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科　　別：工程學

作 品 名 稱：DNA Detection by EGFET using GaN Nanowires Gate

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作者簡介

呂京穎

自幼便對科學研究感興趣，而國中、高中數理資優班的課程，使我在科學研究上得到充足的資源，也不斷開發自己在不同領域的興趣。所參與科展之領域，包含生物、數學、物理等。

陳亭妤

從小便發現對數理的熱忱，一直到現在依然不變，敢於接觸新的知識領域、面對新的挑戰，對於進行實驗研究，從好奇到樂在其中，科學之於我已然成爲不可或缺的一環。

Abstract

DNA-hybridization based detection techniques are widely developed due to their promising applications in genetics, medicine and drug discovery. However, current DNA detection techniques based on labels or reagents are time-consuming, environmentally-harmful and complex to implement. In this study, we have successfully demonstrated a label-free extended-gate-field-effect-transistor (EGFET) sensor utilizing a GaN-nanowires electrode with DNA probes immobilized, capable of specific DNA sequence identification. The principle behind the design is based on the change in surface potential and charge transfer after hybridization. GaN nanowires, being bio-compatible, provide direct transfer path and high surface area, thus offer an unprecedented opportunity of DNA sensing with high sensitivity. In addition, our EGFET design facilitates easy assembly and operation of DNA detection. Comparative studies on complementary and non-complementary DNA were performed to verify the specificity of the sensor. By adapting GaN nanowires structure, the assay time of DNA was shorten to within thirty minutes. Moreover, our sensor displayed an ultra-high sensitivity in the level of attoM: three orders of magnitude higher in resolution than that of other FET-based DNA detection methods.

摘　要

DNA 感測器近年來蓬勃發展，應用層面包括基因工程、醫學及藥物的開發等。然而，目前較常使用的感測方法，需要繁瑣、耗時的標定過程，且其所使用的化學藥劑，對環境易造成傷害。鑒於以上方法之不完善處，我們決定設計一套新的感測系統。

此研究結合了氮化鎵奈米線 (GaN Nanowires) 及延伸式場效電晶體 (EGFET) 的優點，成功的發展出創新的 DNA 感測系統。氮化鎵奈米線的高生物匹配性及高感測面積，能有效提高靈敏度；延伸式場效電晶體的設計，使感測器具由免標定、即時感測的特性，且易於組裝及操作。實驗中，我們將探針 DNA (probe DNA) 修飾在氮化鎵奈米線作爲之延伸閘極上，由於 DNA 在中性水溶液中帶負電，且 DNA 之間具有強烈的互補特性，因此，當目標 DNA (target DNA)與探針 DNA 接合，形成雙股 DNA，氮化鎵奈米線 (閘極) 的表面電位即會有所變化，並造成 FET 特性的改變。藉由此性質，即能成功感測 DNA。

研究結果顯示，此研究所發展出的 DNA 感測器，具有相當高的靈敏度 (10^{-18} M)，相較於其他以 FET 技術設計出的 DNA 感測器，靈敏度提升了三個數量極，此外，此感測器亦具有高選擇性，即使單一鹼基對的突變也能成功辨別。

一、研究動機

生醫感測的研究近年來受到很大的重視，因爲它能使疾病受到早期的發現，進而提升疾病的治癒率，並使有限的醫療資源受到最有效的運用，而要使疾病早期發現、早期治療，主要的關鍵就在於如何建構出一套準確靈敏的疾病感測機制，感測器所能感測到的濃度越低，疾病就能越早受到發現及治療！對此十分感興趣，特別是在 DNA 的感測方面，DNA 是生物體中最重要的基因載體，許多突變型疾病，如癌症等，可以藉由 DNA 偵測而被發現。因此，在廣泛閱讀相關文獻後，決定利用場效電晶體（Field Effect Transistor，即 FET）電學特性，及氮化鎵奈米線（GaN NWs）結構，嘗試設計出一套完整的感測系統，並設法提高它的感測靈敏度，期望未來它將能使疾病的感測更加的準確及迅速，對醫療研究有所幫助。

二、研究目的

1.設計一套 DNA 感測系統，找出最佳感測狀態。

2.藉由氮化鎵奈米線結構及氮化鎵薄膜的比較，找出何者爲最佳感測素材。

3.藉由改變待測 DNA 濃度，試著找出其樣品的感測極限，以及探討 DNA 濃度對樣品電性的影響，並推出其公式。

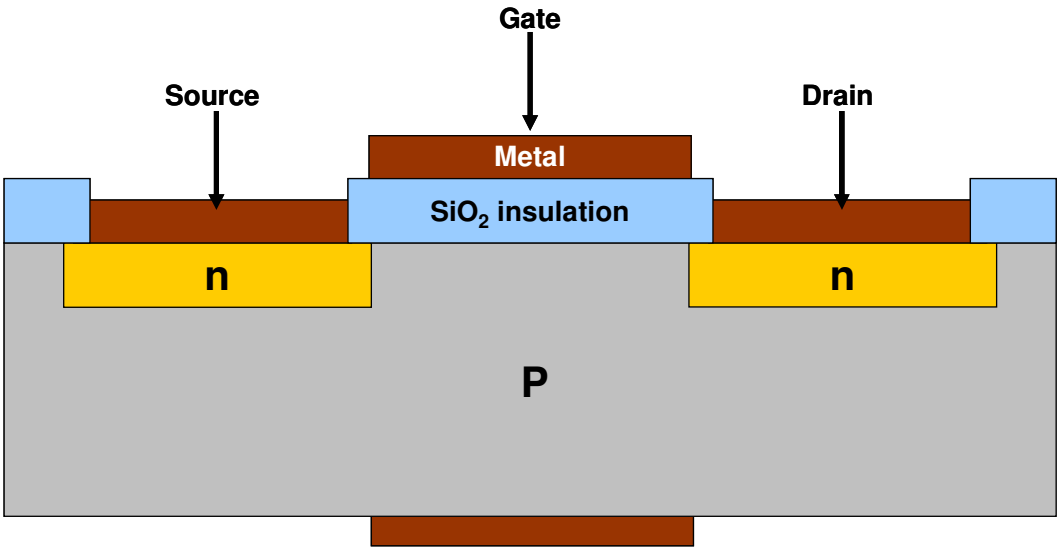
4.設計出一套組裝簡易且操作方便的 DNA 感測系統。

5.設計出具有高靈敏度及高選擇性的 DNA 感測系統。

三、文獻探討

1.延伸式場效電晶體（Extended Gate Field Effect Transistor，即 EGFET）的原理及優勢

場效電晶體總共有三個電極：源極、汲極和閘極，可藉由在閘極外加電壓，來開啓並控制源極和汲極間電子流通道的大小。場效電晶體有兩種類型，本實驗中所採用的爲以電子流來工作的 n 型金氧半場效場效電晶體 (MOSFET)，是由源極提供電子，經過打開的通道，到達汲極，電流方向是由汲極流向源極。



圖一、N-MOSFET的結構圖

何謂延伸式場效電晶體（EGFET）？延伸式場效電晶體即是將 FET 的閘極（Gate）另外外接出來，以便於對閘極表面特性的控制，其優點在於組裝簡易、成本低、延伸閘極的形狀大小有較大調變空間、且對感測靈敏度的影響極低。在本研究中，即是在延伸出的閘極上修飾 probe DNA，由於完全互補 DNA 間的接合，能改變閘極表面特性，因而影響閘極外加電壓，造成源極和汲極間通道大小改變，進而讓流過的電流大小產生差異。

2.氮化鎵奈米線的選用及相關研究

在許多對氮化鎵的研究中顯示，氮化鎵具有以下特點，適合用來作爲本實驗感測素材：

(1).對生物分子非常敏銳且不會對其造成傷害。

(2).相較於許多研究所使用的金，氮化鎵的化學特性較穩定。

(3).以金作爲感測電極製作程序複雜不易掌控，而以氮化鎵奈米線作爲電極相較下易操作許多

在眾多場效電晶體的 DNA 感測研究中，有些著重在電極表面素材的探討，如金薄膜等；有些著重在感測裝置的微型化或不同系統架設的差異，如直接以 FET 閘極作爲感測端或採用延伸式的場效電晶體。而在本實驗中，將對整體感測裝置作較爲完整的探討，在電極表面素材方面，針對氮化鎵薄膜及氮化鎵奈米線進行比較；在沿伸閘極的裝置方面也有所巧思；另外，最重要的是，針對濃度變化做了一系列的探討，這是在別人研究中所沒有的部份。

四、實驗器材與藥品

1. Semiconductor Characterization System （Keithly 4200）

2. Commercial FET （型號：CD4007）

3. 麵包板（Bread Board）

4. probe DNA（序列：SH-5´-ATGGGCCTCCGGTTC-3´）

5. target DNA（序列：5´-GAACCGGAGGCCCAT-3´）

6. Duplex （buffer）

7. 甲醇

8. 3-巰基丙基三甲氧矽烷（MPTMS）

9. 氮化鎵奈米線 （GaN NWs）

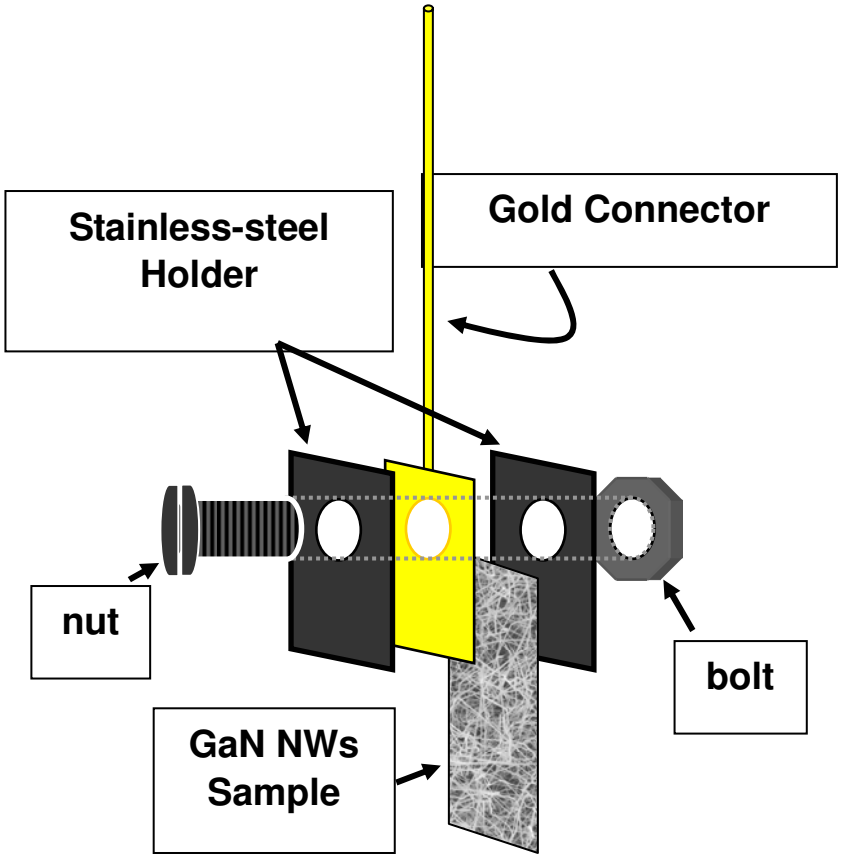
10.氮化鎵薄膜 （GaN thin film）

五、研究方法與過程

1.裝置與實驗條件的確立

(1).架設實驗裝置

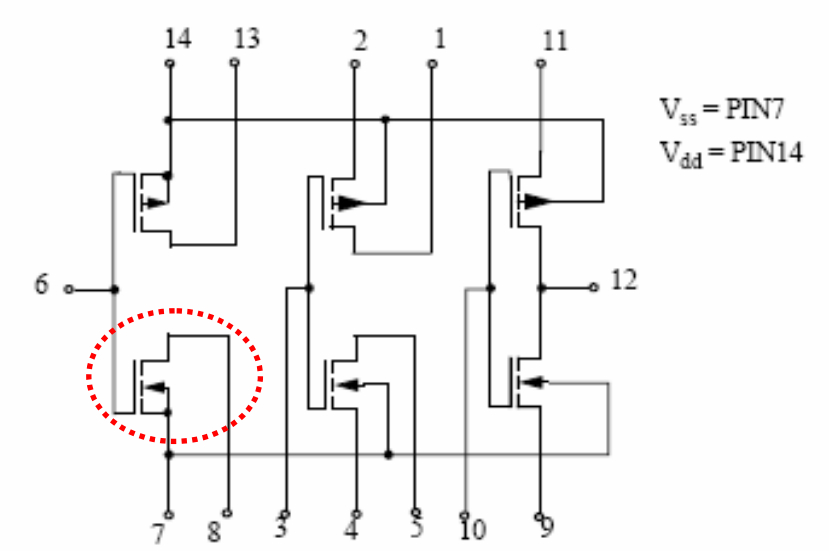
a. GaN 感測電極：以矽基板上的氮化鎵奈米線或氮化鎵薄膜作爲 Extended Gate，將其以 holder 夾住後（圖二），與 FET 的閘極（Gate）連接。



圖二：GaN感測電極示意圖

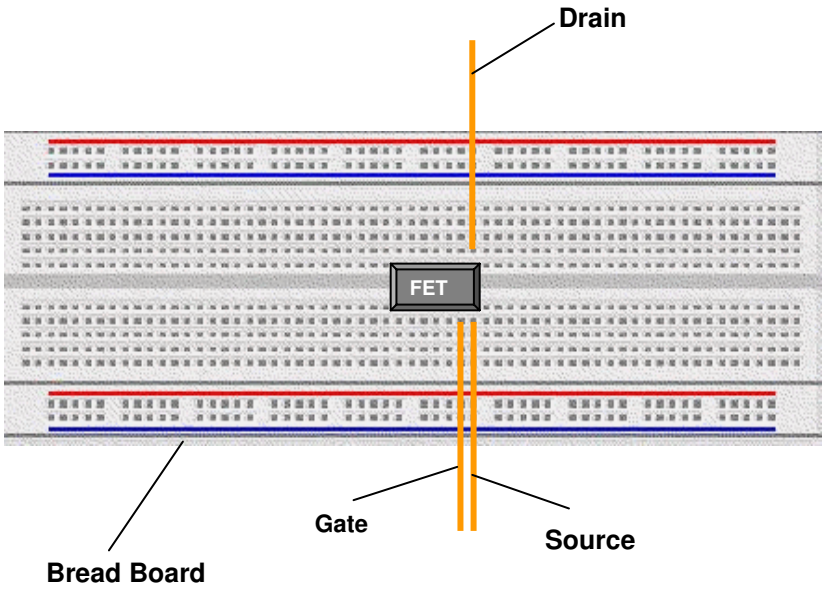
b. 感測系統：選用市面上即可取得的 FET（型號：CD4007）（圖三）作爲轉換器，並將其固定在麵包板上（圖四）。量測時，在 Source 與 Drain 間加一固定電壓（Vds），並藉由改變加在

參考電極（Reference）的電壓（Vg），得 Source 與 Drain 之間電流（Ids）對 Vg 的關係。（圖五）

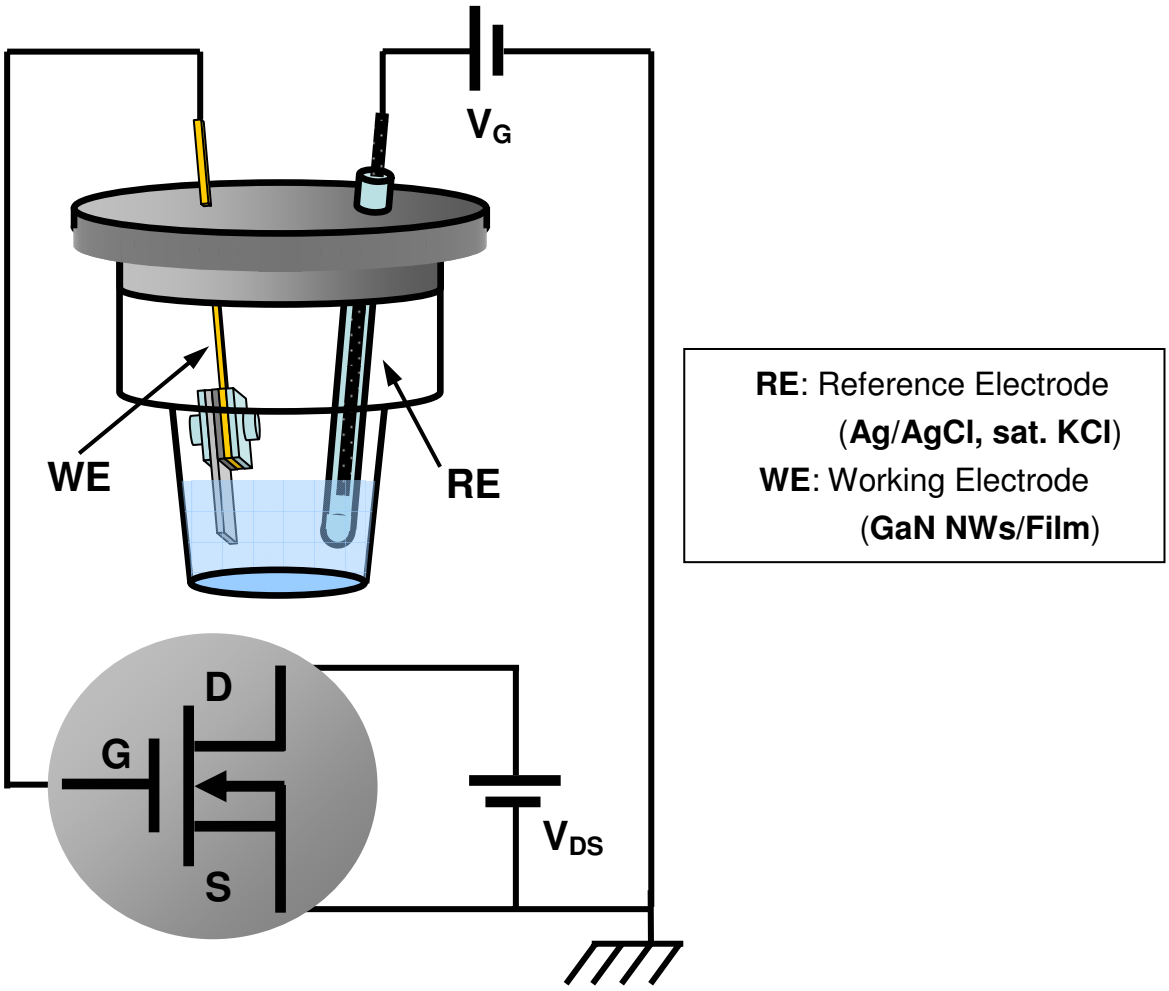


圖三：Schematic Diagram of FET

實驗選用編號 6.7.8 三個腳分別作爲 Gate、Source、Draian （n 型）



圖四：FET與麵包板示意圖



圖五：實驗裝置示意圖

(2).確定實驗條件---Vds 的選擇：

爲了選擇適當的 Vds，在固定 Vg=4 的條件下，量測樣品 Ids 對 Vds 的關係。此後分別測量固定 Vg=2,-2,-4 的條件下，Ids 對 Vds 的關係。藉由此步驟，找出反應區的 Vds。

(3).背景值量測

將未經修飾的樣品，在固定 Vds=1 及 0.1 的條件下，改變加在參考電極的電壓，得 Ids 的變化。爲確認其結果有可重複性，每個濃度都重複作 5 次的量測。將其結果做圖，作爲背景值。

(4).表面修飾

a. 氧化：

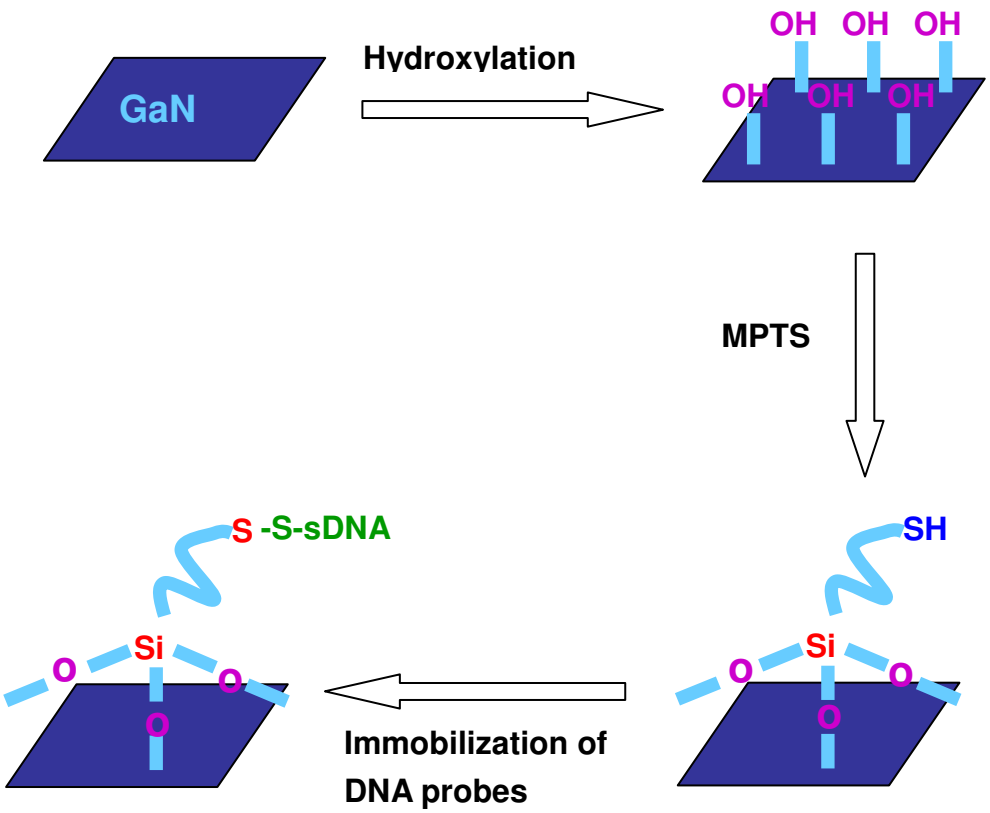
爲了要將氮化鎵表面與 MPTS 鍵結，因此需要將電極置於酸性溶液（0.12 M H_2SO_4 + 0.52 M HNO_3）中使其具有氫氧官能基，並在室溫下靜置 1 小時。

b. MPTS：

將具有氫氧官能基電極置於 3-巰基丙基三甲氧矽烷（MPTMS）與甲醇溶液中（體積比 1：10），並在室溫下靜置一小時。

c. 修飾單股 DNA：

將修飾過 MPTS 的 GaN 置於 ssDNA（序列：SH-5´-ATGGGCCTCCGGTTC-3´）溶液中，並在 4℃的環境下靜置 24 小時，以將 DNA 固定在奈米線的表面。



圖六：表面修飾過程示意圖

(5).ssDNA 電性量測

將已修飾單股 DNA 的樣品，在固定 Vds=1 及 0.1 的條件下，改變加在參考電極的電壓，得 Ids 的變化。爲確認其結果有可重複性，每個濃度都重複作 5 次的量測。將其結果做圖。

2.DNA 感測---靈敏度（sensitivity）

將樣品（分別爲 GaN NWs 及 GaN thinfilm）放入含不同濃度之 target DNA 中，待三十分鐘反應時間後，分別量測當 probe DNA 在接上不同濃度之 target DNA 時，在 Vds 固定的條件下，樣品 Vg 對 Ids 的關係，以找出其感測的極限（能感測的最低濃度）。爲確認其結果有具再現性，每個濃度都重複作 5 次的量測。其後，在固定 Ids 的條件下，找出各濃度所對應的 Vg，並推出其公式。同時，爲確定將樣

品放入含完全互補之 DNA（target DNA）時，電性之改變確實爲接上雙股 DNA 之緣故，亦將樣品放入不含 DNA 之溶液（Buffer）中，觀察其電性。

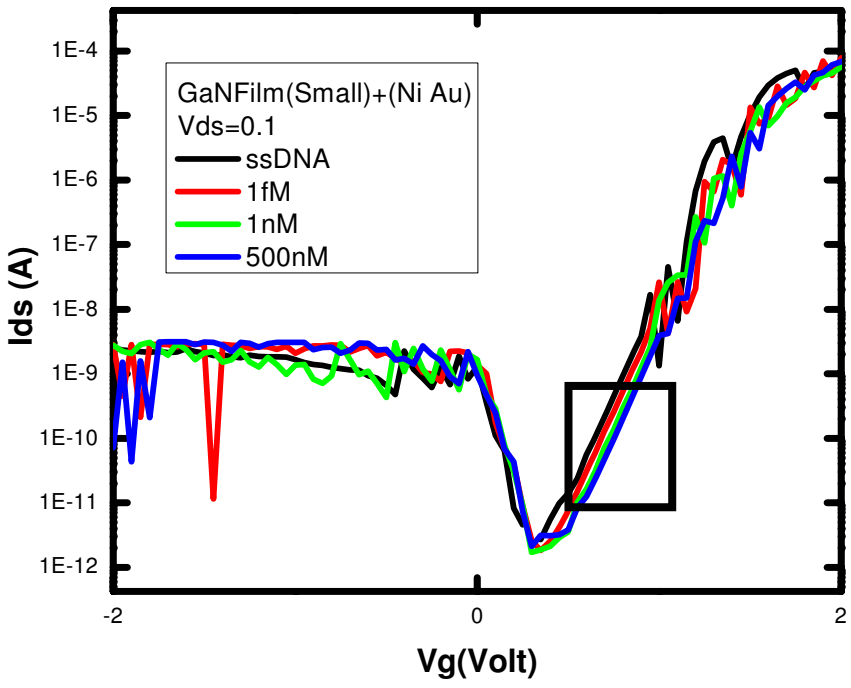
3.選擇性

以此 DNA 感測器，感測不互補 DNA，及一個鹼基對突變的 DNA 序列，以確認此感測器對 DNA 序列具有選擇性。

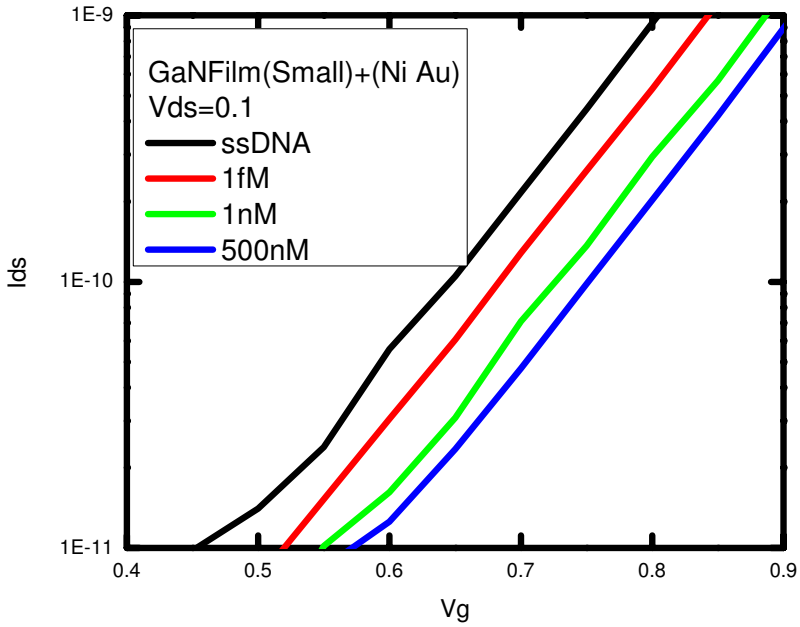
六、研究結果

1.靈敏度

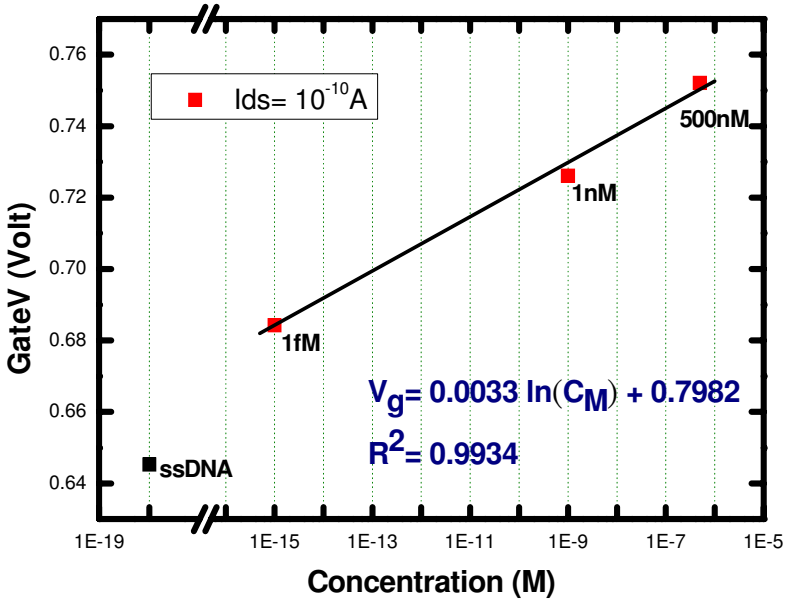
(1).GaN thinfilm



圖七(a)：氮化鎵薄膜在Vds=0.1下，隨hybridization及target DNA濃度變化所表現的電性。

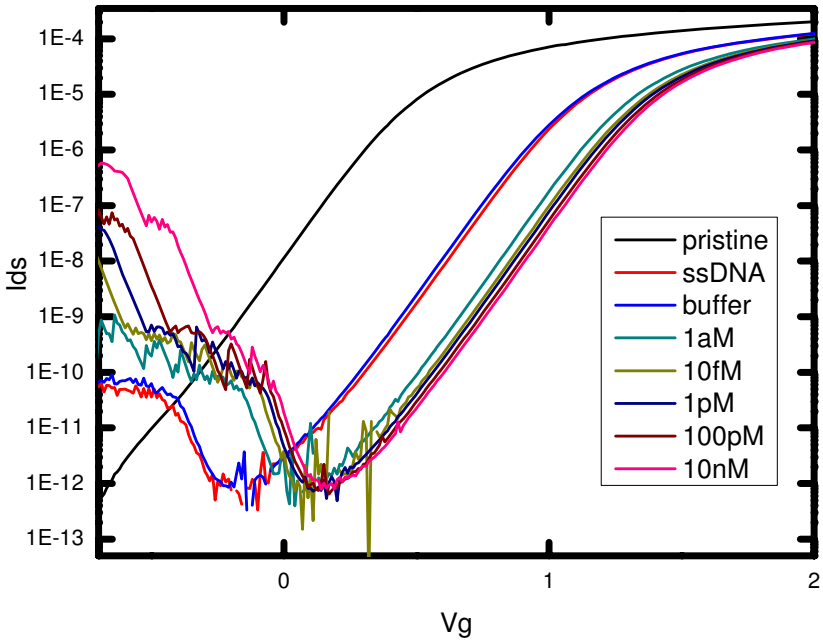


圖七(b)：此爲(a)的局部放大圖（Vg=0.4～0.9)。從圖中可清楚得知，隨著hybridization及target DNA濃度的增加，Ids-Vg圖形會往右偏移。而此次的感測極限爲1fM（1E-15M），已與前人的相關研究達到相同的靈敏度。

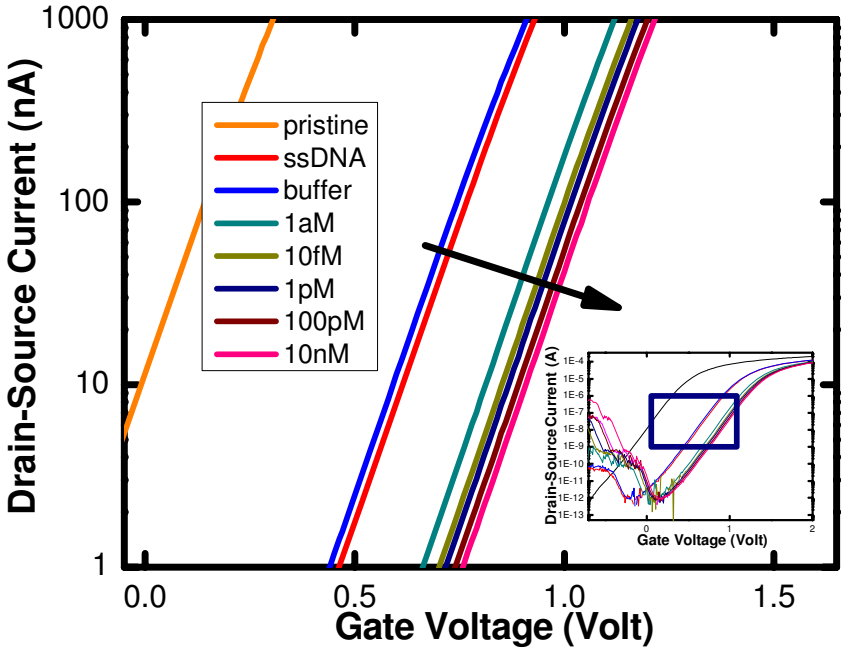


圖八：將Ids固定爲10^{-10}A下，取各濃度所對應的Vg值，將其作圖。其後作一回歸直線，將其公式化，公式爲Vg=0.0033ln(C_M)+0.7982。其中決定係數（表示數據與回歸直線的相關性）爲0.9934。

(2).GaN NWs

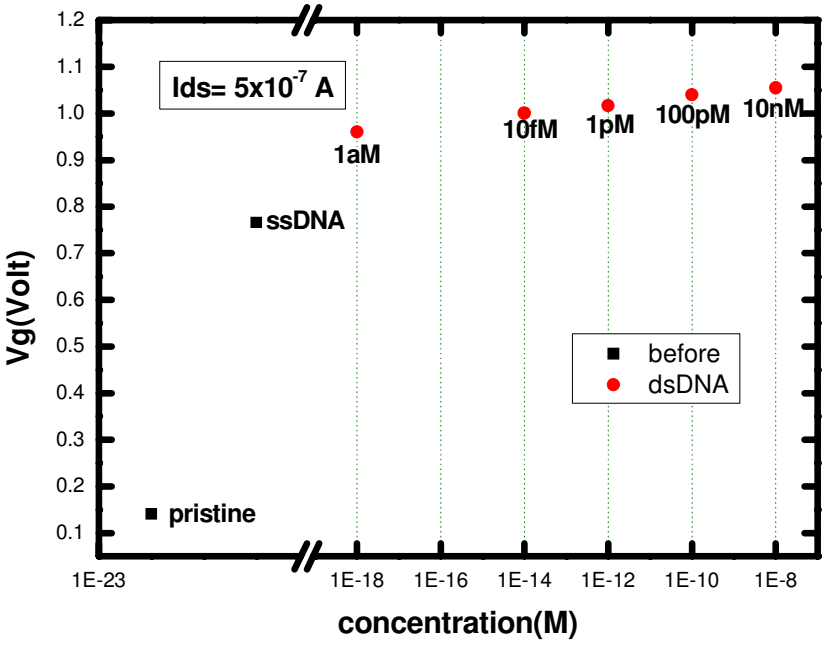


圖九(a)：氮化鎵奈米線在Vds=0.1下，隨hybridization及target DNA濃度變化所表現的電性。

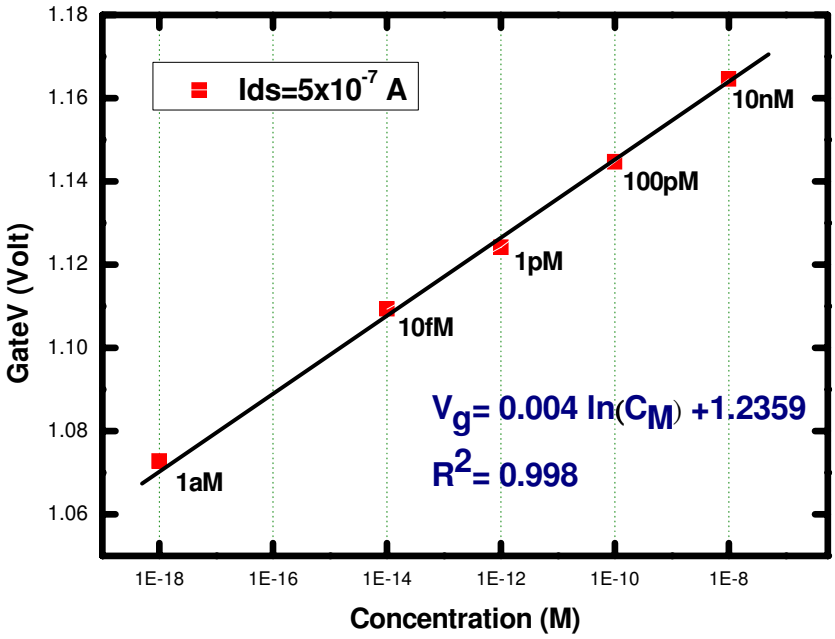


圖九(b)：此爲(a)的局部放大圖。從圖中可清楚得知，隨著hybridization及target

DNA濃度的增加，Ids-Vg圖形會往右偏移。而濃度同樣是0的電性差異，與hybridization後的改變相較微乎其微，因而證實圖形向右偏移確實爲hybridization所造成。而此次的感測極限爲1aM（10^{-18}M），較前人的相關研究（10^{-15}M）突破了3個數量級。

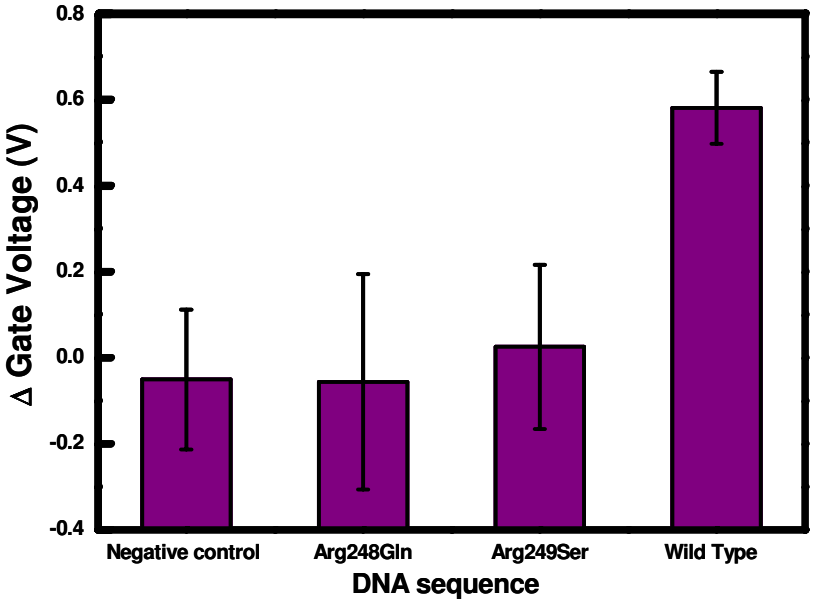


圖十(a)：將Ids固定爲5x10^{-7} A下，取未修飾、修飾單股DNA及各濃度所對應的Vg值，將其作圖。



圖十(b)：將(a)做一次回歸直線，將其公式化，公式爲 Vg=0.004ln(C_M)+1.2359。其中決定係數（表示數據與回歸直線的相關性）爲0.998，相關性相當顯著，其中斜率又較氮化鎵薄膜爲高，可推測奈米線爲較靈敏且穩定的感測材料。

2.選擇性



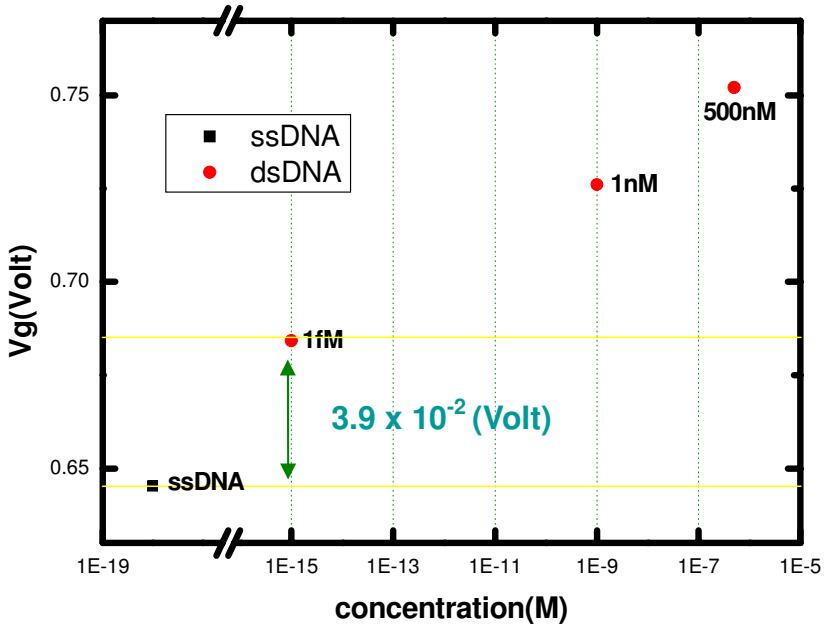
圖十一：分別感測不互補 DNA、單一鹼基對突變 DNA 及完全互補 DNA 後，分析各自之 Vg 改變量。結果中，只有完全互補 DNA 能顯著改變 Vg，顯示此感測器具有高靈敏度，即使單一鹼基對的突變也能夠辨別。

七、討論

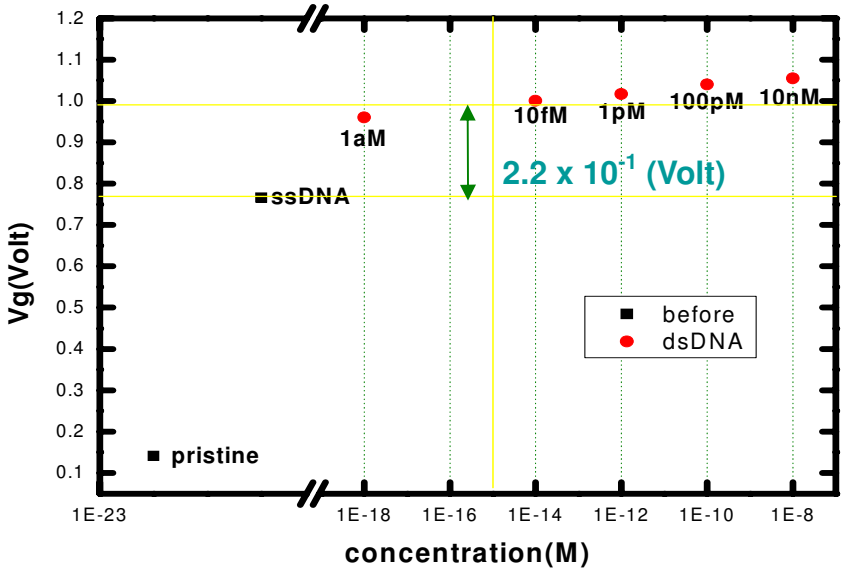
1.hybridization 後及隨濃度變高，Ids-Vg 圖形改變且向右偏移的原因，將前人的研究結果之推論，與實驗結果相對照，將造成 hybridization 後及隨濃度變高，Ids-Vg 圖形改變且向右偏移可能的原因如下：

由於在中性水溶液中，DNA 的磷酸根解離而帶負電。隨著 probe DNA 的修飾，及與 target DNA 的接合，負電荷將固定在感測器的表面，因此將感測器表面的正電荷中和，使加到閘極的有效電壓減少，也就是說在相同 Vg 的情況下，Ids 將下降，因此造成圖形向右位移。

2.氮化鎵奈米線與氮化鎵薄膜的靈敏度比較



圖十二(a)：氮化鎵薄膜經hybridization後，Vg的改變量爲3.9×10^{-2} （Volt）



(b)

圖十二(b)：氮化鎵奈米線經hybridization後，Vg的改變量爲2.2×10^{-1} （Volt）

由圖十二可得知，氮化鎵奈米線經 hybridization 後，Vg 的改變較爲顯著，表示氮化鎵奈米線較氮化鎵薄膜更能靈敏的檢測出 DNA hybridization。推測是因爲氮化鎵奈米線的奈米結構，增加了反應面積，致使反應靈敏度提升。

八、結論與應用

1.隨著 hybridization 及 DNA 濃度的增加，Vg-Ids 圖形會向右偏移，藉此可以用來感測 target DNA。

2.成功製作出 EGFET 之 DNA 感測器，且其靈敏度高達 1aM(10^{-18}M)，較前人的研究成果(10^{-15}M)更提升了三個數量級。

3.此感測器具有高選擇性，即時單一鹼基對的突變也可以成功辨別。

4.固定 Ids 的條件下，Vg 對濃度的關係爲一次曲線；threshold voltage 對濃度亦爲線性關係。

5.氮化鎵奈米線較氮化鎵薄膜的靈敏度爲高，更適合作爲 DNA 感測的材料。

6.未來作疾病檢測時，只要將與疾病 DNA 相互補的 DNA 修飾在氮化鎵奈米線上，即可輕易檢測出人體內是否帶有疾病的 DNA，且可以在其濃度相當小的狀態下就檢測出，使檢測更爲方便快速並且讓疾病能提早得到治療，提升治癒率。

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評語

本作品是研製出以場效電晶體爲主的高靈敏度 DNA 感測器，主要設計包括

1) 以氮化鎵奈米線作爲閘極感測端。

2) 將氮化鎵奈米線表面以 DNA 修飾。

本作品除其 DNA 感測靈敏度可達 10^{-18} M 外，其在創作能力，研究過程及完整性上都具特色，是一件優異的作品。

Abstract

DNA-hybridization based detection techniques are widely developed due to their promising applications in genetics, medicine and drug discovery. However, current DNA detection techniques based on labels or reagents are time-consuming, environmentally-harmful and complex to implement. In this study, we have successfully demonstrated a label-free extended-gate-field-effect-transistor (EGFET) sensor utilizing a GaN-nanowires electrode with DNA probes immobilized, capable of specific DNA sequence identification. The principle behind the design is based on the change in surface potential and charge transfer after hybridization. GaN nanowires, being bio-compatible, provide direct transfer path and high surface area, thus offer an unprecedented opportunity of DNA sensing with high sensitivity. In addition, our EGFET design facilitates easy assembly and operation of DNA detection. Comparative studies on complementary and non-complementary DNA were performed to verify the specificity of the sensor. By adapting GaN nanowires structure, the assay time of DNA was shorten to within thirty minutes. Moreover, our sensor displayed an ultra-high sensitivity in the level of attoM: three orders of magnitude higher in resolution than that of other FET-based DNA detection methods.

1. Introduction

1.1 Evolution of DNA sensor

Various DNA detection methods have been widely established for genetic, pharmaceutical and medical applications, based on semiconductor-assisted sensors. However, the conventional systems suffer from design-complicacy, time-consuming and expensive complicated process, and inefficient sensitivity. In addition, some of the highly sensitive detection techniques, including fluorescence, electrical or electrochemical, or surface plasmon resonance; requires DNA-labeling process, which suffers from unwanted biologically-harmful contamination, high cost, and complicacies in structure and fabrication. However, to facilitate real-time early detection of DNA in minute quantity, a sensor with higher sensitivity, but with simple structure, bio-compatibility, cost-effective and ease of fabrication/technique is favorable.

1.1.1 FET-based sensing technique

FET (Field Effect Transistor) -based biosensors, fabricated by well-established semiconductor integrated circuit technology, have attracted huge attention because of their potential advantages in terms of miniaturization, standardization and mass-production. FET-based techniques provide very high sensitivity for detection of charged bio-molecules such as DNA, without the requirement of expensive instruments and reagents, since it can transduce a small potential change at the gate-surface, due to the presence of the charged bio-molecules, into a detectable electric/voltage signal. EGFET (Extended-Gate FET) is a modification of conventional FET structure, where the gate is extended from the FET device. Its additional advantages include flexibility in gate-shape, isolation of FET-device (from the chemical and biological environment), and hence insensitivity to temperature and light, ease in packaging and passivation of sensing-surface (extended gate).

In this study, an EGFET-based sensing technique has been developed, utilizing GaN nanowires as the extended-gate material. It didn’t require complicated procedures but still possessed very high sensitivity, which could be achieved by few other sensing systems but in expense of time-consuming and expensive techniques.

1.1.2 Properties of GaN nanowires

Gallium nitride (GaN) is a group Ⅲ/group Ⅴ semiconductor material, with a wide and direct band gap (3.4 eV). It has been an important material for high-performance blue LEDs since 1990s. In 2003, M. Stutzmann, et al reported a unique bio-compatibility and non-toxicity properties of GaN, which created a new possibility for biosensing application [22]. Moreover, in 2006, Young et al proposed a promising potential for GaN as a transducer for bio-chip application [24]. In this study, we further evaluated the efficiency of GaN NWs-based DNA sensor, for we believe that nanowires could provide higher detection surface, more biding sites for bio-molecules and direct transfer path, which might increase the sensitivity efficiently. In the present study, our GaN NWs –based EGFET sensor revealed very high sensitivity, 1 aM (10^{-18} M), for the detection of a specific gene sequences (p53)

1.2 Human p53 gene

The chosen DNA sequence, p53, adapted from tumor suppressor gene, encodes a transcription factor that is crucial to human cancer prevention. If the p53 gene is damaged or lost, the cell's tumor suppression function cannot work effectively, which will result in uncontrolled division of cells. Tumor inducing mutations have been observed at more than 100 sites in the p53 gene, among which mutations at five "hot spot," in codons 175, 245, 248, 249, and 273 are the most common ones. In this study, two of these mutational hot spots, in codons 248 and 249, were chosen as sequences for single base mismatch detections[1].

2. Project Goals

The objectives of this project include two major aspects: first, to design a DNA sensor with high sensitivity and specificity; second, to design a DNA sensor with easy assembly and operation.

[1] The mutation in codon 248 (CGG → CAG/Arg → Gln) is associated with lung cancer, while the mutation in codon 249 (AGG → AGT/Arg → Ser) with liver cancer.

3. Mechanism

3.1 Extended-Gate Field Effect Transistor (EGFET)

EGFET has been developed by modifying a conventional MOSFET (metal oxide semiconductor field effect transistor), via a utilization of GaN NWs sample as an extended gate. In traditional EGFET (Fig. 1a), where the sensing materials (2) (e.g. GaN NWs) is directly synthesized on the "Gate" of FET device (1); complicated fabrication process is needed, and maintenance is difficult if part of the EGFET is damaged. As per our EGFET design (Fig. 1b), it is easily assembled with commercial FET (1), bread-board and GaN NWs (2), where the FET is completely isolated from the sensing system by a simple metal wire, and hence contribute to lower cost and easy maintenance, and ease in real-time *in situ* sensing.

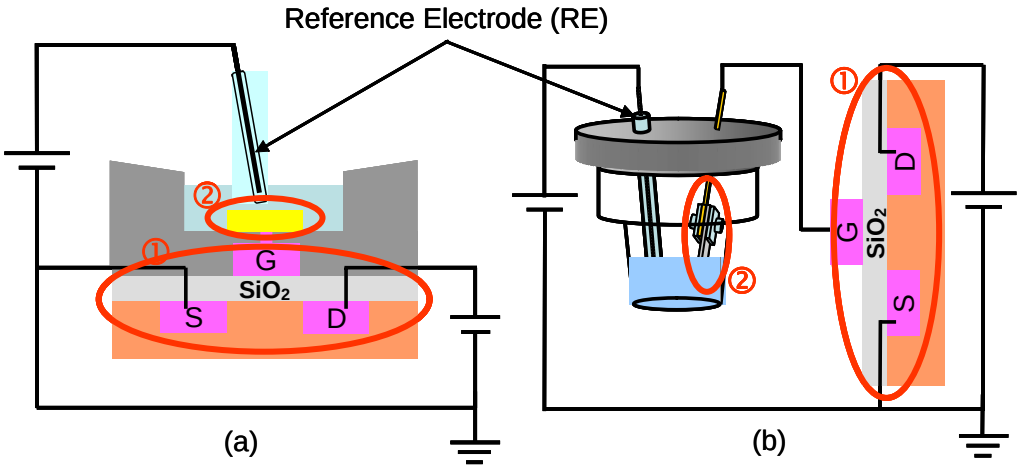
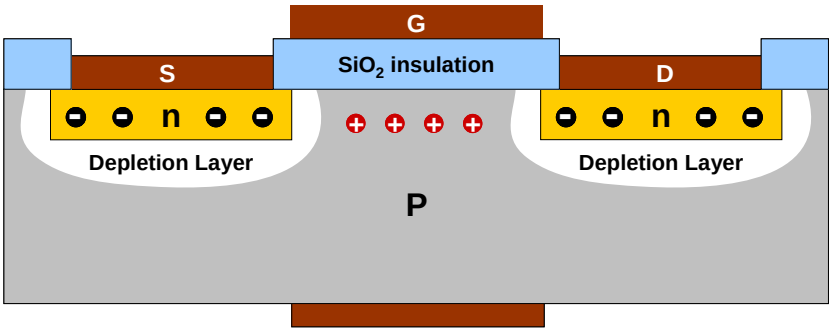


Fig. 1. Comparison of different types of EGFET. (a) Traditional EGFET (b) Our EGFET

The operation mechanism of EGFET is similar to that of MOSFET. The only difference is that gate voltage (V_g) is applied through reference electrode to the working electrode (gate). MOSFET includes two types: N-MOSFET and P-MOSFET. Here we take N-MOSFET as an example to illustrate the working mechanism of MOSFET. V_g controls the conductive channel between drain and source, hence the drain current (I_d). When $V_g = 0$ (Fig. 2a), there is no conduction between drain and source. When $V_g < V_T$ (threshold voltage) (Fig. 2b), because its structure is equivalent to a capacitor, the accumulated positive charges on gate would induce negative charges on the other side of the insulator. However, these negative charges would soon be neutralized by the electron holes around them,

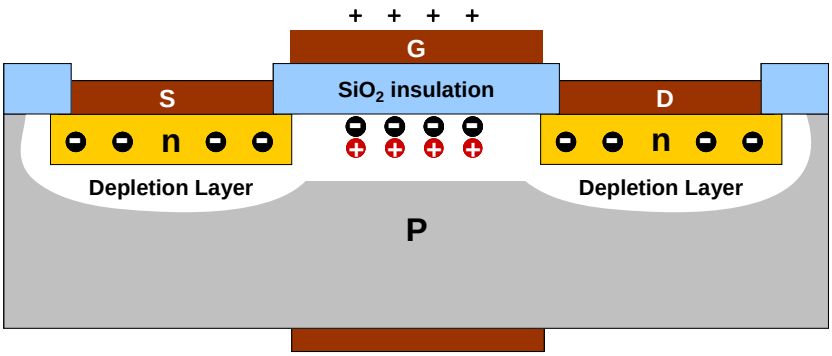
thus depletion layer would form. When $V_g = V_T$ (Fig. 2c), a high concentration negative carriers form in inversion layer that allows current to flow between drain and source. When $V_g > V_T$ (Fig. 2d), the gate voltage is no longer used to change the depletion layer, but serve to control the amount of accumulated electrons. At this time, the density of electrons is proportional to $V_g - V_T$. In conclusion, FET is like a switch controlled by V_g.

(a)



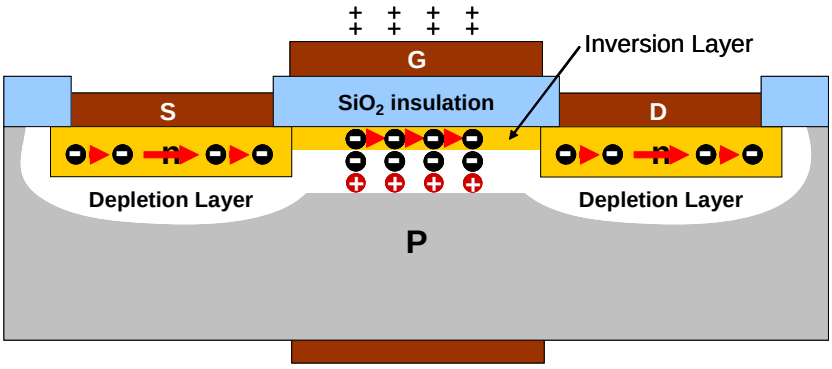
Vg = 0

(b)

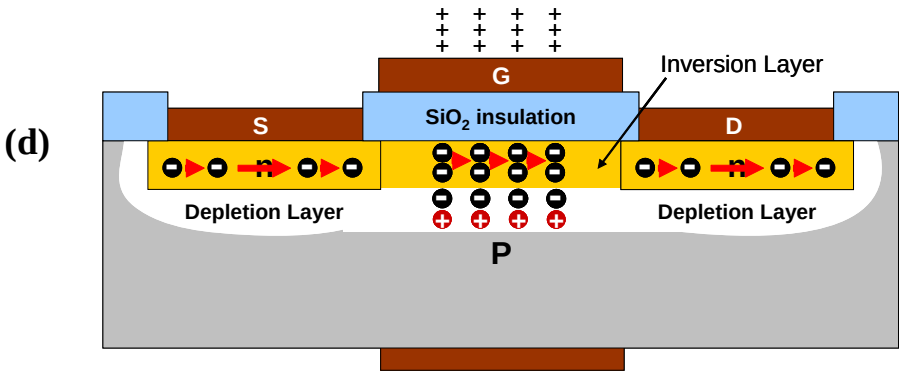


$V_g < V_T$, new depletion layer forms

(c)



$V_g = V_T$, n-type channel (inversion layer) forms



$V_g > V_T$, density of electrons is proportional to V_g-V_T

Fig. 2. Working mechanism of N-MOSFET

3.2 DNA detection

DNA has the characteristics that each type of base on one strand forms a bond with just one type of base on the other strand (A only binds to T, C only binds to G), which is called complementary base pairing. Mechanism of DNA-sensing is based on this specific interaction between two complementary DNA strands. The probe DNA, immobilized on the surface of the sensor (GaN NWs), is subjected to its complementary target DNA. DNA molecules having phosphate groups are negatively charged in aqueous solution (Fig. 3). When hybridization happens, which means two complementary DNA strands bind together and form double helix, the negatively-charged DNAs cause a surface potential change of the sensor, resulting in a change of effective voltage applied to gate. In other words, threshold voltage, V_T, would be changed, which leads to a change in drain current, I_d, at same V_g. From the difference of V_T or I_d, DNA can thus be detected.

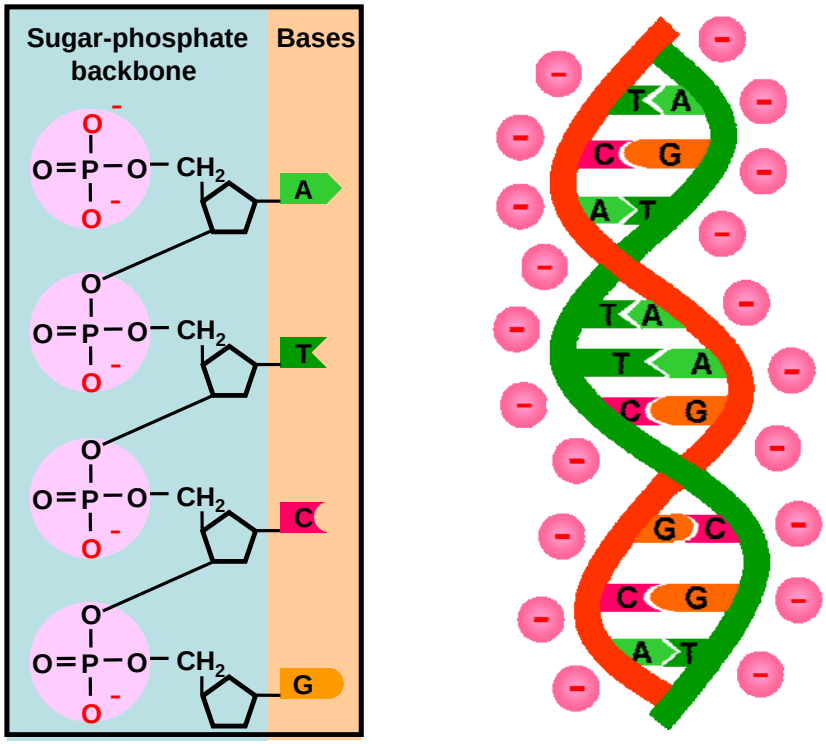


Fig. 3. Schematic Diagram of negatively-charged DNA in aqueous solution.

4. Materials and Reagents

The following chemicals and reagents were used: saline sodium citrate buffer 20X (SSC), sulfuric acid, nitric acid, methanol, (3-mercaptopropyl) trimethoxysilane (MPTMS) from Sigma-Aldrich; Cleland's REDUCTACRYL Reagent from Merck; Duplex buffer (30 mM Hepes + 100 mM potassium acetate), probe oligonucleotide (5'-HS-$(CH_2)_6$-ATGGGCCTCCGGTTC-3'), complementary target (5'-GAACCGGAGGCCCAT-3'), Arg248Gln (5'-GAACCAGAGGCCCAT-3'), Arg249Ser (5'-GAACCGGAGTCCCAT-3') and non-complementary target (5'-CCCCCCCCCGGGGGGGGG-3') from Integrated DNA technologies, Inc.

5. Methods and Device

5.1 Preparation of DNA probes

Prior to the use, the probe oligonucleotides were diluted to 20 μM by SSC. Spin column was centrifuged for 1 minute (3000 rpm.). Reductacryl reagent and probe DNA were next injected into spin column and centrifuged for 2 minutes (3000 rpm.). The purpose of this process was to reduce the disulfide bond that was synthesized to protect probe oligonucleotides.

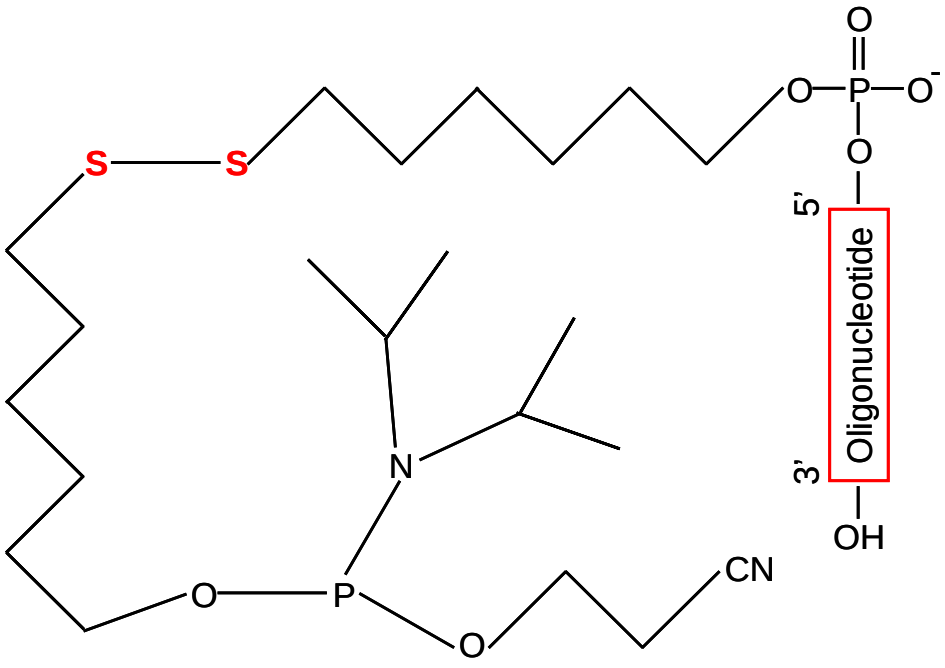


Fig. 4. Thiol-modified probe oligonucleotide.

5.2 Hydroxylation of NWs' surface

Prior to the MPTMS-modification, GaN NWs need to be modified with hydroxyl groups first. Thus, NWs sample was hydroxylated by treating it in acid solution (0.12 M H_2SO_4 + 0.52 M HNO_3) at room temperature for one hour.

5.3 NWs' surface-modification by MPTMS

Since MPTMS can react with water easily, NWs sample must be dried first. It was then kept in MPTMS and Methanol solution (volume ratio 1:10) at room temperature for one hour. Here MPTMS acts as a linker between GaN NWs and probe DNA.

5.4 Immobilization of probe DNA on NWs' surface

The MPTMS-modified NWs were incubated in probe DNA solution at 4 ^{o}C for 24 hours to immobilize DNA probes on NWs' surface.

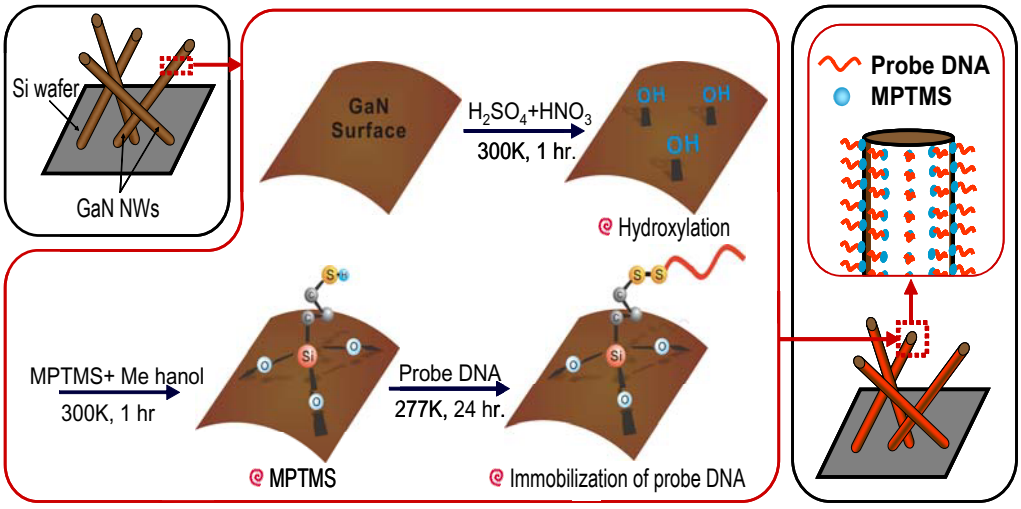


Fig. 5. Surface modification process

5.5 Device set up

GaN NWs samples (Fig. 6), fixed by the metal holder (Fig. 7), were connected to the gate of a commercial FET via metal wire and taken as the extended gate (Fig. 8).

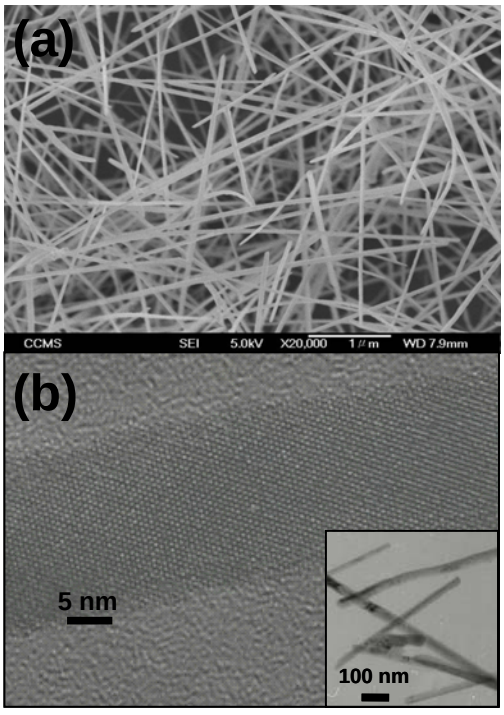


Fig. 6. (a) SEM and (b) TEM image of GaN NWs (Courtesy: CCMS, NTU)

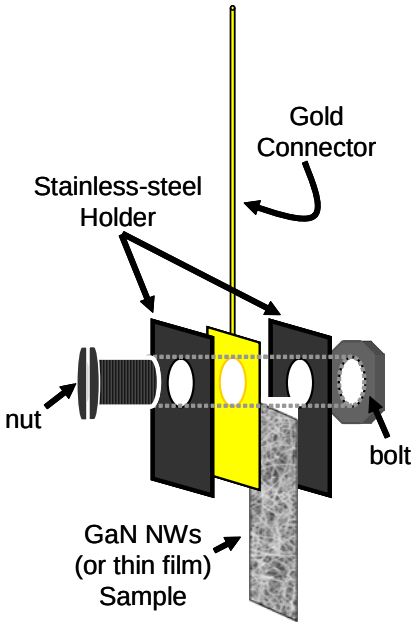
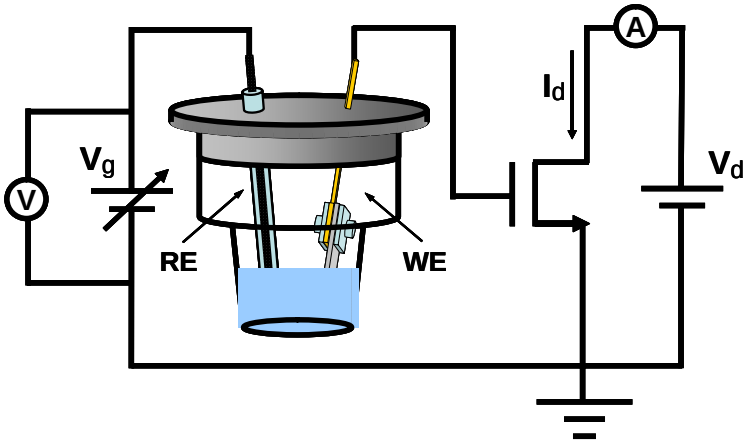


Fig. 7. Diagram of the working electrode (WE)



RE: Reference Electrode (**Ag/AgCl, sat. NaCl**)
WE: Working Electrode (**GaN NWs/Thin Film**)

Fig. 8. Circuit diagram of the GaN NWs-based EGFET

5.6 *in situ* Detection of DNA hybridization

Probe DNA-immobilized GaN NWs sample was subjected to the fully complementary target DNA in Duplex buffer solution for *in situ* DNA-hybridization sensing. By applying a bias to the sensor (gate voltage) through the reference electrode (RE), the $I_d - V_g$ characteristics of the FET sensor has been monitored at different concentration of target-DNA in order to obtain the relationship between DNA-concentration and V_g. The DNA-concentration was increased at a time interval of 30 min.

5.7 *in situ* Single base mismatch detection in human p53 gene sequences

Probe DNA-immobilized GaN NWs was subjected to the target DNA, in a sequence from a non-complementary target to single base pair mutated sequences of p53 gene (Arg248Gln, Arg249Ser), and finally to wild type (fully complementary) target. The measurement procedure was the same as the *in situ* hybridization detection. This characterization was served to evaluate the *in situ* specificity of the sensor.

6. Results and Discussion

6.1 *in situ* DNA hybridization sensing

Fig. 9a represents the $I_d - V_g$ characteristics of the GaN NWs-based EGFET sensor. The curve shifts in positive V_g-direction with increasing concentration (C_M) of target DNA, indicating the enhancement of negative charges on GaN-surface as

a consequence of the hybridization phenomena. Thin film-based sensor was served for the comparison (Fig. 9b).

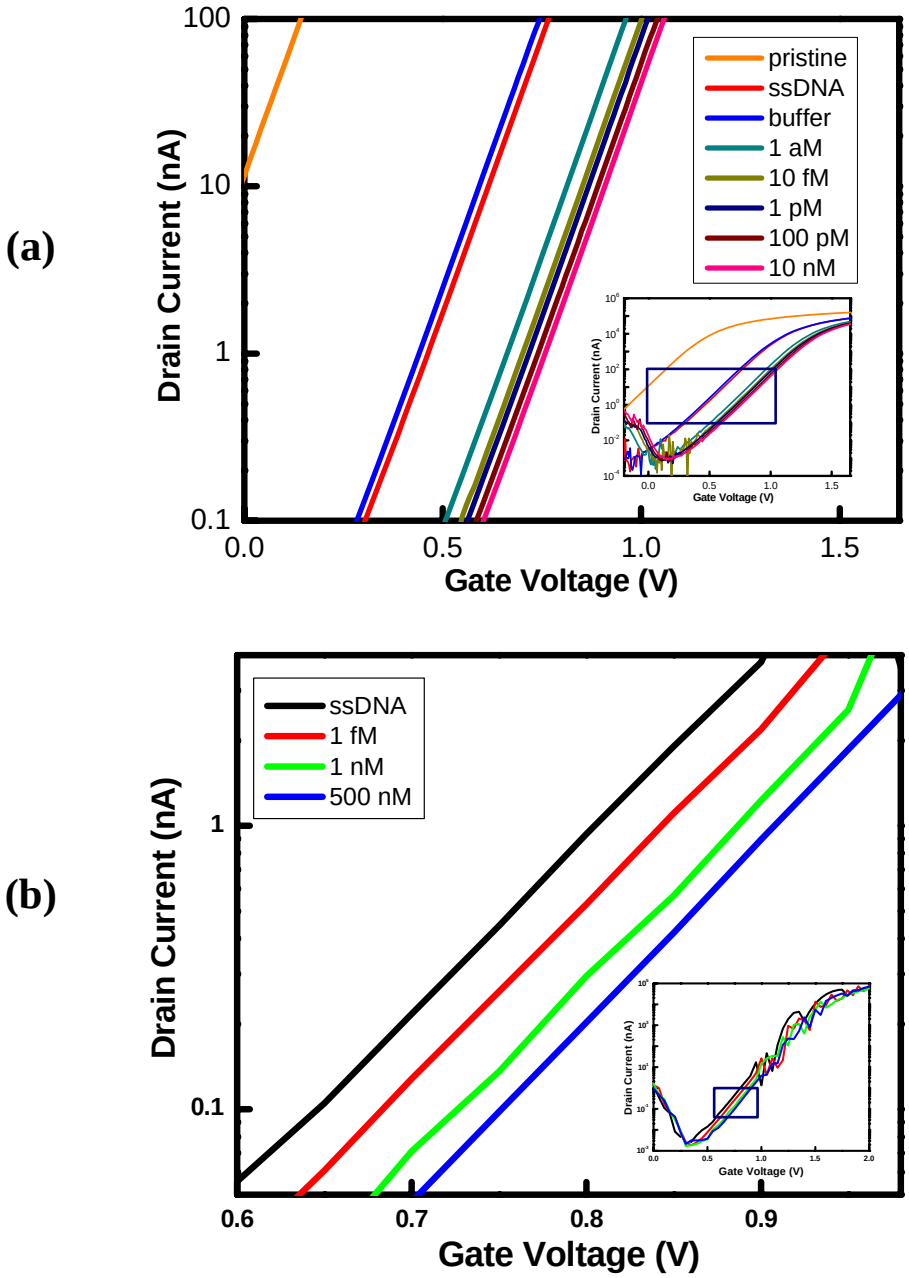


Fig. 9. I_d-V_g curve of (a) GaN NWs-based and (b) GaN thin film-based sensor.

Regarding to the shifts of I_d-V_g curve towards positive V_g with the target DNA concentration, a hypothesis was proposed as depicted in Fig. 10. In aqueous solution under electric field, the mobile-anions would move toward RE with positive potential (Fig. 10a), and mobile-cations toward WE (GaN-surface). As a consequence of the immobilization of negatively-charged probe DNA on WE, the mobile-cations would be neutralized or entrapped (by DNA molecules) before

reaching the NWs, which causes a decrease in distribution of bias-voltage to gate. This result in higher V_T and smaller I_d, and a subsequent shift of I_d-V_g curve towards positive V_g-direction (Fig. 10b). Further hybridization with target DNA would provide more negatively-charged molecules on GaN-surface, and hence extend the shift in more positive V_g-direction (Fig. 10c). The effect would be more visualized from the continuous shift of I_d-V_g curve, with the increase in target DNA concentration.

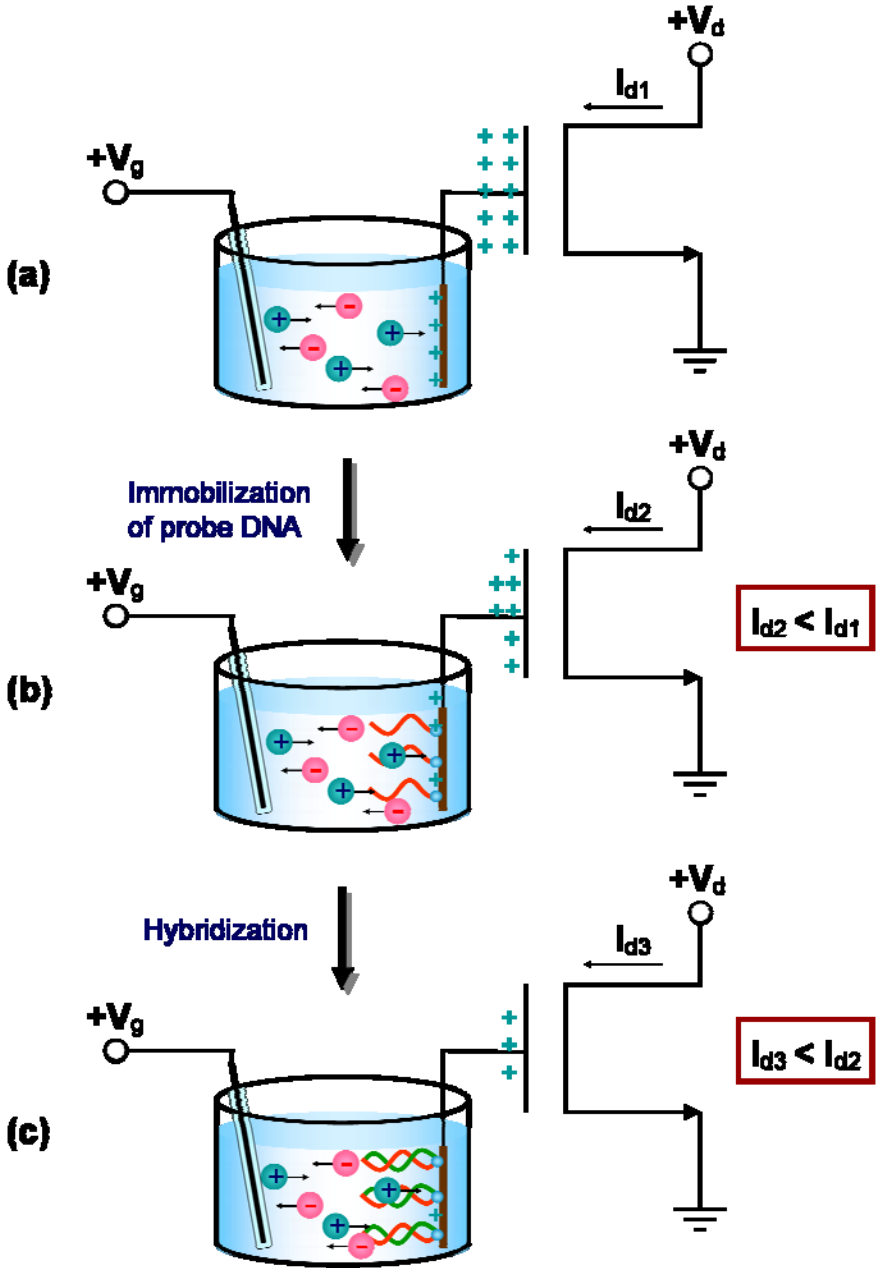


Fig. 10. Proposed schematic illustration of charge distribution, corresponding to DNA-immobilization and hybridization phenomena.

6.2 Dependence of V_g on target DNA concentration

Fig. represents the relationship between V_g and target-DNA concentrations, obtained at fixed I_d (10^{-9} A) (from Fig. 9). Results establish the feasibility of V_g, as a parameter for DNA-sensing based on GaNNWs-EGFET sensor, which shows a linear correlation over a wide range of DNA-concentrations (~ 12 orders). While the same sensor with GaN thin film revealed the similar trend, but at higher concentration.

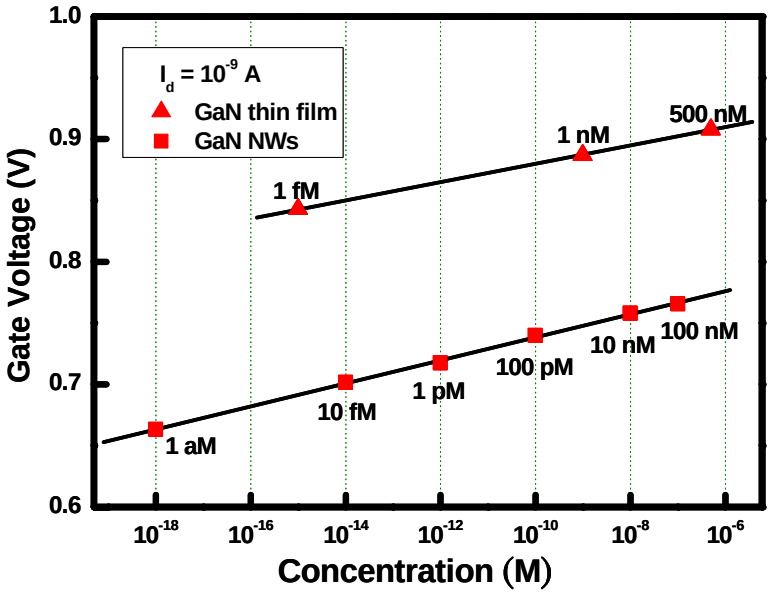


Fig. 11. Relationship between V_g and target DNA C_M

6.3 Advantages of using NWs-based system

As shown in Fig. 11, NWs-based sensor is at least 3 orders of magnitude more sensitive than thin film-based one. For further evaluation of the advantages of NWs-based system, the change in V_g ("Δ Gate Voltage" or ΔV_g) after hybridization for thin film and NWs-based system were compared (Fig. 12). Results show the ΔV_g for NWs-based sensor is about 7 times of that for thin film-based one, suggesting that the unique "surface-dominating" nature of nano-structures possess very high sensitivity to the surface-immobilized biomolecules.

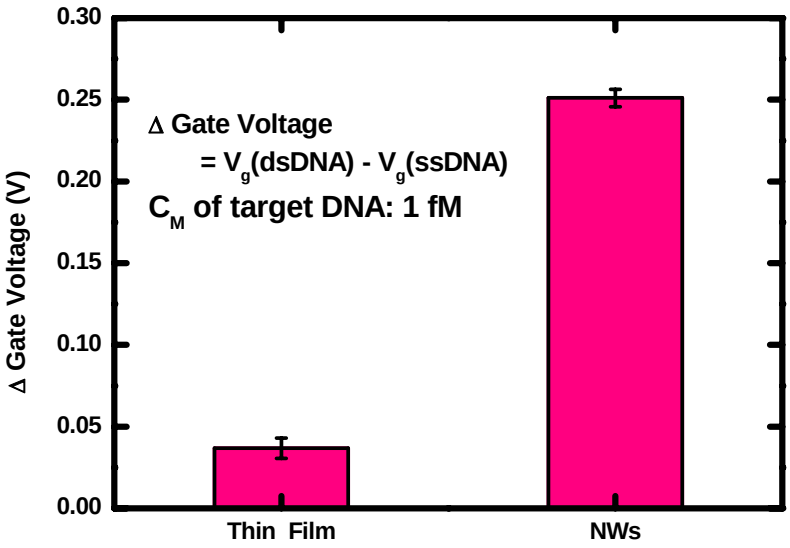


Fig. 12. Comparison of sensitivity between NWs and thin film-based sensors.

6.4 *in situ* detection of single base-pair mutation in human p53 gene

To evaluate the specificity of the sensor, detections of sequences adapted from human p53 gene and its single base-pair mutated sequences were performed. Fig. 13 indicates that the sensor is capable of distinguish single base-pair mutation, suggesting the excellent specificity of the sensor towards the fully complementary target only.

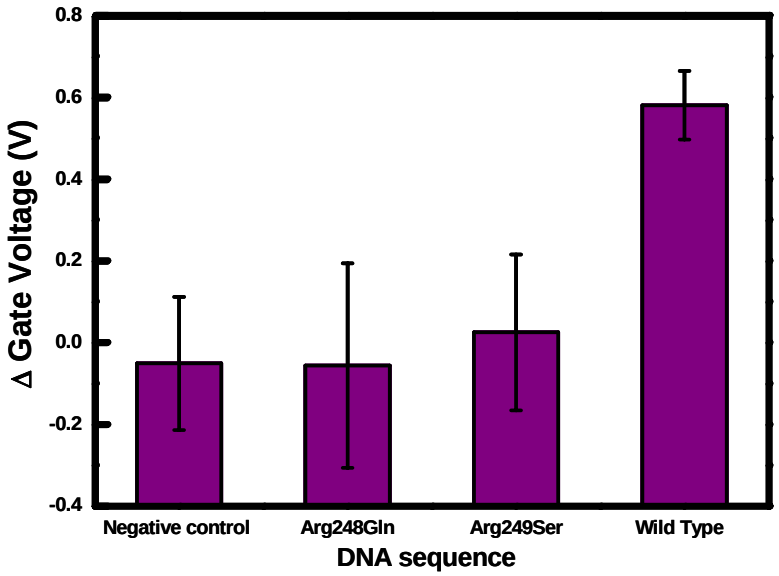


Fig. 13. The *in situ* specificity detection of single base-pair mutations in the p53 genome. Negative control is sequence that non-complementary to probe DNA; Arg248Gln, Arg249Ser are single base mutations in codons 248, 249 respectively; wild type is complementary target.

7. Conclusion

By integrating the advantages of gallium nitride (GaN), nanowire-structure and EGFET, the innovative and efficient DNA sensor was achieved. Owing to gallium nitride, our sensor is chemically stable, bio-compatible and biological affinity, which makes sensor capable of being recycled for further use. Due to the high detection surface of nanowire-structure, our sensor possesses high sensitivity of 10^{-18} M, which is almost three orders of magnitude higher in resolution than other FET-based sensor. The advantages of EGFET-based design provide the ease in device-assembly and sensing-operation, and it can perform real-time detection in aqueous solution without the requirement of any packaging technology. The GaNNWs-based EGFET sensor also showed excellent specificity towards the fully complementary target only, during real time detection of single base-pair mutation.

8. Future Works

The applications of our sensor can be much wider: first, it can be applied to detecting wider classes of bio-molecules such as protein and RNA; second, due to its small size, the sensor could be operate directly within the test tube; third, the relationship between the density of probe DNA and the sensitivity of the sensor is what we would like to investigate; fourth, we will evaluate the possibility of our sensor for applying in post-PCR/RFLP DNA sequence check.

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