NEMS Capacitive Sensors for Highly Sensitive, Label-Free Nucleic-Acid Analysis

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Abstract. A highly sensitive NEMS capacitive sensor with electrode separation in the order of Debye length is fabricated for label free DNA analysis. The use of nano-scale electrode separation provides better insight in to the target-probe interaction which was not previously attainable with macro or even micro scale devices. As the double layers from both the capacitive electrodes merge together and occupy a major fraction of the capacitive volume, the contribution from bulk sample resistance and noises due to electrode polarization effects are eliminated. The dielectric properties during hybridization reaction were measured using 10-mer nucleotide sequences. A 45-50% change in relative permittivity (capacitance) was observed due to DNA hybridization at 10Hz. Capacitive sensors with 30nm electrode separation were fabricated using standard silicon micro/nano technology and show promise for future electronic DNA arrays and high throughput screening of nucleic acid samples.

Keywords: Micro/Nanofabrication, Capacitive Sensor, Biosensors, DNA detection.

1 Introduction

Over a decade of rapid advances in Micro and Nano fabrication technologies has opened up enormous possibilities across various fields of science and technology. The integration of microelectronics technology with molecular biology is having a transforming impact in the development of biosensors with potential applications in future drug and diagnositic development. By the use of miniaturization techniques, the sensing elements or at least parts of them are now getting shrunk down to the same order of dimension as the biomolecules being sensed, resulting in the improvement of many attributes of the molecular detection processes. These nano-scale sensors offer solutions to many problems suffered by conventional signal transduction mechanisms, thereby improving detection sensitivity immensely.

Biosensing, in general involves the detection or quantification of specific biochemical agents such as a particular DNA sequence or protein, using a biorecognition layer for specificity, which is usually immobilized on a transducer surface.

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Several physiochemical signal transduction mechanisms such as optical, magnetic, electrochemical and piezoelectric have been demonstrated over the past decades for the generation of a physical signal from the binding/hybridization events[1][2][3][4]. The dielectric spectroscopic measurements conducted on micro fabricated capacitive structures gained special attraction due to their label free operation and absence of any mechanical motion [5].

Although, several examples of capacitive biosensor have been reported in the literature, many physical and electrochemical properties of these structures and the measurement methods used have significantly limited their commercial full-scale development as a biosensor. The existence of electrode polarization effect and noises from solution conductance limited the earlier dielectric spectroscopic measurements to high frequencies only, which in turn limited its sensitivity to biomolecular interactions, as the applied excitation signals were too fast for the charged macromolecules to respond [6][7][8]. The series parasitic impedance from electrode polarization effect masked the dielectric changes occurring due to biomolecular interactions at low frequencies (<1 kHz) and the proposed methods for minimizing this effect were not compatible with bio sensing applications [9][10].

In an attempt to address the above mentioned challenges, we report a NEMS capacitive sensor with electrode separation in the order of Debye length. The use of a 30nm electrode separation provides better insight into the molecular interactions, which was not previously attainable with macro or even micro scale devices. As the double layers from both the capacitive electrodes merge together and occupy a major fraction of the capacitive volume, the contribution from bulk sample resistance in the measured impedance will be eliminated. The interaction between the electrical double layers due to the space confinement decreases the potential drop across the electrode spacing and allows dielectric measurements at low frequency.

2 Experimental

2.1 Device Fabrication

The most critical parameter for enhancing sensitivity by eliminating the electrode polarization effect is the nanometer separation between the capacitive electrodes. The desired separation of less than 50nm is difficult to achieve with conventional lithographic techniques [11]. To overcome the resolution limit, we have used a sacrificial layer process where the thickness of the SiO_2 spacer layer determines the electrode separation. The process steps are schematically indicated in Fig 1. In the first process step 500nm thick Silicon Nitride is deposited on the single side polished <100> Si wafers after which a 1μm thick photo resist spacers are patterned to act as the sacrificial layer for the formation of the first set of Au electrodes (a). Gold electrodes are deposited using E-beam evaporation under ultra high vacuum conditions. The selective removal of the photo resist sacrificial layer defines the first set of Au electrodes (b). In the next step a very thin and uniform layer of SiO_2 is deposited using Plasma Enhanced Chemical

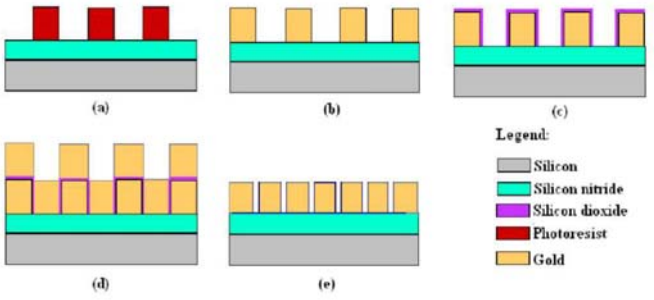

Fig. 1. Schematics of the fabrication process flow. a) photo resist spacers are patterned b) gold electrodes formed by sacrificial method c) deposition of SiO_2 for nanometer spacing d) deposition of gold e) SiO_2 spacer removed.

Vapor Deposition (PECVD), to form the nanometer spacers between the electrodes (c). Followed by the patterning of SiO_2 sacrificial layer, a second layer of gold metallization of 1μm is done using E-beam evaporation (d). The Au electrodes were planarized by CMP and finally the SiO_2 spacer film between the gold electrodes is selectively etched off using HF (e). The deposited Silicon Nitride layer will act as etch stop of this etching process and also serves as an isolator between the gold electrodes and the Si wafer. Here the use of deposited oxide thin film to define the separation between gold electrodes allows the fabrication of capacitive structures with electrode separations lower than the resolution limit of optical or e-beam lithography.

Detection Scheme. The detection is based on the changes in sensor capacitance due to the variation in dielectric properties of the Debye layer resulting from the biomolecular interactions. The Debye capacitance (double layer

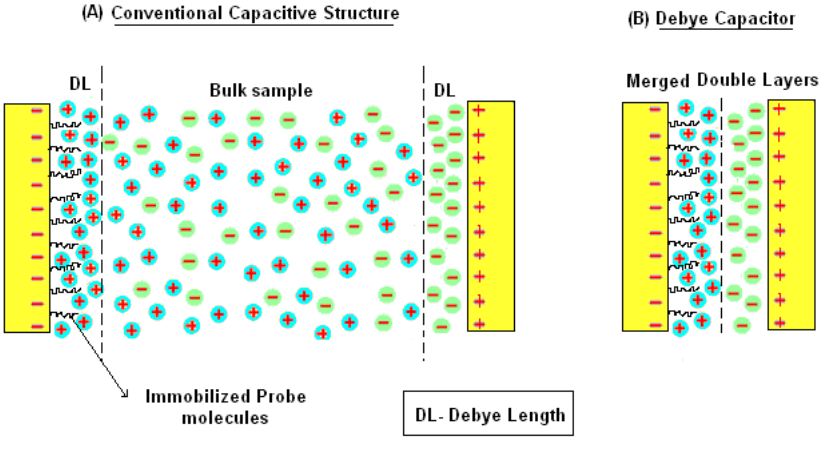

Fig. 2. Comparison between conventional capacitive sensor and Debye Capacitive Sensor

capacitance) formed by the accumulation of counter ions near the electrode surface is highly sensitive to the changes in the dielectric and charge environment in the electrode/electrolyte interface. The characteristic length of this diffuse double layer of charges (Debye layer) is given by the Debye-Hckel parameter and is generally called the Debye length. The calculated Debye length for the buffer solution used in our experiment is 76 nm. The use of capacitive element with electrode separation of 30nm results in the overlapping of the Debye layers of the two electrodes. Fig 2 shows a comparison between the working of a conventional capacitive sensor and the Debye capacitive sensor.

Results and Discussion. The dielectric properties were investigated over a frequency range of 10Hz to 100 kHz, with 0V DC bias and 20mV AC signals using an SR 785, 2 channel dynamic signal analyzer. A Lab View program is used to collect and record data through a GPIB interface. The electrical contacts and the functioning of the entire system including the capacitive element are verified by measuring the dielectric spectrum with air and De Ionized water in between the electrodes. The relative permittivity values of various concentrations of buffer solutions are measured to verify the properties of the Debye layer. Figure 3 shows the relative permittivity values obtained for the different buffer concentrations and are seen to increases with increasing concentration. This can be explained by the fact that the Debye length decreases with increasing concentration which results in an increase in potential drop across the sensor.

DNA oligonucleotides used for the experiments were purchased from IDT (Integrated DNA Technologies). Other chemicals including the buffer solution were purchased from Sigma-Aldrich. Single stranded probe DNA sequences premodified by the thiol linker(5'-CACGTAGCAG/3 Thio MC3-D/-3') were immobilized on the gold electrodes using a concentration of 10μM in 0.05xSSC buffer (7.5mM Sodium Chloride + 0.75mM Sodium Citrate). By taking advantage of the high affinity of sulphur atoms to gold substrate, the DNA molecules with thiol end groups are chemically assembled onto the gold surface from the solution. The oligomer chains are thus tethered to the gold substrate at one end

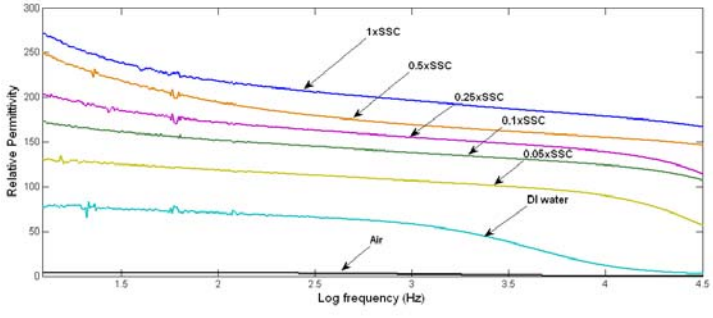

Fig. 3. Relative permittivity as a function of frequency for various concentrations of buffer solutions.The measured permittivity increased as the ionic strengths are increased.

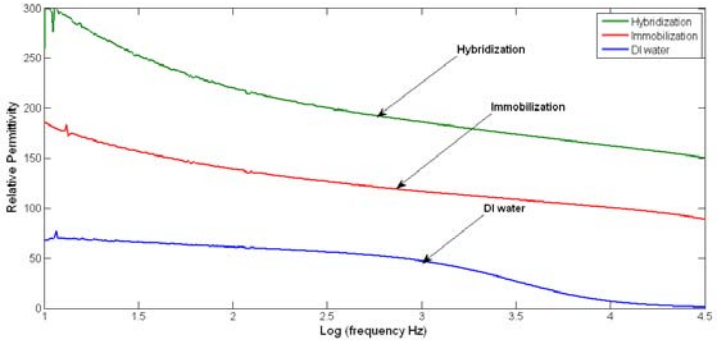

Fig. 4. Dielectric Spectra after hybridization of (10μM) complementary target with the immobilized probe sequence

and the rest of the chain stays fully extended at an angle of approx. 30 degree from the surface. The Van der Waals forces between adjacent chains helps to order the oligomers parallel to each other .The <111> crystal orientation of gold which is obtained by thin film deposition is found to give excellent result for the formation of self assembled monolayers (SAM). Mercapto hexanol (HS- $(CH_2)_6$ OH) SAM layers were immobilized in between the DNA strands in order to passivate the vacant spaces. Prior to immobilization procedure the structure was cleansed using acetone, isopropanol and deionized water. The substrates with immobilized oligomers were then allowed to interact with 0.1μM to 10μM concentration of complementary oligomers (5'-CTG CTA CGT G-3') over a short period of time. After incubation, the substrates were rinsed with deionized water to remove the nonspecifically bound target molecules.

The rel.permittivity changes after probe immobilization and target hybridization are shown in Fig 4a. The increased potential drop across the electrical

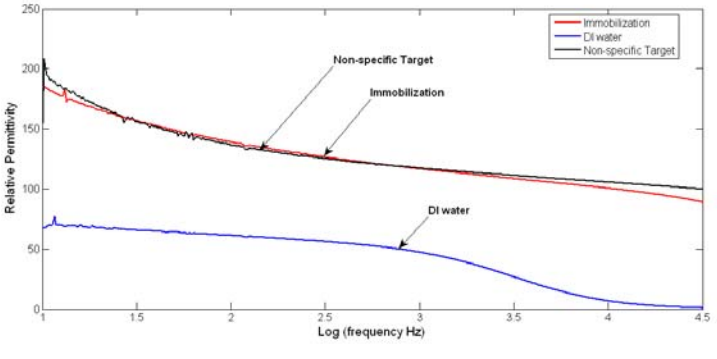

Fig. 5. Dielectric Spectra after the interaction of non-complementary target with the immobilized probe sequence. The slight variation in rel.permittivity value indicates the non-specifically bound oligomers.

double layer due to the additional layer of hybridized oligomers is reflected as the increase in the overall sensor permittivity (capacitance).

As a control experiment, a non complementary target (5'-ATG GCC CTG T-3') solution in the same concentration as the complementary target solution is allowed to interact with the immobilized probe layer. Fig 5 shows negligible change in dielectric property upon exposure to the non complementary sequence. This supports the relationship between capacitance change and specific nucleotide interaction.

3 Conclusion

Capacitive biosensors with electrode separation in the order of electrical double layer width were designed and fabricated using SiO_2 sacrificial layer techniques. The nano scale electrode space confinement is shown to eliminate noises from electrode polarization effect and solution conductivity, permitting the dielectric spectroscopic measurements at low frequencies. DNA hybridization experiments with complementary and non complementary target sequences were performed and a 45-50% change in sensor permittivity (capacitance) was observed after the hybridization of the immobilized probe with the complementary oligomer sequences. Work is presently being carried out in order to optimize the detection mechanism and improve the sensitivity and selectivity of the sensor. Ideally, Single Nucleotide Polymorphism (SNP) could be detected with the current geometries of the device. The improved sensitivity demonstrated by the Debye Capacitive sensor combined with its use of reduced sample volume and low fabrication cost makes it promising for applications such as point of care diagnostics and biowarfare agent detection.

Acknowledgement

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