ELECTRICAL DETECTION OF DNA AND INTEGRATION WITH NANO-FLUIDIC CHANNELS

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A Dissertation
Presented to the Faculty of Princeton University in Candidacy for the Degree of Doctor of Philosophy

Recommended for Acceptance by the Department of Physics

September 2008
Abstract

The elongation of genomic length DNA in confining nanochannels is not only a fascinating exercise in polymer dynamics, but also is of great interest in biotechnology because the elongation of the confined molecule is directly proportional to the actual length of the molecule in basepairs. Precision length measurements of genomic length DNA molecules are useful because most of the mutations are not point mutations, but a rearrangement, insertion or deletion of a variety of lengths of segments of the genome within the genome itself. Conducting such measurements without an expensive optical microscope will be of great value for genomic analysis, and electrical measurements should provide the highest resolution. In this thesis, measurement methods using field-effect transistors (FETs) and electrochemical measurements are presented, and we demonstrate methods to integrate the electronic devices with the nano-fluidics.

Since DNA molecules are negatively charged in an aqueous solution, FETs can be used to detect the electric fields from the molecules. Our studies show that different characteristic I-V curves are seen when DNA molecules are present in the solution, for FET devices made of carbon nanotubes and poly-silicon film. We also demonstrate that some easy electrical measurements can be used to detect DNA or the bases of DNA by their electrochemical properties.

To integrate the electrical detection with the fluidics, we present two different methods to achieve that goal. A channel made with silicon oxide lift-off and anodic bonding can be used to integrate electronics with microchannels, and a self-sealed parylene capped nanochannel can be easily integrated with the electronics. The ability to integrate electronics with nanochannels can eventually lead to an all-integrated single-piece genomic diagnostic system.
Acknowledgements

First of all I would like to thank a former post-doctoral fellow in Austin group, now Prof. Robert Riehn at North Carolina State University. This work is impossible without the generous help from Robert. Teaching me how to do nanofabrication itself already puts him on top of this page, not to mention various help on DNA sample preparations, electronics, and so much more.

I would also like to thank all former and current Austin group members, including Dr. Shuang Fang Lim, Dr. Yang Mei Wang, Dr. Jason Puchalla, Dr. Peter Galajda, Dr. Walter Reisner, Keith Morton, Dr. David Inglis for technical helps and useful discussions.

This work heavily relies on the fabrication processing inside and out of a clean-room. I would like to thank Helena Gleskova, Mikael Gaevski, Joseph Palmer, Dr. Jian Huang of PRISM, Michael Skvarla of CNF, Dr. Magnus Bergkvist and Graham Kerslick of NBTC. I would also thank all of those who have been my cleanroom buddies, that includes but not limited to Kuen-Ting Shiu, Tzu-Ming Lu, and Joe Checkelsky.

We gathered a lot of technical help on the way. I would like to thank Dr. Fei Gong at Will Happer Group for the introduction and help on setting up the anodic bonding system. I would also like to thank David Hsieh at Zahid Hasan Group for the help on glove box, and Dr. Abhay Pasupathy of Ali Yazdani Group for the help on carbon nanotubes CVD. Also the assistance from Shu-Wen Teng of Nai Phuan Ong Group when both of our cameras were broken and up for repair.

Talking about useful discussion, I have to mention Dr. Yuan-Yu Jao of Will Happer Group and Tzu-Ming Lu of Daniel Tsui Group. Discussions with them have greatly benefited my work.

We cherish every collaboration opportunity, that includes the useful discussion about device fabrication with John Mannion of Harold Craighead group at Cornell
University; carbon nanotube samples from John Mannion, Lukas Urban in Ali Yazdani group, Chris Staii of A. T. Charlie Johnson’s group at University of Pennsylvania, and Olgicia Bakajin at Lawrence Livermore National Laboratory.

I would like to thank every member in my Prelims and General study groups. It was impossible to learn so much of material, such as general relativity and high energy physics, without the help from you guys. Finishing both big exams in my first year is one of my most memorable achievements at Princeton. Without that I could not start my research in my second year.

I have to eat to do the work at Princeton, therefore I would like to mention Mr. and Mrs. Wu at Tomo Sushi. I guess it is not all that common that a frequent customer becomes a personal friend? I really appreciate how they broaden my life experience and helped me on various things.

Hard to articulate what they actually did related to this dissertation, but my friends at and outside of Princeton, including a lot of them with Taiwanese root, helped me to maintain a basic sanity while facing the competitive science research world everyday. I thank all of you. Among them I would like to single out Dr. Chia-Han Lee for the supplies of acid-free paper for the bound copies for Mudd Library.

Last but not the least I would like to acknowledge my adviser, Prof. Bob Austin. This is an adviser not only helping you on your research, not just advise you on planning your career, never shy on giving you new ideas, he also advise you on things you do not expect from a physics professor, such as traffic laws. When that happens you know you can ask him about almost everything.
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Chapter 1

Introduction

The ultimate purpose behind this thesis is to employ nanotechnology to develop a method to significantly improve the efficiency of genomic analysis. With the finishing of the Human Genome Project (HGP) [1, 2], the mapping of human genes is completed, and scientists are now focusing on lowering the cost and time for sequencing, and developing efficient methods to study the genetic variations between individuals. There are many ways a genome can be different from one individual to another.

One of the best known genetic differences between individuals are single nucleotide polymorphisms (SNPs), which are the mutations of single bases. Approximately one in 900 basepairs of the human genome are SNPs [2], but that number has been accumulated through out the generations. The average rate for a point mutation to occur is one per $10^9$ per cell generation, and the average number of SNPs between parents and children is roughly 120. Since SNPs happen so rarely, they can be used to identify ancestral trees with very high confidence [2].

Another major type of genomic differences come from mobile segments of DNA sequences, called transposable elements, which can be deleted, copied and pasted, or cut and pasted to other locations in the genome. Such genomic changes will result in changes in the total genomic lengths, which SNPs do not. We will discuss this in
more detail in Section 2.1. Although sequencing is always a good way to study the genomic content, it is difficult for the current technology to study highly repetitive DNA segments [1], and some of the genomic variations due to the moving of the whole segments may go undetected. For that purpose, a precise measurement on the genomic length can be used as a screening tool complimentary to conventional sequencing methods.

Nanofabricated structures are of particular interest in the analysis of biological polymers, such as DNA molecules [3]. Single-molecule DNA analysis with nano structures could in principle analyze the genome from single cells. More than skipping the polymerase chain reaction (PCR), single-molecule studies avoid a lot of issues in ensemble measurements, for instance separating different DNA pieces in a solution [4]. If the technology permits, it will allow us to sequence a DNA molecule with just one scan. A fast and inexpensive method for genomic analysis is essential for studying genomic variances between individuals, and to understand how biology mutates its own genome to adopt the change in the environment.

Nanostructures can be used to analyze the length of DNA optically and electronically. They both have advantages and disadvantages, and we will discuss them respectively.

We first turn to the optical method. One way to study a single DNA molecule is to pull it into a long nanochannel. When the molecule enters the nanochannel, it is elongated due to the spatial confinement of the channel. The elongated molecule has been studied optically by staining the molecules with fluorescence dyes. For the single-molecule studies, the major limitation is the diffraction limit that sets the optical resolution at the scale of 100 nm. Despite that, a lot of progress has been made. The end-to-end length in a channel is proportional to the genomic or contour length, and the fluctuation of the end-to-end length can give us an accurate estimate on the genomic length that beats the diffraction limit of the optical imaging [5].
effects of channel width [6], ionic conditions [7] on the measured end-to-end length is now well understood, and the dynamics of recoiling has been well studied [8]. Other than the studies of the polymer physics, nanochannels with fluorescence microscopy are also useful biological applications. Nanochannels have been used to visualize the restriction enzyme cutting the DNA molecules, and to map the cutting site on the DNA [4]. It can also be used to study how the transcription factors find the binding sites on a DNA molecule [9]. More discussion about nanochannels can be found in Section 2.3. With all the accomplishments, nanochannels have proven to be an excellent tool to study single DNA molecule, but the requirement of a state-of-the-art fluorescence microscopy makes the application outside a research laboratory limited, plus the fluorescence labeling of DNA molecules may change the properties of the molecule. Besides, with the diffraction limit, it takes $10^5$ of repeated measurements to reach single basepair resolution of length measurement by the method laid out in Ref. [5].

Electrical diagnostics is a completely different story. It has been demonstrated that the translocation of a DNA molecule through a nanopore will cause an ionic current blockage through the pore, and the length of the blockage is related to the length of DNA [10]. Although with a promising start, nanopore technology is now facing two major hurdles. First of all, when a voltage is applied across the pore for electrophoresis, almost all of the voltage drop happens right across the pore, and that makes the durations for the translocation short. The translocation time is so short that it is nearly impossible to extract detailed information. Even if the translocation can be slowed down, although the DNA molecule is highly spatially confined inside the pore during the translocation, most of the molecule is still wobbling randomly on the both sides of the pore, thus the two big coils will keep pulling the part inside the pore in a random manner. For a 3 kilo-basepairs (kbp) single-stranded DNA (ssDNA) to translocate through a 4 nm pore, the translocation time has a distribution that
peaks at 170 $\mu$s, but in each individual event, the translocation time can vary from 100 $\mu$s to 350 $\mu$s [11]. A more thorough literature review on nanopore can be found in Subsection 2.2.2.

The problem of the wide distribution of the translocation time can be greatly reduced by further confining the DNA molecule spatially, by a nanochannel. By doing so, we automatically avoid the problems from the two big random coils on the sides by linearizing the molecule inside the channel. Further, the motion of the molecule can be controlled by the voltage across the channel. It has been shown that while there is no voltage driving the DNA, the thermal motion of DNA molecule is less than 3 $\mu$m in 40 seconds [8]. Comparing with the 10-100 $\mu$s translocation events in a nanopore, we can significantly lengthen the translocation duration. Therefore, building a device with electrical detection capability integrated with nanochannel opens the door to many opportunities in DNA analysis.

To achieve this goal, we have to overcome two major issues. First of all, we have to have a method being able to pick up electric signals from DNA molecules, and the electronic device has to be made in nanoscale. The literature review and theoretical discussion on the potential methods can be found in Section 2.2, and our work on potential sensing mechanisms is shown in Chapter 4. Another major issue is the integration of nano-electronics with nanochannels. However a fascinating idea, such an integration is actually a difficult task. Traditionally a nanochannel is etched into a fused silica wafer, then sealed from the top with another piece of fused silica or quartz. That means any electronic device on top of the wafer will break or deteriorate the sealing. In Chapter 5, two methods are presented for the integration of fluidics and electronics. In Section 5.1, a method based on silicon oxide lift-off is discussed. Although it produced some data indicating successful integration of micro-fluidics and nano electrodes, it was impossible to push the technique further down the scale for nanochannels. In Section 5.2, we introduce a method by using a sacrificial PMMA
layer to define the nanochannel underneath a parylene film, so the nano-fluidics can be essentially integrated with all kinds of surface electronics. We use simple metal wires to demonstrate the feasibility of the integration.

As it can be seen, this work heavily relies on nanofabrication. We discuss all the techniques in detail in Chapter 3.
Chapter 2

General background

2.1 DNA and genetic variations in biology

In this section, we will briefly review the biological background related to DNA. Since this thesis is about employing nanotechnology to perform DNA analysis, it is important to know what is really happening in the evolution dynamics and what kind of information is lacking in our current knowledge about analyzing genomes.

DNA, or deoxyribonucleic acid, is a nucleic acid which contains the genetic information for all the cellular organisms and some viruses [12]. The genomic information is coded by four different nitrogenous bases: adenine (A), thymine (T), cytosine (C), and guanine (G). Linked by a phosphate sugar backbone, the sequential appearance of the bases is called the DNA sequence. With the hydrogen bonds between A - T pairs and C-G pairs, two complimentary strands form a double-helix structure [13]. Each phosphate group carries a charge of $2e^-$. With the asymmetric structure of the sugar ribose, all five carbons can be easily labeled by a number, and the two phosphate groups are connected to 3’ and 5’ respectively, therefore a single DNA (or RNA) strand has two distinct ends, 3’ end (pronounced as three prime end) and 5’ end, therefore a sequence and the reverse sequence are not identical. The functions
of a lot of DNA sequences are not understood. For those we do know that they carry genetic information, they are transcribed into messenger RNAs (mRNAs), and the mRNAs are translated into proteins, which perform a lot of biological functions. Every three bases form a codon, which codes for an amino acid. For example, for an RNA codon AAA would be translated into a lysine. There are in principle 64 possible codons, and each of them represents one of 20 amino acids, or to start/stop the translation. Details of such processes can be found in a molecular biology textbook such as Ref. [12].

Conventional theory maintains that evolution happens as a random walk through adaptive fitness landscape, and the genetic change comes from random mutation sources, such as radiation, mutagen chemicals, or from inevitable errors in the replication processes [14]. Since those genomic “damages” are relatively localized, eventually they create a point mutation, where the base nucleotide at one locus is replaced into another nucleotide. Single nucleotide polymorphisms (SNPs) are known as the most frequent type of variation in the human genome occurring once every several hundred base pairs throughout the genome [15]. It is not necessary that a SNP would change anything at the end of the translation. A SNP may happen at where is not transcribed, or a change from CCG to CCC does not change the resulted proline, since they both code for the same amino acid. A SNP may result in changing one amino acid, for example a change from CCC to CGC makes a switch from proline to arginine. The most significant change a SNP can do to the result of translation is at the start and stop codons, so a protein may be missing, or a protein with many more amino acids is produced from the translation. Because SNPs are evolutionarily conserved, it can be used to identify polygenic contributors [16]. SNPs are also found to be related to the development of cancer [17]. There are various ways to detect a SNP (please see examples such as Ref. [16], [18], [19], [20]), but the only way to definitively identify if an individual harbors a genetic mutation is to sequence the
entire gene [21].

Although sequencing techniques do exist [22, 23], most of them are time consuming and labor intensive to sequence a genome, especially like those of human can easily take months or years and a lot of scientists to get sequenced [24, 25]. Different schemes for ultra fast single molecule sequencing have been proposed [10, 26], but none of them have provided any information at single base resolution. In short, if point mutations are the only type of genetic variations in biology, we either need a lot of resources to fully analyze the mutations, or it will take a major technology breakthrough that we are still waiting for.

On the other hand, since every biochemical reaction happening in a living cell is highly regulated by the organism itself, it is not hard to imagine that the genetic changes can be a process guided by the organism itself [14]. Although Barbara McClintock first discovered the natural genetic engineering mechanisms back in 1950 [27], so-called adaptive mutation, when the organism changes its own genome in response to the stress in the environment, has only gained attention in recent twenty years [28, 29]. It is now established that point mutations and adaptive mutations are independent mechanisms [29]. There are many different mechanisms for the organisms to create new DNA structures [30, 31]. Among them, transposable element (TE) is one of the most important system, which make up about 43% of human genome [24]. These TEs include the mobile “controlling elements” discovered by McClintock and they comprise integrated systems of proteins and nucleic acids that interact to mobilize DNA to new locations in the genome [14].

There are many different mechanisms for gene transposition. For example, a DNA sequence called a retrotransposon is first transcribed into RNA, and then is reverse transcribed into a single-stranded cDNA, then is made into a complimentary double stranded fragment, and then the fragment is inserted into the recipient gene [12, 32]. In other words, a segment of gene is first copied, and pasted at another location with
a reverse sequence. Or, for a DNA transposon, a segment of gene can be excised out of the original strand, be duplicated or not, then inserted into another location, i.e. “copy and paste” or “cut and paste” a segment of gene. The boundaries of a DNA transposon are defined by inverted repeats, i.e. the complimentary reverse sequence [12]. It is also possible that a segment of gene is simply deleted [28, 33]. Please note that all of the above scenarios result in a change in the total genome length. Even with the “cut and paste” scheme, the sequence of the target site is duplicated to facilitate the insertion, and there is a 5 or 9 base pairs of genomic length increase [34].

Another situation for genetic materials to be changed is called horizontal gene transfer (HGT). HGT includes all processes that transfer the gene to any cell that is not its offspring, including but not limited to those of other species [35]. In fact, HGT was first discovered in 1950s because different bacteria developed resistance to the same drugs, while the chance for de novo development in each lineage through point mutations is minimal [36]. HGT was first believed limited to prokaryotes (organisms without a cell nucleus, i.e. bacteria and archaea), but more and more examples in eukaryotes (everything with a nucleus, such as animals, plants, fungi, etc) have been later discovered [37, 38]. Hundreds of human genes appear likely to have resulted from horizontal transfer from bacteria at some point in the vertebrate lineage [24].

The existence of HGT has significantly altered scientists’ view of evolution, especially of Charles Darwin’s description of the evolution of species as the tree of life (i.e. a bifurcating diagram showing a common ancestor evolves into different species), since it blurs out the boundary of species [39]. Various transfer mechanisms, including those involving plasmids, phage DNA, or TEs, are responsible for HGT. Please find more details in Ref. [40], [41]. Although the actual transfer events are rarely directly observed (the best evidence for HGT is derived from molecular genetic analysis), sub-

1The original paper, Yamanaka et al, Hihon Iji Shimpor vol: 1861, 34 (1959), is in Japanese.
Substantial amounts of DNA are inserted (to the recipient) and deleted (from the donor) during the process [36].

One of the most common methods used in sequencing is Sanger’s chain termination method [22, 42]. The DNA piece to be sequenced is put in single-stranded form, and a DNA polymerase is used to fill in the complimentary sequence to form double stranded DNA. Unlike PCR, four standard dideoxynucleotides (dATP, dTTP, dCTP, dGTP) are added in the reaction solution separately as chain terminators. As a result, the polymerization is terminated at different lengths. After that, the lengths of the polymerized chain are determined by gel electrophoresis, with different terminators in different lanes. By resolving the length to single base precision, the sequence of the strand is therefore obtained. The whole process is demonstrated in Fig. 2.1.

Although a great technology, the Sanger method is difficult to apply when the
DNA is longer than 1,000 bps, since it is hard to resolve the DNA strands with one base difference in length when the total length is getting longer and longer. We can use restriction enzymes to cut out a piece of DNA from a specific gene, and only sequence that gene. But in most cases, if we would like to sequence a whole genome, this approach is difficult to work since the understanding of the whole genome mapping is not there. As a result, a “shotgun” method can be employed. Shotgun sequencing cuts the whole genomic length DNA into various random pieces, sequences each random pieces, then reconstruct the original sequence by analyzing the repetitive segments [43]. Human genome has been sequenced by this method [25]. As the work on the Human Genome Project has shown, using shotgun sequencing to sequence the whole human genome is possible, but it takes a lot of work to sequence a single human being’s genome, and the regions with tandemly repeats are still unresolved [1].

Other than the shotgun sequencing, there are other high through-put, parallelized sequencing technologies. Among them, the best know method is probably DNA microarray gene chips. The idea of gene chip sequencing is simple, and has been around since 1987 [44]. A gene chip consists of an array of spots of different DNA oligonucleotides probes, and the target ssDNA samples later are flushed onto the chip. If the sequences match, they hybridize and the targets are bound on the probes. With the target DNA pieces labeled by fluorephores, hybridization be easily be observed by a microscope. The usage of gene chips to do sequence related analysis has been successful, such as detecting SNPs [45]. However, they are more useful to analyze known genes, instead of sequencing completely unknown DNA. Part of the reasons is that the oligonucleotides on a gene chips are relatively short, are not efficient enough for obtaining information from methods like shotgun. And with the hybridization scheme, if a DNA sequence repeats itself, and for the above reasons we know it’s a very real possibility, it is impossible to spot such a repeat with a gene chip since it only shows the existence of such a segment, and has no power to demonstrate how
many copies are in there. Please be aware that sensing with hybridization is not a single-molecule mechanism. When the concentration of DNA is too low to yield a measurable signal, polymerase chain reaction (PCR) is used to duplicate the DNA strand in question. First denature a double stranded DNA into two single strands, DNA polymerases are used to synthesize the complimentary strand on the template. Once another denaturation step is applied, the newly synthesized strand is now a template for the DNA polymerase to produce new strands, therefore called chain reaction [46]. PCR has been the standard method for replicating DNA sequences.

## 2.2 Electrical signals from DNA molecules

In this section, I will first discuss the electrostatic effects from a DNA molecule in an electrolyte solution (Subsection 2.2.1), then briefly review various ways people have obtained electrical signals from DNA molecules, including a brief literature review on nanopores (Subsection 2.2.2), a quick review on using field effect transistors (FETs) (Subsection 2.2.3) and electrochemical measurements to detect DNA (Subsection 2.2.4).

### 2.2.1 Electrical potential in an electrolyte solution

Since our work is to obtain electric signals from an elongated DNA molecule in a nanochannel, and our DNA molecules are kept in buffer solution to prevent them from denaturation, it is useful to discuss how the electrical potential is created by such an arrangement.

The theoretical treatment of electrokinetic phenomena is based on the concept of the electrical double layer. When there is a solid-liquid interface, Helmholtz first considered the double layer as two oppositely charged layers at a fixed distance apart [47]. Later, Gouy and Chapman introduced the concept of a diffuse double layer, that
a finite thickness of the liquid at the interface creates a non-uniform distribution of positive and negative charged ions, although ions in that region are free to move. Stern later proposed a two-part double layer, combining Helmholtz and Gouy-Chapman theories. In Stern’s model, a thin layer of molecules, approximately as thick as the molecular diameter, is fixed on the interface, and a diffuse layer surrounds outside. The potential difference between the bulk solution and the boundary of the fixed / diffuse layer is called the zeta-potential [47], and the boundary is called the shear surface.

We now proceed to calculate the potential as a function of distance from a charged DNA molecule. For ease of calculation, we first assume that a DNA molecule is a long charged cylinder with radius $a = 1.2$ nm [48] (end effects are negligible here). This approximation surely does not accurately describe the real configuration of a DNA molecule elongated in a nanochannel, and we will discuss how the numbers we acquire from the following calculations can be of use later in this section.

The fundamentals of this calculation is Poisson equation

$$
\nabla \cdot [\epsilon(\vec{r}) \nabla \psi(\vec{r})] = -\frac{\rho(\vec{r})}{\epsilon_0}.
$$

(2.1)

In our system of interacting ions, the number density of an ionic species $\rho_i(\vec{r})$ may be expressed relative to the density of the same ionic species in the absence of interactions with other ions in the system $\rho_{i0}(\vec{r})$ by [49]

$$
\rho_i(\vec{r}) = \rho_{i0}(\vec{r}) \exp[-ze^2\psi(\vec{r})/k_BT],
$$

(2.2)

where $e$ is the protonic charge and $z_i$ is the valency of the ion $i$, $k_B$ is the Boltzmann constant, and $T$ is the temperature. With

$$
\rho_{i0} = c_i^\infty z_i \lambda(\vec{r})e,
$$

(2.3)
where \( c_i^\infty \) is the number density of the ion \( i \) in the bulk solution and \( \lambda(\vec{r}) \) describes the accessibility to ions at point \( \vec{r} \). Assuming the electrolyte solution naturally has a free charge distribution \( \rho^f(\vec{r}) \), we can write down the full Poisson-Boltzmann equation

\[
\nabla \cdot [\epsilon(\vec{r}) \nabla \psi(\vec{r})] = -\frac{1}{\epsilon_0} \left\{ \rho^f + \sum_i c_i^\infty z_i \lambda(\vec{r}) e \exp \left[ -\frac{z_i e \psi(\vec{r})}{k_B T} \right] \right\}. \tag{2.4}
\]

Assuming the potential is small, we can linearize the equation and get

\[
\nabla \cdot [\epsilon(\vec{r}) \nabla \psi(\vec{r})] = -\frac{1}{\epsilon_0} \left\{ \rho^f - \sum_i c_i^\infty z_i^2 e^2 \psi(\vec{r}) \lambda(\vec{r}) \frac{1}{k_B T} \right\}. \tag{2.5}
\]

We can define

\[
\kappa^2 = \frac{e^2 \sum_i c_i^\infty z_i^2}{\epsilon \epsilon_0 k_B T}, \tag{2.6}
\]

where \( \epsilon \) is the bulk dielectric constant. \( 1/\kappa \) is called the Debye length, which is the natural length scale of screening in an electrolyte solution.

We next further simplify the system into a simple salt solution, that is \( z_1 = 1 \) and \( z_2 = -1 \), \( \rho^f = 0 \), \( c_1^\infty = c_2^\infty \), \( \epsilon(\vec{r}) = \epsilon \), and \( \lambda = 1 \). That significantly reduces Eq. 2.4 into

\[
\nabla^2 \frac{e \psi}{k_B T} = \kappa^2 \sinh \frac{e \psi}{k_B T}. \tag{2.7}
\]

By defining the dimensionless potential \( y = e \psi/k_B T \) and the dimensionless distance \( x = \kappa r \), we can obtain the commonly seen Poisson-Boltzmann equation with cylindrical symmetry [50],

\[
1 \frac{d}{dx} \left( x \frac{dy}{dx} \right) = \sinh y. \tag{2.8}
\]

By employing the Poisson-Boltzmann relation, we ignore the interactions between small ions in the electrolyte solution. Since those interactions are at least one order of magnitude smaller than the interactions between ions and DNA [51], it should be a fine approximation.
Analytic solutions of Eq. 2.8 are generally not available. To obtain the asymptotic behavior, with $y << 1$, we apply Debye-Hückel approximation

$$\sinh y \approx y$$ (2.9)

and obtain the zeroth order modified Bessel equation

$$x^2 \frac{d^2 y}{dx^2} + x \frac{dy}{dx} - x^2 y = 0.$$ (2.10)

To ensure that the potential at infinity is zero, i.e. $(x, y) = (\infty, 0)$, the solution has to be the zeroth order modified Bessel function of the second kind [52]

$$y(DH) = CK_0(x),$$ (2.11)

where $C$ is a constant. One way to figure out the integration constant is to fit the electric field at $r = a$ by the field calculated from Gauss’ law

$$\left( \frac{dy(DH)}{dx} \right)_{x=ka} = -\frac{1}{2\pi\epsilon_0} \frac{Ze^2}{ak_BT}.$$ (2.12)

And from Eq. 2.11, we have

$$\left( \frac{dy(DK)}{dx} \right)_{x=ka} = -CK_1(ka) = -\frac{y_0(DH)K_1(ka)}{K_0(ka)}.$$ (2.13)

where $y_0(DH) = y(DH)(ka)$. Compared with Eq. 2.11, we have

$$y_0(DK) = CK_0(ka) = \frac{2\xi K_0(ka)}{ka K_1(ka)},$$ (2.14)

where

$$\xi = Ze^2/4\pi\epsilon_0 k_BTb,$$ (2.15)
Figure 2.2: Electrostatic potentials in an electrolyte solution under Debye-Hückel approximation. In the regular salt limit, an easily measurable voltage only happens within 10 nm of the DNA. As the salt concentration is reduced, Debye screening length gets longer and potential change at 20 nm might be measurable. \( \xi = 1 \) is the condition set forth by the condensation theory outlined by Manning.

where \( b \) is the average distance between the charged groups, therefore \( \xi \) is a parameter to describe the linear charge density. We then obtain the conventional Debye-Hückel potential [53]

\[
y(DH) = \frac{2\xi K_0(\kappa r)}{\kappa a K_1(\kappa a)}.
\]

(2.16)

The above result is proposed to be used to estimate the upper limit of the electrostatic contribution to the free energy change of DNA synthesis [54]. Plots of Eq. 2.16 are shown in Fig. 2.2.

Since the core of Debye-Hückel approximation is \( y << 1 \), it has to be noted that the result formula is more accurate when \( r >> 1/\kappa \), i.e. when the potential is already highly screened by the electrolyte solution.

When we get closer to the DNA strand, i.e. \( r \sim 1/\kappa \), the Debye-Hückel approx-
imation is no longer valid, and \( y(DK) \) no longer accurately describe the potential. The best way to calculate the electrostatic potential close to the electric double layer region, i.e. Gouy region or the diffuse layer, is to numerically solve the differential equation 2.8, and this approach is referred as Gouy-Chapman. For the numerical result

\[
y = \frac{2\xi}{\beta(\kappa a, y_0)} \frac{\gamma(x, y)}{\gamma(\kappa a, y_0)} \frac{K_0(x)}{K_1(\kappa a)},
\]

where \( \gamma \) is a correction parameter [55], whose number can be found in Ref. [50].

Zeta-potential (\( \zeta \)) is related to \( y_0 \) as

\[
y_0 = \frac{e\zeta}{k_B T}.
\]

Please note that the separation of Gouy and Debye-Hückel regions to describe the potential is more of a mathematical convenience (the validity of Debye-Hückel approximation) than a physical distinction. In fact in both regions the potential is described by Poisson-Boltzmann equation.

The next layer is the Stern layer, where the counterions condense on the DNA. To calculate the exact amount of charge bound on a DNA molecule is a tricky business. The thickness of Stern layer may be at the molecular scale, and the assumption of cylindrical symmetry is not an obvious assumption [51]. However, detailed studies show that a periodic but discrete charge distribution and a continuous charged cylinder yield very similar potentials from theoretical calculation [56]. In Gerald Manning’s condensation theory [57], in order to keep a statistical-mechanical phase integral

\[
I = \int_0^{\rho_0} \exp \left( -\frac{u(\rho)}{k_B T} \right) 2\pi \rho d\rho
\]

convergent, \( \xi \) has to be less then 1. For the physical interpretation, he argued that, when \( \xi \geq 1 \) (equivalent to charge density higher than or equal to \( 0.14e^-/\text{Å} \)), the
system is unstable, and counterions condense and neutralize the “real” charges (which is $2e^−/$bp, $0.6e^−$/Å, or $\xi = 4.3$. The charges are on the phosphate backbone so the sequence does not matter here), and the “effective” charge should be the real charges plus the condensed charges such that effective $\xi^* \sim 1$ [57]. As a result, the effective charge density has an upper limit of $0.14e^−$/Å. However, Stigter maintained that in lower limit of the integral 2.19 should be $\kappa a$ instead of 0, and with that the integral should not diverge, therefore $\xi$ does not have to be below 1 [55]. As unintuitive as it seems, the condensed ions can be in the diffusive Gouy region, not necessarily in the molecular thin Stern layer [55]. Later on, le Bret and Zimm suggested that the counterions do not condense on the charged cylinder, but are “confined” within a radius $R_M$ [53, 58]

$$R_M \approx (2a/\kappa)^{1/2} \exp[(\xi - 2)/2(\xi - 1)], \quad (2.20)$$

while $\kappa a \to 0$. This picture is distinctly different from Manning’s, although the number of confined counterions is the same as Manning’s condensed counterions [58].

Since then, numerical solution to Poisson-Boltzmann equation has become a better way to provide a more accurate picture, though Manning’s condensation theory can still be used as a way of approximation, with errors depending on the situations [58]. A recent molecular dynamics simulation shows that the amount of counterion charge within the Bjerrum layer is comparable to the predicted value by Manning’s theory, but this agreement is weakened since there is no well-defined distinction between condensed and noncondensed charges, so the precise value of condensed charge is impossible to obtain [59]. Bottom line is, Manning’s condensation theory provides an estimate on the distribution of counterions for the start, but it may not be accurate in all experimental systems.

We move back to the discussion of a DNA molecule elongated in a nanochannel. At 50 mM of buffer solution, the Debye length $1/\kappa \sim 1.36$ nm. Even at a low salt limit 1.3 mM, $1/\kappa \sim 8$ nm. Since our channel sizes are around 100 nm, if the DNA
molecule remains at the center or the far end of the channel, the electrostatic potential falls well in the Debye-Hückel range. Because of the thermal fluctuations, it is for sure the DNA molecule do not stay away from the potential sensor. When it goes closer or into the Gouy region, calculation from Eq. 2.16 is not accurate, but it only underestimates the real potential, that is the measured value should be higher than that calculated from Eq. 2.16. Since the thermal fluctuations bring the DNA strand closer and further from the sensing surface from time to time, with a longer time scale, the measurement is an average of the potential from the effective width of the DNA (less than 10 nm) to 100 nm. Referring to Fig. 2.2, although while the distance greater than 20 nm yields almost no potential difference, when the molecule gets really close to the measurement surface, the corresponding voltage can be in the 0.1 V range, which is not a really small voltage to be measured.

Note that the above discussion applies only to simple salt solution. For multivalent ions with $Z \geq 3$, it is actually possible that the condensation results in a charge inversion situation [60, 61].

2.2.2 Nanopore

The nanopore was first demonstrated as a tool for DNA analysis in 1996 [10]. At that time, a trans-membrane ion channel protein, *Staphylococcus aureus* $\alpha$-hemolysin, was used as the nanopore. $\alpha$-hemolysin is a protein produced by bacteria which causes the lysis of red blood cells *in vitro*. The total height of the protein is about 10 nm, with a 7 nm high cap plus rim structure, and a 5.2 nm stem [62]. The “pore” size at the opening at the cap is 2.6 nm [62], but it varies from 3.6 nm in the middle of the cap, 1.5 nm at the junction of cap and stem, to 2.2 nm at the opening on the stem [63]. Those proteins were placed on the lipid bilayer membrane, therefore the electrical conductivity could only happen through the channels. With that kind of pore size, only a single DNA or RNA strand can enter, and it has been shown that
the translocation causes a trans-membrane current blockade, and the length of the blockade is related, and believed to be proportional to the length of the molecules [10].

This opened up the whole field of nanopore DNA analysis. To further its argument as a candidate for single-molecule DNA sequencer, the translocation duration and current blockade was shown capable of revealing some molecular details about the translocating molecule. For example, the translocation of Poly(dA)$_{100}$ molecules resulted in a different statistics than Poly(dC)$_{100}$[64], and the direction of RNA translocation, i.e. 3' → 5' or 5' → 3' yielded different voltage drops [65].

With all the promising results, however, the statistics of the translocation of the same type of DNA molecules demonstrates a broad peak (the longer the DNA piece is, the broader the distribution [66]), or in some situations multiple peaks [10]. As a result, the translocation through an α-hemolysin can not be a real single-molecule DNA analysis, but still a statistical study of many molecules. At the same time, although the pore size of α-hemolysin is small, the length of the channel (the height of the molecule mentioned above) is long, therefore the current blockage being measured is the total effect of 10 nm long of DNA, not anything close to a single-base resolution. Besides, the shape of the channel further complicated the dynamics of translocation.

To improved this, it was believed that a thin, small artificial nanopore with no internal structure is the solution. In 2001, a 1.8 nm artificial nanopore with a tiny pore length was made by focus ion beam (FIB), and the corresponding translocation was studied [67]. Since then, DNA or RNA translocation through a nanopore has been extensively studied on various aspects, including new methods for fabricating the pores [68], theoretical study on the translocation [69, 70], control of the translocation speed [71] and so on. Although the polymer physics of DNA translocation through a nanopore has been an interesting scientific question, since then, more and more problems have been shown for using the nanopore as a tool of detail single molecule
analysis. For example, a DNA molecule can enter the pore with different “hernias” configurations [72], the broad distribution makes the pore as a single-molecule DNA scanner difficult, the short translocation duration generally makes it difficult to read into the details in the translocating DNA, the sizes differences within the two purines (A and G) and Pyrimidines (C and T) are not that much, and once a molecule has translocated through the pore, it was impossible to study the same molecule again.

People have tried various ways to overcome the challenges in nanopore. For example, to control the dynamics of the translocation, a bead can be attached to the DNA, and the motion of the molecule can therefore be controlled, and studied by the bead [73]. Viscous fluid has been used to slow down the translocation [71]. However, the fundamental problem is that, as the length of DNA piece goes to a real genomic length, the broad statistics of the translocation duration makes the result from a single scan useless. Since the broadening is caused by the entropic coiling of DNA outside the pore, unless with some dramatic change in design that stretches the DNA molecule, the basic physics of thermodynamics still prohibits nanopore to be a useful single-molecule DNA scanner.

2.2.3 Field effect transistor

Detecting DNA molecules with their intrinsic properties is a natural way to go. Because DNA molecules are highly negatively charged, and the presence of a negatively charged molecule can change the potential surrounding the molecule, as discussed in Subsection 2.2.1, the potential at the liquid / sensing electrode interface is consequently changed. By using the charges from DNA to gate a transistor, various kinds of FETs sensitive to small gate field changes are natural candidates for electronic detection.

Making the sizes of the semiconducting channel smaller can improve the sensitivity. For that purpose, people have tried to use semiconducting single-walled carbon
nanotubes (SWNTs) for such detection. SWNTs are naturally small, with diameters less than 10 nm. It was first demonstrated that we can use electrolyte to gate carbon nanotube transistors [74], and later it has been shown that the charges introduced by a lot of DNA molecules are enough to change the characteristic drain-source current [75], though the mechanism corresponding to the changes was later shown more related to the Schottky barrier at the metal-tube contact than the change of semiconducting properties of the tube itself [76]. The properties of SWNTs have been well documented. For example, functionalization of SWNTs for biomolecular recognition can be done with the adsorption of Triton on the carbon wall [77], and the effect of humidity on SWNT FETs is also known [78]. The biggest challenge for using single SWNT as sensing FET is that the chemical vapor deposition (CVD) steps used to grow SWNTs yield every tube differently, making the potential for a commercializable product nearly impossible. Statistically, one-third of the tubes are metallic, and two-third of them are semiconducting, and every semiconducting tube may have different band gap positions and widths.

To overcome the statistics, people have tried to use a CNT network for the sensing [79, 76]. In principle, by measuring a network, we average out the differences between each individual tube. On the other hand, by measuring a vast CNT network, the sensitivity to a small amount of molecules is lost. Our measurement results from a CNT network will be discussed in Section 4.3 and 5.1. Because a CNT network is not a spatially well defined device, in order to read into an potential details in the DNA molecules, a hybridization probe needs to be employed, and therefore this kind of devices inherits the problems of biochips: polymerase chain reaction (PCR) is required to duplicate the DNA strand when studying the genome from a single cell, and when a specific sequence repeats itself, which happens often for reasons described in Section 2.1, it is difficult to tell how many copies are actually in the genome, therefore losing a great deal of information from the genome.
Unlike CNTs, electrical properties of silicon nanowires (SiNWs) can be reproducibly controlled by dopant concentration and NW diameter [80]. By decorating the surfaces with peptide nucleic acid (PNA), it has been shown that SiNWs with an average diameter of 20 nm can distinguish wild-type from the ΔF508 mutation in the cystic fibrosis transmembrane receptor (CFTR) gene [81]. With the PNA sequence complementary to the wild type CFTR sequence, conductance of the p-type SiNWs showed a significant increase while 10 fM of the wild type DNA solution was added to the device. For comparison, only a small transient bump was observed while the mutant DNA is introduced, due to the nonspecific interaction of negatively charged oligonucleotides with NW sensors [81]. One of the big advantage of NWs to SWNTs is the reproducibility from different devices, since the electrical properties of the devices are well controlled [81]. As described above, however, detection with hybridization faces the same problems that gene chip has. But since NWs responded to 100 fM DNA solution through nonspecific interaction, using NWs as charge sensor in a nanochannel is a real possibility.

Using amorphous carbon derived from nano patterned negative electron beam resist for biosensing has been shown as an alternative possibility [82]. Since the nanostructures are fabricated by lithography, the carbon structures can be precisely controlled, which is a big advantage to SWNTs and SiNWs, but the properties and surface treatment methods are relatively less known. 40 nm platinum-silicide nanowires have also been used as FET to detect DNA by hybridization [83]. As amorphous carbon devices, platinum-silicide devices are less documented, and the surface treatment and properties are more of an unknown.

Other than using the fancy nanotechnology building blocks, an FET can be built with traditional silicon based fabrication processes. Using electrolyte as the metal gate in the metal-oxide-semiconductor (MOS) FET, it was shown that the drain current vs. drain-source voltage is different when 1.52 μM of DNA molecules is present

23
in the solution [84]. Devices based on MOSFETs and compatible with standard complimentary metal-oxide-semiconductor (CMOS) processes are seen as the best candidates for commercialization. They can be made by the same technology that makes our computers, and can have the best on-chip supporting circuitry to enhance the output signals. Since the conducting channel in a MOS device is formed by band bending, the thickness of the channel can be as small as Å scale [85]. The width of the channel depends on the fabrication processes and design, but generally speaking it is hard to beat the 20 nm limit currently set forth by e-beam lithography, which we will discuss in Subsection 3.1.3. It was shown that a MOSFET based sensing electrode made of standard CMOS processes can see free DNA solution in µM range, and be used to monitor the progress of PCR [86]. To achieve µM concentration of DNA, PCR is once again necessary. Since a MOS device senses only the charge, it is not clear how to tell different DNA strands apart, since the negative charges are distributed on the backbone instead of the bases. On the other hand, a very sensitive charge sensor can be used to detect whether there is any DNA strand in the close area, and combined with nanochannel it can be designed to perform DNA length measurement.

2.2.4 Electrochemical measurements

Other than the negative charges carried by DNA molecules, the electrochemical properties, such as red-ox properties, are another way to obtain signals. DNA can be detected by various chemical reactions. For example, guanine can reduce a Ru(bpy)$^{2+}$ into a Ru(bpy)$^{3+}$, and then the oxidation signals from Ru(bpy)$^{3+}$ can be picked up by the man-made electronics. A review on the possible chemical reactions lead to an electronic signals can be found in Ref. [87]. Here we only focus on the physics models of measurements where no complicated chemical mediators are involved, namely electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Those
two are more relevant to the purpose of this thesis because we employed measure-
ments very similar to EIS is Section 5.2, and CV is regarded as a way to provide us
information about the sequence content.

Before we start the discussion on individual measurement techniques, I would
briefly introduce some basics about electrochemistry. For an electrode immersed in
an electrolyte solution and no current is going in and out of the electrode, it achieves
an equilibrium potential difference between the electrode and the solution (\(\Delta \phi_e\)). And
in a circuit, when charges are moving in and out from the electrode, that potential
difference becomes \(\Delta \phi\). In electrochemistry, the potential that really matters in the
reaction is called the overpotential \(\Delta V = \Delta \phi - \Delta \phi_e\). It can be shown that, if the
rate limiting effect is the charge transfer between the solution and the electrode,
the current density at the cathode and the anode can be described by the famous
Butler-Volmer equation

\[
\begin{align*}
    i_{\text{cath}} &= i_0 \left[ e^{-\beta \Delta VF/RT} - e^{(1-\beta)\Delta VF/RT} \right] \\
    i_{\text{an}} &= i_0 \left[ e^{(1-\beta)\Delta VF/RT} - e^{-\beta \Delta VF/RT} \right],
\end{align*}
\]  

(2.21)

where \(F = 96,485 \text{ C mol}^{-1}\) is Faraday constant, and \(R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}\) is the
gas constant. An electrode is a cathode while \(\Delta V > 0\), an anode when \(\Delta V < 0\),
\(i_0\) is the exchange current density determined by the reaction rate constant, and
\(0 < \beta < 1\), the symmetry factor, is the ratio of the change of activation energy to
the change of electrical energy in the double layer [88]. This is important because all
electrochemical measurements involve current flow at some overpotential.

As its name suggests, electrochemical impedance spectroscopy is a measurement
of the ac impedance of the solution with different ac frequencies. There are various
components that contribute to the impedance, and we will discuss them one by one.
Historically, even before the systematic research on the impedance of the actual elec-
$Z_W$ is the Warburg impedance in Eq. 2.22, $C_{DL}$ is the capacitance of the double layer, $R_{CT}$ is the contact resistance, and $R_{sol}$ is the solution resistance.

trode / electrolyte solution interface began, Warburg published an analysis describing the impedance offered by the diffusive movement of ions under an ac field [88]. Warburg showed that the impedance has a phase angle of $45^\circ$ and is related to the ac frequency as $\omega^{-1/2}$, so that

$$Z_W = \sigma/\omega^{-1/2} - i\sigma/\omega^{-1/2}$$

(2.22)

with

$$\sigma = \frac{1}{2} \frac{RT}{(z_iF)^2} c_i \left( \frac{1}{D_i} \right)^{1/2}$$

(2.23)

where $D_i$ is the diffusion constant of the ion concerned, $c_i$ is its concentration, $z_i$ is its valency. At sufficiently high frequencies and concentration, the Warburg impedance becomes small compared with the other impedances of the electrode / solution interface [88].

Other than Warburg, each electrode should have three other impedance components: solution resistance ($R_{sol}$), interfacial contact resistance ($R_{CT}$), and the capacitance of the double layer ($C_{CT}$). The effective circuit diagram is shown in Fig. 2.3 [89]. When the amplitude of the ac voltage is small (e.g. 5 mV), the contact resistance
can be considered as [88]

\[ R_{CT} = \frac{0.025}{i_0} \text{ohms cm}^{-2}, \]  

(2.24)

where \( i_0 \) is the exchange current density in A cm\(^{-2} \). This is an empirical relation, not a derivation from any first principle.

From the Gouy-Chapman theory, the capacitance of double layer should be given by [90]

\[ C_{DL,GC} = \varepsilon \varepsilon_0 \kappa. \]  

(2.25)

There are various models to get a more accurate capacitance. For example, with a method called mean spherical approximation (MSA) [90],

\[ C_{DL,MSA} = 2 \varepsilon \varepsilon_0 \Gamma, \]  

(2.26)

with

\[ 2\Gamma(1 + \Gamma d) = \kappa. \]  

(2.27)

where \( d \) is the ionic diameter. Generally speaking, for a solution with ionic strength such that \( 1/\kappa = 2 \text{ nm} \), \( C_{DL} \approx 34.5 \mu\text{Fcm}^{-2} \).

With a charged particle smaller than \( 1/\kappa \), it can be shown that the mobility is given by [91]

\[ \mu \equiv \frac{v}{E} = \frac{\varepsilon \varepsilon_0 \zeta}{\eta} \]  

(2.28)

where \( v \) is the velocity of the particle, \( E \) the applied electric field, \( \eta \) the viscosity of the solution, and \( \zeta \) is the zeta-potential described in Subsection 2.2.1. \( R_{sol} \) can be therefore obtained through resistivity

\[ \rho_{sol} = \frac{1}{zc\mu} \]  

(2.29)

where \( c \) is the concentration of the particles and \( z \) is the charges it carries in the unit
of $e$. The results of the impedance spectroscopy are usually plotted as $|Z|$ vs. $\omega$, $\text{Re}[Z]$ vs. $\omega$, $\text{Im}[Z]$ vs. $\omega$, or $-\text{Im}[Z]$ vs. $\text{Re}[Z]$, while the last one is usually referred as Nyquist or Cole-Cole plot.

Although it appears as a simple system, the result of EIS measurements is not always trivial. For electrolyte as simple as KCl or CO$_2$ in water, completely different $Z-\omega$ curves are found [92]. EIS has been used to study the conductivity change when Zn$^{2+}$ ions are bound onto DNA molecules [93], and can be used to detect DNA hybridization [89].

In our application, when a DNA molecule is elongated in a nanochannel, both the $C_{DL}$ and $R_{sol}$ are changed. For a 150 nm long 100 nm x 100 nm channel, the volume is only $1.5 \times 10^{-18}$ L. For a 60% extension of the DNA molecule [6], there are roughly 735 bps of DNA in that volume, equivalent to 1470 $e^-$. For comparison, for a 10 mM saline solution, who has around 9000 ions in the same volume, that is more than 16% of difference on the number of charged sites, corresponding to a 7% change in the Debye length, which surely changes $C_{DL}$ accordingly.

Cyclic voltammetry is more like a DC I-V curve measurements, with a difference that the voltage is sweeping back to the original starting voltage [88]. The purpose of cyclic voltammetry is to oxidize / reduce chemicals at the electrodes, and observe the charge transfer from the chemical reactions. To avoid hydrogen evolution from hydrolysis, voltage across the electrodes should be kept below 1.5 V. The voltage sweeping rate is also critical for a successful measurement. When the sweeping rate is too fast, we simply charge and discharge the double layer capacitance; when the rate is too slow, the convection overtakes diffusion as the main driving force that moves the chemical species in the solution and makes the interpretation of the results difficult.

In a typical cyclic voltammogram, when the voltage is sweeping toward the positive, a peak due to the reduction of the chemical species is observed. As soon as
the potential on the electrode is high enough so that $\Delta V > 0$, the electrode becomes an anode, the electrons are transferred from the solution to the electrode, and the current from the reduction occurs. When $\Delta V > RT/\beta F$, it grows exponentially with the increasing voltage as indicated by Eq. 2.21. As the voltage goes higher, diffusion becomes important since a significant amount of chemicals around the electrodes are already reduced, and the current now starts to be affected by the diffusion rate of the chemical species. The current peaks when diffusion starts to play a dominant role in limiting the reaction rate. As the voltage goes even higher, the chemicals being reduced are depleted close to the electrodes, therefore the rate is completely limited by the diffusion, and the current reaches a limited current set forth by the diffusion. As a result, the voltage when the current starts to exponentially grow reflects the equilibrium potential $\Delta \phi_e$, and the peak position is related to $\Delta \phi_e$ and the concentration at the same time. The dip on the reverse sweep happens for the same reasons, but the chemical reaction is now oxidization instead of reduction. Since different chemical species have different $\Delta \phi_e$, the exponential growth of current happens at different voltages, and resulting in different peak positions, therefore we can tell the chemical components apart by peak positions.

For DNA, it has been shown that the bases adenine and guanine show peaks at different voltages [94]. As for the diffusion limited current density $i_L$, it has been shown that a microelectrode with a size smaller than 50 $\mu$m can have a much higher $i_L$ at the same concentration [95], therefore a miniaturized electrode can be used to carry out the measurements with much lower concentrations. As a standard electrochemical method, CV has been used to acquire signals from DNA. It has been shown that a microfabricated electrode combined with foundry-made CMOS circuitry can be used to perform CV measurements on DNA [96]. When the concentration is low, the modification of a glassy carbon electrode with multi-walled carbon nanotubes can be used to significantly enhance the CV signals [97].
In a typical CV measurement of guanine or adenine, the concentration of the bases is in the range of 10-100 µM [94, 97]. For a single-molecule confined in a nanochannel described above, due to the small volume of the channel, the average concentration of the bases is 4.4 mM, orders of magnitude higher than the concentration necessary for the conventional bulk measurements.

2.3 DNA analysis by a nanochannel

Because of the entropic term of free energy, when no external force is acting upon it, a DNA molecule tends to stay as a random coil in free solution. In the 1990s, scientists started to use a magnetic tweezer [98] or an optical tweezer combined with hydrodynamic flow [99] to stretch DNA molecules and study the elasticity of the molecule as a model polymer chain. Years later, combined with electric field, nanofabricated structures were used to, again stretch the DNA molecules, and to study the elasticity behavior of re-coiling [100]. With a properly designed nano structure, DNA molecules can be stretched only by the spatial confinement [101]. For a period of time, the polymer physics of single-molecule DNA experiments was almost equal to stretching the molecules and seeing it re-coil. The elasticity of single DNA molecule has been one of the most studied properties in the laboratory [8].

Unlike stretching DNA molecules with beads, hydrodynamic flow, or electric field, elongation due to spatial confinement does not maintain a tension along the DNA backbone at all time, which lowers the possibility of breaking the molecule, and appears as a better candidate for any application that requires an elongated DNA molecule. To elongate DNA into a linear configuration, it is natural to pull DNA into a very narrow one-dimensional channel, so the molecule is spatially confined in two directions, can only extend in the channel direction [102].

The extension of DNA molecule in a nano channel can be understood in two
Figure 2.4: Confined polymers in de Gennes ($D >> P$, upper case) and Odijk ($D >> P$, lower case) regimes. In de Gennes regime, the molecule can be sub-divided equally into a series of blobs with contour length $L_b$, the stretch arises from the mutual repulsion of the blobs. In Odijk regime, the molecule undergoes a series of deflections with the wall.

Different regimes, as shown in Fig. 2.4. When the channel width ($D$) is much greater than the persistence length of DNA ($P$), the DNA molecule is free to coil inside the channel, but the chain has to keep away from going through itself, i.e. self-exclusion. Self-avoidance is a property of the DNA molecule, and is not related to the channel width. However, the spatial confinement enhances the effects from self-avoiding polymer. In free space, the effects are relatively small because the exclusion volume is so small compared with the total available volume for the DNA chain. As the space gets tighter, the self-exclusion volume occupies a larger portion of the total available volume, and the effects become more observable.

De Gennes developed the classic model of a confined self-avoiding polymer [103]. He considered the confined polymer chain as a series of blobs, as shown in Fig. 2.4. Contour length of the polymer is uniformly distributed into each blob, and the self-exclusion makes the blobs repel one another as hard spheres. Assuming the Flory-Pincus scaling, de Gennes showed that

$$ r \cong \frac{L (w_{eff}P)^{1/3}}{D^{2/3}}, \quad (2.30) $$
where \( r \) is the extension length, \( L \) is the contour length of the molecule, \( w_{\text{eff}} \) is the effective width of the molecule given by \([7]\)

\[
w_{\text{eff}} = \frac{1}{\kappa} \left[ 0.7704 + \log \left( \frac{\xi^2}{2\epsilon\epsilon_0 k_B T \kappa} \right) \right].
\] (2.31)

On the other hand, when the channel width is smaller than the persistence length of the molecule, the DNA chain cannot be folded back, and is bent only at the channel walls, leaving each DNA segment with length \( \lambda \) and an angle between the wall \( \theta \), as shown in Fig. 2.4. Odijk showed that \([104]\)

\[
\lambda^3 \approx D^2 P,
\] (2.32)

and therefore

\[
r = L \cos(\theta) \approx L \left[ 1 - \left( \frac{D}{\lambda} \right)^2 \right] = L \left[ 1 - A \left( \frac{D}{P} \right)^{2/3} \right],
\] (2.33)

where \( A \approx 0.361 \) \([104]\).

The scaling laws between extension length and the contour length have been experimentally studied and verified \([6]\). Since in both de Gennes and Odijk regimes the extension length is proportional to the contour length, it is obvious that nanochannel can be used to analyze the genomic length of DNA molecules.

If the extension of DNA in a nanochannel is fixed, when we try to obtain the genomic length of a DNA piece by measuring the extension optically, that resolution is limited by the diffraction limit. However, in reality, the extension of DNA is fluctuating inside a nanochannel. From the de Gennes theory, we can obtain that the fluctuation of extension end-to-end length is \([5]\)

\[
\sigma_r^2 = \langle \Delta r^2 \rangle = \frac{4L}{15} \left( P w_{\text{eff}} D \right)
\] (2.34)
which gives us an uncertainty falls into the order of couple microns. Originally introduced as an error on the length measurement, however, opened up another opportunity. Since the fluctuation can be observed optically, we can now do multiple repetitive measurements of the same molecule to reduce the standard deviation of the mean. The uncertainty of the length measurement is now

\[ \delta r = \frac{\sigma_r}{\sqrt{N}}, \]  

where \( N \) is the number of measurements. It has been shown that with one minute of observation, we can achieve \( \pm 400 \) bps of accuracy, equivalent to \( \pm 136 \) nm, which is way beyond diffraction limit [5]. In principle we can carry out the measurement with unlimited number of frames to improve the resolution to single base pair.

The polymer physics of DNA in a nanochannel has been extensively studied. The elongation of DNA can come from not only spatial confinement, but also hydrodynamic flows [105]. The influence of ionic strength on the extension has been measured experimentally and compared with the theoretical results [7]. The relaxation of a compressed DNA molecule inside a nanochannel was studied and modeled [106], and the effects of friction between DNA molecule and nanochannel sidewalls were also studied [8]. On the engineering side, various strategies have been used to fabricate a smaller nanochannel. Although a combination of nanoimprint lithography (NIL) and SiO\(_2\) sputtering can achieve channel width in 20 nm range [102], the smoothness of the channel sidewall emerged as a major problem since a couple nanometers of roughness now has a big influence on the local channel widths. Recently it was shown that atomically smooth channels with sub-20-nm width can be made with NIL and anisotropic etching along crystalline planes [107]. For biological applications, other than measuring the genomic lengths of DNA, restriction enzyme cutting a DNA molecule has been visualized in a nanochannel. Since the length of the cut DNA can be measured
by a nanochannel, this is a single-molecule restriction mapping technique [4]. With the advantage of visualizing an elongated DNA molecule, nanochannel was also used to study the dynamics of protein-DNA interaction [9].
Chapter 3

Methods and techniques

In this Chapter we will discuss the technical details related to this thesis. In section 3.1, we will discuss a wide range of issues related to device fabrication, included the choice of wafers, different lithography strategies, nano electrodes building blocks, wafer bonding techniques and so on. We will discuss our lock-in amplifier based electronic measurement schemes in Section 3.2, preparation of various DNA samples in Section 3.4, and set-up for fluorescence microscopy in 3.3.

3.1 Device fabrication basics

3.1.1 Wafer

Both fused silica wafers and silicon wafers were used in this work. Quartz and fused silica wafers are known for its low auto fluorescence, therefore are ideal substrates for optical analysis. Their insulating properties make them candidates for electrical applications, but the piezoelectricity makes the fabrication of nano electronics on quartz wafers a tough job. A small localized stress applied on the substrate, such as those caused by vibration in the carrying box, may cause enough voltage to induce a current burning a metal nano line. Charging on both fused silica and quartz wafers
makes e-beam lithography more difficult. Static charges are another major problem, that blow drying the chip may be enough to ruin the nano electrodes.

On the other hand, silicon wafers have been widely used in the semiconductor industry, and are known as ideal candidates for electronic applications. Its non-transparency means we have to perform optical imaging on the same side as the electrical contacts, although not a pleasant fact, does not forbid the possibility of optical and electrical detection at the same time. Since doped silicon wafers are usually used, they are by themselves conducting substrates. Another important property about silicon wafers is the ability to cleave the wafers. With the (100) wafers we used\textsuperscript{1}, it can be easily cleaved along (010) and (001) planes into smaller rectangular dice. The direction of the cleavage planes are indicated by a primary flat on the circular wafer from the manufacturers. For surface electronics, it is more than common that a furnace CVD step is used to grow silicon oxide on top of the wafer. Since it is a high temperature process (usually involve a furnace or oven heated to around 1000°C), and growing a uniform layer of thermal oxide requires very clean silicon surface, a standard RCA clean\textsuperscript{2} is required before the furnace process. All Silicon wafers we used in this work were grown with a 100 nm thick dry thermal oxide by MRL Industries Furnace at Cornell NanoScale Science and Technology Facility (CNF). Per CNF rules, wafers have to be RCA cleaned within six hours before loading into the furnace. Loading and unloading are typically operated under 800°C. The furnace is first flushed with nitrogen (N\textsubscript{2}), then ramped to the targeted growing temperature. Once the furnace reaches the growing temperature, oxygen (O\textsubscript{2}) combined with N\textsubscript{2} starts to flow to grow the dry oxide.

The thickness and growing process can be understood with Deal-Grove model

\textsuperscript{1}The top polished surface has a Miller index (100)
\textsuperscript{2}RCA clean includes the first Standard Clean (SC-1) with ammonium hydroxide (NH\textsubscript{4}OH) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) for organic clean and the second Standard Clean (SC-2) with hydrogen chloride (HCl) and H\textsubscript{2}O\textsubscript{2} for ionic clean. Both steps are performed in a heated bath, 75-80°C. If the accuracy of oxide thickness is required, another room-temperature hydrofluoric acid (HF) dip should be added in between.
The model has following assumptions on the transport of the species of oxidant (which is \(O_2\) for dry oxide): (a) it diffuses from the bulk (concentration \(C_0\)) of the oxidizing gas to the outer surface of the wafer (concentration \(C^*\)) with a transport coefficient \(h\) (so the flux can be written as \(F_a = h(C^* - C_0)\)); (b) it diffuses across the existing oxide film toward the silicon with effective diffusion constant \(D\) (flux is given from Frick’s law \(F_b = -DdC/dx\)); (c) it reacts at the silicon / existing oxide interface and forms a new layer of \(SiO_2\) with a rate constant \(k\) (flux is given from the reaction rate equation \(F_c = kC_i\)). By assuming the steady state condition \((F_a = F_b = F_c)\), the flux of oxidant can be written as

\[
F = F_a = F_b = F_c = \frac{kC^*}{1 + k/h + kx_0/D_{eff}},
\]

therefore the rate of growth is

\[
\frac{dx_{ox}}{dt} = \frac{kC^*/N_1}{1 + k/h + kx_0/D_{eff}},
\]

where \(N_1\) is the number of the oxidant molecules needed in a unit volume of oxide. We can then solve the differential equation to get the thickness of the oxide

\[
x_{ox} = \frac{A}{2} \left(1 + \frac{t + \tau}{A^2/4B} \right)^{1/2} - 1,
\]

where

\[
A \equiv 2D(1/k + 1/h),
\]

\[
B \equiv 2DC^*/N_1,
\]

\[
\tau \equiv (x_i^2 + Ax_i)/B,
\]

where \(x_i\) is the initial thickness of the oxide layer.
After the growing, the temperature of the furnace chamber is cooled down to the unloading temperature with N\textsubscript{2} atmosphere. Hydrogen (H\textsubscript{2}) gas can be added in the growing step to accelerate the growth, and such grown oxide is called wet oxide. Although wet oxide has much higher growing rate, fast oxidation forms more dangling bonds on the silicon / oxide interface, which allow current to leak along the interface. The dielectric strength of wet oxide is also lower than that of dry oxide, which means as gate oxide, dry oxide can hold a higher voltage, and it is easier to break down a wet oxide. As a result we chose dry oxide as our gate oxide material.

We also used LPCVD Polysilicon Furnace at CNF to grow polycrystalline silicon for the device mentioned in Section 4.2. Running the poly-Si CVD process is similar to growing oxide, and an RCA cleaning is required before the furnace step. The temperature is not as high as oxide growing, usually between 450 and 670 °C. For growing an undoped poly-Si film, silane (SiH\textsubscript{4}) is used as the processing gas. Since silane is a hazardous gas, the furnace chamber is operated under vacuum.

### 3.1.2 Photolithography

Since the 1960s, photolithography has been used in the production of integrated circuits and other micro features. In photolithography, we first produce the wanted patterns onto a binary intensity amplitude mask, which is transparent to UV light in our designated area and opaque in others. In our practice, we start with a blank 5” chrome mask, with a layer of positive photoresist and chromium on top of Pyrex glass. Photoresist is a kind of light-sensitive material whose solubility can be changed by the exposure of UV light. Pyrex glass is used here because its thermal expansion coefficient is similar to that of silicon, so that enables us to work on silicon based substrates (including oxidated silicon wafers, not including quartz and fused silica wafers) at different temperatures at different steps. This is especially important for overlay exposure, when alignment of the structures on the mask to the structures on
the wafer is necessary. If the mask and the wafer have distinct thermal expansion coefficients, not only scaling errors may happen, it is impossible to align the patterns. This is particularly important for us since CNF cleanroom is kept at 17°C, and PRISM cleanroom is kept at 19-21.5°C among different rooms. We use GCA/MANN 3600F Optical Pattern Generator at Cornell to expose the resist, providing us feature size from 2 µm to 1.5 mm in 0.5 µm increments, and the positional accuracy of exposed images is 0.6 µm. After the exposure, the photoresist is developed, and the exposed area is dissolved. We thereafter perform a chrome etch to remove the chromium layer under the exposed area, while chromium in the unexposed area is protected by the resist. After the chrome etch, remaining photoresist is stripped in a hot solvent bath.

We employ contact lithography to transfer the pattern onto the wafers. Although some theoretical studies showed the possibility of λ/20 (or ~20 nm) resolution with contact lithography [109], with the nonflatness of the masks, wafers, unevenness of the photoresist thickness, dusts and other contaminants, it is almost impossible for contact lithography to reach the near-field optics limit of using only evanescent wave to expose the photoresist. As a result, in real applications, contact lithography can practically achieve resolution in the range of 1-2 µm. 1 and 2 µm wide lines and 2 µm wide gap are shown in Fig. 3.1.

During the process, we first spin coat photoresist on the wafer. Since the viscosity of the resist is carefully tuned by the manufacturer, the thickness of the resist film is controlled by the spin speed. The resist we used, AZ 5214E (Clariant), forms a 1.4 µm thick film while spun at 4000 rpm for 40 seconds. After spin coating, we need to perform a “soft bake” to remove the solvent (which is methoxy-propyl acetate, or PGMEA, for AZ 5214E), so the resist becomes solid and does not stick onto the mask during the contact lithography. AZ 5214E is sensitive to 310 - 420 nm of UV exposure, and our aligner (Karl Suss MA6 at PRISM) provides 365 nm line at 2.0 mW/cm² (CI1) and 410 nm line at 3.5 mW/cm² (CI2). To load the sample on the aligner, the
wafer is first brought to contact with the mask, then the sample is lowered by 20 - 40 μm so the wafer can move relative to the mask. For the first layer exposure, attention has to be paid to ensure that the primary flat is either parallel or perpendicular to the dice boundaries, so when we cleave the wafer the cleavage will happen along the designed boundary lines. For overlay exposure, we have to move and rotate the wafer such that the two alignment marks on the wafer is right under the two corresponding marks on the mask. Once the wafer is properly positioned, it is once again brought into contact with the wafer. The wafer is then exposed with UV source described above through a photo mask, therefore only the area not under the chromium on the mask (where chromium has been etched away) is exposed. Since AZ 5214E is a positive-toned resist (unless gone through image-reversal processes described later in this Subsection), the exposed area becomes soluble in a basic solution, or developer (AZ 312-MIF diluted with 1:1 de-ionized (DI) water or AZ 300-MIF, Clariant).

For contact lithography, the chrome side of the mask is or almost is in contact with the photoresist spun on the wafer. There are four contact modes for exposure,
proximity, soft, hard, and vacuum. Proximity contact leaves a small gap between the wafer and the mask, which does the least damage to the mask, but features smaller than 10 µm is hardly resolved. Since chrome masks are quite robust and we typically have features smaller than 10 µm, this contact mode is never used\textsuperscript{3}. For soft contact, the wafer holder brings the wafer up until it pushes the mask with certain pressure (0.2 bar as the recommended setting at PRISM). The position of the wafer holder and mask remains the same for hard contact, but an air flow is used to bring the wafer closer to the mask during the exposure. In this way the features whose sizes are about single micron can be resolved. For Vacuum contact, the space between the wafer and the mask is evacuated to bring them into really intimate contact. Since the wafer holder for vacuum contact does not support pieces, this contact mode was also never used in our applications.

The exposed wafer is then developed by the developer described above. After the development, the exposed area is dissolved. After that, the pattern on photoresist is transferred onto the wafer by either etching or lift-off. A “hard bake” to harden the resist follows if the wafer is going to be etched afterward.

When photoresist is used as an etch mask, only the portion of the wafer not covered by resist is etched. We etch our wafers for alignment marks. To do that, we employed Reactive Ion Etching (RIE, by Plasma-Therm 790 at PRISM), with fluorine plasma, CF$_4$ for thermal oxide and SF$_6$ for Si. We also use oxygen plasma to pattern parylene. During the parylene etching, a short HF (1:10 Buffered Oxide Etchant, BOE) dip of wet etching is also used to remove the adjacent evaporated SiO$_x$ layer. In general RIE is more directional than wet etching, so RIE is used when we need to etch a thicker film. RIE may etch photoresist and our targeted film at the same time. For example, the etch rate of parylene C to photoresist is about 1:1. In that case

\textsuperscript{3}although staffs at CNF recommend people to use proximity exposure to protect masks, we never observed any permanent mask damage that is a result of our choice of hard exposure mode. On the other hand, staffs at PRISM advise users to avoid proximity exposure due to the worse resolution.

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case, we need to put a thicker resist (e.g. AZ P4330-RS provides a 3.3 \( \mu m \) film at 4000 rpm) at the beginning of the lithography process, i.e. to choose a resist which is itself thicker, and coat it with a less spinning speed.

Lift-off is commonly used when patterning metals. In our case, 5 nm of adhesion Chromium layer and 95 nm of Au were used for micro-electrodes, and 1 nm of iron as CNT catalyst. In the process of lift-off, we first pattern the photoresist, then evaporate (Denton/DV-502A E-Beam Evaporator at PRISM, CVC 4500 Evaporator at CNF, or the thermal evaporator at 125 Jadwin) the desired metal on top of it, so for the exposed area the metal contacts the wafer directly, and there is resist between metal and wafer in the unexposed area. After the evaporation, we remove the photoresist in a heated solvent bath, and therefore the metal on top of the resist is then removed.

To implement the concept, we need to make sure the following details of the process works. First of all, for the exposed area, the wafer has to be clean, i.e. no residual resist on the wafer, otherwise the metal might be removed since the residual resist is dissolved when we remove the unexposed resist and unwanted metal. The residual resist can be easily removed by a short oxygen plasma RIE, and Microposit Remover 1165 (MicroChem Corp.) is used to dissolve the unexposed resist and remove the unwanted metal. Usage of 1165 can effective prevent the re-deposit of the metal after the resist is dissolved. Then, as an empirical rule, we have to make sure that the thickness of the metal film is less then 1/3 of the thickness of the resist, otherwise the metal may not come off clean as the resist is removed.

To improve the quality of the lift-off edge, we can adapt either image-reversal or lift-off resist (LOR) strategy. With the attenuation of UV in photoresist, the development of the resist forms an overcut feature, as shown in Fig. 3.2. As a result, the coated metal tends to form a continuous film. By image-reversal, we reverse the sign of the photoresist before development, i.e. the originally presumed soluble resist
Figure 3.2: Different photolithography strategies for lift-off. The regular one is the easiest to do, but the over-cut resist profile deteriorates the lift-off quality at the edge. Both image-reversal and LOR can create a good under-cut for lift-off, but need different sign of masks. For image-reversal, after the first exposure, a reverse bake is used to reverse the sign of the exposed resist (i.e. soluble becomes insoluble), then a flood exposure is taken to make the rest of the resist soluble.
now stays, therefore we convert the overcut profile into an undercut one, thus the evaporated film is not continuous, and therefore smoother at the the lift-off edge. To do image-reversal we need a mask with the opposite sign. Lift-off resist (LOR) is a kind of polymer that itself is not photosensitive, but soluble in the basic developer, and dissolves faster than the regular photoresist. Once the top overcut photoresist is dissolved, LOR is quickly dissolved, and therefore forms an undercut. Both image-reversal and LOR can easily be used to make nice and fine metal features.

3.1.3 E-beam lithography (EBL)

In the semiconductor industry, photolithography is almost the only way for patterning. With state-of-the-art double patterning technology, Intel and other companies have commercialized the 45 nm manufacturing, but such infrastructure is usually too expensive for academic research, and may not be flexible enough to work on various kinds of pattern designs. In this work, all the sub-micron lithographical features are made by e-beam lithography (EBL), including $\sim 30$ nm wide metal lines and 100 nm wide nanochannels.

Unlike photolithography, the resolution limit of EBL is nowhere close to, or even beats the diffraction limit of the electron beam, whose de Broglie wavelength is $\sim 0.12$ Å for a 10kV beam. Although focusing an electron beam is very different from the light beam, and is usually not as good, electronic optics can usually achieve a 1 Å resolution. Instead, the resolution is decided by the following factors: (a) Coulomb interaction between electrons and the resist is not limited to the e-beam focal point, therefore resist close by but not right at the focal spot can be also exposed; (b) exposure from the secondary electrons; (c) the molecular structure of the resist; (d) the molecular dynamics of the development process [110]. The contribution of each individual factor is hard to evaluate, but for the best-tuned EBL system overall the limit for the resolution on PMMA is in the range of 12-15 nm [110].
Similar to photolithography, we first use a highly focused electron beam to “expose” a kind of e-beam resist, in this case poly(methyl methacrylate) or PMMA, then develop PMMA and transfer the patterns onto the wafers. PMMA is commercially available in various concentrations in two kinds of solvents, anisole (also known as methoxybenzene) and chlorobenzene. Some reports show that chlorobenzene is genotoxic, and the general acute toxicity is low to moderate [111]. Anisole has a strong smell, and is less hazardous [112]. We used 2% PMMA in anisole in our work.

The processes of EBL is way more complicated than the optical counterpart, and a significant amount of time is spent on the calibration of the system. Distinct from the contact lithography described above, the exposure of PMMA to the electron beam is not done through a mask, but a moving electron beam to scan across the exposed area on the wafer. Unlike a light beam, electron beam cannot be focused by well-made lenses. Instead, focusing by electromagnetic devices, the process of focusing is not only tuning the “focal length”, which is called working distance in EBL, we also need to make sure that the beam is focused in a cylindrically symmetric manner by adjusting the stigmations. It can be time-consuming to achieve a well focused e-beam spot in some situations. To make sure we focus the beam properly, we let the beam sit at a fixed spot for a while, ranging from couple seconds to 1 or 2 minutes, to “burn” a so-called contamination dot on PMMA. If properly focused, our system (Raith E-Line at PRISM) can produce a dot smaller than 20 nm. A related consequence is that the resolution of Raith E-Line on PMMA can be as small as 20 nm. With a small focal point, the imaging depth (the tolerance of the working distance) is also short, and since the sample may not be perfectly perpendicular to the e-beam, we employ a three-point calibration on the tilted plane to ensure the writing focal point is on the wafer surface. We focus the beam at three individual points manually, then by assuming a planar sample, the computer calculates the correct working distance at each point. In the Raith system at PRISM, an alternative way to keep the e-beam
focused on the sample is available. Combining a laser beam and position photodiode, the height control function can sense the height changes on the surface, and tune the working distance or z position accordingly. This function is particularly useful when working on a wafer with no mark on it, since three-point alignment does not work on a plain wafer. Three-point alignment is preferred by the PRISM staffs since it is more reliable and less prone to the systematic glitches.

The e-beam writer we used has a writing strategy to stitch the write fields, that the machine scans the e-beam to expose one write field, usually 100 µm x 100 µm in our case, then moves the stage so that the next write field is under the e-beam. With that, we also need to calibrate the beam and stage movements, in a step called write field alignment. Equipped with a laser-positioned stage, the movement of the stage is deemed absolute, and the EBL software applies adjustments to the beam. Write field alignment applies three adjustments to the system: zoom, position, and rotation. Since an e-beam is scanning by an angle, although the distance and angle can be one-to-one mapped, a calibration has to be carried out for this mapping. This is called zoom since this mapping simply scale everything up or down. Just like alignment in photolithography, position and rotation are important for overlay exposures. Since write field alignment is the step when we last align the pattern with the alignment marks, it is important to ensure that the system knows where to write which patterns. It is usually recommended to make the alignment marks by EBL. But since it takes too long for the writing, it is not practical, and our alignment marks are made by photolithography, therefore the position of our alignment marks may not be accurate enough for EBL write field alignment. Please be reminded that the photomask produced by GCA/MANN 3600F Optical Pattern Generator has potentially 600 nm position errors. The exact position of the marks are usually not the problem, since it is very likely that the active photolithographically patterned features are off-positioned by a similar distance. The biggest problem here is the
“zoom” calibration. To overcome this, we scan the alignment marks to obtain the positions information, and correct the zoom factors by correlating four images while the stage is at different positions. This usually gave us a fairly good stitching and position alignment. Raith E-Line does offer a writing mode called Fixed Beam Moving Stage, or FBMS, by not stitching the fields, but writing the whole big pattern with a constantly moving stage. The problem of this writing mode is its lack of focus correction as described above, therefore we need to use a smaller e-beam aperture so the focal point is longer in z direction. As a result, the current of e-beam is smaller, and the write time actually increases.

Since we need a tiny e-beam spot to scan through all the exposure area, EBL is by nature not a fast process, and the calibration mentioned above can easily take an hour or more for one device. It is not a good strategy for the industry to mass produce chips with EBL, but the flexibility and relative low cost (compared with the high numerical aperture, immersion photolithography or double patterning used by the semiconductor industry) enable a lot of academic research.

After the writing, PMMA is developed in a solution with one part of Methyl isobutyl ketone and three parts of isopropyl alcohol (1 MIBK : 3 IPA by volume). The area exposed with electron beam dose is thereafter dissolved.

For a nano metal structure, we first use EBL to define the PMMA patterns, then employ metal evaporation and lift-off to transfer the metal pattern onto the wafers. Because of the secondary electron distribution, EBL developed PMMA automatically has an under-cut profile, as image-reversed photolithography. A pair of 30 nm wide EBL made gold lines are shown in Fig. 3.3. Although 20 nm lines can be written on PMMA, after lift-off the smallest metal structures we have achieved is 30 nm. We also constructed self-sealed nanochannels with EBL, but since it is not a standard EBL process we will leave the detail discussion to Subsection 5.2.1.
Figure 3.3: SEM image of (a) a pair of EBL made 30 nm wide gold lines and (b) 45 nm PMMA lines.
3.1.4 Chemical vapor deposition of carbon nanotubes

To date the most reliable method to produce single-walled carbon nanotubes is through chemical vapor deposition (CVD). It is important that the tubes used as devices are single-walled, because the electronic properties of the tubes are decided by their chirality, and by statistics, 1/3 of the tubes are metallic, and the metallic tubes are more conductive than the semiconducting ones, a multi-walled CNT is more likely than a single-walled tube to have the conduction dominated by a metallic layer [113]. Since semiconducting tubes are more sensitive to the change in the environment, the production of single-walled CNT is crucial to the work.

Catalytic growth of CNTs was first reported in 1993 with iron catalyst [114]. It requires iron, iron oxide, or cobalt nano particles as catalyst, and the diameters of the tubes are similar to the sizes of the corresponding nano particles. Such nano particles can be synthesized in a specialized chemistry laboratory. Without that kind of support, in this work, two different easier methods were used for the catalyst nano particles.

One method we tried was wet chemistry (processes similar to Ref. [115]). The solution is made from ferric nitrate (Fe(NO$_3$)$_3$•9H$_2$O), dioxobis(acetylacetonate) molybdenum (VI) (MoO$_2$(acac)$_2$), and aluminum oxide (Al$_2$O$_3$) nanoparticles (Sigma-Aldrich). MoO$_2$(acac)$_2$ is air sensitive so the operation should be kept in a glove box. Materials such as Al$_2$O$_3$ are called catalyst support, which increases the specific surface area for higher yield. Patterning of the catalyst pads is done by lift-off. Since the solution contains some methanol as solvent, PMMA is recommended for performing lift-off, and PMMA can be patterned by oxygen plasma RIE with a lithographically patterned photoresist mask.

The other method we used is evaporation of iron. When a very thin film is coated, the metal forms grains instead of a continuous film. The yield of this method may be slightly lower, but fewer chemicals are involved, and it is more robust, that it
does not degrade with time. It is important for us since we could not perform the CVD furnace step at Princeton, so the catalysts have to remain active while being transferred to the location that CVD is actually performed. Since we had problems with the degradation of the wet chemistry catalysts during the samples transport, iron evaporation later became our primary method to provide the catalyst for CVD process. The evaporated iron can be easily patterned by photolithography and lift-off.

CVD is done in a furnace, the recipe described below is called flying catalyst by McEuen group at Cornell University. Before inserting the samples, we first need to remove all the carbon residual in the furnace tube by heating it at 900°C with the both ends of the tube remaining open. After the cleaning process, we are ready to insert the samples. To fit the one-inch furnace tube, samples cannot be larger than one inch in both length and width. And limited by the size of the furnace, it is recommended that the samples are not spread more than 3 inches inside the tube. If samples are 1 in x 1 in squares, only three samples can be processed at a time.

Four gases are used in the processes. They are Argon (Ar), hydrogen (H₂), methane (CH₄), and acetylene (C₂H₂), where H₂, CH₄, and C₂H₂ combined are called the process gases. At the beginning of the process, we flow all four gases to make sure there is no problem with the gas flow. The first processing step is called the reduction of the catalyst. We heat the samples to 700°C with H₂ and Ar flowing for 30 minutes. After the reduction, as the samples sitting at the middle of the furnace tube, we pull the tube so the samples are outside of the oven, and heat the oven to 1040°C, and let process gases and Ar flow for 3 min, then turn off Ar flow and switch the temperature to 915°C. While the temperature at the oven is still lowering, we push in the tube, flow for about 10 min, and then turn Ar on and switch off CH₄ and C₂H₂. Turn off H₂ while the oven is less than 500 °C, and keep the cooling going with Ar flowing.

Although a rectangular sample with width smaller than one inch fits the furnace, it is very difficult to process such a device, since the spun photo resist / PMMA film has uneven thickness, therefore the optimal exposure / dose varies a lot on the same piece of wafer. To avoid that lithographical difficulty, a square piece is preferred, therefore limit the sample size to 1 in x 1 in.
Figure 3.4: (a) SEM and (b) AFM images of CNT samples. (c) and (d) show the discharging effect of tubes on a fused silica substrate. A tube is connected to the Cr/Au contact, and in (c), the whole tube can be seen as darker. We used FIB to cut the tube, and in (d), it can be seen that the part of the tube now not connected to the Cr/Au contact became slightly brighter than the background instead of darker in (c).

CNTs can be visualized by either Scanning Electron Microscopy (SEM) or Atomic Force Microscopy (AFM), and the corresponding images are shown in Fig. 3.4.

With its small dimension, CNTs are naturally considered for nano electronics, but the growth process limits its applications. Although the alignment of the growing direction of CNTs in the CVD process is not impossible, the growing direction has been mostly perpendicular to the wafer surface [116, 117], and that is not what we were looking for. To be integrated with other fabricated structure such as contact electrodes and fluidic channels, the tubes have to be laying on the wafer surface, and the control of growing direction in this situation is still relatively poor. It has been reported that the flow direction of the process gases can be used to controlled the direction of CNT growth [118], in practice, it is an art to keep the air flow in a laminar
manner near the wafer. Turbulence in the nano scale results in a random growth. Since the tubes are grown randomly from the catalysts pads, as shown in Fig. 3.4 (a), it is almost impossible to precisely control the position of the tube grown. Even if the direction of growing is controlled as described in Ref. [118], the control at the exact position is still poor.

3.1.5 Wafer bonding

To create a micro/nano fluidic system, some kind of sealing is needed. Our group has been a pioneer in the development of using nanochannels for DNA analysis [6, 5, 4, 119, 9], and the channels have been constructed by first etching into the fused silica or quartz wafer, then sealing with a quartz coverslide by direct quartz-quartz binding. The concept of quartz-quartz binding is obvious, that on a clean, hydrophilic quartz surface, hydroxyl groups (-OH) form on the oxygen sites, and when two clean and flat pieces of quartz meet each other, with the reaction

\[
\text{Si} - \text{OH} + \text{Si} - \text{OH} \rightleftharpoons \text{Si} - \text{O} - \text{Si} + \text{H}_2\text{O},
\]

(3.7)

and the two pieces automatically join together through Si-O-Si bonds to form a new covalently bound crystal\(^5\) [121]. To perform quartz-quartz binding, the two joining pieces are first cleaned by the RCA steps described in Subsection 3.1.1. After blow drying the cleaned surfaces, simply let the two clean and flat surfaces touch each other. The bonding is evident by the disappearance of colored fringes that indicate unbounded regions. After that, the chip is annealed in 1000°C for three hours to make the bonding permanent.

Although such a bonding technique has created a lot of success, it is intrinsically impossible when we have electronic devices on top of the wafer surface. The problem is

\(^5\)This reaction is reversible while temperature is under 400°C [120].
Figure 3.5: Set-up for anodic bonding. A hotplate is used to increase the ionic mobility in the glass, and the high-voltage source drives the ions so that a strong electric field presents at the glass / substrate interface to hold them together.

twofold. Part of the problem is its high-temperature annealing step, which can easily destroy the metal circuitry. Moreover, the metal on the wafer not only complicates the RCA cleaning, when metal is supposed to be removed, but also becomes an obstacle since the protruded metal structures prevent the quartz surfaces to touch each other. Although we played with the idea, this method was eventually not used in the devices particularly detailed in this thesis.

Instead, we employed anodic bonding (also called electrostatic bonding) for our devices described in Section 5.1. Unlike direct quartz-quartz bonding, anodic bonding joins a piece of silicon (or oxidized silicon) to a glass (usually Pyrex or Borosilicate, so the thermal expansion coefficient of the glass matches that of silicon). The set-up for anodic bonding is shown in Fig. 3.5. During the bonding process, the wafer and the glass are first heated on a hotplate to a temperature below the softening temperature of the glass, usually below 500°C (with the hotplate temperature at or around 550°C), to increase the ionic conduction of the glass, then a DC high voltage
(at range of 1 kV) is applied across the glass and the silicon substrate. It has been reported that a sample temperature of 360°C and 750-1000 V of voltage are required for a good bonding result [122]. A graphite plate (GraphiteStore.com) and a metal block are used to contact the Si substrate and Pyrex glass respectively. We chose a ceramic top hotplate and a graphite plate instead of a metallic top hotplate to avoid the high-voltage that interferes with the normal functions of the hotplate. Since the mobile ions in Pyrex are mostly positively charged sodium (Na⁺) or other metal ions, the positive potential is sided with the substrate so Na⁺ are driven away from the interface. The ion migration in the glass creates a large electrostatic field at the glass-substrate interface, and the resulting force brings the two surfaces together. With the strength of the electric field, we do not need high pressure to press the pieces together, but only to make sure the two pieces are in contact. We monitor the current in series with the high-voltage source during the bonding process. Since the electric current is contributed by the ion migration in the glass, and the current exhibits an exponential decay with the time, we can use the current as an indicator on the progress of the bonding. We usually stop the process when the current stops dropping at a fluctuating noise level.

### 3.1.6 Parylene coating

Complimentary with the wafer bonding techniques, we use parylene as the top capping layer for our self-sealed nanofluidic systems described in Section 5.2. Parylene is the generic name for members of a unique polymer series. Parylene C, or poly(chloro-p-xylylene), the polymer we used to fabricate our devices with nanochannels integrated with nano electrodes, has a chemical structure as shown in Fig. 3.6. The deposition of a thin parylene film by a gaseous precursor was first observed by Szwarc in 1947 [123]. By studying the gas permeability and apparent diffusion coefficient, it has been shown that parylene C coating provides a pin-hole free and extremely conformal
film [124]. Parylene coating has been used to provide a dielectric film and protection capping for nano electronics [125]. The quality of parylene film is even good enough for gate insulator [126].

Parylene deposition was done at Nanobiotechnology Center (NBTC) at Cornell, with a process shown in Fig. 3.7. Parylene is coated in a modestly evacuate chamber (no high vacuum gauge is connected to the system, \( \sim 0.1 \text{ torr} \)) near room temperature. The process begins with dimer powders, and we evaporate (or, technically, sublimate) them at 150°C. The dimer vapor is later decomposed into monomer in a process called
pyrolysis at 690°C, and the monomer vapor then enters the coating chamber, which is not heated. It has been shown that the dimer is quantitatively cleaved into two monomer units at temperatures above 550°C and pressures less than 1 Torr [127]. Once the monomers enter the main deposition chamber, which is in room temperature, they diffuse to the substrate (or any other solid surface), and get adsorbed onto the surface. No special initiator chemical is required for the polymerization to occur [128]. Instead, the initiation is for three monomers to form a diradical oligomer (which is a chain of structure of Fig. 3.6 with \( n = 3 \), and both the unbound covalent bonds are radicals) [129], and after that the propagation reaction takes place, that monomers just attach onto the oligomer and form a longer and longer diradical. There is no termination reaction, but the newly generated diradicals might bury the existing ones in the film. A typical molecule has \( n = 2000 - 4000 \) [128]. Since it does not take any specific reaction to initiate the polymerization, parylene film is deposited onto the whole surface in the chamber. In order to remove the polymers from the exhaust so they do not deposit in the pump, a cold trap at -70°C is placed in the pumping line.

It is known that auto-fluorescence can be induced in a parylene film by an active plasma [130] and UV radiation with wavelength 270 - 300 nm [131]. Without the induction, the background fluorescence of parylene is low, and parylene-based devices have been used for fluorescence DNA assays [132] and ssDNA separation [133]. To enable the optical imaging of fluorescently stained DNA molecule, we need to avoid the exposure of the film to UV and plasma treatments. Since \( \text{O}_2 \) RIE is used to pattern parylene film, it is important to have photoresist etch mask thicker than the parylene film. Also to avoid UV radiation the samples have to be kept in dark, covered by aluminum foil during transportation and storage. It is also very helpful to cover the watch window of parylene deposition system during the CVD process.
3.2 Electronic measurements

In this work, we extensively used lock-in amplifiers (Stanford Research SR-830 DSP Lock-in Amplifier) to perform ac measurements. A lock-in amplifier is usually used to pick up small signals from a noisy environment [134]. The measurements by a lock-in amplifier needs a reference signal, \( V_{\text{ref}} \sin(\omega_{\text{ref}} t + \theta_{\text{ref}}) \). Assuming the signal we want to detect has a form \( V_{\text{sig}} \sin(\omega_{\text{sig}} t + \theta_{\text{sig}}) \), the phase sensitive detector (PSD) then multiplies the two signals so that the output of the phase sensitive detector is

\[
V_{\text{psd}} = V_{\text{sig}} V_{\text{ref}} \sin(\omega_{\text{sig}} t + \theta_{\text{sig}}) \sin(\omega_{\text{ref}} t + \theta_{\text{ref}})
= \frac{1}{2} V_{\text{sig}} V_{\text{ref}} \left\{ \cos[(\omega_{\text{sig}} - \omega_{\text{sig}})t + \theta_{\text{sig}} - \theta_{\text{ref}}] - \cos[(\omega_{\text{ref}} + \omega_{\text{sig}})t + \theta_{\text{sig}} + \theta_{\text{ref}}] \right\}.
\] (3.8)

Filtered by a low-pass filter, for all signals with an \( \omega_{\text{sig}} \neq \omega_{\text{ref}} \) vanish, and only for those \( \omega_r = \omega_{\text{ref}} \) become a DC output

\[
V_{\text{psd}} = \frac{1}{2} V_{\text{sig}} V_{L} \cos(\theta_{\text{sig}} - \theta_{\text{ref}}).
\] (3.9)

Since \( V_{L} \) is known, \( V_{\text{sig}} \cos \theta \) can be easily obtained, with \( \theta \equiv \theta_{\text{sig}} - \theta_{\text{ref}} \).

The lock-in amplifier we used has a second PSD, with the reference signal shifted by 90°, so the low pass filter output is

\[
V_{\text{psd2}} = \frac{1}{2} V_{\text{sig}} V_{L} \sin(\theta_{\text{sig}} - \theta_{\text{ref}}).
\] (3.10)

And if we define

\[
X = V_{\text{sig}} \cos \theta, \quad Y = V_{\text{sig}} \sin \theta,
\] (3.11)
we can easily get

\[ R = V_{\text{sig}} = \sqrt{X^2 + Y^2}, \quad \theta = \tan^{-1}(Y/X). \] (3.12)

For more details, please see SR-830 manual [135]. Since a lot of electrochemical reactions, ionic migration, chemical adsorption may happen in the solution, we indeed measure a noisy system, and an ac lock-in measurement can help us to pick up only the signals we want to measure. Other than its sensitivity and the rejection of noise, ac measurements are preferred because a DC voltage is used to move the DNA molecules by either electrophoresis or electro-osmosis, and that voltage certainly interferes with any DC measurement. As a result any DC measurement is impractical.

We had three different kinds of ac measurements carried out. For a FET device, we have \( I_{DS}-V_{DS} \) (Section 4.3) and \( I_{DS}-V_G \) (Section 5.1) measurements, where DS denotes between drain-source, G is the back gate. \( I-f \) scans are also performed (Section 5.2). This is a measurement very similar to the electrochemical impedance spectroscopy described in Subsection 2.2.4. It is not exactly a traditional EIS measurement since we do not have a typical three-probe set-up for this measurement. It is not realistic to have a silver / silver chloride reference electrode nanofabricated in our devices since they are soluble in aqueous solution. A tiny bit of the electrode dissolving is not an issue for a big electrode, but in nano scale it takes very little of salt to have an electrode complete disappear.

A schematics of an \( I_{DS}-V_{DS} \) measurement on a CNT-FET is shown in Fig. 3.8. Cr/Au electrodes are fabricated on top of the tubes, and we used them as our drain and source electrodes. A DC voltage \( (V_{\text{off}}) \) added with an ac ripple \( (\delta V) \) is fed into one lead, and the other lead in virtually grounded, and the current goes through it is turned into an amplified voltage signal by a NIMM op-amp module. The gain is usually set at \( 10^5 \) to \( 10^6 \). A LabView program is used to control the SR-830 through
Figure 3.8: Using a lock-in amplifier to measure $I_{DS}-V_{DS}$. A function generator is first used to create a signal with an ac ripple amplitude $\delta V$ on an offset DC voltage $V_{off}$, and the signal is fed to the drain lead on the device. The source end is virtually grounded, and $I_{DS}$ is amplified by a current preamp, and that amplified signal is read by a lock-in amplifier. As a result, what is measured here is the slope of the I-V curve ($\delta I/\delta V$) at the offset voltage $V_{off}$. We integrate over the differential to get the I-V curves.

A gate scan is relatively trivial. With zero DC $V_{DS}$, an ac signal ($\delta V$) is still fed through the drain-source electrodes, and a DC $V_G$ is at the gate electrode, usually the silicon back gate. We connect the silicon back gate by using carbon paint at the wafer cleaving edge, where unoxidated silicon is exposed, to a piece of metal, usually copper. A LabView program is used to control the $V_G$ and record the results. We read the ac current between drain and source, therefore we measure the two-probe conductance between the electrodes. The arrangement of current-to-voltage conversion op-amp and lock-in amplifier is the same as $I_{DS}-V_{DS}$ measurements.

The wiring for $I-f$ scans is very similar to that of $I_{DS}-V_G$ measurements, with the only change that silicon back gate is now grounded. Since it is a two-probe
measurement, one probe is used to feed the ac voltage, and the other is virtually
grounded, connected to the op-amp module as our current pre-amp, and we read the
voltage output from the current pre-amp by the lock-in amplifier. Here the scanning
parameter is the ac frequency, with a range from \( \sim 100 \) Hz to 102 kHz.

3.3 Epi-fluorescence microscopy set-up

Although our goal is to detect DNA electronically, the comparison with conventional
optical detection techniques is very important for us to understand what we measured
electronically, therefore the single molecule fluorescence microscopy used in optical
analysis [136] was still needed.

Two different light sources were used in our experiments. We used both mercury
arc lamp and Ar/Kr mixed gas ion laser (Coherent, Innova 70C Spectrum). A mercury
lamp is standard for biological imaging, but if a brighter excitation is needed, we used
the Ar/Kr ion laser. The Ar/Kr ion laser we used provides 488, 514, and 647 nm with
strongest lines (0.25 W), lines in 476, 521, 531, 568 nm (0.10-0.15 W), and minor lines
in UV, 458, and 752 nm \(< 0.5 \) W) [137]. An acousto-optic tunable filter (AOTF, from
NEOS Technologies, N48062-2.5-.55) is used to select a single line out of the “white
light” laser. The core of an AOTF is a piezoelectric material. An acoustic wave is
used to generate a periodic pressure pattern in the material, therefore generating a
refractive index wave, and the refractive index wave serves as a sinusoidal grating.
To satisfy the Bragg condition, incident light with different wavelengths is diffracted
with different Bragg angle, therefore light with different colors are separated. For
practical reason, the outlet of the filtered light is at a fixed position, so the alignment
of the optics can remain the same while different color of light is chosen. As a result,
different frequencies of the acoustic wave are applied at the AOTF, so the period
of the grating is changed, and different color of the light becomes the output at
the same position. Other than choosing the color, AOTF can also be used to tune the amplitude of the output light. The intensity of the light is proportional to the acoustic power, and inversely proportional to the square of the optical wavelength [138]. Combining the Ar/Kr ion laser and the AOTF, we have lines at 476, 488, 514, 520, 568, 633, 647 nm, and the 488 nm one is the strongest.

While a laser is used as the light source, a set of scanning mirrors is needed to obtain a full frame of illumination. A scanning mirror is a light-weight mirror connected to a galvo motor, so the angle of the mirror can be controlled by the input current at the motor. Two scanning mirrors are used to provide the X-Y scan. Since the incident light is a fixed beam, the first scanning mirror (the one the light beam hits the first) is smaller, and the second one has an elongated shape along the scanning direction of the first one, so the scanned beam still hits the second mirror. The mirrors are controlled by a function generator, which provides symmetric triangular waves for the fast axis and the sawtooth shape of waves for the slow axis. The ratio of the frequencies should be tuned to provide a uniform illumination, that the two frequencies do not have obvious beats, resonance, or other features to make the frame flickering or with some bright lines. The exact scanning frequencies depend on the application, but it is better for the slow axis to be scanned at a rate faster than the frame rate of the camera. The scanning beam should overfill the back-aperture of the excitation illumination path of the microscope to achieve the best illumination.

The excitation light goes into the microscope through an epi-fluorescence attachment, the setup is shown in Fig. 3.9. Before the beam is focused onto the sample, it first goes through an excitation filter, which ensures only a certain wavelength of excitation is going through, then reflected by a dichroic mirror. When a mercury lamp is used, the excitation filter is the sole mechanism for choosing the excitation wavelength. A dichroic mirror is crucial in fluorescence microscopy, since it splits the excitation and emission light by reflecting the shorter wavelength excitation and
Figure 3.9: Microscope setup for epi-fluorescence microscopy.
transmitting the longer wavelength emission.

After the reflection at the dichroic mirror, the excitation beam is focused by an objective. An objective is a complicated optical device, whose function is solely focusing the light. A major part of the reason it is such a complicated device is that multiple lenses (in some cases mirrors) are used to correct spherical aberration and coma and for chromatic aberration [139]. The objective also sets the resolving power of the microscope. For an incoherent illumination,

\[ R \sim 0.61 \frac{\lambda_0}{N.A.}, \]  

where \( \lambda_0 \) is the wavelength in vacuum, and \( N.A. = n \sin \theta \) is called the numerical aperture, with \( \theta \) the angular semi aperture on the object side. It can be shown that with a coherent illumination, the resolving power is given by [139]

\[ R = 0.82 \frac{\lambda_0}{N.A.}. \]  

For a 60X air objective we used, the resolving power is 570 nm for a 488 nm excitation. The volume of the excitation focal spot is described by the point-spread function (PSF). With the dimensionless coordinates

\[ u = \frac{2\pi}{\lambda} \left( \frac{a}{f} \right)^2 z, \quad v = \frac{2\pi}{\lambda} \left( \frac{a}{f} \right) \sqrt{x^2 + y^2}, \]  

where \( a \) is the radius of the aperture, \( f \) is the focal length, \( \lambda \) is the wavelength of the focusing light, \( z \) is the distance component along the optical axis, and \( x \) and \( y \) are the distances perpendicular to the optical axis. With \( A/f \) as a constant related to the amplitude, for a condenser to focus a parallel beam, by integrating over the
aperture at the objective, it can be shown that the intensity distribution [139]

\[ I(u, v) = \left| -\frac{2\pi i a^2 A}{\lambda f^2} e^{i \left(\frac{1}{2}\right)^2 u} \int_0^1 J_0(v \rho) e^{-\frac{1}{2} i \omega \rho^2} \rho d\rho \right|^2. \] (3.16)

With exceptions of multi-photon microscopy [140] and up-conversion phosphors [141], the emission photons from a fluorephore molecule typically have lower energies than the excitation photons, and the shift of the peaks in the energy spectrum is called the Stokes shift. This shift is usually due to phonon or thermal relaxation during the fluorescence emission process, sometimes related to radiative transitions.

The emitted photons are then collected by the same objective. In most cases the emission is isotropic, but the objective can only collect the photons within a solid angle, and that solid angle should be given from the numerical aperture

\[ \Delta \Omega = 2\pi \int_0^\theta \sin \theta' d\theta' = 2\pi (1 - \cos \theta), \] (3.17)

where \( N.A. = n \sin \theta \). As a result, the ratio of photons being collected by the objective can be given by

\[ \frac{P}{P_0} = \frac{\Delta \Omega}{4\pi} = \frac{1}{2} \left[ 1 - \sqrt{1 - \left( \frac{N.A.}{n} \right)^2} \right], \] (3.18)

so an objective with higher \( N.A. \) collects more light. For Nikon M Plan 60X extra-long working distance objective with \( N.A. = 0.70 \), only 14.3% of light is collected by the objective. For Nikon Plan Apo 100X \( N.A. = 1.4 \) oil-immersion objective, 30.9% of light is collected.

Due to the Stokes shift, the emission light now has a longer wavelength, and transmits through the dichroic mirror. To ensure no excitation light leaks through the dichroic, an emission filter is used to remove the light with shorter wavelength.

After the emission filter, the emitted photons are detected by a camera. To image the fluorescence from the single-molecule system, an intensified CCD (ICCD,
I-PentaMAX, Roper Scientific) or an electron-multiplying CCD (EMCCD, Rolega-MGi Plus, Q-Imaging) was used. An ICCD has an image intensifier to increase the intensity of light. The incident photons first reach a photocathode to generate photo electrons, and the photo electrons are accelerated toward a micro channel plate (MCP). An MCP is an array of miniature electron multipliers oriented parallel to one another [142]. After the multiplication, the electrons are accelerated to a phosphor screen, which once again turns the electrons back to photons, and a CCD is used to image the photon distribution on the phosphor screen. An EMCCD has a completely different strategy for imaging low light. Instead of amplifying the light on the CCD, photons hit the photo sensors on EMCCD directly, and the signals are enhanced by the electronic circuits. For a typical CCD, when the photons hit the photoactive silicon layer, electric charges proportional to the light intensity are generated. The charges induced at each pixel are transferred by a “shift register”, which transport all the charges to the adjacent capacitor, and that is why the device is called charge-coupled device. At the end a charge amplifier is used to turn the charge into a voltage. In this way, the charge induced on the whole array is now turned into a series of voltages. For an EMCCD, a gain register is placed between the shift register and the charge amplifier. The gain register has a large number of amplification stages, and each stage impact ionization (using a high-energy electron to produce two or more lower-energy electrons) is applied to multiply the charges. The electronic gain can be as high as 1000.

3.4 DNA sample preparation

Various DNA samples were used in this thesis. We will briefly explain how those samples were prepared before experiments.

In Section 4.3, ssDNA samples were used. Four different sequences of oligomers
were used in this work. 305.5 µg (or 41.3 nmol) of 24mer 5’-GTT TGA CAA ACA TCA AGA CAG AAG-3’ and 270.3 µg (or 43.1 nmol) 21mer 5’-GAC TAC CTC CTC CAC AGA CTC-3’ were purchased from e-oligos, and their complimentary sequences 5’-CTT CTG TCT TGA TGT TTG TCA AAC-3’ (305.68 µg) and 5’-GAG TCT GTG GAG GTA GTC-3’ (307.04 µg) were purchased from Invitrogen. The sequences were chosen because of historic reasons, that those sequences were used in Ref. [143] to detect volatile molecules in the air. The DNA came in a tube, and we put 100 µL of sterilized distilled water to make the stock solution, which is ~0.4 mM of oligomers or ~8 mM of bases.

We also used double-stranded lambda-DNA. We purchased 50 µg/mL sample in 0.5X TBE from New England BioLabs (N3011S), then diluted the solution to desirable concentration, usually 1-10 µg/mL. The standard 1X Tris/Borate/EDTA, or TBE buffer, contains 89 mM of tris-base, 2 mM of EDTA, and 89 mM of boric acid. The tris-base acts as a buffering component to keep the pH stable, boric acid increases the conductivity, and the role of the EDTA is to protect the nucleic acids against enzymatic degradation [136]. 0.5X TBE has half of the concentration described above. If the sample was used only for optical measurements, it is diluted by 0.5X TBE or DI water. If the DNA was used for imaging, fluorescence stain is necessary. We used two different intercalation dyes for this purpose: TOTO-1 (TOTO-1 iodide (514/533) 1 mM solution in DMSO, Molecular Probes) for green fluorescence and BOBO-3 (BOBO-3 iodide (570/602) 1 mM solution in DMSO, Molecular Probes) for red. Red dye is favored for combination with parylene channel since parylene has lower auto-fluorescence in that spectral range. To stain DNA, we mixed the diluted DNA solution with the dye solution, and the ratio of the two solutions is calculated such that one dye molecule for every 4-10 bps, with assumption that all dye molecules bind onto the DNA. After the mixing, the solution was incubated in dark for at least two hours. If the sample is going to be imaged for an extended
period of time, oxygen scavaging solution is needed, that includes glucose (8 mg/mL \(\beta\)-D-glucose; G7528, Sigma), glucose oxidase (0.2 mg/mL; G-7016, Sigma), catalase (40 \(\mu\)g/mL; C-40, Sigma), and 200 mM \(\beta\)-mercaptopethanol, as used in Ref. [9]. A sample of fluorescently stained lambda-DNA is shown in Fig. 3.10.
Chapter 4

Tests on potential sensing mechanisms

In the following two Chapters, we will discuss the DNA sensor development in detail, some in a historical view. Although knowing our ultimate goal is a device with electronics and nanochannels integrated together, the work focused only on the sensing strategies at the beginning stage. In this Chapter we will first discuss using a lock-in amplifier to perform electrochemical measurements similar to cyclic voltametry and electrochemical impedance spectroscopy (with setup discussed in Section 3.2 in Section 4.1, then we present the measurement with a polycrystalline silicon film and nickel contacts in Section 4.2. In Section 4.3 we will discuss some experiments with a carbon nanotubes network.

4.1 Measurements relying on electrochemical properties

No nanofabricated device was used in the work described in this section. Instead, we simply placed platinum wires as electrodes into the bulk solution to perform the
Figure 4.1: Comparison of 0.5X TBE buffer solution (red) and 5 µg/mL lambda-DNA in 0.5X TBE (green) under the ac current measurements with frequencies scan. (a) plots the absolute value of conductance and (b) shows the phase on lock-in amplifier measurements.

We used $I-f$ measurements scheme laid out in Section 3.2 to simulate the electrochemical impedance spectroscopy. The comparison of 5 µg/mL of lambda-DNA in 0.5X TBE buffer and only 0.5X TBE buffer is shown in Fig. 4.1. Please be noted that the comparison of the absolute numbers on the two curves may not provide any useful information, since the separation between the electrodes, the lengths of the electrodes immersed in the solutions, and the shapes and sizes of the solution droplets being
measured were not identical. However the curve shapes should be quite indicative, since all the factors mentioned above only change the numbers in a proportional way. It can be seen that in the $R$ curves, they possess completely different shapes, that the curve with only the buffer solution is concave down, and the one with DNA is concave up when the frequencies are higher than 300 Hz. The trend in phase shift is more similar, but the curve with DNA in the solution crosses over the 0 phase shift at a higher frequency. Since all the $\theta$ are close to 0, the $y$ components of the lock-in measurement behave very similar to that of $\theta$, and $x$ components resemble more of $R$. As a result, we were led to conclude that the change of the amplitude of the ac conductance is mostly a result of the changes from the real part of the impedance. Comparing with the discussion in Subsection 2.2.4, that means contact resistance $R_{CT}$ and the solution resistivity $\rho_{sol}$ are changing with ac frequencies, and make the major contributions to distinguish the buffer and buffer with DNA. The real part of Walburg impedance $Z_W$ does not appear to be a major source of the differences, since any change in $Z_W$ should follow the same trend in both real and imaginary parts.

Although we are not at a stage to detail the electrochemical mechanism for the measurements, we have demonstrated that a simple ac conductance measurement can be used to distinguish whether there is DNA in the buffer solution.

For the CV-like measurements, a LabView program is used to sweep the voltage between the two electrodes up and down, with an average rate similar to those used for a standard CV measurements. In order to make a solution with 0.1 mM of the bases, we used a potassium phosphate monobasic (0.2 M KH$_2$PO$_4$) based buffer, with sulfuric acid (H$_2$SO$_4$) to make it with a pH $\sim$ 1. The dissolvation of the bases can raise the pH to around 2.75 [94]. The bases are practically insoluble in a pH neutral aqueous environment.

The typical curves are shown in Fig. 4.2. It can be seen that the plot with buffer solution is distinct from the one with guanine dissolved in it. The curve shapes do
Figure 4.2: Ac current measurements with a DC bias across the two electrodes in (a) pH $\sim 1$ phosphate buffer solution and (b) 0.1 mM of guanine in said buffer.
not resemble the CV peaks seen in Ref. [94] and [97], but it has to be noted that although we have a DC bias voltage sweeping like a CV measurement, we do not measure the DC current going through the electrodes, but the amplitude of the ac ripples. The rapid surge of current in Fig. 4.2 (a) above 1 V is likely due to the hydrolysis of water. It is known that water is hydrolysized on platinum electrodes while the voltage is higher than 0.8 V [94]. For Fig. 4.2 (b), the current peaks close to 0.4 V, which is similar to the voltage where the current starts its exponential growth in a typical CV plot, which is around 0.6 V [94]. The current surge is presumably related to the hydrolysis of water and is also seen at the same voltage in (b).

Although this is not a typical CV scan, we do show that it can be used to distinguish whether guanine is dissolved in the buffer solution.

4.2 Poly-Si film

In this section, we used oxidized silicon wafer as substrate, so the silicon could be used as back gate. We tried both aluminum (Al) and nickel (Ni) to form contacts. Although Ni contacts are known for higher contact resistance, since Al shows nearly no contrast on poly-Si film under SEM so it is difficult to image the fabrication results, and eventually we went with Ni contacts. The Ni contacts were first defined by contact lithography, and then we evaporated Ni and performed lift-off. After lift-off, we annealed the wafers at 800 °C for 1 hour under forming gas, which consists of hydrogen (H\textsubscript{2}) and nitrogen (N\textsubscript{2}), so the oxidation of the metal should be minimal. The purpose of annealing is to form a nickel silicide (NiSi\textsubscript{2}) compound at the contact [144]. The schematics for the device is shown in Fig. 4.3.

The device is characterized by a gate scan by sweeping the voltage at the silicon back gate, and the $I_{DS}$ curve is shown in the inset of Fig. 4.4. Although we did not dope the poly-Si film, it can be seen that the bandgap is not at the zero gate voltage.
The liquid is applied onto the chip directly, in contact with the poly-Si film and both contact electrodes. Since the solution is in contact with the electrodes, this is not only an FET measurement, but a combination of FET and electrochemical measurements. The effect of buffer solution and lambda-DNA in buffer solution is shown in Fig. 4.4. Although TBE buffer solution is much more conductive than air, the $I_{DS}-V_{DS}$ curve shows a higher resistance. We suspect that is an effect between water / poly-Si interface. It is common for a silicon surface to form a thin intrinsic oxide film, usually at the order of 5 nm. Also it is known what when a silicon dioxide surface is immersed in an aqueous environment, negative charges appear on the surface because of the forming of silanol (Si-OH$^-$) groups. As a result, with aqueous solution presents on top of the poly-Si film, a negative voltage is introduced to gate the poly-Si film. And as shown in the inset of Fig. 4.4, that result in a higher resistance.

The more interesting results come from the comparison of 0.5X TBE buffer and 5 µg/mL of lambda-DNA in 0.5X TBE buffer. The conductivity is apparently higher than the 0.5X TBE curve, and the shape of the curve process an unmistakenable S-
Figure 4.4: $I_{DS}$-$V_{DS}$ and $I_{DS}$-$V_{G}$ (inset) curves from a poly-Si device. It is clear that the existence of lambda-DNA changes the $I_{DS}$-$V_{DS}$ behavior.

shape, but it worth noting that the S-shape curve is not symmetric. With the molar concentration of DNA only 0.32 nM (for comparison the 0.5X TBE buffer has more than 89 mM of salts), the conductivity change is unlikely to be caused simply by the additional charge carriers. It is not a result of electrochemical charge transfer either. Although the exact peak position is related to the concentrations of the chemical comounds and many other factors, the voltage that the measured current starts to take a rise is determined by the oxidative properties of the chemicals, as described in Subsection 2.2.4. From references, it is shown that the CV curves for adenine and guanine have the current rising around 0.62 V and 0.82 V [94, 97], but our curve shows a continuous rising trend from 0 V. Besides our scanning speed was not tuned for the CV measurements.

To be sure, the asymmetry of the curve must come from the properties of the Ni / poly-Si film contacts, since as long as the two contacts are completely identical, there is no reason a negative $V_{DS}$ should yield anything different from a positive $V_{DS}$ with the same absolute value. We suspect, the mechanism for the curve shape is a result
of the poly-Si film being gated by mobile DNA molecules. When a voltage is applied across the film, negatively charged DNA molecules move toward the positive voltage because of the electrophoresis. Although the salt in buffer solution does the same under electrophoresis, DNA molecules carry much more charges than the simple salt ions, and the gating effects from DNA is conceivably greater than the salt solution, and the change in chemical potential in the semiconductor reduces the impedance at the contacts.

4.3 CNT network

We started with exploring the possibility of electrical detection of DNA in bulk solution. It has been shown by molecular dynamics simulation [145] that single-stranded DNA (ssDNA) molecules are non-covalently bound to the carbon wall through van der Waal interaction, we decided to try CNT as our sensor. As described in Section 3.1.4, the tubes are grown in a random manner, so a high density network is the easiest thing to begin with.

4.3.1 Device fabrication

The device fabrication here is relatively simple. Our collaborators, Dr. Bakajin at Lawrence Livermore National Laboratory and A. T. Charlie Johnson Group at University of Pennsylvania put the high/low-density of CNTs on the fused silica wafer respectively, and then we laid Cr/Au contacts by image-reversal photolithography and metal lift-off, as described in Section 3.1.2. The contacts were made by chance. Since the distribution of the tubes were completely random, and the overlay area of our metal electrodes was large enough, it was quite easy to have tubes connecting our electrodes.
4.3.2 Results

As shown in Fig. 4.5, a high-density, continuous CNT network was used in the experiments. Since 1/3 of the tubes in the network are metallic, and the metallic tubes typically have higher conductances than the semiconducting ones while no apparent gate voltage presents, the drain-source I-V characteristic curves look more ohmic than semiconducting, as shown in Fig. 4.6. As we applied a droplet of ssDNA (3’-GTT TGA CAA ACA TCA AGA CAG AAG-5’) solution on the device, the I-V curves became non-linear. Since the droplet was in an open space, it dried out over time, and when it was dry, we could see that the I-V characteristic curve went back to linear, and the resistance was higher than the bare CNT network. For reference, if a droplet of de-ionized (DI) water was applied onto the device, very limited changed was observed, as shown in Fig. 4.7, therefore we concluded that the changes seen in Fig. 4.6 are from the ssDNA molecules. The effects can come from individual tubes, metal contact of the tubes, or the the contacts between tubes (tube cross-over).

It was encouraging to see any electronic effect from DNA molecules, but to realize a precise DNA length measurement, a highly localized detection method is needed, and
Figure 4.6: $I_{DS}$-$V_{DS}$ curves from a high density CNT network device. The green curve shows an ohmic behavior of the bare CNT network, and the red curve shows a $V_{DS}$ scan while a droplet of ssDNA solution is on the device. Since the scan was done in the open space, the droplet was drying out over the time (from left, or lower $V_{DS}$, to the right), and it dried at the point noted by the arrow. The Blue curve shows the scan while ssDNA solution dries on top of the device, as the conductance got reduced. It can be seen that the dry part of the red curve shows a similar resistance to that in the blue curve.
an extended CNT network runs contrary to the idea of a localized device, therefore if CNT is meant to be our detection building block, a single tube device will greatly enhance our chance of length measurement. Since CNTs are grown randomly under CVD process, the way to achieve a single-tube device is to reduce the tube density so that in the region where two micro electrodes overlap, there is only one tube in between. As a result, our next step is to see how a CNT network with much lower density responds to DNA molecules.

One of such devices is shown in Fig. 4.8. Although there was more than one tube between the two electrodes, there was no tube cross-over between two contact lines, therefore it was useful to clarify whether the conductivity reduction comes from the tubes cross-over, that is DNA molecules separate the tubes further at the cross over region so even the tubes are remained the same, the conductivity of the whole network got reduced.
Figure 4.8: SEM image of a low density CNT network with Cr/Au contact lines on top. Compare with Fig. 4.5, it can be seen that the tube density is much lower, only three tubes in the 50 µm region, and tubes do not cross over.

The results are shown in Fig. 4.9. Similar to the high density network, the addition of DNA solution increases the resistance of the network, and the effect was greatly enhanced when the solution was dry. Actually, the effect was much greater in low density network than the high-density counterpart. With this, we concluded that the effect was not from the tube cross-over, but either from the contact resistance or tube properties.

One interesting aspect from the measurements in the low density network measurements is, if we zoom in the dry DNA (green) curve in Fig. 4.9, as shown in Fig. 4.10, it actually rendered a diode-like I-V curve. If we reversed the voltage scan direction, the same diode-like curve remained, and nearly no hysteresis was observed. In an ideal diode, the curve is described by the following equation [146]

\[ I_{DS} = I_0(e^{V_{DS}/nk_BT} - 1) \]  

(4.1)

where \( I_0 \) is the reverse bias leakage current and the factor \( n = 1 \). \( k_B \) and \( T \) are
Figure 4.9: $I_{DS}$-$V_{DS}$ curves from a low density CNT network. It can be seen that DI water (red) makes almost no effect on the conductivity of the tubes (compared with bare tubes, black), but the addition of ssDNA solution greatly reduces the conductance (blue). Sequence of DNA: 5’ - GTT TGA CAA ACA TCA AGA CAG AAG- 3’. The same as Fig. 4.6, the voltage scan itself is a time series, and once the droplet dries, the conductance is further reduced, in this case lowered by couple orders of magnitude.
Figure 4.10: Diode-like I-V curves when DNA solution dried on a low-density device while a biased DC voltage is applied across drain-source. The green and blue curves show little hysteresis, and the red one is a fit of a diode function. Not being a perfect p-n junction, the fit is only good close to zero voltage.
Boltzmann constant and temperature respectively, and \( n \) is the ideality factor, may be a function of the bias voltage. Parameters used in our fit are \( I_0 = 6 \times 10^{-9} \) and \( n = 4 \), which implying our device is far from an ideal p-n junction, which is not surprising because we never intended to build one. If the p-n junction formed by the uneven gating from the DNA molecules, it is natural that the chemical potential transits from more hole-like to electron-like gradually, instead of a sharp jump in an ideal p-n junction. In high voltage bias level, the deviation of the data from the fit is mostly due to the significant voltage drop not at the p-n junction, but at the other part of the tube and the contact resistance. Very similar deviation was observed when two back gates were used to create a p-n junction in a CNT FET device [146], which led us to believe the cause of the diode-like curve is the drying of DNA solution at a none-zero bias voltage, therefore more negatively charged DNA molecules are accumulated on one side of the tube, therefore change the chemical potential of the tube unevenly and result in the diode.
Chapter 5

Development of nanochannels with nano electronics

Along with the development of the electrical sensing mechanism, we realized that the real challenge is to have a device with electrodes and fluidic channels at the same time, so we began with the integration of a two-micron wide micro fluidic channel with carbon nanotube devices, as detailed in Section 5.1. Unfortunately, that process was not compatible with e-beam lithography, which is necessary for the fabrication of a nanochannel. As a result, we developed a new way to construct an auto-sealed parylene nanochannel which has electrodes embedded in it, by using PMMA as sacrificial layer. At the same time, in order for a length measurement, the randomness of CNT growth is undesirable to us, so we switched to e-beam lithographically made 30-nm metal lines, as detailed in Section 5.2. The parylene channels eventually provide us an opportunity to integrate the nanochannels with almost all kinds of surface electronics.
5.1 Sealing CNTs with a micro channel

It is with great interest to see a CNT network respond to DNA solution, and the dried electrolyte solution can form a p-n junction, as shown in Section 4.3, but since a network is by its nature not a localized structure, it is not likely that it can be used for a precision length measurement. Furthermore, since we need to integrate the nanochannel with the electrical sensing techniques, we need a way to construct electronics in sealed fluidics. In this Section, we detail our first attempt to achieve that goal, by showing how to build single-CNT device with a 2 \( \mu \text{m} \) wide microchannel integrated altogether. Our strategy here is to build electronics on top of the wafer first, and then form the channel side walls by silicon oxide lift-off. The same silicon oxide layer covers part of the electronics on the wafer. After that, we use anodic bonding to seal a piece of Pyrex or Pyrex equivalent borosilicate glass as the top of the sealed channel.

5.1.1 Device fabrication

In order to use the back gate to study the semiconductor properties of CNTs, all the devices discussed in this section were built on silicon wafers with 100 nm of dry thermal oxide. After the furnace oxidation, we first laid down the alignment marks by photolithography and reactive ion etching, then put the iron catalyst pads by photolithography and metal lift-off. We then grew CNTs by CVD steps described in Section 3.1.4, and then put Cr/Au contacts by photolithography and metal lift-off. At this point all the electronics were already made on the wafer.

We then constructed the micro-fluidic channel. As shown in Fig. 5.1, we used photolithography and silicon oxide lift-off to define the channel area. To access the channel, we then used sand-blaster to drill two holes through the wafer at the feeding channel areas. After proper cleaning steps, we sealed the channel with a small piece
Figure 5.1: Fabrication process for a Pyrex sealed micro-fluidic channel integrated with CNT devices.
of Pyrex, or Pyrex equivalent Borofloat, by anodic bonding procedures described in Subsection 3.1.5. A resulted micrograph by an optical microscope is also shown in Fig. 5.1. It is common that the microfabricated gold patterns do not stand heating around 400°C because of melting and other phase transitions, but since our gold patterns were confined in a silicon oxide surrounding, they actually stayed continuous and without deformation.

5.1.2 Results

A single tube device and a sealed channel with two pairs of electrodes (each pair corresponds to one CNT device) are shown in Fig. 5.1. It can be seen that the color of the channel is different from that of the substrate because of the thin air film. Since CNTs were grown randomly, in most pairs of electrodes, there were actually no tube in between. On average, for one chip with 22 pairs of electrodes, there were no more than two pairs connected with CNT(s). Also with tube density this low, it was extremely unlikely to have more than one tube connecting the electrodes.

One of the advantage of a sealed device is the ability of exchanging the liquid. In Fig. 5.2, we demonstrate the conductivity of a CNT device increased while DI water was pushed into the Pyrex-sealed channel. The cause of the conductance change is a combination of the following: the conduction of the water itself, the creation of water-SiO$_2$ interface induces the surface charge that gates the tube, and the interaction between water and CNT that increases the tube conductivity. Except the last factor, which is considered limited and the sign is unclear, the other two should increase the conductivity as shown, with the semiconducting CNT contacted by Cr/Au are naturally slightly n-type because of the band bending caused by the mismatch in chemical potentials.

Another concern about the process is the heating during anodic bonding might affect the electronic properties. Although not measured as identical, CNT devices
retain its semiconducting properties after the bonding, as shown in Fig. 5.3.

Since every tube is different, it is hard to speak generally what effect DNA molecules have on every individual tube. The characteristic curves from one semiconducting device are shown in Fig. 5.4. The green plot, a CNT device with the channel filled with DI water, shows a clear bandgap centered at $V_G \approx -1V$ with lower conductance, and the conductance raises significantly around $V_G \approx 1$ and $-3V$.

It can be observed that, when DNA is added to the solution, the blue plot, has more differences in the positive gate voltage side of the bandgap. The detailed mechanism is not completely understood, but we suspect it is related to the negative charges on DNA. One possibility is that more DNA molecules are attracted to the device surface, therefore more DNA molecules surround the tube, and the negative charges from DNA neutralize the effects from the positive bias on the back silicon gate. On the other hand, when a negative gate voltage is applied, DNA molecules are repelled from the surface, therefore the gate scan renders almost the same characteristics as just DI water.
Figure 5.3: $I_{DS}$-$V_G$ curve of a semiconducting CNT device after anodic bonding process. Since a semiconducting tube is n-type because of the contact chemical potential, the bandgap appears at the negative gate bias. Since we stop the scan at $\pm 3$V, only one edge of the gap is shown.

Figure 5.4: $I_{DS}$-$V_G$ curves of a semiconducting CNT device, before and after the addition of DNA into the solution. ssDNA sequence as 5'-GAG TCT GTG GAG GAG GTA GTC-3'.
5.2 Parylene nanochannel with nano electrodes

To achieve electronic length measurement, there were several challenges to overcome, provided with what we observed in the CNT-microchannel device. First of all, we have to push the channel size small enough so DNA molecules are elongated in the channel. Although it is possible to switch the SiO$_x$ lift-off process to EBL, which enables the nanochannels, the smoothness of the evaporated SiO$_x$ and the pinholes and other structural defects in the film significantly deteriorate the quality of the channels. To solve the problem, we decided to first build our electronics on top of the wafer, then construct the channels with a sacrificial layer. The concept is somehow similar to Ref. [147], but the exhaust produced in Ref. [147] is hazardous, limiting the processes at locations with special ventilation systems. Instead, we developed a way to define the nanochannels by a PMMA sacrificial layer patterned by EBL, then cover it with a transparent layer, and later remove the PMMA to form the channels. For the transparent capping layer, we unsuccessfully tried evaporated silicon oxide (SiO$_x$) and silicon nitride (Si$_3$N$_4$). The tests on oxide eventually failed because of the defects. PECVD of silicon nitride can be used to provide a conformal film [148], but since polymers are generally not allowed in a PECVD chamber, we need to use a metal sacrificial layer instead, which guarantees a much slower removal, and creates a major difficulty for our overall process. We tried to perform nitride coating with thermal evaporation, but still failed to obtain a good quality conformal film. We eventually settled with parylene C for our capping layer. As described in Subsection 3.1.6, deposition of parylene is done in a room temperature chamber, which is compatible with almost all possible previous fabrication processes. Detail of such a construction will be discussed in Subsection 5.2.1.

Other than the channel sizes, the sensing devices made of CNTs are not ideal either. As detailed in Subsection 3.1.4, CVD growing of CNT is a random process, and the location for the tube crossing the channel is completely uncontrollable and
unpredictable. Not only the locations, the variations in the electronic properties of CNTs is another challenge for a wide range of applications. Moreover, once a nanochannel is aligned with a CNT device, when a DNA molecule is going across the tube, only a tiny portion of the tube is exposed to the DNA\(^1\), not to mention only a small portion of DNA overlaps with the tube, which means much less negative charges are influencing the electronic properties of the tube. Although the effects should still be there, they may not be big enough for daily applications. Other than the scientific reasons, since we do not own a CNT CVD furnace our own, we have to rely on other groups to provide us the CNT samples. We tried to work with three different laboratories for that purpose, but our source of CVD grown CNTs has never been truly stable and reliable. With our collaborator, John Mannion of Craighead Group at Cornell, working on sensing by CNTs in a channel, we moved our focus toward EBL made metal nano lines, whose location can be precisely controlled within 30 nm, and can be designed to measure the properties of the solution in the channel instead of the wire itself.

Overall sensing mechanism is also a concern. Although FETs have been a successful sensing device, for the reasons stated above, the combination with nanochannel brings more disadvantages than the advantages on the sensing part. For a successful sensing with nano electrodes, we considered the following structures as possible candidates: (a) a pair of aligned electrodes with a nano gap in the channel; (b) combination of a nanopore and nanochannel, so we utilize a nanochannel to elongate the DNA, and measure the current across the pore, and (c) two electrodes immersed in the channel, as shown in Fig. 5.5. Theoretical studies have shown that the configuration in Fig. 5.5 (a) yields different conductance signatures for different bases [149, 150], but the electrode-electrode spacing of 1.5 nm in the theoretical studies is not immediately

\(^1\)A typical separation between two contact electrodes is 1-2 \(\mu\)m, and the channel width for a reasonable elongation (70%) should be 100 nm or smaller. As a result, only less than 10% of the length of the tube is affected by the DNA molecule.
realistic for our fabrication technologies. Besides the Chou group in Department of Electrical Engineering and Riehn Group at North Carolina State University are also pursuing detection with this configuration, so we decided to explore other opportunities. The setup in Fig. 5.5 (b) solves the entropic coiling and short translocation time problems of nanopore, as discussed in Subsection 2.2.2. Two electrodes immersed in the channel are used to measure the current only through the pore, so we do not need to measure the current all along the channel, which may smear out the signal at the pore. To do that, we need the exact same technology to place metallic nano electrodes in a nanochannel as shown in Fig. 5.5 (c). In (c), we place two electrodes to measure the solution in the channel. As discussed in Subsection 2.2.4, the concentration of bases when a DNA molecule confined in a nanochannel in range of mM is high enough for an electrochemical measurement, we decided to explore the technology for the fabrication of a device described in Fig. 5.5 (c) first, which also lays down the ground for a pore-in-a-channel device.
5.2.1 Device fabrication

Fabrication of this device is a long process, including three photolithography layers and two EBL ones, two RIE and two lift-off steps. The fabrication processes are schematically shown in Fig. 5.6.

To make EBL an easier process, we started with an oxidated silicon wafer (100 nm dry thermal oxide), and we put photolithography alignment marks down by photolithography and RIE, followed by photolithography and Cr/Au (5 nm of Cr and 95 nm of Au) lift-off for micro electrodes. Since the EBL nano electrodes were supposed to be aligned with the micro contacts, e-beam alignment marks were also on the same layer.

We next employed EBL for the 30-nm-wide line pairs. We exposed the line region and made the lines by lift-off. Since the lines were going under the channel, thickness of the metal was controlled at 25 nm, compared with 100 nm of channel height. It was difficult to perform lift-off with metal thinner than this. At this point, all the electronics related components were already fabricated.

We then laid down the channels by a PMMA sacrificial layer. For doing that, we exposed the area surrounding the channels, but left the channels themselves unexposed, so after development PMMA stayed at where the channels were supposed to be. We demonstrated that 100 nm thick of gold patterns have no negative effects on the EBL, has limited influence, if any, on spinning PMMA resist evenly and the secondary electrons distribution during e-beam writing.

Conceptually, we next coat the device with parylene, then remove the PMMA to form the channels. There are two technical challenges for doing that. First, parylene is hydrophobic, so we evaporated SiO$_x$ from both side angles so the channel walls were mostly made of hydrophilic SiO$_x$. Then, since parylene does not stick strongly with the wafer, which results in a channel peel off in later stage, we silanized the wafer with vinyl trichlorosilane right before the parylene coating.
Figure 5.6: Fabrication processes for parylene C sealed nanochannels integrated with EBL nano line pairs devices.
For the removal of PMMA, we first needed to punch through the parylene film so the outside solvent can access PMMA. We etched parylene by oxygen RIE, and the etching process was masked by a layer of photoresist. The etch rate for parylene C and photoresist was nearly 1:1, therefore we needed a photoresist layer thicker than our parylene. After O₂ RIE on parylene, we also needed a short 1:10 buffered oxide etchant dip to remove the evaporated SiOₓ. To ensure the access holes are clear, we performed another short O₂ RIE, so the blue color of 100 nm oxide on silicon wafer showed. The access holes were later used to fill liquid into the channels.

We used solvent to remove (dissolve) PMMA from the channel area, with a three-step soak, consisted with warm (60°C hotplate) Remover 1165, room-temperature 1,2-dichloroethane and room-temperature acetone. The channels were later dried by a critical point dryer, and the acetone also served as the medium for the drying process, since acetone mixes well with liquid carbon dioxide (CO₂). During the process, we used pressurized liquid CO₂ to replace acetone, then heated the system so that liquid CO₂ reaches its critical point, where liquid and gas phases are no different, then slowly released the gaseous CO₂. We used this process to avoid the surface tension to collapse our channels during the drying. Since the refractive index of air is significantly different from that of parylene, we could clearly see color difference after PMMA was removed from the channels. Images of the device at different fabrication steps are shown in Fig. 5.7.

After PMMA removal, we needed to re-wet the channels with water based solution. Since there was a hydrophobic parylene barrier at the access holes, it was common that a bubble blocks the channels for wetting. We could either use a vacuum pump to remove the bubble, or let the chip go through a critical point wetting process [151]. In the later case, we first immersed the chip in water and placed it between two flanges, which were later sealed with a copper o-ring. The whole cell was tightly sealed with the capacity to hold high pressure. We then heated the whole cell in an oven to
Figure 5.7: Images of devices at different steps. (a) SEM image of a pair of 30 nm wide metal nano lines; (b) AFM image of a ∼120 nm thick PMMA sacrificial layer on top of the nano lines; (c) optical micrograph of a parylene stripe covering PMMA sacrificial layer; (d) parylene channels after PMMA removal, the color change from (c) is obvious.
Figure 5.8: Epi-fluorescence image of fluorescent dye in a nanochannel (bright horizontal line at the center) with electrodes designs.

water’s critical point, when water vapor was no different from the liquid water, and the vapor could easily occupy the volume where used to be air. As a result, when the cell was cooled down, the water was filled in the channel.

5.2.2 Results

The realization of electronic devices integrated with auto-sealed nanochannels is a big step forward in the overall progress, and we demonstrated the capability of making it happen. We took a pair of metal lines for example, but since the chip was not subject to any extreme condition such as high temperature PECVD process, our fabrication process does little damage to the electronics on the chip, and is compatible with any surface electronics that leaves the surface mostly flat. Although the parylene and PMMA removal are specialized steps, they can be applied onto the standardized CMOS chips.

In Fig. 5.8, we demonstrate an epi-fluorescence micrograph of a parylene channel
Figure 5.9: Impedance of the liquid between one end of the channel to the other vs. ac frequencies. The impedance of TBE solution is about $10^3$ larger than that of bulk TBE solution, but processes the same frequency response. That result shows the electric current is going through a clean TBE solution in a small channel.

on top of a series of gold electrodes. Fluorescent dye apparently glowed in the channel, which is the line in the middle, showing our channel is continuous. It can be seen that the auto-fluorescence from the parylene strip is no brighter than the signals from the gold. Since our point is to integrate nanochannels with electrodes, metal structures are inevitable on the surface. Although they are less likely to fluoresce, the reflection from the metal may easily leak through the dichroic mirror in the epi-fluorescence microscopy set-up, resulting brighter area on the metal areas.

We also performed the impedance measurements between two ends of the channels, which is shown in Fig. 5.9. In the plot, it is shown that the Tris/Borate/EDTA (TBE) buffer solution in the channel processes the same curve shape as in bulk. Since electrochemical impedance spectroscopy has been established as a way to identify minor chemical differences such as single base mismatch at distal end of DNA duplex [18], this result has two significances. First, since the impedance stopped dropping around 300 Hz, it is self-evident that the current we measured is not the ac capacitive
current through silicon oxide, but from the TBE solution in the channel. Second, the identical shape indicated that the liquid inside the channel was indeed TBE buffer, and no chemical residual from the PMMA removal steps left any observable trace. This is particularly valuable as a proof of principle on our PMMA sacrificial layer removal processes.

The results of measurements from the nanoelectrodes embedded in the nanochannels are shown in Fig. 5.10. Although an electrode in an electrolyte solution can be well modeled, our system is much more complicated than that since it was built on a silicon substrate, especially given we tried to ground the silicon back gate, and the silicon wafer remains to have a 1-100 ohm-cm of resistivity. As a result, a capacitive current dominates the conduction in higher frequencies, although the grounding of back silicon has already significantly reduced the capacitive current. Although we do not fully understand the details about the curve shapes, we would like to point out that different solutions do yield different shapes in $I$-$\omega$ plots.
Chapter 6

Future Perspectives

In this Chapter, we discuss the possibility of the techniques developed in this dissertation to have a real impact in the future biological diagnostics. The idea of using an integrated microfluidic chip, or what has been called microfluidic large-scale integration (mLSI), has been seen as a candidate to be the new generation of automation experiment paradigm [152]. More than building micromechanical control elements such as valves, electronic measurements have been used with microfluidics to perform detection and sorting [153]. At the same time, a lot of interesting experiments have been done with nanofluidics (see Section 2.3), but the ability to perform electrical measurements with nanofluidics is highly limited by the sealing process. Since we integrate the electrodes with a self-sealed nanochannel, and the self-sealed channels can be built onto any relatively flat surface electronics, the problem of integration is solved, and the next step is to find a scientifically interesting target to study with our technology. In the short term, a device to perform DNA length measurements can be made, and we will discuss that in Section 6.1. The ultimate goal is to have an integrated device, that can do genomic analysis with some cells input (such as blood cells). We will discuss the prospect on that in Section 6.2.
6.1 Precise electrical DNA length measurement

With single-molecule fluorescence microscopy, the ability for a nanochannel to perform a DNA length measurement that beats the optical diffraction limit is now with no dispute [5]. For an electronic device embedded in the nanochannel, which can distinguish whether DNA strand is present on top of it and yield an on/off kind of signals, the length measurements can be achieved by an array of such devices, as shown in Fig. 6.1 (a). In this set-up, the size of the devices and the separation between them replace the diffraction limit as the limiting factor for the measurement resolution. In the semiconductor industry, 32 nm process has been demonstrated, and 45 nm process has been used to mass produce some processors, e.g. Intel Core 2 Duo T5000/T7000. Please be noted that a 45 nm process means the average half-pitch is 45 nm, the actual features may have line width smaller than that [154]. For a rough estimate, that enables a length measurement with resolution in the 30-50 nm range, which is already a 5 fold improvement from the $\sim$150-200 nm diffraction limit. To achieve single base pair resolution, it will take roughly 10,000 measurements. Assuming three samplings can be done in one second, it will take nearly one hour of sampling to achieve the single-base-pair resolution. If the spacing between the devices can be improved to 10 nm, which is conservatively predicted by Ref. [154] to be com-
mercialized in 2022, the time scale for a precise length measurement for a genomic length DNA can be reduced to the range of 20 minutes. This may not appear as the fastest measurement that can be done in a laboratory, but for that time it can be told that whether any migration of genes happened, in your body or your experimental system! Given the genetic variations come from the transposition of genes happen much more often than a point mutation, this technology can be effectively used to monitor the genetic change in the evolutionary dynamics.

Furthermore, there is no fundamental reason to believe that a sensing device can only provide an on/off signal. For electrodes with 100 nm separation in a 100 nm x 100 nm channel, one single basepair means a 3 mM change of the base concentration in that volume. If the channel size is reduced to 10 nm x 20 nm as reported in Ref. [107], that single basepair is equivalent to 0.8 M of base concentration change. As a result, the average properties of the solution in that confined volume should be very different. In that case, we should employ a measurement scheme shown in Fig. 6.1 (b). In that situation, we have the both ends of the DNA fall into two pairs of electrodes, and we should be able to read the number of basepairs of DNA fall within the two electrodes in that pair. To be sure the measurement is not done with only two pairs of electrodes, but a whole series of them, with most of them with DNA occupying the full length of the channel between the electrodes or no DNA in between at all, and only two pairs are actively used for single-basepair length measurements. In this case, without multiple measurements, the resolution is related to the linewidth of the electrodes. For a 4.5 nm electrode, only 225 measurements are needed to achieve single-basepair resolution, which means the measurements can be completed in two minutes. If the future technology would allow us to align CVD grown nanotubes or nanowires precisely, the width of the electrodes can be further reduced by a factor of 5, and the time for a single-basepair resolution measurement will be reduced to seconds.
To be clear we do not suggest parylene capped self-sealed nanochannels can be pushed into the 20 nm range. To achieve that kind of feature size with the same self-sealed techniques, PECVD silicon nitride is likely to be the better material in the application. The problems of the high temperature PECVD chamber and metal sacrificial layer is still up to be solved, but the opportunity is certainly there. And please be aware that 20 nm nanochannel is not required in the high precision length measurement. It is mentioned for a possibility to speed up the measurements.

6.2 A fully integrated genomic analysis tool

To be sure, in a real application, the input of the genomic material is not purified DNA molecules, but the cells from the evolutionary experiments, or a mixture of different kinds of cells, such as blood. To extract the genetic content from those cells, some early stages of processing are required. We will briefly discuss how to achieve a single diagnostic tool in this section.

For a blood sample, the first step is to separate the white blood cells, or leukocytes, from the whole blood, since white blood cells are the typical DNA sources for Human Genome Project [24]. Blood content is traditionally separated by different densities of the components, which is typically done with a centrifuge. Microfluidic devices have recently been used for blood separation [155, 156]. Utilizing the leukocyte margination and plasma skimming, a 34-fold enrichment of the leukocyte-to-erythrocyte ratio can be achieved [155]. To achieve a complete fractionation, the separation can be done with a microfluidic device called bump array [156]. With a series of posts with well-designed separation and arrangement, cells (or particles in general) with different sizes can be separated by the hydrodynamic flow. In this way, we can separate white blood cells from the rest of the blood content.

Once the white blood cells are obtained, the next step is to extract the genomic
content from the cells. By mixing the lysis buffer (containing 4 M GuSCN in TE buffer with 1% Triton-X 100, titrated to pH 6.7) with whole blood in a microfluidic system, cells can gradually lyzed and genomic DNA can be released [157]. Since a bump array can be used to steer, refract, and focus streams of biomaterials [158], it can also be used to lyze cells and direct DNA molecules to the next stage of analysis tool.

Since human genome DNA is about one meter long, it is necessary to cut the DNA molecule into shorter pieces by restriction enzymes for further analysis. If the restriction cutting (or restriction digest) happens in a bulk solution, we will have difficulties to distinguish different pieces of DNA since they might have similar lengths, and the length measurement is not all that informational when we do not know which piece of DNA we are looking at. Fortunately, the restriction digest can be carried out in a nanochannel [4]. By doing that, we can measure the length of DNA pieces in sequence to their appearance in the whole genome, and if a segment of DNA is transposed to somewhere else, it can be easily spotted.

Although it will certainly be a difficult job to produce such an integrated micro/nano fluidic chip in an inexpensive manner, the development of the micro/nano fluidics in the past couple years has make the one-piece DNA analysis tool a real possibility. Such a diagnostic tool will be very useful for either medical or scientific purposes.
Bibliography


