Array-Based Electrical Detector of Integrated DNA Identification System for Genetic Chip Applications

Mei Xue[1], Jiong Li[2], Zuhong Lu[2], Ping K. Ko[1], and Mansun Chan[1]

[1]*Department of Electrical and Electronic Engineering*
Hong Kong University of Sci. & Tech., Clear Water Bay, Kowloon, Hong Kong
Tel: (852) 2358 8519, Fax: (852) 2358 1485, e-mail: mchan@ee.ust.hk
[2]*Key Lab of Molecular and Biomolecular Electronics of Ministry of Education*
Southeast University, Nanjing 210096, China PR

Abstract

An electrical detector used for detecting the array-based electrical signal of DNA with nanoparticle probes has been fabricated and experimentally demonstrated. Compared with the traditional approach of optical signal based detection system's, the proposed approach can significantly simplify the fabrication of integrated detection system for biochip applications. Furthermore, it can also produce a more integrated system with higher density. The details of fabrication steps and the procedures of attaching the target probe associated with gold nanoparticles onto the silicon-based fixture are presented in this paper. Experimental results show that a matched and unmatched DNA sample functionalised with gold nanoparticles can be distinguished. By using the silver enhancement and pre-hybridization method, the sensitivity of this array-based electrical signal detection system exceeds that of the traditional fluorescence detection system by two orders of magnitude.

1. Introduction

In the latter half of the 20th century, significant progress has been made in molecular biology, catalyzed by the discoveries of techniques for the synthesis, analysis, and manipulation of deoxyribonucleic acid (DNA). The benefits of this progress include the commercial availability of improved drugs produced by genetic engineering and new techniques for the diagnosis of genetic diseases [1]. The recent advances in DNA "amplification" by the Polymerase Chain Reaction (PCR) technology [2] together with automated methods for DNA sequencing [3] have made success in mapping the entire set of human DNA.

In this paper, we will focus on a particular issue in DNA analysis, which is the identification of whether a known sequence exists in a DNA sample. The process can be used as early disease determination due to the existence of certain gene mutation (e.g. cancer). The process involves the synthesis of the target gene sequence (called probes), applying the sample to be diagnosis to the probes and determining whether a match is found. In this process, an effective method to detect the DNA matching is becoming very important.

A restrictive requirement of most DNA detection systems is a thermal-stringency wash to differentiate target strands from ones with mismatches and thus achieve desired selectivity. Gold particles that are heavily functionalised with oligonucleotides were reported to exhibit extraordinarily sharp thermal-denaturation profiles that translate into higher target selectivities [4]~[7]. Based on this, we propose the resistance detection method that provides an alternative to existing detection methods [8]~[13]. In our approach, an oligonucleotide-modified gold nanoparticle probe is used to label samples instead of traditional fluorescence labels and when the samples match with the probes, they are attached to the probes. All unattached samples are washed away. Afterward, the silver enhancement is performed to fulfil the gap among the gold particles. The resistance of the sensor with matched samples is much smaller than that of the sensor with unmatched samples. The whole procedure is shown in Fig1.

In a conventional detection system, the signal is detected by an external CCD array with an optical filter. Compared with optical ones, DNA sensor array chips with fully electronic readout offer several advantages, since they allow easier operation and avoid expensive set-ups of optical detection system including CCD cameras, lenses, and microscopes. Furthermore, electronic signal processing techniques can be used to analyse the DNA information. In this paper, the effectiveness of the electrical detector for detecting DNA matching is described.

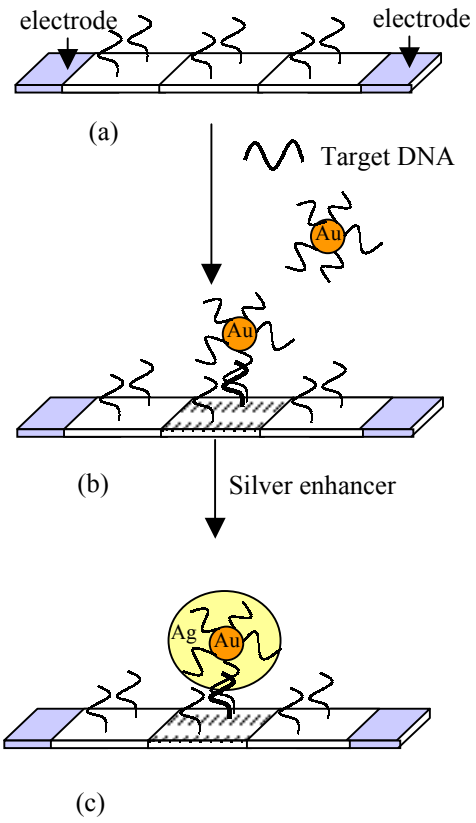


Fig1. Scheme showing the concept behind electrical detection of DNA. (a) attachment of oligonucleotides to the surface of the chip, (b) hybridization of target samples after labelling them with nanoparticles labelling, (c) formation of the conductive line after the silver enhancement.

2. Device fabrication

The cross section of the electrical detector is shown in Fig. 2(a). Modifications to the conventional process are required due to material compatibility. Due to the solution used in the cleaning process, aluminium cannot be used as the interconnect metal and noble metal such as gold or platinum has to be used.

The actual fabrication process begins with a (100) p type substrate. The buffer oxidation is followed by the nitride deposition to insulate the electrical detector with each other. After LTO deposition, standard lithography is performed to define the sensitive region of the electrical sensor. Titanium is deposited on the whole surface of wafer as a gluing metal. Gold is then deposited as the electrodes. The metals are then patterned to form the electrical sensors shown in Fig. 2(b). Using the fabrication process described above, a 4x3 electrical sensor array is fabricated. The area of each sensitive region is 16x50μm^2.

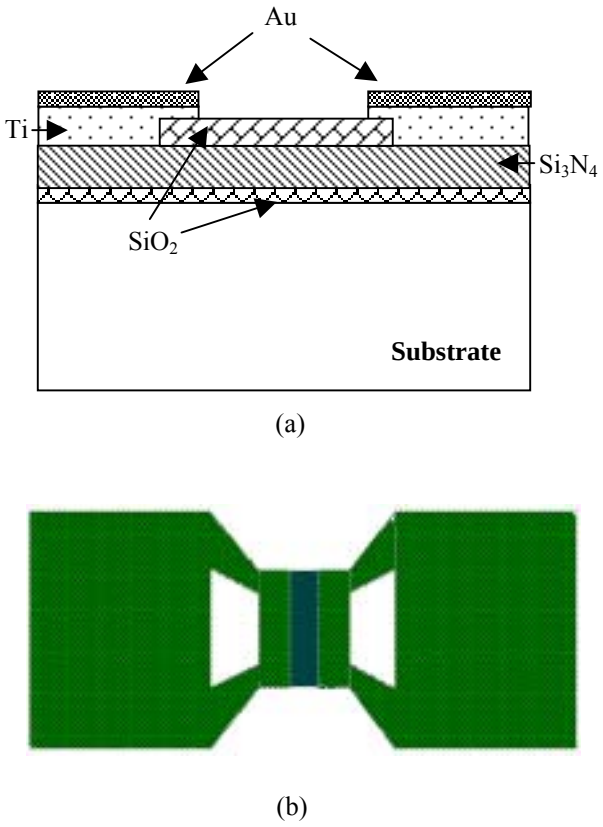


Fig2. (a) The cross section of the detector, (b) the top view of the detector layout.

3. Attaching DNA probes onto Silicon Fixture

The DNA detection process involves attaching a known sequence of DNA strend (or probe) corresponding to an ingredient (such as the DNA of a bacteria) onto the silicon-based fixture. Afterward, a sample (say the blood of a patient) is applied to the probes and if a match is found, the matched DNA will be attached to the fixture (referred as hybridization). By introducing gold nanoparticles to the DNA sample to be detected, the amount of electrical current of the sample remains on the silicon fixture indicated the quantity of the ingredient to be detected. The result can be captured by an electrical detector array and analysed by signal processing techniques. The first step is to achieve integration between the bio-material with the silicon fixture.

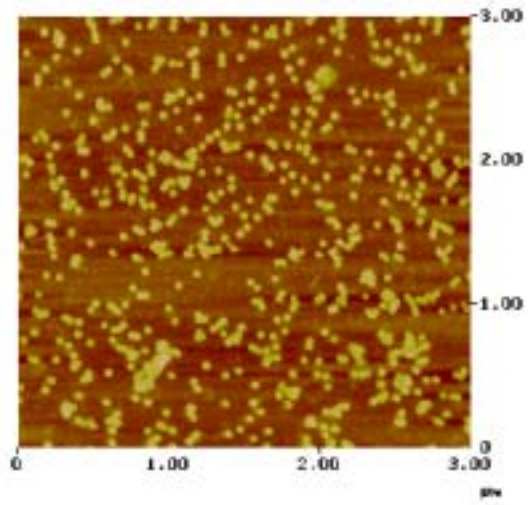
It starts with surface cleaning with strong acid which attacks most of the metal used in silicon processing. Therefore, conventional fabrication process has to be modified to incorporate more inert metal such as gold and platinum. In our experiment, gold is used as the contact electrode. After the cleaning, the surface is

treated with a boiling solution composed of 1:4:3000 of H_2O_2: MPTS (3-mercaptopropyl trimethoxysilane): 2-propanal for 30minutes to form the self-assembly layer. Afterword, DNA probe with known sequence is attached to the MPTS layer (immobilization) by spotting 0.3 M NaCl, 0.3 M Na Citrate buffer (pH 4.5) solution of appropriate oligonuclotide in the electrode gaps by manual pipetting. The chip is kept at room temperature for at least 12 hours. Experimental result shows that the DNA probes can only be attached to silicon dioxide but not silicon nitride. As a result, silicon-nitride is deposited as an insulative material to separate different sensors. The DNA samples attached with gold nanoparticle labels are then applied onto the silicon chip and the entire chip is incubated in a custom-made hybridization chamber in a water bath at 37˚C for 2 hours. After washing the chip with 0.6 M $NaNO_3$, 10 mM phosphate buffer, the chip is placed into a silver enhancer solution (Sigma), which is composed of $AgNO_3$ and hydroquinone, and is incubated at room temperature for 10-20 min. During the silver enhancement procedure, the gold nanoparticles have been significantly increased in diameter and contact with each other. Thus the elelctrically conductive layer is formed.

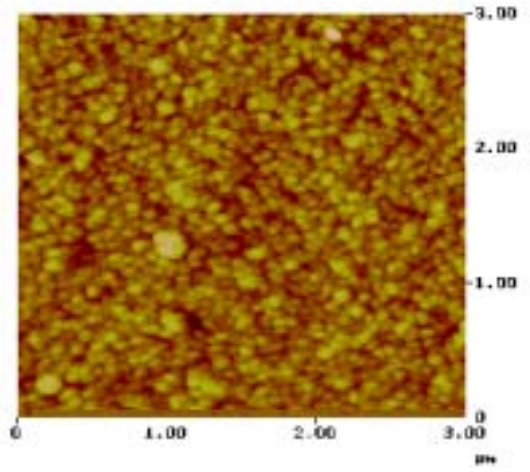
4. Experimental results and discussions

To increase the sensitivity of the chip, we use a signal amplication method in which silver ions are reduced by hydroquinone to silver metal at the surface of the gold nanoparticles. This process also faciliates visualization of nanoparticle labels hybridized to the detector surface. The silver enhancement is studied by Atomic Force Microscopy (AFM) and results are shown in Fig. 3. Before the silver enhancement, the gold particles can be observed to scatter on the surface of the chip(shown in Fig3.(a)). It is an one-layer structure. After the silver enhancement is applied, the gold particles have been increased in diameter. And the small particals on the surface of the gap among the gold particles also can initiate the silver deposition. It results in the uniform particle growth and smaller particles. Thus the multilayer structure can be observed in Fig3(b). If enough particles can fill the gaps, a measureable electrical signal can be provided.

As sliver particles can stick to the surface of silicon nitride to increase the background noise during silver enhancement, the time of silver enhancement is a very crucial parameter in the detection system. This phenomenon is called silver absorption. The resistance of the compartment between two electrodes is measured after the different time of silver enhancement. The results are listed in table1. When the silver enhancement time is smaller than 10 min, the particles on the sensitive region cannot be connected. When it is larger than 20 min, the particles on the insulative surface have grown large enough to form the conductive line. That means the signal cannot be distinguished from the background noise. From the experiment results, to achieve the best signal to noise ratio, the silver enhancement time should be 10~15min.



(a)



(b)

Fig3. AFM measured SiO_2 surface (a) before silver enhancement, (b) after silver enhancement

Table1. The signal and background noise after different silver enhancement time

Sample	Silver enhancement time	Signal (resistance: Ω)	Noise (background resistance: Ω)
1	0min	10^{13}	10^{13}
2	12min	10^{5}	10^{12}
3	12+10min	10^{4}	10^{4}

The IV characteristics of matched and unmatched samples are shown in Fig4 corresponding to 15min silver enhancement time. Smooth IV line proves our

well-controlled experiment procedure. Significant difference between the current of matched and unmatched samples can be observed by 6 orders of magnitudes. From experiment results, a simple electrical detector can be used to distinguish DNA samples, provided a suitable silver enhancement time.

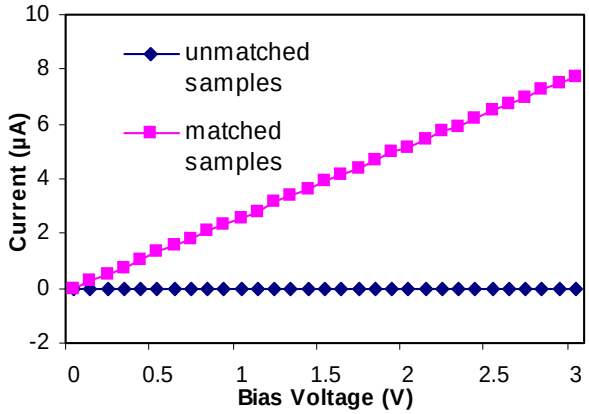


Fig4. The IV characteristics of matched and unmatched samples.

From the experimental results, we can see that silver enhancement time can significantly affect the sensitivity of the detection system through the introduction of unwanted background noise with prolonged treatment. To overcome the silver absorption of background surface, pre-hybridization is used to prevent silver attaching to the silicon nitride surface, thus result in the lower background noises. During the pre-hybridization, an oligonucleotide, which is different from the immobilized oligo and hybridized samples, is applied to the surface of the chip before hybridisation. It will prevent silver absorption on the chip background. From observations, we can see that without pre-hybridization the color of background is white which is the color of silver particles, while it is blue with pre-hybridization, which is the color of silicon nitride. Fig5 shows the

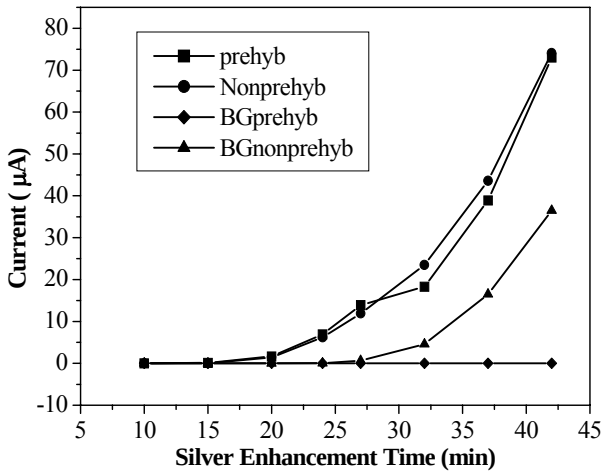


Fig5. The current comparison between with pre-hybridization and without pre-hybridization for the signal and background noise respectively

measured electrical currents of the matched and unmatched samples with pre-hybridization and without pre-hybridization corresponding to silver enhancement time. The current of the matched sample is almost same in these two cases, but the current of background noise greatly reduces after pre-hybridization. By using this method, the electrical detector can measure the concentration of DNA sample as low as 10pM. The sensitivity of this detection system exceeds that of the conventional fluorescence detection system by two orders of magnitude.

5. Conclusion

A resistance detection system for DNA identification has been demonstrated. In order to incorporate the DNA material onto the silicon based material, minor modification to the fabrication process to incorporate inert metals is necessary. A substantial difference in the measured current is experimentally demonstrated. By using the method of silver enhancement and pre-hybridization, the concentration of 10pM has been achieved. It proves that the sensitivity of the resistance detection system exceeds that of the normal fluorescence detection system by two orders of magnitude. This resistance-based identification provides a revolutionary approach for DNA identification.

6. Acknowledgement

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7. References

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