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DNA Single Base Polymerization Detection Using CMOS FET-Based Redox Potential Sensor Array

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Abstract

The world's first DNA single base polymerization detection using FET-based redox potential sensor is presented. Since the accuracy of redox potential sensors is not affected by buffer conditions, DNA single base polymerization can be potentially detected with great accuracy. We specifically demonstrate successful detection using a 32×32 CMOS sensor array.

1. Introduction

DNA detection using FETs has the advantages of low cost, small form factor, and simplicity [1], and two particular FET-based methods, one based on direct charge detection method and the other based on pH-sensing have been well explored in the literature. Unfortunately, such methods are susceptible to buffer conditions, such as salt concentration and pH buffer capacity, which can seriously degrade their accuracy and reliability [2,3].

As an alternative, we herein demonstrate DNA single base polymerization detection using FET-based redox potential sensors for the first time (Fig. 1). Our novel approach detects electron transfer by an enzyme-catalyzed redox reaction of pyrophosphoric acid (PPi) released during DNA polymerization. The electron transfer is detected through electrical potential, as determined by the Nernst equation using an extended gate-FET with a gold electrode [5]. Since this method is not affected by buffer conditions, it has the potential for high-accuracy detection, and is therefore a promising candidate for improved FET-based DNA sequencing.

2. DNA single base polymerization detection using redox potential detection

The reaction system of DNA polymerization detection using enzyme-catalyzed redox potential detection [4,5] is illustrated in Fig. 2. DNA polymerization causes a release of PPi, which is finally converted into redox agents (in this case, hexacyanoferrate(II) and hexacyanoferrate(III)) with help of three enzymes.

The reaction changes the ratio of the oxidant and reductant concentrations, after which the electrode potential, E, changes according to the Nernst equation (1).

$$E = E^0 + \frac{RT}{nF}\ln\frac{[\mathrm{Ox}]}{[\mathrm{Red}]} \quad (1)$$

Here, E^0 is the standard electrode potential, R is the gas constant, T is absolute temperature, F is the Faraday constant, and n is the number of transition electron. At room temperature, the theoretical sensitivity given by eq. (1) is 59.2 mV/decade. The potential change is detected by a CMOS FET sensor equipped with a gold electrode.

3. CMOS Sensor Array and Measurement Setup

Fig. 3 shows the physical implementation of the proposed CMOS FET-based redox potential sensor array. Using a post-process, we formed gold working electrodes on a CMOS sensor array chip, as illustrated in Fig. 3(a). The source-drain follower [6] shown in Fig. 3(b) was implemented and used for low-power and stable voltage transfer.

In order to evaluate the proposed sensor array, we designed and fabricated a test chip in 2P3M 0.6 μm standard CMOS technology, as depicted in Fig. 3(c). The array size is 32×32. The gold electrodes are each 20 μm ×25 μm, and are arrayed with a pitch of 120 μm ×120 μm. The occupied footprint is 7.5 mm ×7.5 mm, including peripheral circuits.

Fig. 4 illustrates the measurement setup for DNA single base polymerization detection using the proposed sensor array. A solution flow cell was used to implement the flow-based sensing [7].

4. Measurement Results

The median of sensor array outputs was used for robust sensing as a countermeasure against device variations. Fig. 5(a) shows the measured potential change dependent on the ratio of hexacyanoferrate (III) to (II). A sensitivity of 57.0 mV/decade was verified, which matches well with eq. (1) (59.2 mV/decade). Fig. 5(b) shows a histogram of sensor array outputs when the ratio of hexacyanoferrate (III) to (II) is 10^0. These results demonstrate that the proposed sensor array can successfully detect changes in oxidant and reductant concentrations with maintaining small potential variations (σ is 7.17 mV).

Fig. 6 is the measured potential change dependent on PPi concentration. This result demonstrates a sensitivity of -12.3 mV/decade to a logarithmic concentration of PPi in the range from 0.05 to 1 mM, along with a high degree of linearity (r^2 = 0.999). The sensitivity which is degraded from eq. (1) (-59.2 mV/decade) can be quantitatively substantiated by taking the reaction equilibrium constant into account.

Fig. 7 shows the measured time course of potential change in DNA single base polymerization. Note that there is a 5.65 mV difference between the reaction solution with a mismatched base (dCTP) and that with a matched base (dTTP). This voltage difference is quantitatively reasonable given the PPi detection result, which achieves sufficient SNR of more than 20dB. Thus, we conclude that the proposed sensor array successfully detected the DNA single base polymerization.

5. Conclusion

This is the first time DNA single base polymerization detection has been performed using a CMOS FET-based redox potential sensor array. Because we use an enzyme-catalyzed redox reaction, the sensor array is not affected by buffer conditions, yielding more stable and reliable DNA sequencing results. In experiments, a prototype of the proposed sensor array in 0.6 μm CMOS successfully performed DNA single base polymerization detection.

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References

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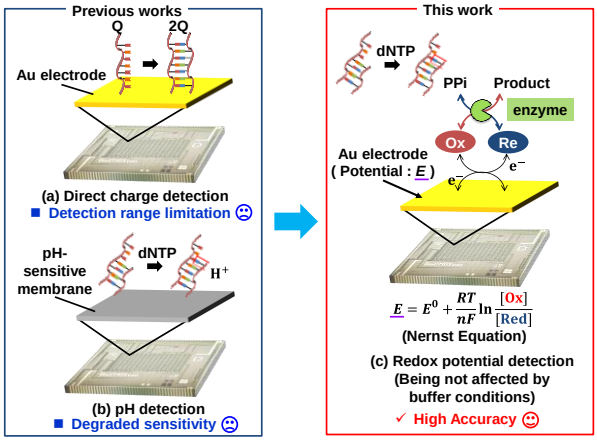

Fig. 1. Comparison of this work to previous works.

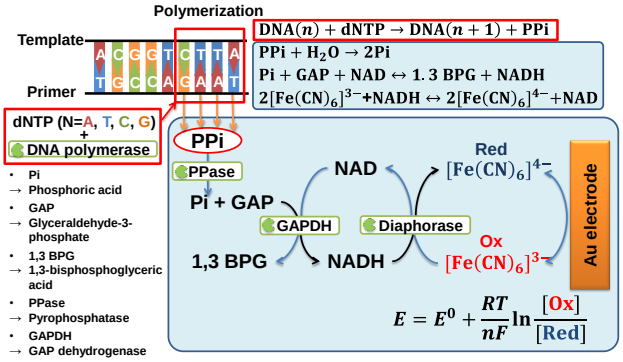

Fig. 2. Enzyme-catalyzed reaction of DNA polymerization.

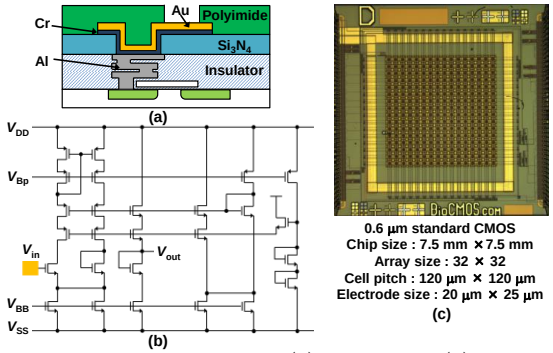

Fig. 3. Proposed sensor array (a) Electrode, (b) Schematic of source-drain follower, (c) Chip microphotograph.

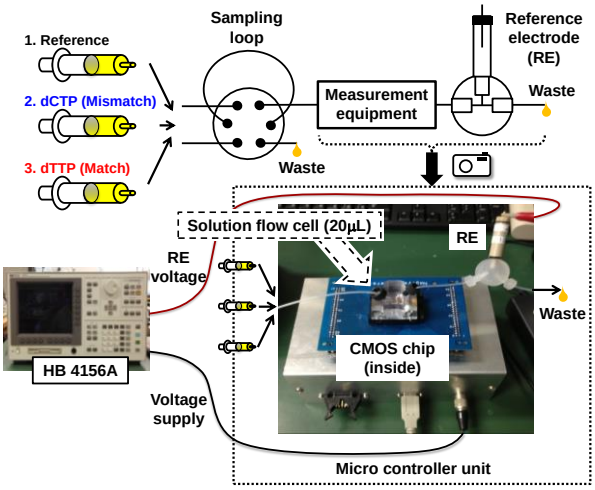

Fig. 4. Measurement setup for DNA single base polymerization detection.

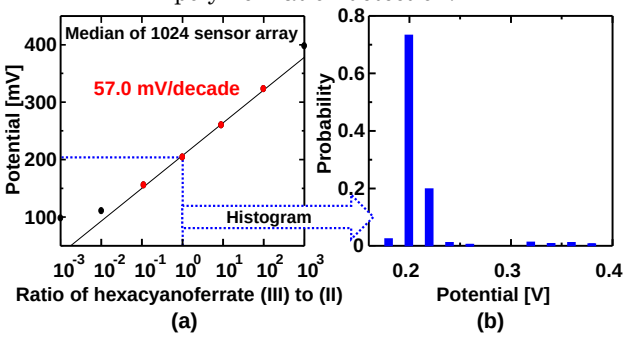

Fig. 5. (a) Measured potential change dependent on the ratio of hexacyanoferrate (III) to (II). (b) Histogram when the ratio of hexacyanoferrate (III) to (II) is 10^0.

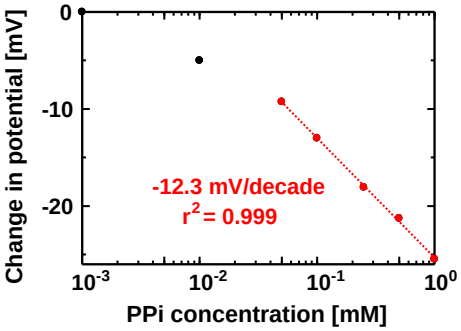

Fig. 6. Measured potential change dependent on PPi concentration.

Tested base sequence
Template : 3'- GTGTG AGTGT CAAAA GTGAA ATGAG ATAGC – 5'
Primer : 5'- CACAC TCACA GTTTT CACTT – 3'

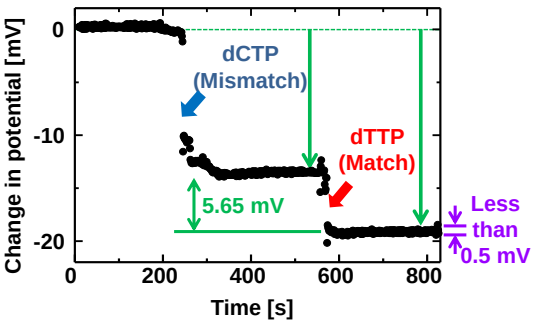

Fig. 7. Measured time course of potential change in DNA single base polymerization.