Sequence Specific Label-Free DNA Sensing Using Film-Bulk-Acoustic-Resonators

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***Abstract*—A label-free biosensor (for detection of DNA sequences) based on film-bulk-acoustic-resonator (FBAR) is presented in this letter. The FBAR's resonant frequency shifts to a lower value when a complementary single-strand DNA sequence is hybridized with a DNA probe sequence on an Au-coated FBAR surface. The sensor is capable of distinguishing a complementary DNA that is mismatched to a probe DNA by a single nucleotide. The label-free, highly sensitive and selective, and real-time detection of DNA sequence could easily be made into an array for combinatory DNA sequencing, and could possibly help geneticists to detect specific DNA sequences accurately and fast, without any expensive optical scanning or imaging.**

***Index Terms*—Biosensor, DNA hybridization, film-bulk-acoustic-resonator (FBAR), mass sensor.**

I. Introduction

DNA-based biosensors represent a promising tool with wide applications, such as in gene sequence analysis, gene profiling and mutation studies, virus and bacteria detection, clinical diagnostics, drug discovery, and counter bioterrorism. A number of DNA-based biosensors (electrochemical, optical, mechanical, and piezosensors) have been developed in recent years [1], [2], and their uses are demonstrated by sequence-specific oligonucleotide detection, identification of genetically modified organisms, gene mutation studies, etc. Unlike traditional gel electrophoresis and other methods, DNA-based sensors can be used without labeling (e.g., ^{32}P) or fluorescent tags.

In this letter, we introduce a film-bulk-acoustic-resonator (FBAR) [3] as a novel microelectromechanical system (MEMS) for sequence specific and label-free detection of DNA molecules from the resonant frequency shift measurement. The discussed FBAR device consists of a thin piezoelectric ZnO film sandwiched between two thin Al-electrode layers, which are supported on a SiN diaphragm. The area of the Al/ZnO/Al sandwich is typically tens to tens of thousands μm^2. The resonant frequency of the FBAR is determined by the thickness of its four layers, and drops linearly with added mass on the surface.

A typical FBAR has a resonant frequency near gigahertz (GHz), and its mass-sensitivity is tens times higher than a typical quartz crystal microbalance [4]. For sensor applications, a very thin-gold layer ($\sim$800 Å) is deposited on the top electrode for chemical modification with probe DNA molecules using thiolation chemistry so that selective sensing is possible. When the target ssDNA molecules hybridize with the surface bound DNA probes, the mass load of the resonator is increased, and the resonant frequency proportionally drops. Thus, hybridization event can be followed in real-time by observing the resonant frequency shift. Since the transduction mechanism is mechanical, this technique does not need fluorescent tags or ^{32}P label.

TABLE I
DNA Probe and Targets Used in the Experiments Properties

Entry	Sequence	Comments
1)	5'-(HS-C_6)-CGCCAAGCAGTTCGT-3'	Probe
2)	3'-GCGGTTCGTCAAGCA-5'	Complement
3)	3'-GCGGTTC<u>A</u>TCAAGCA-5'	1 Mismatch
4)	3'-GCG<u>T</u>TTCGGCA<u>G</u>GCA-5'	2 Mismatch
5)	3'-GCGG<u>C</u>TCG<u>G</u>CAA<u>T</u>CA-5'	3 Mismatch
6)	3'-<u>A</u>CG<u>C</u>TTA<u>G</u>G<u>C</u>AA<u>T</u>C<u>G</u>-5	6 Mismatch

II. Experiments and Results

The oligonucleotide sequences shown in Table I were tested in a setup similar to that described in [3]. The 15-mer probe nucleotide (entry 1) was functionalized at the 5'-end with a HS-$(CH_2)_6$-group for immobilization on the Au-coated FBAR device. Selected targets were complementary (entry 2), single-mismatched (entry 3) and more than one mismatched (entry 4–6).

For maximum hybridization (for maximum resonance shift that leads to highest signal-to-noise ratio), the probe DNA molecules must be arranged on the surface at an optimum concentration; After reviewing a number of published data, we decided to treat an Au-coated FBAR device with HS-ssDNA (2.0 μM/1.0 M KH_2PO_4, pH 4.2) for two hours followed by immersion for 1 h in 1.0 mM mercapto-hexanol (MCH) in aqueous solution forming a mixed-monolayer [5]. Under this condition of surface treatment, the number of probe DNA molecules is expected to be in the range of $\sim 10^{12}$/cm^2, and $\sim$100% hybridization could be possible when treated with $\sim$1.0 μM target DNA in 1.0 NaCl-TE buffer [5].

Gold surfaces treated only with HS-ssDNA usually contain a significant amount of nonspecifically bound DNA molecules due to electrostatic attraction between the metal surface and anionic DNA molecules. Such surface-bound molecules negatively affect the number of surface-bound DNA probes and the degree of hybridization with target sequences. MCH treatment is known to remove those molecules from the surface by competitive covalent bond formation with the gold surface, and

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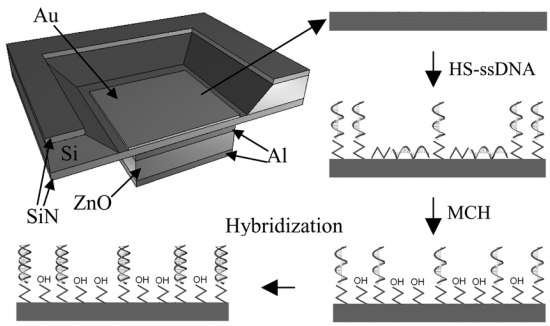

Fig. 1. Illustration of FBAR device being treated with HS-ssDNA and MCH for mixed monolayer formation and hybridized with a target DNA sequence. The resonant frequency of the HS-DNA immobilized FBAR decreases when hybridized with the target DNA complement.

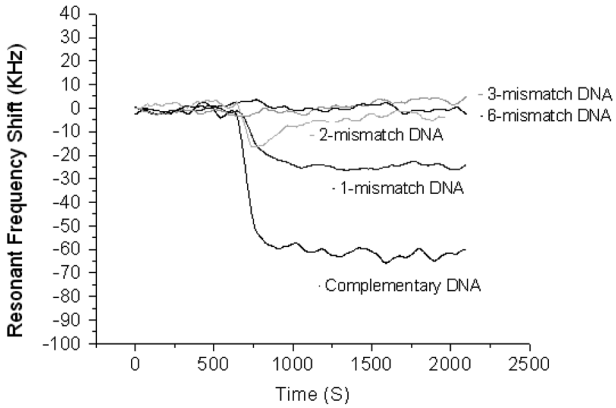

Fig. 2. The resonant frequency shift of the FBAR on whose surface is immobilized with DNA.

helps reorganization of the probe molecules for better hybridization with complement strand by occupying the empty space [5]. The effect of MCH treatment on probe DNA organization and hybridization is depicted in the Fig. 1.

For hybridization, 1 μM target DNA solution was prepared by dissolving 1.0 M NaCl-10 mM Tris-HCl-1.0 mM EDTA in pH 7.5 buffer solution. To observe real-time hybridization, the FBAR device was initially immersed in the same buffer solution used to dissolve the target DNA samples, and stable resonance was obtained in about 20 min. Then, the device was exposed to target DNA solution and the resonant frequency shift was recorded in real-time using a desktop computer connected to the network analyzer (that measured the impedance of the FBAR, giving the information on the resonant frequency shift). The resonant frequency (about 1.0 GHz) dropped significantly ($\sim$70 kHz) when the DNA-probe-immobilized FBAR was exposed to the target complementary sequence (1 μM, entry 2). The change of resonant frequency was fast, and reached to saturation point quickly. By stepwise and controlled addition, stepwise changes of the resonant frequency (ladder like) were observed until the stable endpoint was reached. The result can be reproduced by removing the target DNA sequence by dehybridization (washing with hot TE buffer above the melting temperature) and treating with a fresh target solution. To rule out any possibility of nonspecific or false resonance shifts, a MCH-only-coated FBAR was treated with the same complementary DNA strain. No resonance shift was observed, supporting the interpretation that the resonance shift is due to a specific probe-target DNA interaction. With the single-mismatch sequence, the resonant frequency shift was $\sim$35% ($\sim$25 kHz) of that ($\sim$70 kHz) obtained with the complementary sequence. Therefore, the FBAR is capable of distinguishing the complementary DNA from a single-nucleotide mismatch DNA sequence. It was possible that the single-mismatch sequence could hybridize with the probe to a certain extent using one of the two regions bordering the mismatched nucleoside base. A similar result was obtained with other methods of DNA sequence detection. On the other hand, with two or more mismatches, no noticeable resonance shift was observed from the baseline resonant frequency (Fig. 2).

Therefore, using a thin-gold layer coated FBAR device with an immobilized 15-mer oligonucleotide probe, we have demonstrated selective, label-free and real-time detection of oligonucleotides sequences, and DNA match/mismatched determination. The FBAR has been shown to be able to distinguish oligonucleotides differing by only one nucleobase (single-nucleotide mismatch).

Since the target complement can be removed from the surface by "melting" (dehybridization) after detection, the device can be reused over many cycles to save time and expensive reagents. The DNA sensor can be optimized for specificity, depending on the target sequences or target organisms' DNA fragments, by changing the immobilized probe sequence. Thus, this class of sensor is expected to have wide applications, ranging from food safety tests to clinical applications. The main advantages that FBARs have added are micron size (that would require a minute amount of sample), label-free and real-time analysis capability, and adaptability in array formats for simultaneous analysis of multiple targets. These advantages make FBAR an attractive candidate for DNA-biosensing.

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