DESIGN, FABRICATION, AND CHARACTERIZATION OF FIELD-EFFECT AND IMPEDANCE BASED BIOSENSORS

DISSERTATION

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ABSTRACT

Highly sensitive biological sensors are important to the development of biological and medical science. The purpose of this work is to develop highly sensitive AlGaN/GaN heterostructure field-effect transistors (HFETs) and silicon on insulator (SOI) nanowire biosensors. Impedance based lipid membrane characterization is also discussed.

Due to chemical inertness in biological buffer solutions and highly localized carriers, AlGaN/GaN heterostructures are ideal for high-sensitivity field-effect biosensors. The AlGaN/GaN HFET biosensor is firstly designed based on modification of conventional AlGaN/GaN heterostructure high electron mobility transistors (HEMTs) by substituting the metal gate electrode for biomolecule immobilization (ssDNA or ssPNA for ssDNA detection and antibody for protein detection) and the formation of a reservoir for applying solutions. A silanization and biotinylation procedure was developed to immobilize the streptavidin (SA) on the AlGaN surface. The devices show reasonable performance prior to any optimization. With feasibility demonstrated, the device sensitivity is further improved in three aspects. The first is to optimize AlGaN oxidization methods. Inductively coupled plasma (ICP) plasma has been found to produce the highest surface protein coverage and the best electrical properties (i.e. less surface trap density). The second is to operate devices in the subthreshold regime. In this regime, the drain current versus the gate voltage follows a semi-log relationship. The biomolecule introduced an effective voltage shift that results in much higher current change. The results with subthreshold regime
operation have shown a sensitivity improvement of seven orders of magnitude. The third method is to recess the AlGaN barrier so that a much smaller gate voltage is necessary to bias the device at the subthreshold regime. With this strategy, the noise induced by the gate current and ion movements is reduced while signal-to-noise ratio is increased. The subthreshold swing is 74.4 mV/decade, which is largely improved. The SA detection limit is lowered one order of magnitude compared to the subthreshold regime operation.

To extend the application of AlGaN/GaN protein sensors, anti monokine-induced interferon gamma (MIG) IgG is immobilized on silanized AlGaN surfaces for MIG detection. The sensors have shown reasonable detection limits for clinical applications. To model and improve the device performance, a two-dimensional analysis has been developed for planar AlGaN/GaN biosensors. Because analytical solutions are not available, numerical simulations are needed.

Besides the AlGaN/GaN heterostructure, an SOI structure was also developed for nanowire biosensors. To avoid ion drifting in silicon dioxide, oxide-free surface modification process was developed and characterized for better chemical stability in biological buffer. For fabrication, e-beam lithography and plasma dry etching processing have been developed. The minimum nanowire width is 30 nm. Theoretical analysis has been developed for modeling ideal three-dimensional cylindrical nanowires. Numerical simulations with Silvaco software were used to verify the effects of device dimension and the doping level. Both theoretical and numerical simulation show that the nanowires with lower doping levels, smaller widths (or diameters) have higher sensitivities. Theoretical analysis also shows that lower buffer ionic concentration has higher sensitivity. Numerical modeling shows that longer nanowire widths have higher sensitivity.
In addition to the field-effect biosensors, impedance based measurements are very sensitive to the surface molecular structure change. Electrochemical impedance spectroscopy (EIS) was used to characterize the qualities of tethered bilayer lipid membranes (tBLMs) on planar gold surfaces and gold surfaces with nanopores. Nanopores fabricated from top-down technologies are with well-defined shapes and dimensions, which benefits the understanding of dimension-related EIS characteristics. tBLMs with artificially-introduced defects were characterized to simulate the channel opening in the cell membranes. Equivalent circuit models have been developed to explain the EIS behavior of gold surfaces with well defined nanopores.

In this work, field-effect AlGaN/GaN HFET biosensors have been designed for the detection of proteins. With the optimization of oxidization methods and operating the device in subthreshold regime, the sensitivity is largely improved. SOI based nanowire biosensors are also being developed. The fabrication process and the surface modification procedure have been established. Theoretical and numerical analysis have been developed to predict and improve the device performance. Besides, EIS characterizations of tBLMs with well-defined nanopores are developed to study the cell membrane channel opening, which will be used in drug/gene delivery applications.
This is dedicated to the my dearest wife and parents.
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CHAPTER 1

Introduction

1.1 Definition of Biosensors

A sensor is a device that measures/responds to a physical quantity (heat, light, sound, pressure, motion, etc.) and converts it into a signal which can be read/processed by an observer or by an instrument. Sensors have a wide variety of applications, ranging from daily use, such as humidity and temperature sensors, to research labs, such as biomarkers for cardiac and infectious diseases as well as cancers, to military applications, such as infrared night vision goggles.

Chemical and biological sensors (referred as biosensors hereon) are more complex extensions of physical sensors. Biosensors are defined as measurement devices which utilize chemical or biological reactions to detect and quantify a specific analyte or event.

Qualification and quantification of chemical and biological processes are of great importance for life science and pharmacy. Biosensors have been receiving tremendous research efforts due to the capability of converting biological information, such as the concentration of biological species and reaction rate, into signals that people can read. Biosensors are important in monitoring environmental contaminants, identifying toxicity and biological contaminants in food and pharmaceuticals, and security and biodefense for public safety. The market demand for chemical and biological sensors keeps growing in the last
few years. Market revenue increased to $12.5 billion in 2009 and is estimated to reach $19.0 billion in 2016 [6]. For chemical sensors, the US market demand is expected to rise 8.6% annually through 2014 [7].

1.2 Principles of Field-Effect Electrical Biosensors

There are different biosensing mechanisms such as fluorescent imaging [8], surface plasmon resonance (SPR) [9], ellipsometry [10], quartz crystal microbalance (QCM) [11], ionic channel [12], micro-/nano-electromechanical system (MEMS/NEMS) [13, 14], scanning probe microscopy (SPM) [15] and electrical methods [16, 17, 18, 19, 20].

The working mechanism of electrical biosensors is to identify or quantify chemical and biological species or processes by measuring and amplifying related electrical signals, such as charge, electrical field, or current. Due to the inherent convenient interface to subsequent reading and processing units, electrical biosensors received tremendous research interests especially in the last two decades. With the help of well-developed microelectronic technology, and emergent material and nanotechnologies, electrical biological sensors are being developed for high sensitivity, low cost, real-time, and clinical-oriented applications.

The components and working flow of electrical biosensors are shown in Fig. 1.1. An electrical biosensor system includes analytes, a biological/electrical interface, a transducer unit, a signal processing unit, and a display unit. The analytes are extracted from objects to be monitored (such as tissues and blood from patients) and pre-treated (such as purification and concentrating). The specific segments from the analytes, such as proteins and nucleic acid, are captured at the biological/electrical interface, and the transducer detects the capture processes by providing an electrical signal. The signal is then amplified and processed by the processing unit, and the detection result is displayed to the end-user.
The focus of this work is to develop the biological/electrical interface and the transducer unit. Categorized by the signal capture methods, the electrical biosensors can work in \textit{amperometric} mode, which is to measure the generation of current; the \textit{potentiometric} mode, which is to measure potential of charge accumulation; the \textit{conductometric} mode, which is to measure the conductance change between electrodes; the \textit{impedimetric} mode, which is to measure the change of impedance, including resistance and reactance \cite{5, 21}; and the \textit{field-effect} mode, which uses the transistor mechanism to measure the effective gate voltage modulation on the source/drain current \cite{17, 20, 22}.

In this work, research efforts are focused on developing high sensitivity AlGaN/GaN and silicon-on-insulator (SOI) based field-effect biosensors and impedance based membrane characterization methods.
1.2.1 The Electron Energy Levels of Aqueous Solutions

To utilize semiconductors for the purpose of field-effect biosensors, it is important to understand the electronic properties of aqueous solutions and organic molecules. This section provides a brief overview of the electron energy levels for liquid water and biomolecules.

The Band-Diagram of Liquid Water

Similar to semiconductors, liquid water has conduction and valence bands [23]. However, due to the movements of water molecules in liquid phase and the non-crystal molecule structure, the band-diagram of liquid water can not be accurately calculated or measured. Fig. 1.2 shows a set of calculated results. As shown, the band edge definition is not as clear as crystalized solid materials such as Si and Au.
The Electrons Energy Level of Aqueous Solutions

Ions in water solution can be oxidized or reduced. The reaction can be expressed as

\[ \text{Red} \rightleftharpoons \text{Ox} + e_{\text{RedOx}}. \] (1.1)

The energy levels of electrons and ions in liquid solutions fluctuate in the range of a reorganization energy \( \lambda \) because of thermal vibration. The electron levels of oxidants and reductants in aqueous solutions are discrete energy levels, similar to the donor and acceptor energy levels in semiconductors. However, due to ion movements and interactions with surrounding ions and molecules, the discrete energy levels expand to energy bands. The distribution of density vs. energy level follows a Gaussian distribution. The most occupied electron levels of reductants (donors) and oxidants (acceptors) are \( \epsilon_{\text{Ox}} \) and \( \epsilon_{\text{Red}} \) respectively.

The electron levels follow a Gaussian normal distribution \[ W(\epsilon) = \frac{1}{\sqrt{4\lambda kT}} \exp\left(\frac{-(\epsilon - \epsilon_0)}{4\lambda kT}\right). \] (1.2)

The electron density of states of the reductants and acceptors can be obtained by the product of the probability distribution and concentrations \( N_{\text{Red}} \) and \( N_{\text{Ox}} \)

\[ D_{\text{Red}}(\epsilon) = W_{\text{Red}}(\epsilon)N_{\text{Red}} = \frac{N_{\text{Red}}}{\sqrt{4\lambda kT}} \exp\left(\frac{-(\epsilon - \epsilon_0)}{4\lambda kT}\right), \] (1.3)

\[ D_{\text{Ox}}(\epsilon) = W_{\text{Ox}}(\epsilon)N_{\text{Ox}} = \frac{N_{\text{Ox}}}{\sqrt{4\lambda kT}} \exp\left(\frac{-(\epsilon - \epsilon_0)}{4\lambda kT}\right). \] (1.4)

Fig. 1.3 shows a schematic of electron density states of a RedOx system.

The energy level \( \epsilon_F \) at which the donor state density equals the acceptor state density is called Fermi level of the redox electron. Combining Equations (1.3) and (1.4) results in

\[ \epsilon_F(\text{RedOx}) = \frac{1}{2}(\epsilon_{\text{Ox}} + \epsilon_{\text{Red}}) + kT \ln \frac{N_{\text{Red}}}{N_{\text{Ox}}} = \epsilon_0^F(\text{RedOx}) + kT \ln \frac{N_{\text{Red}}}{N_{\text{Ox}}}, \] (1.5)

where \( \epsilon_0^F(\text{RedOx}) \) is the standard Fermi level, at which \( N_{\text{Red}} = N_{\text{Ox}} \).
1.2.2 The Electron Energy Level of Organic Molecules

Similar to the valence and conduction bands of semiconductors, the highest occupied and lowest unoccupied molecular orbits are defined as HOMO and LUMO, respectively. The electron orbits of molecules can be in a way similar to the band diagram engineering of semiconductors. The electron affinity and ionization potential of molecules have similar effects to semiconductor doping. Conductive polymer chains can be used as Ohmic material and interconnections.

1.2.3 The Electrical Double Layer

During the process of integrating solid state devices with aqueous solutions for the purpose of electrical biosensors, new phenomena arise at the solid/liquid interfaces. Driven by electrostatic force and concentration gradient, ions and molecules can be adsorbed onto or repelled from the solid surface, which results in the formation of a electrical double
Figure 1.4: An EDL model of a metal/aqueous solution interface [21]. Anions are selectively adsorbed on the metal surface. $x_1$ and $x_2$ are the locations of the inner Helmholtz plane (IHP) and outer Helmholtz plane (OHP) respectively. $\phi$ is the potential, and $q^M$, $\sigma^i$, and $\sigma^d$ are the charge densities at the metal surface, in the inner Helmholtz layer, and the rest regions.

layer (EDL). EDL is important to electrical biosensors because selective adsorption of target biomolecules occurs at the solid/liquid interface. This section gives a brief review of EDL to complete the understanding of the electrical properties of the biosensor system, consisting of the aqueous solution, the solid/liquid interface, and the solid.

**The Electrical Double Layer Structure**

As shown in Fig. 1.4 [21] (slightly modified), the double layer contains an inner layer (also named compact, Stern, or Helmholtz layer) with specifically adsorbed on the solid surface, and an outer layer (also named a diffusion layer).
The Potential Distribution of EDL

Consider an ion species \( i \) in the aqueous solution side of an EDL as shown in Fig. 1.4. The non-uniformity of electrostatic potential \( \phi \) in the solution along the \( x \) direction causes charge density differences, following a Boltzmann distribution

\[
\rho(x) = \sum_i n_i^0 z_i q \exp\left(-\frac{z_i q \phi}{kT}\right),
\]

(1.6)

where \( n_i^0 \) is the ion bulk concentration, \( z_i \) is the signed charge per ion, \( q \) is the charge of a positive electron, \( k \) is the Boltzmann’s constant, and \( T \) is the absolute temperature.

From electrostatics, the charge density is related to the electrostatic potential by Poisson’s equation

\[
\rho(x) = -\epsilon \epsilon_0 \frac{d^2 \phi}{dx^2},
\]

(1.7)

where \( \epsilon \) is the dielectric number of the solution and \( \epsilon_0 \) are the dielectric constant of the vacuum. Combining Equations (1.6) and (1.7) results in a Poisson-Boltzmann equation for the aqueous solution

\[
\frac{d^2 \phi}{dx^2} = -\frac{e}{\epsilon \epsilon_0} \sum_i n_i^0 z_i q \exp\left(-\frac{z_i q \phi}{kT}\right).
\]

(1.8)

Fig. 1.5 shows an example of the potential distribution across a semiconductor/aqueous solution interface [25].

1.2.4 Charges of Biomolecules

Biological molecules, such as single-strand Deoxyribonucleic acid (ss-DNA), in physiological buffers carry positive or negative charges. Amphoteric molecules such as proteins carry both positive and negative charges at different segments. The net charge of protein molecules varies with the environmental pH values. The pH value, at which the net charge
of the molecule reaches zero, is defined as an isoelectric point (pI). Similarly, the pH value at which the net charge at the EDL reaches zero is defined as the pI point of the interface.

### 1.2.5 The Working Principle of ChemFETs

As shown in Equation (1.8), when the charge distribution changes in the EDL, the potential distribution also changes. If the change of potential occurs at the gate area of a transistor, the potential change will modulate device $I_d - V_d$ characteristics. If the gate area of a transistor is functionalized to specifically immobilize a certain type of chemical/gase/molecule, the device characteristics will respond when the specific adsorption happens. This is the working principle of a Chemical field effect transistor (ChemFET).

When the gate area of a ChemFET is modified to be sensitive to a certain ion, it is defined as a ion-sensitive field-effect transistor (ISFET). For example, SiO$_2$, Si$_3$N$_2$, Al$_2$O$_3$, and Ta$_2$O$_5$ are used for the measurement of pH values (the concentration of H$^+$) [26, 27].
Similarly, when the gate surface of a FET is functionalized to detect specific biomolecules with enzymes, or a certain type of biomolecules, the device is referred as an ENFET, or a bioFET. ISFETs, ENFETs, and bioFETs are subtypes of ChemFETs.

1.2.6 The Working Principle of Impedance Methods

When an AC voltage (e.g., a sine wave) is applied to a circuit, if the electrons cannot simultaneously follow the change of electrical, there is a phase different between the applied electrical field and the measured current. Impedance is defined as the ratio of the applied voltage and the measured current, which has an imaginary term. The phase delay is resulted from the contributions of capacitors and inductors, which have a $\pm 90^\circ$ difference from a pure resistor in the complex plane.

When an electrical field is applied at an electrode/aqueous solution interface, there could be two different types of currents [21]. If there are charges transporting through the interface, there will be reduction and/or oxidization reactions. This type of process is called a faradiac process or a charge transfer process. This type of current is called faradaic current. The electrode/solution interface structure could change through adsorbing and/or desorption of ions on the solution side and forming an accumulation or a depletion layer on the electrode side. This process is called a nonfaradaic process, and the current charges the EDL on the solution side and the accumulation or depletion layer on the electrode side. The electrode/solution interfaces with nonfaradaic processes behave analogously to capacitors.

With applying a series of single-frequency stimulations or a multi-frequency stimulation, the impedance of an electrode/aqueous solution system can be measured and analyzed. As will be discussed in Chapter 5, the impedance contribution from individual components
of the system (such as the charge transfer resistance and the EDL capacitance) can be extracted from a measured impedance spectra and an equivalent circuit. Because EDL is sensitive to the change of the electrode surface structure, the impedance method can be utilized to monitor the adsorption/desorption processes on electrode surface.

1.3 Analytes

The primary interest of biosensors is to detect specific biological species and reaction processes for research and diagnosis purposes. Electrical biosensors have shown huge potential for future clinical applications due to a demonstrated capability of detecting fundamental biological molecules. Through different approaches, electrical biosensors have proved successful for the detection of a variety of analytes. These analytes include $\text{H}^+$ for pH values [28, 29, 30], polar liquids [31], ionic solutions [32, 33, 34], protein [29, 30, 35, 36], DNA [29, 37], enzyme [38, 39], cells [40, 41, 42, 43] and viruses [44].

1.4 Transducer Material

The selection of transducer material is critical for electrical biosensors. The materials need to fit the needs for high sensitivity performance and to be compatible with available fabrication technologies. Also the materials need to be stable in biological environments and to have good interface to biological molecules. People have been using silicon as biosensor material for the convenience of sophisticated silicon processing technologies [30, 33, 45, 46]. The development of semiconductor material technology has introduced more candidates for electrical biosensors, such as GaN [28, 31, 35, 36, 40, 43, 47], Al-GaAs [48], ZnO [49] and graphene [50]. Due to the emergent nanotechnology, bottom-up carbon nanotubes or semiconductor nanowires have been demonstrated to be applicable...
in biosensing with high sensitivities [51, 52, 53, 54, 55, 56, 57]. With the development of organic electronics, a number of conductive polymers have been used as low cost and disposable candidate materials for electrical biosensors [22].

1.5 Surface Modification

The surface modification step is the biological/electrical interface. Without modifying the transducer surface, the sensor shows inherent ion selectivity due to the native transducer material properties. For example, (Al)GaN shows native selectivity to anions due to positively charged surface [32, 34]. To be designed for specific target ions, the sensing area of transducer must be modified. For pH value detection, people have been using oxynitride [33], glass, Si$_3$N$_4$, Ta$_2$O$_5$, and other oxide films [58] to detect H$^+$. Most of the transducer materials listed above are inorganic, which is not natively biocompatible. Some of them even could be toxic to biological species, e.g. native arsenic oxide. Hence, the surface preparation is crucial for the attachment of biological species [59].

To achieve biocompatibility on different transducer material, there are a few frequently used methods. Firstly, by physical adsorption. The transducer surface can physically adsorb the first linker molecule layer. For example, people demonstrated the deposition of DNA molecules onto GaN surface by physical adsorption [60]. The drawback of this method is that the uniformity and coverage is not well-controlled. It is not widely used for biosensors. Secondly, by covalent binding. For silicon, the surface can be oxidized and linked to saline molecules through valence bonding [35, 36]. H-terminated diamond surface can be used to bind with unsaturated amine by UV illumination [61, 62]. Thirdly, by forming self-assembly monolayers (SAMs). The transducer surface can be deposited with gold film
and thiol-gold chemistry can be used to bind the first link molecule layer [43]. Once biocompatibility is achieved, the surface can be modified further by other linker molecules to be specific for the target molecules.

1.6 Organization

Chapter 2 will give an introduction to the AlGaN/GaN heterostructure and the development of the first generation of AlGaN/GaN heterostructure field effect transistor (HFET) biosensors, which were used to detect streptavidin (SA) and ssDNA.

Chapter 3 describes the optimization of AlGaN/GaN HFET Biosensors for the detection of proteins. The first part introduces the optimization of oxidization methods to achieve highest biomolecule coverage and retain good electrical properties. The second part describes the sensitivity improvement by operating the devices in the subthreshold regime. The third part describes the efforts on recessing the AlGaN barrier to shift the device threshold voltage to near zero voltage. With a lower gate bias, the background current is reduced, so that the signal-to-noise ratio is increased. Hence, the sensitivity is improved. The model of AlGaN/GaN is included for a better understanding to the device design.

Chapter 4 describes the design and fabrication of silicon-on-insulator (SOI) based biosensors for ssDNA detections. To avoid ion drifting in the Si oxide, an oxide-free surface modification technology is developed and characterized. Analytical modeling of cylindrical nanowires is developed. To verify the model, numerical modeling of Si nanowire biosensors are developed using Silvaco.

Chapter 5 introduces the efforts on characterization of tethered bilayer lipid membranes (tBLM) using electrochemical impedance spectroscopy (EIS). The EIS spectra of both planar tBLM and tBLMs with nanopores are measured and modeled with equivalent circuits.
The understanding to nanopore affected EIS spectra will be used to explain the cell channel opening.

Chapter 6 suggests the future developments based on the finished work described in previous chapters.
CHAPTER 2

Introduction to AlGaN/GaN Heterostructure and the First Generation of AlGaN/GaN HFET Biosensors

AlGaN/GaN HFETs have demonstrated excellent power and high frequency performance for wireless and microwave applications \([63, 64]\) because of the high sheet carrier concentration in GaN channel at the AlGaN and GaN interface, high saturation velocity, and high electrical breakdown field. Transistors fabricated on the AlGaN/GaN material system can also be used as high-sensitivity gas, chemical, and biological sensors because of the unique material and device properties such as chemical inertness, non-toxicity, and high transconductance \((g_m)\) \([28, 35, 36, 65]\).

AlGaN/GaN HFET biosensors/chemical sensors had been demonstrated prior to this work. Researchers at University of Florida have demonstrated detection of SA \([36]\), DNA \([43]\), halide ions \([32]\). Two other group researchers reported recording cellular behaviors with cells immobilized on the gate area of AlGaN/GaN HFETs \([40, 42]\). A group of Greece researchers found that native AlGaN surface selectively attracts anions \([34, 66, 67, 68]\). However, the reported sensitivity is much lower than Si-based biosensors. The reported SA and ssDNA detection concentrations are 5 mg/ml \([36]\) and 1 \(\mu\)M \([43]\) respectively, whereas the reported detected SA and ssDNA concentrations for Si sensors are both on the order of pM \([69]\). The difference indicates that the performance of AlGaN/GaN biosensors could
be improved largely. In this work, we first developed the first generation of AlGaN/GaN HFET biosensors. Subsequently, surface/interface characterization technologies and device physics are used to improve the device performance.

For AlGaN/GaN HFET sensors, the properties of the open gate surface, which is the sensing area, are critical for selectivity or specificity. For gas sensors, a catalytic metal thin film layer such as Pt or Pd is deposited as the catalyst for hydrogen detection [65]. For ISFETs, the selectivity of ions is determined by the selection of the open gate surface materials [28, 58]. For biological sensors, surface modification and functionalization is critical for biomolecular immobilization and sensing specificity [35, 59, 70]. To immobilize biomolecules on AlGaN/GaN surfaces, two typical strategies have been developed. One strategy is to deposit a thin metal film on the AlGaN surface, e.g. Au or Ag, and link probe molecules through a self-assembly process. A widely-used process is to link the gold surface to thiol-modified molecules [43]. The other strategy is to oxidize the AlGaN surface and link the next layer of functional molecules to surface oxygen atoms by covalent bonding, i.e. silane molecules [35]. These two strategies can be designed for detection of different target molecules.

This chapter introduces the AlGaN/GaN heterostructure, the design and fabrication of the first generation of AlGaN/GaN HFET biosensors, and primary SA and ssDNA detection results. With the feasibility validated, the optimization of device design and improvement of sensitivity will be introduced in chapter 3.
2.1 Introduction of AlGaN/GaN Heterostructure and AlGaN/GaN HFETs [1, 2]

2.1.1 Crystal Structure

Because only Wurtzite AlGaN/GaN is used in this work, only this AlGaN/GaN structure will be discussed. Wurtzite AlGaN/GaN heterostructures can be grown on c-plane sapphire and SiC substrates by both metal-organic chemical vapour deposition (MOCVD) and molecular beam epitaxy (MBE) methods. However, due to the nonsymmetry of the wurtzite structure, there are two different atomic layering in two opposite directions along the growth axis, as displayed in Fig. 2.1.

2.1.2 Spontaneous and Piezoelectric Polarization

Without applying an external electrical field, there is a spontaneous polarization electrical field \( P_{SP} \) in the equilibrium AlGaN and GaN lattices. For heterostructures, deformation occurs at the interfaces due to the lattice constant mismatch. The deformation of lattice
induces piezoelectric polarization $P_{PE}$. The spontaneous $P_{SP}$ and piezoelectric $P_{PE}$ polarizations can be expressed as:

\[
\vec{P}_{SP} = P_{sp} \vec{z},
\]

\[
\vec{P}_{PE} = e_{33} \varepsilon_z + e_{31}(\varepsilon_x + \varepsilon_y),
\]

where $\vec{z}$ is the direction of crystal growth, $\vec{x}$ and $\vec{y}$ are in the plane perpendicular to the growth direction, $\varepsilon_z = (c - c_0)/c_0$ is the strain in $c$-axis, and $\varepsilon_x = \varepsilon_y = (a - a_0)/a_0$ are the in-plane strain, $c_0$ and $a_0$ are the lattice constants in $\vec{z}$ and $\vec{x}$ $\vec{y}$ directions in equilibrium, and $e_{33}$ and $e_{31}$ are the piezoelectric constants. The values of AlN and GaN lattices are shown in Table 2.1.

<table>
<thead>
<tr>
<th>Material</th>
<th>AlN</th>
<th>GaN</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$ (Å)</td>
<td>3.1526</td>
<td>3.175</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>5.0961</td>
<td>5.158</td>
</tr>
<tr>
<td>$C_{13}$ (N/cm²)</td>
<td>$1.1285 \times 10^{12}$</td>
<td>$1.0 \times 10^{12}$</td>
</tr>
<tr>
<td>$C_{33}$ (N/cm²)</td>
<td>$3.69 \times 10^{12}$</td>
<td>$3.55 \times 10^{12}$</td>
</tr>
<tr>
<td>$e_{31}$ (C/cm²)</td>
<td>-0.58</td>
<td>-0.4900</td>
</tr>
<tr>
<td>$e_{33}$ (C/cm²)</td>
<td>1.55</td>
<td>0.7300</td>
</tr>
<tr>
<td>$P_{sp}$ (C/cm²)</td>
<td>-0.081</td>
<td>-0.0290</td>
</tr>
<tr>
<td>$E_g$ (eV)</td>
<td>6.13</td>
<td>3.42</td>
</tr>
</tbody>
</table>

The relation between the lattice constants can be expressed as

\[
\frac{c - c_0}{c_0} = -2 \frac{C_{13}}{C_{33}} \frac{a - a_0}{a_0},
\]
where $C_{13}$ and $C_{33}$ are elastic constants, which is shown in Table 2.1. Inserting Equation (2.3) into (2.2) results in:

$$\vec{P}_{PE} = 2 \frac{a - a_0}{a_0} \left( e_{31} - e_{33} \frac{C_{13}}{C_{33}} \right) \hat{z}. \quad (2.4)$$

For Ga-face AlN and GaN, $[e_{31} - e_{33}C_{13}/C_{33}] < 0$. The piezoelectric polarization is negative for tensile (the lattice is stretched, $a > a_0$ and $c < c_0$) and positive for compressive (the lattice is compressed, $a < a_0$ and $c > c_0$) strained layers. The spontaneous polarization of GaN and AlN is negative, which means the piezoelectric and spontaneous polarization are in the same direction for tensile strain and in the opposite direction for compressive strain. For N-face AlN and GaN, both spontaneous and piezoelectric polarizations change the direction. The polarization directions with different strains are shown in Fig. 2.2.

![Figure 2.2: The directions of spontaneous and piezoelectric polarizations in Ga- and N-faces of strained and relaxed AlGaN/GaN heterostructures.](image)
2.1.3 Sheet Charge Density

Gauss’s law describes the relationship between the gradient of the electrical field and the distribution of charges:

\[ \vec{D} = \varepsilon_0 \vec{E} + \vec{P}, \]  
\[ \nabla \cdot \vec{D} = \rho. \]  

(2.5) \hspace{1cm} (2.6)

Without an external electrical field, Gauss’s equation becomes:

\[ \vec{D} = \vec{P}, \]  
\[ \nabla \cdot \vec{P} = \rho. \]  

(2.7) \hspace{1cm} (2.8)

Inserting Equations (2.1) and (2.4) into Equation (2.8) results in the sheet charge introduced by the discontinuity of the polarization for planar structures:

\[ |\rho(x)| = |P_{PE}(Al_xGa_{1-x}N) + P_{SP}(Al_xGa_{1-x}N) - P_{SP}(GaN)| \]
\[ = \left| 2a_0 - a(x) \left[ e_{31}(x) - e_{33} \frac{C_{13}(x)}{C_{33}(x)} \right] + P_{SP}(x) - P_{SP}(0) \right|. \]

(2.9) \hspace{1cm} (2.10)

The constants of different Al fractions can be calculated using Vegard’s law and Table 2.1.

2.1.4 Sheet Carrier Concentration

Due to the high polarization electrical field, the electrons in the proximity region are induced and collected at the AlGaN/GaN interface, resulting in a high concentration of two-dimensional electron gas (2DEG) without intentional doping. The sheet carrier concentration \( n_s(x) \) can be expressed as:

\[ n_s(x) = \frac{\rho(x)}{q} - \left( \frac{\epsilon_0 e(x)}{dq^2} \left[ e\Phi_b(x) + E_F(x) - \Delta E_C(x) \right] \right), \]

(2.11)
Figure 2.3: The schematics of (a) an AlGaN/GaN/AlGaN structure, (b) its band-diagram and polarization charge distributions, and (c) simulated band-diagram and carrier concentration distributions.
where $q$ is the unit positive electron charge, $d$ is the thickness of the AlGaN barrier, $q\Phi_b$ is the Schottky barrier height (SBH), and $E_F$ is the Fermi level from the GaN conduction band edge, $\Delta E_C$ is the conduction band offset at the AlGaN/GaN interface. Fig. 2.3 shows an example structure of AlGaN/GaN/AlGaN heterostructure, the schematic of conduction band edge and distribution, and the calculated band-diagram and sheet carrier distribution from the Bandeng software [71], assuming the change density coming from the surface trap donors.

Figure 2.4: The $I_d - V_g$ family curves (a) and the DC transfer characteristics (b) of a typical Al$_{0.35}$Ga$_{0.65}$N/GaN HFET with a gate length of 0.25 $\mu$m and a gate width of 100 $\mu$m [2]. In subfigure (a), the gate was biased from 1 V to -5 V in a step of -1 V.

2.1.5 The Operation Principle of AlGaN/GaN HFETs

Similar to metal-oxide-semiconductor field-effect transistors (MOSFETs) with metal gate electrodes, the drain current of AlGaN/GaN HFETs in the subthreshold and linear
regimes can be expressed as

\[ I_{\text{sub}} = \mu C_{\text{barrier}} (m - 1) \frac{W}{L} \left( \frac{k_B T}{q} \right)^2 \times \exp \left( \frac{q (V_g - V_{\text{th}})}{m k_B T} \right) \left( 1 - \exp \left( - \frac{q V_d}{k_B T} \right) \right) \] (2.12)

\[ I_{\text{lin}} = \mu C_{\text{barrier}} (V_g - V_{\text{th}}) V_d \frac{W}{L}, \] (2.13)

where \( \mu \) is the carrier mobility, \( C_{\text{barrier}} \) is the barrier capacitance, \( m = 1 + C_Q/C_{\text{barrier}} \) where \( C_Q \) is the quantum capacitance, \( W \) and \( L \) are the gate width and length respectively, \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, and \( q \) is the electron charge. Typical \( I_d - V_g \) family curves, transfer characteristics are shown in Fig. 2.4 [2].

### 2.2 AlGaN/GaN Biosensor Device Design and Fabrication

As described in Fig. 2.4, the AlGaN/GaN HFET \( I_d - V_d \) characteristics demonstrates effective modulation from the gate electrode. Similar to traditional AlGaN/GaN HFETs, AlGaN/GaN HFET biosensors have fiducial (optional), mesa, Ohmic, and overlay layers. The difference is that there is no gate Schottky metal. Instead, the gate AlGaN surface is functionalized for capturing target molecules. This is a baseline design of fabrication processes. When the target molecule varies, the surface functionalization protocol requires modifications of fabrication procedure. The variation of fabrication will be introduced with the target molecules as needed.

#### 2.2.1 Fabrication Processes

A few standard processes (such as photolithography) and a few specific procedures (such as GaN dry etching) for AlGaN/GaN heterostructure processing are used in the fabrication of AlGaN/GaN HFET biosensors. The details of all standard procedures are introduced in Appendix A to avoid redundant information.
**Fiducial Layer**  The fiducial layer usually is a lift-off process for the definition of alignment markers for the following steps. The formation of a fiducial layer has four steps: 1) an image reversal step with the photoresist AZ 5214; 2) oxygen descum; 3) deposition of metal layers (e.g. Ti (200 Å)/Au (2000 Å); and 4) lift-off. For large size planar devices when the alignment is not critical (tolerance $\gg 1 \mu m$), this layer can be neglected. Instead, markers in the mesa layer can be used for this purpose.

**Mesa Layer**  The mesa layer is used to electrically isolate the devices on the same chip. The fabrication of this layer has three steps: 1) photolithography with the photoresist Shipley 1811; 2) plasma dry etching; and 3) removal of photoresist residue in organic solvent.

**Ohmic Layer**  The Ohmic layer is used to electrically connect the source/drain electrodes to the carriers in the channel. The formation of an Ohmic layer has five steps: 1) an image reversal step with the photoresist AZ4156; 2) oxygen descum; 3) deposition of Ti/Al/Ti/Au (350 Å/900 Å/600 Å/1000 Å); 4) lift-off in solvent; 5) rapid thermal annealing at 850 °C for 30 sec. With the complete of Ohmic layer, the $I_\text{d} - V_\text{d}$ characteristic measured from the source and drain electrodes should be linear at near 0 V bias.

**Overlay Layer**  An overlay layer is used to extend the electrode area for lower probing resistance during electrical measurements. The formation of an overlay layer has four steps: 1) an image reversal step with the photoresist AZ4156; 2) oxygen descum; 3) deposition of Ni/Au (200 Å/2000 Å); 4) lift-off in solvent.

**Device Package**  Different from traditional AlGaN/GaN HFETs, the AlGaN/GaN biosensors work with physiological solutions which contain a high concentration of salts. It is
very important to package the devices so that the biological components are isolated from the electrical components and improve the reliability of electrical measurements.

An ideal packaging material is expected to be [72]:

- highly adhesive to the sensor chip surface;
- not reactive or adsorptive with target chemical/bimolecular solutions;
- highly electrically insulating;
- good mechanical stability in air and aqueous ambient;
- good compatibility with micro-/nano-fabrication processes for mass production;
- cost-effective.

In addition, integration of a micrometer-scale reference electrode is beneficial to the reliability of a biosensor system.

Different packaging material and strategies have been applied during the development of ChemFETs. There are a few commonly used methods:

- micro-fluidic channels with polydimethylsiloxane (PDMS), etc. [73];
- photo-curable resins to form a mixing reservoir (SU8, polyimide, silicone, etc.) [74];
- inorganic films such as a Si$_3$N$_4$ layer. [75];

Inorganic materials such as Si$_3$N$_4$ have the advantage of good compatibility with microfabrication processes. However, contamination from the ions in physiological solutions causes chemical/electrical instability. SU8 and polyimide have the advantage of compatibility with micro-fabrication process, but were found to have adhesion problems after being
rinsed in aqueous solutions for a few tens of hours. Hence, devices packaged with SU8 or polyimide can only be tested for the first few times before the package layer fails. Photo-patternable silicone does not work well with transparent substrates [76]. PDMS was found to have adhesion problems with AlGaN surfaces.

As a result, silicone paste was selected to package the AlGaN/GaN biosensors in this work. The AlGaN/GaN biosensors in this work have a spacing of 2 mm between the source/drain electrodes. The spacing allows applying silicone manually to form a mixing reservoir. The procedure is as follows:

- Cover the active area with 1 mm wide PDMS stripe.
- Apply silicone onto the sides of the PDMS stripe to define the active area, make sure the source and drain electrodes are not completely covered.
- Remove the PDMS stripe.
- Wait 24 hours for silicone to cure.

2.2.2 Device Performance with PBS

Fig. 2.5 shows a series typical \( I_d - V_d \) family curves in the subthreshold regime and an \( I_d - V_g \) curve measured on an AlGaN/GaN HFET biosensor fabricated as described in the previous subsection. PBS solution (Sigma) is applied in the reservoir and a Pt electrode is used to applied the gate bias. Compared with Fig. 2.4, the AlGaN/GaN HFET shows similar gate voltage modulation on \( I_d - V_d \) characteristics to Fig. 2.4.
2.3 AlGaN/GaN Protein Sensors

2.3.1 Motivation

Proteins are biological molecules consisting of one or more polypeptides in a globular or fibrous form. Proteins contain transferred genetic code information, are essential parts of the cells, and participate in every process within cells. High sensitivity protein sensors could largely assist the development of gene/drug delivery for new medicines, understanding cellular behaviors, etc. Real-time detection of proteins can improve the accuracy of diagnosis and make it possible for physicians to make early-stage (e.g. before patients can feel any symptoms) decisions. Miniaturization of devices can reduce the amount of sample, which could save patients from discomfort.

This section shows the developments of protein sensors for two specific types of proteins, which are SA and monokine induced by interferon γ (MIG/CXCL9). The focus of this work is to develop device structures and measurement strategies to improve the sensitivity for clinical applications. The design of surface molecular structure for SA and MIG...
immobilization and protein handling are conducted by Dr. Stephen C. Lee’s group at the Department of Biomedical Engineering, The Ohio State University.

### 2.3.2 Introduction to SA and MIG

Avidin-biotin binding is one of the strongest non-covalent interactions in nature with high specificity, a binding rate of $10^{-7}$ M, and a very low disassociation constant ($10^{-13} - 10^{-14}$ M) [77]. It is a widely used scheme for specific molecular recognition. The pI value of SA molecules is around 5. In physiological buffer solution (pH=7.4), SA molecules are negatively charged. In this work, SA-biotin binding is used to verify the device work principles. SA is a tetramer molecule. The binding of two biotin molecules and a SA molecule is shown in Fig. 2.6 [78]. In the later part of this work, we present a SA molecule with a four-packet sphere.
MIG belongs to a family of about 50 related, cell-cell signaling proteins called chemokines that regulate migration and tissue infiltration by inflammatory cells [35]. In a healthy human body, the concentration of MIG is on the order of picomolar ($1 \times 10^{-12}$ M). The detection of MIG concentration in patients with transplants is important because a nanomolar ($1 \times 10^{-9}$ M) concentration of MIG indicates transplant rejections. A sensor that can quantify MIG in vivo can be used to predict transplant rejection and therefore to more appropriately manage immunosuppression treatments. The MIG molecule is charged with 20 positive charges/molecule at physiologic pH, which benefits the detection using field-effect biosensors. Continuous real-time monitoring the MIG concentration could help physicians and researchers to study mechanisms of transplant rejection and adjust immunosuppressive therapy to fit different patients in a very early stage.
2.3.3 Fabrication Processes for Planar AlGaN/GaN HFET Protein Sensors

The designed planar AlGaN/GaN HFET structure has a gate area of \(2 \, \text{L mm} \times 2 \, \text{mm (W).}\) The fabrication processes for planar AlGaN/GaN HFET protein sensors are exactly same as in the description of Section 2.2.1 except that there is no fiducial step because the device dimension is large and the designed tolerance for alignment is 5 \(\mu \text{m.}\) The detailed fabrication process is shown in Fig. 2.8.

![Figure 2.8: An overview of fabrication procedure of AlGaN/GaN protein sensors. a) The original AlGaN/GaN heterostructure. b) After mesa patterning, dry etching, and removal of photoresist. c) After Ohmic layer patterning, metal deposition, and lift-off, Overlay layer patterning, metal deposition, and lift-off. d) After applying sealing material (polyimide, silicone, etc.) to form reservoir.](image-url)
An overhead view of a completed device is shown in Fig. 2.9. As shown, only the sensing area is exposed, with the source and drain electrodes partly sealed to form the reservoir. The other parts of the source and drain electrodes are exposed for probing.

![Figure 2.9: The top view of finished AlGaN/GaN HFET protein sensors.](image)

### 2.3.4 Surface Modification

#### Surface Modification for SA Detection

The whole surface modification procedure for SA immobilization is shown in Fig. 2.10. Firstly, the AlGaN surface is oxidized with oxygen plasma or wet chemicals (i.e. piranha solution), as shown in Fig. 2.10(a). After oxidation, the samples are boiled in de-ionized (DI) water for 30 min at 100 °C so that the surface ends up with hydroxyl groups. The samples are then blown dry by a nitrogen gun. 2% 3-Triethoxysilylpropylamine (3-APTES) in acetone was used to treat over the device active regions, rinse the samples for 30 min.
The silanized samples are washed in acetone and then propanol to remove un-bound silane molecules, dried by nitrogen blow and baked at 120 °C for 5 min. After this step, the AlGaN surface is modified with amine groups, as shown in Fig. 2.10(b). Sulfo-NHS-biotin (Pierce Biotechnology, Rockford, IL) is dissolved in DI water at a concentration of 1 mg/ml. The solution is then dropped onto silanized AlGaN surfaces and covered by a glass slide to spread the SA solution over the entire sample surface. The samples are then stored in a humid chamber to avoid evaporation at room temperature. After 2 hours, the samples are rinsed in phosphate buffered saline (PBS) three times to remove un-bound sulfo-NHS-biotin molecules (Fig. 2.10(c)) and then immersed in superblock solution (Pierce Biotechnology, Rockford, IL) for 1 hour (Fig. 2.10(d)). After the biotinylation, the modified surfaces are ready to react with SA molecules. The molecular structure with captured SA molecules is shown in Fig. 2.10(e).

However, without any optimization, APTES polymerizes on the oxidized AlGaN surface to form a network structure through side hydroxyl groups, as shown in Fig. 2.11(b) [80]. With changing the APTES concentration, the silane layer structure can be very close to Fig. 2.11(a). This optimization was developed independent of the device development. The silanization protocol will be described below, as needed.

**Surface Modification for MIG Detection**

Two strategies were developed to bond MIG to the AlGaN surface. The first one is to use the same surface modification procedure for SA immobilization and saturate the surface with SA molecules. Because each SA molecule has four biotin binding pockets, SA can bind to both biotinylated APTES layers and biotinylated MIG. The MIG molecules are biotinylated with an EZ-Link biotinylation kit (Pierce inc.). The biotinylated MIG molecules have a biotin group to bind with the available sites on the surface SA molecules.
Figure 2.10: The surface modification procedure for SA immobilization. a) Oxidization the AlGaN surface. b) Silanization. c) Biotinylation. d) Application of blocker. e) The final molecular structure of modified AlGaN surface bonding with SA molecules.
A hypothetical surface molecular structure resulting from this strategy is shown in Fig. 2.12. Because SA can only bind biotinylated molecules, this strategy will not detect native (unbiotinylated) MIG.

The second strategy starts with an AlGaN oxidization. Triethoxy silane aldehyde (TSA) is used to modify the surface with aldehyde groups. The exposed aldehyde groups then react with the primary amines on the end of anti-MIG/CXCL9 immunoglobulin G (IgG) (humane or murine) [81]. Anti-MIG/CXCL9 IgG is the receptor for MIG molecules. The whole process is shown in Fig. 2.13. As shown in Fig. 2.13, because the MIG molecules are polyclonal, the receptors cannot differentiate the epitopes on MIG. The orientation of MIG is expected to be random. This method does not require the MIG molecules to be modified and is appropriate for clinical applications.
Figure 2.12: The surface molecular structure for binding biotinylated MIG.

Figure 2.13: The AlGaN surface modification procedure to bind MIG molecules [82].  

a) Oxidized AlGaN surface is modified with TSA.  
b) Aldehyde groups on TSA reacts with anti-MIG IgG.  
c) MIG molecules bound to anti-MIG IgG.


2.3.5 Effects of PBS Concentrations

Fig. 2.14 shows the device structure, the fabrication, and dimension of which is introduced in section 2.3.3, with a floating gate test setup. After the active gate area has been modified with biotin groups, the device is ready for detections. To sense the presence of SA molecules, two $I_d - V_d$ curves are measured. The first $I_d - V_d$ curve is measured with only PBS in the reservoir, before application of the target SA solution. The second $I_d - V_d$ curve is measured after the target SA solution has been applied, wait for 30 min for binding to happen, and triple rinsed by fresh PBS solutions to remove unbond target molecules.
The comparison of measured drain currents with gate floated before and after SA binding in 1× PBS. The target SA concentration is 2.5 µg/ml (47.3 nM).

Fig. 2.15 shows the measured \( I_d - V_d \) characteristics for the detection of 2.5 µg/ml (47.3 nM) SA solution. As expected, the binding of negatively charged SA in 1× PBS causes the drain current decrease. Fig. 2.16 summarizes the measured currents with \( V_d = 1 \) V at different PBS concentrations or ionic strengths. Before SA binding, the drain current decreases with the increase of ion concentration. In this case, the biotinylated AlGaN surface is positively charged and is sensitive to negative charges. As the buffer ion concentration increases, more negative ions are attracted onto the surface, which depletes more carriers in the channel; hence the drain current \( I_d \) decreases. After SA binding, the
drain current increases with the increase of buffer ion concentration due to the ion shielding effect. A higher ion concentration, or a smaller Debye length as shown in Fig. 2.16, causes greater shielding effect. As a result, the sensitivity at high ion concentrations is attenuated. The measured sensitivity is 15.2% with 0.1 × PBS, 11.3% with 0.25 × PBS, and 5.8% with 1 × PBS, respectively. The Debye length in an electrolyte is defined as

\[ \lambda_D = \sqrt{\frac{\epsilon_0 \epsilon_r k_B T}{2 N_A q^2 I}} \]

(2.14)

where \( I = \sum_{i=1}^{n} c_i z_i^2 \) is the ionic strength, \( c_i \) is the molecular concentration of ion \( i \), \( z_i \) is the charge number of ion \( i \), the sum is taken over all ions in the electrolyte, \( \epsilon_0 \) is the permittivity of free space, \( \epsilon_r \) is the dielectric constant, \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature in kelvins, \( N_A \) is the Avogadro number, and \( q \) is the elementary charge. Table 2.2 shows the Debye length of different PBS concentrations. Because the SA molecules are located around 1.6 nm away from the AlGaN surface, in principle the modulation from the SA molecules can not be detected by the devices when the PBS concentration is above 0.25×. However, due to conformation of the SA layer, some segment of the SA still can be detected as shown in Fig. 2.16. When the PBS concentration is much higher than 0.25×, such as 0.5×, the potential change only in the region within 1.01 nm to the AlGaN surface can be detected. The presence of SA molecules are too far to be sensed.

### 2.4 AlGaN/GaN ssDNA Sensors

#### 2.4.1 Introduction

DNA is a long polymer molecule consists of repeating nucleotides. DNA contains the genetic instructions to construct other components of the cells, such as proteins and RNA molecules. Four bases in DNA are adenine (A), cytosine (C), guanine (G), and thymine (T), which are attached to the sugar/phosphate to form the complete nucleotide. A only
Figure 2.16: The channel current measured at $V_d = 1$ V at different concentrations of PBS or ion strengths before and after biotin-SA binding.
Table 2.2: Calculated Debye length ($\lambda_D$) for different concentrations of PBS.

<table>
<thead>
<tr>
<th>PBS</th>
<th>$\lambda_D$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1×</td>
<td>0.7</td>
</tr>
<tr>
<td>0.75×</td>
<td>0.82</td>
</tr>
<tr>
<td>0.50×</td>
<td>1.01</td>
</tr>
<tr>
<td>0.25×</td>
<td>1.43</td>
</tr>
<tr>
<td>0.10×</td>
<td>2.30</td>
</tr>
<tr>
<td>0.01×</td>
<td>7.30</td>
</tr>
<tr>
<td>0.001×</td>
<td>23.2</td>
</tr>
</tbody>
</table>

bonds to T, and G only bonds to C, which is called complementary base pairing. The bases of a ssDNA molecule are not paired up. When two DNA molecules with complementary sequence are bonded, a helix structure will be formed as shown in Fig. 2.17 [78]. The bonding between two complementary DNA molecules can be broken at their melting temperature ($T_m$ value). When all the base pairs in a DNA double helix melt, the strands separate and have a random coil conformation.

Selective and sensitive detection of DNA hybridization has important applications in the areas of DNA sequencing, genetics of diseases, and drug development. This section introduces the development of ssDNA hybridization sensors with AlGaN/GaN HFETs.

2.4.2 AlGaN/GaN Heterostructure

MOCVD is used to grow the AlGaN/GaN heterostructure on a (0001) sapphire substrate. A 40 nm AlN nucleation layer was firstly grown on the sapphire substrate, followed by a 3 $\mu$m undoped GaN layer. A 20 nm undoped Al$_{0.3}$Ga$_{0.7}$N layer is grown on the GaN layer to form the heterostructure. Hall measurements at room temperature showed a sheet carrier density of $1.3 \times 10^{13}$ cm$^{-2}$ and an electron mobility of 1175 cm$^2$/V·sec. The carrier
Figure 2.17: The structure of a DNA double helix. Complementary base pairing is shown in the bottom right [78].

The carrier concentration peak is around 20 nm from the surface.

### 2.4.3 Device structure and fabrication

The AlGaN/GaN HFET ssDNA sensor fabrication procedure is shown in Fig. 2.19. The procedure starts with an AlGaN/GaN substrate (Fig. 2.19(a)). The electrical isolation between devices was performed by an ICP dry etching process with Cl₂, BCl₃, and Ar by using an Oxford PlasmaLab100 system (Fig. 2.19(b)). A lift-off process of Ti(350 Å)/Al(900 Å)/Mo(600 Å)/Au(1000 Å) layers was implemented to define Ohmic pattern (Fig. 2.19(c)). Ohmic contacts were formed by annealing at 850 °C for 30 seconds in a rapid thermal annealing (RTA) system in N₂ ambient. The electrodes were isolated from the solution by a polyimide layer which was defined by optical lithography (Fig. 2.19(d)).
Figure 2.18: Carrier concentration profile vs. depth of an AlGaN/GaN heterostructure extracted from Ni/Au Schottky diodes.

The polyimide thickness is around 10 µm after curing at 320 °C for 1 hour. The sizes of gate area are on the order of hundreds of micrometers. After that, Ti/Au was deposited to be the immobilization area for thiol-modified ssDNA probes (Fig. 2.19(e)). Ti layer is used to increase the adhesion between gold and AlGaN surface. The thicknesses of Ti and Au are 10 Å and 50 Å, respectively. A schematic top-view of a completed device is shown in Fig. 2.19(e). The cross-sectional view of devices is shown in Fig. 2.20. After the gate metal deposition, the current levels of the devices were observed to decrease by around 10%.

2.4.4 ssDNA Immobilization and Hybridization on Au surfaces

The processes of immobilization and hybridization of ssDNA on Au surfaces were firstly studied by fluorescent imaging and atomic force microscopy (AFM) measurements.
Figure 2.19: The fabrication procedure of AlGaN/GaN HFET ssDNA sensors. a) The original AlGaN/GaN heterostructure. b) After mesa layer patterning, dry etching, and photore sist removal. c) After Ohmic layer patterning, metal deposition and liftoff, and annealing. d) After the deposition of Ti (20 Å)/Au (50 Å) for probe ssDNA immobilization. e) After applying the packaging layer. f) The top-view of an AlGaN/GaN HFET ssDNA sensor.
before they were transferred to AlGaN/GaN surfaces. The study was performed on Si substrates with Au patterns. 12 mer desalted oligonucleotides (Sigma-Aldrich) were used in this study. Table 2.3 shows the sequences of probe and target ssDNAs. There are two copies of each specific sequence, which are with and without fluorescent labeling. p1 and t2 are matched ssDNA sequences, while t1 is mismatched sequence to p1 for control study. TE (Tris and EDTA) buffer with pH 8.0 was used to make solutions with proper concentrations.

Table 2.3: Oligonucleotide sequences and nomenclature for electrical detection and fluorescent imaging.

<table>
<thead>
<tr>
<th>Function</th>
<th>Name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>probe</td>
<td>p1</td>
<td>5’-CCT AAT AAC AAT 3’-C2-thiol</td>
</tr>
<tr>
<td>probe</td>
<td>fp1</td>
<td>Cy5-5’-CCT AAT AAC AAT 3’-C2-thiol</td>
</tr>
<tr>
<td>target</td>
<td>t1</td>
<td>5’-AAA AAA AAA AAA-3’</td>
</tr>
<tr>
<td>target</td>
<td>ft1</td>
<td>Cy3-5’-AAA AAA AAA AAA-3’</td>
</tr>
<tr>
<td>target</td>
<td>t2</td>
<td>5’-ATT GTT ATT AGG -3’</td>
</tr>
<tr>
<td>target</td>
<td>ft2</td>
<td>Cy3-5’-ATT GTT ATT AGG -3’</td>
</tr>
</tbody>
</table>
2.4.5 Fluorescence Study

Fluorescent imaging was implemented on a Nikon ECLITSE TE2000-S fluorescent microscope. The exposure time was set to 5 seconds. The fluorescent images are shown in Fig. 2.21. The left side of each picture shows the fluorescent images with a sampling bar. The right side shows the fluorescent intensities vs. position across the sampling bar. Devices were submerged in PBS buffer for imaging.

Fig. 2.21(a) shows gold and silicon emission background under the illumination of 649 nm wavelength light (the excitation wavelength of Cy5). The sample was firstly rinsed in 1 µM fp1 solution for 12 hours, washed by water and taken out for imaging. Fig. 2.21(b) shows the fluorescence intensities of the sample with Cy5 excitation wavelength. Compared with Fig. 2.21(a), the increase in fluorescence intensities indicates the probe ssDNA molecules had been bonded to the Au surface. After that, the substrate was rinsed in 1 µM mercaptoethanol (MCE) solution for 2 hours to block the area without any probe ssDNA molecules to avoid physical adsorption of target ssDNAs and help probe ssDNAs to have better conformation for hybridization. Followed by that, the substrate was washed in water and cleaved into two pieces. One piece was rinsed in 1 µM ft1 solution and the other piece was rinsed in 1 µM ft2 solution. There is 1 M NaCl in target ssDNA solution to improve hybridization. The two samples were taken out after 1 hour waiting, washed with water, and fluorescent images were taken. Fig. 2.21 (c) shows the gold and Si emission background with the excitation wavelength of Cy3, which is 550 nm. Fig. 2.21 (d) and (e) show the fluorescent intensities of the sample rinsed in ft1 and ft2 separately. By comparing the fluorescent intensity changes, the increase of fluorescent intensities on ft2 solution rinsed sample confirms that hybridization indeed occurred while the fluorescent intensities on the sample rinsed in ft1 solution are slightly higher than the background intensity level.
Figure 2.21: The fluorescent images of Si substrates with Au patterns in the process of ssDNA immobilization and hybridization. (a) background image of Cy5 excitation wavelength, (b) image after probe ssDNAs immobilization, (c) image of Cy3 excitation wavelength with probe ssDNA immobilized, (d) image after treated with mismatched ssDNA solution, (e) image after treated with matched ssDNA solution.
2.4.6 AFM Study

AFM is a powerful tool to characterize surface morphology of self-assembly monolayers (SAMs). A Veeco Dimension 3100 system was used in this study. The AFM images of Au surfaces during the immobilization and hybridization process are shown in Fig. 2.22. The grain sizes in Fig. 2.22 confirmed the immobilization and hybridization of ssDNA molecules. In Fig. 2.22 (a), there are no circular grains on the Au surface and the feature pattern sizes are small, which is the initial condition for thiol-modified ssDNA molecules to attach. Fig. 2.22 (b) shows the Au surface morphology after the sample had been rinsed in 1 mM probe ssDNA solution for 12 hours. Circular grains appeared all over the Au surface due to the attachment of probe ssDNAs. Fig. 2.22 (c) shows that the circular grain sizes were increased further by the attachment of target ssDNA molecules. The surface roughness of Au surfaces did not change significantly in the three cases, indicating that the uniformity of SAM is good.

2.4.7 Electrical Detection

After the surface modification and immobilization/hybridization processes were performed on Si surfaces, they were transplanted to AlGaN/GaN HFETs for electrical detection. After the device fabrication, the sample was rinsed in 1 mM p1 solution for 12 hours and followed by a rinse in 1 mM MCE solution for 2 hours. The device was then biased at 0.5 V, and the current was monitored by an Agilent 4156C semiconductor parameter analyzer. Comparison measurements were performed by applying 1mM t1 solution and t2 solution to two different devices respectively. The measurement results are shown in Fig. 2.23.
Figure 2.22: AFM images of Au surfaces (a) after Ti/Au deposition, with a surface roughness is 0.989 nm (b) after probe ssDNA immobilization, with a surface roughness is 1.265 nm, and (c) after hybridization with matched target ssDNAs with a roughness of 1.172 nm.
Fig. 2.23 (a) shows the drain current vs. time plot during the hybridization process with matched ssDNA target. The hybridization was not observed until around 150 sec later after the target ssDNA solution was applied, which was also observed by Kang et al [43] but the reason for this is still unclear. As shown in Fig. 2.23 (a), in the first few tens of seconds, the decrease of current is sharp and most of the current change happened in this period. In this time range, there were enough target ssDNA molecules to hybridize with the surface probes, which indicate the current change rate is dominated by ssDNA hybridization process. As the target ssDNA molecules in the surface area being consumed by hybridization, the current change rate was dominated by mass transfer process for target ssDNA molecules to diffuse to the surface area. As all the probe ssDNA molecules on Au surface are hybridized with target molecules, the current change rate saturates. The three processes discussed above determine the hybridization rate in the short time range (e.g. first few tens of seconds), the middle time range, and long time range. The last two ranges are difficult to isolate in the current vs. time curve. Fig. 2.23 (b) shows the control measurement with mismatched target ssDNA solution applied. There is no appreciable current change observed, which means the hybridization and physical adsorption of target ssDNA molecules to the modified Au surface is minimal.

2.4.8 Summary

A design of AlGaN/GaN HFET biosensor was demonstrated for the detection of ss-DNA hybridization. The probe ssDNA was modified by thiol groups and was immobilized on Au surface. The target ssDNA molecules were then hybridized with immobilized probe ssDNA molecules. This process was studied by both fluorescent imaging and AFM. The AlGaN/GaN HFETs gate area was deposited with thin Ti/Au film. The immobilization and
hybridization processes were repeated on modified AlGaN/GaN HFETs. While hybridization, the device was biased and the current was being monitored. A decrease of current was observed during the hybridization process. In the first few tens of seconds, the hybridization process was dominated by the conjugation between matched ssDNA sequences. After that, the hybridization process was dominated by mass transfer process and saturation of the immobilized probe ssDNA molecules. As a comparison, no obvious response was observed with the non-complementary sequence.
CHAPTER 3

Sensitivity Improvement of AlGaN/GaN protein sensors

Chapter 2 introduced the first generation of AlGaN/GaN HFET biosensors developed for SA and ssDNA detection. This chapter introduces the improvement of device performance, which includes the optimization of oxidization methods, operating the device in the subthreshold regime, and recessing the AlGaN barrier to shift the threshold voltage to zero/near zero. The detection of MIG is also introduced for developing real clinical applications. The last part of this section introduces the modeling of planar AlGaN/GaN HFET biosensors.

3.1 Optimization of Oxidization Methods

3.1.1 Introduction to the Methodology

In this section, the AlGaN surface oxidization methods are optimized for the purpose of higher surface SA molecular coverage. Reactive ion etching oxygen plasma, inductively-coupled oxygen plasma, and wet chemicals are used to oxidize AlGaN surfaces. After oxidation, X-ray photoelectron spectroscopy (XPS) and water contact angle measurements are used to check oxidization effectiveness. Labeled SA molecules are bound to the oxidized surfaces through linker molecules for comparison of surface modification effectiveness. Schottky diodes are fabricated to investigate the impacts of oxidization processes.
on electrical properties, such as Schottky barrier heights, sheet carrier concentrations, and interface trap densities. The results show that the inductively-coupled plasma oxidation process has a superior behavior compared to the reactive ion etching oxygen plasma and wet chemical oxidation processes. AlGaN/GaN HFET protein sensors fabricated using the inductively-coupled plasma oxidation process have exhibited improved sensitivity compared to a previously reported GaN-based biosensors.

To immobilize probe molecules on electrical biosensors by covalent bonding to oxygen atoms, an ideal surface oxidization process is expected to increase the surface reactivity to biological molecules, improve device electrical performance, and be compatible with the microelectronic fabrication process. To oxidize the AlGaN surface, there are a few candidate methods that can be implemented for evaluation. As a traditional wet chemical approach, piranha solution treatment on AlGaN surface has demonstrated the capability of achieving oxidization and hydrophilicity at the same time, which is essential for silanization [83]. On the other hand, plasma treatment has also been studied for biocompatibility. Reactive ion etching (RIE)/inductive-coupled plasma (ICP) treatments are widely used in semiconductor device fabrication. RIE plasma generally creates a high bias to the substrate or a high ion kinetic energy while ICP plasma generated under a high coil power has a higher plasma density. With different gases and power conditions, the plasma can be used for different purposes such as ashing photoresist residues and etching materials with designed selectivities [84]. When oxygen plasma is used, it can also be used for oxidizing semiconductor surfaces, such as AlGaN [85]. The surface properties, such as morphology, hydrophilicity, charges, and traps could change largely by these processes. The surface charges and surface potential can be changed by the introduction of charged oxygen atoms.
The barrier height of Schottky diodes fabricated on these materials can be increased or decreased, which will affect the gate leakage current. The 2DEG carrier concentration will also be affected [86]. The properties of AlGaN surface states can also be changed due to the plasma ion bombardment, which has a large impact on the device performance because surface and interface states can respond to the gate potential by charging and discharging. For sensors, the chemical/biological species induced signals could be weakened, resulting in a decreased sensitivity. The dynamic response of the sensors is also affected by traps that are introduced during the processing [87]. To achieve high sensor sensitivity, it is very important to oxidize AlGaN surface with minimum degradation of device performance.

In this section, we evaluate the effectiveness and impact of techniques for oxidizing III-nitride materials for biosensing applications. Three different methods of oxidation are characterized by XPS, water contact angle, and capacitance-voltage (C-V), and current-voltage (I-V) measurements. The chemical structures of oxidized AlGaN surfaces are analyzed by XPS study [88, 89]. Water contact angle measurement is used to compare the change of AlGaN surface hydrophilicity which is important for silanization. The specificity and surface coverage are characterized by fluorescence images. C-V and I-V characteristics of AlGaN/GaN Schottky diodes are for analysis of the electronic properties such as 2DEG densities, and surface and interface trap densities and lifetimes etc. The discussion of oxidation and trap properties. The optimized silanization protocol has not been developed at this work. The conclusions are summarized at last.

3.1.2 Material and Oxidization Methods

Materials

For XPS, water contact angle, and fluorescence imaging characterization, AlGaN wafer (TDII Inc.) with a thickness of 0.5 \( \mu \text{m} \) and a Si doping concentration of \( 1 \times 10^{17} \text{ cm}^3 \) on a
SiC substrate. For Schottky diodes and biosensor devices, AlGaN/GaN heterostructure layer structure was used. The heterostructure was grown by MOCVD on a (0001) SiC substrate. A 40 nm AlN nucleation layer was first grown on the substrate, followed by a 3 \( \mu m \) undoped GaN buffer layer. A 23 nm undoped \( \text{Al}_{0.3}\text{Ga}_{0.7}\text{N} \) layer is grown on the GaN layer to form the heterostructure. Hall measurements at room temperature showed a sheet carrier density of \( 1.3 \times 10^{13} \text{ cm}^{-2} \) and a carrier mobility of \( 1175 \text{cm}^2/\text{V} \cdot \text{sec} \).

**Oxidation Methods**

For wet chemical treatment, the sample was rinsed for 20 minutes in a piranha solution (PS) made from \( \text{H}_2\text{SO}_4 : \text{H}_2\text{O}_2 = 3 : 1 \) [83], rinsed in de-ionized (DI) water with ultrasonic agitation for 5 minutes, and blown dry by \( \text{N}_2 \). An Oxford PlasmaLab 100 system was used to oxidize AlGaN surfaces at RIE and ICP modes separately. For the RIE method, 25 Watts radio frequency (RF) power with 5 mTorr chamber pressure, 50 sccm \( \text{O}_2 \) and 0 Watts ICP plasma coil power was applied. The plasma-induced bias to substrate was -110 V. For ICP method, 1000 Watts ICP plasma coil power with 20 mTorr chamber pressure, 50 sccm \( \text{O}_2 \), and 0 Watts RF power was applied. The duration of both RIE and ICP treatments was 30 seconds. For comparison, four samples were studied by XPS, water contact angle, fluorescence and diode measurements. The four samples are referred later in this paper as REF, PS, RIE, and ICP respectively corresponding to non-treatment, piranha solution, RIE plasma, and ICP plasma treatments.

**XPS Measurements**

For XPS study, the samples were transferred into an ultrahigh vacuum (UHV) Kratos Axis Ultra XPS system chamber immediately after oxidation. Overnight vacuum pump was performed to achieve a base pressure of \( 1 \times 10^{-10} \text{ Torr} \) before measurements. A
monochromatic Al anode X-ray gun was used for detection of C 1s, Al 2p, Ga 3d and Ga 2p peaks. C 1s peak is aligned to 284.5 eV as a reference peak. Mg anode X-ray gun was used to measure O 1s and and Ga 2p peaks. Because the deep level Ga 2p peak is not sensitive to oxidization and the peak position can be better defined compared to the multiple C 1s peaks from contamination in the air and the immobilized silane molecules, it is used as a reference peak to align Mg anode activated peaks to Al anode activated peaks.

**Water Contact Angle Measurements**

During measurements, four samples were held separately on a horizontal flat stage. A syringe was used to apply the same volume of DI water drops onto the samples. The water profile image was captured immediately after the application of DI water, without noticeably evaporation. The contact angle is extracted from the images with a developed Matlab program.

**Diodes Fabrication and Measurements**

For device fabrication, Ti/Al/Ti/Au metal layers were deposited with a lift-off process followed by RTA to make Ohmic contacts. The devices were then deposited with a 200 nm SiO$_2$ layer. Schottky patterns were opened by a negative photoresist photolithography step and etching of oxide layer in diluted HF. Photoresist were then removed and three of the samples were oxidized by piranha solution, RIE and ICP methods separately while the fourth sample was kept as the reference. During the treatment, only the open area that is to be deposited by Schottky contacts was oxidized while the rest area remains protected by the thick SiO$_2$ film. The SiO$_2$ protection layer was then removed by diluted HF after the Schottky metal Ni/Au was deposited by another lift-off process. The fabricated diodes have a dimension of 120 $\mu$m in diameter. After fabrication, I-V measurements were performed
using an Agilent 4156C semiconductor parameter analyzer and C-V measurements were performed using an Agilent 4284 LCR meter. For C-V measurements, the frequency range was from 100 Hz to 1 MHz and the applied AC signal amplitude was 100 mV.

### 3.1.3 Results and Discussions

**XPS Characteristics**

The core level electron spectra shown in Figs. 3.1 and 3.2. Ga 3d peaks are deconvoluted into Ga-O, Ga-N and N 2s peaks and Al 2p peaks are deconvoluted into peaks of Al-O and Al-N bonds [90]. The de-convoluted peak positions and the area ratios are summarized in Table 3.1. As shown in the table, both the Ga 3d(O) and Al 2p(O) peak areas increase for all the oxidized samples compared to the REF sample. The ratios of O 1s/N 1s, Ga 3d(O)/Ga 3d (N) and Al 2p(O)/Al 2p(N) increase compared to the REF sample, suggesting that the oxide fraction increases while nitride fraction decreases and oxidization of both Ga and Al elements happen for all the oxidation methods. From Table 3.1, the peak positions of Ga 3d(O) and Al 2p(O) shift to higher bonding energy because the newly-formed oxides introduces more high energy bonds. The Ga 3d(N) and Al 2p(N) positions remain constant
Figure 3.1: XPS Ga3d spectra and de-convoluted Ga-O, Ga-N, and N 2s peaks of samples oxidized by three different methods. The increase of Ga-O peak areas compared to REF sample indicates that surface Al atoms are oxidized by all the methods.
for all samples. As shown in Fig. 3.3, the O 1s spectra are de-convoluted into O 1s(Ga) and O 1s(Al) bonding peaks. As shown in Table I, the peak area ratio change between O 1s(Al) and O 1s(Ga) bonds shows clear differences for different oxidization methods, suggesting that surface Al and Ga atoms are oxidized at different ratios for different oxidization mechanisms. For ICP and piranha solution treated samples, O 1s(Al)/O 1s(Ga) ratio decreases while for RIE treated sample, O 1s(Al)/O 1s(Ga) ratio increases. This suggests that on the ICP and piranha solution treated samples, there is more gallium oxide formed while on the RIE treated sample there is more aluminum oxide formed. For ICP treatment, there is no bias between the plasma and substrate.

Under the coupling between ICP coil and plasma, since the plasma induced substrate bias is essentially zero at zero RF power, there is no physical ion bombardment process during the oxidization process by the high density plasma. Hence, the oxidization is more chemical and isotropic [84], which is somewhat similar to the wet chemical treatment process. For RIE treatment, the bias between plasma and substrate is induced. The oxygen ions in plasma are accelerated by the bias to substrate to gain kinetic energy. Therefore, the AlGaN surface experiences ion bombardment as well as oxidization. Because gallium oxide is more volatile than aluminum oxide, with high kinetic oxygen ions bombarding, some of the gallium was sputtered and evacuated. As a result, there exists more Al oxide on the surface.

**Water Contact Angle Measurement**

Water contact angle measurement results show the change of hydrophilicity after the oxidizations. The images of water drop profiles on AlGaN surfaces were taken with a video camera in Dr. David Tomasko’s lab in the Department of Chemical and Biomolecular Engineering. The water contact angles were then calculated from the curvature of the...
Figure 3.2: XPS Al 2p spectra and de-convoluted Al-O and Al-N peaks of samples oxidized by three different methods. The increase of Al-O peak areas compared to REF sample shows that surface Al atoms are oxidized by all the methods.
Figure 3.3: XPS O1s spectra and de-convoluted into O-Al and O-Ga peaks of oxidized samples by three different oxidation methods.
water profile. Fig. 3.4 shows the images taken for a reference AlGaN surface and differently oxidized AlGaN surfaces. The water contact angle on the reference, ICP, RIE and PS samples are 50°, 15°, 15°, and less than 10° respectively. The comparison of water contact angles means that all the oxidization methods are effective to generate a hydrophilic surface. For ICP/RIE plasma treatments, the water contact angle changes are slightly less than the wet chemical treated sample due to the presence of hydroxide on wet chemical treated samples [83]. The difference between ICP and RIE treatment is not appreciable.

![Figure 3.4: The comparison of water drop profiles on different surface for water contact angle measurements.](image_url)
Electrical Properties

Thin oxide films have been used to passivate AlGaN/GaN heterostructure surface and suppress gate leakage current [91, 92]. It has been shown that the SBH is increased in oxide passivated AlGaN/GaN diodes [91].

In this work, the SBHs are extracted using the model with an ideal diode in parallel with a resistor to account for the effects of tunneling or surface leakage current [93]. The thermionic current of a diode is given by

\[
I = I_s \exp \left[ \frac{q(V_a - IR_s)}{nkT} - 1 \right],
\]

\[
I_s = AA^{**}T^2 \exp \left( -\frac{q\phi_b}{kT} \right),
\]

where \( V_a, R_s, n, q, k, T, A, \phi_b, \) and \( A^{**} \) are the applied voltage, series resistance, ideality factor, electronic charge, Boltzmann’s constant, measurement temperature in Kelvin, the Schottky metal area, the SBH, and the Richardson constant for GaN, respectively. \( V_a - IR_s \) is the voltage applied on the Schottky barrier. A parallel resistance \( R_p \) is added to model the leakage current. The total current can expressed as

\[
I_{total} = I_s \exp \left[ \frac{q(V_a - IR_s)}{nkT} - 1 \right] + \frac{V_a - IR_s}{R_p}.
\]

Equation (3.3) can be used to fit measured diode I-V curves to extract SBH, ideality factor, and \( R_s \).

The extracted values of SBHs are shown in Table II. Compared with the REF devices, ICP and piranha solution treated samples have higher or similar values of SBH while devices treated by RIE plasma have a decreased value of SBH. The 2DEG sheet carrier concentrations determined by C-V characteristics [94] are also shown in Table II. Compared to REF devices, all oxidized devices show a slight decrease in carrier concentration, while
RIE plasma treated sample shows a slightly greater decrease compared with ICP and piranha solution treated samples. This is understood as ICP and piranha solution treatments are more chemical processes and the increase of SBH is the result of thin surface oxide. While RIE treatment is both chemical and physical, the decrease of SBH is caused by the damage of AlGaN/GaN crystal by ion bombardment. Due to the oxidization of the surface of AlGaN barrier layer, from C-V characteristics, we have observed a shift of threshold voltages toward to the positive direction [63, 95]. This is likely due to the decrease of 2DEG sheet carrier concentration as shown in Table II after surface oxidization and RIE plasma damage to the crystal.

There are varieties of methods to extract surface and interface states properties. For metal-oxide-silicon (MOS) structures, extraction methods based on C-V and conductance-voltage (G-V) characteristics are widely used [96]. Because metal/AlGaN/GaN structure is similar to MOS structure, the methods developed for silicon devices have also been used for AlGaN/GaN devices. In this work, we used the conductance method to extract surface and interface state density and time constant based on G-V characteristics of AlGaN/GaN diodes [97, 98]. The equivalent circuits for measurement and modeling is shown in Fig. 3.5 [97]. In Fig. 3.5(a), the $C_m$ and $G_m$ are measured parallel capacitance and conductance. In Fig. 3.5(b), $C_b$ is the barrier capacitance, $C_s$ is the space layer capacitance, $C_{it}$ and $R_{it}$ are the interface trap capacitance and loss term. $R_s$ is the series resistance. Fig. 3.5(b) is simplified into Fig. 3.5(c), where $C_p$ and $G_p$ can be extracted from measured $C_m$ and $G_m$. 

63
values by equations (3.4) and (3.5):

\[
C_p = \frac{-C_b[(C_m^2 - C_m C_b)\omega^2 + G_m^2]}{\omega^4 C_m C_b^2 R_s^2 + \omega^2(C_b^2 R_s^2 G_m^2 + C_m^2 + C_b^2 - 2C_b^2 R_s G_m - 2C_b C_b) + G_m^2}, \tag{3.4}
\]

\[
G_p \frac{\omega}{\omega} = \frac{-\omega C_b^2(R_s C_m^2 \omega^2 + R_s G_m - G_m)}{\omega^4 C_m C_b^2 R_s^2 + \omega^2(C_b^2 R_s^2 G_m^2 + C_m^2 + C_b^2 - 2C_b^2 R_s G_m - 2C_b C_b) + G_m^2}. \tag{3.5}
\]

Figure 3.5: The equivalent circuits for the extraction of interface trap characteristics. (a) circuit model for measurement, (b) circuit model for trap states, and (c) simplified circuit model for extraction.

By comparing Fig. 3.5 (b) and (c), the interface trap density \(D_{it}\) and time constant \(\tau\) can be extracted. For the case that the traps at only a single energy level, \(C_p\) and \(G_p/\omega\) are given by:

\[
C_p = C_s + \frac{C_{it}}{1 + (\omega \tau)^2}, \tag{3.6}
\]

\[
G_p \frac{\omega}{\omega} = \frac{q \omega \tau D_{it}}{1 + (\omega \tau)^2}. \tag{3.7}
\]
For the case of a continuum of energy levels:

\[
C_p = C_s + \frac{C_{it}}{\omega \tau \tan(\omega \tau)}, \quad (3.8)
\]

\[
\frac{G_p}{\omega} = \frac{qD_{it}}{2\omega \tau} \ln[1 + (\omega \tau)^2]. \quad (3.9)
\]

Typical measured conductance \( G_p/\omega \) curves are shown in Fig. 3.6. It turned out that the single trap level model fits the measured \( G_p/\omega \) curves. As shown in Fig. 3.6, compared to REF curve, the peak positions of the conductance \( G_p/\omega \) curves for the oxidized devices shift to lower angular frequency direction, which suggests longer time constants of surface and interface states. The peak admittance values of all oxidized samples are lower than that of the REF sample, which indicates a decrease of trap density. The average values and deviations of extracted trap densities and time constants from randomly picked devices are shown in Table 3.2. The trap densities of oxidized samples decrease slightly compared to REF samples. The trap time constants of RIE devices increase slight compared to REF samples, while the trap time constants of ICP and piranha solution treated devices are significantly longer, which suggests that the shallow traps with small time constants on the AlGaN surface are passivated during the oxidization processes. Though unlikely, based on these results, it is hard to tell if there are any new deep traps introduced during the oxidization processes for ICP and piranha solution treated samples.

**Fluorescence Imaging and ELISA Analysis**

Fluorescent images of REF, ICP, RIE and PS AlGaN samples with immobilized Alexa Fluor 488 labeled SA molecules are shown in Fig. 3.7. The intensity ratios of biotinylated AlGaN samples to correspondingly oxidized unbiotinylated samples are 167/1, 182/1, and
Figure 3.6: Typical measured $G_\phi/\omega$ characteristics. The inset shows the equivalent circuit which is used for parameter extractions.

152/1 for samples oxidized by, RIE, ICP and PS methods, respectively, and 80/1 for a sample with a native oxide (REF). The intensity ratios between biotinylated samples and unbiotinylated AlGaN samples indicate that there is little physical adsorption of SA molecules to 3-APTES films on the oxidized AlGaN surfaces. The enzyme-linked immunosorbent assay (ELISA) provided absorbance data for each oxidization protocol correlated to the SA binding for the biotinylated AlGaN chip surface, which was averaged (after normalization) and the standard error was calculated (Fig. 3.8).
Table 3.2: SBH, threshold voltage, and trap density change. Extracted from I-V and C-V characteristics.

<table>
<thead>
<tr>
<th></th>
<th>REF</th>
<th>ICP</th>
<th>RIE</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBH (eV)</td>
<td>0.71±0.07</td>
<td>0.94±0.04</td>
<td>0.57±0.04</td>
<td>0.78±0.12</td>
</tr>
<tr>
<td>$V_{th}$ (V)</td>
<td>-4.26±0.19</td>
<td>-4.16±0.15</td>
<td>-3.90±0.10</td>
<td>-3.96±0.05</td>
</tr>
<tr>
<td>$n_s$ ($\times10^{-13}$ cm$^{-2}$)</td>
<td>1.10</td>
<td>1.07</td>
<td>0.93</td>
<td>0.97</td>
</tr>
<tr>
<td>$D_{it}$ ($\times10^{12}$ cm$^{-2}$eV$^{-1}$)</td>
<td>3.53±0.85</td>
<td>2.65±0.01</td>
<td>3.11±0.55</td>
<td>2.98±0.15</td>
</tr>
<tr>
<td>$\tau$ (µsec)</td>
<td>3.55±0.37</td>
<td>95.3±31.8</td>
<td>9.50±2.54</td>
<td>188±92.5</td>
</tr>
</tbody>
</table>

Figure 3.7: Fluorescent images of (a) REF, (b) ICP, (c) RIE, and (d) PS samples. The inset pictures are the fluorescent images of the corresponding reference samples. The inset of small pictures on top right corner of each picture are the fluorescent images of samples without labeled SA bonding.
In both ELISA and fluorescence experiments, comparison between biotinylated samples suggests that ICP oxidized samples were most efficiently silanized and biotinylated. While ELISA is a more widely accepted method for protein quantification, the fluorescence method can reveal differences in distribution of biotinylated silanes on AlGaN surfaces as a function of oxidization protocol. This difference can not be revealed by ELISA, as ELISA assays do not provide position related SA concentration differences. Nonetheless, ELISA data corroborates rank order efficiency of differentially oxidized surfaces to support derivatization that was established in the fluorescence experiments. The comparisons of the fluorescent coverage homogeneity shown in Fig. 3.7 and the normalized total adsorption shown in Fig. 3.8 indicate again that ICP is the best oxidization protocol for APTES functionalization of the AlGaN device surface.

Figure 3.8: Graph from ELISAs comparing SA binding to silane treated, biotinylated AlGaN chips oxidized by ICP, RIE and PS methods using absorbance normalized by surface area in $\mu$m$^2$. Data indicate that ICP treatment is the best oxidization protocol for APTES functionalization of AlGaN devices when compared to RIE and PS treatments.
3.1.4 Results

By the comparisons of previous characterization methods, ICP oxidized surface showed good oxidization effectiveness, reduced trap densities, better fluorescent intensity for SA bonding, and also good compatibility with microelectronic device fabrication process. This process was deployed in AlGaN/GaN HFET biosensors for SA detection.

3.2 Improvement of Sensitivity with Subthreshold Regime Operation with A Control Gate Electrode

3.2.1 Introduction

Without any electrochemical side effects (e.g., oxidize or reduce the AlGaN surface and SAMs), a gate voltage is applied through a Pt control electrode to the solution so that the device operates sensitively in the subthreshold regime. Due to the logarithmic relationship between the channel current and gate voltage in the subthreshold regime, at a concentration of 4.73 pM streptavidin, the device exhibits 9.97% current change in the subthreshold regime compared with the current in phosphate buffered saline solution. In the linear regime, the current change is 0.49% at the same streptavidin concentration.

3.2.2 The AlGaN/GaN HFET Protein Sensor Fabrication

The device fabrication and surface modification processes are identical to the descriptions in subsections 2.3.3 and 2.3.4. The AlGaN/GaN heterostructure is slightly different with a Fe-doped GaN buffer layer for the compensation of background carriers. The sheet carrier concentration is $1.0 \times 10^{13} \text{ cm}^{-2}$ and the Hall mobility is $2100 \text{ cm}^{2}/(\text{V} \cdot \text{sec})$. Fig. 3.9 shows the cross-sectional view of the HFET device and subthreshold measurement setup. As shown, a Pt reference electrode (a 0.5 mm diameter platinum wire from Aldrich,
St. Louis, MO, 99.99% purity) was dipped into the PBS solution with the relative height set at 1.5 mm to the AlGaN surface by a precisely controlled XYZ probe arm.

### 3.2.3 Measurement Results

$I_d - V_d$ measurements were performed in both subthreshold and linear regimes. In the subthreshold regime, a gate voltage of -3.4 V was applied via the floating Pt electrode and in the linear regime, a gate voltage of 0 V was applied. For $I_d - V_g$ measurements, the gate voltage was swept from 0 V to -5 V while the drain voltage was fixed at 0.5 V. The measured $I_d - V_g$ and $I_g - V_g$ characteristics and device transconductance as a function of gate bias are shown in Fig. 3.10. The $I_d - V_g$ curves show typical HFET-like behavior. The
device exhibits excellent pinch-off (the drain side channel carriers are depleted by the gate and drain biases) characteristics. The drain current at off-state is on the order of nA/mm. The gate leakage current is on the order of nA/mm or lower. There was no observable gate current peak during \( V_g \) sweep, indicating that no reduction/oxidization reaction occurred at the AlGaN/PBS interface, and the modified surface properties remained unaffected. The HFET device has a threshold voltage of -3.64 V, which is determined by the intersection of linear extrapolation of drain current at the maximum transconductance gate bias. Re-write equations and (2.12) and (2.12):

\[
I_{	ext{sub}} = \mu C_{\text{barrier}}(m - 1) \frac{W}{L} \left( \frac{k_B T}{q} \right)^2 \times 
\exp \left( \frac{q(V_g - V_{\text{th}})}{m k_B T} \right) \left( 1 - \exp \left( - \frac{qV_d}{k_B T} \right) \right),
\]

(3.10)

\[
I_{\text{lin}} = \mu C_{\text{barrier}}(V_g - V_{\text{th}}) V_d \frac{W}{L}.
\]

(3.11)

As shown, in subthreshold regime, \( I_d \) exhibits an exponential relationship with \( V_g \). The measured \( \log_{10}(I_d) \) vs. \( V_g \) slope is 3.208 decade/V, whereas \( I_d \) is in a linear relationship with \( V_g \) in the linear regime. Hence, with identical threshold voltage shifts, the ratio of current change in the subthreshold regime is much greater than in the linear regime.

### 3.2.4 Discussion

The results of measured \( I_d - V_d \) characteristics for the detection of 4.73 pM SA in 0.25 PBS are shown in Fig. 3.11. We define the sensitivity in percentage as

\[
S = \frac{\Delta I}{I_0} \times 100\%.
\]

(3.12)

A sensitivity of 9.97% is determined at the subthreshold regime while a sensitivity of 0.49% is determined at the linear regime. Because SA molecules in PBS (pH 7.4) are negatively
charged with -4 electrons [99, 100], some electrons in the channel at the AlGaN/GaN interface are depleted by SA molecules bound to the AlGaN surface. The threshold voltage is shifted to the positive direction. Although the current decrease in both subthreshold and linear regimes validate the specific binding of SA molecules to the biotinylated AlGaN surface, there is significant (20×) difference in sensitivities.

Fig. 3.12 shows an illustrative explanation of subthreshold regime working principle. As shown in both Fig. 3.12(a) and (b), the carrier density in the channel has a minimum value on the source side due to a lower potential. The depletion edge in the linear regime is farther from the AlGaN/GaN interface than that in the subthreshold regime. With the SA molecules immobilized on the AlGaN surface, the carriers in the channel are depleted;
Figure 3.11: $I_d$-$V_d$ characteristics of AlGaN/GaN HFET biosensors for the detection of 250 pg/ml (4.73 pM) SA in 0.25× PBS at (a) subthreshold ($V_g = -3.4$ V) and (b) linear regimes ($V_g = 0$ V).

Figure 3.12: The depletion region change of an AlGaN/GaN HFET biosensor before (black solid) and after (red dash) SA molecule immobilization working at a) the subthreshold regime and b) the linear regime.
hence, the depletion edge moves towards the AlGaN/GaN interface for both cases. However, because in the linear regime the carrier density is much larger than in the subthreshold, the change of ratio is more sensitive for the subthreshold regime case.

To illustrate the sensitivity dependence on $V_g$, Fig. 3.13 shows the current decrease ($\Delta I_d$) and sensitivity vs. $V_g$ for 4.73 pM SA solution. The measured $I_d - V_g$ characteristics are shown in the inset of Fig. 3.13. Because the transconductance in the linear regime is higher than that in the subthreshold regime, greater current decreases are observed with the same threshold voltage shift. However, due to the greater log10($I_d$) vs. $V_g$ slope as shown in Fig. 3.10, the sensitivity is much higher in the subthreshold regime. The summarized sensitivities for all tested SA concentrations in both subthreshold and linear regimes are shown in Table I. The test results for both the pre-saturated and 0.473 pM SA show a current decrease of about 6%. However, the biotin-SA binding has a certain disassociation rate, though it is very small. As a result, there are always some SA molecules that are not biotinylated in a pre-saturated solution. Therefore, we believe that the 6% current decrease in 0.473 pM SA solution could be a real signal. Nevertheless, at the conservative side, we claim a device sensitivity of 4.73 pM. As the SA solution concentration increases further, the sensitivity increases significantly to a few tens of percent, e.g. 67.26% for 4.73 nM SA. As comparisons, for all tested SA concentrations, the sensitivities in the linear regime are less than 1%, which are two orders of magnitude lower than in the subthreshold regime. The threshold voltage shifts extracted from $I_d - V_g$ characteristics before and after SA binding are shown in Table I. Negative control solution and 473 fM SA solution introduce a 10 mV threshold voltage shift, whereas greater threshold voltage shifts are observed at higher SA concentrations. With 4.73 nM SA solution, the threshold voltage shift is 44 mV.
The densities of transferred charge from bound SA molecules to the device channel in Table I are calculated by \( Q = C_{\text{barrier}} \times \Delta V_{\text{th}} \), where the barrier capacitance was measured to be \( 3.18 \times 10^{-7} \text{ F/cm}^2 \). In the SA concentration range of 4.73 pM to 4.73 nM in 0.25× PBS, the transferred charge density ranges from \( 3.78 \times 10^{10} \text{ cm}^{-2} \) to \( 8.75 \times 10^{10} \text{ cm}^{-2} \), which is about one order higher than the reported charge detectable in an AlGaN/GaN HFET hydrogen gas sensor [65]. The reasons are mainly attributed to the following: (a) the binding site density is higher in hydrogen sensor as both biotin and streptavidin are much larger molecules than hydrogen; (b) in hydrogen sensors, disassociated hydrogen binds directly on the semiconductor (AlGaN) and the catalytic metal interface forming a dipole layer whereas in the devices reported here the distance between the binding sites to the semiconductor surface is about 3 nm, which is about double distance of Debye length in 0.25× PBS solution used in this work. Nevertheless, the device sensitivity demonstrated in this work is 7 orders higher than the reported AlGaN/GaN HFET SA sensors [36]. To further improve the device performance, our current efforts are being directed to the following. Since the gate bias in PBS results in the movements of ions and introduces noise in the measurements, we are recessing the AlGaN barrier for the devices to operate at the subthreshold regime without applying any gate voltage. Furthermore, the device sensitivity can be correlated with the sheet carrier concentration-mobility product values of devices recessed at different AlGaN barrier thicknesses. Another factor is the non-uniformity of the APTES layer. Since APTES forms a network instead of a smooth and uniform layer on the AlGaN surfaces [80], the non-uniformity of APTES also introduces noise to the tests. To minimize this effect, a more uniform 3-aminopropyldimethylethoxysilane (APDMES) layer is being used to replace the non-uniform APTES layer.
Table 3.3: The measured sensitivities in the linear and the subthreshold regimes, threshold voltage shifts, and effective transferred charge densities dependence of SA concentrations.

<table>
<thead>
<tr>
<th>Concentrations (pM)</th>
<th>$S_{\text{lin}}$ (%)</th>
<th>$S_{\text{sub}}$ (%)</th>
<th>$\Delta V_{\text{th}}$ (mV)</th>
<th>$\Delta Q$ (cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.C.</td>
<td>0.45</td>
<td>6.42</td>
<td>10</td>
<td>$1.99 \times 10^{10}$</td>
</tr>
<tr>
<td>0.473</td>
<td>0.58</td>
<td>6.00</td>
<td>10</td>
<td>$1.99 \times 10^{10}$</td>
</tr>
<tr>
<td>4.73</td>
<td>0.49</td>
<td>9.97</td>
<td>19</td>
<td>$3.78 \times 10^{10}$</td>
</tr>
<tr>
<td>47.3</td>
<td>0.57</td>
<td>37.82</td>
<td>23</td>
<td>$4.57 \times 10^{10}$</td>
</tr>
<tr>
<td>473</td>
<td>0.18</td>
<td>43.28</td>
<td>25</td>
<td>$4.97 \times 10^{10}$</td>
</tr>
<tr>
<td>4730</td>
<td>0.78</td>
<td>67.26</td>
<td>44</td>
<td>$8.75 \times 10^{10}$</td>
</tr>
</tbody>
</table>

Figure 3.13: The drain current change due to SA binding at a concentration of 250 pg/ml (4.73 pM) in 0.25 × PBS and device sensitivity as a function of gate bias at $V_d = 0.5$ V. The inset shows two measured $I_d$-$V_g$ characteristics before and after SA binding.
Figure 3.14: The subthreshold $I_d - V_d$ characteristics measured before (blue solid) and after (red dash) MIG applied.

Detection of Biotinylated MIG

Detection of MIG is also developed using the subthreshold regime measurements. The surface modification procedure for biotinylated MIG immobilization is described in section 2.3.4. The target MIG concentration is 10 ng/ml (880 pM), which is close to the MIG concentration in patients. As shown in Fig. 3.14, the drain current is increased by 11%. The feasibility of MIG detection on AlGaN/GaN HFET biosensors is approved.

3.2.5 Conclusion

In conclusion, we have investigated the sensing performance of AlGaN/GaN HFET biosensors at the subthreshold regime for protein detection. The detection sensitivity in the subthreshold regime is significantly higher than that in the linear regime. At the concentration of 250 pg/ml (4.73 pM), the device sensitivity are 9.97% and 0.49% in the subthreshold
regime and the linear regime, respectively. Also, the detection of biotinylated MIG demonstrate the feasibility of possible clinical application of AlGaN/GaN HFET biosensors.

3.3 High Sensitivity AlGaN/GaN HFET Protein Sensors with Recessed AlGaN Barrier

3.3.1 Introduction

As shown in section 3.2, AlGaN/GaN HFET biosensors shows the maximum sensitivity at the subthreshold regime. However, to achieve a carrier density of $1 \times 10^{13} \text{cm}^{-2}$, the thickness of AlGaN barrier is usually around 20 nm. To bias the channel into the sub-threshold regime, $V_g = -3$ - $-4 \text{ V}$ needs to be applied. This gate voltage will cause ions in the physiological buffer solution to move and result in measurement noise. In addition, $-4 \text{ V}$ is sufficient for many electrochemical reactions to take place so that the measurement setup is not in a stable status. To avoid side effects from a high gate voltage, we adopted the gate recess process from the development of enhancement-mode AlGaN/GaN high electron mobility transistors (HEMTs) to shift the subthreshold gate voltage to zero/near zero volt and retain high sensitivity.

3.3.2 Recession Procedure

The recession of the AlGaN barrier is implemented with an Oxford Plasmalab100 system. The recession process contains two steps. The first step is to use BCl$_3$ to etch the AlGaN barrier, and the second step is to use Cl$_2$/O$_2$/N$_2$ gases with zero voltage biasing to passivate the etched surface to obtain high quality surfaces [84]. The details of the processes are show in Table A.1 in Appendix A.5.1. To shift the threshold voltage to near zero volt, the AlGaN barrier needs to be etched to a certain thickness. The threshold voltage shift is more sensitive to the first step duration. To find the right thickness, diodes
were fabricated with varied first step durations to find the necessary condition to shift the threshold voltage to 0 volt.

### 3.3.3 Diode Fabrication

The fabrication procedure of recessed diodes is shown in Fig. 3.15. The AlGaN/GaN heterostructure used in this study is from the same batch which is used in section 3.2. Five diode samples were fabricated with the recession etching step duration to be 50, 55, 60, 65, and 70 seconds respectively. AFM measurements were implemented in step e), after the removal of SiO$_2$ and before the patterning of Schottky layer.

### 3.3.4 Threshold Voltage Shift and Carrier Profiling

The C-V characteristics of diodes without recession and the etching step durations of 50, 55, 60, 65, and 70 seconds are shown in Fig. 3.16. There are a few major differences. Firstly, the capacitance at $V_a=0$ V increases ($V_a=0.7$ V for 70 sec), which is summarized in Table 3.4. Because $C = \varepsilon/d_{\text{AlGaN}}$, the increase of accumulation capacitance with the increase of recession duration indicates that the decrease of the AlGaN barrier thickness. Secondly, the threshold voltage, which is extracted from the extrapolation of the C-V curves in the depletion regime, shifts to the positive direction with the increase of recession duration. As shown in Table 3.4, with the etching duration of 70 sec, the diodes with Ni/Au Schottky metals reaches a slight positive threshold voltage. At $V_g=0$ V, the device is at sub-threshold regime. However, because the AlGaN surface of HFET biosensors is in contact with PBS, the real device threshold voltage may be slightly different. The carrier concentration vs. depth profiles are extracted from the C-V characteristics. Similar to MOS structures, for AlGaN/GaN heterostructures, carrier concentration vs. depth profile at the AlGaN/GaN interface can be extracted from measured C-V characteristics [1, 101]:

79
\[
\frac{1}{C'} = \frac{1}{C_{AlGaN}} + \frac{1}{C_s},
\]  \hspace{1cm} (3.13)

\[
n(z) = \frac{C_s^3}{q\epsilon_{GaN}\epsilon_0} \frac{dV}{dC_s},
\]  \hspace{1cm} (3.14)

\[
z = \frac{\epsilon\epsilon_{GaN}}{C_s},
\]  \hspace{1cm} (3.15)

where \(C\) is the measured differential capacitance normalized by the Schottky area, \(C_{AlGaN} = \epsilon_0\epsilon_{AlGaN}/d\) is the AlGaN barrier capacitance, \(C_s\) is the depletion capacitance in the channel, \(V\) is the applied voltage, \(q\) is the unit electron charge, \(\epsilon_0\) and \(\epsilon\) are the dielectric constant of the vacuum and AlGaN barrier respectively. The sheet carrier concentration can be obtained by integrating the carrier concentration along \(z\) direction:

\[
n_s = \int_{-\infty}^{+\infty} n(z)dz.
\]  \hspace{1cm} (3.16)

The extracted carrier concentration vs. depth profile is shown in Fig. 3.17. The integrated sheet carrier concentration is shown in Table 3.4.
Figure 3.15: An overview of fabrication procedure of AlGaN/GaN diodes with the Al-GaN barrier recessed. a) The original AlGaN/GaN heterostructure. b) Photolithographical patterning of the Ohmic layer, deposition and lift-off of Ohmic metals, and annealing. c) After deposition of 500 Å SiO$_2$ layer, a photolithography step to open a circular window with a diameter of 120 µm window to remove the SiO$_2$ layer by a diluted buffered oxide etch (BOE) dip, and photoresist is removed by acetone. d) Recession of the AlGaN barrier with an Oxford Plasmalab100 system. e) Removal of the SiO$_2$ layer with diluted BOE, photolithographical patterning of the Schottky layer, Ni/Au deposition, and lift-off.
Figure 3.16: The measured C-V curves of diodes recessed by 50, 55, 60, 65, and 70 sec respectively.

3.3.5 Recessed AlGaN/GaN HFET Biosensor

The recessed AlGaN/GaN HFET biosensor fabrication process is shown in Fig. 3.18. All the steps are identical to the process described in section 3.2 except the recession steps d) and e). In step d), a 500 Å SiO$_2$ is deposited, patterned, and etched for masking the non-recession areas. After recession, the SiO$_2$ layer is removed. Fig. 3.19 shows the top view of a finished device. As shown, the recessed area is exposed for surface treatment and detection, as well as part of non-recessed area. Fig. 3.20 shows the cross-sectional view of a recessed AlGaN/GaN HFET biosensor with the test setup and an equivalent circuit. With the test setup, the Pt gate electrode is used to measure the $I_d - V_g$ curve, and it is removed for open-gate measurements. The equivalent circuit has five components, which are the Ohmic contact resistances on the source ($R_s$) and the drain ($R_d$) sides, the channel resistances between the source/recession ($R_{chs}$) and drain/recession ($R_{chd}$) regions, and the active channel.
Figure 3.17: The extracted carrier vs. depth profile. The 0 nm plane is at the AlGaN/GaN interface.

Table 3.4: The extracted parameters of AlGaN/GaN Ni/Au diodes with the AlGaN barrier being recessed for 0, 50, 55, 60, 65, and 70 seconds respectively.

<table>
<thead>
<tr>
<th>Property</th>
<th>0 sec</th>
<th>50 sec</th>
<th>55 sec</th>
<th>60 sec</th>
<th>65 sec</th>
<th>70 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitance (nF/cm²)</td>
<td>386.6</td>
<td>693.6</td>
<td>710.2</td>
<td>734.1</td>
<td>853.3</td>
<td>956.4</td>
</tr>
<tr>
<td>( n_s \times 10^{12} \text{ cm}^{-2} )</td>
<td>10.7</td>
<td>6.73</td>
<td>6.21</td>
<td>4.71</td>
<td>2.62</td>
<td>0.52</td>
</tr>
<tr>
<td>AlGaN thickness (Å)</td>
<td>24</td>
<td>120</td>
<td>116</td>
<td>112</td>
<td>97</td>
<td>88</td>
</tr>
<tr>
<td>Threshold Voltage (V)</td>
<td>-3.69</td>
<td>-1.66</td>
<td>-1.63</td>
<td>-1.12</td>
<td>-0.71</td>
<td>0.0196</td>
</tr>
</tbody>
</table>

\(^a\) At \( V_a = 0.7 \text{ V} \)

\(^b\) ICP oxygen plasma treated Schottky surface.
resistance under the recessed region (R_{act}). Because \( R_{act} \gg \max\{R_s, R_d, R_{chs}, R_{chd}\} \), the total resistance can be approximated as:

\[
R_{tot0} = R_s + R_d + R_{chs} + R_{chd} + R_{act}
\]

(3.17)

\[
\approx R_{act}.
\]

(3.18)

With SA or other target molecules bond to the surface, \( R_s \) and \( R_d \) do not change, whereas \( R_{chs}, R_{chd}, \) and \( R_{act} \) change to the same direction. Because the channel between source and drain to the recessed active area is working at linear regime, the percentage change of \( R_{chs} \) and \( R_{chd} \) is much smaller than that of \( R_{act} \) (\( \Delta R_{act} \gg \max\{\Delta R_s, \Delta R_d, \Delta R_{chs}, \Delta R_{chd}\} \)) as has been demonstrated in section 3.2. As a result, the percentage change of total resistance can be expressed as:

\[
S = \frac{\Delta R_{tot}}{R_{tot0}} = \frac{\Delta R_{act} + \Delta R_s + \Delta R_d + \Delta R_{chs} + \Delta R_{chd}}{R_s + R_d + R_{chs} + R_{chd} + R_{act}}
\]

(3.19)

\[
\approx \frac{\Delta R_{act}}{R_{act}} \times 100\%.
\]

(3.20)

As shown in Equation (3.20), \( R_s, R_d, R_{chs}, \) and \( R_{chd} \) do not have appreciable affects on the device performance. The recessed active region dominates the device performance.

The measurement set-up is shown in Fig. 3.20. It is similar to the subthreshold regime measurement described in the previous section. The only difference is the recessed AlGaN barrier. The comparison of \( I_d - V_g \) curves of non-recessed and recessed AlGaN/GaN HFET biosensors are shown in Fig. 3.21. As shown, there are two major differences. The first is that the threshold voltage is shifted to the positive direction, which is around -0.5 V. Only a small gate voltage is needed for the device to work at subthreshold regime (e.g. \( V_g = -0.3 \) V). Secondly, the off-state drain current is reduced by two orders, which is slightly higher than \( 1 \times 10^{-10} \) A. The decrease of the off-state drain current could increase the signal-to-noise ratio.
Figure 3.18: An schematic overview of the AlGaN/GaN HFET protein sensors fabrication procedure with recessed AlGaN barriers. a) The original AlGaN/GaN heterostructure. b) After mesa patterning, dry etching, and removal of photoresist. c) After Ohmic layer patterning, metal deposition and lift-off, annealing, Overlay layer patterning, metal deposition and lift-off. d) After deposition of 500 ÅSiO$_2$ layer, a photolithography step to open a 100 µm wide window to remove the SiO$_2$ layer by BOE dip, and photoresist removal. e) After recess the AlGaN barrier and removal of the SiO$_2$ layer. f) After applying sealing material (polyimide, silicone, etc.) to form reservoir.
Figure 3.19: A topview of an AlGaN/GaN HFET protein sensor with recessed AlGaN barrier.

Figure 3.20: The cross-section view of a recessed AlGaN/GaN HFET biosensor, the electrical test setup, and the equivalent circuit.
To demonstrate the device behavior, a recessed AlGaN/GaN HFET protein sensor is modified with APTES and biotinylation for SA detection. Fig. 3.22 shows the measurement results of 25 pg/ml SA. The current change is 22.7%, which is improved by one order compared to section 3.2.

### 3.4 Detection of MIG

The recessed AlGaN/GaN HFET biosensors are also used in MIG detection. The Al-GaN surfaces are firstly modified with APTES, then treated with IgG with anti-human MIG (anti-huMIG) or anti-murine MIG (anti-muMIG) receptors. In this process, all amines on the end of the IgG will react with the aldehyde on the surface to link the IgG molecules to the surface. IgG will bind to the specific MIG protein. Figs. 3.23 and 3.24 show the measurements on devices modified with anti-huMIG and anti-muMIG receptors response to human and murine MIG (huMIG and muMIG). As shown, the device with shows an
increase of drain current with the correct target molecules, indicating the binding of positively charged MIG molecules. On the other hand, the drain current does not respond to the wrong target MIG molecules.
Figure 3.23: The schematic of anti-muMIG selectivity to muMIG (upper left) and huMIG (upper right) molecules (courtesy of Ms. Patricia Casal). The currents responses of anti-muMIG modified AlGaN/GaN HFETs to muMIG (lower left) and huMIG (lower right) molecules.
Figure 3.24: The schematic of anti-huMIG selectivity to huMIG (upper left) and muMIG molecules (upper right) (courtesy of Ms. Patricia Casal). The currents responses of anti-muMIG modified AlGaN/GaN HFETs to huMIG (lower left) and muMIG (lower right) molecules.
3.5 Modeling AlGaN/GaN HFET Biosensors

To derive an accurate relationship between the captured biomolecule charges (e.g. SA) and the $I_d$ of the biosensors, the Poisson’s equation will be applied to all involved domains, including both the semiconductor region and the electrolyte region, together with necessary boundary conditions. A schematic of highly idealized AlGaN/GaN surface molecular structure with immobilized SA molecules is shown in Fig. 3.25. For devices with large dimensions compared to the molecule sizes, the derivation is simplified into a two-dimensional problem by neglecting the orientation on the gate width direction. As shown in the figure, the $x$-axis is set to be parallel to the norm of the AlGaN/GaN surfaces, pointing to the PBS solution, whereas $y$-axis is parallel to the 2DEG channel. The whole system can be divided into five different material domains. They are labeled from $\Omega_1$ to $\Omega_5$, which are GaN, AlGaN, organic linker molecules, SA, and PBS solution respectively. The boundaries are labeled from $BC_1$ to $BC_6$.

**The Poisson Equation and Boundary Conditions in GaN Domain** The GaN layer is undoped. The Poisson’s equation in GaN domain is

$$\nabla \cdot (\varepsilon_{\text{GaN}} \nabla \phi_{\text{GaN}}(x, y)) = 0.$$  (3.21)

The boundary condition for the bottom GaN layer fits Neumann boundary condition, which means that there is not potential change at the boundary $BC_1$:

$$\Delta \phi_{\text{GaN}}|_{x=BC1} = 0.$$  (3.22)

At the AlGaN/GaN interface, the discontinuity of spontaneous and piezoelectric polarizations introduce a layer of fixed charges [1, 102]:

$$|\sigma(x)| = |P_{PE}(Al_mGa_{1-m}N) + P_{SP}(Al_mGa_{1-m}N) - P_{SP}(GaN)|,$$  (3.23)
Figure 3.25: The structure of AlGaN/GaN structure with immobilized SA molecules. This figure is not drawn according to scale.

where $P_{PE}(Al_mGa_{1-m}N)$ and $P_{SP}(Al_mGa_{1-m}N)$ are the piezoelectric and spontaneous polarizations in AlGaN and $P_{SP}(GaN)$ is the spontaneous polarization in GaN.

**The Poisson Equation and Boundary Conditions in AlGaN Domain**  
Since the AlGaN layer is also undoped, the Poisson equation for AlGaN layer is

$$\nabla \cdot (\epsilon_{AlGaN} \nabla \phi_{AlGaN}(x,y)) = 0. \quad (3.24)$$

The 2DEG carriers reside close to the AlGaN/GaN interface on the GaN side. The 2DEG sheet carrier concentration at the AlGaN/GaN interface is given by [1, 102, 103]:

$$n_s = \left\{ 1 + \frac{\epsilon_{AlGaN}}{\epsilon_{GaN}} \frac{d_{GaN}}{d_{AlGaN}} \right\}^{-1} \left\{ \frac{\sigma}{q} - \left( \frac{\epsilon_{AlGaN}}{d_{AlGaN}q^2} \right) \times [q\phi_b^{eff} + E_F - \Delta E_C] \right\}$$

$$= \frac{\sigma}{q} - \left( \frac{\epsilon_0 \epsilon_{AlGaN}}{d_{AlGaN}q^2} \right) [q\phi_b^{eff} + E_F - \Delta E_C], \quad (3.25)$$
where the $\epsilon_{\text{AlGaN}}$ and $\epsilon_{\text{GaN}}$ are the permittivities of AlGaN and GaN respectively, $d_{\text{AlGaN}}$ and $d_{\text{GaN}}$ are the thicknesses of AlGaN and GaN respectively, $\sigma$ is the bound sheet charge from spontaneous and piezoelectric polarization, $q$ is the unit positive electron charge, $q\phi^\text{eff}_b$ is the effective Schottky barrier height, $E_F$ is the Fermi level, and $\Delta E_C$ is the conduction band discontinuity. To be in the same form as other boundary condition equations, this equation can be re-organized into Gauss’s law form by:

$$\nabla \cdot D|_{x=BC2} = \nabla \cdot P|_{x=BC2} + \epsilon_0 \nabla \cdot E|_{x=BC2} = n_s. \tag{3.26}$$

In equation (3.25), except $q\phi^\text{eff}_b$, all other parameters are GaN and $\text{Al}_m\text{Ga}_{1-m}\text{N}$ material properties. $q\phi^\text{eff}_b$ is decided by the Fermi levels of AlGaN/GaN system and the organic and solution molecules and ions in contact with the AlGaN surface. The Fermi level $E_F$ in equation (3.25) is given by

$$E_F = E_0 + \frac{\pi \hbar^2}{m^*} n_s, \tag{3.27}$$

where $E_0$ is the ground subband level of the 2DEG, $\hbar$ is the Planck’s constant divided by $2\pi$, and $m^*$ is the effective electron mass in GaN. As can be seen in equations (3.25) and (3.27), $n_s$ and $E_F$ can be calculated iteratively by numerical methods. No analytical expression is available. However, C-V measurements can be used to extract the 2DEG sheet carrier concentration.

At the saline/AlGaN interface, there are no free charges. Hence the boundary condition at this interface can be derived by Gauss’s law. Since the saline and carbon chain of sulfo-NHS-biotin linker molecules are in PBS solution, the dielectric constant for the linker side is between the dielectric constants of the carbon chains ($\sim 1.5$) and water (80).

$$\epsilon_{\text{AlGaN}} \nabla \phi_{\text{AlGaN}}(x,y)|_{x=BC3} = \epsilon_{\text{Linker}} \nabla \phi_{\text{Linker}}(x,y)|_{x=BC3}. \tag{3.28}$$
The Poisson Equation and Boundary Conditions in Linker Domain  

Since linker molecules do not carry free charges in PBS solutions, the Poisson’s equation:

\[ \nabla \cdot \left( \epsilon_{\text{Linker}} \nabla \phi_{\text{Linker}}(x, y) \right) = 0. \]  \hspace{1cm} (3.29)

At the linker/SA interface, there is no free charges. The boundary condition is

\[ \epsilon_{\text{Linker}} \nabla \phi_{\text{Linker}}(x, y) \bigg|_{x=\text{BC}_4} = \epsilon_{\text{SA}} \nabla \phi_{\text{SA}}(x, y) \bigg|_{x=\text{BC}_4}. \]  \hspace{1cm} (3.30)

The Poisson Equation and Boundary Conditions in SA Domain  

Since SA molecules carry amine groups, which are negatively charged in PBS solutions, the Poisson’s equation for SA domain is

\[ \nabla \cdot (\nabla \phi_{\text{SA}}(x, y)) = \rho_{\text{SA}}. \]  \hspace{1cm} (3.31)

Researchers have studied the charges introduced by SA molecules [99, 100]. Based on the reported results, there are 100 amine groups on the envelope of each SA molecule and each amine group contributes 0.04 electron. The diameter of a SA molecule is 5 nm. Based on these data, each of the SA molecules can be modeled as a sphere with 5 nm diameter and the surface charge density to be \(0.04 \times 100 / (4\pi \times (2.5 \text{ nm})^2) = 0.0509 \text{ q/nm}^2\). The SA layer can be modeled as densely packed with such spheres.

At the SA to bulk electrolyte interface, there is no free charge. The boundary condition again can be expressed as

\[ \epsilon_{\text{SA}} \nabla \phi_{\text{SA}}(x, y) \bigg|_{x=\text{BC}_5} = \epsilon_{\text{PBS}} \nabla \phi_{\text{PBS}}(x, y) \bigg|_{x=\text{BC}_5}. \]  \hspace{1cm} (3.32)
The Poisson Equation and Boundary Conditions in PBS Domain

The relationship between the electrolyte potential and charge concentrations can be expressed by Poisson-Boltzmann equation [104]:

$$- \nabla (\varepsilon_w \nabla (\phi(x,y))) = \rho(x,y) = \sum_{i=1}^{N} z_i q c_{i,0} \exp \left( \frac{-z_i q \phi(x,y)}{k_B T} \right),$$

(3.33)

where $z_i$ is the ion valency, $c_{i,0}$ is the bulk concentration of species $i$, $k_B$ is the Boltzmann’s constant, and $T$ is the absolute temperature in Kelvin.

When a Ag/AgCl reference electrode is applied, the potential of the solution is fixed at the Ag/AgCl electrode surface. Hence, the boundary condition of this system on the solution side is a Dirichlet boundary condition, which can be expressed as

$$\phi_{PBS} |_{x=BC6} = V_G \text{ (vs. Ag/AgCl)},$$

(3.34)

where $V_G$ is the applied gate voltage.

Charge Transfer Effects on $I_D$ 

There are 5 equations (equations (3.21), (3.26), (3.29), (3.31), and (3.33)), 6 boundary conditions (equations (3.22),(3.23),(3.28), (3.30), (3.32), and (3.34)) together with source and drain bias to be solved simultaneously. The one unknown in these equations is the potential distribution $\phi(x)$. Once $\phi(x)$ is obtained, it can be inserted into equation (3.23) to solve $n_s$ value. $I_D$ can be expressed as

$$I_D = qW \mu_n \left( n_s(y) \frac{\partial V_c(y)}{\partial y} + \frac{k_B T}{q} \frac{\partial n_s(y)}{\partial y} \right),$$

(3.35)

where $W$ and $L$ are the width and length of the 2DEG channel respectively, $V_c(x)$ is the channel potential, and $\mu_n$ is the electron mobility in GaN. The first term in the bracket represents the drift current component and the second term represents the diffusion current component. When $n_s$ value is available, it can be inserted into equation (3.35) for the $I_D$ value.
Analytical solutions cannot be achieved for this series of equations. Numerical methods have to be used to find $\phi(x)$. A few papers have been published on Si device simulations [104, 105, 106, 107, 108]. The spontaneous and piezoelectric polarization raises difficulties for the simulations of AlGaN/GaN biosensors. Numerical simulations for AlGaN/GaN biosensors are seldom reported until now.
CHAPTER 4

SOI Nanowire ssDNA Sensors

4.1 Introduction

The advantage of silicon over AlGaN/GaN is the maturity of processing and low cost of material, as well as convenient integration with complementary metal oxide semiconductor (CMOS) processing circuits. Table 4.1 summarizes the major differences between Si and AlGaN/GaN heterostructures. Bottom-up silicon nanowires (SiNWs) have been used for biosensor applications with low costs. However, the device uniformity and assembly remain problematic [109]. On the other hand, top-down technologies (i.e. e-beam lithography and ICP/RIE dry etching) can be used to fabricate SiNWs with a feature size bigger than 30 nm, suffering from higher costs. However, the SiNWs fabricated from top-down technologies are with well controlled device dimensions and parameters, which benefits the fundamental investigations. In this chapter, top-down technologies are used to develop SiNW biosensors.

In the past, Si-based biosensors suffered from the ion diffusion in the oxide layer. In recent years, new technologies allow surface modifications on Si with oxide-free surfaces [62, 111, 112, 113], which could improve the Si/liquid interface electrical properties largely. We developed a surface modification procedure for immobilization of ssDNA
Table 4.1: The Material and Device Property Comparisons Between Si and AlGaN/GaN heterostructures used in this work [1, 102, 110]

<table>
<thead>
<tr>
<th>Property</th>
<th>Si</th>
<th>AlGaN/GaN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal structure</td>
<td>Diamond</td>
<td>Wurtzite</td>
</tr>
<tr>
<td>Energy gap (eV)</td>
<td>1.12</td>
<td>3.4-6.1</td>
</tr>
<tr>
<td>Dielectric constant</td>
<td>11.7</td>
<td>9-9.5</td>
</tr>
<tr>
<td>Carrier polarity</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Carrier Mobility (cm²/V · s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electron:</td>
<td>1430</td>
<td>2100</td>
</tr>
<tr>
<td>Hole:</td>
<td>470</td>
<td>—</td>
</tr>
<tr>
<td>Carrier concentration</td>
<td>10¹⁷ cm⁻³ a</td>
<td>10¹² — 10¹³ cm⁻² b</td>
</tr>
<tr>
<td>Oxide quality</td>
<td>Good</td>
<td>amorphous</td>
</tr>
<tr>
<td>Biocompatibility</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Stability in physiological conditions</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

a Bulk Si doping.
b AlGaN/GaN 2DEG concentration.

probes onto Si surfaces and used XPS to characterize the surface properties, especially the ratio of oxygen peak.

Well-developed Si device theory has been applied to predict the performance of Si biosensors, for both 2-D planar devices and 3-D nanowire devices. At present, the most discussed cases are at equilibrium. When a bias is applied, the device is working at steady-state. In this chapter, the simulation of SiNW biosensors with Silvaco is used to study the steady-state performance for better understanding of the work principle and for improve the device sensitivity.
4.2 Design and Fabrication of SiNW Biosensors

4.2.1 The SOI Substrate

As shown in section 3.2, the subthreshold regime shows highest sensitivity. To apply this strategy to SiNWs, we used a SOI substrate, shown in Fig. 4.1. The oxide thickness of this substrate is 150 nm, the handle layer is 500 $\mu$m thick and heavily p-type doped, and the device layer is 40 nm thick with a doping of $5.0 \times 10^{15}$ cm$^{-3}$. The handle layer works as a back-gate electrode to bias the device layer into different regimes (accumulation, depletion, subthreshold, and inversion). The choice of low device layer doping is in the favor of achieving the subthreshold regime without applying a large backgate bias.

4.2.2 The Fabrication Procedure

Fig. 4.2 shows the device process flow. This process starts with a SOI substrate described in the previous section. In step b), a Leica EBPG 5000 e-beam system and an Oxford PlasmaLab 100 RIE/ICP system were used. The Leica system is used to pattern the nanowires with a high resolution e-beam negative-tone resist hydrogen silsesquioxane (HSQ) [76]. A chlorine based chemistry is used with the SLR therm plasma system to transfer the nanowire patterns from the resist layer to Si layer. The resist layer is then stripped off by a diluted HF dip. A scanning electron microscopy (SEM) image is shown.
in Fig. 4.4, which shows a few SiNWs with different designed width. The smallest feature size shown in this picture is around 30 nm. Because the original Si device layer is with a low doping level, an extra step to improve the doping level of Ohmic areas is necessary for good Ohmic contacts. In step c), a 180 nm thick spin-on-glass (SOG) [114] is applied and patterned to open windows for the Ohmic areas. Because the SOG is amorphous, the mobility of the doped ions are highly limited, which makes it a perfect shielding layer for the ion implantation. The details of the ion implantation process is shown in Appendix A.6. Transmission line method (TLM) structures were fabricated and annealed at different temperatures with Al metal layers to minimize the contact resistance on the doped Si device layer. Fig. 4.5 shows that 3 min at 320 °C in a N₂ ambient has contact resistance of $2.2 \times 10^{-7} \, \Omega \cdot \text{cm}^2$. In step d), the vias were open for backgates. The Ohmic metals were then deposited and annealed. The sealing material SU8 or patternable silicone were then applied and patterned to finish the fabrication process. A top-view of a SOI SiNW biosensor is shown in Fig. 4.3.
Figure 4.2: The procedure of fabrication of SiNW biosensors. a) The SOI substrate. b) MESA patterning (e-beam lithography) and dry etching. c) SOG application and patterning, boron implantation, and removal of SOG and oxide. d) Open vias for backgates. e) Ohmic metal deposition and annealing. f) Application and patterning of sealing material (SU8 or patternable silicone).
4.3 Si Surface Surface Modification for ssDNA Hybridization

4.3.1 Ion Drifting in Silicon Dioxide

Most frequently used Si surface modification methods are reviewed in reference [19]. Because Na\(^+\) and K\(^+\) ions from physiological buffer can diffuse into SiO\(_2\) and be driven by the applied electrical field, the methods with oxide surface are not ideal for electrical devices. Fig. 4.6 shows a comparison of normalized time-dependent DC current measured from a Si ISFET with an oxide gate area and an AlGaN/GaN HFET with the gate area exposed to 1× PBS solution. As shown, the AlGaN/GaN HFET current did not change (< 3%), whereas the current from the Si ISFET decreased by 60%. Researchers developed methods to compensate the drifting. For example, S. Jamasb and his coworkers developed a method to counteract the drifting by superimposing a well-established negative drift component [115].
Figure 4.4: An SEM of SiNW fabricated with e-beam lithography and dry etching.

Figure 4.5: The extracted contact resistance vs. annealing temperature of B-doped Si device layer with Al metals. The annealing duration is 3 min.
4.3.2 Oxide-free Si Surface Modification for ssDNA(ssPNA) Detections

In recent years, researchers developed new surface modification technologies based on oxide-free Si surface, which in principle could solve the drifting problem. This oxide-free technology was firstly developed in diamond [62, 112], and then transferred to Si because of the similarities between C and Si [37, 111, 113, 116]. Fig. 4.7 shows the process of immobilizing probe ssDNA molecules onto an oxide-free Si surface we followed. The first step is to create a H-terminated Si surface with 1% HF for 50 sec and NH$_4$F for 60 sec. The H-terminated Si surface is then treated with 10-\textit{N}-Boc-amino-dec-1-ene (Step (2)) in an oxygen free ambient with a ultra-violet (UV) illumination ($\lambda = 254$ nm). In this step, the C=C bonds are broken by the UV light, and the dangling carbon atoms re-bind with the surface Si atoms by replacing H atoms in the Si-H bonds. The surface is then rinsed in chloroform and methanol. To de-protect amine groups by breaking bonds between the CF$_3$-CO- and -NH- groups, the surface is then rinsed into 25% trifluoroacetic acid (TFA) in methylene chloride for 2 hours followed by 10% ammonium hydroxide and DI (step (3)).
surface amine groups react with sulphosuccinimidyl-4- (N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC) (step (4)) and finally reacted with thiol-modified ssDNA or ssPNA (step (5)).

4.3.3 Characterization of Modified Si Surfaces

XPS was used to characterize the modified Si surfaces before step (1), (2), and (3) to confirm the presence of amine groups. A Kratos Axis Ultra XPS system with a monochromatic Al anode X-ray gun is used. Figs. 4.8, 4.9, and 4.10 show the O 1s, Si 2p, and N 1s peaks of a native Si surface, a H-terminated Si surface, and a Si surface modified with 10-N-Boc-amino-dec-1-ene. There are a few differences that can be observed. The extracted elemental percentages are summarized in Table 4.2. Compared to Fig. 4.8(b) and (c), the O 1s peak in Fig. 4.8(a) is smoother, the oxygen percentage of which is 33.75%. The H-terminated Si surface shows much lower oxygen concentration. In the Si 2p curves, the Si dioxide peak is on the high binding energy side (at around 103.0 eV). As shown in Fig. 4.9, the native oxide has the highest SiO₂ peak, the H-terminated Si surface does not have an obvious SiO₂ peak, and the modified Si surface has a low SiO₂ peak. It indicates that the Si surface got oxidized during the incubation step. Fig. 4.10(a) and (b) show that the nitrogen peak on the native and H-terminated Si surfaces have low nitrogen percentage. With the amine groups immobilized on the Si surface, Fig. 4.10(c) has a well-defined N 1s peak, which confirmed the presence of amine groups on the modified Si surface.
Figure 4.7: The process of immobilizing ssDNA probe molecules onto an oxide-free Si surface. (1) HF and NH$_4$F dip to turn a Si surface with native oxide to a H-terminated surface. (2) Incubation with 10-$N$-Boc-amino-dec-1-ene with 254 nm UV light in an oxygen-free ambient for 12 hours. (3) De-protect amine groups with TFA in methylene chloride, ammonium hydroxide and DI rinse. (4) Incubation with SSMCC. (5) Incubation with thiol-terminated ssDNA (or ssPNA).

Table 4.2: XPS peak area percentages of a native Si surface, a H-terminated Si surface, and a modified Si surface.

<table>
<thead>
<tr>
<th></th>
<th>O 1s</th>
<th>Si 2p</th>
<th>N 1s</th>
<th>C 1s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Si surface</td>
<td>33.75</td>
<td>51.47</td>
<td>0.54</td>
<td>14.57</td>
</tr>
<tr>
<td>H-terminated Si surface</td>
<td>8.00</td>
<td>61.26</td>
<td>1.50</td>
<td>29.28</td>
</tr>
<tr>
<td>Modified Si surface</td>
<td>16.90</td>
<td>47.40</td>
<td>3.00</td>
<td>32.70</td>
</tr>
</tbody>
</table>
(a) The O 1s peak on a native Si surface. (b) The O 1s peak on a H-terminated Si surface.

(c) The O 1s peak on a Si surface modified with 10-N-Boc-amino-dec-1-ene.

Figure 4.8: The O 1s peaks at different steps during the immobilization of amine groups.
(a) The Si 2p peak on a native Si surface. (b) The Si 2p peak on a H-terminated Si surface. (c) The Si 2p peak on a Si surface modified with 10-N-Boc-amino-dec-1-ene.

Figure 4.9: The Si 2p peaks at different steps during the immobilization of amine groups.
(a) The N 1s peak on a native Si surface.  
(b) The N 1s peak on a H-terminated Si surface.  
(c) The N 1s peak on a Si surface modified with 10-N-Boc-amino-dec-1-ene.

Figure 4.10: The N 1s peaks at different steps during the immobilization of amine groups.
Figure 4.11: The fluorescent images of Si surfaces modified with Cy5-modified ssDNA probe molecules, and after hybridization with Cy3-modified complementary ssDNA target molecules.

Fig. 4.11 shows the fluorescent images after the probe ssDNA and complementary target ssDNA have been applied. The modified surfaces show obvious fluorescent intensities from the immobilized probe ssDNA and target ssDNA molecules.
Figure 4.12: Schematic of nanoscale biosensor. (a) The nanowire biosensor device structure with a cylindrical coordinate. (b) A cross-section view of the sensor.

### 4.4 Analytical Modeling Si Nanowire Biosensors [3, 4]

In the Si regime, the current density of a nanowire has a diffusion (driven by the carrier concentration gradient) and a drifting (driven by the applied electrical field) component, which can be expressed as

$$J_{e,h} = q\mu_{e,h}n_{e,h}\nabla\phi \pm qD_{e,h}\nabla n_{e,h}.$$  \hspace{1cm} (4.1)

The distribution of potential and carrier concentrations follows the Poisson’s equation

$$-\frac{1}{r}\frac{d}{dr}\left(\varepsilon_{Si}r\frac{d}{dr}\phi(r)\right) = \begin{cases} q(n_h - N_A) & \text{p-type} \\ q(-n_e + N_D) & \text{n-type} \end{cases}$$  \hspace{1cm} (r \leq R)  \hspace{1cm} (4.2)

$$= \begin{cases} q\left[n_i\exp\left(\frac{q(\phi_i - \phi_f)}{kT}\right) - N_A\right] \\ q\left[-n_i\exp\left(\frac{q(\phi_f - \phi_i)}{kT}\right) + N_D\right] \end{cases}.$$  \hspace{1cm} (4.3)

For simplicity, only the p-type case will be discussed hereon. The equations for the n-type case are similar. For a small disturbance induced by the immobilization of target biomolecules ($q\Delta\phi \ll kT$), by neglecting the non-linear terms of the Taylor expansion ($e^x = \sum_{i=0}^{\infty}(x^i/i!)$ where $i$ is a non-negative integer) of the exponential term, Equation \hspace{1cm} (4.3)
(4.3) becomes
\[- \frac{1}{r} \frac{d}{dr} (\varepsilon_{Si} r \frac{d}{dr} \Delta \phi(r)) = \frac{\Delta \phi(r)}{\lambda_{Si}^2}, \quad (4.4)\]

where
\[\lambda_{Si} = \sqrt{\frac{\varepsilon_{Si} k T}{pq^2}} \quad (4.5)\]
is the Debye length of Si with a hole density of \(p\).

The boundary conditions are
\[\Delta \phi(R) = \Delta \phi_0, \quad (4.6)\]
\[\frac{d}{dr} \Delta \phi(0) = 0, \quad (4.7)\]
\[\Delta \phi(0) \text{ is finite.} \quad (4.8)\]

The solution of Equation (4.4) is the modified Bessel function of the first kind (Appendix B [117]):
\[\Delta \phi(r) = \Delta \phi_0 \frac{I_0(r/\lambda_{Si})}{I_0(R/\lambda_{Si})}. \quad (4.9)\]

The hole concentration in non-degenerated Si nanowires follows Boltzmann distribution:
\[p(r) = n_i \exp \left( \frac{q(\phi_i - \phi_f(r))}{k_B T} \right). \quad (4.10)\]
A small disturbance of Equation (4.10) yields

\[
\Delta p(r) = \Delta \left( n_i \exp \left( \frac{q(\phi_i(r) - \phi_f)}{k_B T} \right) \right) \quad (4.11)
\]

\[
= n_i \exp \left( \frac{q(\phi_i - \phi_f(r))}{k_B T} \right) \frac{q \Delta \phi(r)}{k_B T} \quad (4.12)
\]

\[
= p(r) \frac{q \Delta \phi(r)}{k_B T} \quad (4.13)
\]

\[
= p(r) \frac{q \Delta \phi_0}{k_B T} \frac{I_0(r/\lambda_{Si})}{I_0(R/\lambda_{Si})}. \quad (4.14)
\]

The total charge change of a uniformly doped (a doping level of \( p_0 \)) Si nanowires is:

\[
p_{tot} = \int_0^R 2\pi r \Delta p(r) dr = \int_0^R 2\pi r p_0 \frac{q \Delta \phi_0 \frac{I_0(r/\lambda_{Si})}{I_0(R/\lambda_{Si})}}{k_B T} dr \quad (4.15)
\]

\[
= 2\pi p_0 \lambda_{Si} R \frac{q \Delta \phi_0 \frac{I_0(R/\lambda_{Si})}{I_0(R/\lambda_{Si})}}{k_B T} \quad (4.16)
\]

The capacitance of the nanowire is

\[
C_{NW} = \frac{d p_{tot}}{d \Delta \Phi_0} = 2\pi p_0 \lambda_{Si} R \frac{q \frac{I_1(R/\lambda_{Si})}{k_B T I_0(R/\lambda_{Si})}}{} \quad (4.17)
\]

The sensitivity defined by \( \Delta G/G \) can be expressed as the hole concentration change:

\[
S = \frac{\Delta G}{G} \quad (4.18)
\]

\[
= \frac{\int_0^R 2\pi r \Delta p(r) dr}{\int_0^R 2\pi r p(r) dr} \quad (4.19)
\]

\[
= \frac{2 \Delta \phi_0 q \lambda_{Si} \frac{I_1(R/\lambda_{Si})}{k_B T R I_0(R/\lambda_{Si})}}{} \quad (4.20)
\]
Figure 4.13: The $\lambda_{Si}/R \times I_1(R/\lambda_{Si})/I_0(R/\lambda_{Si})$ vs. $R/\lambda_{Si}$ relationship.

Fig. 4.13 shows a plot of the $\lambda_{Si}/R \times I_1(R/\lambda_{Si})/I_0(R/\lambda_{Si})$ vs. $R/\lambda_{Si}$ relationship. As shown, the sensitivity decreases with the $R/\lambda_{Si}$ with a log-log relationship for $R/\lambda_{Si} > 1$. However, because the measurement error, low sensitivity may not be detected. The physical meaning is that if $R > \lambda_{Si}$, as the doping level of Si nanowire decreases, the surface potential change can more effectively modulate the carrier concentration. The sensitivity stopped increasing as $\lambda_{Si}R \ll 1$. The physical meaning is that $R < \lambda_{Si}$, all the cross-sectional area of the Si nanowire can be modulated by the surface potential change, decreasing the doping level cannot effectively improve the sensitivity.

For a large $R/\lambda_{Si}$, from Equations (B.14), with $\alpha = 0$:

$$I_0 \approx \frac{1}{\Gamma(\alpha + 1)} \left(\frac{x}{2}\right)^\alpha$$  \hspace{1cm} (4.21)

$$\approx 1.$$  \hspace{1cm} (4.22)
Inserting Equation (4.22) into (4.20) yields:

\[ S = \frac{q\Delta\phi_0}{k_B T}. \]  

(4.23)

In the electrolyte regime, the Poisson-Boltzmann equation is:

\[-\frac{d}{dr}\left[ \epsilon_w r \frac{d}{dr} \phi(r) \right] = \rho(r) = \sum_{i=1}^{N} z_i q c_{i,0} \exp\left(-\frac{z_i q \phi(r)}{k_B T}\right) \quad (r \geq R). \]  

(4.24)

The boundary conditions are:

\[ \phi(R) = \phi_0, \]  

(4.25)

\[ \phi(\infty) = 0. \]  

(4.26)

For symmetrical ionic solutions, Equation (4.24) can be simplified into

\[-\frac{d}{dr}\left[ \epsilon_w r \frac{d}{dr} \phi(r) \right] + \frac{q\kappa^2}{k_B T} \sinh(\beta\phi(r)) = 0. \]  

(4.27)

The analytical solution to Equation (4.27) is

\[ \sigma_{DL} = -\frac{2k_B T \epsilon_W}{q\lambda} \sinh\left(\frac{q\phi_0}{2k_B T}\right) \left(1 + \frac{\beta^{-2} - 1}{\cosh^2\left(\frac{q\phi_0}{2k_B T}\right)}\right)^{\frac{1}{2}}, \]  

(4.28)

where

\[ \beta = \frac{K_0(R/\lambda)}{K_1(R/\lambda)}, \]  

(4.29)

\(K_n\) is the \(n\) order modified Bessel function of the second kind (Appendix B).

For small perturbation, Equation (4.27) can be linearized by using only the linear terms of the Taylor series of the \(\sinh\) function \((\sinh(x) = \sum_{i=0}^{\infty} x^{2i+1}/(2i+1)!\) where \(i\) is a non-negative integer) for \(q\Delta\phi(r) \ll k_B T:\n
\[ \frac{d}{dr}\left[ \epsilon_w r \frac{d}{dr} \phi(r) \right] + \left(\frac{\phi(r)}{\lambda_w^2}\right) = 0, \]  

(4.30)
where

\[ \lambda_w = \sqrt{\frac{N}{\sum_i \epsilon_i k_B T}} \]  \hspace{1cm} (4.31)

is the Debye length of the electrolyte. Equation (4.31) is known as Debye-Hückel equation.

The solution of Equation (4.31) is

\[ \phi(r) = \frac{K_0(r/\lambda_w)}{K_0(R/\lambda_w)} \]  \hspace{1cm} (4.32)

Equations (4.1), (4.2), and (4.24) can be combined to solve the potential distribution and current density of the system.

The total charge of the double layer is

\[ \sigma_{DL} = \int_{R}^{\infty} -2\pi c_{i,0} \sinh \left( \frac{e\phi(r)}{k_B T} \right) dr \]  \hspace{1cm} (4.33)

\[ \approx \int_{R}^{\infty} -2\pi c_{i,0} \frac{e\phi(r)}{k_B T} dr \quad \text{for} \ \phi(r) \ll k_B T \]  \hspace{1cm} (4.34)

\[ = \int_{R}^{\infty} -2\pi c_{i,0} \frac{e}{k_B T} \times \frac{K_0(r/\lambda_w)}{K_0(R/\lambda_w)} dr. \]  \hspace{1cm} (4.35)

Inserting Equation (B.20) yields

\[ \sigma_{DL} = -2\pi c_{i,0} \frac{q\phi_0}{k_B T} \frac{R}{\lambda_w} \frac{K_1(R/\lambda_w)}{K_0(R/\lambda_w)}. \]  \hspace{1cm} (4.36)

The capacitance of the double layer is

\[ C_{DL} = \frac{d\sigma_{DL}}{d\phi_0} \]  \hspace{1cm} (4.37)

\[ = 2\pi c_{i,0} \frac{q}{k_B T} \frac{R}{\lambda_w} \frac{K_1(R/\lambda_w)}{K_0(R/\lambda_w)}. \]  \hspace{1cm} (4.38)

Fig. 4.14 shows a plot of \( R/\lambda_w \times K_1(R/\lambda_w)/K_0(R/\lambda_w) \) vs. \( R/\lambda_w \) relationship. As shown, for a fixed radius, the capacitance decrease as increase of \( \lambda_w \). Because \( \Delta V = \)
Figure 4.14: A plot of $R/\lambda_w \times K_1(R/\lambda_w)/K_0(R/\lambda_w)$ vs. $R/\lambda_w$ relationship.

$\Delta Q/C$, for a smaller capacitance, for the same change of charge density, the change of surface potential is greater, which means a higher sensitivity.

The conductance change of a NW can be derived from an effective capacitor model. The charge densities of the adsorbed target biomolecules, the double layer, and the nanowires follow the conservation law:

$$\sigma_T = -(\sigma_{DL} + \sigma_{NW}),$$

(4.39)

where

$$\sigma_T = \sigma_S N(t),$$

(4.40)

$\sigma_S$ is the effective charge introduced by a single target molecule. To calculate the effective charge density from the target molecule charge density, the Poisson-Boltzmann equation need to be solved.
4.5 Numerical Modeling SiNW Biosensors with Silvaco

To verify the developed theory, we simulate the device behavior with Silvaco [118]. Two-dimensional devices were firstly simulated for large width devices. Because the available version of Silvaco software limits the amount of mesh nodes, two-dimensional simulations were firstly implemented to extract the behavior of the backgate electrode, which behave similarly for the three-dimensional structures.

4.5.1 Simulation of Planar SOI Biosensors

Fig. 4.15 shows the two-dimensional SOI biosensor structure used for Silvaco simulations. The SOI structure is the same with the structure we have (section 4.2.1). The Si layer under the Ohmic metals are with a doping level of $1 \times 10^{19} \text{cm}^{-3}$, which is decided by the boron-implantation. The bottom electrode simulates the back-gate electrode. The air/Si interface is used to set interface charges, which simulates the appearance of charged biomolecules. For simplicity, the effects from the physiological buffer solution is not considered. Only the effective deposited charges from the immobilized biomolecules are included.

Fig. 4.16 shows the effect of the equilibrium hole distribution with negative charges deposited on the active surface. As shown, the hole concentration on the active region increases under the deposited charge area. The $I_d - V_g$ characteristic with $V_d = 15 \text{V}$ is show in Fig. 4.17. As shown, the threshold voltage is around 18 V. The deposited charges induce $I_d - V_g$ curve shifts to the negative position. The highest sensitivity is in the subthreshold regime.
Figure 4.15: The two-dimensional SOI biosensor device structure used for Silvaco simulations.

Figure 4.16: The surface charge induced hole distribution in equilibrium.
Figure 4.17: The $I_d - V_g$ characteristics with/without deposited charges [(left) $V_g = -10 \text{ V} - 30 \text{ V}$, (right) $15 \text{ V} - 20 \text{ V}$].

### 4.5.2 Simulations of SiNW Biosensors

**Simulation Set-ups**

The following assumptions are made without any loss of the validity of the model. 1) The substrate of the Si nanowire is neglected, which is not a critical factor to the sensor performance. 2) The doping levels of the Si nanowires are set to variable values, which in reality the nanowire conductance can be controlled by applying the back-gate electrode for depletion modulations on SOI devices. 3) The effect of deposition of individual charged biomolecules is simulated by adding a 5 nm by 5 nm charged interface at the Si/air interface, the location of which is randomly distributed. An example of simulated device structure is shown in Fig. 4.18. The coverage of deposited biomolecules (charged area) varied from 0% to 100%. The sensitivity is defined by the introduced S/D current changes normalized by the S/D current at 0% coverage. For reasonable accuracy and affordable CPU time, the grid is set to 5 nm on the active Si regimes only. The source and drain regions are heavily doped. The thickness of the Si nanowire is fixed at 40 nm. Doping levels of $1 \times 10^{17} \text{ cm}^{-3}$, $1 \times 10^{18} \text{ cm}^{-3}$, and $1 \times 10^{19} \text{ cm}^{-3}$ are simulated for comparisons.
Doping Effects

Fig. 4.19 shows the effect of doping levels. The Debye lengths \( \lambda = \sqrt{kT\varepsilon_{\text{Si}}/qN_d} \) of three doping levels are 13.0 nm, 4.1 nm, and 1.3 nm respectively. As shown in Fig. 4.13, with the same structure dimension, the sensitivity shows a reverse relationship with the Debye length. With lower doping levels, deposited charges have larger effective regions, which promises higher sensitivities. Figs. 4.20 and 4.21 show the cross-sectional hole and potential distribution of different Si doping levels. As shown in Equation (4.14), for a large \( R/\lambda_{\text{Si}} \) value, the hole distribution close to a constant. With small \( R/\lambda_{\text{Si}} \) values, the hole distribution follows a near exponential distribution.
Figure 4.19: The simulated sensitivity vs. coverage characteristics of SiNW of a length of 200 nm, widths of from 5 nm to 125 nm, doping levels of (a) $1 \times 10^{17}$ cm$^{-3}$, (b) $1 \times 10^{18}$ cm$^{-3}$, and (c) $1 \times 10^{19}$ cm$^{-3}$. 
Figure 4.20: The potential distribution in the y-z planes.
Figure 4.21: The hole concentration distribution in the y-z plane.
Figure 4.22: (a) The cut-plane. The potential (b) and hole concentration (c) distribution of a SiNW biosensor with L=200 nm and W=25 nm. The doping level is $1 \times 10^{17} \text{cm}^{-3}$. 
Figure 4.23: The potential (a) and hole concentration (b) distribution of a SiNW biosensor with L=200 nm and W=125 nm. (c) The potential distribution normalized by the width of the SiNW. The doping level is $1 \times 10^{17}$ cm$^{-3}$.
The Effects of Width

Fig. 4.19 also shows the sensitivity vs. SiNW widths. As shown, the sensitivity increases as the width decreases. Figs. 4.22 and 4.23 show the potential and hole concentration distributions. Similar to the charge-sharing model of short-channel effects in the MOSFET theory, the modulation in the active Si region is fractionally established from the charges on the two side walls. As shown in Fig. 4.23 (c), the charges on the side walls modulate the SiNW. Smaller widths have greater modulation effects, hence, SiNWs with smaller widths have higher sensitivity.

The Effects of Length

Fig. 4.24 shows that the longer SiNWs have less sensitivities. Figs. 4.25 and 4.26 show the hole and potential distribution in the z-x plane. The effects of channel length can be explain using the narrow gate-width effects of MOSFET theory. The deposited charges modulate both the active Si region and also the neighboring source/drain regions. As shown in Figs. 4.25(d) and 4.26, the regions outside the active region are modulated as well. The modulated charge change in active Si region divided by the total modulated charge change is 17.8% and 96.9% for a length of 5 nm and 125 nm respectively.

4.5.3 Conclusions

The simulation results in this section verified the theoretical modeling in section 4.4. Fig. 4.13 shows that as the devices with smaller $R/\lambda_{Si}$ values have higher sensitivity. Small $R/\lambda_{Si}$ values can be resulted from the decrease of the SiNW radius or the increase of the Debye length in Si, which is resulted from the decrease of the doping level. Fig. 4.19 shows the simulated sensitivities from different widths or doping. Comparisons show that devices with lower doping levels and smaller widths have higher sensitivity, which is
Figure 4.24: The simulated sensitivity vs. coverage characteristics of SiNWs with a width of 200 nm, lengths from 5 nm to 125 nm, doping levels of $1 \times 10^{17}$ cm$^{-3}$.

consistent with the previously stated results. Furthermore, Figs. 4.24, 4.25, and 4.26 show the sensitivities from different SiNW lengths, which cannot be derived from the equilibrium analysis in section 4.4. As a result, SiNWs with greater lengths have higher sensitivities. However, because the Poisson-Boltzmann equation for aqueous solutions is not included in Silvaco, the simulation of the whole system is not available.
Figure 4.25: (a) The cut-plane. The (b) carrier and (c) potential distribution of a SiNW with a length of 5 nm, and a width of 200 nm. The red lines are the cut-line. The (d) carrier and (e) potential distribution along the cut-line.
Figure 4.26: (a) The cut-plane. The (b) carrier and (c) potential distribution of a SiNW with a length of 125 nm, and a width of 200 nm. The red lines are the cut-line. The (d) carrier and (e) potential distribution along the cut-line.
4.6 Summary

The fabrication procedure of SiNW biosensors have been established. The e-beam lithography and dry etching test structures have been successfully fabricated. The devices are under fabrication and testing. The surface modification protocols with on oxide-free Si surfaces were designed and characterized with XPS and fluorescent imaging. The theoretical and numerical modeling of SiNW biosensors have been developed to understand working principle and optimize the device design. The modeling results need to be validated with real device performance.
CHAPTER 5

Characterization of tBLM Using Impedance Method

5.1 Introduction

Biological membrane properties determine the intercellular and extracellular functions of cells. However, due to the complexity of native cell membranes, biomimetic membranes with better known molecular structures and functions are widely studied. As one of the approaches, biological membranes immobilized on solid surfaces have developed to allow applications of surface characterization techniques, such as ellipsometry, neutron reflection, EIS and AFM, etc. tBLMs have been used for the study of membrane process, including ion transport and the interaction of integral and peripheral proteins. EIS has been proved to be a reliable and sensitive method for characterizing the electrical properties of tBLMs when immobilized on conductive substrate, such as gold. People have studied EIS measurements and the tBLMs properties. However, under certain circumstances, the structures of tBLMs change dynamically in real time, such as during the electroporation process when high voltage electrical fields are applied to open pores on tBLMs. Study of EIS characteristics with well-defined defects has not been reported. On the other hand, AFM provides a direct method of physical imaging the molecular structures, including defects. Hence, better understanding of tBLM structures can be obtained by combining EIS and
AFM measurements. In this chapter, defective tBLM on Si substrates is characterized by AFM and EIS to simulate channel openings. Two methods are used to create defects on tBLM. The first method is to use diluted solution when forming the second lipid layer. The second method is to use defective Si substrates. The two methods are described in the follow two sections respectively. The formation of tBLM and a part of the measurements are conducted by Dr. L. James Lee’s group in the Department of Chemical and Biomolecular Engineering, The Ohio State University.

5.2 Fundamentals to the Electrochemical Impedance Method

5.2.1 Impedance with the Faradaic Current [5]

Heterogeneous electrochemical reactions on the electrode surface shown in Fig. 5.1 can be expressed as:

\[ \sum_i s_i M_i^{z_i} \rightleftharpoons ne^- . \]  

(5.1)

With the electrode provides or consumes electrons, the boundary condition at the electrode surface is

\[ D_i \frac{\partial c_i}{\partial x} \bigg|_{x=0} = \frac{s_i i_f}{nF} \text{ at } x = 0 , \]  

(5.2)

where \( F \) is the Avogadro constant. The total current density is

\[ i_f = f(V, c_i(0)) . \]  

(5.3)

For impedance measurements, sinusoidal oscillations with certain frequencies are applied. Oscillating quantities can be expressed as

\[ X = \tilde{X} + Re \left\{ \tilde{X} \exp(j\omega t) \right\} , \]  

(5.4)
where symbols with $\bar{\text{ }}$ and $\tilde{\text{}}$ mean the DC and AC components respectively. When the oscillation magnitude is small for the first order expansion

$$\tilde{\text{i}}_f = \left( \frac{\partial f}{\partial V} \right)_{c_i(0)} \tilde{V} + \sum_i \left( \frac{\partial f}{\partial c_i(0)} \right)_{V,c_j,j\neq i} \tilde{c}_i(0),$$

(5.5)

where the charge-transfer resistance is defined as

$$\frac{1}{R_t} = \left. \frac{\partial f}{\partial V} \right|_{c_i(0)}. $$

(5.6)

Equation (5.5) can be reorganized into

$$\tilde{V} = R_t \tilde{i}_f - R_t \sum \left( \frac{\partial f}{\partial c_i(0)} \right)_{V,c_j,j\neq i} \tilde{c}_i(0).$$

(5.7)

The oscillation of concentration gradient is

$$\left. \frac{d\tilde{c}_i}{dx} \right|_{x=0} = \frac{s_i \tilde{i}_f}{nFD_i}. $$

(5.8)
Equation (5.7) becomes
\[ \tilde{V} = \tilde{i}_f (R_t + Z_D), \] (5.9)

where
\[ Z_D = R_t \sum_i \left( \frac{\partial f}{\partial \tilde{c}_i} \right)_{V,c_{j\neq i}} \frac{s_i}{nF D_i} \frac{-\tilde{c}_i(0)}{\frac{d\tilde{c}_i(0)}{dx}} \bigg|_{x=0}. \] (5.10)

When the current density consists of Faradaic reactions and charging of a bias-independent double layer, the current can be expressed as:
\[ i = i_f + C_{dl} \frac{dV}{dt}. \] (5.11)

The oscillation current can be written as
\[ \tilde{i} = \tilde{i}_f + j\omega C_{dl} \tilde{V}. \] (5.12)

The total impedance is
\[ \tilde{Z}(\omega) = \frac{\tilde{U}}{\tilde{I}} = R_e + \frac{R_t + Z_D(\omega)}{1 + j\omega C_{dl}(R_t + Z_D(\omega))}, \] (5.13)

where \( R_e \) is from the resistance of the bulk electrolyte.

### 5.2.2 Impedance with Ion Diffusion

For a large area electrode in aqueous solution with uniform surface properties as shown in Fig. 5.1, the one-dimensional convection-diffusion equation is:
\[ \frac{\partial c_i}{\partial t} + v_x \frac{\partial c_i}{\partial x} - D_i \frac{\partial^2 c_i}{\partial x^2} = 0, \] (5.14)

with the following boundary conditions:
\[ c_i \to c_{i,\infty} \quad \text{for} \quad x \to \infty, \] (5.15)
\[ f \left[ c_i(0), \frac{\partial c_i}{\partial x} \bigg|_{x=0} \right] = 0 \quad \text{for} \quad x = 0. \] (5.16)
Neglecting natural convection term in Equation (5.14) results in Fick’s second law:

\[
\frac{\partial c_i}{\partial t} - D_i \frac{\partial^2 c_i}{\partial x^2} = 0,
\]

(5.17)

with the following boundary conditions:

\[
c_i \to c_{i, \infty} \quad \text{for} \quad x \to \infty,
\]

\[
c_i = c_i(0) \quad \text{at} \quad x = 0,
\]

(5.18)

\[
c_i = c_{i, \infty} \quad \text{at} \quad t = 0.
\]

Oscillation of concentration:

\[
c_i = \bar{c}_i + Re \{ \tilde{c}_i \exp(j\omega t) \}.
\]

(5.19)

Inserting Equation (5.19) into (5.17):

\[
j \omega \tilde{c}_i \exp(j\omega t) - D_i \frac{d^2 \tilde{c}_i}{dx^2} - D_i \frac{d^2 \bar{c}_i}{dx^2} \exp(j\omega t) = 0.
\]

(5.20)

Canceling the steady-state and exponential terms:

\[
j \omega \tilde{c}_i - D_i \frac{d^2 \tilde{c}_i}{dx^2} = 0.
\]

(5.21)

Introduce a dimensionless concentration \( \theta_i(x) = \tilde{c}_i / \bar{c}_i(0) \):

\[
j \frac{\omega}{D_i} \theta_i - \frac{d^2 \theta_i}{dx^2} = 0.
\]

(5.22)

The solution is

\[
\theta_i = A \exp(y \sqrt{j \frac{\omega}{D_i}}) - B \exp(-y \sqrt{j \frac{\omega}{D_i}}).
\]

(5.23)

The boundary conditions are

\[
\theta_i \to 0 \quad \text{as} \quad x \to \infty,
\]

\[
\theta_i = 1 \quad \text{at} \quad x = 0.
\]

(5.24)
Thus, A=0 and
\[ \theta_i = \exp(-x\sqrt{j\frac{\omega}{D_i}}). \] (5.25)

The inverse of the derivative with respect to \(x\) is
\[ \frac{-1}{\theta'_i(0)} = \frac{1}{\sqrt{j\frac{\omega}{D_i}}}. \] (5.26)

The of impedance is defined as Warburg impedance:
\[ \frac{Z_D(\omega)}{Z_D(0)} = \frac{-c_i(0)}{\frac{dc_i}{dx}}_{x=0} = \frac{1}{\sqrt{j\frac{\omega}{D_i}}} = \sqrt{\frac{D_i}{2\omega}}(1 - j). \] (5.27)

The Cole-Cole plot is given by:
\[ \frac{Re(G)}{\omega} = \frac{Im(G)}{\omega} = \frac{\sqrt{2}}{2} \frac{1}{Z(0)\sqrt{\omega D_i}}. \] (5.28)

On the Cole-Cole plane, it is a straight line with a 45° slope. Considering Equation (5.13), \(Z_D(\omega)\) is large at small \(\omega\) values. The 45° slope only presents at low 45° slope in the low frequency regime. At high frequencies, the capacitance \(C_{dl}\) dominates.

### 5.2.3 Equivalent Circuits

The electrical behavior of the electrode can be analogous to a combination of lumped electrical circuit components (resistors, capacitors, and/or inductors). For the electrode discussed in the previous subsection, the impedance from the surface in Equation (5.13) can be re-organized into
\[ \frac{1}{Z_{surf}} = j\omega C_{dl} + \frac{1}{R_t + Z_D(\omega)}. \] (5.29)

The total impedance \(\tilde{Z}(\omega)\) is analogous the circuit shown in Fig. 5.2.

Besides usually used resistors, capacitors, and inductors, constant phase element (CPE) is a circuit element that is introduced for modeling non-ideal time-constant dispersion. A
few examples of the non-ideality are the finite area of the electrode, the electrode surface morphology is not uniform, etc. The impedance of CPE is given by

\[ \frac{1}{Z} = Y = Q(j\omega)^\alpha, \]  

(5.30)

where \( Q \) is the amplitude, with the unit siemens \( \times \) sec\(^{\alpha} \), and \( \alpha \) is a unitless quantity within the range \([0, 1]\). For \( \alpha = 1 \), the CPE is an ideal capacitor; whereas for \( \alpha = 0 \), the CPE is an ideal resistor.

### 5.3 Characterization of tBLMs with EIS

Previously, we have published characterizations with defective tBLMs created from the control of solution concentration during tBLM formation [119]. The characterized tBLMs are with undefined localizations and sizes. To characterize tBLMs with well defined pore sizes, locations, and amount of pores, e-beam lithography and plasma dry etching are used to create well-defined nanopores in Si/SiO\(_2\) substrates. The nanopores are introduced from the substrate into tBLMs. EIS characteristics are then measured for modeling.
5.3.1 Device Fabrication and tBLM Preparation

Device Fabrication

The process flow of device fabrication is shown in Fig. 5.3. 100 nm oxide was thermally grown on Si(100) substrates. Four device samples were patterned with single diameter nanowell arrays by a Leica EBPG5000 e-beam lithography system with ZEP520A e-beam resist, as shown in Fig. 5.3(a). The diameters of the four device samples are 30 nm, 50 nm, 70 nm, and 100 nm respectively. To expose dimensions of tens of nanometers, a beam current of 100 pA with acceleration voltage of 100 kV was used. The spacing between two nanowells in both x and y directions are 33.3 µm so that the amount of nanowells is 10,000 with a circular testing area with the diameter of 4 mm. The nanowell arrays were developed in ZED-N50 developer for 1 min and rinsed in isopropanol for 30 sec (Fig. 5.3(b)). The devices were etched by an SLR plasma etcher to transfer the nanowell patterns into the SiO₂ layer using a combination of SF₆ and O₂ gases (Fig. 5.3(c)). The residue of ZEP520A was removed by N-methylpyrrolidone (NMP) and isopropanol rinse. Device fabrication were completed with the deposition of Ti(10 Å)/Au(200 Å) metal layers with a CHA e-beam evaporator (Fig. 5.3(d)). Finished surface roughness is within 0.2 nm.

Materials

Synthesis and purification of the 1-thiahexa(ethylene oxide) lipidic anchormolecule WC14 [20-tetradecyloxy-3,6,9,12,15, 18,22-heptaoxahexatricontane-1-thiol], was described previously [120]. The phospholipid, 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhyPC), was used as supplied from Avanti Polar Lipids (Birmingham, AL). β-Mercaptoethanol (βME) from Sigma-Aldrich (St. Louis, MO) was distilled before use. Distilled water was obtained from a HQ grade water purification system at The Ohio State University.
Figure 5.3: The fabrication process of nanopore devices. (a) Spin-coating of ZEP520A e-beam resist and e-beam patterning. (b) Development of exposed ZEP520A for nanowell patterns. (c) Plasma dry etching to transfer nanowell patterns into SiO$_2$ layer and strip off ZEP520A resist. (d) Deposition of Ti/Au metal layers.
(Columbus, OH). An aqueous solution of PBS with 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, and a pH of 7.4 was used after dilution from 10× PBS (Thermo Scientific, Rockford, IL). Thin Au layers (30 nm) over a Ti adhesion layer (2 nm) were deposited at a deposition rate of 5 Å/sec on Si/SiO₂ <100> wafer with a CHA vacuum E-beam evaporator. The Au layers typically had a surface roughness (rms) of 0.2 nm, as measured by AFM (Asylum Research, Santa Barbara, CA).

**Preparation of the tBLM**

The anchoring membrane mixed SAMs were formed on the devices as described previously [120]. The freshly prepared Au films were exposed to solutions of WC14:βME (0.60 mM: 0.14 mM) in 99.5% ethanol for >12 hours. The bilayer lipid were formed by 10 min incubation in 10 mM 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhyPC) (Avanti Polar Lipids) in 1× PBS (Thermo Scientific) with a pH of 7.4 and flashed by fresh PBS solution for 10 sec.

**Atomic Force Microscopy**

AFM was performed in the PBS buffer solution using a MFP-3D-Bio-AFM (Asylum Research, Santa Barbara, CA) equipped with an iDrive Magnetic Actuated Cantilever holder. The fluid cell was sonicated in ethanol for 5 min before use, immediately followed by extensive rinsing with ethanol and drying under a stream of N₂ gas. Images were acquired using the AC Mode in fluid, collected using a scan rate of 0.5 Hz and a resonant frequency of 5-6 kHz with the set point of 0.1-0.2 V (90-80% reduced from free amplitude of the AFM cantilever) unless specified otherwise. Au-coated Si₃N₄ pyramidal cantilevers with a nominal spring constant of 0.09 N/m (AR-iDrive-N01, Asylum Research) were mounted in the fluid cell. SPIP software (Scanning Probe Image Processor, Image
Metrology A/S, Denmark) was used to analyze the surface roughness and characterize the particles and pores based on their size and shape.

**Electrochemical Impedance Spectroscopy Measurements**

EIS measurements were performed using an electrochemical impedance system (Gamry Instruments, Warminster, PA, model Series G300 potentiostat, EIS300 software). The measured frequency range is from 300 kHz to 0.1 Hz with 10 points per decade. The test area on the devices was defined by a circular PDMS reservoir with a diameter of 4 mm ($A_{\text{eff}} = 0.126 \text{ cm}^2$) to confine around 10,000 nanoholes. Another device with a perfect Au layer was also measured for reference. The schematic of device and measurement configuration is shown in Fig. 5.4. As shown, the Au surface on the device is the working electrode. Each of these four parallel cells had a geometric surface area ($A_{\text{eff}}$) of 0.126 cm$^2$. All EIS data were normalized to $A_{\text{eff}}$. The reference electrode was a saturated silver-silver chloride (Ag/AgCl/PBS) microelectrode (1004G, Koslow Scientific Co., Englewood, NJ), and the auxiliary electrode was a 0.5 mm diameter platinum wire (Aldrich, St. Louis, MO, 99.99% purity) coiled around the barrel of the reference electrode. All measurements were carried out at 0 V DC bias and 30 mV AC amplitude versus reference electrode at 20 °C.

**5.3.2 Results and Discussion**

**SEM Images of Fabricated Nanopores**

Fig. 5.5 shows fabricated nanopores on the gold layer. As shown, the average grain size of gold on the SiO$_2$ surfaces is in the range of 20 - 30 nm. The edge definition of 32 nm and 51 nm nanopores are not clear. The 75 nm and 104 nm nanopores have good edge definition.
EIS Characteristics of Planar Au Surfaces

The EIS characteristics of planar SAM and tBLM surfaces have been well studied [119, 120, 121]. The measured EIS characteristics are shown in Fig. 5.6. The equivalent circuit used for analysis are shown in Fig. 5.7. At high frequencies, the Warburg impedance is negligible, and the $R_{lk}$ is much greater than the CPE resistance ($1/j\omega C$). Because CPEs in this work behave like capacitors, for the convenience of discussion, CPE will be replaced with a capacitor hereon.

The conductance of the equivalent circuit in Fig. 5.7(b) follows

$$Y = \frac{C_0 + j\omega C_0^2 R_e}{1 + \omega C_0^2 R_e^2}. \quad (5.31)$$

The real and imaginary parts follow the equation:

$$\left( Y_{re} - \frac{C_0}{2} \right)^2 + Y_{im}^2 = \left( \frac{C_0}{2} \right)^2,$$

$$143$$
Figure 5.5: The SEM images of a 32 nm (upper left), 51 nm (upper right), 75 nm (lower left), and 104 nm (lower right) nanopores.
Figure 5.6: (a) The measured EIS characteristics of a Au surface, SAM surface, and tBLM surface. (b) An enlarged view of the EIS characteristics.
Figure 5.7: (a) The complete equivalent circuit. (b) The equivalent circuit at high frequencies. (c) The equivalent circuit at low frequencies. $R_e$ is the solution resistance, $Z_w$ is the Warburg impedance, CPE is the non-ideal surface capacitance (double layer, SAM, and tBLM), and $R_{lk}$ is the leakage resistance.
which is a circular function, with the center at \((C_0/2, 0)\) and diameter of \(C_0/2\). The interceptions to x-axis are at \((0, 0)\) on the high frequency end and \((C_0, 0)\) on the low frequency end. From this property, the extracted capacitance from the bare gold surface, SAM surface, and tBLM surface are around \(80 \, \mu F/cm^2\), \(10 \, \mu F/cm^2\), and \(1 \, \mu F/cm^2\) respectively. These values are close to the published results [119, 120, 121].

As shown in Equation (5.27), the Warburg impedance increases with the decrease of frequency. In the low frequency regime, the Warburg impedance is larger than \(R_e\). The impedance of the CPE also increase with the decrease of frequency. At low frequencies, the impedance of the CPE is negligible when it is much greater than the impedance of \(R_{lk}\). The equivalent circuit is shown in Fig. 5.7(c). When \(Z_w \gg R_{lk}\), the diffusion impedance dominates and in the Cole-Cole plot, the curve turns into a straight line with \(45^\circ\). Introducing Equation (5.27) yields:

\[
|Z_w|^2 \gg R_{lk}^2, \quad (5.33)
\]
\[
Z_D(0)^2 \frac{D_i}{\omega} \gg R_{lk}^2, \quad (5.34)
\]
\[
\omega \ll \frac{Z_D(0)^2 D_i}{R_{lk}^2}. \quad (5.35)
\]

In Fig. 5.6, the EIS characteristic measured from the bare gold surface does not show a \(45^\circ\), which indicates that the Warburg impedance does not dominate in the frequencies higher than 0.1 Hz. The EIS characteristics from the SAM and tBLM surfaces have a straight tail. As will later be shown in other measurements, the tail in the tBLM characteristics is not from the Warburg impedance. In general, the surface with extra layer(s) of non-conductive polymer molecules will decrease the leakage current. In other words, \(R_{lk}\) will increase. As a result of Equation (5.35), the Warburg impedance of the SAM and tBLM surfaces
will appear at lower frequencies compared to the bare gold surface. Hence, the Warburg impedance will be neglected hereon.

The equivalent circuit model is shown in Fig. 5.9. With negligible $R_e$ and CPEs are close to capacitors, the conductance of the circuit is

$$Y = j\omega C_1 + \frac{j\omega C_2}{1 + j\omega C_2 R_2}. \quad (5.36)$$

**EIS Characteristics of Nanopore Devices with SAM**

Fig. 5.8 shows the Cole-Cole plots of EIS measurements for devices without nanowell arrays and with nanowell arrays with the diameter of 30 nm, 50 nm, 70 nm, and 100 nm respectively. Fig. 5.9(a) shows the equivalent circuit for modeling electrode coated with defective non-conductive polymer molecules. As the area of defects are much smaller than the area of coated regions, CPE$_1$ is much higher (usually one order or more) than CPE$_2$, and $R_2$ is on the order of $10^4 \ \Omega/cm^2$. At high frequencies, the impedance of the CPE$_2$ and $R_2$ branch is much higher than the CPE$_1$ branch so that it can be neglected. With this assumption, the equivalent circuit for high frequencies can be simplified into Fig. 5.9(b). The curve shape of this circuit is a semicircle as described in Equation (5.32). Similar from the previous discussion, the SAM capacitance increases from 0.55 $\mu F$ to 1.5 $\mu F$, as the diameter of the nanowells increases from 0 nm to 100 nm. The increase may be resulted from two factors. The first one is that the three-dimensional surface structure. The derivation of the theories described in this chapter assumes the surface is infinite planar surfaces. This assumption is not valid when the surface presents abundant three-dimensional structures. Secondly, the plain area is used to normalized the measured impedance/admittance. With three-dimensional surfaces, the area is not accurate. Due to these reasons, the discussion hereon only remains qualitative. Because of this reason, Dr. L. James Lee’s group is
developing finite element method (FEM) based numerical simulations for better modeling nanopore impedance behaviors. At low frequencies, $R_e$ is negligible and the equivalent circuit can be simplified into Fig. 5.9(c). The admittance of this circuit can be expressed as

$$Y = j\omega C_1 + \frac{j\omega C_2}{1 + j\omega C_2 R_2},$$

(5.37)

which can be organized into the following two parts for Cole-Cole plots:

$$Y_{re} = R e \left( \frac{Y}{j \omega} \right) = C_1 + \frac{C_2}{1 + \omega^2 C_2^2 R_2^2},$$

(5.38)

$$Y_{im} = I m \left( \frac{Y}{j \omega} \right) = -\frac{\omega R_2 C_2^2}{1 + \omega^2 C_2^2 R_2^2}.$$  

(5.39)

$Y_{re}$ and $Y_{im}$ have the following relationship:

$$\left[ Y_{re} - \left( C_1 + \frac{C_2}{2} \right) \right]^2 + Y_{im}^2 = \left( \frac{C_2}{2} \right)^2,$$

(5.40)

which is a semicircle with the interceptions to the x-axis are at $(C_1,0)$ at low frequency limit and $(C_1 + C_2,0)$ at high frequency limit. In these measurements, the second semicircles do not present at the applied frequency range.
Figure 5.9: (a) The complete equivalent circuit for a defective membrane, and the simplified equivalent circuit for (b) high frequencies and (c) low frequencies.
Figure 5.10: The measured EIS characteristics for (a) a planar device, and the devices with nanoparticles of (b) 30 nm, (c) 50 nm, (d) 70 nm, and (e) 100 nm.
EIS Characteristics of Nanopore Devices with tBLM

Fig. 5.10 shows the Cole-Cole plots of EIS measurements with tBLMs on the devices with and without nanopores. As shown in the insets, the diameters of high frequency semi-circles indicate that the tBLMs capacitances are around $0.872 \, \mu F/cm^2$ for all the devices, which is close to previously reported results [119, 121]. In all the measurements, the second semicircle on the low frequency side shows up, which corresponding to the CPE$_2$ branch which is discussed in the previous section. Except Fig. 5.10(a), there appears to be a intermediate frequency non-obvious semicircle in the range of 100 Hz - 1 kHz. The reason for this phenomenon is still under investigation. In the derivation of Equation (5.40), the simplification is made with the assumption that the branch with CPE$_2$ or $R_e$ may be neglected at certain frequencies. However when the values of CPE$_1$ and CPE$_2$ are not largely different, the two semicircles may merge. This phenomenon can be observed from Fig. 5.10(a)-(e). As the nanopore area increases, the amplitude of CPE$_2$ increases. With the amplitude difference between CPE$_1$ and CPE$_2$ decreases, the two semicircles start merging. As a result, the completeness of the high frequency circle is destroyed. As shown, the radian of the high frequency semicircle reduces from close to $180^\circ$ in Fig. 5.10(a) to around $120^\circ$ in Fig. 5.10(e).

5.4 Conclusions

In this chapter, the EIS characterization of nanopore devices with SAMs and tBLMs are discussed. We have shown a well-established fabrication process for devices with 30 nm, 50 nm, 70 nm, and 100 nm nanopores using Si wafers. The EIS measurements of bare planar gold surface, and planar gold surfaces with SAMs and tBLMs are firstly measured and equivalent circuits are used to model EIS behaviors. The equivalent circuit models
are then extended to explain the EIS characteristics of the nanopore devices. As a result, the tBLM capacitance is well characterized. And the low frequency semicircle behavior is explained. Due the three-dimensional structure of the nanopore surfaces, it is not accurate to model the EIS behavior with current equivalent circuit models. Three-dimensional FEM simulation is necessary for more accurate curve fitting.
CHAPTER 6

Further Developments

6.1 AlGaN/GaN HFET Biosensors

The detection of MIG is in progress, which has shown a detection level appropriate for clinical applications. To develop commercialized products, the following developments are necessary.

1. Packaging. For easier operations, the device need to be wire-bonded and packaged for on-site detections so that there is no need for probe station.

2. Miniaturization. The device chip size in this work are on the order of mm². To embed the device in a needle size extraction devices, the device dimension needs to be miniaturized into sub-millimeter dimensions.

3. Sampling and post-processing circuitry. Sampling circuits are necessary to extract the device $I_d - V_d$ characteristics. Post-processing circuits are needed for display the detection results for professional judgements.

Besides planar structures, AlGaN/GaN nanowire devices are being developed for sensitivity improvement.
6.2 SiNW Biosensors

The development of SiNW biosensors are not completed yet. The next steps will be to finish the fabrication of devices and integrate with the surface modification for ssDNA detection. Secondly, the device performance can be combined with the device modeling for better understanding to device principles and for further improvement of device performance. Except ssDNA detections, the surface modification procedure can be modified for protein detections. For example, after the de-protection of amine groups, the surface can be biotinylated for SA detections or treated with anti-MIG IgG for MIG detections. The surface modifications can be extended for multi-target recognition arrays. For example, some of the nanowires are selectively modified for SA detection while the rest are modified for MIG detection. This could result in multi-target detections with a single extraction for clinical applications.

6.3 Membrane Study

As discussed in Chapter 5, the equivalent circuit model is not valid for modeling surfaces with nanopores. To understand the EIS behavior of nanopores, FEM modeling are being developed.

Other than bio-mimic membranes, the electroporation of living cells are of more importance for drug/gene delivery applications. A three-dimensional device with nanopores with 100 nm nanopores in a 3 μm thick Si membrane are being developed. The cells will be immobilized on the nanopores. An electrical field will be applied so that the cell membrane will be electroporated and drug/gene will be delivered. During this process, the EIS characteristics will be monitored to study the process dynamics.
The same structure with a single pore with a much smaller diameter (< 5 nm) will be fabricated for micro-RNA detection. To make nanopore diameter smaller than 5 nm, an e-beam lithography with a dry etching process will be implemented for a nanopore with a diameter at around 30 nm. A subsequent oxidization or SiNx deposition process will be used to shrink the nanopore diameter. A target cell (e.g. a breast cancer cell) will be immobilized to the nanopore. An electrical field will be applied to open the membrane of the cell and extract the micro-RNA from the nucleus. Because the passage of a single micro-RNA (diameter ≈ 2 nm) will block the nanopore and cause the resistance and impedance change, both DC and EIS measurements will be used to monitor this dynamic process and for comparisons. The extracted micro-RNA will be used for sequencing and further to study the relevance to the disease (e.g. breast cancer) and develop gene recipes.
All the cleanroom processes are performed with the facilities in the cleanroom at the Department of Electrical and Computer Engineering (ECE cleanroom) and the OSU nanotech west cleanroom (NTW cleanroom). The details of individual processes are introduced in the following subsections.

A.1 Photolithography

The following photolithography processes are developed with the ECE cleanroom Karl Suss MJB3 photomask aligner.

A.1.1 Image Reversal Process with AZ5214

1. Clean AlGaN/GaN sample with acetone.

2. Clean a Si carrier sample with a size of around 2 cm × 2 cm.


4. Spin coat AZ4156 photoresist on Si carrier sample.

5. Soft bake the Si sample on hot plate.
6. Spin coat sample with HMDS followed by baking.

7. Spin coat sample with AZ4156 followed by soft baking.

8. Align the sample and the mask with align markers (when necessary) and expose.


10. Flood exposure.

11. Develop the pattern in MF319 and rinse the sample in DI.

12. Check with microscope to see the development is completed.

A.1.2 Shipley 1811 Process

1. Clean AlGaN/GaN sample in Acetone.

2. Clean a Si carrier sample with a size of around 2 cm × 2 cm.


4. Spin coat S1811 photoresist on Si carrier sample and load the AlGaN sample in the center area.

5. Bake sample on the hot plate.

6. Spin coat sample with HMDS followed by baking.

7. Spin coat sample with S1811 followed by a soft bake.

8. Align the sample and the mask with the MJB3 aligner followed by exposure.

9. Develop the pattern in MF319 and rinse in DI.

10. Check with microscope to see the development is completed.
A.2 E-Beam Lithography

The following e-beam lithography writing procedure were developed with the Leica EBPG 5000 system at NTW cleanroom. E-beam lithography mainly is performed with e-beam resists (such as ZEP520A, HSQ, PMMA, AZPN 114, and SU8, etc.).

General e-beam procedure is as follows:

1. design the layout (e.g. using Advanced Design System (ADS), Cadence, or LEI Edit).
2. design the dose (range), beam current, and beam step size;
3. fracturize the layout with CATS® (Version X-2005.09) into .IWFL format, and upload to the e-beam workstation;
4. clean the substrate(s);
5. dehydration baking;
6. spin-coat e-beam resist;
7. pre-exposure baking;
8. (optional) spin-coat Espacer300 (Showa-Denko Inc.);
9. (optional) sputter 50 nm thick Al film;
10. (optional) align e-beam markers using the optical microscope and record the locations of the e-beam markers;
11. (optional) record exposure area range;
12. measure heights from different part of the substrate, ensure the height difference within one substrate is less than 10 µm;
13. load the substrate and pump down the chamber;

14. calibrate the holder;

15. prepare beam current, ensure the aperture alignment;

16. (optional) e-beam marker searching:\(^3\);

17. (optional) measure heights on the reflective area and extrapolate the heights for the writing area:\(^5\);

18. prepare command files;

19. execute command files for e-beam exposure;

20. unload the substrate(s);

21. (optional) strip off Espacer300\(^1\);

22. (optional) strip off the Al film\(^2\);

23. (optional) post-exposure baking\(^6\);

24. developer rinse;

25. stopper rinse.

\(^1\) For insulating substrates.

\(^2\) For insulating and/or transparent substrates.

\(^3\) When alignment is necessary.

\(^4\) When alignment is not necessary.

substrates with part of the surface is covered by a reflective film.
The e-beam processes are completed and the substrate(s) are ready for the subsequent deposition and lift-off, dry etching, or imaging processes.

A.3 General Metal Deposition Processes

The following metal/dielectric deposition procedure is based on the ECE cleanroom CHA evaporator.

1. Vent and open the chamber.

2. Load the samples onto the loading tray and place metal source/crucibles into the heating pockets.

3. Pump the chamber and wait until the chamber pressure reaches $3 \times 10^{-7}$ Torr.

4. Start heating the source for the first deposition. If the interface is important, the deposition rate of the first 50 nm should start at around 0.5 Å/sec. If the finishing surface is important, the deposition rate of the last 50 nm should be around 0.5 Å/sec.

5. Change the source and continue deposition of following layers (optional).

6. Cool and vent the chamber, unload samples and metal sources.

A.4 Liftoff

Liftoff is applied to remove resist area after metal deposition of image reversal process.

1. Rinse sample in acetone.
2. When the metal layer over resist starts to crack, spray acetone on sample surface to accelerate this process.

3. If the device surface is clean observed by naked eyes, dip it in IPA for 30 sec and blow dry with \(N_2\) gun.

4. Check with microscope to see if the surface is clean or not. If it is clean, this process is finished.

5. If surface cannot be cleaned by rinse, put it into ultrasonic oven for 10 min. Rinse in IPA and dry. Check with microscope to see if there is any improvement. If it is still not cleaned, go to next step.

6. Boil sample in Remover PG at 180 °C for 5 min. Wait the beaker cool and remove the sample for IPA dip. Dry the sample and check with microscope. If there is improvement, keep doing it until liftoff is clean. Otherwise, the liftoff may fail.
A.5 Plasma Processes

The plasma systems are part of the facilities available within the Electrical and Computer Engineering Department Cleanroom and the nanotech west cleanroom, the Ohio State University.

A.5.1 Plasma Processes with the ECE Oxford Plasmalab 100 System

Table A.1: The Plasma Processes with the ECE Cleanroom Oxford Plasmalab 100 ICP/RIE System.

<table>
<thead>
<tr>
<th>Recipe name</th>
<th>Gases</th>
<th>Pressure (mTorr)</th>
<th>P\textsubscript{RF (W)}</th>
<th>P\textsubscript{ICP (W)}</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen Descum \textsuperscript{a}</td>
<td>O\textsubscript{2}</td>
<td>5</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>(Al)GaN Deoxidization</td>
<td>BCl\textsubscript{3}</td>
<td>10</td>
<td>15</td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td>(Al)GaN Mesa Etching</td>
<td>BCl\textsubscript{3}/Cl\textsubscript{2}/Ar</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>20</td>
</tr>
<tr>
<td>(Al)GaN Recession Passivation</td>
<td>Cl\textsubscript{2}/N\textsubscript{2}/O\textsubscript{2}</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>N.A.</td>
</tr>
<tr>
<td>Oxygen Plasma Passivation</td>
<td>O\textsubscript{2}</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>AlGaN Surface Oxidization</td>
<td>O\textsubscript{2}</td>
<td>100</td>
<td>0</td>
<td>1000</td>
<td>20</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Optional for organic resist only.

A.5.2 Plasma Processes with the NTW Plasmatherm SLR System

Table A.2: Plasma Processes with the NTW Plasmatherm SLR System.

<table>
<thead>
<tr>
<th>Recipe name</th>
<th>Gases</th>
<th>Pressure (mTorr)</th>
<th>P\textsubscript{RF (W)}</th>
<th>P\textsubscript{ICP (W)}</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin Si Layer Etching</td>
<td>Cl\textsubscript{2}</td>
<td>50</td>
<td>15</td>
<td>500</td>
<td>20</td>
</tr>
<tr>
<td>ZEP/SiO\textsubscript{2} Etching</td>
<td>CHF\textsubscript{3}/O\textsubscript{2}</td>
<td>100</td>
<td>150</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>
### A.5.3 Plasma Processes with the ECE Anatech Seren System

<table>
<thead>
<tr>
<th>Recipe name</th>
<th>Gases</th>
<th>Pressure (mTorr)</th>
<th>$P_{RF}$ (W)</th>
<th>$P_{ICP}$ (W)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Al)GaN Oxidization</td>
<td>$O_2$</td>
<td>75</td>
<td>75</td>
<td>0</td>
<td>N.A.</td>
</tr>
</tbody>
</table>
A.6  Shallow Boron Doping Procedure

1. Pre-furnace clean.

   (a) Piranha solution 10 min;
   
   (b) HF dip till oxide removed;
   
   (c) SC-1;
   
   (d) SC-2;

2. Load device samples with Techneglas GS-126 boron sources next to each other on the load boat. The device sides face the sources.

3. Boron diffusion furnace operations is shown in Table A.4.

<table>
<thead>
<tr>
<th>step #</th>
<th>Initial T (°C)</th>
<th>Final T (°C)</th>
<th>Nitrogen Flow (sccm)</th>
<th>Time (hh:mm)</th>
<th>T.C. Controller</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>300</td>
<td>300</td>
<td>15</td>
<td>00:01</td>
<td>Furnace</td>
</tr>
<tr>
<td>01</td>
<td>300</td>
<td>855</td>
<td>15</td>
<td>01:30</td>
<td>Process</td>
</tr>
<tr>
<td>02</td>
<td>855</td>
<td>855</td>
<td>15</td>
<td>00:08</td>
<td>Process</td>
</tr>
<tr>
<td>03</td>
<td>855</td>
<td>855</td>
<td>15</td>
<td>00:22</td>
<td>Process</td>
</tr>
<tr>
<td>04</td>
<td>855</td>
<td>300</td>
<td>15</td>
<td>01:30</td>
<td>Process</td>
</tr>
<tr>
<td>05</td>
<td>300</td>
<td>300</td>
<td>15</td>
<td>End</td>
<td>Furnace</td>
</tr>
</tbody>
</table>

4. Wet Oxidation oxidation at 800 °C for 20 min.

5. Deglaze in HF Dip. 5 min. nominal Deglaze etch is completed when entire wafer surface is hydrophobic. If a hydrophilic surface is left, then all B-Si phase has not been oxidized and oxidation must be repeated. HF Dip range of 5:1 to 10:1 is acceptable.
APPENDIX B

Bessel Functions

B.1 Bessel Differential Equation

Bessel functions, also known as cylinder functions or cylindrical harmonics, are the solutions of Bessel’s differential equation [78]

\[ x^2 \frac{d^2 y}{dx^2} + x \frac{dy}{dx} + (x^2 - \alpha^2) y = 0 \]  \hspace{1cm} (B.1)

for any \( \alpha \) values. However, the most common \( \alpha \) values are integers or half-integers.

B.2 Bessel Function of the First Kind \( J_\alpha \)

Bessel function of the first kind is when \( \alpha \) is an integer and the Bessel’s equation is finite at \( x=0 \). The Bessel function of the first kind can be expressed by its Taylor series at \( x=0 \):

\[ J_\alpha = \sum_{m=0}^{\infty} \frac{(-1)^m}{m! \Gamma(m + \alpha + 1)} \left( \frac{x}{2} \right)^{(2m+\alpha)} \]  \hspace{1cm} (B.2)

where \( \Gamma(n) = (n - 1)! \). The Bessel function of the first kind with orders 0-5 is plotted in Fig. B.1 [117].
Figure B.1: 0-5 orders Bessel function of (top) the first and (bottom) the second kind.
B.3 Selected Properties of Bessel Function of the First kind

When \( \alpha \) is an integer \( n \), the following relationship is valid:

\[
J_{-n}(x) = (-1)^n J_n(x)
\]  \hspace{1cm} \text{(B.3)}

The derivative of the Bessel function of the first kind can be obtained by the following relationship:

\[
\frac{d}{dx} J_n(ax) = \frac{a}{2} [J_{n-1}(ax) - J_{n+1}(ax)].
\]  \hspace{1cm} \text{(B.4)}

For \( n = 0 \) and \( a = 1 \), the above equation becomes:

\[
\frac{d}{dx} J_0(x) = \frac{1}{2} [J_{-1}(x) - J_1(ax)].
\]  \hspace{1cm} \text{(B.5)}

From Equation (B.3), \( J_{-1}(x) = -J_1(x) \). Equation (B.5) becomes

\[
\frac{d}{dx} J_0(x) = J_1(x).
\]  \hspace{1cm} \text{(B.6)}

At \( x=0 \), \( J_1(x) = 0 \) (Fig. B.1):

\[
\frac{d}{dx} J_0(x) = 0.
\]  \hspace{1cm} \text{(B.7)}

B.4 Bessel Functions of the Second Kind \( Y_\alpha \)

The Bessel functions of the second kind, also named Neumann function, are the solutions of the Bessel differential equation, with a singularity at \( x=0 \). For non-integer \( \alpha \):

\[
Y_\alpha(x) = \frac{J_\alpha(x) \cos(\alpha \pi) - J_{-\alpha}(x)}{\sin(\alpha \pi)}
\]  \hspace{1cm} \text{(B.8)}

For an integer order \( n \),

\[
Y_N(x) = \lim_{\alpha \to n} Y_\alpha(x)
\]  \hspace{1cm} \text{(B.9)}
**B.5 Modified Bessel Functions: \( I_\alpha \) and \( K_\alpha \)**

When the argument \( x \) of Bessel functions are imaginary numbers, the bessel functions are called modified Bessel functions of the first and the second kind:

\[
I_\alpha(x) = i^{-\alpha} J_\alpha(ix) = \sum_{m=0}^{\infty} \frac{1}{m! \Gamma(m + \alpha + 1)} \left( \frac{x}{2} \right)^{2m+\alpha} \tag{B.10}
\]

\[
K_\alpha(x) = \frac{\pi}{2} \frac{I_{-\alpha}(x) - I_\alpha(x)}{\sin(\alpha \pi)} \tag{B.11}
\]

**Asymptotic Forms**

For large \( x \) (\( x \gg |\alpha^2 - 1/4| \)), the modified Bessel functions become:

\[
I_\alpha(x) \approx \frac{e^x}{\sqrt{2\pi x}} \left( 1 + \frac{(1-2\alpha)(1+2\alpha)}{8x} + \ldots \right) \tag{B.12}
\]

\[
K_\alpha(x) \approx \sqrt{\frac{\pi}{2x}} \exp(-x) \tag{B.13}
\]

For small \( x \) (\( 0 < x \ll \sqrt{\alpha + 1} \)):

\[
I_\alpha \approx \frac{1}{\Gamma(\alpha + 1)} \left( \frac{x}{2} \right)^\alpha \tag{B.14}
\]

\[
K_\alpha \approx -\ln \left( \frac{x}{2} \right) - \gamma \quad \text{if} \quad \alpha = 0 \tag{B.15}
\]

\[
\frac{\Gamma(\alpha)}{2} \left( \frac{\alpha}{2} \right)^\alpha \quad \text{if} \quad \alpha > 0 \tag{B.16}
\]

**B.5.1 Derivatives**

For \( y = J, Y, I, H^{(1)}, H^{(2)} \):

\[
\frac{d}{dx} y_p(\alpha x) = \alpha y_{p-1}(\alpha x) - \frac{p}{x} y_p(\alpha x) \tag{B.17}
\]

For \( y = K \):

\[
\frac{d}{dx} y_p(\alpha x) = -\alpha y_{p-1}(\alpha x) - \frac{p}{x} y_p(\alpha x) \tag{B.18}
\]

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Figure B.2: 0-5 orders modified Bessel function of the first (top) and the second (bottom) kind.
With $\alpha = 1$ and $p = 1$, Equation (B.17) can be transformed into:

\[
\frac{d}{dx} [xy_1(x)] = xy_0(x) \quad \text{for } y = J, \ Y, \ I, \ H^{(1)}, \ H^{(2)} \tag{B.19}
\]

\[
\frac{d}{dx} [xy_1(x)] = -xy_0(x) \quad \text{for } y = K \tag{B.20}
\]


[71] http://my.ece.ucsb.edu/mgrundmann/bandeng.htm


[76] http://www.dowcorning.com


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