Noise Suppression with Additional Reference Electrode for Time-Dependent Protein Sensing Tests with Si Nanograting FETs*

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Abstract— We propose a method to suppress the electrolyte potential noise in time-dependent protein sensing tests with an additional reference electrode, which doesn't have the challenging requirements as for the reference FET (REFET). The noise is recorded by the additional electrode and then suppressed in the sensing results. This noise is likely due to the electrochemical reaction at the electrolyte – solution gate interface. Results suggest increased readability with reduced signal level variation and increased credibility. The limit of detection (LOD) decreases by 50% ~ 70%.

I. INTRODUCTION

Over the past few decades, the development and improvement in nanoelectronic biosensors have made them promising candidates in the applications of disease diagnose, new drug discovery, health care, etc. Emerging among them are the Si nanowires (SiNWs) biologically-sensitive field effect transistors (bio-FETs), which has demonstrated great potential as ultrasensitive, label-free, rapid and miniature electronic sensors to detect chemical and biological species [1-3]. In certain applications such as protein sensing where the analyte concentration is extremely low ($\sim$ fM), enhanced sensitivity is preferred, which can be achieved in the form of exponential gain by biasing the bioFET in the subthreshold regime [4]. However, while benefiting from the high sensitivity, the noise in the electrolyte potential, which is amplified along with the small analyte signals to detect, also starts to emerge as a problem.

A widely accepted solution is to incorporate a second reference FET (REFET), which doesn't respond to the analyte to be detected [3]. Differential measurement is applied between the bioFET and REFET, eliminating the effects of unstable electrode/electrolyte potential which appears as a common signal in the output of both FETs. A well-known example is the direct detection of penicillin with enzymatically modified ion-sensitive FET (ISFET) by Caras and Janata [5]. Nevertheless, a few challenges still remain regarding the REFET. First, the differential measurement requires that the REFET is electrically identical to the bioFET [3]. Second, the REFET requires careful surface modification in order to stay chemically inert to the analyte, while at the same time equally sensitive as the bioFET to other electrolyte changes (e.g., ionic strength, pH and temperature) [6]. In this study, we investigated a different approach. Instead of a second FET, we used a second

electrode to monitor and suppress the electrolyte potential noise. In comparison to REFET, the second electrode is easier to make, reduces overall system complexity, and doesn't have the challenging requirements as for the REFETs. This approach was studied in the time-dependent protein sensing tests of our interest. The additional-electrode measurement scheme with ISFET has been reported before in transfer-characteristics-based tests [7], but to our best knowledge, time-dependent sensing test has not yet been reported, nor has the noise suppression method proposed in this study.

II. EXPERIMENTAL

A. Device Fabrication and Experimental Setup

Si nanograting FETs (NGFETs) each with 100 nanowires were used for the improved reliability and uniformity [8]. Detailed fabrication process has been reported previously [8, 9]. Fig. 1a illustrates the schematic of protein sensing setup with a Si NGFET and dual Ag/AgCl electrodes. The Si NGFETs were fabricated on a silicon-on-insulator (SOI) wafer with top-down e-beam lithography (nanograting channel) and photolithography (S/D pads, leads, probe pads, etc.). Each nanowire in the grating channel is $\sim$ 50 nm in width, 30 nm in height and 20 μ m in length. The nanograting channel has a low boron concentration of $10^{15}/\text{cm}^2$ from the substrate doping, and the source/drain junctions were highly doped with phosphorous by ion implantation. The electric connections were made from nickel silicide, and a silicon nitride layer was deposited on top to provide insulation and protection. Fig. 1b and 1c show an optical image of a typical Si NGFET and an electron micrograph of the nanograting.

Fig. 1d shows an image of the 3D-printed sensing clamp. A fluidic channel (500 μ m in both width and height) defined in polydimethylsiloxane (PDMS) was connected to a pair of inlet/outlet for solution delivery, and sealed on the Si NGFET chip by the sensing clamp. Two identical Ag/AgCl electrodes were mounted at the top of the fluidic channel, one as the solution gate (that sets the electrolyte potential) and one as the reference electrode (to monitor the electrolyte potential). The source/drain probe pads were located near the chip edges and could be easily probed.

B. Gate Oxide Surface Modification

The dual-electrode setup was utilized in time-dependent protein sensing tests, and for this purpose the channel gate oxide surface was modified with antibody. Fig. 1e shows the surface species after the modification. Bare SiO₂ gate

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Figure 1. a) Schematic of protein sensing with a Si nanograting FET (NGFET) and dual Ag/AgCl electrodes. The oxide-electrolyte surface of the nanograting is functionalized with antibody. The FET is biased in subthreshold region by a solution gate at the top of the fluidic channel. The other electrode monitors the bulk solution potential. b) Optical image and c) electron micrograph of a typical Si NGFET with 100 nanowires in the grating. Each nanowire is of 50 nm in width, 30 nm in height and 20 μ m in length. d) 3D-printed clamp for protein sensing with dual Ag/AgCl electrodes. e) Species at the gate oxide surface after modification with TESU, antibody (mouse IgG) and passivation (with BSA). The corresponding antigen used in this study is anti-mouse IgG with gold.

surface was first cleaned in Piranha solution (sulfuric acid : hydrogen peroxide = 3 : 1) for 30 seconds, converting surface siloxane bonds to hydroxyl groups. After that, 0.1% 11-triethoxysilylundecanal (TESU) in toluene was applied to the chip for 5 hours, forming self-assembled monolayer (SAM) on the surface. The chip was then treated with 50 μ g/mL mouse IgG (dissolved in 2 mM KPB with NaCNBH₃ as reducing agent) for 3 hours. Finally, the chip was treated similarly with ethanolamine or bovine serum albumin (BSA) for 3 hours to passivate the remaining unreacted aldehyde groups on TESU.

C. Sensing Solution Preparation

Anti-mouse IgG with gold was used as the analyte or target protein to be detected. The antibody-antigen binding was verified in separate control tests with scanning electron microscopy (SEM). Protein solutions were prepared by cascade dilution in PBS (with salt concentration $\sim$ mM level). Because the stock protein solution also contains BSA, extra BSA was added to equalize the BSA concentration in all protein solutions. Two PBS buffer solutions were prepared, one with added BSA and one without. To minimize the effects from changes in pH, ionic strength and temperature, the pH and conductivity of all solutions were measured and adjusted, and all solutions were stored under the same condition. Table 1 lists the solutions prepared for a sample protein sensing test. The details of protein sensing is not further discussed here because the mV range of signal induced by pH variance is still comparable to protein signal, and therefore the repeatability of results is being studied and verified.

TABLE I. SOLUTIONS FOR A SAMPLE TEST

Solution	Protein Concentration	BSA (w/v)	pH	Conductivity
PBS	None	0%	7.14	204 μ S/cm
PBS with BSA	None	0.01%	7.14	207 μ S/cm
Protein 1	1 pM	0.01%	7.15	208 μ S/cm
Protein 2	10 pM	0.01%	7.13	210 μ S/cm
Protein 3	100 pM	0.011%	7.12	228 μ S/cm

III. RESULTS

To understand and take advantage of the additional electrode in the noise suppression, data from a sample test (with solutions as shown in Table 1) are presented in this section. First, the transfer characteristics of the Si NGFET were extracted to determine the appropriate biasing point of solution gate (V_{SG}) in the subthreshold region. Then, the time-dependent protein flow test was performed. The results are discussed in the next section.

A. Transfer Characteristics ($I_{DS} - V_{SG}$ and V_{Ref})

Antibody-modified n-channel Si NGFETs were biased with $V_{DS} = 0.1$ V and source terminal grounded. A bidirectional voltage sweep was performed on the solution gate and the drain current was measured, covering the regions of accumulation, depletion (subthreshold) and inversion in the transfer characteristics. Within the subthreshold region, the biasing point of the solution gate (V_{SG}) was determined and later used in the time-dependent sensing test. The reference electrode was configured as zero current (actual current $< fA$), and its voltage (V_{Ref}) was recorded at the same time. Fig. 2 shows the transfer characteristics for the sample test, in 2 mM PBS. The sensor

Figure 2. Transfer characteristics ($I_{DS} - V_{SG}$) and reference electrode voltage (V_{Ref}) by the Si NGFET modified with TESU and mouse IgG, tested in 2 mM PBS

Figure 3. Time-dependent measurement of drain current ($I_D - t$) and reference electrode voltage ($V_{Ref} - t$), for different sensing solutions. The actual solution gate voltage (V_{SG}) is also recorded and Figure 1. a)

performed well with zero hysteresis, good subthreshold swing (SS) of 75 mV/Dec and large on/off ratio of 6 orders of magnitude. V_{SG} was chosen to be 0.62 V for the time-dependent test. V_{Ref} followed well the sweeping voltage of solution gate with a nearly constant voltage gap (~ 20 mV), indicating that the reference electrode was reflecting the bulk solution potential set by the solution gate.

B. Time-Dependent Protein Flow Sensing Test ($I_{DS} - t$)

The Si NGFETs were biased and operated under similar conditions as the transfer characteristics test, with the exception that the solution gate was biased at the constant voltage of pre-determined V_{SG} . As a time-dependent measurement, different sensing solutions were delivered to the sensor in a flowing state during different time windows. Besides the drain current, V_{Ref} was also recorded during the whole test. Fig. 3 illustrates the time-dependent sensing results of the sample test (with solutions in Table 1), including totally 9 time windows. Baseline solutions were tested before and after each protein solution of different concentrations. Applied V_{SG} was also measured at the same time which stayed perfectly constant ($\Delta V_{SG} < 10 \mu V$).

IV. DISCUSSION

Prior to any formal discussion of the results, a key assumption must be clearly stated. At the electrolyte – reference electrode interface, the bulk solution potential U_{Sol} and reference electrode voltage V_{Ref} are related as

$$U_{Sol} = V_{Ref} + \Psi_{Ref}, \quad (1)$$

in which Ψ_{Ref} represents the surface potential at the electrode – electrolyte interface. As the reference electrode was configured as zero current, there should be no electrochemical reaction at the interface that gave rise to any change in Ψ_{Ref} . On the other hand, the pH and conductivity of all sensing solutions were controlled, and thus unable to cause change in Ψ_{Ref} . Therefore, we assume that Ψ_{Ref} was constant during the tests, which suggests that the change in V_{Ref} truly reflects the change in U_{Sol} .

In the following context, first the electrolyte potential noise in the results is discussed. Then a method to suppress the noise is presented, based on the discussion.

Figure 4. Noise suppression for the sample test results in Fig. 3. The relative surface potential and scaled V_{Ref} are plotted in the upper graph, with $\alpha = 0.4$. The lower graph shows the results after noise suppression, with the time windows labeled.

A. The Electrolyte Potential Noise

Results in Fig. 3 suggest that despite the constant V_{SG} applied to the solutions, V_{Ref} varied dramatically (~ 30 mV) during the test. To understand this noise, we apply similar analysis to the electrolyte – solution gate interface:

$$U_{Sol} = V_{SG} + \Psi_{SG}, \quad (2)$$

in which Ψ_{SG} is the surface potential at the solution gate. Similar to the reference electrode, pH or conductivity weren't the cause of this noise. However, there was current (> 0.1 nA) flowing across the solution gate – electrolyte interface, which was necessary to maintain the constant V_{SG} . Because the charge carriers in electrode (electrons) and electrolyte (ions) are inherently different, this current is a sign of electrochemical reactions at the interface, which enabled the conversion between the different carrier types. Combining (1) and (2):

$$V_{Ref} + \Psi_{Ref} = U_{Sol} = V_{SG} + \Psi_{SG}, \quad (3)$$

since Ψ_{Ref} and V_{SG} are constant, the observed noise on V_{Ref} can be attributed to Ψ_{SG} and is very likely due to the electrochemical reactions at the electrolyte – solution gate interface. Another clue is the tiny hysteresis of V_{Ref} in Fig. 2, which indicates that Ψ_{SG} changed slightly when the solution gate voltage swept back.

As shown in Fig. 3, the remarkable resemblance of drain current (I_{DS}) to V_{Ref} in shape indicates that I_{DS} was also affected by the same noise. This is not surprising, as the solution gate biased the Si NGFET via the electrolyte, and its potential U_{Sol} was directly perceived by the sensor.

B. Noise Suppression with V_{Ref}

The advantage of the additional electrode is that V_{Ref} can be used to suppress the same noise present in I_{DS} , which will be used for protein signal analysis. From (3) we can write

$$\Delta V_{Ref} = \Delta U_{Sol} = \Delta \Psi_{SG}. \quad (4)$$

Notice that V_{Ref} (V) and I_{DS} (A) have different units, so the first step is unit conversion. Since I_{DS} responds exponentially to the channel surface potential Ψ_0 , it's

Figure 5. Average signal level and standard deviation (error bar) of Ψ_0 (the sample test) within each time windows, before and after noise suppression

preferred to convert the former to the latter which changes linearly, for the simplicity of data manipulation in future steps. For the sample test, the measured $V_{Ref} - I_{DS}$ relation from Fig. 2 is used for the conversion and the resulting Ψ_0 is plotted in the upper graph of Fig. 4. Because only the relative change of surface potential matters, the starting point of Ψ_0 is set as the reference point (zero point). Also plotted on the same graph is the scaled ΔV_{Ref} (relative to the starting point as well), or V_{Noise} . The reason for the scaling is that the noise amplitude of V_{Ref} is greater than that of Ψ_0 . Although U_{Sol} stays constant for its DC component in the bulk solution, ΔU_{Sol} may not as the AC components will attenuate during propagation in the solution. As a result, ΔU_{Sol} seen by the reference electrode and the sensor may have attenuated differently, depending on their locations relative to the solution gate. This is the reason that a scaling factor α of 0.4 is applied to ΔV_{Ref} in Fig. 4, which, at this moment, can only be determined empirically. Finally, the noise signal V_{Noise} is subtracted from Ψ_0 , and the result is plotted on the lower graph of Fig. 4, with labeled time windows. Compared to the one without the noise suppression, this result is more readable, and more credible as explained below.

Fig. 5 illustrates the averaged signal level and standard deviation (as error bar) of Ψ_0 within each time window, before and after the noise suppression. One prominent improvement after the noise suppression is that the error bars shrink dramatically, indicating a huge decrease in noise level. The second improvement is that all signal levels of PBS with BSA (even window #s) are closer to each other, which increases the credibility of this result, as ideally these levels should be the same. In Fig. 6, the average standard deviation of Ψ_0 for this test is calculated, before and after the noise suppression, together with results from two other tests. These results show 50% ~ 70% decrease in the average standard deviation. The limit of detection (LOD) for a biosensor can be defined as the smallest detectable change of analyte (protein) concentration with a reasonable certainty [10]. Practically, setting three times of standard deviation as LOD yields a confidence level of 99.86%. Despite the lack of improvement in protein sensitivity due to unaltered relative difference between signal levels, results in Fig. 6 suggest the LOD of the Si NGFET protein sensors has decreased by 50% ~ 70% after the noise suppression.

Figure 6. Average standard deviation of Ψ_0 for the sample test (test 1) and two other tests, before and after noise suppression

V. CONCLUSION

In summary, we propose a method to suppress the electrolyte potential noise in time-dependent protein sensing tests with an additional reference electrode. This noise is likely due to the electrochemical reaction at the electrolyte – solution gate interface, implied by the solution gate current. Results after the noise suppression suggest increased readability with reduced signal level variation as well as increased credibility. The limit of detection (LOD) decreases by 50% ~ 70% as confirmed by several test results.

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