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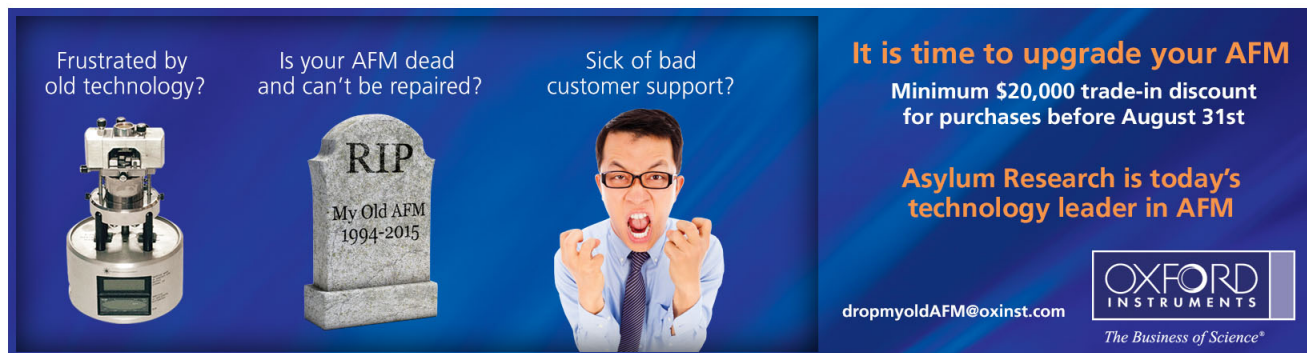
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Real-time label-free quantitative monitoring of biomolecules without surface binding by floating-gate complementary metal-oxide semiconductor sensor array integrated with readout circuitry

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We report a label-free field-effect sensing array integrated with complementary metal-oxide semiconductor (CMOS) readout circuitry to detect the surface potential determined by the negative charge in DNA molecules. For real-time DNA quantification, we have demonstrated the measurements of DNA molecules without immobilizing them on the sensing surface which is composed of an array of floating-gate CMOS transistors. This nonimmobilizing technique allows the continuous monitoring of the amount of charged molecules by injecting DNA solutions sequentially. We have carried out the real-time quantitative measurement of 19 bp oligonucleotides and analyzed its sensitivity as a function of pH in buffer solutions. © 2007 American Institute of Physics. [DOI: 10.1063/1.2803848]

Real-time detection and quantification of the products generated by polymerase chain reaction (PCR) process have drawn attention as an emerging technology for numerous applications.¹ Various optical sensing methods have been introduced to monitor the amount of PCR products after each cycle. One of them is to detect fluorescent signal from Taq-Man probes or intercalating dyes.² These techniques require many complex optical components, so that it is difficult to implement them in a miniaturized analysis system. Miniaturization is important for genetic testing and medical diagnostics at the point of care. Numerous researches have been reported on the realization of PCR process in microchips for efficient consumption of power and reagents, and fast response.^{3,4}

Recently, various electrochemical techniques have been investigated for detecting deoxyribonucleic acid (DNA) molecules based on sensing their intrinsic properties.⁵ Among these methods, the detection of the intrinsic charges in DNA molecules is one of the most attractive approaches because they provide many advantages. Typically, ion-sensitive field-effect devices fabricated in complementary metal-oxide semiconductor (CMOS) process are used for label-free detection with a high sensitivity in a small form factor. These sensors can be easily miniaturized at low cost using standard integrated circuit fabrication processes and provide the capability of monolithic integration with readout circuitry to enhance the signal-to-noise ratio.^{6,7} However, in order to detect DNA molecules, it is required to immobilize single-stranded

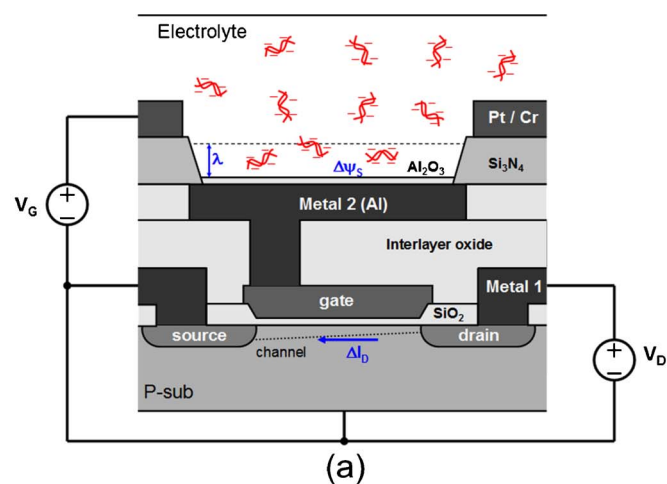
probes on the surface of the ion-sensitive devices for the hybridization of single-stranded complementary target molecules in the electrolyte solution⁸⁻¹⁰ or to deposit a positively charged layer such as poly-L-lysine on the sensing electrodes to attract the negatively charged nucleic acid molecules.^{11,12} These additional processes increase reaction time and the complexity in detection protocols. Furthermore, it is difficult to incorporate the continuous monitoring for repetitive assays (e.g., real-time quantitative PCR) for these protocols because the regeneration of the sensing surface requires a complicated rinsing sequence.

We have explored a nonsurface binding detection technique for the real-time sequential monitoring of DNA molecule concentrations. The charges of DNA molecules in a buffer solution can directly affect the potential of the field-effect transistors (FETs) where the gates of FETs are floated. The potential at the interface between electrolyte and electrodes depends on the amount of charges in a buffer solution within Debye length which is determined by the ionic strength of the electrolyte solution.¹³ The Debye length is given by

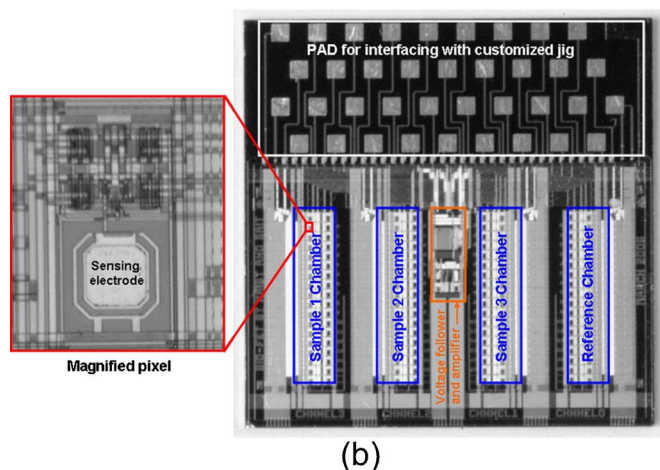
$$\lambda_D = \sqrt{\frac{kT\epsilon_e}{2z^2q^2n_0}}, \quad (1)$$

where k is the Boltzmann constant, T is the absolute temperature, ϵ_e is the electrolyte permittivity, z is the valence of the ions in the electrolyte, q is the elementary charge, and n_0 is the ionic strength of the electrolyte. The density of DNA molecules within the Debye length should be proportional to the DNA concentration in a bulk solution. Therefore, it is

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(a)



(b)

FIG. 1. (Color online) (a) Schematics of a floating-gate field-effect transistor sensing device and label-free detection. The surface potential change ($\Delta\psi_s$) is determined by the amount of DNA molecules in the Debye length (λ) of the solution. (b) Microphotographs of the fabricated sensor chip and the magnified view of the sensing element.

possible to quantitatively measure the concentration (or charge) of DNA molecules in a solution without any immobilization process. Accordingly, any surface modification process for immobilization is not necessary. Sensing surface can be easily regenerated by rinsing with a buffer solution. Thus, various sample solutions can be continuously monitored by injecting them sequentially to a microchannel where the sensing field-effect devices are located.

Figure 1(a) shows the device structure of a floating-gate FET sensing device in a DNA solution. Unlike the conventional ion-sensitive field-effect transistor,¹⁴ we implement the sensing surface with the top-layer metal ($90 \times 90 \mu\text{m}^2$ native $\text{Al}_2\text{O}_3/\text{Al}$) electrode which is connected to the floating gate of the sensing transistor. This allows the device to be fully compatible with standard CMOS process. Followed by CMOS processes, Pt (5000 Å)/Ti (500 Å) layer is deposited and patterned on the passivation layer to form a reference electrode to apply reference potential to the solution. For direct measurement of the surface potential change due to DNA molecules, we have adapted an operational amplifier circuit scheme by replacing a floating-gate sensing transistor in one of the input transistors in a differential input stage.¹⁵ The field-effect transistor connected to the sensing electrode is integrated into the differential input stage of the folded-cascade amplifier to configure a voltage follower. From this

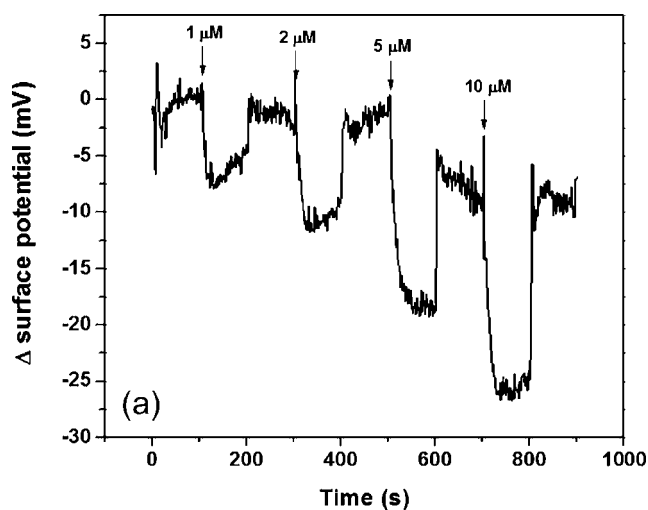


FIG. 2. Surface potential change between the sample and reference chamber as a function of time for the sequential injection of various 19 bp oligonucleotides solutions (1, 2, 5, and 10 μM , respectively). A 0.01 mM phosphate buffer solution is introduced before and after injecting oligonucleotides solutions for regenerating the sensing surface.

circuit configuration, we can acquire the output voltage equal to the surface potential of the sensing electrode.

A prototype sensor has been fabricated using 1 μm double-poly double-metal standard CMOS process. The sensor is composed of four 1×16 sensor arrays in each chamber, as shown in Fig. 1(b). In this prototype, the three pixel arrays are incorporated in their respective sample flow chambers defined by silicone rubber, so that three different bio-samples can be loaded and monitored simultaneously. One pixel array is exposed to a calibrated buffer solution in a reference chamber in order to compensate any background noise or signal drift in the electrolyte and electrode interfaces. The variable gain amplifier integrated on chip with the sensor performs the subtraction of signals (between the sample and the reference signal) and amplifies the difference.

We have detected and quantified DNA molecules in a buffer solution by measuring the change in the surface potential of the floating-gate sensing transistor. For the introduction of liquid samples, a customized plastic jig has been designed and assembled on the top of the fabricated chip. Liquid samples were injected by external syringe pumps. To increase a detectable range, we chose a phosphate buffer (PB) solution with very low concentration (0.01 mM) which consists of 0.001 mM Na_2HPO_4 and 0.009 mM NaH_2PO_4 . The pH and the Debye length of the PB solution are around 6 and 100 nm,¹⁶ respectively. Figure 2 shows the measured surface potential difference between the sample and reference chambers from a sequential injection of the sample solutions containing 19 bp oligonucleotides with various concentrations of 1, 2, 5, and 10 μM , respectively. The sensing electrodes were rinsed with the PB solution before and after injecting oligonucleotides solutions to the sample chamber. All the fluids were introduced at a flow rate of 3 ml/min and switched to different sample concentrations in every 100 s. The surface potential was decreased by injecting oligonucleotides solution and recovered by injecting buffer solution very rapidly. The transition of the potential can be measured below 30 s, realizing real-time quantification at high throughput.

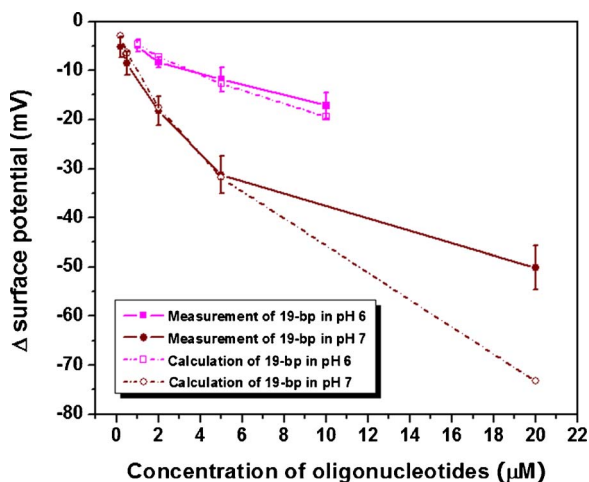


FIG. 3. (Color online) Surface potential for various oligonucleotides concentrations. Solid and dotted lines show the measurement results and the theoretical calculations as a function of various oligonucleotides concentrations for the two different pH buffer solutions of pH 6 and pH 7, respectively. The error bar represents the standard deviation from the measured responses from 16 elements in the sensor array.

According to the Debye-Hückel theory, the relationship between the surface charge and the potential at the electrolyte-electrode interface is described by using the Graham equation,¹⁶

$$\sigma = \sqrt{8\epsilon_e k T n_0} \sinh\left(\frac{zq\Psi}{2kT}\right), \quad (2)$$

where σ is surface charge and ψ is the difference between surface and bulk potential in solution. We can calculate the change in the surface potential due to the negative charge of oligonucleotides in the solution using the following equation:⁹

$$\Delta\Psi = \frac{2kT}{zq} \left[ar \sinh\left(\frac{\sigma_0 + \sigma_{\text{DNA}}}{\sqrt{8\epsilon_e k T n_{0+\text{DNA}}}}\right) - ar \sinh\left(\frac{\sigma_0}{\sqrt{8\epsilon_e k T n_0}}\right) \right], \quad (3)$$

where σ_0 and σ_{DNA} are initial surface charge density and negative charge density of DNA molecules, respectively. The initial surface charge of the sensing electrode can be estimated by pH sensitivity of aluminum oxide. The pH sensitivity was measured about -50 mV/pH and the pH value at the point of zero charge on the surface (pH_{pzc}) is known to be 8.¹⁷ Therefore, in the case of pH 6 solution, the initial surface charge (σ_0) is approximately 124 nC/cm^2 . In order to calculate the amount of negative charges (σ_{DNA}) in oligonucleotides, we assumed that charges are uniformly distributed in the solution and fully activated within the Debye length which should be changed by the ionic concentration of the solution. Furthermore, we assumed that the additional ionic strength of oligonucleotides solution ($n_{0+\text{DNA}}$) is by 19 times higher than that of a 1:1 electrolyte because each oligonucleotide has 19 sequential molecules. The charges (σ_{DNA}) of $1 \mu\text{M}$ 19 bp oligonucleotides solution in the Debye length of 58.7 nm will be -10.7 nC/cm^2 . The surface potential can be calculated from this charge and plotted in dotted lines in Fig. 3. The calculation is in good agreement with the experimental results, as shown in Fig. 3.

Figure 3 also shows the quantitative measurement compared with the theoretical calculation of the change in surface potential by oligonucleotides in two buffer solutions with pH of 6 and 7, respectively. From Eq. (3), the change of surface potential depends on the initial surface charge. For investigating the possibility of increasing the sensitivity, we have measured the sensor response with 0.01 mM PB solution which has different compositions (0.006 mM Na_2HPO_4 and 0.004 mM NaH_2PO_4) to change the pH level of the solution from pH 6 to pH 7. The surface potential change is significantly enhanced (absolute potential is decreased) in the solution of pH 7 as compared with that in the solution of pH 6. Actually, we could detect 200 nM oligonucleotides in the solution of pH 7; that is, the sensitivity is increased fivefold. This is because the initial surface charge in pH 7 solution becomes smaller at the given fact that pH_{pzc} of our sensing electrode is 8. Therefore, the total surface charges [$\sigma_0 + \sigma_{\text{DNA}}$ in Eq. (3)] can be affected more significantly by the same amount of DNA molecules. Moreover, the improvement of the sensitivity leads a higher dynamic range by a factor of 10, so that our sensor can measure all the range of PCR products, which is typically from 1 to 100 ng/ μl .⁴ The 200 nM oligonucleotides of 19 bp is equivalent to 1.1 ng/ μl .

In summary, we have demonstrated the real-time label-free quantitative measurement of DNA molecules by using a fully integrated CMOS sensor array. Oligonucleotides with various concentrations have been successfully measured by the direct detection of DNA negative charges without any immobilization process on the sensing electrodes. The surface of the sensing electrodes can be easily regenerated by rinsing with a buffer solution followed by DNA injections, allowing the continuous monitoring of DNA samples. Furthermore, we have investigated the sensitivity enhancement in different buffer conditions and theoretically analyzed the effect.

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