Nanoporous Gold: A High Sensitivity and Specificity Biosensing Substrate

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Abstract

Unambiguous identification of analyte biomolecules with high quantitative sensitivity and specificity continues to be a biosensing challenge. In this work, we demonstrate a micromachined quartz resonator array with nanoporous gold electrode as a highly sensitive substrate for biosensing applications. Furthermore, using surface enhanced Raman spectroscopy (SERS) the nanoporous gold substrate can be used to obtain label free detection of biomolecules with very high selectivity. Enhanced gravimetric sensitivity to 24-mer oligonucleotide binding reaction was achieved due to the increased electrode surface area. This dual mode sensing of biomaterials allows for unique identification of the attached biomolecules on the sensor surface via SERS whereas the quartz resonator provides a very accurate quantitative measure of the analyte. This paper presents the first results on the application of nanoporous gold (np-Au) electrode micromachined quartz resonators for detection of DNA hybridization reaction using simultaneous gravimetric and SERS measurements.

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1. Introduction

The three main challenges in the development of biosensors include: (i) Development of sensor platforms capable of high sensitivity, quantitative bioassays with the ultimate capability of detecting single molecules, (ii) Engineering a sensor configuration that enhances the occurrence of interactions

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between the analyte molecules and the sensor active area at low concentrations, and (iii) Achievement of high degree of molecular specificity to avoid false positives. Using micromachined 66 MHz quartz resonator arrays, we have previously demonstrated ~4.25 times improvement in the sensitivity (signal to noise ratio) in biosensing applications [1]. However, the selectivity in these gravimetric devices was achieved only through specific binding reactions and is often complicated to achieve. To address these challenges, we have developed micromachined quartz crystal resonator arrays in which the conventional gold electrode is replaced with a nanoporous gold electrode which offers an increase in the effective adsorption surface area, and hence enhanced sensitivity without sacrificing the Q-factor of the device [2]. Furthermore, the nanoporous gold electrode is a Raman spectroscopy (RS) active layer and therefore produces an enhanced signal-to-noise ratio for RS identification of adsorbed molecules, i.e. a unique way to fingerprint the adsorbed biomolecules [3, 4].

2. Materials and Methods

2.1. Materials

Nuclease free Duplex Buffer (NDB; 30mM Hepes pH 7.5, 100mM KAc) was used as received from Integrated DNA Technologies, Inc (IDT). Single strand DNA (ss-DNA) containing thiol modifiers placed at the 5’-end of the oligo was synthesized by IDT and used as received. A random sequence 24-base oligonucleotide (SH-5’ AGC ACA CCA TCA CCA AAG CAA CAG 3’), named PK DNA, and the target with a sequence complementary to the 24-base ss-DNA probe, labeled with 6-FAM fluorescent dye (Ex. 495 nm, Em. 520nm) on the 5’-end were also purchased from IDT. All oligonucleotide stock solutions were diluted with 18MΩ-cm DI water (Millipore Milli-Q system; Barnstead International), and again diluted with NDB to prepare 1μM ss-DNA probe and target solutions for the use in experiments.

2.2. Fabrication of nanoporous structure: Selective etching

Figure 1(a) shows a schematic drawing of a quartz crystal resonator consisting of a nanoporous gold top electrode. Application of sinusoidal stimulus to the quartz results in the set-up of shear horizontal waves propagating through the thickness of quartz. When the half-wavelength of the acoustic wave in quartz equals the thickness of the quartz, resonance condition is satisfied. To form np-Au electrode on micromachined quartz crystal microbalance (QCM) arrays, selective chemical etching of silver from silver-gold (Ag-Au) alloy film is used. A silver-gold alloy film is prepared using ion-beam sputtering on the micromachined QCM. A silver-gold alloy target containing 70 atomic % of silver was used for the ion-beam sputtering of the film. This was followed by photolithographic patterning and chemical etching in iodide-based silver etchant to pattern the silver-gold electrodes. The sample was thereafter immersed into 70% concentrated nitric acid for 6 hours, resulting in the formation of sponge-like, discontinuous nanoporous gold with less than 5% of silver left-off. The device is packaged in a modified 24 pin, dual-in-line ceramic package in which a 6 mm x 6 mm square hole is cut using waterjet machining, and the fabricated resonator array is attached using silicone adhesive and cured at room temperature for 24 hours to avoid build-up of thermally induced stresses in the quartz and the individual pads of each resonator are wire bonded (see Fig 1(b)).

2.3. Measurement Setup

All measurements are carried out using Agilent 4395A impedance analyzer inside a special aluminum die-cast (4.7” x 4.7” x 3.54”) to prevent RF interference and to control the temperature during the
experiments at 25 (±0.1) °C. Impedance analyzer was initially calibrated to obtain accurate QCM resonance parameters and was set to simultaneously measure the magnitude $|Z|$ and phase $\theta$ of impedance as a function of frequency at the first and third resonance modes. 801 data points were acquired in a specified frequency span around the resonance frequency. The device was clamped between customized Teflon test blocks to hold solutions containing ss-DNA probe and the target complementary DNA, as shown in Fig. 1 (c). All the experimentally measured data is recorded by specifically coded Labview software.

3. Results and Discussion

The formation of nanoporous gold film involves not only a selective dissolution of one of the components in the deposited alloy, but also a critical film thickness. This was clearly observed when two Ag-Au alloy films of thicknesses 50nm and 100nm were deposited using ion-beam sputtering from the same target (silver-gold alloy containing 70 atomic % of silver). Following an identical selective etching process in 70% concentrated nitric acid on the two films for 6 hours, nanoporous gold structure was observed only on the 100 nm thick Ag-Au alloy film whereas no change could be seen on 50nm Ag-Au alloy film even with additional 18 hours of immersion in etching solution (total 24 hours of etching time) that is consistent with the results in the literature [3].

Selective etching is the technique which is selective dissolution of one or more components from a metallic alloy. In this process, the less noble components are oxidized and dissolved into etching solution whereas the more noble components remain in its stable metallic form. Since gold is an inert material, it does not oxidize or form compounds and complexes which perturb surface diffusion and formation of a nanoporous structure [2]. Phenomenologically, formation of nanoporous gold can be thought to arise as a consequence of the atomic rearrangements at the interface between the metal and the solution. As gold atoms are physically released from the Ag-Au alloy due to the dissolution of silver into solution, they nucleate into clusters initially laying the foundation for the formation of three dimensional porous

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Fig. 1. (a) Schematic illustration of a quartz crystal resonator with np-Au top electrode. (b) Optical photograph of the packaged quartz resonator array in a 24-pin modified dual-in-line ceramic package (c) Optical photograph of the fabricated device with a Teflon set-up.

Fig. 2 Optical photographs of (a) 50 nm thick Ag-Au alloy film after selective etching for 24 hours (Inset shows SEM picture of a small region of the film) shows only clustering of gold atoms without the formation of nanoporous structure (b) 100 nm thick Ag-Au alloy film after selective etching for 6 hours (Inset shows SEM picture of a small region of the film) clearly shows the formation of the nanoporous structure.
structure at the surface as additional atomic layers of gold atoms are released during the dissolution process. Thus the selective etching (corrosion) process can be considered as diffusive redistribution of gold and vacancies on a randomly nucleated surface template layer [3]. If the amount of gold in the film is below a critical amount, the admobility of the gold atoms causes a rearrangement of atoms which results in a solid film rather than the filamentous nanoporous layer. Figure 2 shows the optical and SEM pictures of the nanoporous gold films formed for the two thicknesses.

24-base single-stranded thiolated DNA probe was immobilized on QCM arrays with both conventional solid-gold electrode and nanoporous-Au electrodes. This was followed by hybridization with, 6-FAM fluorescent dye (Ex. 495 nm, Em. 520 nm) labeled, complementary sequence DNA molecules, and the frequency and $Q$-factor shift were measured by impedance analyzer for micromachined QCM with both solid-gold electrode and np-Au electrode. The frequency shift upon injection of the target ss-DNA solution was measured until the hybridization process reached saturation. The results show nearly two times greater frequency shift, 2500Hz for np-Au electrode as compared to a frequency shift of 1290Hz for solid-Au electrode, even though the resonance frequency of solid-Au QCM was 13MHz higher than that of np-Au QCM. The shift in $Q$-factor for both solid-Au and np-Au electrodes was almost negligible [5].

100 – 200 nm thick np-Au films have been seen to highly reproducible SERS substrates with the maximum enhancement factors reaching $\sim 10^4$ for 632.8 nm excitation and good spot to spot reproducibility [3]. SERS response of np-Au electrode with single stranded DNA and hybridized DNA was experimentally measured and clearly showed distinguishing peaks in the 1200 -1600 cm$^{-1}$ wavenumber range. However, it is difficult to distinguish Raman spectra, resulting from oligonucleotides with different sequences due to the structural similarities.

Conclusions

In conclusion, using np-Au electrode micromachined quartz crystal resonators, coupled with SERS, we have demonstrated a dual mode sensor with enhanced gravimetric sensitivity achieved through sensor design and the large surface area of np-Au and fingerprinting capability for the unique identification of adsorbed biomolecules.

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