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Nanogap Dielectric Biosensor for Label Free DNA Hybridization Detection

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ABSTRACT

Sensors based on nanogap capacitance changes are being developed for genomic and proteomic applications because they offer label-free detection on platforms amenable to high throughput configurations. This paper presents the development of fabrication and characterization of lab-on-a-chip methodology for deoxyribonucleic acid (DNA) hybridization detection. The electrodes made of amorphous silicon (a-Si) separated by a narrow gap whose width is comparable to the size of a DNA molecule are used as a trap. During hybridization, trapped DNA change from single stranded DNA (ssDNA) to double stranded DNA (dsDNA) cause the change of charge density of molecules structure. This change results in an effective change of capacitance that can be monitored electronically by using dielectric spectroscopy, a type of impedance spectroscopy that measures the dielectric properties of a medium as a function of frequency.

Keywords: *Nanogap, DNA hybridization, Lab-on-a-chip, Spacer Patterning Technology, Reverse Spacer Lithographic.*

INTRODUCTION

Background

Scientists today can identify or sequence any DNA molecule using conventional method such as Polymerase Chain reaction (PCR), however this method is time consuming with respect to cope up with the rapid alterations or mutations that occur in the gene's DNA and cause certain diseases, such as breast cancer. It is inherently difficult of extremely small size of DNA bases by the conventional method. Determining a suitable DNA base requires a base that can interact individually, requiring a detector of a similar size [1]. Traditional technology avoids this problem by repeated copying, chemically generating a multitude of labeled polymers for every based present and determining the presence or absence of a big group of polymers is trivial compared to determining what a single molecule is [2]. However recent development of nanotechnology fabrication could make another way in single organic molecule detection; by observing their electrical behavior using nanogap between the electrodes.

Any tiny size sample like DNA should be trap in a gap or space between two electrodes and connecting the electrodes. When molecules are fixed between electrodes, it can change the charge carrier density of the electrodes [3]. This change of charge carrier density results in an effective change of conductance that can be monitored electronically. The sensor structure allows for direct conversion of molecular recognition and binding events to electronic signals.

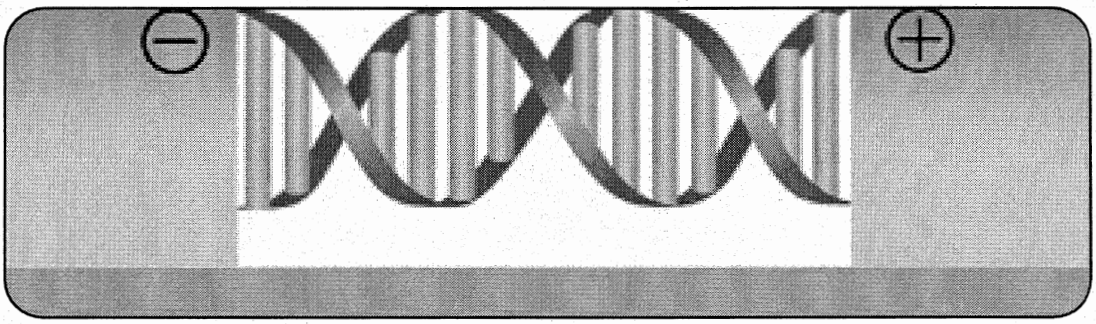

Figure1. Cross-sectional electrodes of nanogap filled with DNA.

A number of contradicting findings were reported in the experiments regarding the charge transport properties of DNA. The experiments appeared to indicate metallic [4], semiconducting [5], and insulating [6] electronic properties. These transport experiments were inspired by electron transfer experiments where one attaches donor and acceptor groups at both ends of DNA molecules and characterizes their electronic coupling through the DNA. Several experimental and theoretical studies have demonstrated the conducting behaviors of DNA molecules by direct electrical conductivity measurements. Nevertheless, the exact charge transport mechanism is still unknown and controversies are ongoing [7]. One of the uncertainties in these conduction measurements is electrical contact between DNA molecules and electrodes. It is not even easy to confirm, by using conventional microscopy techniques, whether nanometer-scale DNA molecules are really in between electrodes.

In this study, hybridization of DNA detection will be done by using dielectric spectroscopy, a type of impedance spectroscopy that measures the dielectric properties of a medium as a function of frequency. It is based on the interaction of an external field with the electric dipole moment of the sample, and it is gaining importance as label-free detection tool for biomolecular structure and binding events. Binding event studies are often performed by immobilizing the recognition element at the electrode surface, thereby amplifying the signal from small analyte concentrations in the bulk solution. In these cases the signal measured after the recognition event is a modification of the electrical double layer capacitance at the interface.

The Structure of DNA

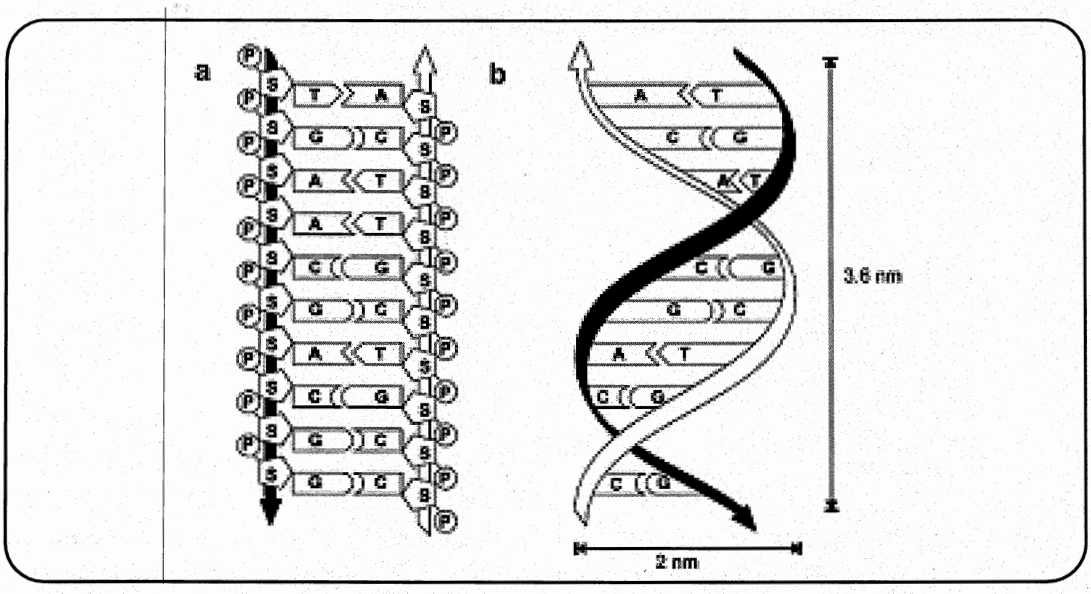

Figure 2. Schematic structure of double-strand DNA; (a) double-strand DNA; (b) double-helical structure of double-strand DNA.

Watson and Crick [8] were the first to determine the double-helical structure of DNA. They found that DNA consists of two strands, running anti-parallel as shown in Figure 2(a). The pentagons labeled with an `S' denote the sugar rings and the `P'-labeled circles are negatively-charged phosphate groups. One of the four bases (Adenine, Thymine, Guanine or Cytosine) is attached to each sugar. The genetic information is carried in the sequence of these four bases in the molecule. This structure can be exists as double-helical structure of double-strand DNA as shown in Figure 2(b). It forms a polymer with a diameter of about 2 nm and contains one full helical turn for each 3.6 nm or 10.4 base pairs. An intriguing property of double-strand DNA is its moderate flexibility: It can be smoothly bent or twisted with very little influence on the helical properties. An important property for our work is the fact that DNA in solution is highly charged at neutral pH. Each phosphate group on the backbone has a negative charge, resulting in a linear charge density of 5.9 e- per nanometer. The effective charge density however is considerably lower due to counterion condensation [9].

DNA Hybridization

It is possible for two complete single-stranded molecules to hydrogen bond at every base-pair to form a double-stranded molecule (called dsDNA); this process is termed hybridization. For this to occur, two requirements need to be met: (1) The two dsDNA molecules need to be of opposite "orientation" and: (2) The two ssDNA molecules need to have the appropriate "base-pairs". In ssDNA one end is necessarily terminated with phosphate group (called the 5' end), and other a sugar (called the 3' end). This determines a strand's orientation: either 5'-3' or 3'-5'.

Complete hybridization requires both condition (1) and (2) described above to be met, but incomplete hybridization is still possible if there exists only a small percentage of inappropriate base pairs. If two ssDNA molecules are capable of complete hybridization they are called complimentary.Nucleotides will bind to their complement under normal conditions, so two perfectly complementary strands will bind to each other readily [10]. This is called annealing. However, due to the different molecular geometries of the nucleotides, a single inconsistency between the two strands will make binding between them more energetically unfavorable.

Measuring the effects of base incompatibility by quantifying the rate at which two strands anneal can provide information as to the similarity in base sequence between the two strands being annealed. Annealing may be reversed by heating the double stranded molecule of DNA (or RNA or DNA:RNA) to break the hydrogen bonds between bases and separate the two strands. This is called melting or denaturation [11].

MATERIALS AND METHODS

Nanogap Fabrication and Assembly

The process begins with deposition silicon nitride (Si_3N_4) on clean silicon wafer following by amorphous silicon and silicon oxide on top of it. After that, photo resist (PR) solution will be load on the SiO_2 layer before making the proper mask alignment on top of PR. By using exposure system (by applying UV light through a mask), pattern from mask can be transferred on PR. Later on the development and etching process, the same pattern of mask finally moved on SiO_2 and Si_3N_6 layers.

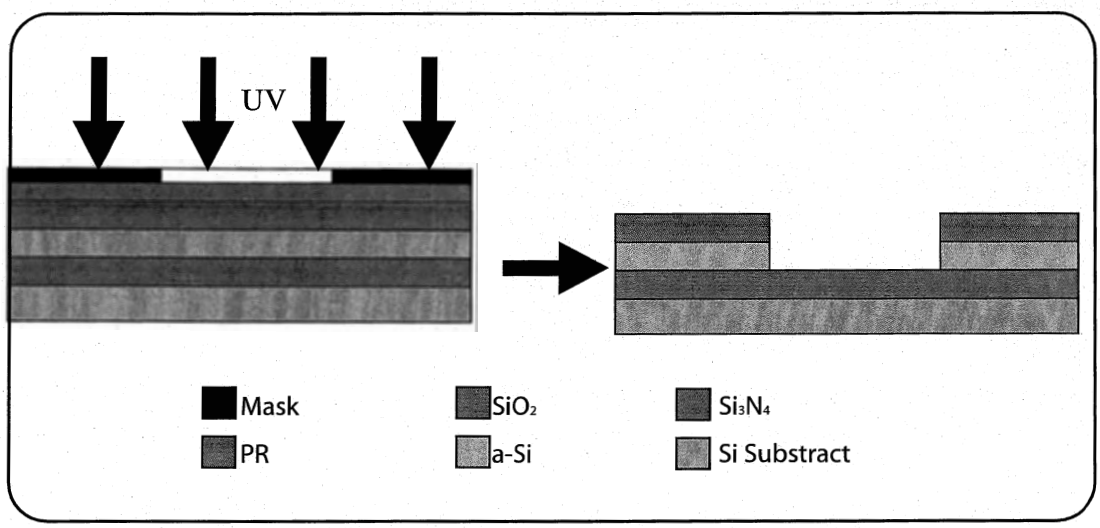

Figure 3. Layer by layer alignment and patterning method.

The layer of silicon nitride is deposited by conventional chemical vapor deposition (CVD) to isolate two electrodes from the crystalline silicon substrate. A layer of amorphous silicon (a-Si) is deposited to make the first electrodes of the vertical capacitor. Next, a thick layer of silicon oxide is deposited. The oxide layer serves as a hard mask to protect the electrode during subsequent chemical-mechanical polishing (CMP) because the polishing rate is faster at the protruded patterns than at the flat patterns [12]. The first electrode is then defined using standard photolithography process and anisotropic etching. Next, a SiO_2 layer of sacrificial is deposited. This process is the critical step on fabrication process, since it determines the nanogap size or dimension.

The sacrificial layer on the top of the a-Si is then removed by etch-back, which does not require any masks. Thus, the nanowidth residue or spacer of sacrificial SiO_2 is left along the first electrode as shown in Figure 4. A layer of a-Si is deposited to make the second or grounded electrodes of the vertical capacitor. After CMP, the top a-Si and the hard mask is completely etched-back and planarized as shown in figure. Next, the spacer is removed by wet etching. During this release process, the nitride insulator underneath the a-Si (first and second electrodes) is not etched, thus avoiding any isotropic undercut profiles.

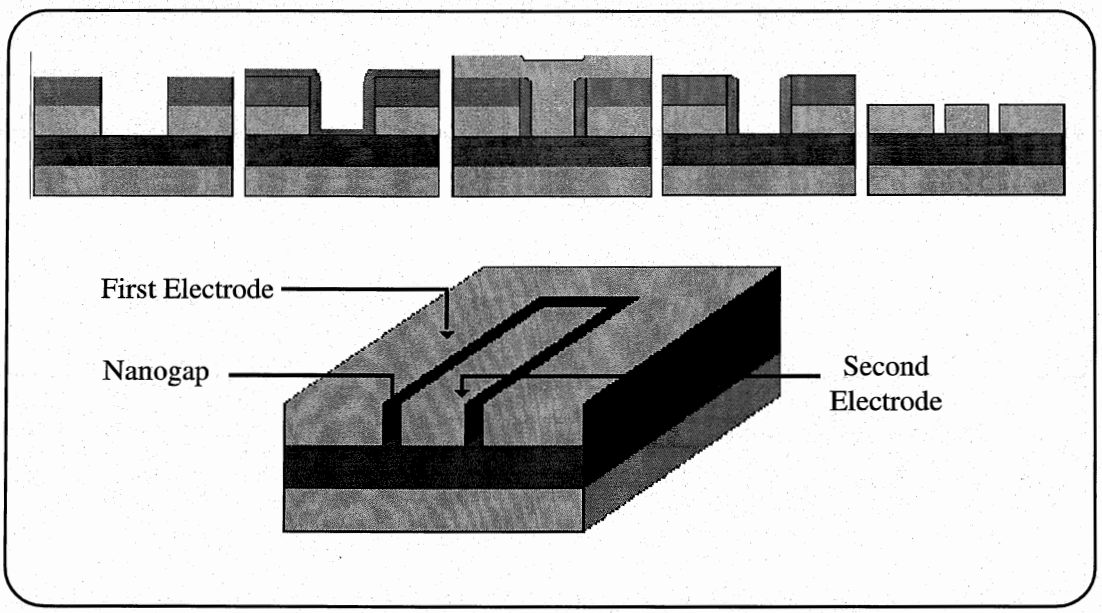

Figure 4. Fabrication process for nanogap.

Imaging and Detection

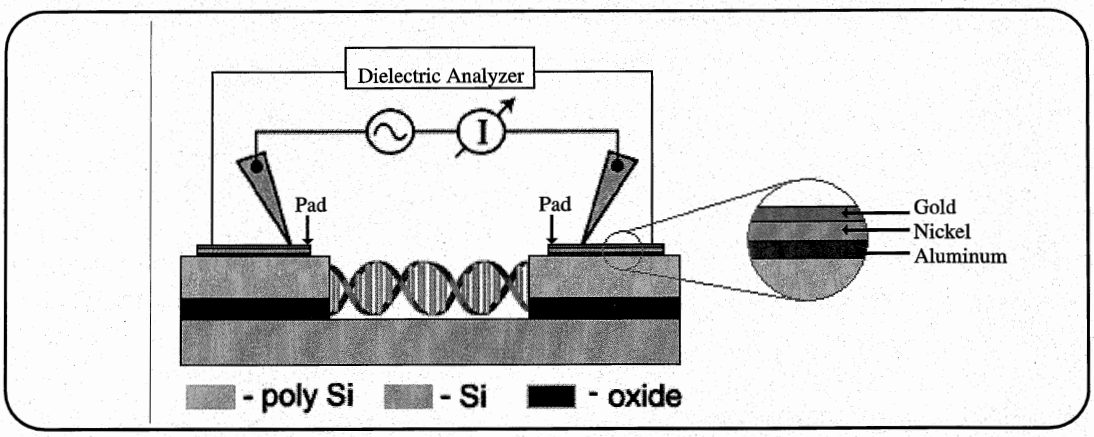

Figure 5. Schematic representation of materials used in the device fabrication process.

The nanogap dielectric biosensors consisted of a capacitor between two parallel amorphous silicon electrodes. The capacitance of the nanogap indicates the permittivity of the medium filling in the gap. The nanogap sensor schematically shown in Figure 5, features a vertical air nanogap bordered at the sidewalls of a-Si electrodes. These electrodes will be connected to dielectric analyzer to probe station employed a two-wire impedance measurement set-up. There two contact points to the sensor:

i. To gold pad that is connected to the first electrode, and
ii. To another gold pad that is connected to second electrode.

Dielectric measurements for spectroscopy imaging are taken using probes between the first electrode (left) and the second electrode (right). The measured space consists of a silicon oxide spacer as well as an under etched nanocavity for sample introduction.

Sample Preparation

There are two types of sample ;

i. Preparation of various ionic strength solutions by diluting phosphate-buffered saline or PBS buffer solution in DI water
ii. Preparation of various concentration target molecule (single stranded DNA) solution by dissolving and diluting the DNA in DI water. This concentration must be suitable to the size of nanogap capacitor.

Detection of existence ssDNA in Aqueous Solution

Each sample will be label in order to measure the response of nanogap sensor to de-ionized water and varying concentrations of buffer or DNA solution. The response monitoring can be done by placing samples in nanogap by using a pipette either by increasing or decreasing concentration. The measurements will be taking from 1 MHz down to 1 Hz with certain spacing factor. At each frequency, permittivity, ε , the parallel capacitance, C_p , and lost tangent, tan δ , will be record. The loss tangent is a measure of the conductivity of the sensor under test and is used to monitor the performance of the sensor. A loss tangent

higher than 100 implied that sensor's conductance is too high and no longer behave as capacitance, and hence, the permittivity of the sample [13]. The spectrum can be record as measured permittivity as a function of the frequency.

Detection of DNA Hybridization

A DNA molecule is positioned between the nanoelectrodes by electrostatic trapping from a dilute aqueous buffer containing about one molecule per $(100\ nm)^3$. This technique was developed for the trapping of single molecules, and has been shown to be successful for a variety of nanoparticles [8]. After a DNA molecule trapped from the solution, the device will be dry up in a flow of nitrogen and response of nanogap can be measured in same way as described above.

Figure 6 Shows that, as the input frequency decreases, the capacitance increases more rapidly after hybridization than before hybridization. However, there is no significant difference of the measured capacitance before and after hybridization in the case of non-conjugated DNA strands.

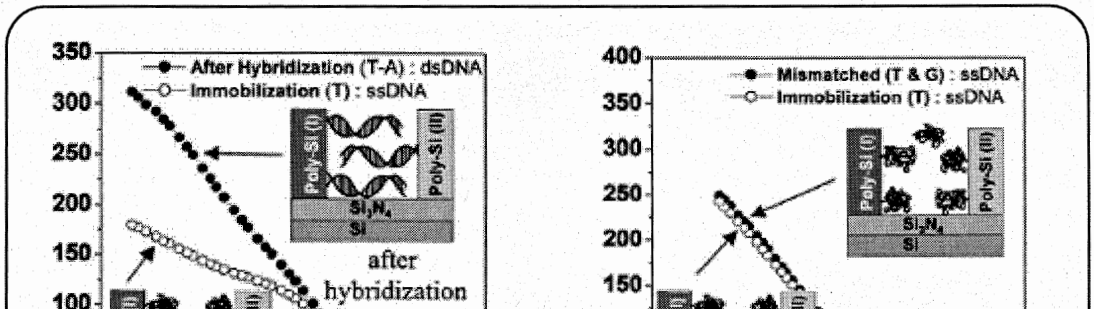

Figure 6. Measured capacitance versus input frequency after immobilization and hybridization. Capacitance increases as the frequency decreases in the conjugated base pairs (T–A) and there is no significant difference of capacitance in the nonconjugated base pairs (T–G) [12].

SUMMARY

In the present paper we have reviewed the development of methods and techniques for the production, immobilization and electrical characterization of DNA hybridization detection. Fabrications of electrodes separated by a nanogap whose width is comparable to the size of a DNA molecule are demonstrated with micro lithography process and Spacer Patterning Technology. The DNA hybridization on the silicon surface causes change of capacitance that can be monitored electronically by using dielectric spectroscopy. The real-time measurement on capacitance can monitor the surface hybridization process. One main advantage of this impedance approach is that there is no need for a fluorescence labeling to either target or probe. Since the impedance measurement is made on silicon substrate, the fabrication in the future applications will be more feasible because of the maturity of the microelectronics industry.

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