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Interface Layering Phenomena in Capacitance Detection of DNA with Biochips

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Abstract: Reliable DNA detection is of great importance for the development of the Lab-on-chip technology. The effort of the most recent projects on this field is to integrate all necessary operations, such as sample preparation (mixing, PCR amplification) together with the sensor user for DNA detection. Among the different ways to sense the DNA hybridization, fluorescence based detection has been favored by the market. However, fluorescence based approaches require that the DNA targets are labeled by means of chromophores. As an alternative label-free DNA detection method, capacitance detection was recently proposed by different authors. While this effect has been successfully demonstrated by several groups, the model used for data analysis is far too simple to describe the real behavior of a DNA sensor. The aim of the present paper is to propose a different electrochemical model to describe DNA capacitance detection.

Keywords: DNA Biochip, capacitance detection, ion layering, constant phase elements

1. Introduction

Arrays for gene-based tests, known as DNA microarrays, have drastically changed the way genetic analysis and research are performed, by enabling the user to perform a huge number of analyses in parallel. They are reliable, fast, and powerful. For example, they can test the whole human genome as they achieve densities of a million sites per square centimeter [1]. Moreover, they have also been employed for population genotyping [2] and for cancer predisposition [3]. DNA microarrays, allowing highly parallel and low-cost analysis, exploit the capability to fabricate a large number of

miniaturized detection sites on a substrate and to extract information from each of them after exposure to the solution containing the target DNA. Nevertheless, the high cost of the scanner, the sensibility of optical systems and the processing steps needed to label the samples pose critical limits to widespread deployment in point-of-care usage. For these reasons, significant research effort is being devoted to develop devices that are suitable for low-cost mass production, and can be used outside highly specialized laboratories. A solution implementing direct electrical read-out and avoiding labeling of the DNA target molecules would significantly enhance portability while maintaining high-parallelism, as well as on-site sensing and data processing. The use of highly integrated microsystems may provide new diagnostic tools for the point-of-care diagnostics. The main technical advantage of monolithically integrated, fully electronic DNA sensor devices is the capability of signal processing in the direct proximity of the sensor. This results in the highest sensitivity with respect to the transducer signal. Furthermore, modern CMOS fabrication technologies allow the integration of a large number of sensors on a single die requiring only few electrical connections to the outside world, which significantly eases the packaging of the devices. It is, therefore, possible to envision a CMOS based DNA biochip that features a vast array of sensing sites which implement a label-free fully electronic DNA detection technique. In addition, such a biochip may integrate all system blocks required for data post-processing on the same die. In such a system, individual sensors can be independently selected by means of on-chip addressing circuitry, the sensor output can be directly converted by using on-board analog-to-digital converters, and the resulting digital signal can be directly elaborated on the same chip. Such a system can also be enhanced to include other required biotechnology procedures close near the sensing sites such as, DNA amplification by means of PCR. The surfaces of the sensing site are usually made by interdigitated [4] or square [5] gold electrodes and they are bio-modified (functionalized) by covalent binding of single-stranded DNA probes. Among the different approaches used to detect DNA hybridization between probes and a related DNA target, label-free techniques offer significant advantages in terms of costs, since they avoid the expensive reagents and pretreatment steps required to attach labels. Recently, a number of label-free approaches based on mass changes [8] or electrical properties of electrode/solution interfaces induced by DNA hybridization [9] have been proposed. When compared to other detection alternatives, measuring the capacitance is a simple and straightforward solution. The functionalized sensor surface forms a capacitor when exposed to a physiological solution. This capacitance changes when hybridization occurs between complementary target DNA strands in the solution and the probe strands on the sensor surface. The change can be measured by a circuit below the sensor electrode pair. Under proper electrochemical conditions, bio-modified metal interfaces in a saline solution exhibit an almost ideal capacitive behavior. This is the assumption usually considered for gold electrodes modified with short DNA strands immobilized by means of alkanethiol [10, 11] or modified only with alkanethiol [12]. Under this assumption, the electrode/solution interface can be modeled by the equivalent circuit considering just a conventional capacitance as the parameter to be measured. This assumption is usually considered good enough because it has been observed that when a complementary DNA strands bind with the surface probes, the capacitance varies [10]. The explanation is that when the DNA duplex is formed, the solution ions attracted to the polarized metal surface are displaced [10, 11]. This increases the distance between the charge inside the electrode and the ions in the electrolyte, thus decreasing the interface capacitance. However, a more precise characterization of the interface with DNA functionalized gold surface realized on a biochip, has shown quite different behavior with respect to that expected from a conventional capacitor [5]. In this work, based on measurements on an actual biochip, we show that the frequency characterization of gold electrodes functionalized with DNA strands can not be accurately described by a conventional capacitance model and we propose a different electrochemical model that can be used to improve data interpretation in capacitive DNA sensors.

2 Materials and Methods

In this work, measurements were performed on a prototype biochip using gold electrodes on a glass substrate. The gold sensor electrodes were functionalized with DNA single strand molecules having an alkanethiol group in order to obtain a proper DNA probe surface. The DNA hybridization was tested by using complementary and non-complementary single strand target DNA molecules Details of the fabrication, functionalization, and hybridization experiments are reported in the following.

2.1 Chip Fabrication

The biochip used in this work was fabricated in the clean room facilities at the Center of Micro and Nanotechnologies (CMI) of Ecole Polytechnique Fédérale de Lausanne (EPFL). The biochip was developed as part of a Lab-on-Chip project aimed on investigating cost-effective solutions for DNA sensors. A standard lift-off process is used to pattern the gold electrodes on the glass substrate. To improve adhesion between the substrate and the electrodes first a 20 nm layer of Cromium is deposited, followed by a 200 nm layer of Gold using thermal evaporation.

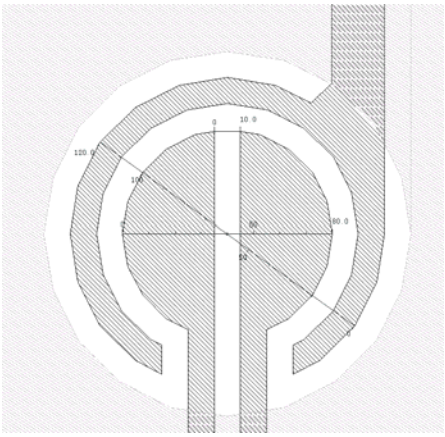

Fig. 1. Layout of the sensing electrodes in the biochip. The rulers show dimensions in μm**.**

The entire surface is covered by a thick (10μm) layer of SU8 photoresist that acts as a passivation layer. Individual sensor spots are exposed by developing SU8. The chip contains a total of 64 electrode pairs arranged in groups of four electrode pairs. Figure 1 shows one electrode in detail. The two electrodes are separated by 10μm and are surrounded by a third reference electrode. The total sensing area of both sensing electrodes is 4,800 μm^2

2.2 DNA Probe Immobilization

Prior to measurements the gold electrodes on the biochip have been cleaned by exposing the chip to oxygen plasma for 20 minutes at 200 W. Following this step, single stranded DNA molecules modified with alkanethiol groups were immobilized on the gold electrodes by covalent S-Au bonds (a 3 μM DNA 1 M Na_2HPO_4 solution is spread on the electrode surface for 18 hours). Two different probe molecules of the same length (25-mer) and thiol modified with a chain of 6 carbon atoms as a spacer are bound to different electrodes on the same chip. To this aim, two separate droplets are placed by mean of microliter pipettes to obtain sites which will and will not experience hybridization reaction. Before measurements, the gold surfaces are extensively rinsed with ultra

pure water to remove molecules that are not covalently bound to the gold electrodes or to the passivation layer of the chip.

2.3 The DNA Target Hybridization

Target DNA solution (3 μM DNA 30-mer and TE 0, 3 M NaCl pH 7) is heated up to 80◦C, spread on the electrodes and cooled down to room temperature (for about 30 minutes). Finally the sample is rinsed in the same saline solution (TE 0, 3 M NaCl pH 7) in order to remove the unbound DNA target. In order to verify the biological steps previously described, we have performed, in the past, an independent standard optical detection test based on fluorescence molecules bound to DNA molecules [5]. In that case, we have tested the efficiency of the hybridization reaction in the case of complementary and non complementary target molecules: the first one indicates that the 80% of the probes react with target forming the double helix while the second one indicates that less than 10% of probe react with target, demonstrating the good quality of our process.

2.4 DNA Target Detection

DNA detection is demonstrated by comparing measurements from electrode pairs subjected to the same reaction but with different DNA strands bound on the surface, complementary and non complementary to target molecules, respectively (the latter for negative control). All measurements were performed in the same saline solution used during the hybridization step (TE 1X 0, 3 M NaCl pH 7). Since capacitances exhibit significant mismatches, a measurement after functionalization was performed and these values are used as a reference to be compared with the results obtained after (tentative) hybridization.

2.4 Capacitance Measurements

A standard Carl Suess prober was used to contact the biochip with two electrical probes. A HP4284A Impedance Meter was used to collect data. The instrument was controlled over the GPIB interface using the Matlab Instrumentation toolbox. The resulting data was transferred into files, that were later parsed using customized Perl scripts to tabulate results and plots were made using gnuplot. The impedance meter can be used to record the impedance in several different configurations. For these measurements two separate configurations were used one after another: by modeling the impedance by a capacitor (*Cs*) and a resistor (*Rs*) in series. In this case, the complex impedance was then calculated as:

$$Z_s = R_s + \frac{1}{j\omega C_s} = R_s - j\frac{1}{\omega C_s} \tag{1}$$

or by modeling the impedance by a capacitor (*Cp*) and a resistor (*Rp*) in parallel. In this case, the complex impedance was calculated as:

$$Z_P = \left(\frac{1}{R_p} + j\omega C_p\right)^{-1} = \frac{R_p}{1 + j\omega R_p C_p} = \frac{R_p}{1 - \omega^2 R_p^2 C_p^2} - j\frac{\omega R_p^2 C_p}{1 - \omega^2 R_p^2 C_p^2} \tag{2}$$

A simple Matlab script was used to control the HP4284A. The device was configured to use a potential difference of 50mV, and from 100 Hz to 1 MHz, impedance was sampled 10 times per decade. The integration (aperture) time was kept at MEDIUM. Recording both impedance value pairs took around 32 s. The estimated C values were equivalent by using the parallel or the series capacitance measurements configuration.

3. Results and Discussion

Data on DNA biochip capacitance measurements are reported in figures 2, 3, and 4. In particular, figure 2 reports the average capacitance trend upon the frequency of 30 different un-functionalized sensing areas. Figure 3 and 4 are, indeed, related to the frequency behavior of the DNA probe and target layers, respectively. Therefore, data in figure 2 is related to the frequency behavior of the bare gold electrodes, while data in figures 3 and 4 are related to the frequency behavior of DNA single strand and double strands layers, respectively. From these figures it is easy to observe that the frequency trend does not follow the generally accepted simple model presented in section 2.4. In particular, the standard simple model uses a conventional capacitor which has a constant frequency behavior while the results in figures 2-4 clearly show a capacitance that decreases with the increasing frequency. It can be seen that data in figures 2, 3, and 4 follows the curve that was obtained by modeling the biochip with the equivalent circuit presented in figure 5 [13].

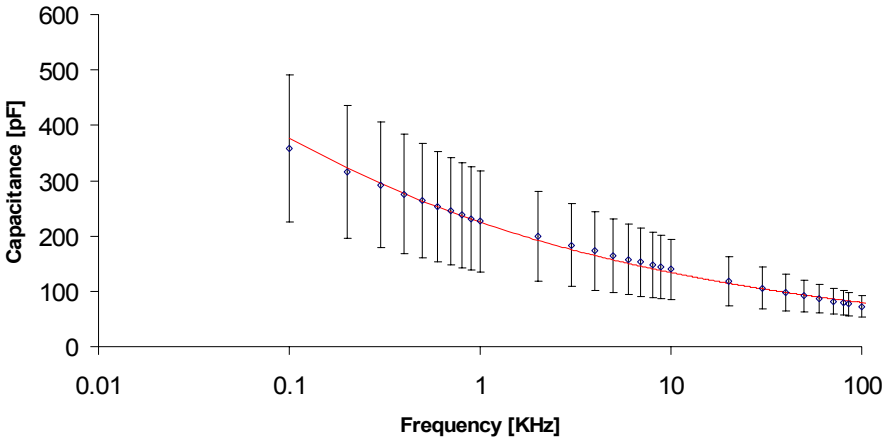

Fig. 2. Average capacitance vs. frequency for the bare electrodes. The bars represent the standard deviation calculated on 30 different biochip sensing areas.

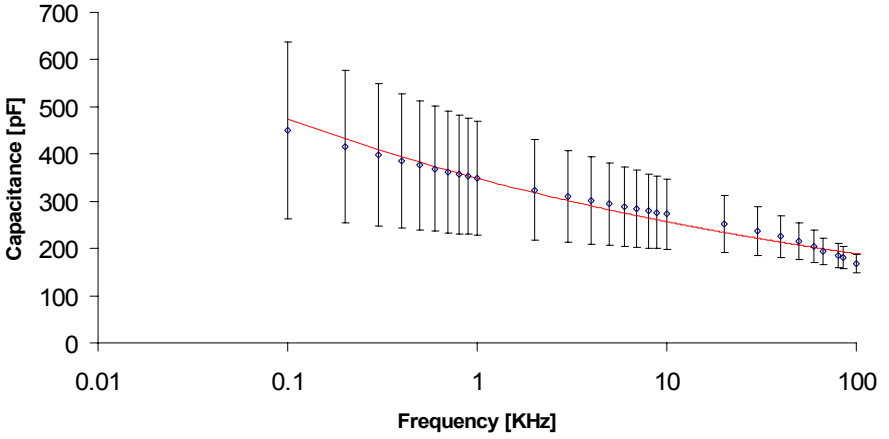

Fig. 3. Average capacitance vs. frequency for the DNA probes immobilized on the gold electrodes. The bars represent the standard deviation calculated on 30 different biochip areas.

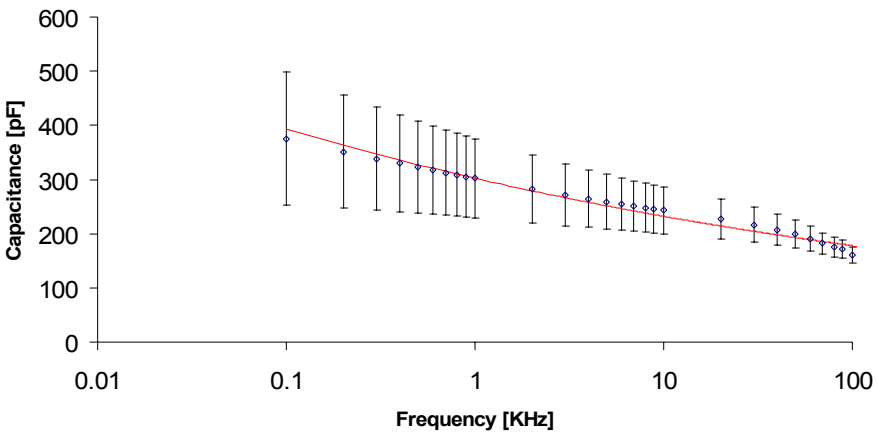

Fig. 4. Average capacitance vs. frequency for the DNA target hybridized with the probes. The bars represent the standard deviation calculated on 30 different biochip areas.

Originally this electrochemical model was proposed to describe the interface behavior of thiols coated with gold nanoparticles and the present paper is the first attempt to use it for describing the Capacitive DNA detection.

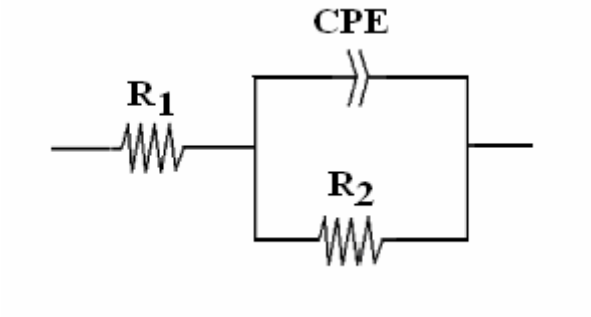

Fig. 5. Electrochemical model including a CPE element to account of the ions layers behavior at the biochip/solution interface.

This model replaces the simple capacitor with a Constant Phase Element (CPE) for describing the behavior of the ion layers at the gold/solution interface. The same model is now proposed for fitting the frequency dependent capacitive behavior of gold plated DNA sensor electrodes used in DNA biochips. In fact, the main idea in capacitive DNA sensors is that the hybridization of DNA on the gold surface changes the ion layering displacement at the interface. However, the measured frequency behavior shows that some ions conduction is occurring by through the DNA layers. Therefore, this new model describes the biochip behavior much better, since the CPE element takes the resistive behavior of the DNA layer in account as well. In fact, figures 3 and 4 show a very good agreement with the model, but a small discrepancy can be observed for a few data points close the frequency of 100 KHz. This phenomenon is present in the case of functionalized chip, and so may be related to the DNA layer behavior. This means that to model the high frequency behavior of the layering phenomenon on the DNA biochip, a more complex model may be considered. For example, a second CPE element may be introduced to describe the interface after the organic layer formation [13]. Note that, the model we propose in this paper is more than sufficient to describe the biochip behavior in the frequency range that we have considered. Therefore in this study we will concentrate on the new electrochemical model that includes only one CPE element, and examine how the changes in the model parameters are correlated with the electrochemical variations of the

interface following DNA immobilization and hybridization. The impedance of the CPE element depends on two fitting parameters, *Cp* and α, following the equation given in [14]:

$$Z_{CPE} = \frac{1}{C_p(j\omega)^{\alpha}} = \frac{1}{\omega^{\alpha} C_p}\sqrt{1-\alpha^2} - j\frac{1}{\omega^{\alpha} C_p}\alpha \tag{3}$$

Tables 1 and 2 show that, as expected, the *Cp* parameter changes depending on the DNA state on the gold electrodes. However, at the same time, the tables also indicate that the α parameter is changing as well. Interestingly, α increases after DNA immobilization on the electrodes. This is an evidence of the increased insulating properties of the gold/solution interface due to the presence of an organic. In practice, the increase of the α parameter means that the CPE element has become increasingly capacitive.

Table 1. Absolute variations of the CPE parameters.

CPE parameters	Bare electrode	DNA Probes	DNA Target
Cp[pF]	706	1095	950
α	0.775	0.867	0.885

Table 2. Relative variations of the CPE parameters.

CPE parameters	Bare electrode	DNA Probes	DNA Target
Cp	-	55%	-13%
α	-	12%	2%

Table 3. Absolute variations of the resistance and reactance of the CPE element.

CPE parameters	Bare electrode	DNA Probes	DNA Target
R(GΩ)	45	177	177
X(GΩ)	123	837	970

As reported in table 3, the resistance of CPE is increased accounting for the fact that less of the gold electrode surface has access to the solution ions, due to the presence of the immobilized DNA probes. The reactance is also increased accounting for the presence of DNA probes at the electrode surface, which is an intrinsic charged molecule [9]. This reactance is further increased after the target hybridization. The target is entering the free space between the DNA probe molecules and therefore thereby increases the insulating behavior of the double strand DNA monolayer. At the same time, the value of the *Cp* parameter changes as a result of the functionalization of the biochip. Its relative variation, presented in table 2, provides us information on the interface spacing. Moreover, the decreasing of the *Cp* value during the target hybridization is a confirmation that the layering ions are moved away from the gold surface due to longer double stranded DNA molecular chains. The variation of the α parameter suggests that the dynamic movement of ions into the DNA layer has to be considered as well.

Another observation from the data in table 3 is that the reactance is increasing during DNA hybridization while the resistance remains practically constant. This shows us that the movements of ions are not significantly affected by the formation of DNA double strands, unlike in the case of

functionalized single strand DNA molecules on gold electrodes where a large increase in resistance is observed. Therefore, the increase in reactance confirms that the DNA capacitance detection is due to differently displaced ions after the hybridization, while the constancy in the resistance shows that the formation of double strand DNA molecules does not reduce the number of conductive paths through the organic layer for the ions in the solution.

Conclusions

The aim of this paper was to identify a proper model that can be applied to DNA sensing biochips using capacitance measurements. It was shown that the presented model was able to accurately describe the frequency dependent capacitance characteristics obtained from actual measurements from biochips. The new model introduces two different fitting parameters and enhances the widely employed simple capacitive model significantly. These two parameters model the displacement and the movements of layering ions at the electrode/solution interface, as due to different states of DNA on the sensing electrodes of a biochip. The model not only describes the insulating properties of the DNA layer, but also describes its conducting behavior. Moreover, this model also accounts for the right frequency behavior of the DNA biochip. Further improvements of the present work will be focused on reducing the standard deviation measured in single capacitance measurements per fixed frequency. In particular, this goal will be achieved by following slightly different immobilization techniques, namely by using three-glycole thiols, which have already been considered in protein biosensors using Surface Plasmon Resonance detection [15], or by modifying other repelling lipoates [16]. In both cases, the aim will be to improve the ideal capacitive behavior of the gold surface.

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