METHOD OF DETERMINING A SENSITIVITY OF A BIOSENSOR ARRANGEMENT, AND BIOSENSOR SENSITIVITY DETERMINING SYSTEM

Cross Reference to Related Application

[0001] This application claims priority to US provisional application no. 61/241,572 filed on 11 September 2009, the content of which is incorporated herein by reference in its entirety for all purposes.

Background

[0002] The present invention relates generally to a method of determining a sensitivity of a biosensor arrangement, and a biosensor sensitivity determining system.

[0003] Field-effect semiconductor devices can serve as a basis for chemical and biological sensors. In such a sensor, the presence of chemical or biological substances changes the conductance of the field-effect semiconductor device. Thus, the amount of change of conductance reflects the amount of chemical or biological substances put onto the sensor.

[0004] For example, a planar field effect transistor (FET) can be configured as a chemical or biological sensor by modifying the gate dielectric (on which no gate electrode is present) with molecular receptors or a selective membrane for the analyte of interest. The binding of a charged species to the molecular receptors then results in depletion or accumulation of carriers within the transistor structure, see for example document "P. Bergveld, *IEEE Trans. Biomed. Eng.*, vol. BME-19, p. 342, 1972".

[0005] Different ways of calibrating such a sensor are known. For example, in document "M. Abe, K. Murata, T. Ataka, K. Matsumoto, *Nanotechnology*, vol. 19, 2008", a calibration technique is presented in which a set of test solutions having different concentrations of target species is used to construct normalized characteristic curves for the sensor. This approach might be useful for certain applications such as pH and ion concentration measurement that detects reversible process. However, it cannot be used for detection of antibody-antigen binding process, for example, which is practically irreversible due to the extremely small dissociation constant of the process. Moreover, considering that a whole set of test solutions has to be prepared for calibration curve construction and the calibration should be performed for every single biosensor chip, this method is expensive and not suitable for point-of-care applications where the cost effectiveness is very important.

[0006] It would be desirable to provide a calibration method of chemical or biological sensors which is applicable to both reversible and irreversible processes and only needs minimal effort to be carried out.

Summary of the Invention

[0007] The invention provides a method of determining or adjusting the sensitivity of a biosensor arrangement comprising at least one field effect biosensor, each of the at least one field effect biosensor comprising: a semiconductor substrate comprising a source region, a drain region and a channel region disposed between the source region and the drain region; a gate isolation layer covering the channel region; and a reference electrode disposed over the gate isolation layer such that a electrolytic solution to be sensed can be

provided between the reference electrode and the gate isolation layer. The method comprises the following processes carried out for each field effect biosensor: providing an electrolytic solution between the reference electrode and the gate isolation layer; applying a source/drain voltage between the source region and the drain region; varying a reference voltage supplied to the reference electrode over a voltage range; measuring a resulting drain current while varying the reference voltage in order to obtain a corresponding drain current function; and determining the sensitivity of the field effect biosensor based on the reference voltage supplied to the reference electrode and the corresponding drain current function.

[0008] The invention further provides a biosensor sensitivity determining system for determining or adjusting the sensitivity of a biosensor arrangement comprising at least one field effect biosensor, each of the at least one field effect biosensor comprising: a semiconductor substrate comprising a source region, a drain region and a channel region disposed between the source region and the drain region; a gate isolation layer covering the channel region; and a reference electrode disposed over the gate isolation layer such that a electrolytic solution to be sensed can be provided between the reference electrode and the gate isolation layer. The biosensor sensitivity determining system comprises the following functional units for each of the field effect biosensors: a source/drain voltage applying unit adapted to apply a source/drain voltage between the source region and the drain region; a reference voltage applying unit adapted to apply a varying reference voltage which varies over a predetermined voltage range to the reference electrode; a drain current measuring unit adapted to measure a drain current resulting when varying the reference voltage in order to obtain a corresponding drain current function; and a

sensitivity determining unit adapted to determine the sensitivity based on the reference voltage supplied to the corresponding reference electrode and the corresponding drain current function.

Brief Description of the Drawings

[0009] In the drawings, like reference characters generally refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the following description, various embodiments of the invention are described with reference to the following drawings, in which:

FIG. 1A shows a schematic view of a biosensor which may be calibrated according to an embodiment;

FIG. 1B shows a schematic view of a biosensor sensitivity determining system according to an embodiment of the present invention which uses a biosensor according to Figure 1A;

FIG. 2 shows an energy band diagram and charge distribution occurring in the biosensor shown in Figure 1A;

FIG. 3 shows an equivalent circuit of the biosensor shown in Figure 1A;

FIG. 4 shows an equivalent circuit of the biosensor illustrating the case where a sensitivity of the biosensor with regard to a change of molecular charge is determined;

FIG. 5 shows an equivalent circuit of the biosensor illustrating the case where a sensitivity of the biosensor with regard to a change of the reference voltage is determined according to an embodiment of the present invention;

FIG. 6 shows a diagram indicating a measurement of the sensitivity of the biosensor over the reference voltage as well as the soure/drain current over the reference voltage;

FIG. 7 shows measurement results of pH testing with and without applying the inventive method;

FIG. 8 shows measurement results of DNA testing with and without applying the inventive method;

FIG. 9 shows a schematic view of a biosensor which may be calibrated according to an embodiment;

FIG. 10 shows a schematic drawing of a circuitry usable in conjunction with the biosensors in order to carry out the inventive method according to an embodiment of the present invention;

FIG. 11 shows a schematic drawing of a circuitry usable in conjunction with the circuitry shown in Figure 10 according to an embodiment of the present invention; and

FIG. 12 shows experimental results obtained with the circuitry shown in Figure 10.

Description

[0010] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the invention may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention. Other embodiments may be utilized and structural, logical, and electrical changes may be made without departing from the scope

of the invention. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

[0011] In the scope of the present invention, the term "biological sensor" also includes the meaning of a chemical sensor. That is, the "biological sensor" according to the present invention may sense biological material as well as chemical material.

[0012] Figure 1 shows a biosensor 100, the sensitivity of which can be determined or adjusted according to embodiments of the present invention. The biosensor comprises a field effect transistor. The field effect transistor comprises: a semiconductor substrate 102 comprising a source region 104, a drain region 106 and a channel region 108 disposed between the source region 104 and the drain region 106; a gate isolation layer 110 covering the channel region 108; and a reference electrode 112 disposed over the gate isolation layer 110 such that an electrolytic solution 114 to be sensed can be provided between the reference electrode 112 and the gate isolation layer 110. The biosensor further comprises a source/drain voltage applying unit 116 adapted to apply a source/drain voltage V_{DS} between the source region 104 and the drain region 106 and a reference voltage applying unit 118 adapted to apply a reference voltage V_{REF} (gate voltage) to the reference electrode 112. Further, the biosensor comprises a source/drain current measuring unit 120 which measures a soure/drain current I_D. The source region 104 is connected via a source contact 122 to a first terminal of the reference voltage applying unit 118, and the reference electrode is connected to a second terminal of the reference voltage applying unit 118. The drain region 106 is connected via a drain contact 124 to a first terminal of the source/drain voltage applying unit 116. A second terminal of

the source/drain voltage applying unit 116 is connected to a first terminal of the source/drain current measuring unit 120. A second terminal of the source/drain current measuring unit 120 is connected to the first terminal of the reference voltage applying unit 118 and to a bulk electrode 126 provided on the bottom surfacre of the substrate 102.

[0013] The biosensor 100 works as follows: An substance to be examined in put on the gate isolation layer 110 such that the bottom part of the reference electrode reaches into the substance. Then, the reference voltage V_{REF} is applied to the reference electrode 112. Depending on the substance, the strength of the soure/drain current I_D will vary (This is due to the fact that the gate insulation layer 110 is covered by a cover layer 128 (not shown in Figure 1) comprising receptors adapted to bind a particular kind of molecules ("target molecules")). If the substance to be analyzed comprises such molecules, the influence of the effective charge of the molecules on the channel region 108 is different, compared to the case where the molecules do not correspond to the receptors). The strength of the soure/drain current I_D therefore indicates which substance has been put onto the gate isolation layer 110, i.e. indicates whether the substance to be analyzed comprises the target molecules or not.

[0014] Figure 1B shows a biosensor sensitivity determining system 150 according to one embodiment of the present invention which determines the sensitivity of the biosensor 100. The biosensor sensitivity determining system 150 comprises a controlling/processing unit 160 which is connected to the reference voltage applying unit 118, the source/drain voltage applying unit 116, and the drain region 106. The controlling/processing unit 160 controls the reference voltage applying unit 118, the source/drain voltage applying unit 116, and the drain region 106 in the following manner:

The reference voltage V_{REF} is varied while the a source/drain voltage V_{DS} is kept constant. The controlling/processing unit 160 then monitors the resulting soure/drain current I_D and calculates the sensitivity based on the variation of the reference voltage V_{REF} and the corresponding soure/drain current I_D.

[0015] Figure 2 shows a corresponding energy band diagram and charge distribution 250 ocurring in the biosensor 100 shown in Figure 1. As can be derived from Figure 2, the binding of target molecules 200 to the receptors located on the cover layer 128 generates an amount of charge q_m which influences the energy band structure, in particular within the channel region 108. This change in the energy band structure can be detected by the change of the soure/drain current I_D routed through the channel region 108. Aspects of Figure 2 will be explained later in more detail.

[0016] Figure 3 shows an equivalent circuit 300 of the biosensor 100 shown in Figure 1. As can be derived from Figure 3, the effect of electrolyte 114 on the channel region 108 can be modeled by an arrangement of capacitors and resistors. Aspects of Figure 3 will be explained later in more detail.

[0017] Figure 4 shows an equivalent circuit 400 of the biosensor 100 illustrating the case where a sensitivity S_{qm} of the biosensor 100 with regard to a change of molecular charge Δq_m is determined. This is a conventional way of determining the sensitivity of the biosensor 100: a change of charge Δq_m is induced by providing a known testing substance onto the cover layer 128 (the effective charge of molecules of the testing substance effect the change of charge Δq_m). The change of charge Δq_m effects a change of the soure/drain current ΔI_D routed through the channel region 108. In this way, the sensitivity of the biosensor 100 on the testing substance can be examined.

[0018] Figure 5 shows an equivalent circuit 500 of the biosensor 100 illustrating the case where a sensitivity S_{cal} of the biosensor 100 with regard to a change of the reference voltage ΔV_{REF} is determined: a change of the reference voltage ΔV_{REF} effects a change of the soure/drain current ΔI_D routed through the channel region 108. This approach is used by embodiments of the present invention.

[0019] Figure 6 shows a diagram indicating a measurement of the sensitivity S_{cal} of the biosensor 100 over the reference voltage V_{REF} as well as the soure/drain current I_D over the reference voltage V_{REF}. As can be derived from Figure 6, the sensitivity S_{cal} reaches a maximum at low reference voltages V_{REF} (subthreshold region). However, at low reference voltages V_{REF}, the soure/drain current I_D becomes very small. At very low reference voltages V_{REF}, the soure/drain current I_D may thus be too small in order to yield reproducible results. Thus, the reference voltage V_{REF} should be chosen such that the both the sensitivity S_{cal} and the soure/drain current I_D have acceptable values.

[0020] Figure 9 shows a further possible biosensor 900 which may be calibrated according to embodiments of the present invention. In contrast to the biosensor 100 which is a field effect transistor (FET) biosensor, biosensor 900 is a nanowire biosensor. Instead of the channel area 108, the biosensor 900 uses nanowires 902 which extend between one or several source areas 104 and one or several drain areas 106. On the nanowires 902, receptors 202 are located which are adapted to bind target molecules 200 of a particular kind. The substrate 102 is isolated against the source areas 104, the nanowires 902 and the drain areas 106 via an isolation layer 904. The measuring of the sensitivity of the biosensor 900 is carried out in an analogue manner as described in the context of Figure 1B. In this context, it should be noted that, in the scope of the present

invention, the term "field effect biosensor" includes both biosensors as shown in figure 1 ("classical" filed effect biosensor) and biosensors an shown figure 9 (nanowire biosensor). The nanowire biosensor as shown in figure 9 is a filed effect device in the sense that an electrical field controls the transmissivity through the nanowires.

[0021] In Figure 10, a possible embodiment 1000 of the processing/controlling unit 160 is given. It is assumed in this example that the processing/controlling unit 160 controls a plurality of biosensors at the same time. The plurality of biosensors are indicated by block 1001. In this example, the processing/controlling unit 160 comprises an ASIC unit 1002 and a FPGA unit 1004. The ASIC unit 1002 comprises a plurality of channels, each channel being connected to one of the biosensors. Each channel further comprises a pixel circuit which processes the output (a plurality of soure/drain currents I_D) of the biosensors. The FPGA unit 1004 controls the interaction between the ASIC unit 1002 and the plurality of biosensors 1002. Further, the FPGA unit 1004 processes the data collected by the ASIC unit 1002 and generates and outputs calibrated biosensor data.

[0022] An example of a pixel circuit 1100 which may be employed in the ASIC unit 1002 is shown in Figure 11.

[0023] According to an embodiment of the present invention, determining the sensitivity of a field effect biosensor comprises: determining an effective transconductance function by differentiating the measured drain current function with respect to the reference voltage supplied; dividing the determined effective transconductance function by the measured drain current function.

[0024] According to an embodiment of the present invention, the sensitivities of the different field effect biosensors are compared among each other, wherein a normalization

process is carried out in which an avarage sensitivity for all of the field effect biosensors is calculated.

[0025] According to an embodiment of the present invention, the working point of each field effect biosensor is shifted into a region of maximum sensitivity.

[0026] According to an embodiment of the present invention, the working point of each field effect biosensor is shifted into a region of increased sensitivity in which the sensitivity is not maximum, in which however the drain current would still be large enough to yield reliable measurement results.

[0027] According to an embodiment of the present invention, the sensitivities of the field effect biosensors are compared among each other, wherein biosensors showing a sensitivity falling below a first threshold value or exceeding a second threshold value are disabled.

[0028] According to an embodiment of the present invention, the sensitivity determining unit is further adapted to determine an effective transconductance function by differentiating the measured drain current function with respect to reference voltage supplied to the reference electrode; and dividing the determined effective transconductance function by the measured drain current function.

[0029] According to an embodiment of the present invention, the sensitivity determining/adjusting system comprises a processing unit adapted to compare the sensitivities of the field effect biosensors among each other, wherein a normalization process is carried out in which an average sensitivity for all of the field effect biosensors is calculated.

[0030] According to an embodiment of the present invention, the sensitivity determining/adjusting system comprises a calibration unit adapted to shift the working point of each field effect biosensor into a region of maximum sensitivity.

[0031] According to an embodiment of the present invention, the sensitivity determining/adjusting system comprises a calibration unit adapted to shift the working point of each field effect biosensor into a region of increased sensitivity in which the sensitivity is not maximum, in which however the drain current would still be large enough to yield reliable measurement results.

[0032] According to an embodiment of the present invention, the sensitivity determining/adjusting system comprises a disabling unit adapted to compare the sensitivities of the field effect biosensors among each other, wherein biosensors showing a sensitivity falling below a first threshold value or exceeding a second threshold value are disabled.

[0033] In the following description, further background information/aspects of the present invention will be given.

[0034] The calibration technique according to embodiments of the present invention originates from the finding that the biosensing sensitivity is well presented by the effective transconductance parameter ($\Delta i_d / \Delta v_{ref}$) of the field-effect sensor device, which is measured as a drain current change (Δi_d) when the reference electrode voltage increments (Δv_{ref}). This observation will be proven by the following analysis, and the theoretical background and operational details of the proposed calibration scheme will be explained as well.

[0035] The simplified diagram of a planar field-effect sensor device and its biosensing steup is depicted in Fig. 1A/1B. Note that the sensor device is very similar to a typical MOSFET device, except the conductive gate of the MOSFET is replaced by the combination of an electrolytic solution and a reference electrode. The surface of the exposed gate insulator is functionalised, then probe molecules are immobilized on this surface. On successful binding with the target molecules, depending on the effective charge of the target molecules, a positive or negative shift in the threshold voltage of the FET is detected.

[0036] Figure 2 shows a 1-D model of the device together with the charge distribution and energy band diagram [see reference 1]. The charge balance between the semiconductor and electrolyte bulk required by Gauss' law is given by

$$q_s + q_0 + q_{md} = 0 \qquad (1)$$

[0037] wherein a charge density q_{md} can be split into a molecular charge density q_m from the molecules on the exposed gate insulator surface and q_d due to the ion distribution in the bulk electrolyte, q_0 is the surface charge density at the insulator surface due to the proton exchange at amphoteric sites, and q_s is the semiconductor charge density. Several characteristic electrostatic potential drops linked with the distribution of aforementioned charge components can be noticed: (a) ψ_d due to q_d, (b) ψ_{md} due to q_{md}, (c) ψ_0 due to q_{md} and q_0, (d) ψ_s due to q_s, (e) ψ_{cl} and ψ_{ins} due to q_{md}, q_0, and q_s. These associations between the electrostatic potentials and charge components can be modeled as a set of capacitances connected to each other as shown in Fig. 3. $C_{g,FET}$, C_{cl}, C_{stern} and C_{dl} are the gate capacitance of the field-effect biosensor, cover layer capacitance, Stern

layer capacitance and Guoy-Chapman double layer capacitance, respectively [see reference 1] and are defined as follow:

$$C_{g,FET} = \frac{C_{ins} C_s}{C_{ins} + C_s} \quad (2)$$

$$C_s = \frac{\partial q_s}{\partial \psi_s} \quad (3)$$

$$C_{ins} = \frac{\partial q_s}{\partial \psi_{ins}} \quad (4)$$

$$C_{cl} = \frac{\partial (q_{md} + q_0)}{\partial \psi_{cl}} \quad (5)$$

$$C_0 = \frac{\partial q_0}{\partial \psi_0} \quad (6)$$

$$C_{stern} = \frac{\partial q_{md}}{\partial (\psi_{md} - \psi_0)} \quad (7)$$

$$C_{dl} = \frac{\partial q_{md}}{\partial \psi_{md}} . \quad (8)$$

[0038] A Warburg impedance R_{dl} of the insulator-electrolyte interface and the bulk electrolyte resistance R_{be} have been neglected in this model. Generally, this omission is justified at low frequencies and at low bias potentials [see reference 2]. The $g_{m,FET}$ represents the transconductance of the field-effect device.

[0039] Based on the derived model, the sensitivity of biosensor to the molecular charges can be analyzed (Fig. 4). The body and source terminals of the device are grounded, the reference electrode is biased at a certain fixed voltage, and the drain terminal is biased with a small voltage to induce the flow of drain current i_d. When molecular charge Δq_m is added, the gate potential of the device changes through the

capacitive network, and in turn the i_d changes to $i_d + \Delta i_d$. The sensitivity to the molecular charge S_{qm} is defined by

$$S_{qm} \equiv \frac{\Delta i_d / i_d}{\Delta q_m} \tag{9}$$

[0040] and can be expressed as follows:

$$S_{qm} = \frac{\Delta v_g}{\Delta q_m} \times \frac{\Delta i_d}{\Delta v_g} \times \frac{1}{i_d} = \frac{\Delta v_g}{\Delta q_m} \times \frac{g_{m,FET}}{i_d} \tag{10}$$

[0041] Assuming that $C_{g,FET}$ and C_{cl} are much smaller than C_0 and C_{stern} as is typically true, the gate voltage change due to the molecular charge increment becomes

$$\frac{\Delta v_g}{\Delta q_m} \approx \frac{1}{C_{dl} + \frac{C_{stern} C_0}{C_{stern} + C_0}} \cdot \frac{C_{stern}}{C_{stern} + C_0} \cdot \frac{C_{cl}}{C_{cl} + C_{g,FET}}$$

$$= \frac{C_{stern}}{C_{dl} C_{stern} + C_{dl} C_0 + C_{stern} C_0} \cdot \frac{C_{cl}}{C_{cl} + C_{g,FET}} \tag{11}$$

[0042] As a result, the S_{qm} is given by

$$S_{qm} = \frac{C_{stern}}{C_{dl} C_{stern} + C_{dl} C_0 + C_{stern} C_0} \cdot \frac{C_{cl}}{C_{cl} + C_{g,FET}} \cdot \frac{g_{m,FET}}{i_d} \tag{12}$$

[0043] In the calibration process, however, the actual sensitivity to the molecular charge cannot be measured directly. Embodiments of the present invention propose using a small-signal change in the reference voltage instead of the real molecular charge attachment to estimate the sensitivity to the molecular charge so that the sensor device sensitivity can be calibrated. Figure 5 shows the setup for measuring the sensitivity to the reference voltage change Δv_{ref}. This sensitivity characterized for calibration purpose S_{cal} is defined by

$$S_{cal} = S_{vref} \equiv \frac{\Delta i_d / i_d}{\Delta v_{ref}} \tag{13}$$

[0044] and can be written as

$$S_{cal} = \frac{\Delta v_g}{\Delta v_{ref}} \times \frac{g_{m,FET}}{i_d} = \frac{g_{m,eff}}{i_d} = K_{cal} \cdot S_{qm} \tag{14}$$

[0045] where the effective transconductance $g_{m,eff}$ is approximated by

$$g_{m,eff} = \frac{\Delta v_g}{\Delta v_{ref}} \times g_{m,FET} \approx \frac{C_{cl}}{C_{cl} + C_{g,FET}} \cdot g_{m,FET} \tag{15}$$

[0046] with assuming $C_{g,FET}$ and C_{cl} are much smaller than C_0 and C_{stern}. The K_{cal} which is the proportionality factor between the S_{cal} and S_{qm} can be written as

$$K_{cal} = \frac{S_{cal}}{S_{qm}} \approx \frac{C_{dl}C_{stern} + C_{dl}C_0 + C_{stern}C_0}{C_{stern}} \tag{16}$$

[0047] Note that of C_{dl}, C_{stern}, and C_0 are all very weak function of v_{ref} compared to $g_{m,FET}$ in the operation range of interest [see reference 2], and therefore K_{cal} can be regarded as a constant. It implies that S_{cal} based on the measurement of the effective transconductance $g_{m,eff}$ and drain current i_d provides a good representation of the actual biosensing sensitivity S_{qm}, and can be effectively used for the calibration process.

[0048] Based on the observation discussed above, according to an embodiument of the present invention, a new calibration technique as follows is proposed: (a) For a biosensor or a multitude of biosensors, i_d is measured with sweeping v_{ref} over the range of interest, e.g. –0.5 to +0.5 V. (b) For each sensor, $g_{m,eff}$ is calculated as a function of v_{ref} by differentiating the measured i_d with respect to v_{ref}. (c) For each sensor, S_{cal} is calculated by dividing the calculated $g_{m,eff}$ by the measured i_d. (d) The calculated S_{cal} can be used for normalization of the results among different sensors, or for the calibration by using the

result from the biosensor placed in the separate control chamber where the known concentration of the analyte is applied. Note that this technique can be used not only for the normalization or calibration but also for the optimization of the sensor sensitivity.

[0049] As shown in Fig. 6, $S_{cal} = g_{m,eff} / i_d$ increases as v_{ref} decreases and comes saturated in the subthreshold region of the device, meaning that if the biosensor is operated in the subthreshold region, the sensitivity can be maximized. However, it should be considered that in the deep subthreshold region, i_d becomes too small to be measured reliably, and therefore, according to an embodiment of the present invention, the optimum v_{ref} may be decided considering both the maximization of S_{cal} and the current measurement limit of the readout system used. Another application of the proposed technique is the screening of the biosensor devices among many. For example, if the measured S_{cal} of a certain device is lower than the preset threshold, the result from this device can be discarded for better overall statistics.

[0050] In order to validate the proposed calibration technique, pH testing and DNA testing were performed on nanowire biosensors, one popular type of nano-scale field-effect biosensors [see reference 3]. In the pH testing, the pH level changed from 7 to 4 and the corresponding nanowire resistance change was measured from a total of 30 nanowire biosensors. As shown in Fig. 7, by applying the calibration technique, the data statistics becomes much improved. As an indicator of the measured data spread, the ratio of the standard deviation and the mean of resistance change was calculated. This measure of data spread is reduced from 0.2 to 0.13 with applying the calibration technique. The same improvement was observed in the DNA testing performed, see Fig. 8. In this experiment, after applying the 1ng/mL of the target DNA, the corresponding nanowire

resistance change was measured again from 30 nanowire biosensors. The ratio of the standard deviation and the mean of resistance change decreases from 1.1 to 0.4 with the proposed technique applied. In figures 7 and 8, box 700 indicates the nanowire resistance change in % measured without calibration, and bars 702 the corresponding error bars, box 704 indicates the nanowire resistance change in % measured with calibration, and bars 706 the corresponding error bars, square dots 710 the SD/mean of change, arrows 712 indicate the improvement of the error bars, arrow 714 the improvement of the SD/mean of change.

[0051] According to an embodiment of the present invention, the calibration factor S_{cal} is based on the measurement of the effective transconductance $g_{m,eff}$ and the drain current i_d, wherein the calibration technique is as follows: For a biosensor or a multitude of biosensors, i_d is measured with sweeping v_{ref} over the range of interest, e.g. –0.7 to +0.3V; the $g_{m,eff}$ is calculated as a function of v_{ref} by differentiating the measured i_d with respect to v_{ref} and the value of S_{cal} is calculated as for each biosensor. Subsequently, S_{cal} can be used for normalization of the results among different sensors. Note that this technique can be used not only for the normalization or calibration but also for the optimization of the sensor sensitivity. The value of S_{cal} increases as v_{ref} decreases and peaks in the subthreshold region of the biosensor, hence operating in the subthreshold region can maximize the sensitivity. However, it should be considered that in the deep subthreshold region, i_d becomes too small to be measured reliably, and therefore the optimum v_{ref} may be decided considering both the maximization of S_{cal} and the current measurement limit of the readout system used.

[0052] Returning to Figure 10, an embodiment of an overall readout system is shown which comprises 256 channels readout ASIC (application specific integrated circuit) 1002 and an FPGA (Field Programmable Gate Array) 1004. Each channel in the ASIC is assigned with a dedicated pixel circuit block containing low current sensing circuits and a 10bit single slope ADC. The FPGA is implemented to provide the timing controls for proper operation of the ASIC and to be used during calibration to calculate for the factor $g_{m,eff}$, S_{cal} and the resultant sensitivity factor S_{qm}. Figure 11 shows a possible embodiment of each pixel circuit. Low current sensing is achieved with the single-ended charge integrator followed by the correlated double sampling (CDS) circuit which helps to reduce the offset and the 1/f noise contribution from the charge integrator. The CDS circuit setup with the comparator is combined with a 10-bit SRAM and counter circuits to form a single-slope ADC architecture to provide the digital readout for the current measured at the input of the charge integrator. Both the charge integrator and the comparator are implemented with an offset cancellation scheme using the switch S_1. When switch S_1 is closed, the offset voltage from the charge integrator and the comparator is stored on the capacitor C_{off} and $C_{o,comp}$, respectively. The function of capacitor C_{ramp} is to capture the offset from the ramp input. Subsequently, when switch S_1 is opened, the offset is cancelled. The output voltage of the charge integrator is directly proportional to the input current i_{int} integrated across the capacitor C_{int}. The period of the integration as represented by Phase 1 (Figure 11) is adjustable externally to fit different input current ranges. Switch S_2 is kept closed during integration period (Phase 1) and open in the Phase 2. Phase 2 is the analog to digital conversation period where a rising ramp signal is applied via the capacitor C_{ramp}. At the same time, the counter is also

initiated. The rising ramp signal toggles the output of the comparator at the point where the voltage at the negative input of the comparator is larger than V_{cm}. Consequently, the SRAM will capture the count on the counter. The count captured on the SRAM is the digital representation of the input current I_{int}.

[0053] Figure 12 shows initial electrical measurements made with the ASIC. The electrical measurements were obtained by sweeping the input current range from 100pA to 8nA for an integration period of 1.5 ms. V_{out} is the converted digital output with respect to the different input current I_{int}. The results show good linearity output response across the 40dB of input current range. The dynamic range tested is sufficient for the intended application of interest but is not limited to only 40dB as it can be further increased by the adjustment of the integration period as mentioned previously. The diagram on the right of Figure 12 shows the output referred noise characteristic for the measured input current at 2.3nA. The 95% percentile or 3 standard deviation concludes an input noise variation of less than 0.5% or ± 11.5pA variation. This implies that the input referred noise floor for the measurement is $11.5pA_{rms}$ for the signal bandwidth of interest.

[0054] According to an embodiment of the present invention, a 256 channels readout system is used. It comprises a microfludic housing that holds the 256 nanowire biosensors, a special electrical probe connector that allows connection to the nanowire biosensors and our readout ASIC. The ASIC chip was fabricated using Chartered 0.18μm 1P6M standard CMOS techology with MIM capacitor option. Approximately 120,000 transistors are integrated on 4.6mm x 4.6mm die area for the 64-channels of the

amplifiers and A/D converters. The chip is powered up with 1.8V VDD and consumes 1.8mW of power.

[0055] According to one embodiment of the present invention, the nanowire biosensor was convalently immobilized with peptide nucleic acid (PNA), a bifunctional linker, glutaraldehyde, was employed to bind its one end to the SiNW amines, leaving the other end to bind amine-terminated PNA. The nanowire biosensors were treated with 1% glutaraldehyde in H_2O for 1 h, and rinsed with pure H_2O. Ten micromolars of PNA in 1 x SSC were incubated with the SiNWs in a humid atmosphere at room temperature overnight. The chips were washed three times with 1xSSC, 5 min each after immobilization of the PNA. The freshly prepared chips were used for measurements immediately. Figure 8 shows the results of the DNA binding on the nanowire biosensor. The results obtained are statistical averages measured across 20 nanowire biosensors. The consequence of the binding results in the change in the nanowire biosensor resistance. The spread of the measured resistance change is larger for case where no calibration was implemented where the coefficient of variation (CV) is 1.1. However, the CV value for case where calibration was implemented recorded a reduction to 0.4. This indicates that the calibration algorithm is able to improve on the measurement by first taking into account on the variability of the individual nanowire biosensor sensitivity and normalizing their final sensitivity with the appropriate S_{cal} coefficient.

[0056] As has become apparent from the foregoing description, according to embodiments of the present invention, a new calibration technique based on the measurement of the effective transconductance parameter of the field-effect biosensors is provided. The technique can be used for normalization of the results among different

sensors to mitigate the variability of their electrical properties, or for the calibration by using the result from the biosensor placed in the separate control chamber where the known concentration of the analyte is applied. The technique can be also used for the optimization of the sensor sensitivity, considering both the maximization of sensitivity and the current measurement limit of the readout system used. Another application of the proposed technique is the screening of the poor-quality biosensor devices among a plurality. In order to validate the proposed calibration technique, pH testing and DNA testing were performed on nanowire biosensors, and it was verified that the data statistics become much improved by applying the calibration technique.

[0057] As has become apparent from the forgoing description, embodiments of the calibration method according to the present invention use the effective transconductance parameter of field-effect sensor devices. This method is very effective because it directly calibrates the sensor sensitivity, and does not require any addition of substantial complexities – e.g. a set of calibration solutions for calibration curve construction, complicated on-chip calibration circuitry, special device structure, or complex calibration algorithm.

References

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[0058] While the invention has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. The scope of the invention is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

Claims

What is claimed is:

1. 1. A method of determining/adjusting the sensitivity of a biosensor arrangement comprising at least one field effect biosensor, each of the at least one field effect biosensor comprising:

- a semiconductor substrate comprising a source region, a drain region and a channel region disposed between the source region and the drain region;
- a gate isolation layer covering the channel region; and
- a reference electrode disposed over the gate isolation layer such that a electrolytic solution to be sensed can be provided between the reference electrode and the gate isolation layer,

the method comprising the following processes carried out for each field effect biosensor:

- providing an electrolytic solution between the reference electrode and the gate isolation layer;
- applying a source/drain voltage between the source region and the drain region;
- varying a reference voltage supplied to the reference electrode over a voltage range;
- measuring a resulting drain current while varying the reference voltage in order to obtain a corresponding drain current function; and
- determining the sensitivity of the field effect biosensor based on the reference voltage supplied to the refernce electrode and the corresonding drain current function.

2. The method according to claim 1,

wherein determining the sensitivity of a field effect biosensor comprises:

- determining an effective transconductance function by differentiating the measured drain current function with respect to the reference voltage supplied;
- dividing the determined effective transconductance function by the measured drain current function.

3. The method according to any one of the claims 1 or 2,

further comprising comparing the sensitivities of the different field effect biosensors among each other, wherein a normalization process is carried out in which an avarage sensitivity for all of the field effect biosensors is calculated.

4. The method according to any one of the claims 1 to 3,

further comprising shifting the working point of each field effect biosensor into a region of maximum sensitivity.

5. The method according to any one of the claims 1 to 3,

further comprising shifting the working point of each field effect biosensor into a region of increased sensitivity in which the sensitivity is not maximum, in which however the drain current would still be large enough to yield reliable measurement results.

6. The method according to any one of the claims 1 to 5,

further comprising comparing the sensitivities of the field effect biosensors among each other, wherein biosensors showing a sensitivity falling below a first threshold value or exceeding a second threshold value are disabled.

7. A biosensor sensitivity determining system for determining/adjusting the senitivity of a biosensor arrangement comprising at least one field effect biosensor, each of the at least one field effect biosensor comprising:

- a semiconductor substrate comprising a source region, a drain region and a channel region disposed between the source region and the drain region;
- a gate isolation layer covering the channel region; and
- a reference electrode disposed over the gate isolation layer such that a electrolytic solution to be sensed can be provided between the reference electrode and the gate isolation layer,

the biosensor sensitivity determining system comprising the following fuctional units for each of the field effect biosensors:

- a source/drain voltage applying unit adapted to apply a source/drain voltage between the source region and the drain region;
- a reference voltage applying unit adapted to apply a varying reference voltage which varies over a predetermined voltage range to the reference electrode;
- a drain current measuring unit adapted to measure a drain current resulting when varying the reference voltage in order to obtain a corresponding drain current function; and

- a sensitivity determining unit adapted to determine the sensitivity based on the reference voltage supplied to the corresponding reference electrode and the corresonding drain current function.

8. The biosensor sensitivity determining system according to claim 7,

wherein the sensitivity determining unit is further adapted to:

- determine an effective transconductance function by differentiating the measured drain current function with respect to reference voltage supplied to the reference electrode;
- dividing the determined effective transconductance function by the measured drain current function.

9. The biosensor sensitivity determining system according to any one of the claims 7 or 8, further comprising a processing unit adapted to compare the sensitivities of the field effect biosensors among each other, wherein a normalization process is carried out in which an avarage sensitivity for all of the field effect biosensors is calculated.

10. The biosensor sensitivity determining system according to any one of the claims 7 to 9, further comprising a calibration unit adapted to shift the working point of each field effect biosensor into a region of maximum sensitivity.

11. The biosensor sensitivity determining system according to any one of the claims 7 to 9,

further comprising a calibration unit adapted to shift the working point of each field effect biosensor into a region of increased sensitivity in which the sensitivity is not maximum, in which however the drain current would still be large enough to yield reliable measurement results.

12. The biosensor sensitivity determining system according to any one of the claims 7 to 11, further comprising a disabling unit adapted to compare the sensitivities of the field effect biosensors among each other, wherein biosensors showing a sensitivity falling below a first threshold value or exceeding a second threshold value are disabled.