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A review on the electrochemical sensors and biosensors composed of nanogaps as sensing material

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In the past two decades, the biological and medical fields have seen great advances in the development of biosensors and biochips capable of characterizing and quantifying biomolecules.To understand the important relationship between the biosensor and nano structure we introduce this proposal to fabricate and characterize the nanogap biosensor using size reduction technique for ss-DNA immobilization and hybridization detection . In this review, 2 masks designs are proposed. first mask is the lateral nanogap with gold electrode a1nd the second mask is for pad gold electrode pattern, and lateral nanogap is introduce in the fabrication process using silicon, and gold as electrode. Conventional photolithography technique is used to fabricate this nanogap (NG) based on the standard CMOS technology and characterization of its conductivity together with it's effect during sensing is investigated in this research.

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1. Introduction

The development and application of nanogaps for electrochemical sensors and biosensors are reviewed in this article. As the potential threat of bioterrorism increases, there is a great need for a tool that can quickly, reliably and accurately detect contaminating bio-agents in the atmosphere. Biosensors can essentially serve as low-cost and highly efficient devices for this purpose in addition to being used in other day to- day applications. A biosensor is a sensing device comprised of a combination of a specific biological element and a transducer. A "specific biological element" recognizes a specific analytic and the changes in the biomolecule are usually converted into an electrical signal (which is in turn calibrated to a specific scale) by a transducer. In this article we present the basics of biosensing devices which can serve as an introductory tutorial for readers who are new to this field. Subsequently we provide high-level descriptions of a few representative biosensors as case studies, followed by a brief discussion of the major difficulties the biosensor research communities normally encounter.

Nanotechnology is playing an increasingly important role in the development of biosensors. The use of nanogap has allowed the introduction of many new signal transduction technologies in biosensors. The sensitivity and performance of biosensors is being improved by using doping process for their construction.

Sensors represent a most plausible and exciting application area for nanotechnology; and nanosensors based on advanced nanomaterials are expected to emerge in the marketplace in significant volumes over circa the next ten years. Sensors constructed at the molecular scale have promise for being extremely sensitive, selective, and responsive [1].

Moreover, nanotechnology can be applied to create sensors of minuscule size. The reduction in sensor size can result in lower materials cost, reduced weight, and lower power consumption, which are key factors driving opportunities for sensors in the marketplace. Nanosensors of highly reduced power consumption are very suitable for integration into wireless communication devices to enable widespread distributed monitoring and control. Very low-power nanosensors would also be beneficial for use as battery-operated handheld or wearable sensors. Logical and promising sensing application areas for nanosensors include medical (e.g., blood gas monitoring/ blood analysis, patient monitoring, diagnostic testing), biowarfare detection, genetic analysis, drug screening or discovery, food inspection/testing, environmental monitoring, and industrial chemical process monitoring/leak detection.

Nanogap is not observed spontaneously in nature and must be produced in a laboratory. Nanogaps can be suspended, deposited or synthesized from the elements. This research fabrication process will focus on the essential feature of a nanogap. Once optimized, the technology could be used to many area of application.

Fig. 1. shows AFM characterization of the nanogap.

A biosensor can be generally defined as a device that consists of a biological recognition system, often called a bioreceptor, and a transducer. The interaction of the bioreceptor with the analyte is designed to produce an effect measured by the transducer, which converts the information into a measurable effect. Nanosensors and nano-enabled sensors have applications in many industries, among them transportation, communications,

building and facilities, medicine, safety, and national security, including both homeland defense and military operations. Consider nanoparticles and nanogap sensors that detect chemicals and biologics, nanosensors placed in blood cells to detect early radiation damage in astronauts, and nanoshells that detect and destroy tumors.

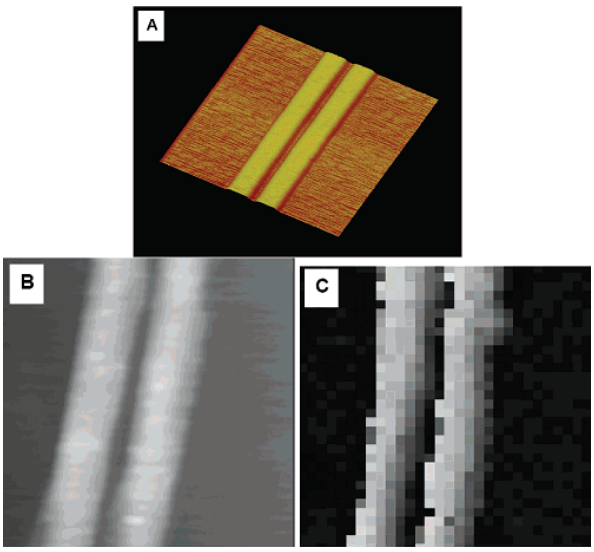

Fig. 1. AFM characterization of the nanogap: (A) topographic image (2 μm-2 μm) recorded in air, (B and C) adhesion force map (3 μm-3 μm) recorded in aqueous solution using a hydrophobic tip. Brighter levels in the images correspond to higher height (B) and higher adhesion (C).

In resent years, electrical methods for cell separation and identification such as electrophoresis, dielectrophoresis and impedance method are having more and more important applications. These methods have made fundamental improvements in separation resolution, cell purity, sample size, device cost and portability compared with traditional techniques.

As one of the most important methods for biological detection, AC impedance methods have a wide and potential application in the field of biomedical engineering; it can provide information about the growth, motility, and physiology of cells growing on the electrodes. However, there are still two factors decreasing the sensibility.

First, the planar electrodes are difficult to obtain useful signal. Second, AC impedance methods are limited at low frequency by the parasitic noise due to the electrical double layer impedance.

Recently, the impedance methods develop rapidly because of the introduction of nano technology. Mingqiang Yi, Ki-Hun Jeong and Luke P. Lee reported in [2] that electrodes with nanogap can measure the impedance spectroscopy of the liquid more precisely. They detected the existence of single stranded DNA (ssDNA) oligonucleotides (20-mer) in l00nM aqueous solutions using a 20 nm gap of 1.2 pl in volume. At present, most nanogap structures reported are planar structure and the signal is too weak to sense. Few 3D structures of nanogap which take silicon or polysilicon as sacrificial layer are reported in [3]-[4]. These structures haven't good compatibility with biological sample as organic polymer-based Au electrodes. N.C.Das released multi-layer metal structure by dry etching technologynique. Though this method is difficult to produce electrodes stronger enough to load liquid sample, it provide a new idea for fabrication of 3D nanogap metal electrode. A detection method of biological cells or bacteria by using a dielectrophoretic impedance measurement (DEPIM) has been developed.

This result makes it possible to develop a system by which cell separation and detection can be processed within a single chip. In this research, a 3-D nanogap Au electrode grid array biosensor is designed. This sensor is expected to separate and identify cells by DEPIM. In order to make the fabrication of biochip more applicable, the process scheme is designed as simple as possible. However, NEMS technology must be introduced to fabricate thin metal cantilevers that configuration the array of microelectrodes with nano gaps. In the paper, Au layer is taken as mask and organic polymer coating on substrate is taken as planarizing layer, and the sidewall etching of RIE is made use of to form Au electrode cantilevers with nanogaps. This method is easy to apply and cantilevers formed are expected to be strong enough to load the liquid.

Essentially, there are two approaches towards the fabrication of structures at or near the atomic level. The first is the `top down' approach where the precision of existing macroscopic techniques is improved. This concept has been demonstrated in semiconductor industry, where lithographic processes are nowadays used to make integrated circuits with critical dimensions smaller than 100 nm. This precision will be improved further, but true atomic precision can not be obtained with this approach. The second 'bottom up' approach strives to build structures using atoms or molecules as building blocks. Most striking are experiments where individual atoms are positioned on an atomically flat substrate using scanning-probe techniques. Patterns of atoms have even been demonstrated to act as simple logic gates. Such scanning-probe techniques however are not very practical: Assembly by placing a single atom at a time is a very time consuming process.

2. Nanogap biosensor mask design

In this project, SOI wafer is used as starting material to fabricate a nanogap biosensor. The first step is to fabricate two designs as two masks, whish is the two mask design is proposed and the silicon nanogap with gold electrode process flow is designed. This Research is mainly focus on the issue related to the fabrication of the biosensor and the development of a new technology. The sidewall etching using RIE to form thin nanogap metal cantilevers which configured the 3-D nanogap electrode grid array structure. Anisotropy of RIE is modeled and the etching profiles are simulated. This method is proved to be applicable by analysis and experiments [5].

The starting material use in this project is SOI wafer, 100mm in diameter (4 inch wafer) as shown in Fig. 2.

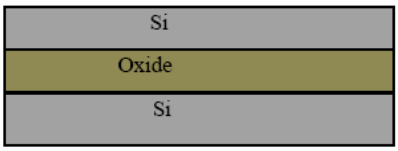

Fig. 2. SOI wafer

The first process is to check the wafer type from its specification, measure wafer thickness (SOI thickness), and measure the sheet resistance . After that, lightly scribe the backside of the wafer, and protect the top surface, using the sribe tool provided. Mark gently but make it visible and then place scribed wafer in container.Wafer is cleaned before each processes.

As for the lithography process, two photomasks are employed to fabricate the nanogap using conventional photolithography and E-Beam lithography techniques. Commerical chrome mask is expected to be used in this research for better photomasking process. This mask is used to develop the gold electrode with silicon nanogap. The photomasks are designed using AutoCAD software and then printed onto a chrome glass surface.

Fig. 3 is the first mask for nanogap electrode formation whish the length and width of 5000µm and 2500µm respectivily.

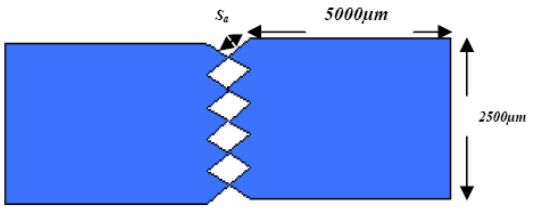

Fig. 3. Design Specification of the Mask.

The proposed angle length of the end electrode are shown in Table 1. This is simply to check the best angle for the best nanogap formation after etching process.

Table 1. Difference dimensions for $\boldsymbol{S_d}$.

S_d	µm
1	1100
2	1000
3	900
4	800
5	700
6	600

The symbol S_d refer to the dimension for Side angle of the design for nanogap formation. Its show that when S_d is large this mean the nanogap become very sharp and less sharp with less dimension of S_d.

Fig. 4 shows the actual arrangement of device design on chrome mask. Its consist of 160 dies with 6 different design.

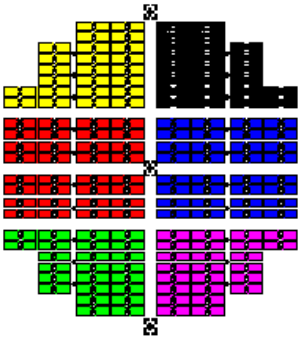

Fig. 4. Schematic design of the actual mask on chrome glass.

Fig. 5 is a schematic device design of mask 2 with 5000μm length and 2500μm width. The distant between two rectangles is indicated as S_a bearing the same dimension with S_d according to the theory of Pythagoras, and the dimension of S_a can be defined mathematically as shown in Fig. 6.

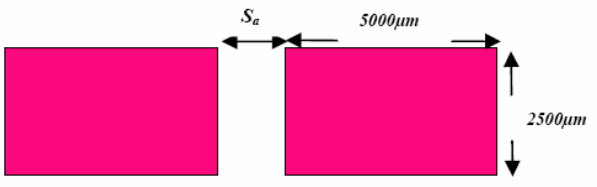

Fig. 5. Design Specification for Mask 2.

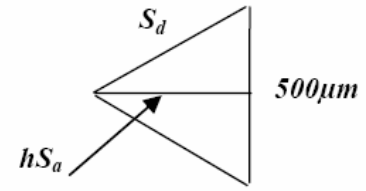

Fig. 6. schematic representation S_a, *where* $S_a=2hS_a$

Table 2. Variance Dimensions for $\boldsymbol{S_a}$ *follow To the Dimension for* $\boldsymbol{S_d}$.

S_d (µm)	$hS_a=((S_d)^2-(250)^2)^{1/2}$ (µm)	$S_a=2\ hS_a$ (µm)
1100	1071	2139
1000	968	1936
900	864	1729
800	759	1519
700	653	1307
600	545	1090

From the above table the dimension for S_a depend on dimension of $S_{d,}$. The calculated S_a is based on $S_a=2hS_a$.

Fig. 7 is a schematic mask on a chrome glass.

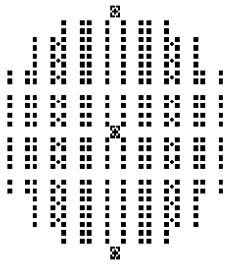

Fig. 7. Schematic Mask on chrome glass

3. Biosensor fabrication

The process steps of gold electrode with silicon nanogap fabrication are shown in Fig. 7, starting by cleaning the wafer SOI before performing a layer of resist coating on the surface of the wafer, then exposed mask 1, which can be seen in step (c), after developing the resist, an etching process of a layer of silicon is performed, then removing the resist. In Fig. 8(e) a deposit Ti/Au process is done on the surface. Next is photolithography process, a layer of positive photoresist is applied to ultraviolet light through a mask 2, after the development only unexposed resist will be remained. As in Fig. 8(g) an etching process of a layer of Ti/Au is performed before removing the resist. Finally a structure of the gold electrode with silicon nanogap is obtained as in Fig. 8(h).

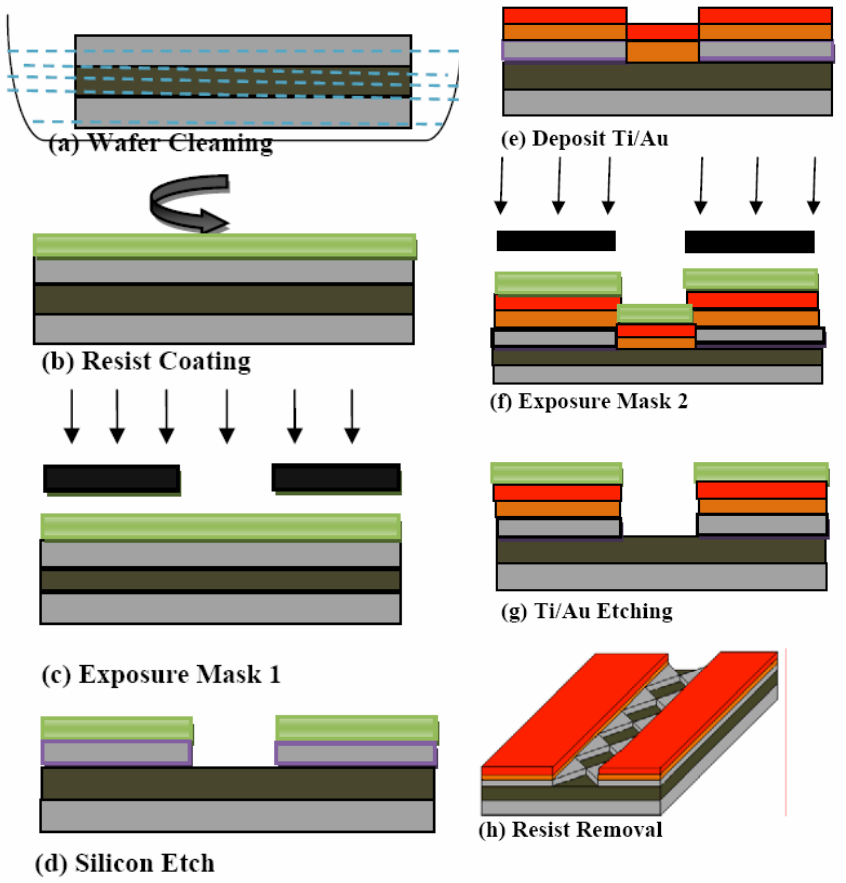

Fig. 8. Gold/Ti –Silicon/Oxide-Silicon Structure Process Flow.

4. DNA Immobilization

Low-cost portable DNA biochips are in high demand in many areas including medical diagnostics, genomics and forensics. Label-free electrical detection promises to fulfill this demand, but a key requirement in the development of a wide range of DNA biosensors is the ability to immobilize DNA probes onto gold substrates at an optimum surface density. Maximum sensor signals are generally obtained when the amount of target DNA hybridizing with immobilized probe DNA is maximized. This increases as the density of probes increases up to 4×1012 probes/cm2; however, at higher probe densities the amount of hybridized target decreases rapidly [6]. In addition, fast hybridization kinetics are desirable for DNA biochips, requiring a probe density of 3×1012/cm2 or below [7].

Probe surface density is extremely important for the success of detection mechanisms that rely upon the intrinsic charge of the DNA phosphate backbone. Hybridization results in an increased negative charge at the sensor surface. This causes a change in the potential of the surface, which is used to transduce hybridization in field effect detection [8, 9]. The increased negative charge also hinders charge transfer to negatively charged redox molecules in solution. This causes a change in charge transfer resistance that can be detected using electrochemical impedance spectroscopy (EIS) [10]. Detection of these effects requires that the DNA charge is not significantly screened by counter ions, requiring that probes are closely spaced compared to the Debye charge screening length in the solution.

When determining the surface density of oligonucleotide probes, the density on the microscopic area as opposed to the geometric area should be determined. The ratio between the microscopic and geometric surface areas is given by the roughness factor. This varies between approximately 1–3 with different types of gold electrodes and with different cleaning protocols [11, 12]. Not accounting for the roughness factor results in an overestimation of the microscopic DNA surface density, an increase in the variance in densities determined and difficulty in comparison between studies.

Gold electrodes are commonly functionalized with DNA using the method reported by Herne et al 1997 [13]. The gold electrode is first incubated with thiol-modified DNA at a high ionic strength, and then subsequently exposed to a mercaptohexanol (MCH) solution. The MCH displaces nonspecific interactions between the DNA and gold and forms a self-assembled monolayer (SAM) that resists non-specific adsorption of target DNA. The DNA surface density is controlled by the incubation time with DNA; however, the immobilization of DNA is rapid, so short incubation times are required to achieve densities of 3×1012/cm2 or below, and there is a significant variation in the densities obtained. In addition, the DNA surface density has been shown to be sequence and length dependent, and the MCH not completely effective at removing unmodified DNA non-specifically adsorbed on gold [14]. [15] Satjapipat et al 2001 used the reverse approach, immobilizing thiol modified DNA onto gold regions freshly exposed by reductive desorption of the mercaptopropionic acid (MPA) in a mixed MPA/hexanethiol or MPA/MCH SAM.

Mixed SAMs are commonly formed by the simultaneous co immobilization of the different components. The mole ratio of constituents in a SAM is mainly determined by the mole ratio of thiols in the deposition solution; however, this also depends upon the time of deposition, temperature, relative solubilities of the thiols, and thermodynamic considerations of the SAM formed. The co-immobilization of thiol-modified DNA with a thiol spacer has been previously reported [16, 9, 17, 18, 19]. However, a systematic study of the immobilization conditions and accurate determination of the associated probe and target densities are still warranted. We report on the optimization of DNA attachment to gold electrodes by the co-immobilization of thiol modified single-stranded DNA (ssDNA) and mercaptohexanol. The DNA surface density was determined accurately using a chronocoulometric method based upon that reported by Steel et al 1998 [6]. Electrodes were also characterized using EIS in the presence of the negatively charged ferri/ferrocyanide redox couple, and the charge transfer resistance and its variation upon hybridization correlated to the microscopic DNA surface density.

5. Summary

Nanogap are not observed spontaneously in nature and must be produced in a laboratory. Nanogaps can be suspended, deposited or synthesized from the elements. This research fabrication process will focus on the essential feature of a nanogap. Once optimized, the technology could be used to many area of application.

The aim of this research is to develop a sensor for specific DNA sequences, using non-complex synthetic single-stranded DNA as a model system. A conductance - based sensor for the direct detection of DNA fabrication and sequences is described. Hybridization of analyze DNA with immobilized DNA between the nanogap induces charge effects, altering the electrical properties of the biolayer, and can be detected by the associated change in the measured conductance and capacitance which is can be monitored electronically by using semiconductor parameter analyzer, spectroscopy and oscilloscope. Using the electrical detection mechanism of a nanogap sensor promises fast and direct real-time monitoring of DNA hybridization of DNA without time consuming and at low cost.

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