# A 3-Dimensional Nanobiosensor Based on an Anodic Aluminum Oxide Template

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## ABSTRACT

In this paper, we propose a high sensitive nanobiosensor based on a 3D sensing element that has uniformly deposited gold nanoparticles. The barrier layer of an anodic aluminum oxide (AAO) film was used as the template; a reducing agent and stabilizer-free method where the electrochemical deposition was utilized to synthesize uniformly distributed gold nanoparticles on the surface of the barrier layer; the distribution density and size of the nanoparticles can be well controlled by the potential applied during electrochemical deposition.; following, receptors or antibodies with respect to a certain biomarker are attached to the gold nanoparticles.

***Keywords***: nanobiosensor, electrochemical deposition, anodic aluminum oxide template

## 1 INTRODUCTION

Earlier detection of cancer cells markedly increases the cure rate of tumors. The biomarker based sensing method is the commonly used approach for cancer cell detection [1]. When the cancer cells in a living body come into being tumors, the concentration of a certain protein in blood gradually increases. The increasing of alpha-fetoprotein (AFP) concentration in blood can be detected for a patient having liver cancer. Brest cancer raises the concentration of CA15-3. Although the symbolization of a biomarker to a certain tumor is not unique, the discovery of a certain biomarker can tell the possibility of tumor forming. Usually the concentration of a specific biomarker for a certain disease is relatively low in blood. A high sensitive biosensor for precise detection is desired. The enzyme-linked immunosorbent assay method (ELISA) [2] has been the commonly used sensing technique. In addition to the ELISA, electrochemical analysis, surface plasmon resonance technique (SPR), and piezoelectric frequency sensing are others feasible approaches. The key issue involved in the biosensing techniques is how to attach as many as analytes to the transducer to have better sensing quality. Recent progresses in micro/nano technologies have enhanced the operations of attachment [3].

Zisman initially discovered that molecules with amino-group could attach to a platinum substrate [4]. This self assembled monolayers (SAMs) technique can be conducted simply by adding a solution of the desired molecule onto the substrate surface and washing away the excess. Nuzzo *et al*. [5] found monolayers of organic disulfides could stably adhere to a gold substrate. Rubinstein *et al*. [6] was the pioneers using the SAMs technique to develop a gold electrode biosensor. Dubois *et al*. [7] reported that gold is difficult to react with others materials due to its low activity. Everett *et al*. [8] discovered that the SAM attached to a gold electrode could maintain its stable condition when the applied electric potential was between +0.8 V and -1.4 V. Consequently, gold is commonly used as the transducer for the SAM based biosensors. Gold nanoparticle that can provide a substantially larger surface area than that of bulk material or thin films has been an extraordinarily useful material of transduce for the SAM based biosensors.

The intrinsic characteristics of gold nanoparticles are primarily dependent on their size and shape. This of course means that different synthesis methods are required to produce the shape and size of particles which give the desired characteristics for different applications. In general, gold nanoparticles are produced in an aqueous solution through the reduction of chloroauric acid ($HAuCl_4$). Over the past decade, great progress has been made in the synthesis of gold nanoparticles of diverse sizes and shapes [9]. Of these methods, Turkevich *et al*.'s water based [10] and Brust *et al*.'s organic liquid based methods [11] are the most widespread used approaches. Both the Turkevich and Brust methods require a reducing agent to reduce $Au^{3+}$ ions to neutral gold atoms, and a suitable stabilizer to prevent the synthesized gold nanoparticles from aggregating. In the Turkevich method, sodium citrate is usually used as both the reducing agent and the stabilizer, although other reductants, such as amino acids have also been successfully used. Spherical gold nanoparticles around 10-20 nm in diameter suspended in water can be produced. The particle size can be increased by reducing the amount of sodium citrate. In the Brust method, sodium borohydride ($NaBH_4$) and tetraoctylammonium (TOAB) are used as the reducing agent and the stabilizer, respectively. The synthesized gold nanoparticles are around 5-6 nm.

In this research, a high sensitive nanobiosensor based on a 3D sensing element that has uniformly deposited gold nanoparticles was proposed. The barrier layer of an anodic aluminum oxide (AAO) film is used as the template; a reducing agent and stabilizer free method based on the electrochemical deposition technique to synthesize uniformly distributed gold nanoparticles on the surface of the barrier layer was conducted. The distribution density and size of the nanoparticles can be well controlled by the

applied potential for the electrochemical deposition; following, receptors or antibodies with respect to a certain biomarker were attached to the gold nanoparticles for further sensing applications.

## 2 SENSOR DESIGN AND FABRICATION

### 2.1 Sensor Design

Figure 1 schematically illustrates the scheme of the proposed transducer of gold nanoparticles. The sequential synthesis processes include: preparation of an AAO film; modification of the surface of its barrier-layer; deposition of an Au thin film; annealing; and electrochemical deposition of Au nanoparticles. Since the Au thin film for the electrode is shaped by the structure of the surface of the barrier-layer, the procedures involving the modification of the barrier-layer surface are crucial.

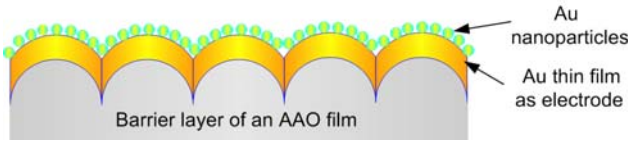



Figure 1: Schematic drawing of the proposed reductant and stabilizer free approach for gold nanoparticle synthesis

### 2.2 Sensor Fabrication

(1) AAO film preparation

The AAO films were prepared using the well-known anodizing process. Aluminum foils were cleansed and electropolished before anodization. AAO films, with a nanopore diameter of around 60 nm and a thickness of 50 μm, were obtained by anodizing the polished aluminum foil in a 0.3 M phosphoric acid solution under an applied voltage of 90 V at 0°C for 2 hours. The remaining aluminum beneath the barrier layer was dissolved in an aqueous $CuCl_2$ • HCl solution that was prepared by dissolving 13.45 g of powdered $CuCl_2$ into 100 ml of 35 wt% hydrochloric acid solution.

(2) Modification of the barrier-layer surface

During the first stage, the remaining aluminum covering the barrier-layer was removed using a mixed solution of $CuCl_2$ and HCl, to reveal the honey-comb like surface of the barrier-layer. The honey-combs had an average convex diameter of 80 nm. Following this, the barrier-layer surface was immersed in a 30 wt% phosphoric acid for 40 minutes to modify the surface structure.

(3) Deposition of an Au thin film

The modified barrier-layer surface was then used as the template for depositing the 3D nanostructure Au film through radio frequency (RF) magnetron sputtering. The experimental conditions during deposition were: pressure = $4.0\times10^{-3}$ torr; temperature = room temperature; argon = 20 sccm; power = 80 W; processing time = 2 min. A 3D nanostructure Au film with thickness being 30 nm can be obtained.

(4) Annealing

Usually, an RF magnetron sputtered thin film shows a higher electrical properties and poorer crystalline structure. To further modify the surface structures of the 3D nanostructure of the Au film and increase the conductance of the sample, we utilized an additional annealing process. The annealing procedures included: heating the sample to 400 °C at a rate of 20 °C/sec and staying for 40 min; then cooling the sample in air to room temperature.

(5) Electrochemical deposition of Au nanoparticles

A picoammeter (Sversa Stat II, Princeton Applied Research) was used to conduct the electrophoretic deposition. The 3D Au sample was placed at the working electrode (WE) with the Au thin film being the electrode. The counter electrode was a Pt film while the reference electrode (RE) was Ag/AgCl. The deposition processes included:

(*i*) *Electrolyte preparation*:

The electrolyte was prepared by dissolving 1 mL of 0.02 M $HAuCl_4$ (Aldrich Inc.) solution in 40 mL of deionized water.

(*ii*) *Reducing potential measurement*:

The cyclic voltammetry method [12] was implemented using an electrochemical analyzer (Model 627C, CH Instruments) to conduct the measurement of the reducing potential of the electrolyte $HAuCl_4$ solution. The range of scanning was from +0.8 V to -0.8V. It was detected that -0.8 V was a feasible reducing potential for the $HAuCl_4$ solution used in this research.

(*iii*) *Electrochemical deposition*:

To eliminate the possibility of unstable depositions at the transient period due to a larger applied voltage, a DC -0.34 V initial potential that was detected by the open-circuit potential method was applied. Following, a DC -0.8 V electric potential was applied for 300 sec at room temperature to conduct the process of electrochemical deposition.

### 2.3 Results and Discussions

Figure 2 shows an SEM image of the modified barrier-layer surface after being etched by a 30 wt% phosphoric acid for 40 minutes. Due to the stress concentration effect during anodization, the phosphoric acid etched out more alumina at the borders between the cells than from the cell surfaces, resulting in an orderly hemispheric barrier-layer surface.

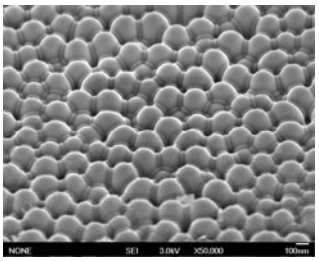


Figure 2: SEM image of a modified barrier-layer surface

The deposition results of an Au thin film on the modified barrier-layer surface are illustrated in Figure 3. The Au thin film took its form from the shape of the barrier-layer surface.

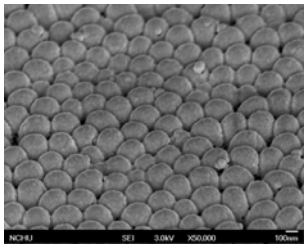
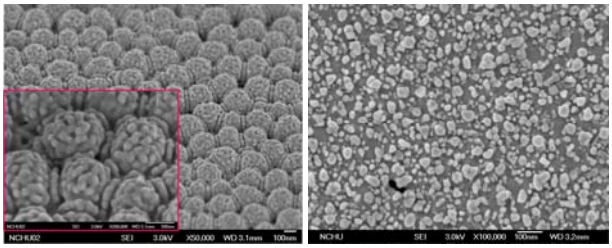


Figure 3: Au thin film electrodes formed according to the shape of the barrier-layer surface

Figure 4 shows the results of the electrochemical deposition of gold nanoparticles. The gold nanoparticles deposited using an orderly hemispheric electrode array (Figure 4) have an average diameter of about 10 nm and are uniformly and compactly deposited on the hemispheric electrode array. However, gold nanoparticles were disorderly distributed when a flat electrode was implemented and a sodium citrate solution was used as the stabilizer.



(a) (b)

Figure 4: SEM images of gold nanoparticles synthesized by electrochemical deposition; (a) 3D elsctrode (b) flat electrode and sodium citrate solution as the stabilizer.

The working principle of the proposed electrochemical deposition is depicted in Figure 5. The uniformly propagated electric flux ( $\vec{E}$ ) perpendicular to the hemispheric Au thin film electrode has pulled the negative charges carrying Au nanoparticles in the electrolyte so that they can be densely deposited onto the surface of the Au thin film electrode without the necessity of any reducing agent and stabilizer.

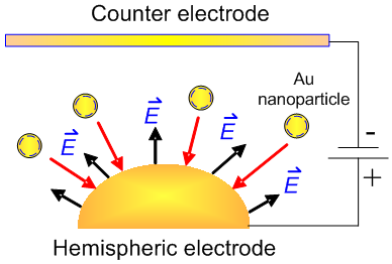



Figure 5: Illustration of the working principle of the electrochemical deposition process

Since the morphology of the deposited gold nanoparticles is a function of the electric flux density, which is determined by the applied potential, it is worthwhile to investigate the dependencies of the deposition morphology on the applied potential. Figure 6 shows the deposition morphologies for different applied potentials. The deposition duration was 300 sec.

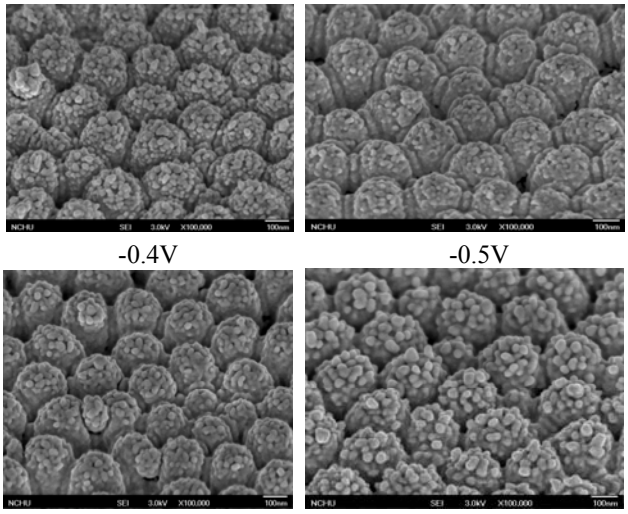



Figure 6: Morphologies of the gold nanoparticles deposited under different applied potentials

## 3 SENSING EXPERIMENTS

The detection of biotin was carried to examine the sensitivity of the proposed 3D gold-nanoparticle based nanobiosensor. The surface of the sensor chip (Figure 5a) was cleaned by soaking in ethanol, acetone, and deionized (DI) water in turn, and shaken by ultrasonic wave for 5 min, respectively. The sensor chip was then dispensed with 1ml of a 5mM 11-MUA (11-mercaptoundecanoic acid) solution to form a self-assembled monolayer of 11-MUA as an anchor membrane. The carboxylic groups of the 11-MUA layer on the sensor chip were activated by immersing the sensor chip in a mixed NHS (N-hydroxysuccinimide) and EDC (1-Ethyl-3-(3-dimethyl- aminopropyl)-carbodiimide) solution (molar ratio, 1:2) for 50 min. After washing with DI water, the sensor chip was dispensed with 1mg/ml of avidin solution. The sensing samples were prepared by immersing the sensor chips in biotin solutions with different concentrations (10 ng/ml, 8 ng/ml, 6 ng/ml, and 4 ng/ml) for 12 hours [13].

### 3.1 Cyclic Voltammetry (CV)

Cyclic voltammetry is usually used to examine the electrochemical properties of an analyte in solution. In a cyclic voltammetry experiment a potential of triangular wave is implemented as the working electrode potential. The current measured at the working electrode is plotted versus the applied voltage to provide the cyclic voltammogram trace. The cyclic voltammogram trace can be to estimate the real area of a sensing device which is effective for the adhesion of the analyte. Accordingly, the sensitivity of the sensing device can thus be examined.

The diameter of the AAO/Au/GNP device as shown in Figure 5(a) is 8mm. Since the GNPs were uniformly

deposited on the hemispheric electrode array, the effective area of the electrode can not be straightly calculated by geometric analysis. In this research, the device was put into a 0.5 M $H_2SO_4$ solution and used the cyclic voltammetry scanning from 0.0 V to 1.6 V at a scan rate of 100 mV/sec to have a cyclic voltammogram as shown in Figure 7. The reducing potential of AuO was detected to be around 0.9 V. Electric charges of 1.22 mC can be accumulated by integrating the reducing peak. Since the charges required to form AuO per 1 $cm^2$ of Au electrode is 386 μC, the effective area of the device was estimated to be 3.17 $cm^2$ (1.22 mC/386 μC). When compared with the area (0.502$cm^2$) of the ϕ=8mm substrate, the effective area was enhanced by 6-fold.

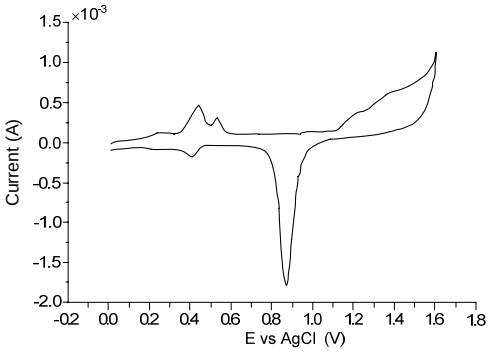



Figure 7. Cyclic voltammogram of the device in a 0.5 M $H_2SO_4$ solution

## 3.2 Electrochemical Impedance Spectroscopy (EIS)

The method of EIS is implemented in electrochemistry based on the information regarding the interface. By applying a periodic small amplitude AC signal to the interface and measuring the actual system response, the impedance parameters of the system can be estimated. Figure 8 is the electrochemical impedance spectroscopy (using Versa stat II 273A) for biotin with various concentrations. It is observed that the impedance of the biotin binding device increases with the increasing of the biotin concentration. Biotin is small molecular with its molecular weight being 244.3. When a biotin molecular binds with an avidin molecular, impedance due to resistance is larger than that is from capacitance. Although the sensitivity of the proposed AAO/Au/GNP device was 4 ng/ml using a less sensitive Versa stat II 273A, a better result is believed to be feasible if a more sensitive machine is implemented.

## 4 CONCLUSIONS

In this paper, we propose a high sensitive nanobiosensor based on a 3D sensing element that has uniformly deposited gold nanoparticles. The sequential synthesis processes include: preparation of an AAO film; modification of the surface of its barrier-layer; deposition of a thin film of gold on the surface of the barrier layer; annealing; and electrochemical deposition of gold nanoparticles on the gold thin film; receptors or antibodies with respect to a certain biomarker were immobilized on the gold nanoparticles. The sensitivity of the proposed 3D gold-nanoparticle based nanobiosensor was verified using cyclic voltammetry and electrochemical impedance spectroscopy through the sensing experiments on biotin detection.

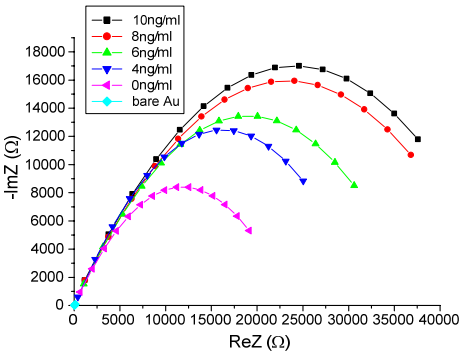



Figure 8: EIS for biotin with various concentrations; working electrode: AAO/Au/GNP device, counter electrode: Pt, reference electrode: Ag/AgCl, buffer solution: mixing of 2.5 mM $Fe(CN)_6^{4-}$ and 2.5 mM $Fe(CN)_6^{3-}$in 50 mM MES, DC power: 0.23 V, AC power: 5mV, AC frequency: 100 kHz~0.1Hz

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