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A third-order mode high frequency biosensor with atomic resolution

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ABSTRACT

An atomic resolution ultra-high sensitivity surface acoustic wave (SAW) biosensor for DNA sequences and cells detection is proposed. Interdigitated transducers (IDTs) fabricated on $LiNbO_3$ substrate achieve a high quality factor (*Q*) of over 4000 at a frequency of 6.4 GHz (third-order harmonic mode) using an optimized design and process. The biosensor shows excellent linear responses to target DNA in the range from 1 μg/ml to 1 ng/ml with a high sensitivity of 6.7×10^{-16} g/cm^2/Hz, hence the difference of a single hybridized DNA base can also be distinguished. With such a high mass resolution, the biosensor is capable of quantitative detection of living cancer cells. The frequency responses of single mouse mammary adenocarcinoma (EMT6) cell and mouse fibroblast (3T3) cell are studied. The interferences in the experiments show insignificant influence on the frequency shift, which verifies the high selectivity of the biosensor. The biosensor is also able to repeat the sensing ability after rough cleaning, therefore cost reduction is achieved from the recycling process in practical applications. The detection limit is defined from the noise analysis of the device, atomic resolution is realized according to the calculation, thereby initiating a potential tool for high-precision medical diagnoses and phenomena observation at the atomic-level.

1. Introduction

High sensitivity, high selectivity and rapid response label-free biosensors suitable for field use or point-of-care applications are extensively used in disease diagnoses and other fields (Alivisatos, 2004; Giljohann, et al., 2009; Rosi et al., 2005). It is imperative to develop techniques capable of detecting ultra-low concentration samples or even single molecules and atoms (Jensen et al., 2008; Naik et al., 2009; Yang et al., 2006). This will enable the reliable detection of certain biochemical reaction mechanisms, such as DNA hybridization, intracellular mutations, etc. Numerous biosensors have been made by utilizing novel materials (Heller et al., 2009; Lu et al., 2011; Mannoor et al., 2012; Peng et al., 2009), structures (Dong et al., 2010; Feng et al., 2008; Li et al., 2007; Venkatesan et al., 2011) and methods (Aebersold et al. 2003; Anker et al., 2008; Lee et al., 2008; Sun et al., 2013) or combinations of them to break previous performance limitations. However, these works contain various drawbacks for real-world applications. For example, some of these devices contain mechanical parts which lack durability, or which cannot sustain repeated immersion in liquids for reliable detection, etc. Other approaches either need complicated device fabrication process or require complex sample purification and subsequent amplification steps, which make the detection very costly and time-consuming. SAW devices are an exciting alternative for biomedical applications. These devices do not contain any mechanical parts and are therefore a robust sensing platform. SAW devices have previously been used with a resonant frequency of over 1 GHz for highly sensitive detection (Krishnamoorthy, et al., 2008; Kim et al., 2013). In order to obtain an even higher sensitivity, it is desirable to increase the resonant frequency (Petrie et al., 2011). Super high frequency (SHF) resonators are able to provide unprecedented sensitivity for mass sensing and real-time measurement. Additionally, these devices are also able to ease the requirements of large complex equipment and therefore enable a greater portability and cost effectiveness. Various complex nano devices have been fabricated which have demonstrated great potential for chemical and biomedical sensing applications (Chen et al., 2011; Dow et al., 2012; Palankar et al., 2013; Shi et al., 2010). SAW devices have a relatively short and straightforward fabrication process and are a simple sensing platform with a high frequency and *Q*. Nevertheless, it is still challenging to fabricate high-performance SAW devices due to a number of factors. The use of non-conducting substrates

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introduces charging during electron beam lithography (EBL) which needs to be corrected using conductive top-coating (Muhammad et al., 2011; Peters et al., 2013). The dense IDTs are adversely affected due to proximity effect during EBL which needs to be corrected by software or avoided by careful process parameter selection.

In this paper, a SAW biosensor with an atomic resolution has been developed. IDTs of 500 nm width and a 2 μm period provide a super high resonant frequency of 6.4 GHz (third order mode) and a *Q* of over 4000. For the purpose of biosensing, using such a high frequency is unprecedented. When the samples are immobilized on the delay area of the biosensor, the resonant frequency of the device is decreased in response to the adsorbed mass. By measuring the frequency shifts, the immobilized mass is obtained. In this way, highly sensitive and selective measurements for ultralight mass can be performed with very simply processed samples. By employing these devices, a single DNA base difference can be detected. Meanwhile, linear frequency response for a varying number of EMT6 cells and 3T3 cells in a wide number concentration range is achieved. The capacity of single cell detection is expected to realize accurate diagnoses of individual cellular mutation, lesion, dynamic change, etc. The biosensor is an ideal solution to sequence entire genomes, detecting markers of disease at a very low concentration and understanding the molecular basis of disease. For instance, by distinguishing the mass differences among the four kinds of DNA bases, the base type in a DNA chain is easily known. The detection accuracy is guaranteed and the cost reduction is realized. It is difficult to diagnose the existence of antigen, bacteria or virus at an early stage because of the low concentration in organisms. This, however, is detrimental to the health of the organisms and causes irreparable harm. The biosensor has the ability to detect very atomic-level mass changes caused by specific binding to detect the lesion. Moreover, the blood glucose, protein or other biological and chemical substances and their concentration are also detectable on account of the reaction or consumption of them on the biosensor. These applications demonstrate that the SAW biosensor offers exciting possibilities for ultra-high-precision mass discrimination.

2. Material and methods

2.1. Device fabrication

The resist was spin-coated on a $LiNbO_3$ substrate and a thin layer of 5 nm Cr was sputtered on top to improve the substrate conductivity. EBL (JEOL 6300FS, 100 keV) was used to pattern the SAW IDTs and delay area. This is followed by removing the thin Cr layer, sputtering 50 nm gold and performing lift-off. (See supplementary materials)

2.2. FEM simulation and principle analysis

The design of the SAW biosensor is optimized by Finite Element Method (FEM) simulation. Firstly, the three-dimension model is created with the practical device parameters. Frequency sweep signal is added on the device to show the spectrum. Secondly, the parameters such as the thickness of the metal, the number and the space of IDTs are gradually changed to optimize the performance of the device. Representative simulated results are shown in Fig. 1a. Left panel shows the surface wave transmission map of the device, it is the resonance of IDTs on the substrate, and the red part shows where the resonance is the strongest. The right panel shows the harmonic analysis results. In the right panel, the displacement amplitude (normalized to the maximum value) is a function of the applied AC signal frequency and the resonant modes can be clearly observed. The third-order harmonic, which still has a large resonant amplitude and an excellent *Q* of over 4000, is applied for detection and the measured resonant frequency is about 6.4 GHz. The *Q* is calculated from the measured reflection coefficient S_{11} using the equation $Q=f/BW$, where f is resonant frequency, and BW is 3 dB bandwidth.

The surface acoustic waves generated by the IDTs propagate along the surface of the substrate at a certain depth. Upon reaching the delay area, these waves will partially reflect. When target substances are immobilized and hybridized on the delay area, a thin superimposed molecular film is formed, which effectively alters the film thickness and density. This causes the velocity of the reflected signal to change, which in turn shifts the resonance frequency (Fig. 1b) according to the equation $f=v/\lambda$, where f is resonant frequency, v is the velocity of surface wave, and λ is wavelength of surface wave. Furthermore, due to the addition of the molecular film on the delay area, there is an increased dissipation of energy causing a reduction of the biosensor *Q*-factor.

Fig. 1c shows the Scanning electron microscope (SEM) of the fabricated SAW IDTs, bonding pad and partially amplified views of the SAW device. The IDT array of the SAW devices are 60 μm wide and 1000 μm long. These IDT arrays attach to two electrodes (60 μm by 200 μm), one of which is connected to the signal port and the other to ground. The size of the delay area used for sample immobilization is 1 mm × 1 mm. Each IDT has a finger width and spacing of 500 nm, which indicates that the wave length λ of the device is 2 μm and the calculated resonant fundamental frequency is about 2 GHz. Fig. 1d shows the probe DNA's immobilization on the gold delay area surface followed by hybridization of the target DNA with the probe DNA. Both attachment steps yield mass and resonant frequency changes.

2.3. Biological treatment

The DNA detection is based on the probe–target DNA binding mechanism (Fig. 1d) and the immobilization of DNA on delay area is shown in Fig. 2a and b. When DNA is immobilized and hybridized on the delay area, the surface has an obvious change that indicates the existence of DNA molecules.

The probe DNA is first immobilized on the gold surface of the delay area, which causes a frequency shift. This is due to the reduction of velocity of the surface acoustic wave through the immobilized molecular film. Then the target DNA is hybridized with the probe DNA under specific conditions to cause an additional frequency shift. By measuring the frequency shifts, the immobilized mass that indicates the DNA solution concentration can be calculated.

A method that improves the immobilization of DNA molecule with gold surface was applied for reliable analysis of the sensing effect (Lucarelli et al., 2008; Peterson et al., 2001; Petrovykh et al., 2003; Smith et al., 2001). To exclude the defects in the DNA hybridization processes, several contrast experiments that exclude the influence of other substances were also conducted to guarantee the validity of the results. The basic DNA immobilization and hybridization flow is described below: (1) The thiol-modified probe DNA (pDNA) solution for immobilization is prepared by mixing with the Phosphate Buffered Saline (PBS, 0.01 M PH=7.4). (2) The gold delay area is rinsed with a Piranha solution (70% H_2SO_4 and 30% H_2O_2) to clean the surface and strengthen the molecular adhesion. (3) The pDNA mixed with a 0.1 M KNO_3 solution is injected on the delay area, and then stored in a 4 °C chamber with proper humidity for 24 h to allow the pDNA to fully immobilize. (4) The delay area is washed with Milli-Q water and then dried with N_2 followed by covering a 1ng/ml bovine serum albumin (BSA) to block the uncovered gold surface. (5) The target