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CMOS ISFET device for DNA Sequencing: Device Compensation, Application Requirements and Recommendations

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Abstract

Ion sensitive field-effect transistor in DNA sequencing application is gaining popularity because of its capability to work massive parallel sensor arrays, especially when integrated in CMOS technology fabrication with inherent scalability from silicon at low cost. This paper reviews methods used in the last decade to design different topologies of ISFET sensor array device for DNA sequencing. Non-idealities of CMOS ISFET device introduced and its compensation in terms of the device being more robust than the system. Application and design requirements of DNA sequencing based on ISFET have been extracted from the literature as a process flow for designers to use. Further, several recommendations mentioned as concluded from previous works that would be useful for future research. After the invention of ISFET by Bergveld, more review articles have been reported but not for specific applications. Hence, this paper will showcase the importance of ISFET application in terms of point-of-care diagnostics and may result in biosensors evolution.

Keywords: ISFET; CMOS Technology; DNA sequencing; Device Compensation; Biosensor; Point-of-Care Diagnostics.

INTRODUCTION

Improvement of a portable gene test in terms of a point-of-care (POC) system is imperative because of the increasing concern on issues, such as human-service-based care, tailor-made prescription, and diseases. Traditional DNA chips depend on optical recognition, in which the target DNA is marked with fluorophore and recognized by optical light scanner. However, this technique requires an administrator and a costly, non-portable device and is thus unfavorable. On the contrary, next-generation (NG) and whole-genome sequencing offer an appealing selection because it analyzes all loci and provides data reflecting minor and major changes in the genome [1,2]. Generally, NG is a method for recognizing putative resistance that cause mutations and managing patients with drug resistance to administer suitable medication regimen [3,4]. The most important benefit of which is biomarker detection for tumor and cancer. The molecular detection (DNA molecule) at the fluid interface is of interest for a wide array of applications, from clinical medicine, biosensors for biomedical drugs, DNA chips, and microarray innovation. Furthermore, an investigation on the binding of charged macromolecules onto charged surfaces is likewise imperative to understand several physiological procedures. For example, microarrays, on which many proteins are printed, provide a profitable stage of utilitarian investigation for proteome. Generally, these are recognizable proofof biomarker disease and utilized in examination of protein–protein interactions [5].

Today, a vast majority of the procedures for DNA hybridization and protein detection utilize different labels and reagents, such as fluorescent, enzymatic, or radiochemical [6]. Disregarding their high sensitivity and low detection restrictions, each of these systems are tedious, costly, complex to actualize, and usually need highly skilled people in the field [7]. Therefore, sensitive, reliable, robust, and high-throughput assay that could recognize biomolecules without labeling (i.e., label-less biosensor) is of great interest. Such method could altogether decrease the time and costs required for test preparation and eliminate procedures identified with the preparation and change of the target molecule. In contrast with other methods, label-less molecular biosensors offer vast potential as clinical instrument, halfway in view of ongoing and multi-target assay, high-throughput diagnosis, automatic, and low cost [8]. Among the transducer ideas proposed for label-less detection of biomolecules, the binding of biomolecules with semiconductor field-effect transistors (FET) device based on chemical sensor (i.e., float gate transistor) outstands most methodologies. Ion-sensitive FET (ISFET) is considered a typical example of a highly sensitive biological sensor.

More than 40 years has passed since ISFET was introduced by Bergveld in the early 1970s [9]. Bergveld, who developed the utilizing a floating gate as a chemical sensor, is one of the references in the field of Isfetology [10]. Large-scale reconciliation of the sensors have been shown from that point onward by considering lab-on-chip device organized as numerous microarrays and compact ISFET pixels, as shown in Figure 1 [11]. Although the sensor was first created for neurophysiological measures, biochip utilization has expanded to various applications, such as DNA sequencing, which has motivated researchers in the last 10 years. ISFET allowed the

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implementation of a unique program that significantly lowered the cost of genome sequencing from $100 million in 2002 to $1000 in 2010 [12].

Several sensing design topologies featured in articles were published in the last decade for classical configuration, as well as novel implementation. The large number of articles published in the field of Isfetology (around 1500 to 2000 articles in WOS and Scopus resources) is difficult to cover. Therefore, the design topology implementation and the physics behind a DNA sequencing device were used for molecular detection of POC portable devices focused in this review. In addition, advancement in ISFET sensors mostly published in the last 10 years was summarized. Specifically, we focus on ISFET array sensors that considered the most promising structure in DNA sequencing instrumentation. The device sensor is more robust than sensor systems due to actual measurements and high result validation.The theory behind ISFET and its structure, as well as ISFET operation macro-model, and the pH of the analyte based on the natural logarithm is summarized in **Section 2**. In **Section 3**, several non-idealities is presented, which the ISFET suffers from in device compensation methods that overcome these non-idealities reported in the literature, as discussed in **Section 4**. A process flow that simplified design and application requirements for DNA sequencing based on ISFET is introduced in **Section 5**. Finally, several recommendations for future research is presented in **Section 6**.

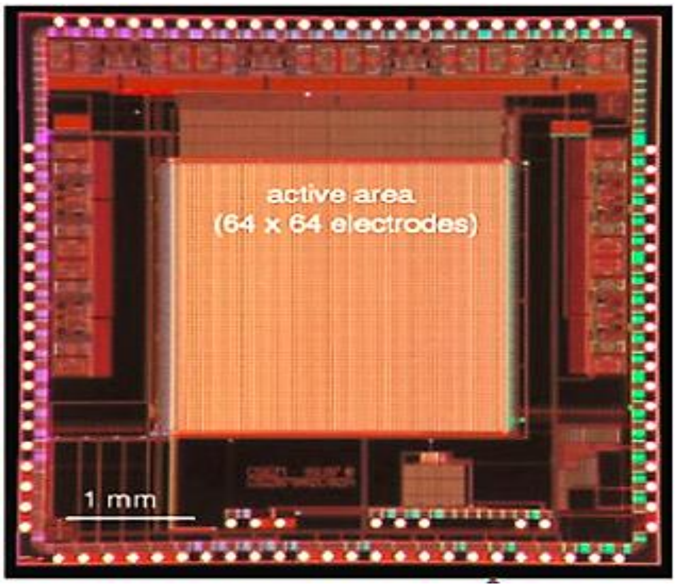


Figure 1: Examples of 64 × 64 ISFET sensing array [11].

THEORY AND PRINCIPLE BEHIND ISFET

The guideline of DNA detection utilizing ISFET was first presented in [13], which demonstrates that when a match between a DNA target and a DNA probe exists, hydrogen particles or protons (H+) are released, changing the pH of the fluid. The ISFET, being particular to H+, can recognize the changes in pH as a result of DNA detection based on the rule of DNA chain extension, and in this way, can be used for applications, such as DNA sequencing.

ISFET was initially actualized using a standard metal–oxide–semiconductor FET (MOSFET) by replacing the gate metal with a remote gate [standard fluid junction Ag/AgCl reference electrode (VG)] to set a steady potential or gate bias to the fluid bearing a chemical-sensitive membrane insulator to the electrolyte, as shown in Figure 2.

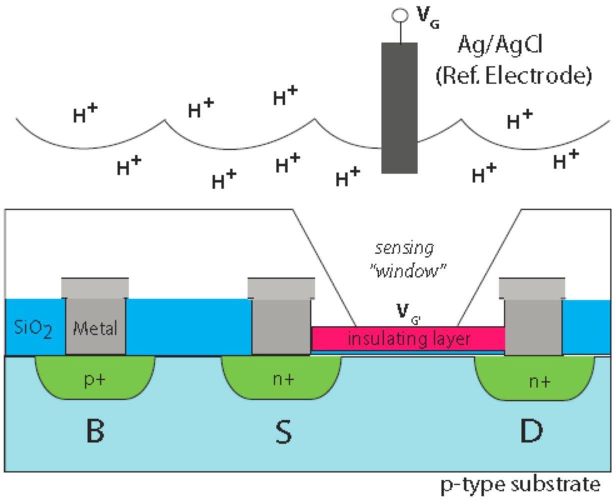


Figure 2: ISFET**.**

Originally, the sensing membrane used silicon dioxide (SiO_2) exposed to an electrolyte solution [14]. However, SiO_2 proved to be an unstable insulating layer because of the effect of hydrogen, causing breaks in the surface membrane of the gate. To overcome this problem, the single layer of SiO_2 is replaced by a double layer of SiO_2 or by a single layer of other insulating membrane, such as silicon nitride (Si_3N_4), aluminum oxide (Al_2O_3), and tantalum oxide (Ta_2O_5), placed on the surface of the SiO_2 layer [15]. In 1999 Bausells et al. [16] presented a new method of pH-sensitive ISFET fabrication in unmodified CMOS technology. The method allows ISFET to integrate with system-on-chips without any post-processing fabrication, providing advantages of scalability, small size, and low cost [17].

The activity of protons and insulating membrane used to trap protons on its surface is related to the ions in the fluid. The pH of the analyte based on the natural logarithm definition of H+ and ion concentration, which demonstrates acidity (least pH=1) or alkalinity (most extreme pH=14), can be expressed as follows:

$$\boldsymbol{pH} = -\mathbf{log}([\boldsymbol{H}^{+}]) \qquad \textbf{(2.1)}$$

Changes in ionic concentration can cause changes in the charge dissemination in ISFET channels, which can be identified as changes in the threshold voltage *Vth* of the sensor. A depiction of the properties of ISFET as a pH sensor alongside its physical and operational properties is now clarified.

In the ISFET model side, a primary macro-model was introduced based on the models describing sensitivity [18,19], as shown in Figure 3.

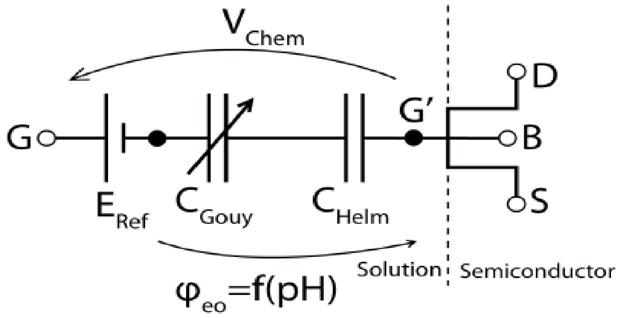


Figure 3: ISFET macro-model redrawn from [18,19].

The change in the reference electrode to the insulator can be written as :

$$\boldsymbol{V_{Chem}} = \boldsymbol{\gamma} + \boldsymbol{S_{pH}} \cdot \boldsymbol{pH} \qquad \textbf{(2.2)}$$

where $\boldsymbol{\gamma}$includes all the non-pH-dependent parameters related to electrode–electrolyte, S_{pH} denotes pH sensitivity, and V_{Chem} in the primary macro-modelling of the ISFET operation is used as a modulator of the FET threshold voltage.

$$\boldsymbol{V_{th-ISFET}} = \boldsymbol{V_{th-MOSFET}} + \boldsymbol{V_{Chem}} \qquad \textbf{(2.3)}$$

Other models have been used for the capacitive characteristic of the sensing membrane [20], as shown in Figure 4. In these models, a capacitor representing the passivation layer is included for the unmodified CMOS ISFET. C_{pass}, C_{ox} and C_d are the passivation, oxide, and depletion capacitances, respectively.

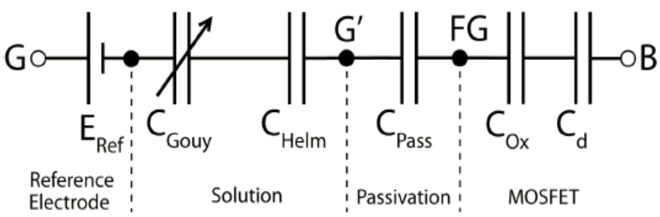


Figure 4: ISFET capacitive model including unmodified CMOS passivation and gate capacitances [20].

Despite all the promising advantages of the ISFET sensor, some non-idealities and deficiencies (e.g., offset, drift, temperature, noise, attenuations, and other issues) limit its implementation characteristics and applications. Some of the issues may be alleviated by changing the advancement, such as the sensing membrane material and deposition. Other issues may require design approaches for the commercial unmodified CMOS process. These issues will be discussed in detail in the next sections.

In summary, this section first introduces the mechanism of ISFET as a chemical sensor for DNA molecule detection. Second, utilizing the standard MOSFET to present an ISFET sensor is discussed to consider the innate scalability of silicon technology at low cost. Additionally, it raised the need of post-processing fabrication and advertised integration by using unmodified CMOS technology and historical practical methods to overcome the disadvantages of this technology. The natural logarithmic definition used by ISFET to detect H+ is then presented. Finally, the typical macro-model of ISFET operation is demonstrated to introduce ISFET methods. Non-idealities specific to ISFETs will then be discussed in the next section.

ISFET ISSUES AND NON-IDEALITIES

In the last decade, majority of research conveyed several non-idealities of ISFET [21]. Here, we focus on non-idealities that are generally worked out by designers but now has become notable for scalability, reliability, and robustness of front-ends design for ISFET sensor device in the future. Specifically, we consider the effects that require divergence from the typical ISFET model.

ISFET fabrication is composed of two groups, one of which works on post-processing technology to deposit new membranes insulator that result to more usable and reliable device [22] but with additional cost and complex design. This type of fabrication, not considered in this review, tend to have low cost and have portable device for new era of sensors that satisfy these issues. The other group uses unmodified CMOS technology with inherent CMOS passivation material without any modification. However, this layer is not perfectly insulating [20], and the result has challenges. The passivation layer is skewed to degrade when the solution is touched. In addition, it can be used for a short period only and suitable for a one-time assay, and then disposed. CMOS technology can now be made inexpensively; hence, its usage at present is practical.

The non-idealities considered in this review start from the effect of temperature on the operation of the sensors, followed by the most important issue of trapped charges induced during fabrication that can leave an offset at the coating gate of the ISFET. Next, drift defined as the monotonic shift in the ISFET output is discussed. Another issue is the sensing membrane insulator that certainly plays a key role in ISFET performance. Lastly, similar to temperature, noise can occur in electrode–electrolyte–insulator. Although eliminating all the problems and creating an ideal ISFET device simultaneously may not be possible, their effects can be suppressed and improve ISFET performance. These issues will now be described in further detail.

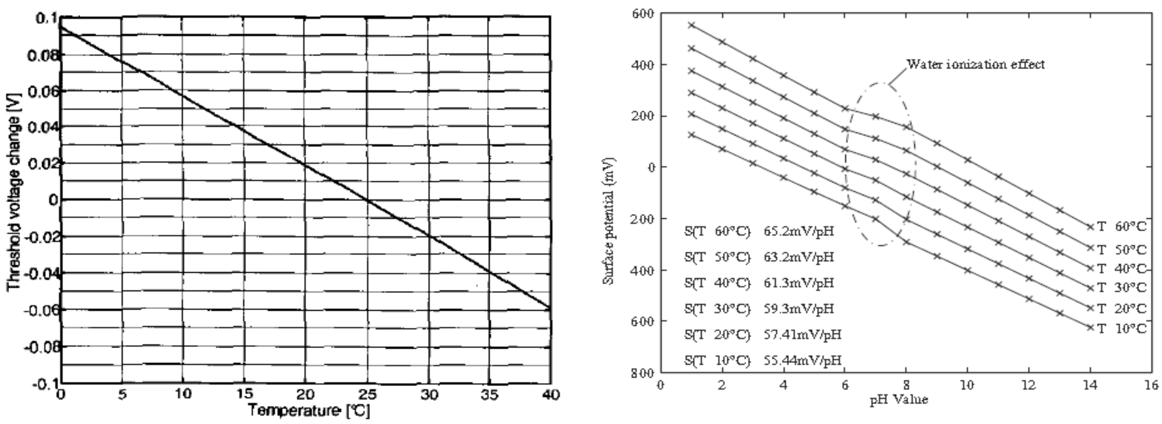
Temperature effect

The influence of temperature on the operation of ISFET sensors is due to the effects of temperature on the intrinsic FET threshold and the electrode–electrolyte–insulator. In other words, ISFET is affected by temperature due to the variations in thermal agitation affecting the electron and the variation in the pH solution. The effectiveness of temperature on the operation of these sensors on the intrinsic FET threshold is shown in Figure 5a [23], and that of the electrode–electrolyte–insulator is shown in Figure 5b [24].

Other research that proposed the effectiveness of temperature on the ISFET can be seen in [25,26]. Similar to drift, the focus of past research was to determine robust compensation methods to improve the accuracy of analytical temperature reliance for the sensor.

Offset

CMOS ISFET fabrication face the same challenges as floating-gate MOSFETs, which is considered the standard. Both suffer from trapped-charge-induced DC offset and attenuation of the input because of capacitive division of the transconductance by the floating-gate capacitor [27]. The effect is illustrated in Figure 6. As reported in [20], a threshold voltage variation of −1.32 V due to trapped charge and a loss of half transconductance efficiency were observed. Other reference [21] approximated the variation in threshold voltage as regular between −4 V and −1 V for standard AMS 0.35 mM, with discrepancies between processes, dies, and throughout the array [28]. Nevertheless, charge is trapped on the floating gate of the ISFET due to charge transfer during chip production, causing an offset in its threshold voltage.



(a) (b)

Figure 5: Influence of temperature variations on IFSET,(a) Threshold voltage change with temperature [23] and (b) Measured and calculated data of the drain-to-source current versus gate-to-source voltage under different [24].

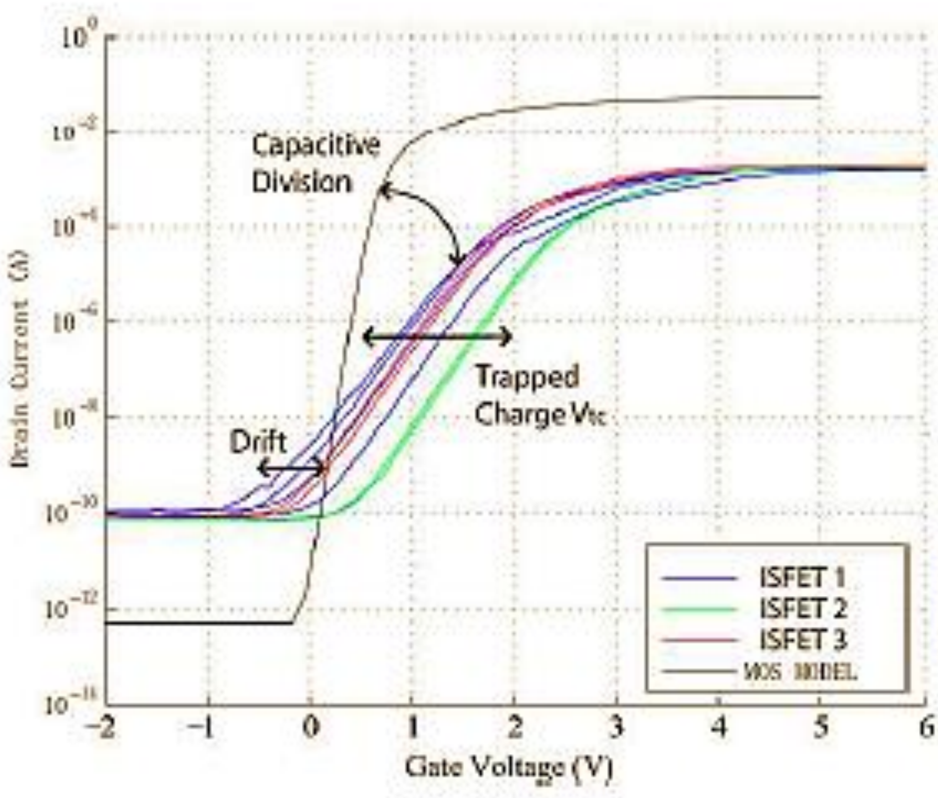


Figure 6: Non-ideal effects present in CMOS-based ISFETs[27].

Drift

Technically, drift is the slow monotonic shift in the ISFET readout output in pH and temperature constant, as shown in Figure 7a. Consequently, it adds uncertainty to the measurements.

In [29], an ISFET fabricated by an unmodified commercial CMOS process was proven to display significant threshold voltage drift, which is attributed to the diffusion of hydrogen ions into the passivation layer, as shown in Figure 7b. Another research [31–33] approved the ISFET drift based on different reasons.

Membrane insulator

The sensing membrane insulator plays a key role in the performance of ISFET. In [33], the pH sensitivity of ISFETs was proven to emerge from cooperation of protons with ISFET gate surface sites, as shown in Figure 8. Comparison of the theoretical sensitivities of different oxidation methods (i.e., SiO_2, ALO_2, and Ta_2O_5) is based on the following equation:

$$\frac{\delta\psi_0}{\delta pH_B} = -2.3\,\frac{kT}{q}\,\alpha$$

With $\alpha = \frac{1}{\left(\frac{2.3kTC_{dif}}{q^2\beta_{int}}\right)+1}$ **(3.1)**

Where, (α) is a dimensionless sensitivity parameter. The value of (α) varies between 0 and 1 depending on the intrinsic buffer capacity and the differential capacitance.

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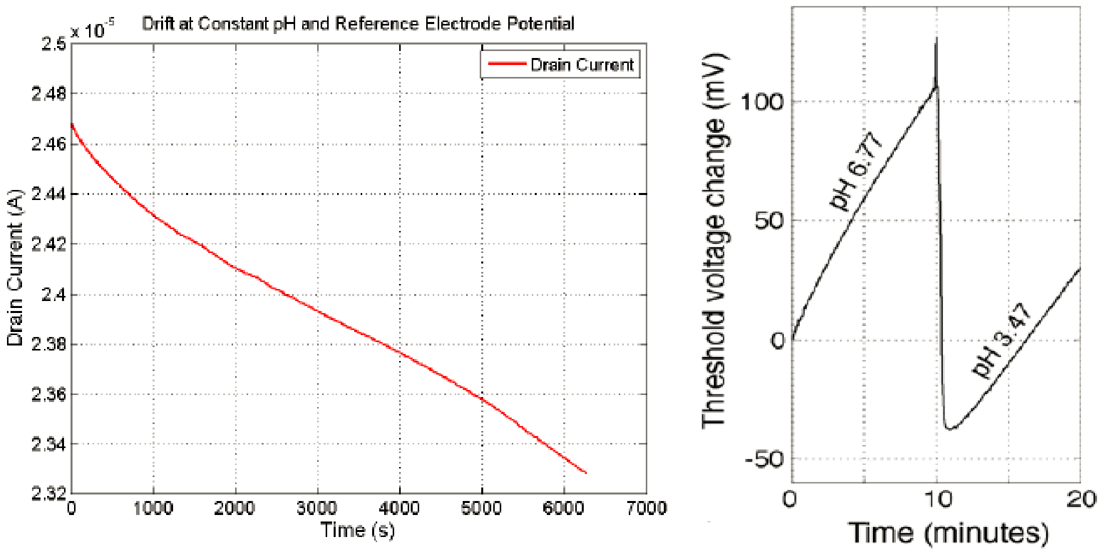

Figure 7: Drift in ISFET,(a) Under a condition of constant pH, temperature, and reference electrode potential, the ISFET signal may drift,b) Graphs of the ISFET threshold voltage response to a change in pH solution [29].

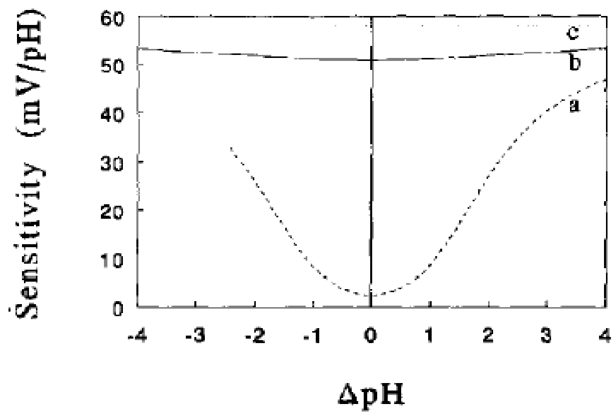

Figure 8: Comparison of the theoretical sensitivities of (a) SiO_2, (b) ALO_2, and (c) Ta_2O_5 [33].

More details on this issue in [34] discussed the basic concepts and current trends in the area of electrolyte–insulator-semiconductor chemical sensors with inorganic gate dielectrics.

Noise:

In this paper, noise in ISFET is highlighted for two reasons. First, any source of noise introduced in the sensor will force a major limit on the resolution and accuracy of the estimated measurements, and consequently, the sensitivity of ISFET. Second, an investigation of noise can provide a minuscule probe and additional knowledge on the fundamental physical mechanisms of the sensor. Although the significance of noise is settled and has been widely considered in MOSFETs and additional electronic devices[35],the noise in sensor appoints the resolution of analog readout, which comprises its basic analysis. Moreover, the substance interface to the standard MOSFET commitment adds noise to the sensing system [36].

DEVICE COMPENSATION OF NON-IDEALITIES IN ISFET DEVICE

In 2016, Nicolas et al. [21] introduced an extensive review of CMOS ISFET as an emerging trend. They divided the compensation into two categories, namely, system and device compensations. We will focus on device compensation because in our viewpoint, the device is more robust than the system. Notably, most articles combine two or more methods to compensate for two or more non-idealities; hence in this section, we will recall the most important contributions of each non-ideal compensation.

Temperature

In the case of temperature, no more solutions for device adaptation methods are presented. One that worked in this issue [38,39] presented a nonlinear temperature compensation method based on the theoretical work (i.e., off-chip implementation) for formulating a body-effect-based ISFET drain current expression. The method used in [39] is a multichannel system that controls temperature to an accuracy of 0.5 °C with the trimming of the temperature sensors to a precision of 0.1 C. The method was performed using an array of 10 temperature sensors distributed across the chip outputting the differential signal. This method is also suitable for offset cancelation of temperature compensation technique, as shown in Figure 9. In 2014, Sohbati et al. [40] presented the readout converter, which is a simple compensation technique that eliminates the effect of temperature on measurements and is also compatible with offset compensation.

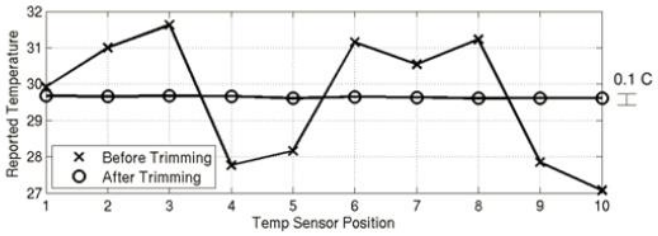

Figure 9: Trimming of the temperature sensors to a precision of 0.1 C [39].

Offset

DC offset is the most promising issue in the literature because it is the most limiting non-ideality in ISFET. The most promising method in the literature is the programmable gate-ISFET (PG-ISFET). It is a device operating point that can be tuned by applying a voltage bias to a control gate capacitively coupled to the floating gate using a capacitor [42–47]. Georgiou and Toumazou (2009) introduced a device that uses a capacitively coupled floating gate to allow tunability of its operating point in counteracting the presence of trapped charge, thereby allowing operation within a tolerable gate voltage range. Feedback allows integration of the device, as well as cancellation of reduced sensitivity due to additional capacitance of a programmable gate [43,44]. Liu and Toumazou (2010) used the same method coupled with chemical signal sensitivity, which can be enhanced due to the feedback system on the sensing node by using auto-zero amplifiers with a ping-pong structure; operation protocol has been developed to realize the circuit, reduce error, and achieve continuous measurement [43]. Wong Jr. et al. (2010) used PG-ISFET sensor integrated with standard MOSFETs to form a chemical inverter that switches when a chemical reaction reaches a certain threshold. By employing feedback to the electrical gate, rapid switching is achieved for changes in pH level, as low as 0.1 unit [44]. Clinton et al. (2011) used auto-calibration algorithm by biasing PG-ISFET to eliminate sensor mismatch in CMOS-based chemical imagers [45]. Miscourides and Georgiou (2016) used a linear PG-ISFET array operating in current mode and biased in velocity saturation to provide linear pH–current conversion while providing high linearity and transconductance (intrinsic pH gain) [46]. PG-ISFET compensation involves several phases of operation that may increase system complexity.

Other methods to compensate for offset issue were distributed to hot-electron injection method for programming the threshold voltages by Georgiou and Toumazou (2009) [47], Fowler–Nordheim electron tunneling used to program charges in the floating gate of an ISFET to control its threshold voltage by Al-Ahdal and Toumazou (2011) [48], and reset switch on the floating gate for offset reset [27], [40], [49].

Drift

As previously mentioned, most compensation methods (e.g., offset or temperature compensation) are suitable for drift compensation. Hence, the method in [49] and [27], in addition to offset compensation, are used to eliminate accumulated drift. Shah and Christen (2013) introduced pulse width modulation (PWM) circuit to cycle the vertical electric field in ISFET that controls the inversion layer in FET, which can be used to reset the inherent drift behavior of ISFET [51,52]. Sohbati and Toumazou (2014) used the dimension and shape effects in terms of different issues, one of which is drift, by increasing the ISFET sensing area; as a function of the perimeter of the gate extension metal layers, decoupling capacitors may degrade the coupling efficiency of the ISFET.

Membrane insulator

Insulator thickness may also influence the binding interactions that occur between the sub-surface layer and electrolyte, with low sensitivity for approximately 1-nm-thin membranes [32]. Amorphous membranes may provide high sensitivity; their effect (summarized in the sub-Nernstian parameter) adds nonlinearity over the working range. More work is needed to overcome this problem.

Noise

Regarding noise non-idealities, Huang et al. introduced a pixel-to-pixel ISFET threshold where voltage mismatch is reduced by correlated double-sampling (CDS) readout [53–55]. Kim et al. proposed a sensor that generates a differential signal between bio-samples and reference buffer solution with significant reduction in offset and gains fixed pattern noise by employing on-chip CDS circuits [55], [56]. Moser et al. (2015) performed PWM to encode the pH in time based on APS [57].

DESIGN AND APPLICATION REQUIREMENTS FOR DNA SEQUENCING BASED ON ISFET

ISFET is popularly used in DNA sequencing because of its capability to work massive parallel sensor array (DNA microarray). However, different factors need to be considered when building a system depending on a specific application, as well as design requirements. Moser at. al. (2016) [21] introduced an excellent guideline for most custom ISFET DNA applications. We will focus on the requirements of DNA sequencing design and application based on ISFET and why ISFET sensor array is used for DNA sequencing.

DNA microarrays have increased across the board arrangement for genomic diagnosis considering their high throughput detection[58]. DNA molecules with known capacities or successions are immobilized on various locations in a DNA chip, and obscure DNA molecules are removed. By spreading a solution containing obscure DNA onto the chip, the formation of double-stranded DNA is possible if the obscure DNA molecules have coordinated arrangements with the known DNA molecules. This method determines the DNA capacities in a cell by counting double-stranded DNA locations in the chip. Conventional DNA microarrays depend on optical detection, whereas the target DNA is labeled with fluorophores and distinguished by optical laser scanner. This method, nonetheless, is complicated because it requires a skilled person and a costly and non-portable device. For a portable and accurate system, another biosensor chip must be created. Electrical or electronic detection utilizing CMOS-integrated circuits has incredible potential because it decreases the labeling procedure, accomplishes high accuracy and real-time results, and offers a low cost and small size alternative device. Different methods have been explored in view of detecting the change in capacitance, current, and electric potential by interactions in molecules. The detection of electric potential change based on ISFET indicates remarkable sensitivity for ion concentrations in DNA sequence, including single-nucleotide polymorphisms [60–64]. Although several recently announced ISFET arrays are devoted to DNA sequencing applications [17],65–69], the majority of these studies only present the application rather than the execution of their framework in a genuine sequencing situation. The mission for the fast and inexpensive genome sequencing has fundamentally added to ISFET works.

From the aforementioned statements, DNA sequencing is extremely broad, and this study cannot give full advice on the subject. However, the intent of this research is to identify the requirements of DNA sequencing based on ISFET, which are listed as follows:

- Faster sequencing: large sensor array
- High resolution: by dense pixels with small pixel size
- Symmetry architecture: compact pixel (rows and columns)
- High pH resolution: effect of different factors on this point (i.e., DNA sample concentration)
- Time: short measurement time
- Accuracy: by applying contact image

The above requirements are clear and discussed in this review except for the measurement time. Short measurement time is required for DNA sequencing application. Measurement time is incorporated into the drift compensation; however, DC offset cancellation is also needed. The time window, among which measurement must be considered, is basic to decide if a reset plan can be utilized. Reset schemes that include a switch to the gate eliminate the measurement time because of leakage. More tests should be completed on these structures to promote characterization. Generally, these solutions aggregate to utilizing in-pixel sensor adaptation for offset and drift, which, as expressed recently, tend to prompt a more robust and scalable array sensor [21].

Different topologies have been used in the literature for DNA sequencing. Thus, it is difficult to obtain a guideline for designing a topology for DNA sequencing device. Moser et al. (2016) laid a simplified guideline for ISFET applications [21] and provided a detailed process flow for designing a DNA sequencing device to be considered when designing the circuit. Figure 10 shows the steps of the process that simplifies design and application requirements for DNA sequencing based on ISFET.

RECOMMENDATIONS

Based on extensive studies from the literature, several recommendations useful for future research are presented below.

- Introduce new compensation for ISFET non-idealities in terms of DC offset, drift, and temperature, especially on device level, to achieve robust system performance.
- On the design side, we recommend the compact pixel with militarized pixel size, as well as large-array device, to cancel noise and capacitive scaling, thereby enhancing SNR.

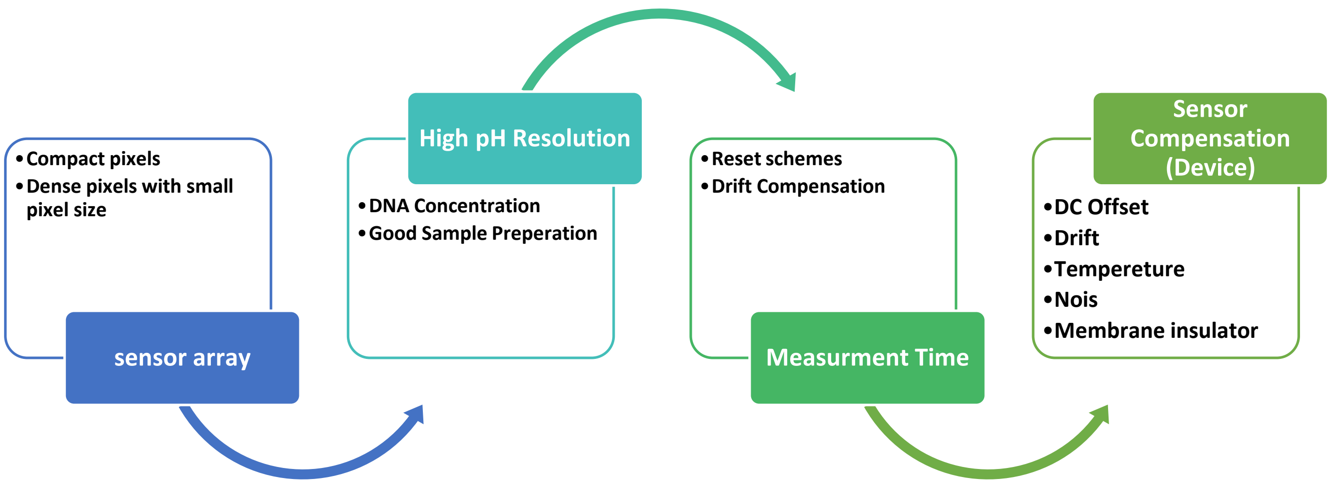

Figure 10: Design and application requirements for DNA sequencing based on ISFET.

- Support the ISFET pH concentration report by image sensor to achieve molecule contact image.
- On the application side, gradually introduce a new application in DNA diagnostics that deal with DNA biomarkers to detect and diagnose cancers and tumors and the effect of epigenetic factors. This suggestion synchronizes with the recommendations by health organizations that Gen (i.e., DNA sequencing) is one of the most important methods satisfying early cancer detection.
- More importantly, we found that the literature lacks clinical validation of the suitability of ISFET devices for DNA molecule detection and its suitability with POC concepts.

Nevertheless, the above list of recommendations is not exhaustive.

SUMMARY

DNA sequencing based on ISFET is gaining importance because of its capability to work massive parallel sensor arrays, especially when integrated in CMOS technology fabrication with inherent scalability of silicon technology at low cost. In the last decade, methods used to design different topologies of ISFET sensor array device have been introduced. In addition, we discussed the non-idealities in CMOS ISFET device and its compensations in terms of device level. Furthermore, application and design requirements of DNA sequencing based on ISFET have been extracted from the literature as a process flow for designers' use. We also mentioned several recommendations useful for future research (as concluded from previous works). We believe in the importance of this application in terms of POC diagnostics as a portable, low cost, and non-technical operator device, which may encourage evolution in the biosensors.

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