A nano-metallic-particles-based CMOS image sensor for DNA detection*

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In this paper we report on a study of the CMOS image sensor detection of DNA based on self-assembled nano-metallic particles, which are selectively deposited on the surface of the passive image sensor. The nano-metallic particles effectively block the optical radiation in the visible spectrum of ordinary light source. When such a technical method is applied to DNA detection, the requirement for a special UV light source in the most popular fluorescence is eliminated. The DNA detection methodology is tested on a CMOS sensor chip fabricated using a standard 0.5 μm CMOS process. It is demonstrated that the approach is highly selective to detecting even a signal-base mismatched DNA target with an extremely-low-concentration DNA sample down to 10 pM under an ordinary light source.

Keywords: CMOS image sensor, nano-metallic particles, DNA detection, 0.5 μm CMOS process

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1. Introduction

Compact chip-based DNA detection methods have been applied to clinical diagnosis to detect genetic diseases (e.g. Thalassaemia), the presence of virus (e.g. bird-flu virus) and kinship or forensic relation as juridical evidence[1−4] to reduce the cost and the time spent on analysis for a decade. At the same time, the international semiconductor industry has improved into the nano-scale regime to result in high performance and low cost very large scale integrated (VLSI) chip production. If combined with the advanced integrated circuit (IC) technologies, DNA chips will have the potential to become lower cost, more sensitive, and more selective. However, the DNA signal itself is not friendly to electronic detection. Some mediums are needed to transduce it into an electronic-detectable signal. A developed detection method is based on fluorescence property of modified DNA targets.[5] However, as a traditional fluorescence based DNA detection method, the use of an organic fluorescence label results in signal intensity degradation with time due to fluorescence quenching.[6,7] It also requires external excitation such as laser or UV light source to excite the fluorescent labels that reduce the portability of such a system. Moreover, the colour information is needed for fluorescence based detection, thereby making it difficult to be fabricated in ordinary IC foundries.

Nano-particles can also be used as transducer for electronic detection.[8] Compared with fluorescence based detection, this method has the advantages of (1) a friendly bio-electronic interface; (2) a time-stable signal; (3) no need of an external optical system. The three advantages lead to more consistent experimental results. In addition, the method is fully compatible with standard CMOS technology without the need of any modification. In this paper, a new DNA detection sensor, combining the optical property of nano-metallic particles and a CMOS Active-Pixel Sensor (APS), is introduced. In this sensor gold nano-particles are used to transduce the bio-signal of

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matched DNA targets on the top surface of CMOS image sensor into electronic-detectable signal, and silver nano-particle deposition is used to amplify the signal. The opacity change of the nano-metallic particle can be easily detected by the APS. The optical sensor design, experimental procedure, optical properties of the gold nano-particles, and experimental results based on a 0.5 μm CMOS technology are reported.

2. CMOS image sensor design

To study the optical property of nano-particles and their application in DNA detection on an IC platform, a CMOS APS is first designed. The structure of each sensor cell is composed of a traditional 3-transistor structure as shown in Fig. 1. Transistor M1 is the reset transistor of the charge-sensing node N1, transistor M2 is the in-pixel amplifier, and a switch transistor M3 is used to select the pixel to be connected to the readout bus. The photodiode is formed with an n^+/p^- structure as a charge sensing element. A metal layer is used as a light shield to cover the signal processing circuits leaving an optical window for detection as shown in Fig. 2. The top silicon dioxide layer, which is opaque to the visible light, is used as a solid support for the following DNA immobilization and hybridization. The cell size is 15 μm×20 μm when implemented in a 0.5 μm CMOS process with a fill factor of 50%. The targeted operation voltage is 3V (V_{DD}).

The opacity is converted into current by the photo diode, and the voltage at the node N1 is integrated and amplified to output voltage by the APS. After reset, the voltage begins to drop down, and the rate is proportional to the photocurrent and thus the light intensity. The hybridization of matched DNA is detected by measuring the opacity change at the site with DNA probes. A nano-metallic layer at the location with matched DNA samples is formed on the top of the photodiode after hybridization and silver enhancement, which results in the changes of light intensity and voltage-drop rate.

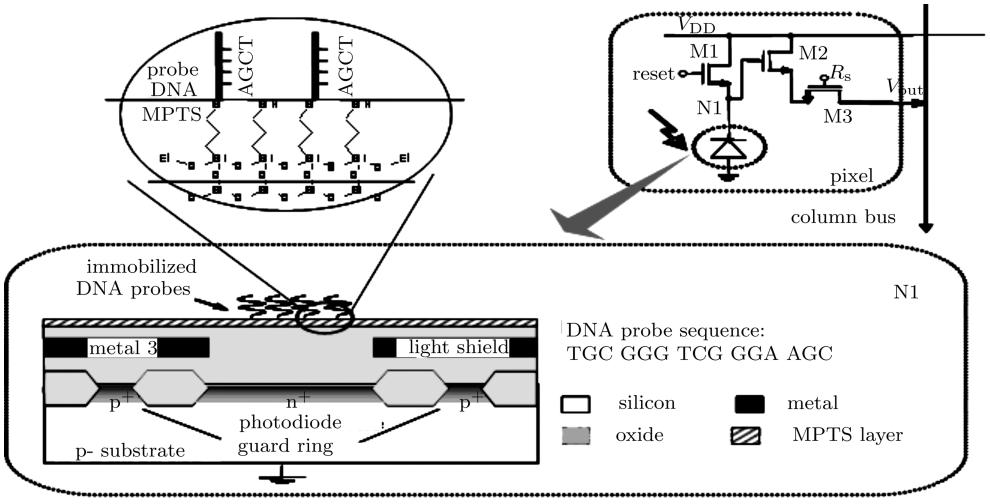


Fig. 1. Schematic and cross-section of an APS cell in the chip. On the top of the detection region are mercaptopropyltrimethoxysilane (MPTS) layer and immobilized DNA. V_{DD} is the power supply voltage, R_s is the source resistance, and V_{out} is the output voltage.

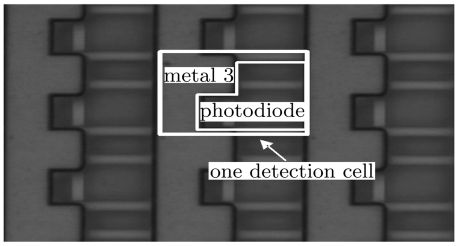


Fig. 2. Micrograph of a small array of a DNA detection cell. The signal process circuits are covered by a metal layer with an optical window uncovered for detection.

3. Surface treatment and DNA detection process

After the sensor fabrication, the surface must be modified before it is used to detect the DNA targets. The treatment process begins with surface cleaning by acetone, 95% ethanol, and deionized water, sequentially. After descum in a oxygen plasma oven for 5 min, the top SiO_2 passivation layer is treated with 1%

MPTS 95% EtOH, and a 16 mM acetic acid mixture solution for 30 min at room temperature to form covalent bonds with the DNA probes.[9] The methoxyl groups on one end of the MPTS molecule can react with the SiOH groups on the SiO_2 surface. On the other end, the thiol group could react with another thiol group, which has been chemically labeled on the 5-terminal of DNA probes. Then different kinds of 15 mer DNA probes with known sequences for interesting DNA targets are diluted in citrate buffer to a final concentration of 40 μM and attached to the chip surface by pipetting. After the chip is incubated in a moist chamber overnight for immobilization and rinsed by washing buffer, it is ready for detection. The structure of the sensor with immobilized DNA probes is also illustrated in Fig. 1.

After surface treatment, the sensor is ready for DNA target detection. The detection process is illustrated in Fig. 3, which includes two hybridization steps and one silver enhancement step. In the first hybridization, the DNA targets, with special added DNA sequence tails, are applied to the DNA array in solution form by pipetting for 2 h at 37 °C. Because of the intrinsic selectivity of DNA sequences, only the DNA samples that match one of the immobilized probes can be captured, while the mismatched DNA targets will be washed away. However, this biosignal of DNA itself cannot be electronically detected easily. Gold nanoparticles are used to transduce it into an electronically detectable signal.

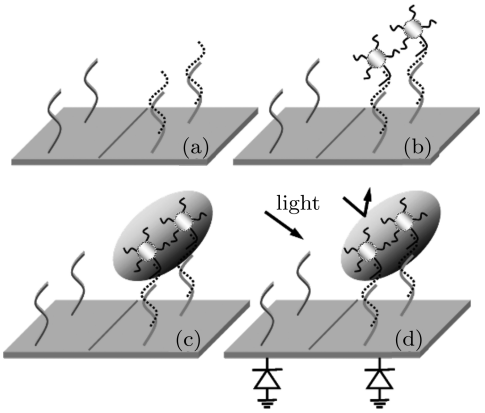


Fig. 3. Illustration of detection. a) hybridization of the DNA targets, b) hybridization with the gold nanoparticle labeled DNA sequence, c) silver enhancement, where a metallic layer is formed at the location with matched DNA probes, d) readout of the signal chain from the underneath APS array. The length of DNA target sequence is 15 bases.

In the second hybridization, the chip is immerged into the solution of gold nanoparticles, which is modified with DNA molecules complementary to the specially added tails of the DNA targets. After a 4-hour hybridization at 37 °C, the gold nanoparticles will be captured by the complementary tails and form a thin gold layer, only at the location with the captured DNA targets. However, the gold particles themselves are too sparse to block the light effectively, thus to be reliably detected. A silver enhancement step, using a silver nitrate solution allows silver nanoparticles to be deposited only at the site with the gold nanoparticles and self-assembled into a larger and thicker metallic layer to amplify the electronically detectable signal.

Figure 4 shows the scanning electron microscope (SEM) images at the site with the matched and mismatched DNA after an 8-minute silver enhancement, indicating a successful enlargement of the gold nanoparticle and the opaqueness change at the location with matched DNA samples. The opaqueness at a location with a particular DNA probe can be detected using the underneath CMOS image sensor under room light.

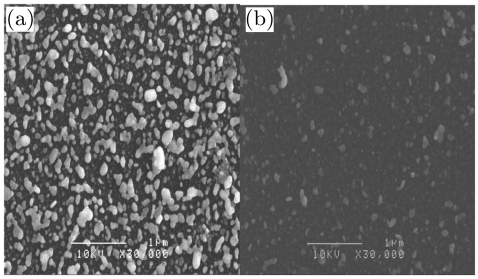


Fig. 4. SEM images of the DNA sensor surface after an 8 min silver enhancement at the location: (a) with matched DNA, (b) without matched DNA.

4. Results and discussion

The characteristics of the CMOS APS, the optical property of the self-assembled nanoparticles and the performance for the application of DNA detection are measured and discussed in this section. To study the effectiveness of the optical detection, the characteristics of the photodiode used in the CMOS APS was first calibrated. The dark current and the current level under illumination with different intensities are shown in Fig. 5, indicating a reasonable optical response. The optical current is six orders of magnitude more than that of the dark current under normal indoor light, which indicates that the sensor can provide sufficient dynamic range for optical detection.

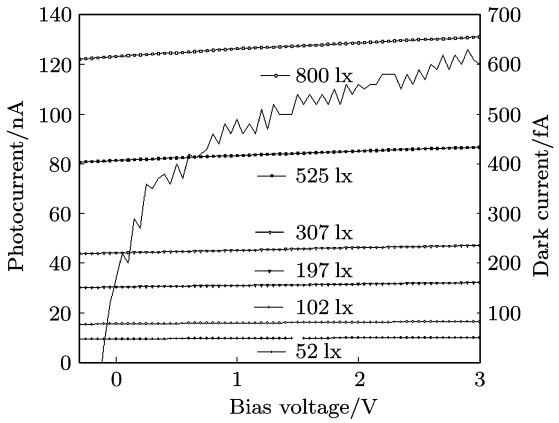


Fig. 5. Dark current and photocurrent characteristics of a photodiode in the detection cell under illumination with different intensities. The ratio of signal current to dark current is more than six orders, indicating the sufficient dynamic range for optical detection.

As the opaqueness of the region with matched DNA probes and sample may depend on the wavelength of the light source, the spectral responses of the sensor at the location with matched and mismatched DNA samples are measured and shown in Fig. 6. It is observed that the deposited gold nano-particles and silver precipitates can effectively block optical radiations of all wavelengths in the visible light region. At the same time, the region with single base mismatched DNA probes, thus no metal nanoparticle deposition is transparent to most optical radiations in the visible spectrum as indicated by its small differences in respect to the reference (the region without DNA materials). Therefore, an ordinary light source can be used in the detection process without the need of using expensive UV light sources. It should be noted that the detection method is very selective so that even a mismatch with a single base in the DNA chain can be easily identified.

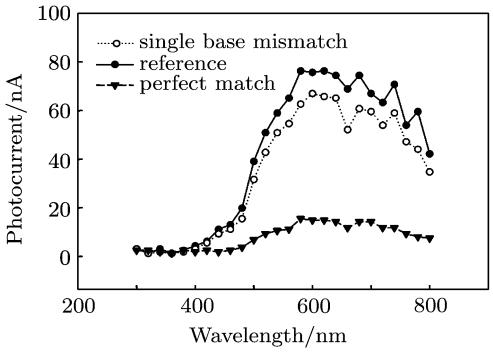


Fig. 6. Spectral responses of the detection cell matched and single base mismatched DNA samples. The reference indicates the characteristics of the original photo diode without any material on top.

The time of silver enhancement can affect the error margin for detecting matched and mismatched DNA samples. The relationships between silver enhancement time and the amount of transmitted light detected by the optical sensor are shown in Fig. 7, indicating that the silver enhancement time is not critical. Hence, by increasing the silver enhancement time, the sensitivity of the optical detection can be increased as shown in Fig. 8. It has been demonstrated that an extremely low DNA concentration down to 10 pM can be detected using the described method.

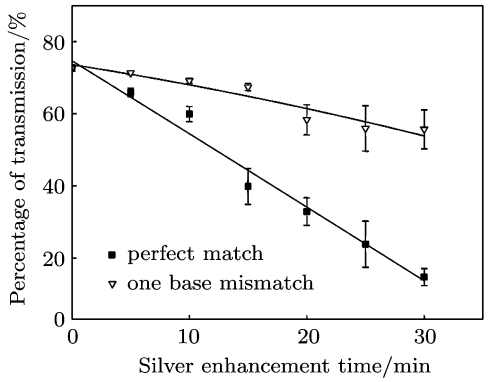


Fig. 7. Measured transmission coefficients at the location of matched and mismatched DNA samples, as a function of the silver enhancement time, indicating the silver enhancement time is not critical.

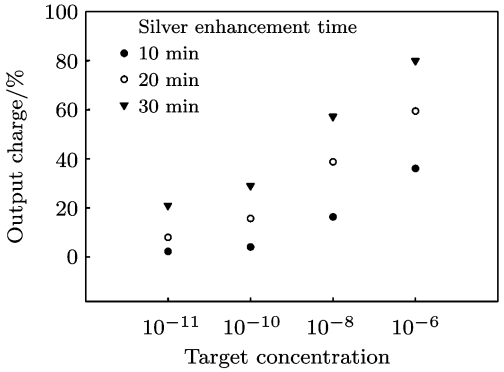


Fig. 8. Relationship between the electrical current and target DNA concentration, indicating a detection of an extremely low DNA concentration down to 10 pM.

The actual output from the CMOS APS is shown in Fig. 9. After the bio-signal is converted into an output voltage by the APS, the matched and mismatched DNA information can be distinguished by the slope of the output. The voltage has been measured under different light sources from a dedicated halogen light to a common room light. Experimental results show that the system can be used in a wide range of light

sources unlike existing detection systems that rely on a UV light source for fluorescence excitation.

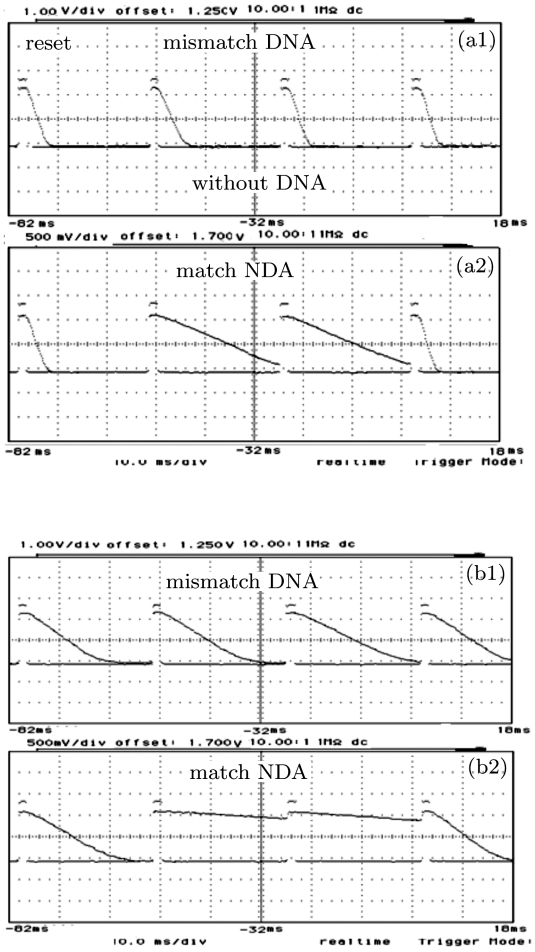


Fig. 9. Output voltage waveforms from underneath APS (one DNA probe covers two detection cells) with (a) a halogen 80 W light source, (b) a common room light source of 450 lx.

5. Array integration

The individual sensor cells designed in the previous section have been integrated into a 64×64 detection APS array with A/D converter (ADC) and correlated double sampling (CDS) circuit that reduces the process variations induced fixed pattern noise (FPN). A guard ring is included to prevent any crosstalk from the neighboring pixels. The fabricated array is then packaged and the exposed metal wiring is covered by an encapsulant to protect it from the chemical used in the detection process. The die photo and the package array are shown in Fig. 10.

The DNA probes are directly applied to the exposed sensor area as a 4×4 DNA array by a pin spotter machine, microprinting system (MicroSysTM 5100). Each spot covers an area of 100 μm in diameter, covering about 20 pixels to provide redundancy and reduce error reading. Some dummy rows/columns remain uncovered for background subtraction and dynamic range adjustment. The voltage readout under common room-light from the pixel is converted into an 8-bit image as shown in Fig. 11, together with the magnitude plot after adjusting the dynamic range by a built-in circuit. In the image, the three columns on the left have single-base mismatched DNA probes while the one on the right has perfectly matched probes with the sample. The matched DNA signal is clearly distinguishable even with a single-base mismatched one. The pictures show that the signal-to-background ratio has sufficient margin for detection even with an ordinary light source and a single base mismatched sample can be distinguished.

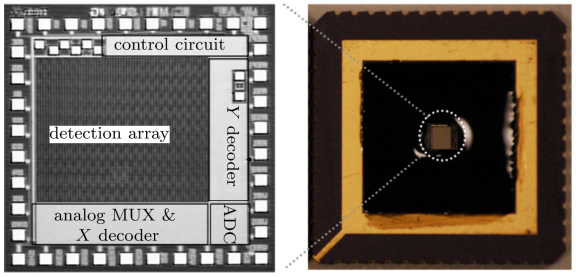


Fig. 10. (colour online) The die photo and the packaged chip with an encapsulant to protect it from the chemical used in the detection process.

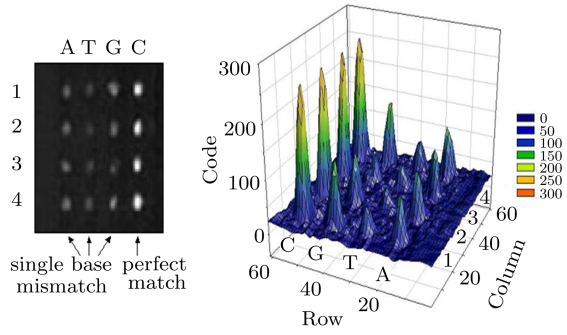


Fig. 11. (colour online) Captured image and relative intensity for matched (the most right) and single-base mismatched (the three columns on the left) DNA samples. The signal-to-background ratio has sufficient margin for detection even with an ordinary light source, and a single base mismatched sample can be distinguished.

6. Conclusions

In this paper, a standard CMOS technology based DNA sensor detection method is developed. This method is based on integrated APS to detect the

change in opacity when a nano-particle layer is self-assembled at the location with perfectly matched DNA sample. The protocol is demonstrated by DNA chip fabricated with a standard 0.5 μm CMOS process. The optical properties of the deposited nanoparticles are carefully studied and calibrated, indicating that more than a five-times difference can be measured under most optical spectrums in the visible light region. With the built-in dynamic range adjustment circuit, the DNA chip can work with a variety of light sources with different intensities. The sensitivity of the system can be enhanced by using a longer silver deposition time, and we have another enhancement so that a low concentration of 10 pM can be detected.

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