.3. The following images (Figures 85-88) are the final chip captured by Scanning Electron Microscope at the SDSU electron microscope facility. The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

Figure 83: Spectrum analysis of different materials exists in the DNA wire chip.

Figure 84: Scanning Electron Microscope at SDSU Electron Microscope Facility

21 the pattern of image storage and transmission used in most computer bitmap image systems.
Figure 85 to Figure 88 exhibit the isometric view of the electrode which includes two layers of SU-8 and gold on top of a Silicon oxide substrate. The red circle shows λDNA attached and suspended between two electrodes. Through this attachment protocol DNA does not contact the substrate. This suspension method will be used in the next chapter to measure electrical characteristics of the DNA wire. The electrical characters of the DNA wire chip is our second positive control experiment to demonstrate the existence of a DNA bridge between two gold electrodes.

Figure 85: Focus on the electrodes
Figure 86: λDNA attached on electrode gold surface and suspended between two electrodes.

Figure 87: 3D view of the electrodes on top of SiO2 substrate.

Figure 88: 3D view of λDNA bridge between two gold electrodes.
CHAPTER 5

ELECTRICAL CHARACTERIZATION OF DNA NANOWIRES IN 2-D AND 3-D PLATFORMS

In this chapter the electrical characterization of suspended DNA between two gold electrodes on 2-D and 3-D platforms will be investigated. The basis for electrical characterization of double-stranded DNA is its inherent electrical properties and potential use as an electronic component. It is established and commonly known that the DNA double helix is stabilized by hydrogen bonds between its bases which structurally bind the two helix strands. DNA contains four bases: adenine (A), thymine (T), guanine (G), and cytosine (C). (A) forms a base pair with (T) and (G) with cytosine (C). (A+T) sequences show n-type properties and (G+C) shows p-type properties [81]. Guanine is the base with the lowest oxidation potential [82]. It loses an electron during oxidative stress and becomes positively charged. This positive charge does not stay at the base where it was formed, but keeps moving along G-rich sequences. Indeed, G-rich sequences lower the molecule’s oxidative potential, so a positive charge can move from a single guanine towards a multiple guanine sequence that attracts such electron holes [83]. In a DNA molecule the guanine/cytosine sequences would accept electrons from the negative pole that could, beyond a certain breakthrough voltage, move through the adenine/thymine barriers to allow electron flow to the positive pole [84]. Upon DNA attachment, a wire bridge like forms on the electrodes. Because of the formation of this bridge the electrical resistance between the electrodes drops from infinite to a few megohms. The change in resistance value is the measure of presence of DNA.
Knowing the electrical characteristics of DNA is important for different sectors of industry, especially for miniaturizing electronic components and solving some of the issues with existing biology research. In biology, for instance, detection of a specific DNA molecule via its electrical characteristics is much easier compared to surface stress based methods [85]. In the electronic industry, DNA-based single electron transistors and quantum-bit elements have already been proposed. Creation of these logic components is based on DNA base pairs, since just a short sequence of DNA base pairs may be enough to create all the combined n- and p-type properties [86].

5.1 DNA Wire Chip with Four Integrated Electrodes

The DNA wire feature achieved in this study consists of four bump pads/electrodes (Figure 90). The four point probes work by applying current via a pair of force connections (current leads). A voltage drop is then generated across the impedance of the DNA wire to be measured according to Ohm's law V=RI (Figure 91). This current also generates a voltage drop across the force wires. To avoid including the voltage drop in the measurement, a pair of sense

\[ V = RI \]

---

22 In general, n-type material uses negatively charged electrons to carry current, whereas positive p-type material uses electron holes to do so. Joining the two types allows the current to flow in a specified direction, which is the basis for all semiconductors, such as diodes, triodes and transistors.
connections (voltage leads) are placed adjacent to the target impedance. The accuracy of the technique comes from the fact that almost no current flows in the sense wires, so the voltage drop given by $V=RI$, is extremely low [87].

![Diagram of voltage and current connections](image1)

**Figure 90:** DNA wire chip consists of 4 electrodes

**Figure 91:** Four-point measurement of resistance between voltage sense connections 2 and 3. Current is supplied via force connections 1 and 4.

In brief, the key difference between 4 probe (Figure 93) and 2 probe (Figure 92) sensing is that the separation of current and voltage electrodes. The 4 point probes allow the ohmmeter to eliminate any contribution of impedance in the wiring and contact resistances given that the voltage electrodes have high enough input impedance [88,89].

![Diagram of 4 and 2 probes sensing](image2)

**Figure 93:** 4 probes sensing

**Figure 92:** 2 probes sensing
5.2 Experimental Setup for Electrical Measurements of DNA on 2-D and 3-D platforms

The following steps detail the laboratory setup to perform electrical measurements of DNA on a 2-D and 3-D wire platform:

5.2.a. Place DNA wire chip in a Petri dish.
5.2.b. 1µl of λDNA is dispensed to the gold electrodes of the chip. The dish is set aside for 10 min at room temperature.
5.2.c. The chip is rinsed with Milli-Q water and dried under an incandescent microscope light for 1 min. During this step DNA is suspended between two gold electrodes by electrostatic force and covalent bonding (see 4.3, method 2).
5.2.d. Following DNA attachment, four probes contact the four electrodes on top of the Signatone probe tip holder. To improve accuracy, the installation is carried out under a microscope (Contour) with a fiber light source (Dolan-Jenner).
5.2.e. The opposite end of the four probes is attached to the voltage source/meter and Amp source/meter (Keithley 2400 Source Meter) front panel.
5.2.f. Select voltage source and current meter. The voltage is applied across two electrodes and current is monitored from the other two electrodes with the amp meter (Figure 94).
5.2.g. To capture the current and voltage data the Keithley 2400 source meter is connected to a laptop PC through a RS232 port and Teraterm web 3.1 software is used to sample the raw voltage and current data (Figure 95). DNA attachment experiments and the electrical measurement set up for both 2-D and 3-D DNA platforms with their I-V curves and other results will be discussed in this chapter.
Figure 94: Voltage source and current amp meter probes across the DNA wire sample.

Figure 95: Electrical Measurement Setup at SDSU MEMs lab. Four probes contact the DNA wire electrodes through the Signatone probe holders. The opposite end of the probes connect to the front panel of the Keithley 2400 voltage source/ampere meter. A RS232 port on the back panel of Keithley is connected to a laptop PC. Teraterm web v3.1 software installed on the laptop is used to collect the raw voltage and current data.
5.2. h. To obtain the voltage and current raw data from the DNA wire chip Teraterm 3.1 software was programmed to automate the data sampling. This was accomplished via a short script which ran in the Teraterm terminal window. The following script was used to apply voltage from 1 to 10 volts in 10 steps over 10 seconds and sample the related current output.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>*RST</td>
<td>Resource GPIB defaults.</td>
</tr>
<tr>
<td>:SOUR:VOLT 0</td>
<td>Set bias level to 0V.</td>
</tr>
<tr>
<td>:SOUR:DEL 1</td>
<td>Set delay to 1s.</td>
</tr>
<tr>
<td>:SOUR:VOLT:MODE SWE</td>
<td>Select the sweep source mode.</td>
</tr>
<tr>
<td>:SOUR:SWE:SPAC LIN</td>
<td>Select the linear sweep scale.</td>
</tr>
<tr>
<td>:SOUR:VOLT:STAR 1</td>
<td>Set start level to 1V.</td>
</tr>
<tr>
<td>:SOUR:VOLT:STOP 10</td>
<td>Set stop level to 10V.</td>
</tr>
<tr>
<td>:SOUR:VOLT:STEP 1</td>
<td>Set step level to 1V</td>
</tr>
<tr>
<td>:TRIG:COUN 10</td>
<td>Set trigger count to 10</td>
</tr>
<tr>
<td>:OUTP ON</td>
<td>Turn on output</td>
</tr>
<tr>
<td>:READ?</td>
<td>Trigger sweep and acquire data.</td>
</tr>
</tbody>
</table>

5.2.i. The data received was copied to an Excel spreadsheet, sorted, and plotted as I-V, R-V, and $\sigma$-V curves.

*Note: Keithley 2400 measurement range limitations:
For the 200V source range selected, the highest current measurement range is 100mA. With the 1A source range selected, the highest voltage measurement range is 20V.
5.2.1 Electrical Measurement of DNA Nanowire Attached on 2-D Platforms

Following the steps 5.2.a through 5.2.i., the voltage is applied across two electrodes and the current is monitored from the other two electrodes with an amp meter (Figure 96). Before dispensing the DNA to the gold electrodes, the conductivity of the gap is measured by the Keithley ohmmeter. The resistance between the two electrodes was infinite and conductivity was zero.

Figure 96: Electrical set up for 2-D DNA chip. DNA attached between 2 electrodes. Voltage source and voltage meter are parallel to the electrodes while the Current meter (I-Meter) is in series with the electrodes.
5.2.1.1 RESULT 1: (I-V) CURVE OF DNA NANOWIRE ATTACHED ON 2-D PLATFORM

DNA attachment between two gold electrodes on the 2-D platform is based on 4.3 method and electric field attraction\(^{23}\). If input voltage increased from 1 to 10 volts across the DNA sample, the current output increased from 7.5nA to 0.154 µA. The following I-V curve displays a S-shape transition where each data point follows Ohms law \(V=RI\) where, \(V\): voltage (V), \(I\): current (A), \(R\): resistance (Ω) (Figure 97).

\[\text{I-V Curve for } \lambda \text{ DNA on 2D Platform}\]

\[\begin{array}{c|c}
\text{Voltage (V)} & \text{Current (µA)} \\
\hline
0.0075 & 0.00 \\
0.0209 & 0.02 \\
0.0425 & 0.04 \\
0.0718 & 0.07 \\
0.1110 & 0.11 \\
0.1250 & 0.12 \\
0.1410 & 0.14 \\
0.1510 & 0.15 \\
0.1540 & 0.15 \\
\end{array}\]

Figure 97: I-V curve for \(\lambda\)DNA attached across 2-D gold electrodes of Chip#7 (012310)
If voltage increases from 1-10v then current increases from 7.5nA to 0.0154 µA.

\(^{23}\) Since DNA is negatively charged, movement of the target DNA molecule towards the metal electrodes under an electric field is much faster than relying on diffusion alone. In addition, the electric field also increased target concentration at the surface.
5.1.2.2 Result 2: (R-V) Curve of DNA Nanowire Attached on 2-D Platform

When the voltage input increases from 1 to 3.5 volts across the DNA sample, the resistance output decreases from 131MΩ to 60MΩ. By increasing voltage from 3.5 to 10 volts, the resistance of the DNA wire stays at approximately 60MΩ (Figure 98).

Figure 98: R-V curve for λDNA attached across 2-D gold electrodes. If voltage increases from 1-10v then, resistance decreases from 131MΩ to 60MΩ and stays around 60MΩ.
5.1.2.3 RESULT 3: ($\sigma^{24}$-V) CURVE OF DNA NANOWIRE ATTACHED ON 2-D PLATFORM

The lambda DNA molecule has a length=150,000 Å and diameter=500 Å [91]. Based on these dimensions the conductivity of a DNA wire is calculated below. The electrical resistance of a DNA wire would be expected to be high due to its long length in comparison to a small cross sectional area. Conversely, the $\lambda$DNA conductivity is low (Figure 99). When voltage increases from 1 to 4 volts the conductivity increases from 58 to 137 ($\text{S} \cdot \text{m}^{-1}$). If voltage increases from 4 to 10 volts the average conductivity stays around 130 ($\text{S} \cdot \text{m}^{-1}$) based on the following calculations.

$$D=500\text{Å}=50\text{nm}, \quad r=25\text{nm}$$
$$L=15000\text{Å}=15\mu\text{m}$$

$$A = \pi r^2 = 625\times10^{-18} \times \pi = 1.96\times10^{-15}$$

$$R = \frac{\rho L}{A}$$

Which, $\rho$=resistivity, $L$=length, $A$=cross sectional area

$$R=\rho \times 15\times10^{-6} / 1.96\times10^{-15} = \rho \times 7.65\times10^9$$

$$R= \rho \times 7.65\times10^9 \Rightarrow \rho=R/7.65\times10^9$$

Electrical conductivity = $\sigma = 1/\rho = 7.65\times10^9 /R$ ($\text{S} \cdot \text{m}^{-1}$)

![Figure 99: $\sigma$-V curve for $\lambda$DNA attached across 2-D gold electrodes. From 1 to 4V, conductivity increases from 58 to 137 ($\text{S} \cdot \text{m}^{-1}$). Then, by increasing voltage to 10 V, the conductivity stays around 130 ($\text{S} \cdot \text{m}^{-1}$).](image)

---

24 $\sigma$: Conductivity: is the reciprocal of electrical resistivity, $\rho$, and has the SI units of siemens per metre ($\text{S} \cdot \text{m}^{-1}$)
5.1.3 Electrical Measurements of DNA Nanowire Attached on 3-D Platforms

λDNA solution is dispensed on a 3-D chip using the same method described in section (5.3.2). The Voltage Source and Amp meter are connected to the four electrodes of the chip. The voltage is applied across two electrodes and the current is monitored from the other two electrodes with amp meter (Figure 100). Before depositing the DNA solution on the gold electrodes, the resistance of the gap was measured by the Keithley ohm meter which showed no conductivity and resistance was infinite.

Figure 100: Electrical set up for 3-D DNA chip. Voltage source and voltage meter are parallel to the electrodes and the Amp meter is in series with the electrodes.
5.1.3.1 (I-V) Curve of DNA Nanowire Attached on a 3-D Platform

To receive I-V curve from 3-D DNA chip sample. 0.1µl λDNA solution was deposited on the 3-D chip electrode gap (see 4.3, method 2). After 4min, 5v voltage applied to the electrodes for 10s. The reasons for doing this step is, since DNA is negatively charged, movement of the target DNA molecule towards the metal electrodes under an electric field is much faster than relying on diffusion alone. In addition, the electric field increases target concentration of DNA between two electrodes [87]. Subsequently voltage increased from 1 to 10V across the DNA sample. By monitoring the output current, we observed an increase in current from 0.4µA to 5.1µA. The I-V curve below shows each data point follows Ohms law V=R×I (Figure 101). Therefore we can conclude the DNA wire is bridged between the electrodes.

![I-V Curve for λDNA on 3D platform](image)

Figure 101: I-V curve for λDNA attached across 3-D gold electrodes of chip#8 (8.18.10). When voltage increased from 1-10v the related current increased from 0.41µA to 5.16 µA. Chip 8, created by negative lithography, is constructed of SU8(100)+Au with a gap=3µm and thickness=50 µm.
5.1.3.2 (R-V) Curve of DNA Nanowire Attached on a 3-D Platform

When the voltage input increases from 1 to 6 volts across the DNA sample the resistance output decreases from 4.9MΩ to 2.16MΩ. By increasing voltage from 6 to 10 volts, the resistance of the DNA wire stays at approximately 2MΩ (Figure 102).

![R-V Curve for λDNA on a 3D platform](image)

**Figure 102**: R-V curve for λDNA attached across 3-D gold electrodes. If voltage increases from 1-10v then resistance decreases from 4.89 MΩ to 2MΩ and stays around 2MΩ.
5.1.3.3 Result 3: (σ-V) Curve of DNA Nanowire Attached on 3-D Platform

Based on the electrical conductivity equation from section 5.1.2.3, \( \sigma = \frac{1}{\rho} = 7.65 \times 10^9 / R \) (S·m\(^{-1}\)) when voltage increases from 1 to 4 volts conductivity increases from 1565 to 3177 (S·m\(^{-1}\)). If voltage increases from 4 to 10 volts, the conductivity remains in the range of 3500 (S·m\(^{-1}\)) (Figure 103). In comparison, \( \lambda \)DNA attached on a three dimensional platform shows higher conductivity than the \( \lambda \)DNA attached on the two dimensional platform.

Figure 103: σ-V curve for \( \lambda \)DNA attached across 3-D gold electrodes. From 1 to 4v, conductivity increases from 1565 to 3177 (S·m\(^{-1}\)). Then, by increasing voltage 1 to 10 v, the conductivity remains in the range of 3500 (S·m\(^{-1}\)).
5.2 CONTROL EXPERIMENTS

In this section two series of control tests will be discussed. The first series, an electrical set up control test, will check the accuracy of our electrical set up before running any DNA test. The second test, a negative control test, was run to prove existence of a DNA wire bridged between the two gold electrodes for both 2-D and 3-D platforms.

5.2.1 Electrical set up control test

To ensure that our electrical set up was calibrated before running the DNA attachment test the (I-V) curve measurement system was tested with four different resistors. These resistors with resistance of 100Ω, 1KΩ, 100KΩ and, 600KΩ were connected to the voltage source/current meter (Keithley 2400) through 4 probes. The Keithley instrument is then connected to a laptop running Teraterm v3.1 software (terminal emulator program). From the front panel of the Keithley 2400 the 4 probes are connected to the four chip electrodes. Once the probe connections are made voltage is supplied (1v to 10v) in 10 steps to two probes while current is monitored from the other probes (Figure 104). I-V measurements and curve data are shown in Figure s 111 though 113.
Figure 105: Voltage and current measurement for $R=600K\Omega$ and their I-V curve

<table>
<thead>
<tr>
<th>R=600KΩ</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>V(V)</td>
<td>I(A)</td>
</tr>
<tr>
<td>1.00E+00</td>
<td>1.69E-06</td>
</tr>
<tr>
<td>2.00E+00</td>
<td>3.38E-06</td>
</tr>
<tr>
<td>3.00E+00</td>
<td>5.08E-06</td>
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<td>4.00E+00</td>
<td>6.77E-06</td>
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<tr>
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<td>1.01E-05</td>
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<td>1.18E-05</td>
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<td>1.52E-05</td>
</tr>
<tr>
<td>1.00E+01</td>
<td>1.69E-05</td>
</tr>
</tbody>
</table>

Figure 106: Voltage and current measurement for $R=100KΩ$ and their I-V curve

<table>
<thead>
<tr>
<th>R=100kΩ</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>V(V)</td>
<td>I(A)</td>
</tr>
<tr>
<td>1.00E+00</td>
<td>1.01E-05</td>
</tr>
<tr>
<td>2.00E+00</td>
<td>2.02E-05</td>
</tr>
<tr>
<td>3.00E+00</td>
<td>3.04E-05</td>
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<tr>
<td>4.00E+00</td>
<td>4.05E-05</td>
</tr>
<tr>
<td>5.00E+00</td>
<td>5.06E-05</td>
</tr>
<tr>
<td>6.00E+00</td>
<td>6.07E-05</td>
</tr>
<tr>
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<td>7.08E-05</td>
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<td>8.10E-05</td>
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<tr>
<td>9.00E+00</td>
<td>9.11E-05</td>
</tr>
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</table>

Figure 107: Voltage and current measurement for $R=1KΩ$ and their I-V curve

<table>
<thead>
<tr>
<th>R=10kΩ</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>I</td>
</tr>
<tr>
<td>1.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>2.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>3.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>4.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>5.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>6.00E+00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>7.00E-00</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>8.00E-00</td>
<td>1.03E-04</td>
</tr>
<tr>
<td>9.00E-00</td>
<td>1.03E-04</td>
</tr>
<tr>
<td>1.00E-01</td>
<td>1.05E-04</td>
</tr>
</tbody>
</table>
The I-V curves determine a linear relationship between each step of voltage and its related current for the three standard sample resistors. These results confirm that our electrical set up is working accurately and is ready to measure DNA wire I-V curves.

5.2.2 Negative Control (2-D platforms)

To run the negative control test we first obtained a 2-D DNA chip. Before attachment of DNA on the chip resistance between the two electrodes was measured at infinite ohms ($\infty$) and no current was detected across its gap (Figure 108). After dispensing the DNA solution on the gap and applying voltage in steps from 1 to 10v for a few seconds the I-V curve fluctuates (10 seconds) and does not follow ohm’s law (Figure 109). After DNA attachment, voltage was applied again in steps from 1-10v. At this stage we see the I-V curve is following Ohms relationship and the resistance between the gap drops from infinite ($\infty$) to 40M$\Omega$ (Figure 110). The reason for this change is any DNA bonded to the gold electrodes forms a electrical bridge. Because of the bridge formation the electrical resistance between the electrodes decreases from infinite to a few mega ohms. The change in resistance value is a way to prove the presence of a DNA wire between two electrodes [92].

![I-V curve before dispensing λDNA](image.png)

**Figure 108:** Prior to DNA attachment while increasing electrode gap voltage no current is sensed by the electrodes thus, $I=0$ and the $R=\infty$.  

Figure 109: After dispensing DNA the I-V curve fluctuates for 10 seconds and does not follow the Ohms relationship.

Figure 110: After DNA attachment, the I-V curve is follows the Ohms relationship and the resistance between the gap drops from infinite $\infty$ to 40M$\Omega$. 
5.2.3 Negative control (3-D platforms)

To run the negative control test we first obtained a 3-D DNA chip. As in the negative test for 2-D platform, we measured the resistance between the two gold electrodes of the DNA chip. The resistance was infinite and no current was sensed across the gap (Figure 111). For the second test, Figure 112, DNA solution was pipetted onto the gap and voltage was applied from 1-10v. In this scenario the I-V curve fluctuated and did not follow Ohms relationship. After one minute, voltage was applied from 1v to 10v, at this time the I-V curve data followed Ohms relationship and the resistance decreased from infinite to 2MΩ (Figure 113). This change in resistivity is a confirmation there is a DNA bridge across the electrode gap.

![I-V Curve before attaching λDNA on 3D platform](image)

Figure 111: Before attachment of DNA by increasing voltage no current is sensed across the electrode gap, I=0 and the R=∞.
Figure 111: After DNA attachment, the I-V curve demonstrates an Ohms relationship and the electrode gap resistance decreases from infinite $\infty$ to 2MΩ.

Figure 112: Immediately after dispensing DNA the I-V curve fluctuates for a few seconds and does not follow Ohms relationship.
5.3 Mathematical and Circuit Models of DNA

Hodniz et al. have developed a PSpice model of the electrical behavior of DNA molecules in 2007 for use in nano-electronic circuit design [93]. To describe the relationship between the current through DNA and the applied voltage they leveraged results from Kutnjak et al. and their direct measurements of electrical conduction through DNA molecules [94]. The experimental dc current–voltage (I –V) curves show a nonlinear conduction mechanism (S shape curve). The results show the least-squares polynomial fit to the experimental data at 304.5 K (31.35°C). The function used was a fifth-order polynomial as follows;

\[ I=A_1 + A_2 V + A_3 V^2 + A_4 V^3 + A_5 V^4 \]

Which,

I=Current (pA)
V=Voltage (V) (Independent DC source)
A= Coefficient

This equation was then used as a mathematical model for electrical behavior of DNA. Based on this equation Hodniz and her group created an equivalent electrical circuit in PSpice software where DNA was modeled as a voltage-controlled current source component, GPOLY ("G" stands for current output and "POLY" stands for polynomial). After running their tests at different temperatures they also described their model, a temperature dependent component GPOLY(T). This result is important because having models of DNA molecules in the form of equivalent electronic circuits would be useful in the design of nano-electronic circuits and devices.

Since we also obtained a "S" shape curve in our I-V experiments, the Hodniz study inspired us to create a mathematical model for our DNA I-V curves as well. Although Hodniz generated I –V curves at different temperatures, our DNA I-V results were exclusively captured at room temperature (304.5K=31.35°C).
5.3.1 Mathematical Model of DNA

To achieve a mathematical model for the DNA I-V curve in this study we obtained the current equations related to five specific voltages from one of our I-V curve data sets. Based on \( I = A_1 + A_2 V + A_3 V^2 + A_4 V^3 + A_5 V^4 \), we obtained the following equations:

For \( V=1 \rightarrow I = -0.116 \times 10^{-6} = A_1 + A_2 - A_3 + A_4 - A_5 \)
For \( V=2 \rightarrow I = 0.409 \times 10^{-6} = A_1 + 2A_2 - 4A_3 + 8A_4 - 16A_5 \)
For \( V=3 \rightarrow I = 0.894 \times 10^{-6} = A_1 + 3A_2 - 9A_3 + 27A_4 - 81A_5 \)
For \( V=4 \rightarrow I = 1.49 \times 10^{-6} = A_1 + 4A_2 - 16A_3 + 64A_4 - 256A_5 \)
For \( V=5 \rightarrow I = 2.08 \times 10^{-6} = A_1 + 5A_2 - 25A_3 + 125A_4 - 625A_5 \)

To find the unknown coefficients, \( A_1, A_2, A_3, A_4 \) and, \( A_5 \), we can either use EXCEL or MATLAB software. In the Excel program, we used the equation \( V \times A = I \). Then, the MMULT (MINVERSE (V),I) command was used to find the inverse of the 5×5 matrix. This inverted matrix was then multiplied by a 5×1 matrix. Through this method we determine the results for the coefficients \( (A_n) \) as follow;

\[
\begin{bmatrix}
1 & 1 & 1 & 1 & 1 \\
1 & 2 & 4 & 8 & 16 \\
1 & 3 & 9 & 27 & 81 \\
1 & 4 & 16 & 64 & 256 \\
1 & 5 & 25 & 125 & 625
\end{bmatrix} \times \begin{bmatrix}
A_1 \\
A_2 \\
A_3 \\
A_4 \\
A_5
\end{bmatrix} = \begin{bmatrix}
-0.116 \\
0.409 \\
0.894 \\
1.49 \\
2.08
\end{bmatrix} \times 10^{-6}
\]

Witch, \[ \begin{bmatrix}
A_1 \\
A_2 \\
A_3 \\
A_4 \\
A_5
\end{bmatrix} = \begin{bmatrix}
-1.1 \\
1.42 \\
-0.56 \\
0.137 \\
-0.011
\end{bmatrix} \]

By inserting the found \((A_1 - A_5)\) coefficients into the fifth-order polynomial, \( I = A_1 + A_2 V + A_3 V^2 + A_4 V^3 + A_5 V^4 \), we have find the following result;

\( I = -1.1 \times 10^{-6} + 1.42 \times 10^{-6} V^2 - 0.56 \times 10^{-6} V^3 + 0.137 \times 10^{-6} V^4 - 0.01 \times 10^{-6} V^5 \)

This is our mathematical model of the electrical behavior of a DNA wire at room temperature.
5.3.2 Circuit Model of DNA

After determining the DNA mathematical model we can model this behavior as an electrical circuit by using PSpice software. According to our mathematical model DNA functions as a fifth-order polynomial, its equivalent electrical PSpice component is a GPOLY. GPOLY is an analog behavioral component which acts as a voltage-controlled current source (Figure 114).

5.3.2.1 PSpice Electrical Circuit Model

Step 1: Create a PSpice circuit with capture option
1. Create a new Analog project.
2. Insert circuit parts from “Controlled Sources”.
3. Choose Analog Behavioral Modeling (ABM) and locate GPOLY (a voltage Controlled Current Source component)
4. Connect the parts with traces and ground them.

Step 2: Specify type of simulation
1. Create a simulation profile.
2. Apply DC sweep
3. Run Pspice

Figure 114: GPOLY standard textbook symbols

Figure 115: Creation of the Gpoly circuit in Pspice
The schematic model of DNA without displaying the internal impedances is obtained by running the Pspice program (Figure 116). After completing the circuit, voltage and amp meters are added.

This model is similar to the DNA schematic model introduced by Hodniz et al in 2007. One difference between our model and the Hodniz model is the Hodniz model GPOLY(T) is a temperature dependence component (Figure 117). In this study we ignore the temperature dependency since most of our experiments were done at room temperature.

Figure 116: DNA Pspice Schematic Model without Internal Resistances @ 32ºC

Figure 117: Hodniz et al. DNA electrical model, 2007.
5.3.2.2 RESISTANCE MEASUREMENT OF DNA WIRE PLATFORMS FOR CIRCUIT MODEL

The DNA electrical circuit model consists of internal and external resistances. These resistances include the resistance of four gold electrodes on the chip and internal resistance of the DNA wire. The internal DNA resistances on 2-D and 3-D electrodes are already measured in sections 5.1.2.2 and 5.1.3.2 of this study. The resistance measurements of the electrodes are explained below.

A. Measuring resistance of the 2-D electrode platforms:

The resistance is measured with a Keithley 2400 ohmmeter for six 2-D chips. The resistance value is 1.5 to 6 kΩ between the connected electrodes. The resistivity output is based on the differences in thickness of the gold layer on these six chips. The impedance between the gap electrodes is infinite in all chips (open circuit).

B. Measuring resistance of 3-D electrode platforms:

The resistances are measured from 1.4 to 4 kΩ between the connected electrodes on four 3-D chips. This variety in resistivity depends on the gold and SU-8 layer thickness. The impedance between the gap electrodes is infinite in all chips (open circuit).
5.3.2.3 Electrical Circuit Modeling of Lambda DNA Wire Attached on 2-D and 3-D Platforms

At this point we have all the data to design our electrical model. The schematic models of a DNA wire on 2-D and 3-D platforms were designed with PSpice.

**A: Electrical Circuit Model of Lambda DNA Wire Attached on 2-D Platform**

$\lambda$DNA average internal resistance according to 5.1.2.2 is 60 M$\Omega$. The resistance between the pair of electrodes on the 2-D chip according to 5.3.2.2 is 12k$\Omega$ (series of two 6k$\Omega$). Based on this data we plot the schematic of DNA as a GPOLY component with an output impedance containing $R_{\text{chip}}=12\text{K}\Omega$ plus $R_{\text{DNA}}=60\text{M}\Omega$.

![Electrical Circuit Model of Lambda DNA Wire Attached on 2-D Platform](image)

Where, $R_{\text{eq}}=R_{\text{chip}} (12\text{K}\Omega) + R_{\text{DNA}} (60\text{M}\Omega)$

Figure 120: Electrical Circuit Model of Lambda DNA Wire Attached on 2-D Platform @ 32°C
B: Electrical Circuit Model of Lambda DNA Wire Attached on 3-D Platform

Similar to the above, λDNA internal resistance average is 2MΩ (see 5.1.3.2). The resistance between the pair of electrodes on the 3-D chip according to 5.3.2.2 is 8kΩ (series of 4kΩ+4kΩ). The following schematic includes the GPOLY component and its output impedance in Pspice.

![Electrical Circuit Model](image)

Where, \( R_{eq} = R_{chip} \ (8\,\text{kΩ}) + R_{DNA} \ (2\,\text{MΩ}) \)

Figure 121: Electrical Circuit Model of Lambda DNA Wire Attached on 3-D Platform @ 32°C
CHAPTER 6

CONCLUSION

In this study we have investigated the electrical characterization of double-stranded DNA (Lambda DNA) through experiments, theoretical models, and equivalent circuit simulations. Based on the evidence presented we conclude that for this particular type of DNA (λDNA) - there is a measurable and significant conductivity that could establish it as a semi-conductor. In general, we have been cognizant of the fact that knowledge sets from highly interdisciplinary areas are required. These areas include nano engineering, electronics, biology, physics, chemistry, computer science, and other engineering realms to firmly establish the unique electrical characteristics of the DNA molecule. The following are what were achieved in this thesis work:

Attached DNA to gold electrodes on 2-D platforms using electrostatic and electrical field attachment methods. Attached DNA to gold-sputtered electrodes made from SU-8 pillars on a 3-D platform using electrostatic attachment, covalent bonding, self-assembly of Oligos (at the DNA ends), and electric field attachment. We had used the following controls in these experiments:

Negative control in which the resistivity of the gap across the two metal electrodes was measured before DNA assembly, the resistivity was infinite. Then, resistance was measured after DNA attachment and the result showed the resistivity dropped from infinite to a few mega Ohm. This change in resistivity is a reason for presence of DNA wire between two electrodes.

During the second control test, positive control, the DNA was removed from the chip by separation of the coverslip on top of the chip. The cover slip glass was fixed on the DNA wire chip with an adhesive-like liquid (mount). This was to avoid any movement of the glass on top of the chip during visualization under a fluorescent microscope. After removal of the DNA, the gap was observed under a fluorescent microscope and no glowing DNA wire was detected. At this step the resistance across the gap was measured again. The resistance was raised from a few mega ohm to infinite which means there is no connection between the two metals.
After attachment, we used three laboratory instruments (3-D microscope, fluorescent microscope, and SEM) for optical characterizations. Repeated measurements of the electrical properties of DNA wire in this study showed DNA acting as a semiconductor. As a result, a semiconductor resistivity measurement device (Keithley 2400) was used to sample I-V data. Later, these I-V curves used to design an equivalent circuit models for electrical characterizations of DNA wire.

To optimize our results from the two dimensional DNA wire chip, DNA assembly on a three dimensional structure was tried for first time at SDSU MEMs lab. In general, the 3-D DNA chip showed lower resistance and higher conductivity and better stability compared to the 2-D DNA chip structure. The stability of 3-D structure after the DNA attachment was tested and monitored for a month during which the top gold layer and the DNA remained intact. Moreover, this was beneficial as the 3-D DNA wire chip did not necessarily need packaging after manufacturing. Additionally, the 3-D electrodes made of two layers of SU-8 and gold is raised more than 15µm above the substrate. This distance circumvents the suspended DNA from contacting the substrate which ensures more accurate resistance measurements.

Other achievements in this study include developing a viable protocol for self assembly of DNA to a gold surface by use of oligos and direct attachment of DNA by electrostatic and covalent bonding. Although this method is not commonly used in other studies, we found it to be a fast and economical way to obtain DNA assembly between two metal posts for performing electrical tests.

In regards to the chip design, the narrow and tapered electrode tips in this study (similar to actual electronic circuit traces) electrostatically concentrates positive charges which aids attraction of negatively charged DNA to selectively bridge between two metal posts. Additional design benefits include a chip with four electrode pads; This allows a measuring technique using separate pairs of current-carrying and voltage-sensing electrodes. Consequently, almost no current flows in the sense wires, so the voltage drop is extremely low. This makes more accurate measurements than traditional two-probe sensing.
6.1 Future Studies and Recommendations

During this study, we came across complimentary research studies that can help to further development in DNA electronics. For instance, design the DNA template feature with different gap sizes from 15µm to smaller than 0.5µm to assemble different lengths of DNA onto these gaps. Additionally, to cut DNA to different lengths we can use a restriction enzyme. Attachment of various DNA lengths between two electrodes can help us to understand the mechanism of charge transfer through a DNA molecule.

In addition to the control tests featured in this study, we can run more accurate tests. For instance, a positive control test can be run to visualize the chip after oligo attachment (before DNA hybridization) by addition of SYBR gold solution. As a negative control, the chip with attached DNA can be exposed to SYBR gold and then visualized.

A new design recommended for future study, which uses four point probes pads instead of four stationary probes, is shown below.

Figure 122: DNA Wire Chip with 4 bump pads (Gen III)
APPENDIX

[Appendix 1] Hummer V Gold Sputter Coater System Function

When a target is bombarded with fast heavy particles, erosion of the target material occurs. The process, when occurring in the conditions of a gaseous glow discharge between an anode and cathode is termed sputtering. If an inert gas (argon) is included in a cathode gas tube, the free ions and electrons are attracted to opposite electrodes and a small current is produced.

Figure 123: Circuit to determine the current-voltage characteristics of a cold cathode gas tube

As voltage is increased some ionization is produced by collision of electrons with gas atoms, named the "Townsend" discharge. When the voltage across the tube exceeds the breakdown potential, a self sustaining glow discharge occurs - characterized by a luminous glow. The current density and voltage drop remains relatively constant, the increase in total current being satisfied by the area of the glow increasing. Increasing the supply voltage increases current density and voltage drop, this is the abnormal glow region. Further increase in supply voltage concentrates the glow into a cathode spot and arc discharge is apparent. The operating parameters of sputter coaters are within the glow discharge regions of the characteristic described.

Table 9: Positive lithography fabrication process flow for 2-D DNA Chip
### Positive Lithography fabrication Process Flow (2D DNA chips)

<table>
<thead>
<tr>
<th>Chip-ID</th>
<th>Shipley</th>
<th>Chip</th>
<th>Dehydration</th>
<th>Photore sist</th>
<th>Soft Bake</th>
<th>Expose</th>
<th>Develop</th>
<th>Gold</th>
<th>Ultrasonic</th>
<th>Fixt ure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Z1)</td>
<td>1ml</td>
<td>3x1 cm</td>
<td>65°C-60s</td>
<td>2000rpm, 45s</td>
<td>115°C, 60s</td>
<td>6.14mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>10s</td>
</tr>
<tr>
<td>2</td>
<td>(A1)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-60s</td>
<td>2000rpm, 45s</td>
<td>115°C, 80s</td>
<td>6.14mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>10s</td>
</tr>
<tr>
<td>3</td>
<td>(M1)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-60s</td>
<td>2000rpm, 45s</td>
<td>115°C, 80s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>20s</td>
</tr>
<tr>
<td>4</td>
<td>(M2)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-60s</td>
<td>2000rpm, 45s</td>
<td>115°C, 60s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>20s</td>
</tr>
<tr>
<td>5</td>
<td>(Z2)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-60s</td>
<td>2000rpm, 45s</td>
<td>115°C, 80s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>18s</td>
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<tr>
<td>6</td>
<td>(M1)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-50s</td>
<td>2000rpm, 45s</td>
<td>115°C, 60s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>10s</td>
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<tr>
<td>7</td>
<td>(M2)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-50s</td>
<td>2000rpm, 45s</td>
<td>115°C, 60s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>25s</td>
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<td>8</td>
<td>(Z2)</td>
<td>1ml</td>
<td>1x1 cm</td>
<td>65°C-50s</td>
<td>2000rpm, 45s</td>
<td>115°C, 60s</td>
<td>6.25mW/cm²</td>
<td>40s</td>
<td>1.5</td>
<td>15s</td>
</tr>
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</table>
## Table 10: Negative lithography fabrication process flow for 3-D DNA Chip

<table>
<thead>
<tr>
<th>Chip # (Si+SiO2)</th>
<th>SU8 Viscosity</th>
<th>Dehydration Bake</th>
<th>Photore sist Coat</th>
<th>Soft Bake</th>
<th>Expose</th>
<th>Post Bake</th>
<th>Gold Sputtering</th>
<th>Ultra Sonic Develop</th>
<th>Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>65°C-60s</td>
<td>3000rpm, 45s</td>
<td>75°C, 5min, 100°C, 20min</td>
<td>16mW/cm², 85s</td>
<td>100°C, 10min</td>
<td>9V, 10mA, 70 Tr, 10min</td>
<td>&gt;15min No gap</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>65°C-60s</td>
<td>3000rpm, 45s</td>
<td>75°C, 5min, 100°C, 20min</td>
<td>16mW/cm², 85s</td>
<td>100°C, 10min</td>
<td>9V, 10mA, 70 Tr, 10min</td>
<td>&gt;15min No gap</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>89°C-60s</td>
<td>3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6mW/cm², 35s</td>
<td>65-95°C 10min</td>
<td>9V, 10mA, 70 Tr, 10min</td>
<td>&gt;15min No gap</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>65°C-60s</td>
<td>3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6mW/cm², 30s</td>
<td>65-100°C 30min</td>
<td>9V, 10mA, 70 Tr, 10min</td>
<td>1min Gap=7μm</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>65°C-60s</td>
<td>3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6.16mW/cm², 30s</td>
<td>65-100°C 30min 120°C,5min</td>
<td>9V, 10mA, 70 Tr, 8min</td>
<td>2min Gap=8μm</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>65°C-60s</td>
<td>2000-3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6.16mW/cm², 30s</td>
<td>65-100°C 30min 120°C,5min</td>
<td>9V, 10mA, 70 Tr, 8min</td>
<td>3min Gap=8μm</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>65°C-60s</td>
<td>2000-3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6.16mW/cm², 30s</td>
<td>65-100°C 30min 120°C,5min</td>
<td>9V, 10mA, 70 Tr, 8min</td>
<td>3min Gap=8μm</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>65°C-60s</td>
<td>2000-3000rpm, 45s</td>
<td>65-95°C Gradually 80min</td>
<td>7 mW/cm², 30s</td>
<td>65-100°C &gt;30min 120°C,5min</td>
<td>9V, 10mA, 75 Tr, 8min</td>
<td>30min Gap=0.5μm</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>65°C-60s</td>
<td>3000rpm, 45s</td>
<td>65-95°C Gradually 80min</td>
<td>6 mW/cm², 30s</td>
<td>65-100°C &gt;30min 120°C,5min</td>
<td>9V, 10mA, 75 Tr, 8min</td>
<td>12min Gap=3μm</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>65°C-60s</td>
<td>3000-2000-3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6 mW/cm², 30s</td>
<td>65-100°C &gt;30min 120°C,5min</td>
<td>9V, 10mA, 75 Tr, 8min</td>
<td>No gap Over developed , lost the feature</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>65°C-60s</td>
<td>2000-3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6 mW/cm², 30s</td>
<td>65-100°C &gt;30min 120°C,5min</td>
<td>9V, 10mA, 75 Tr, 8min</td>
<td>4min+30s Gap=7 μm</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>65°C-60s</td>
<td>2000-3000rpm, 45s</td>
<td>65-95°C Gradually 20min</td>
<td>6 mW/cm², 30s</td>
<td>65-100°C &gt;30min 120°C,5min</td>
<td>9V, 10mA, 75 Tr, 8min</td>
<td>6min Gap=7 μm</td>
<td></td>
</tr>
</tbody>
</table>
Many laboratory strains of lambda are derivatives of the strain \( \lambda c1857 \text{ind1 Sam7} \), which contains four point mutations relative to the wild type strain. The \text{ind1} mutation in the \text{cI} gene creates a new HindIII site at 37584 not present in the wild type. All lambda products sold by NEB are \( \lambda c1857 \text{ind1 Sam7} \). Numbering of the genome sequence begins at the first (5’-most) base of the left end bottom and continues rightward from late genes \text{nuI} and \text{A} towards the early genes. Table 11 shows the positions of all known ORFs larger than 200 codons. Enzymes with unique restriction sites are shown in bold type and enzymes with two restriction sites are shown in regular type. Restriction site coordinates refer to the position of the 5’-most base on the top strand in each recognition sequence.

Table 12: Numbering of the genome sequence

Source: New York Cold Spring Harbor Press
## Chemicals used for λDNA attachment to the gold electrodes

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambda DNA # NS011L 500 u/µl</strong></td>
<td><strong>Buffer fast AP (fermentas)</strong></td>
<td><strong>T4 DNA ligase</strong></td>
</tr>
<tr>
<td><strong>ATM: 1.25 ml</strong></td>
<td><strong>Thermosensitive</strong></td>
<td><strong>5 weiss u/µl, 200 u</strong></td>
</tr>
<tr>
<td><strong>New England Biolabs</strong></td>
<td><strong>Alkaline phosphatase</strong></td>
<td>(<strong>~1000 coh. end lig. u/µl 4000 u</strong>)</td>
</tr>
<tr>
<td></td>
<td><strong>1u/µl, 1000u</strong></td>
<td><strong>Store at -20°C</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Store at -20°C</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Buffer T4 DNA Ligase</strong></td>
<td><strong>Buffer A (fermentas)</strong></td>
<td><strong>PEG 6000 (Fermentas)</strong></td>
</tr>
<tr>
<td><strong>Fermentas 10XT4 DNA Ligase Buffer 0.5ml</strong></td>
<td><strong>PNK</strong></td>
<td><strong>24% (w/v), 0.2ml</strong></td>
</tr>
<tr>
<td><strong>store @ 20°C</strong></td>
<td><strong>(for forward reaction 0.4mL)</strong></td>
<td><strong>Store @ 20°C</strong></td>
</tr>
<tr>
<td><strong>Oligo 1</strong></td>
<td><strong>Buffer B (fermentas)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>IDT (5’-GGG CGG CGA OCT β Thio MC3-O/-3’)</strong></td>
<td><strong>PNK</strong></td>
<td><strong>Kinass 10 u/µl, 500 u</strong></td>
</tr>
<tr>
<td><strong>Tm=55.5 °C  MW=3,931.7 22.40 D, 204.2nm, 0.8mg</strong></td>
<td><strong>T4 Polynucleotide</strong></td>
<td><strong>Store -20°C</strong></td>
</tr>
<tr>
<td><strong>Oligo 2</strong></td>
<td><strong>PNK 10× Buffer B</strong></td>
<td><strong>Mount</strong></td>
</tr>
<tr>
<td><strong>IDT (5’-AGGTCGCCGCCC/3 Thio MC3- O/-3’)</strong></td>
<td><strong>For exchange return 0.2ml</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Tm=55.5°C  MW=3,851.6 21.50D, 202.6nm, 0.78mg</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
[Appendix 6]

Revised Procedure for DNA Attachment SDSU MEMs lab,

by Anson Hsu and Mohamad Rayatparvar

Chip preparation
Sputter coat a generation II CMEMS chip with a 60/40 Au/Pd mixture (which is the target currently available in the Electron Microscope facility).
First pass: Sputter coat at 80mTorr, 9kV, 10mA with flow perpendicular to the functional face of the chip. Source is on for 8 minutes.
Second pass: Repeat coating process with same parameters with the functional face approx. 45-50 degrees off normal. This is an attempt to coat the sidewalls of the pillar electrodes rather than just the tops. Source is on for 3-4 minutes.
Third pass: Repeat step B with chip face oriented 45-50 degrees in the other direction. Source is on for 3-4 minutes.

Oligo Preparation
Form a solution 100µM of both oligo sequences (Integrated DNA Technologies) by combining the lyophilized contents (dry, powdery substance) with 2mL of Tris-EDTA solution. T.E. is commonly used as a stable buffer solution and remains viable through multiple freeze/thaw cycles. Vortex contents for 5 seconds to mix thoroughly. Use these two oligo solutions as stock for further testing.
Perform a 100:1 dilution on both solutions to attain a concentration of 1µM in each container (separate microcentrifuge tubes)
Mix 18uL of solution attained from Step 2 with 1uL of T4 Polynucleotide Kinase (Fermentas) and 1uL of enclosed buffer solution. This modifier phosphorylates the oligos in preparation for attachment and hybridization. Repeat for both oligo solutions. Allow reaction to take place by placing both oligo solution tubes into a water bath at 37°C for 30 minutes.
The Polynucleotide Kinase reaction is stopped by chloroform extraction. This is done the same time as a 10:1 dilution to attain a 100nM concentration of oligos in both vials. 20uL of 100µM solution is added to 25uL of EDTA/Chloroform mixture and 155uL of water. Final solution is spun in a centrifuge for 2-3 minutes and set aside in ice until needed.
Reduction of Oligo Prep

10nM solution of Oligos by mixing 1µL Oligo with 9µL reducing agent solution. Incubate solution @RT for 2-3hrs

Pass samples through the BioRad Spin-6 column using the procedure outlined in the insert

Collect flow through and keep on ice till needed.

λ. DNA Preparation

λ. DNA (New England Biolabs) is linear, mailed suspended in a TE solution (10mM Tris-HCl, 1mM EDTA). A new solution is formed by mixing 2µL of λ DNA (~1ug) with 42µL of water, 1µL of Fast-AP** (Fermentas), and 5µL of enclosed buffer solution.

** Fast-AP is a form of alkaline phosphotase that cleaves the phosphate groups from the λ DNA.

This is used in the place of Shrimp Alkaline Phosphotase.

Incubate the mixture at 37°C for 10 minutes.

In order to stop the phosphotase reaction, heat the mixture in a water bath at 75°C for 5 minutes.

Set product aside on ice until needed.

DNA Attachment

Using a small-volume pipette, deposit a 2µL droplet*** of oligo A onto a section of the chip by hand, viewing the electrodes through an optical microscope for better reference. Soak chip for approximately 4-5 minutes. The sulfur groups on the functional ends of the oligos will selectively attach to the gold substrate, a technique used by research teams investigating self-assembling monolayers.

*** Note: Braun uses a droplet that is 10⁻⁴uL

Rinse chip with TE solution.

Deposit a 2µL droplet of oligo B onto an adjacent section of the chip (again, by hand). Soak chip for 4-5 minutes.

Rinse chip with TE solution.

Introduce 50uL of λ DNA solution onto the chip, approximately covering the region where the oligos are placed. Introduce bulk flow by pipette-suctioning the DNA perpendicular to the border between soak areas for oligos A and B.
Place chip in petri dish with MilliQ H2O or TE buffer solution and parafilm tightly. Place in 45°C oven for 4-8hrs. Remove Chip after 4-8Hrs. and wash thoroughly with MilliQ H2O or TE solution.

Chip Visualization
Prepare 2x SYBR Gold solution from 1000x stock solution (10mL total solution volume). Immerse Chip in SYBR Gold for 10mins then wash in MilliQ H2O for 10mins. Mount and visualize under fluorescent microscope.

[Appendix 7]

SYBR Gold Nucleic Acid Gel Stain

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount</th>
<th>Concentration</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYBR* Gold nucleic acid gel stain</td>
<td>500 µL</td>
<td>Solution in high-quality, anhydrous DMSO*</td>
<td>≤-20°C</td>
<td>When stored as directed, stain stock solution is stable for 6 months to 1 year.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Desiccate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protect from light</td>
<td></td>
</tr>
</tbody>
</table>

* DMSO stock solution is a 10,000X concentrate.

Number of labelings: Sufficient dye is provided to stain at least 100 agarose or polyacrylamide minigels.

Approximate fluorescence excitation/emission maxima: 300, 495/537 nm, bound to nucleic acid
ABSTRACT OF THE THESIS

The electrical conductivity of DNA remains to be controversial with various studies which report DNA properties varying from insulator to extreme conductivity (superconductive). This study investigates experimentally the electrical behavior and performance of a double-stranded Lambda DNA wire on two and three dimensional electrodes by suspending the DNA thereby eliminating the effect of substrate that is thought to be the culprit of inconsistent results. The two dimensional DNA platform design developed in this study helped to obtain an optimized three dimensional platform. The 3-D chip architecture is made of layers of a negative photoresist (SU8) and gold layered on silicon dioxide substrate. The DNA attachments on 3-D and 2-D electrodes were demonstrated based on the following methods: Oligo-DNA self assembly, electrostatic and, electrical field attractions. Electrical results based on I-V and R-V curves showed measurable and significant conductivity through the DNA wire that we believe could establish it as a semi-conductor. A mathematical model based on I-V data as well as an electrical circuit model for lambda DNA are also developed in this study. An equivalent electrical circuit was created in PSpice where DNA is modeled as a voltage-controlled current source. This is important because having models of DNA molecules in the form of equivalent electronic circuits would be useful in the design of nanoelectronic circuits and devices.

The research presented here is characterized by a significant departure from previous studies and made unique contributions by (i) DNA assembly on three dimensional structures which showed lower resistance and higher conductivity in comparison to 2-D or flat electrodes. (ii) 3-D DNA platform structure demonstrated better stability than 2-D structure. (iii) Additionally, these high aspect-ratio 3-D electrodes prevented the suspended DNA from contacting the substrate. This helps to collect more accurate resistance measurements. (iv) design of narrow and tapered electrode tips helped to guide and attract DNA electrostatically between two gold posts. (v) Furthermore, design of four electrodes in this study had an advantage that almost no current flowed in the sense wires, thus the voltage drop was extremely low. This allowed more precise measurements than traditional two sensing probes.
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