Research Article

Fabrication of Silicon Nitride Ion Sensitive Field-Effect Transistor for pH Measurement and DNA Immobilization/Hybridization

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Received 10 July 2013; Accepted 10 September 2013

Academic Editor: Hongchen Chen Gu

The fabrication of ion sensitive field-effect transistor (ISFET) using silicon nitride (Si_3N_4) as the sensing membrane for pH measurement and DNA is reported. For the pH measurement, the Ag/AgCl electrode was used as the reference electrode, and different pH values of buffer solution were used in the ISFET analysis. The ISFET device was tested with pH buffer solutions of pH2, pH3, pH7, pH8, and pH9. The results show that the IV characteristic of ISFET devices is directly proportional and the device's sensitivity was 43.13 mV/pH. The ISFET is modified chemically to allow the integration with biological element to form a biologically active field-effect transistor (BIOFET). It was found that the DNA immobilization activities which occurred on the sensing membrane caused the drain current to drop due to the negatively charged backbones of the DNA probes repelled electrons from accumulating at the conducting channel. The drain current was further decreased when the DNA hybridization took place.

1. Introduction

There are some traditional pH measuring methods in the market such as the litmus papers and glass pH electrodes. These measuring methods are simple and giving considerably quick measurement results. However, they do have their disadvantages. Litmus papers rely on color indication which is not convenient for people with color blind difficulties. Moreover, litmus papers are not reusable and are disposed of after each use. Litmus paper also cannot be used for continuous monitoring of a process because the color change can be affected by the chemical processes [1]. On the other hand, glass pH electrodes are too bulky and fragile. Thus, it is avoided to be used in food industries. Moreover, glass pH electrode cannot be operated at temperature higher than 60°C [2]. It requires frequent maintenance and high setup cost. Therefore, ISFET is gaining popularity as it overcomes the disadvantages of using litmus papers and glass pH electrodes. ISFET is a highly sensitive and small size sensor for rapid detection of the ionic activity in an electrolyte solution which enables it to be used in various applications such as medical, agriculture, environmental monitoring, and food industry.

ISFET was first developed by Professor Bergveld in 1970 using SiO_2 as a sensing layer [3]. The device structure of an ISFET is almost similar with that of a MOSFET, but the gate electrode is being replaced by a sensing membrane [4]. The material used for the gate region of an ISFET is a very important factor that decides the sensitivity and selectivity of the device. Besides functioning as a passivation layer for the ISFET, it needs to be able to avoid hydration and prevent ion migration to the semiconductor surface [5]. To enable ISFET to become functional, electrolyte and a reference electrode are required. The structure of an ISFET is shown in Figure 1.

On the other hand, ISFET fabrication can be fabricated easily using the existing CMOS technology and process. According to Matsuo et al., the silicon nitride is more suitable than the silicon oxide and other inorganic oxides to be used as ISFET sensing membrane [6]. It is because a nonlinear response of pH demonstrated by ISFET is caused by fast hydration of silicon oxide layer [7]. Alternatively, silicon nitride is an easily accessible material and has been used in the existing CMOS technology for many years. In order to measure pH, ISFET is used together with a reference electrode [8]. The function of the reference electrode is to

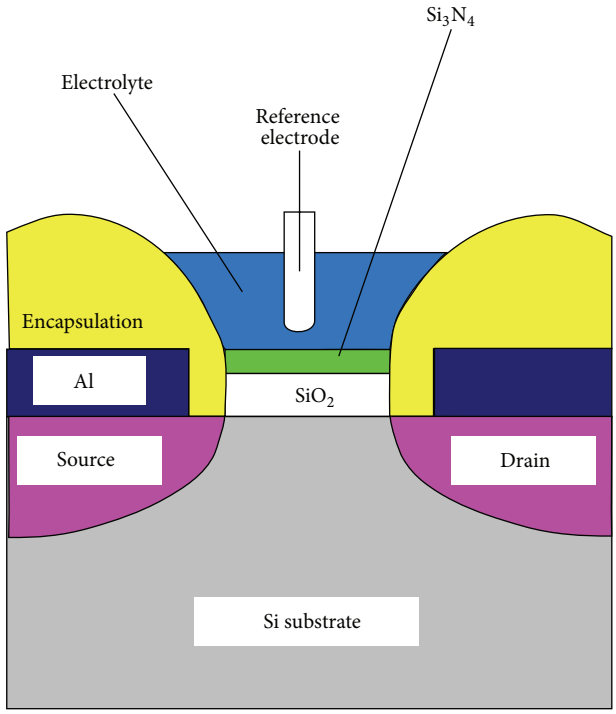

Figure 1: Structure of an ISFET.

provide steady reference potential to meet the requirement of normal FETs to have gate voltage. In this research, we fabricated the highly sensitive ISFET for pH detection using conventional photolithography method. The surface morphology and material of ISFET were characterized using X-ray diffraction (XRD), high power microscope (HPM), and atomic force microscopy (AFM). The pH measurement was carried out using source measurement unit.

The BIOFET can be constructed by using the ISFET device with some surface modification performed chemically to bind with DNA targets. In DNA hybridization, the target (unknown single-stranded DNA (ssDNA)) is identified by a probe molecule with which it forms a double-stranded (dsDNA) helix structure with its complementary nucleic acid with high efficiency and specificity in the presence of a mixture of many different noncomplementary nucleic acids. The unique complementary nature of the base pairing, which is the adenine-thymine and cytosine-guanine, is the basis for the extremely high specificity of the biorecognition process. A DNA-modified FET or GenFET, schematically shown in Figure 2, can also be very useful for hybridization detection.

A GenFET can be obtained by immobilizing well-defined sequences of ssDNA onto a transducer, which converts the specific recognition process of two DNA single strands through the hybridization event into a measurable signal. The inherent miniaturisation of such devices and their compatibility with advanced microfabrication technology can make them very attractive for DNA diagnostics. Unfortunately, there are only a few publications that are related to ISFETs for DNA sensing. A first and successful attempt in the direct detection of a hybridization event with a GenFET was reported by Souteyrand et al. [9]. Noncomplex synthetic single strands of homooligomers have been chosen as a model system to demonstrate the feasibility of using an ISFET for the detection of a DNA hybridization event. Therefore, homooligomers were immobilized on the previously modified SiO_2-gate of a GenFET. To detect the hybridization process in real time, in situ measurements were performed by adding a certain volume of the complementary DNA solution directly to the buffered electrolyte.

In this study, single strands of DNA were immobilized and hybridized onto the silicon nitride sensing membrane surface of the GenFET. Chemical modifications were conducted on the silicon nitride surface before being ready for DNA immobilization and hybridization. The process and results are shown in the following sections.

2. Methodology

2.1. Fabrication of ISFET. The starting material used in this project was a P-type of silicon substrate with a diameter of 100 mm. Prime silicon wafer was used as starting material to fabricate the electrode for the biomolecules detection biosensor. The specification of silicon wafer such as thickness and resistance were determined. Then, the back of silicon wafer was scribed lightly using a scribe tool to mark several lines for cutting the silicon wafer into desired dimension. The silicon wafer was cleaned using acetone before keeping in a clean place.

In this research, the normal transparency sheets were used as the material for the four photomasks to fabricate the ISFET. The photomasks were designed using AutoCAD and printed onto transparency sheets. The design is shown in Figures 3(a), 3(b), 3(c), and 3(d). All dimensions were designed in μm. After the above steps are done, the fabrication process takes place as in Figure 4.

The process was started by depositing a 300–500 nm layer of silicon oxide onto the clean silicon wafer using Modu Lab dry oxidation furnace. After that, silicon nitride thin film was deposited onto the silicon oxide layer using direct current (DC) sputtering. Then, a layer of photoresist (PR) was coated onto the silicon surface using a Laurell WS-400B-6NPP-Lite Resist spin coater. Softbake was conducted for 90 s to remove moisture after PR was coated onto the silicon surface. After that, the titanium was deposited as the hardmask to protect the layers during the diffusion process. The pattern from the photomask was then transferred onto the wafer using mask aligner exposure system (MIDAS MDA-400M) with ultraviolet wavelength of 365 nm and an exposure time of 10 s. Next, the RD6 developer solution (Futurrex) was used to develop the pattern after the UV exposure and continued by the hardbake process for 90 s.

Once the source-drain channel openings are obtained, drive-in diffusion was performed using phosphorus dopant. Annealing process was conducted after the diffusion process to allow the dopant to penetrate better into the diffusion channels. Then, the gate masking was used in the photolithography process to create the gate of the ISFET and followed by

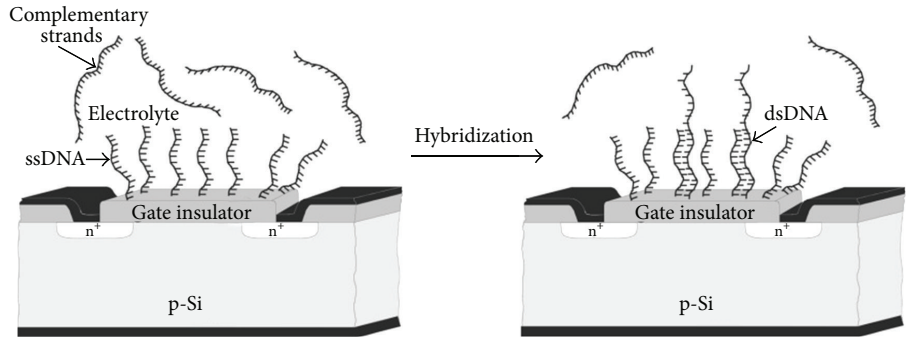

FIGURE 2: Schematic structure of a DNA-FET (GenFET) and the principle of DNA hybridization detection (reproduced with permission from [10]).

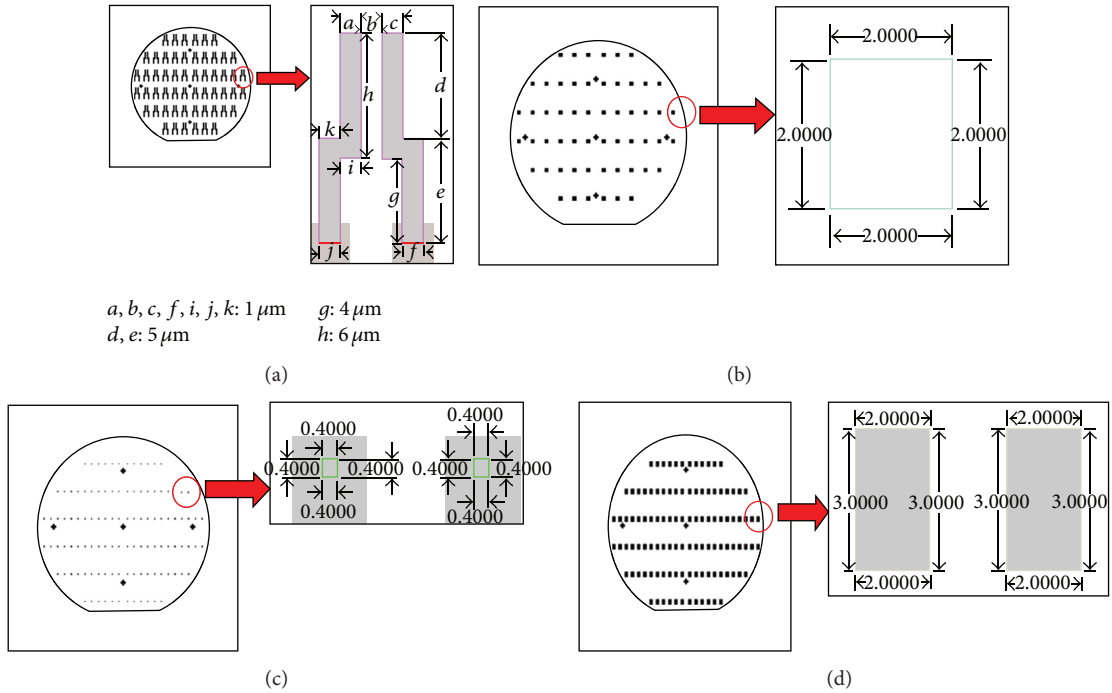

FIGURE 3: The design of photomask (a) source and drain mask; (b) gate mask; (c) contacts mask; (d) metallization mask.

the metallization masking to pattern the metal electrodes for the device. Lastly, a layer of epoxy resin was applied onto the device areas except the gate to function as the encapsulation layer leaving only the gate sensing membrane to be exposed to the solution under test.

2.2. ISFET Characterization. The sensing membrane was analyzed using XRD system (LabX XRD-6100). Intensity was measured by step scanning in the 2θ range of 10–90° with a step of 0.02° and scanning rate of 2° per minute. The morphology of ISFET was inspected using HPM system (Olympus EX51). The surface roughness of ISFET pH sensor was measured using AFM system (SPI 3800N AFM). To measure the sensing performances of the ISFET pH sensor, both the ISFET pH sensor and a silver-silver chloride reference electrode were dipped into the detection solution with pH 2, 3, 7, 8, and 9. The source electrode and the drain electrode were connected to *I-V* measurement solutions unit (LabTracer 2.0). The equipment setup is as seen in Figure 5.

2.3. Surface Modification of Silicon Nitride. The purpose of surface modification was to prepare a suitable platform at the silicon nitride for DNA immobilization and hybridization. The modification was started by cleaning the surface of silicon nitride layer using 1 M NaOH for 1 hour at room temperature and silanizing in toluene which contained 2 wt% of 3-aminopropyltriethoxysilane (APTES) to introduce reactive amino groups at the silicon nitride surface. Then, the amino-silanized silicon nitride surface was rinsed in toluene to remove excess APTES and dried at 120°C for 1 hour. To immobilize DNA probes at the silicon nitride surface, glutaraldehyde was used as the bifunctional cross-linking agent. Thus, to prepare the bifunctional cross-linking agent at the silicon nitride surface, it was soaked for 1 hour at

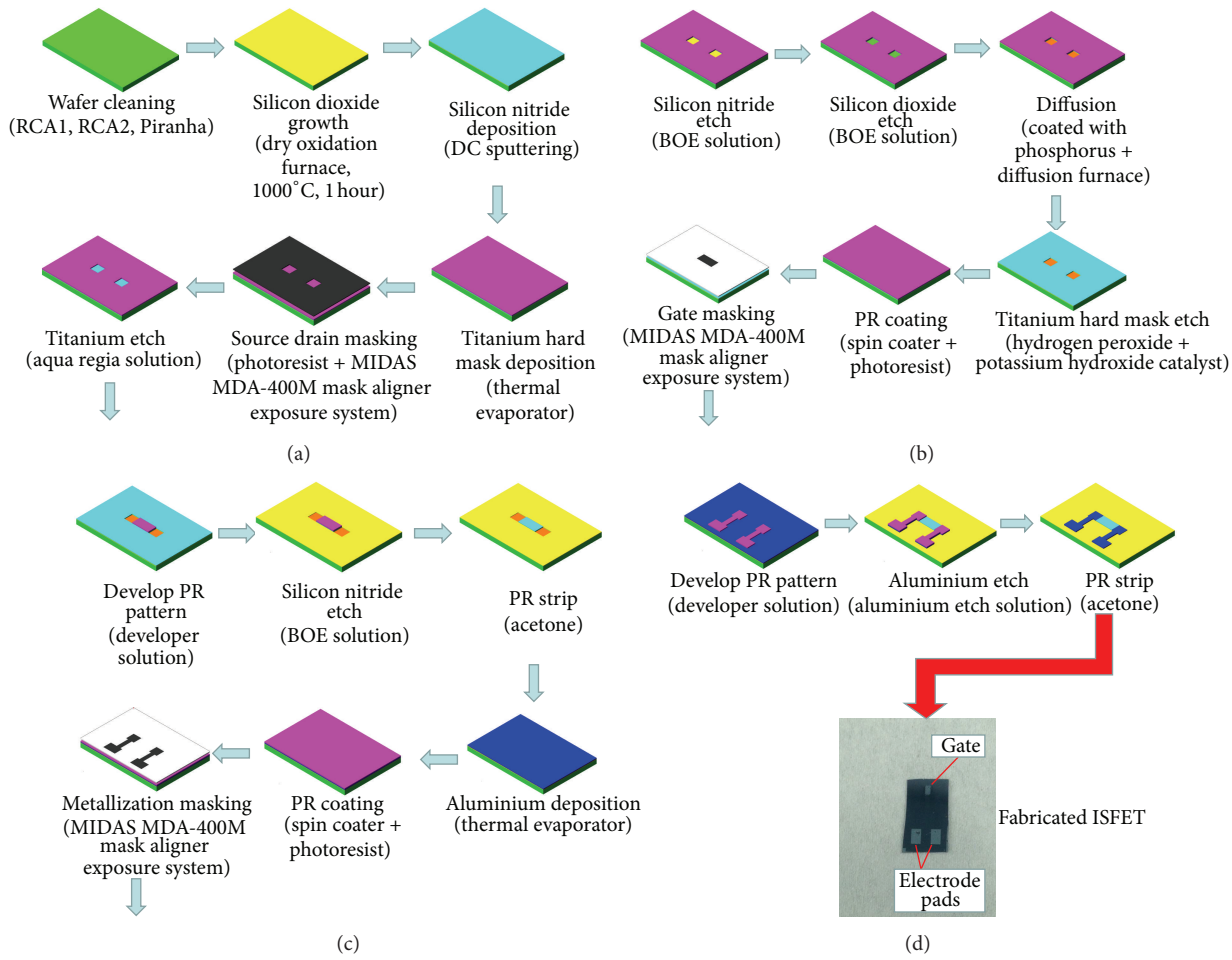

Figure 4: The flow of ISFET device fabrication: (a) part 1; (b) part 2; (c) part 3; (d) part 4.

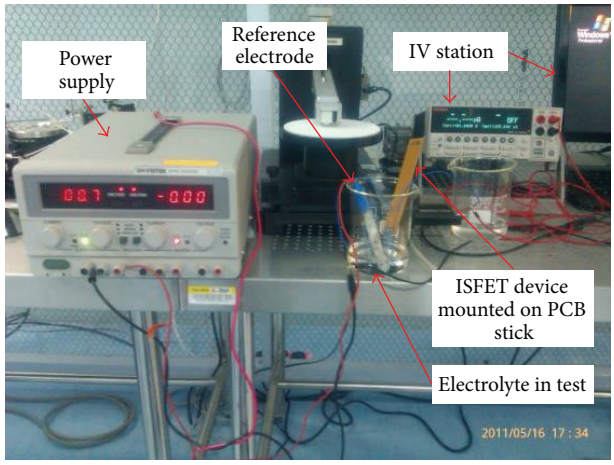

Figure 5: Experimental setup for pH measurement.

room temperature in a 50 mL of mixture solution which consisted of 25 wt% glutaric dialdehyde solution and 0.5 g of sodium cyanoborohydride. After that, silicon nitride surface was rinsed in deionized water and dried at room temperature for 1 hour to finish the surface modification process. The attachment of reactive amino groups at the silicon nitride surface was ready for the next DNA immobilization process. The ISFET was renamed as GenFET after the surface modification was conducted.

2.4. DNA Immobilization Process. For the immobilization process, the DNA probes were purchased from First Base Laboratories. The DNA probes were synthesized with an amino group at 5′-end for attachment to the silicon nitride surface. The base sequence of the probe was 5′-CCACTACCAGGGCACGT-3′ (17 mer). The DNA probes were dissolved first in a TE (Tris-EDTA) buffer (pH 8.0) at a concentration of 100 μM. To couple amino-modified DNA probes with the glutaraldehyde-treated silicon nitride surface, the silicon nitride surface was kept at 50°C for 4 hours in a 50 mL of mixture solution which consisted of 25 wt% glutaric dialdehyde solution and 0.5 g of sodium cyanoborohydride to complete the coupling reaction. The silicon nitride surface was then soaked in a PBS with 1 M glycine at 50°C for 1 hour to block any remaining glutaraldehyde groups. The silicon nitride surface was washed with the PBS (pH 7.0) and

with DI water and then dried in room temperature for 1 hour [11].

2.5. DNA Hybridization Process. For hybridization process, 10 μL of complimentary target DNA was dropped onto the immobilized gate for 1 hour at temperature of 37°C. After that, the sample was rinsed with PBS and deionized water before device electrical characterization was carried out.

3. Results and Discussion

3.1. Visual Inspection Results. Figure 6 is the powder XRD pattern of the freshly prepared sensing membrane of ISFET. The representative for silicon nitride is denoted in the XRD spectra with high intensity at scanning angle of 29°. This is in good agreement with the result reported by Velasco [12] that silicon nitride was the material deposited on the silicon wafer to be used as sensing membrane in this work.

Figure 7 shows the HPM image of sensing membrane (red violet color). The thickness of the sensing membrane was 250 nm. It was observed that the surface of sensing membrane was quite rough which could be attributed to overetching of titanium nitride during the fabrication process. It is because titanium was used as a hard mask for the protection of membrane surface in the diffusion process. The formation of titanium oxide and titanium nitride occurred after the diffusion process due to the presence of oxygen and nitrogen gas. Thus, titanium nitride was etched away for 12 hours using hydrogen peroxide and potassium hydroxide. After that, the Buffered Oxide Etch (BOE) solution was used to etch away the unwanted areas of silicon nitride and silicon oxide layers.

Figure 8 shows the AFM image for sensing membrane. The surface roughness and grain size of silicon nitride film were measured using AFM. The silicon nitride film had surface roughness and grain size of 1.369 nm and 3.817 × 10^2 nm^2, respectively. RMS value of the silicon nitride thin film was 1.755 nm.

3.2. Electrical Characterization Results. The electrical characterization has shown that the ISFET has good characteristics as field-effect transistors. An example is shown in Figure 9. The drain current versus the gate-to-source voltage for different pH values of the liquid solution are shown. Buffer solutions with pH of 2, 3, 7, 8, and 9 were used for the pH measurement of ISFET. The result shows that the voltage was proportional to the current which is in agreement with the finding by Matsuo and Wise [13] and Bergveld [14].

The reference electrode was grounded in the testing circuit throughout the testing of the ISFET. The reason behind the grounded reference electrode is that the reference electrode is in fact nothing more than a contact between a metal wire and an aqueous solution to determine the electrical potential of the electrolyte solution [15]. The reference electrode should not be causing any influence to the electrolyte because the curves can be obtained by changing the pH of the electrolyte solution itself [14].

All the area of ISFET except the sensing membrane of ISFET was covered with silicon glue as a protective layer to

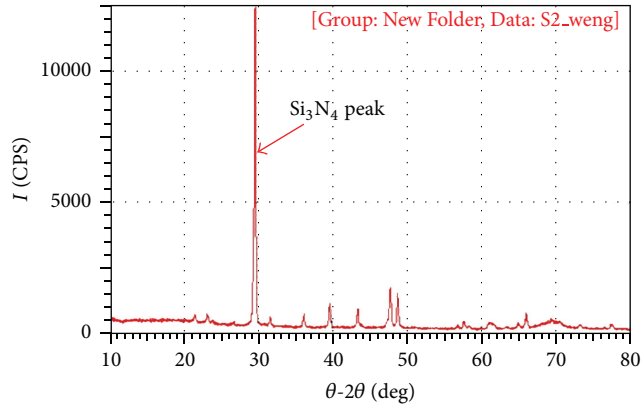

Figure 6: XRD pattern of silicon nitride thin film.

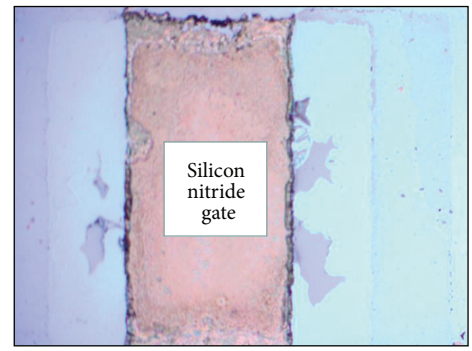

Figure 7: HPM image of sensing membrane.

expose the sensing membrane only before it was immersed into the pH buffer solution. From the IV curve obtained, it can be seen that there was an increase of the drain current with acid solution and a decrease with basic solution. This is due to the accumulation of the positive charge, H^+, in the acidic solution at the interface between the sensing membrane and the pH buffer solution which increased the value of drain current [16–18]. The H^+ ions accumulated on the sensing membrane attract electrons towards the conducting channel, hence, lowering the channel resistance and increasing the drain current. Vice versa, for a basic solution, the negative charge OH^- which accumulated at the sensing membrane decreased the value of drain current [16–18].

The pH response of the ISFET was influenced by the electrical potential at the membrane surface [18]. The electrical potential was originated from the interaction between the insulator surface and the ions presented in the electrolyte. The active sites play an important role for this interaction which can act as proton donor or acceptor. The active sites involved at the silicon nitride surface were the silanol (A–OH=SiOH) and amine ($BH=SiNH_2$) groups [19]. The presence of the hydroxyl group, which can donate or accept proton from the solution, caused the phenomena where the neutral surface hydroxyl group can became negatively charged or positively charged site for maintaining charge neutrality [19]. The surface charge was counter balanced by an equal and opposite

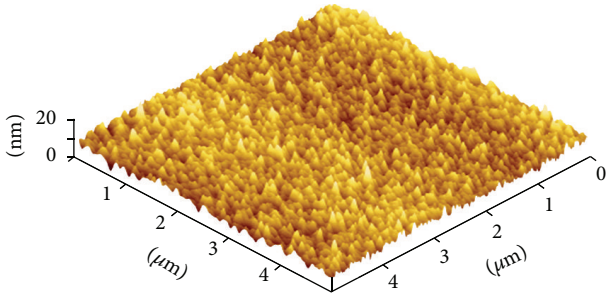

Figure 8: AFM image for sensing membrane.

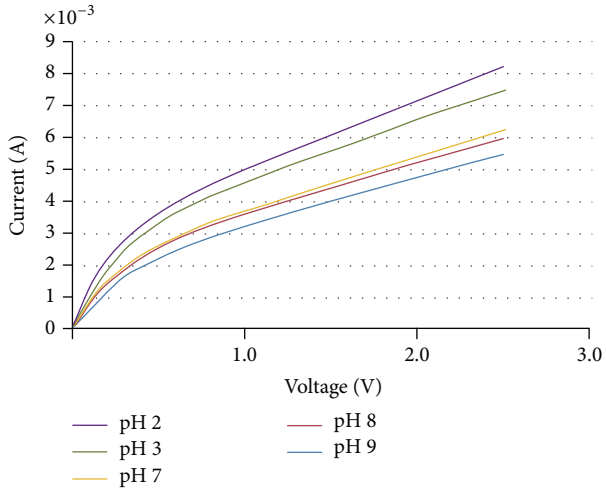

Figure 9: I/V measurement of ISFET for pH 2, 3, 7, 8, and 9.

charge in the solution, resulting in charge distribution that produced double layer potential difference at the gate [19].

3.3. Sensitivity of the ISFET Device. The pH sensitivity of the ISFET was determined by measuring the shift in the threshold voltage of the device. The threshold voltage was obtained from the IV curves as in Figure 9. The V_{th} and sensitivity values of the ISFET were recorded and tabulated in Table 1. When the I_D and V_D were kept constant, the variation of the threshold voltage induced by the variation of pH of the test buffer solutions caused an identical variation in the gate voltage. Thus, the sensitivity of the ISFET can be expressed by the following equation [20]:

$$S = \frac{\nabla V_G}{\nabla \text{pH}} = \frac{\nabla V_{th}}{\nabla \text{pH}} \quad (1)$$

The V_{th} was measured as a function of pH, with pH buffer solutions using the Ag/AgCl reference electrode as the gate electrode. The change of V_{th} of the ISFET due to pH was plotted as shown in Figure 10. The pH sensitivity of ISFET was calculated from the normalized curve plotted in Figure 10. These results are in good agreement with the values found in the literature [6, 13, 16] which show that the ISFET exhibited a satisfactory linear response with pH. The sensitivity value obtained of 43.13 mV/pH did not exceed

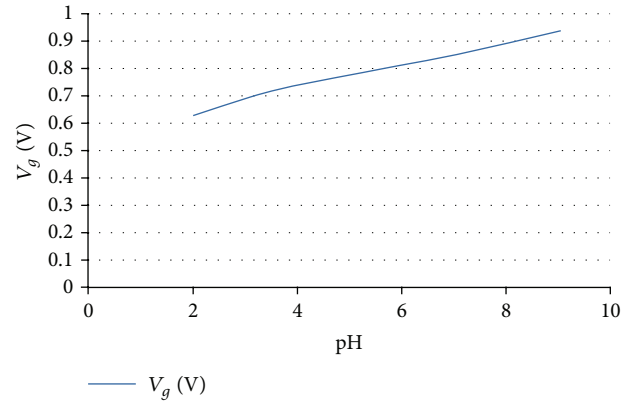

Figure 10: V_g versus pH of ISFET.

Table 1: Drain current measured at different concentrations of complimentary target DNA.

DNA target concentrations (μM)	I_D at V_D = 2 V (mA)
10	2.47
5	1.81
1	1.34
0.1	1.30

the maximum sensitivity as predicted theoretically of 59 mV/pH [18].

The pH sensitivity is determined by the material used as the gate membrane [21]. The dependence of the I_D on the pH value of the testing pH buffer solutions is related to the variation of the threshold voltage of ISFET due to the charge variation on the membrane-solution interface as explained in Gouy-Chapman-Stern theory [22]. This theory explains that when a membrane exposed to an aqueous solution interacts with H^+ ions, it causes charge redistribution in the solution. The surface charges create an electrostatic field that affects the ions in the liquid. This electrostatic field combined with thermal motion of the ions creates a counter charge below the sensing membrane surface. The magnitude of the surface charges and the counter charge is equal but of opposite polarity to be electrically neutral. This variation is detectable through a shift of the threshold voltage of the device.

Besides, the sensitivity of pH measurement is also affected by the thickness of silicon oxide and silicon nitride layers. It is known that the thickness of silicon oxide can affect the direct tunneling current in which the tunneling current increases exponentially with decreasing thickness and restricts the application of silicon oxide as a gate dielectric [23]. As an example, direct tunneling current mechanism becomes dominant when an oxide with thickness of <3 nm which is defined as ultrathin oxide film is used [23]. One of the ways to reduce the tunneling current is to deposit an insulator layer with dielectric constants higher than silicon oxide onto a silicon oxide layer as carried out in this work. Therefore, it is expected that the sensitivity of pH measurement is increased as the thickness of both silicon oxide and silicon nitride

layers increased. Further study and experimental works will be conducted in the future.

3.4. Electrical Characterization for DNA Immobilization and Hybridization. Figure 11 shows the GenFET that is ready for DNA immobilization which consists of ISFET, sensing membrane, copper wire, and printed circuit board (PCB) stick. The performance of GenFET was characterized electrically after the DNA immobilization and hybridization process. The results for before and after DNA probes immobilization and DNA hybridization were plotted into graph as shown in Figure 12.

In Figure 12, it can be observed clearly that, after the DNA immobilization process was completed, the drain current was much lower than the drain current measured before DNA immobilization. At $V_D = 2V$, the I_D value was dropped by 3.82 mA from 7.18 mA to 3.36 mA. This decrease was caused by the attachment of DNA probes at the silicon nitride sensing membrane surface after the immobilization process was carried out. During the immobilization, the DNA probes interacted with the silicon nitride sensing membrane by pushing away electrons to form a depletion region because the DNA probes backbone is built from anion phosphate. When the depletion region was formed, it increased the conduction channel electrical resistance and reduced the drain current. Therefore, when more anions of DNA probes accumulated at the gate surface, this caused less drain current flow at the conduction channel. In addition, the DNA immobilization also decreased the surface area of the silicon nitride sensing membrane which reacted with ions in the PBS electrolyte, and therefore, it reduced the ionic activity at the membrane-electrolyte interface and subsequently decreased the drain current flow in the conduction channel as well.

In the hybridization process, the GenFET was tested again with the PBS electrolyte, and the graph is shown in Figure 12. From the graph, we can clearly see that the drain current was decreased as compared to the drain current recorded after DNA immobilization when applying 0–2 V. This decrease was attributed to the hybridization between DNA probes and complementary target DNA which has the same structure of backbone that consists of anion phosphate only [24]. Therefore, the interaction between DNA and silicon nitride becomes much stronger by creating a thicker depletion region as compared to depletion region before DNA hybridization and further increased the resistance of conduction channel and subsequently reduced the drain current.

3.5. Effect of Different Concentration of Complementary Target DNA. The effect of different concentration of complementary target DNA on the performance of GenFET was investigated. The concentrations of complementary target DNA were 10 μM, 5 μM, 1 μM, and 0.1 μM. The results were tabulated in Table 1 and plotted into graph in Figure 13.

From Figure 13, it is observed that when the concentration of complimentary target DNA decreased, the drain current decreased as well. It is because less amount of complimentary target DNA was hybridized with DNA probes when the concentration of complimentary target DNA was high. At higher concentration of DNA target, the electrostatic repulsion between the DNA complementary strands has reduced the probability of hybridization [25]. At 0.1 μM of DNA target concentration, there were more hybridization events that took place compared to 10 μM of DNA target concentration because during successful hybridization process, more DNA complementary target strands were bound to the DNA oligonucleotide probes increasing the negative charges on the surface. Hence, electrons in the depletion region were further repelled forming thicker layer of depletion region. This thicker layer of depletion region creates higher resistance which reduces the drain current flowing in the device.

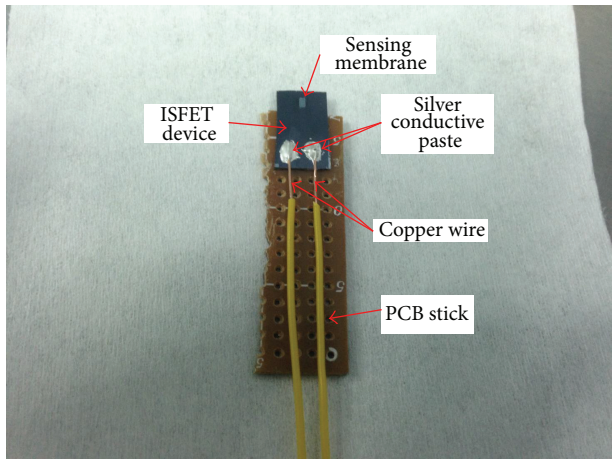

Figure 11: The fabricated GenFET device.

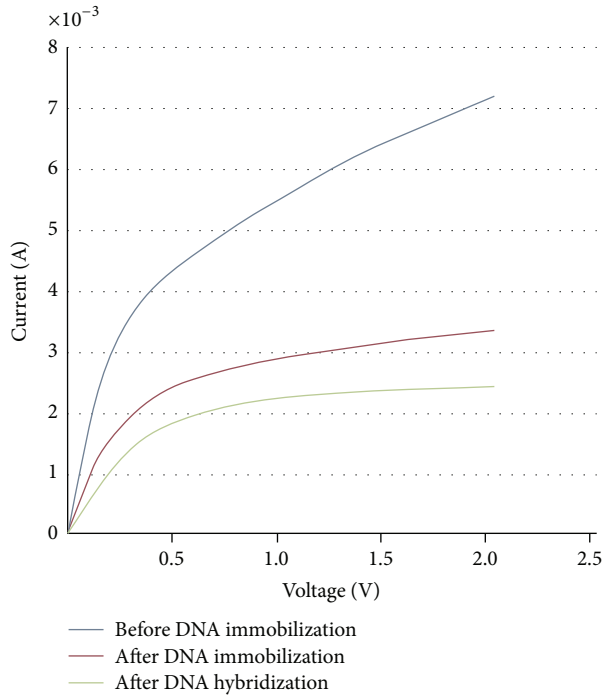

Figure 12: Before and after DNA immobilization and hybridization for current versus voltage.

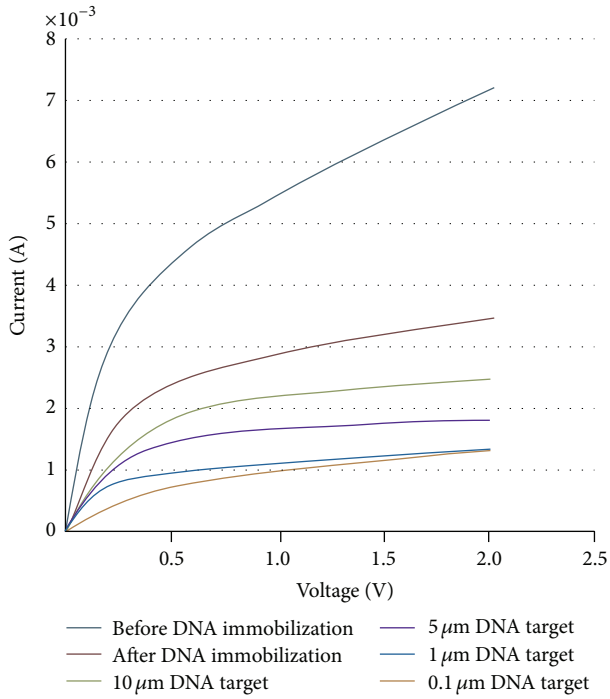

Figure 13: GenFET hybridization by varying different concentrations of complimentary target DNA.

4. Conclusions

In this work, ISFET sensor fabricated by using silicon nitride as the sensing membrane has been shown to measure pH and the DNA immobilization and hybridization event satisfactorily. The ISFET sensor exhibited a linear pH response with a sensing sensitivity of 43.13 mV/pH. The high sensitivity could be attributed to the improvement performance of sensing membrane fabricated using silicon nitride in this work. The drain current was found to decrease after the DNA immobilization and hybridization. In addition, the hybridization between different concentrations of DNA complementary targets with DNA probes decreased the drain current as well. In the next few years, ISFET may have advanced performance and extraordinary properties when accompanying with nanomaterials such as nanowire, nanorod, nanoparticles, and nanotubes. The working principle of GenFET is almost similar to that of the ISFET which still depends on the charge adsorption on the sensing membrane surface of the gate. The GenFET can be used in detection of human inherited diseases such as heart diseases and other sorts of information from living organisms.

Acknowledgments

The authors wish to thank Universiti Malaysia Perlis (UniMAP), FRGS Grant (9003-00203), and Grant MOA Sciencefund 050-01-15-SF1009 for giving the opportunities to do this research in the Micro & Nano Fabrication Clean room. The appreciation also goes to all the team members in the Institute of Nano Electronic Engineering (INEE) especially Nano Biochip Research Group members.

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