High Resolution Electronics for Label Free Capacitive DNA Detection

Daniela De Venuto  
DEE Politecnico di Bari, Italy

Sandro Carrara  
EPFL Lausanne, Switzerland

Bruno Riccò  
DEIS Università di Bologna, Italy

1 Introduction

Surface-based methods for the detection of biological molecules, such as DNAs and proteins, have revolutionized biological detection. However, only limited use has been made of the electrical properties of biologically modified interfaces as a basis for biological sensing (Martelet et al., 2000; Park & Park, 2009).

The wealth of available DNA sequence data makes identifying many diseases as well as biological threats, such as the presence of an infectious agent in the environment, possible. Improving the sensitivity, selectivity, speed, and simplicity, and reducing the cost of such assays are important goals that will significantly affect the administration of healthcare in various locations ranging from the patient’s bedside to the battlefield.

Alterations in gene expression have profound effects on biological functions. These variations in gene expression are at the core of altered physiologic and pathologic processes. DNA array technologies provide the most effective means of identifying gene expression and genetic variations.

DNA is prepared from a wide variety of samples such as tissue, bacteria, saliva, and so on. For genotyping analysis, the sample is genomic DNA. For expression analysis, the sample is cDNA, the DNA copies of RNA. DNA samples are tagged with a radioactive or fluorescent label and applied to the array. Single-stranded DNA binds to a complementary strand of DNA. In positions on the array where the immobilized DNA recognizes a complementary DNA in the sample, binding or hybridization occurs.

The labeled sample DNA marks the exact positions on the array where binding occurs, allowing automatic detection. The output consists of a series of hybridization events, indicating the presence or the relative abundance of specific DNA sequences present in the sample. Conventional arrays often rely on the detection of fluorescence from a molecular fluorophore.

Electronic detection of hybridization is expected to require less complicated instrumentation and feature similar detection limits compared with the traditional optical methods. An approach completely different and suitable for a wide diffusion in less specialized laboratories is based on the interface capacitance measurements of electrodes.

When a conductor is placed in an electrolytic solution, a potential is generated due to an unequal distribution of charges across the interface. Two oppositely charged layers, one on the electrode surface and
one inside the electrolyte, form a “double layer”, that behaves as a parallel plate capacitor. When an additional layer is present at the electrode, such as an oxide or a Self-Assembled Monolayer (SAM), an additional capacitance or resistance associated with that layer is added to the circuit.

In principle, the introduction of molecules, such as DNA, to the interfacial region, affects the measured complex impedance through changes of the local geometry, the dielectric constant, and the amount of charges at the electrode/electrolyte interface. A robust sensor must be able to measure the impedance change with a Signal-to-Noise (S/N) ratio that yields the desired sensitivity (Berggren, 1999; Berggren, 2001; Carrara, 2009; Janata, 1984; Hosseinkhani, 2006).

A perfect bimolecular recognition layer, for example, a SAM of a single-stranded DNA (a SAM of ssDNA), would cover the electrode completely, and the selective binding of complementary bimolecular to that layer (i.e., hybridization in the given example) would be the only contributing impedance element.

Impedance-based sensors can also give a clear detection in the presence of non-selective binding. To remove the non-specific signal, an experimental set-up including a reference sensor can be used. This sensor, functionalized and implemented in exactly the same way as the working sensor (except for the non-complementary DNA or a non-binding protein), is expected to have the same behavior as the working sensor towards non-specific interactions, therefore allowing a specific differential measurement.

For label-less impedance sensing of bimolecular binding, both functionalized insulators and metals have been attempted.

The feasibility of using electrochemical impedance measurements on functionalized hetero-structures (i.e., semiconductor/dielectric/electrolyte) to detect hybridization directly between complementary homo-oligomer DNA strands without labels was demonstrated.

When metal electrodes, such as Pt and Au, are used as the substrate for biolayers, they show stronger signals. A typical example is the work by Berggren et al. (1999) who demonstrated the feasibility of a biosensor for the direct detection of DNA hybridization on gold, but even in this study the detection capability was not very high, and the reproducibility of the sensors was poor.

Janata et al. (1984), Hosseinkhani (2006) and Thompson et al. (2003) showed how small variations in the electrolyte could affect the impedance signal not because of a double-layer capacitance variation but because of electron transport resistance instability.

The latter gives an indication of the typical signal level one can expect when detecting biomolecules using a differential impedance method without the use of any label. With suitable signal amplification methods, much higher impedance differences (orders of magnitude) can be obtained. Charge transfer effects always confound label-less impedance results and often dominate the total system impedance. To reduce this effect, one must attempt to use a well-polarized electrode.

The ability to fabricate a perfectly-polarized membrane (Thompson, 2003) is still in the exploratory phase. With the introduction of different grafting techniques, such as SAMs or polypyrrole functionalization, reaching a higher level of electrode polarization has been demonstrated to be possible, avoiding the Faradic or diffusive current variations to a significant degree. However, commercial products remain elusive because reproducibility and selectivity remain to be major obstacles.

Recently, a sequence-specific DNA biosensor based on capacitance monitoring of the hybridization event has been developed.

The approach is based on the detection of capacitance changes produced by the DNA hybridization events at the sensing interface. This approach is particularly suitable for the realization of a complete, single-chip solution through the integration of the sensing element with the micro-fluidic and electronic parts that complete the system. The hybridization of targets increases the quantity of biological material that insulates the gold electrode from the electrolyte solution, hence the thickness of the capacitance dielectric. Furthermore, it also changes the relative dielectric constant of the insulating layer.

Variation of the sensor capacitance in the order of 30–50% is expected as a result of DNA hybridization. However, the sensing system capability depends on the appropriate choice of electronics readout. For the
sensors geometry considered in our experiments, the sensor capacitance values can range between 20n and 100 pF, depending on the electrodes area, solution concentration, and biological material quantity.

Since a single board has been realized to measure different kinds of sensors, the electronics is tunable and can be optimized to follow the capacitive swing and achieve the needed linearity and resolution (>10 bit).

Moreover, the readout system has been designed to offer the possibility of performing an absolute capacitive measurement and also to compare the value of the active sensor with a dummy one, achieving the fast detection of the amount of the capacitive shift and then of the hybridization occurrence.

In this paper, the focus is on the readout system for the sensor. The designed electronic system can detect the absolute value of the capacitance of the sensor with a resolution better than 1% and the capacitance shift with a resolution better than 0.01%. The electronic systems described here are suitable for integration, allowing a complete single-chip solution together with the micro-fabricated sensor.

2 Biosensor and electrical model

Figure 1 shows the principle scheme of the biosensor under study. One of the two gold electrodes (Thompson et al., 2003) of the sensing capacitors is covered with a oligonucleotides layer, realized using of alkanethiols, and the interface exhibits the ideal capacitive behavior in the 10–100 Hz frequency range. Figure 1 also shows the bottom part of the electric equivalent model as proposed by Randles, where \( C_{dl} \) is the double-layer capacitance in the electrodes, and the resistor represents the solution resistive effect.

With regard to the effects of DNA hybridization, a capacitance decrease before and after the insertion of a long target molecule is observed. Comparisons between surface capacitance values reported in the literature can be misleading because they are strongly affected by the electrolyte parameters and the surface preparation. Nevertheless, the capacitance of bio-modified gold electrodes has been reported in the cited works to vary between 1 and 20\( \mu \)F/cm\(^2\).

The basic processes involved in the bio-functionalization of gold electrodes for DNA detection are the following. First, a careful pre-cleaning must be performed to allow the efficient attachment of the tethered probes. Gold is extremely reactive to binding with biologic molecules and has to be freed from organic and inorganic molecules. Cleaning can be attained by oxygen plasma, chemical etching with hot piranha solution baths, ultrasonic bath, and electrochemical stripping techniques.

Mechanical polishing with alumina or silicon carbide powders is sometimes employed to improve surface cleaning and to provide better electrode flatness.

Immediately after cleaning, the surface must be bio-functionalized with the sensing layer of the probe molecules. Several biochemical procedures have been proposed and tested to bind covalently short oligonucleotide chains on gold surfaces and create dense and homogeneous biomolecular layers. The most efficient techniques employ an anchoring alkanethiol chain attached to an extreme of the sequence of the nucleic acid. The thiol group provides a covalent bond with the gold atoms, and the small alkanic chain (depending on its length) acts to form a compact layer. Molecules can be spotted by micro-spotting, inkjet printing, or deposition on the surface by immersion (dipping) in a micromolar concentration solution. The solvent in which the molecules are dissolved should contain salts to avoid electrostatic repulsion between charged molecules during layer formation.

The surface must then be rinsed with ultra pure water or buffers to remove non-covalently attached probe molecules and to obtain a layer of ordered and well-oriented receptors.

Successively, gold can be dried and briefly stored. DNA bio-affinity recognition reaction is performed by immersing the electrodes modified with different probe sequences in a buffer solution with suitable physical and chemical parameters (i.e., temperature, ionic strength, pH). The solution conditions are extremely important to ensure efficient and specific sensing. The formation of the double helix is a reversible reaction; thus, the target can be eventually desorbed from the surface by heat treatment, which involves rinsing the electrode with
a hot solution of low ionic strength to observe an increase in capacitance (opposite signal). At this stage, hybridization-specific binding can be repeated. Aside from biochemical procedures, electrical characterization also involves the immersion of gold electrodes in a conductive (ionic) solution and polarization at low-voltage levels. Electrical measurements are usually performed before and after complementary sequence recognition.

**Figure 1**: Schematic diagram of an electrode/electrolyte interface in a faradaic sensor and its exemplary model circuit (Park & Park, 2009)

An electrode/electrolyte interface can be simplified as shown by the schematic diagram in Figure 1. When a DC potential is applied to one electrode, solvated counter ions form an electrical double layer by aligning along the electrode surface, which is represented by the so-called double layer capacitor with a capacitance of \( C_d \). The electron transfer to/from the electro-active species, which may approach the electrode as close as two solvent molecules away in the outer Helmholtz plane (OHP), takes place across the inner Helmholtz plane (IHP) by overcoming the activation barrier, \( R_p \), which is called polarization resistance, and the solution resistance, \( R_s \). Once the electron transfer begins, the Warburg impedance (\( W \)) due to the mass transport begins to play a role in determining the electrode kinetics. In the non-faradaic EIS detection with no redox indicator added, the capacitance or the dielectric constant of the probe layer can be utilized as a main sensing signal. The charge-transfer resistance (\( R_{ct} \)), which is the polarization resistance at an equilibrium potential, is utilized as a main indicator in the faradaic EIS detection. A redox pair, such as \((\text{Fe(CN)}_6)^{3-}/4-\) or \((\text{Ru(NH}_3)_6)^{2+}/3+\), is frequently used as a redox indicator for the electrode kinetics at the interface, which is modified by a substrate layer as well as the probe and target DNAs on the electrode surface. Thus, the \( R_{ct} \) values indicate how crowded the electrode surface is when it is modified by a functional molecule, which is capable of selectively capturing a given analyte. The \( R_{ct} \) values are determined by how selective binding has taken place with the analyte and how much analyte is in the test solution.

The changes in resistances or capacitances of the interface are induced by the DNA hybridization events with a single-stranded target DNA on a suitably designed probe platform. To improve the performance of the
DNA sensor, the probe layer should be constructed using a well-defined surface chemistry preventing the non-specific binding the other side reactions to exhibit high selectivity for a specific target DNA. As a result, various DNA sensors are embodied on the electrodes modified by the various platforms, such as self-assembled monolayers (SAMs), mixed SAMs, conducting polymer films, various nanomaterials such as gold nanoparticles, and peptide nucleic acids. The design of the probe layer depends on whether the sensor has a faradaic or non-faradaic nature. For example, a compact SAM or an insulating layer without a leakage current is required for non-faradaic signal processing, whereas a less-packed SAM or a conductive layer accessible to the redox species is more desirable for a faradaic sensor. Changes in electrical properties occurring in the DNA probe layer are usually extracted using a best fitting model. Each circuit element obtained by fitting impedance responses to an electrical circuit can be utilized for analyzing the type and the amount of target DNA and its conformational changes (Tang et al., 2009; Patel et al., 2009). In general, the changes in impedances are linearly related to the surface coverage by a target analyte in a low concentration region, but sometimes it is logarithmically related to the amount for the target analyte in the case of high concentration ranges or heterogeneous binding between the probe and the target molecules. In a faradaic sensor, the charge-transfer resistance, $R_{ct}$, is associated with the energy barrier for electron transfer to/from the redox indicator approaching the electrode surface, which is determined by the change in the crowdedness of the probe layer caused by its binding with the target DNA. In a non-faradaic sensor, the capacitance of the probe layer is a main indicator exhibiting the conformational changes of double-stranded DNA due to its hybridization. Katz and Willner published an excellent review on various biosensors, including immunosensors, enzyme based sensors, and DNA sensors employing faradaic and non-faradaic impedance spectroscopy as a detection tool (Park & Park, 2009). Berggren et al. also introduced the non-faradaic capacitive biosensors (Berggren et al., 1999; Berggren et al., 2001).

The equivalent circuit in Figure 1 can also include an additional series capacitor representing the so-called constant phase element (i.e., the capacitor CPE, not shown in Figure 1) used for diffusion at low frequency. This parameter is connected to the surface roughness (Carrara et al., 2009). In the considered sensor, the high values of the CPE makes the impedance negligible.

In the experiment described in this study several different geometries for such a sensor have been designed and tested (Carrara et al., 2009; Stagni et al., 2007) to improve the performance of the detection system.

The more suitable appears to be interdigitated, because results in a high overlap between neighboring fingers and consequently in a magnified response to the solution and then capacitive, changes. The sensor has been implemented on silicon using gold electrodes. Several geometries for the fingers have been investigated. A very interesting implementation is the one allowing a small array (5 in total) of sensors as shown in Figure 2. It is considered to implement 10 different geometries (Figure 2):

- Finger length of 2 mm for all geometries
- Spacing of 6 $\mu$m for all geometries
- Finger width of 6, 20, 50, 200, or 500 $\mu$m
- Finger height of 5600 or 2800 $\AA$ for each width

To suit the solution droplets better, a circular passivation 2 mm in diameter above each electrodes pair is made.

The sensor connected to one channel of the board, after a cleaning step is set by depositing a single strand of DNA (reference probe). Afterwards, it is covered by a solution; first, the non-complementary and, subsequently, after a washing step, the complementary sequence of DNA is introduced. A reduction of the sensor capacitive value is detected when the complementary strand is present inside the solution (hybridization occurrence). The detected capacitance variation is around 40% (Figure 2).
Figure 2: Sensor array and details of one sensor spot for the interdigitated geometry. Each finger has a length of 2 mm and a variable thickness and width; the distance between fingers (gray) is 6 μm; the circle in the middle, with a diameter of 2 mm is the area directly exposed to the solution; the remaining part of the electrodes is passivated.

Figure 3: Electrical equivalent model of the sensor in Figure 2.

Figure 3 shows the electrical model used to design the amplification electronics. Based on the experimental data on chips with a similar equivalent surface, a capacitance of the order of $10^{-7}$ Farad is expected. The nature of the electrical double layer tries to change its parameters, such as ion adsorption or hydration shell thickness. Only the solution with Na+ hydrated and Cl- adsorbed is given capacitance values with the expected order of magnitude.

Once the disposition of the ions at the interface becomes clear, the nature and thickness of the dielectric are determined:

- Sodium ions are found to have two hydration shells. The distance of the center of charge of the ion from the metal interface is calculated by adding the molecular diameter of two water molecules to the ionic radius of Na+. The relative dielectric constant of water is used in the calculation of the capacitance.
- In chlorine, the distance from the ion center of charge to the metal interface is assumed to be equal to its ionic radius. As Cl- is specifically adsorbed, the vacuum dielectric constant is used to calculate the capacitance.

The capacitor $C_A$ models the effect of the label-free electrode, $C_B$ is the capacitance of the functionalized electrode. The value of $C_B$ is an order of magnitude less than $C_A$, the probe acts as a dielectric, reading the
double layer capacitance between the electrode and the solution. The capacitance is given by the following relation:

\[ C = \varepsilon \frac{A}{d} \]  

(1)

where \( \varepsilon \) is the dielectric permittivity constant of the material between the two plates, \( A \) is the surface of each plate and \( d \) the distance between them, i.e., the dielectric material thickness.

The occurrence of hybridization between the ssDNA analyte and receptor, with the consequent generation of the dsDNA, determines an increase in thickness of the biological material deposited over the electrode and then a reduction of the capacitance. However, in detecting the chance of the \( C_B \), detecting the analyte inside the solution is possible. Not all ions are available near the electrodes; some of them generate a small current. This phenomenon is conducted inside the electrical model by introducing the resistors \( R_A \) and \( R_B \). \( R_s \) represents the resistance of the electrolytic solution, dependent on the kinds of ions and their concentration. It has been demonstrated in (Carrara et al., 2009) that the impact of the current in such resistors is negligible if ethylene-glycol monolayers are used, explaining the high values given to the resistors.

To determine the value of the capacitors inside the model, simplifying the model with a single capacitor is possible. The signal applied to the electrodes has a frequency that makes possible to neglect the resistors in parallel to the two capacitors and the solution resistor. That such approximation is valid inside the range 10–10,000 Hz can be demonstrated. Inside the experiment, frequencies in the range of 100 Hz up to 4 kHz are used. The value of the capacitor associated to the sensor is given by the series \( C_A \) and \( C_B \). If the analyte is not present inside the solution, then hybridization does not occurred, and the capacitor \( C_B \) does not change. The maximum value of the equivalent capacitor is given by:

\[ C_{eq_{max}} = \frac{C_A C_{B_{max}}}{C_A + C_{B_{max}}} \approx 9.52 \text{nF} \]  

(2)

It should be noted that the capacitor \( C_A \) introduces a non-linearity, even if its amount is negligible. Thus, it is neglected during the detection process analysis. Nevertheless, the evaluation of the overall linearity of the system is performed.

3 Electronics for the capacitive biosensor

Due to the peculiarity of the biosensor described above, read-out systems can detect the occurrence of DNA hybridization. To quantify the amount of such event, recognizing the target concentration using this method is required.

Hybridization determines the biosensor capacitance value reduction. The read-out system should be able to detect capacitance shifts from 30%–50% of the nominal value.

To this end, the configuration for the read-out electronics has been proposed (Venuto & Riccò, 2005) to detect the sensor capacitance changes. The system consists of an analogue section, including the front-end, the conditioning electronics, and the digital section that can be constituted by a binary counter. A micro-controller carries out the human interface; i.e., it allows the calibration of the tuning parameters, namely, clock frequency, feedback discharging impedance, and so on to determine the performance.

The system takes the absolute measurement of the value of the biosensor capacitance before and after hybridization. By storing the information in an external memory and comparing the absolute value associated with the measurement in the absence of and when the hybridization affects the probe sensor, the respective capacitive shifts are determined.
Figure 4: Read-out electronics for the capacitive sensor

Figure 4 shows the schematic of the proposed read-out electronics. The first block is a CSA; the integrated charge is digitalized by a comparator and a counter. Each block is described in detail in the following paragraph.

Using the same principle to perform directly the comparison with respect to a reference (dummy) sensor is possible. In this case, the analog block can contain a differential amplifier that works on the signals coming from the CSAs to convert them, first from the working sensor and second from the dummy one. In this measurements, which allows the direct capacitance shift detection the achieved resolution can grow since also the common mode of the op-amp is reduced. The drawback of this second electronics is that it requires two sensors to perform the differential reading. One operates as the probe sensor performing the chemical detection, and the other, the dummy one, gives the capacitive reference value to evaluate the shifts. The condition to make it working is that the temperature and the amount and the overall condition of the solution covering the dummy electrode should remain constant during the complete set of measurements. Otherwise, the noise floor will change, producing the wrong set of detection.
Figure 5 shows the differential system. The differential detection is performed by an instrumentation amplifier followed by the 1 bit conversion.

Additional modification can be conducted by obtaining the difference using an EXOR at the output of the two preamplifiers (CSAs), simplifying the post processing procedure (Venuto & Riccò, 2009; Venuto & Riccò, 2010).

All systems require an accurate calibration procedure for offset and noise compensation to allow a linear detection.

The following paragraph provides a more detailed description of the systems.

4 Charge-sensitive amplifier and the comparator

The common interface between the sensor and the read-out system in the proposed solution is based on a CSA (Figure 6).

As described in paragraph 2, the sensor converts a chemical reaction in a sensor capacitive change ($C_{\text{sensor}}$ in Figure 4).

If a voltage step ($V_{\text{pulse}}$) is applied at one electrode of the cell, the relation:

$$Q = C_{\text{sensor}} \cdot V_{\text{pulse}}$$  \hspace{1cm} (3)

gives the equivalent charge that the front-end has to convert into a voltage and that contains the information that hybridization of the DNA has occurred.

![Figure 6: Operating principle of a Charge-sensing-amplifier](image)

As the charge and the capacitive value are linearly link by the relation (Ameur et al., 2000), the detection of the capacitance is made available through the detection of the charge. Consequently, the front-end can be a CSA. A voltage signal proportional to the charge at the input can be given at the output.

The CSA scheme is depicted in Figure 6. The feedback loop has a capacitance $C_{\text{feed}}$ that stores the charge and a reset resistance $R_{\text{discharge}}$.

The feedback capacitance stores the charge transported by a thin current pulse; the output voltage amplitude is proportional to such charge amount. The reset resistance allows the capacitance $C_{\text{feed}}$ to discharge, giving the output voltage with a finite falling time.
An easy method to detect the charge is to convert it into voltage. A charge preamplifier is useful for this action (Figure 6). When a square wave is applied at one electrode of the sensor, the derivative of the signal generates narrow pulses at the opamp input because of the capacitive behavior. The area below the current pulse is the charge stored by the sensor.

As the current flowing at the opamp input is null, the pulse can only flow in the feedback path. By assuming the virtual short circuit at the opamp input, the following relation is obtained:

\[
V_o(s) = -Z_f(s)I(s) = -\frac{R_{\text{discharge}}}{1+sR_{\text{discharge}}C_{\text{feed}}} Q \xrightarrow{\text{L}^{-1}} V_o(t) = -\frac{Q}{C_{\text{feed}}} e^{-\frac{t}{R_{\text{discharge}}C_{\text{feed}}}}
\]  

(4)

In summary, the current pulse at the input of the opamp is converted into an output voltage proportional to the charge. The voltage exponentially decreases with the time constant \(\tau = R_{\text{discharge}}C_{\text{feed}}\). The time interval needed to allow the output voltage reach the peak value \(Q/C_{\text{feed}}\), is shorter if the charge \(Q\) decreases.

Nevertheless, the exponential behavior reduces the dynamic range and does not allow the detection of small variation in the charge \(Q\). To overcome this limit, the feedback capacitor should be discharged at a constant current by shunting it with a FET operating an active reset continuously. In this way, a linear falling edge is obtained. The feedback capacitance value is calculated to obtain the output’s full range when the maximum value of the charge is at the input:

\[
V_{o_{\text{max}}} = \frac{Q_{\text{max}}}{C_{\text{feed}}} \Rightarrow C_{\text{feed}} = \frac{Q_{\text{max}}}{V_{o_{\text{max}}}} = \frac{C_{\text{eq}_{\text{max}}} V_{\text{pulse}}}{V_{o_{\text{max}}}}
\]  

(5)

**Figure 7:** The adopted Charge-sensitive-amplifier scheme
Due to the inverted configuration of the opamp, the output voltage signal generates a negative ramp during the input rise time and a positive ramp during the input falling time. The slope of the ramp is proportional to the capacitance of the sensor. The negative ramp is neglected, and the positive ramp is considered for the next step. To discriminate better the time constant of the integral during the ramping up, two different paths are induced during the ramping up and down. Feedback to the charge coming from the sensor with two different charging and discharging paths, and different time constants is implemented. To force the current to go through the different feedback paths, diodes are inserted inside the operational amplifier feedback double loop. The negative charge stored is reset by $R_2$. The positive one is reset by the JFET TR1, which is forced to be biased in the saturation region during the discharge, giving a constant current $I_{OFF}$. By regulating the bias voltage of a twin JFET, $T_1$ (Venuto & Riccò, 2009) (not shown in Figure 7), and by trimming the capacitor $C_3$, obtaining a time constant $\tau_{OFF}$ for the CSA is possible, such that the discharging transient of the positive stored charge is given by:

$$\tau_{OFF} = \frac{C_{feed}}{I_{OFF}} v_{out}$$  \hspace{1cm} (6)

where $C_{feed} = C_2 + C_3$ in Figure 7.

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<th>Figure 8: CSA output</th>
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Figure 8 shows the CSA output when the current pulses of different amplitudes are applied at the input. Evidently, the positive slope is suitably slow, allowing the discriminator to perform easily the comparison with the reference. The slope at the output of the CSA (Figure 8) defines the window width at the comparator output as shown in Figure 9.

The comparator output width can be measured by a counter with a suitable clock frequency $f_{clk}$. The counting value $N$ is given by

$$N = \frac{V_{pulse}}{I_{OFF}} f_{clk}$$ \hspace{1cm} (7)

Formulas (3) and (4) show that the discharge transient time affects the read-out system resolution.

The hysteresis comparator determines the counter observation window. The two comparator threshold voltages determine the falling and rising time of the counter clock window.
5 Differential system: Capacitive change detection

The system in Figure 5 measures the amount of the difference in charge between the active sensor and the dummy one. The solution of the active sensor electrodes contains the target molecular, not the dummy sensor solution.

To cancel the common mode and the noise signal, the two sensors have to be similar to each other and both must be covered by the same electrochemical solution. To detect the capacitance shift before measurement, the two channels have to be equalized; i.e., the output of the instrumentation amplifier has to be set to zero.

A calibration phase has to be performed; i.e., the observation range of the system has to be defined. To achieve these results, the two channels have to be regulated to have a different slope. The amount of this mismatch is the maximum capacitive reduction expected in the active cell when hybridization occurs. Consequently, the instrumentation amplifier will be regulated to have the minimum gain to avoid saturation. The output of the instrumentation amplifier (top portion of Figure 10) crosses the comparator thresholds, determining the window width (bottom portion of Figure 10) to trigger the counter.
6 Experimental results

A board implementing the electronics described above is implemented and characterized. A micro-controller allows the selection of the single channel read-out or the differential read-out and the storing and processing of data.

A perfect correspondence between the simulation results is achieved by the board characterization (Venuto & Riccò, 2009; Venuto & Riccò, 2010).

For the single-channel measurement, the system shows a resolution of 1 pF for the absolute measurement of the capacitance, whereas for the differential detection, a resolution better than 0.01pF of variation on the nominal value of capacitance is experimentally demonstrated after an accurate calibration.

The system can be adapted to measure capacitive value in different ranges of values (from uF to pF) only by suitably tuning the capacitor and resistor of the feedback path of the CSA.

For simplicity and precision of the schemes, the methods can be easily implemented on-chip and can even reach better performance in sensors array application.

The merit of the high resolution is in the linearity of the electronics that has been evaluated in the first step. This is done by considering the maximum amplitude at the output of the CSA and evaluating the number of the clock pulses counted by the counter at the output of the electronics when discrete capacitances of 33 nF are used to simulate the sensor and the pulse used as stimulus on one electrode of the capacitor has an amplitude ($V_{\text{pulse}}$) varying from 760-30 mV. The change in $V_{\text{pulse}}$ simulates a variation in the sensor accumulated charge due to the eventual hybridization (Venuto & Riccò, 2010).

Figure 11 shows the results of the linearity investigation. On top, the maximum value at the output of the CSA is plotted as a function of $V_{\text{pulse}}$. At the bottom, the number of the clock periods counted by the counter is plotted as a function of $V_{\text{pulse}}$.

![Figure 11](image.png)
In each plot, the curves are compared with the regression line to evaluate the linearity of the system. In this way, the CSA is shown to have a percent error of linearity of around 0.01% when the sensor electrode is stimulated by a pulse of voltage amplitude ($V_{\text{pulse}}$) of more than 300 mV, where the relative error is plotted. For the counter, the limit is given by its resolution, i.e., 11 bits.

Once the electronics is fully characterized, the measurements of the board with sensors and the solution with DNA are performed.

**Figure 12:** Layout of the capacitive sensors (not in scale)

In Figure 12 shows the layout of the first topology of the sensor. The golden electrodes used are interdigitated with an inter-finger gap of 50 um. The preliminary measurements are shown. The values of the capacitances can change three orders of magnitude once they are inside the solution, depending on the quality of the golden electrodes surface, the solution concentration, and how much the sensor can be introduced into the solution.

Figures 13 and 14 show some results from the measurements with the functionalized electrodes in a solution where a non-complementary strand has been introduced. Figure 13 shows the input stimulus of 13.7 mV of amplitude and 100ms of width for a frequency of 1 Hz, and the CSA output pulses.

**Figure 13:** CSA output and sensor input stimulus in the presence of DNA without a complementary strand
The amplitude of the input pulse is chosen, such that electrolysis is not allowed inside the solution. The amplitude of the detected CSA output signal (as shown in the bottom line of the oscilloscope Figure 14) was 970 mV. With a CSA feedback capacitance of 3.3nF, evaluating a sensor capacitance of 238.5nF is possible. Figure 14 shows the CSA and the comparator output. Distinguishing the window defined by the comparator is possible by fixing a suitable reference voltage of 160mV. In this case, the window width is 50.5ms.

To perform the differential measurements as explained above, a tuning procedure is performed on the two read-out channels, as shown in Figure 15. Once the same measurement is performed on the twin sensor, it has to be calibrated to be ready for the differential measurement. This means that the feedback JFET of one of the two has to be biased to have a different current flowing and to give a different slope to the CSA output even when the amplitude is the same. In this way (Venuto & Riccò, 1999), the differential output will generate a negative signal calibrated to allow the maximum dynamic range of the instrumentation amplifier (Figure 16).
Figure 16: Instrumentation amplifier output in the calibration phase

Figure 16 shows the output of the instrumentation amplifier when the two channels are tuned (the oscillations are due to the interference on the electrical line in the biochemical laboratory where the DNA measurements were performed).

The sensor connected to one channel of the board, after a cleaning step is covered by the complementary sequence of DNA. A reduction of the CSA output amplitude (900mV) is detected and shown in Figure 17. The sensor capacitance detected in this case is 216nF, i.e., a relative variation of 10% with respect to the presence of a non-complementary strand (Figure 18).

The differential system can detect such variations with a resolution of 0.01%.

The system can of course be adapted to measure capacitive values in different ranges (from uF to pF) only by suitably regulating the capacitive value on the CSA and the resistive trimmers on the board. If just one channel is used to measure the absolute value of the capacitance, a resolution of 0.1% is demonstrated.

Figure 17: CSA output and sensor input stimulus in the presence of DNA with a complementary strand
To improve the performance of the detection system, several different geometries for such a sensor have been designed and tested (Stagni et al., 2007).

The best performances are the ones that reached the interdigitated array as shown in Figure 19.

The sensors have been implemented on silicon, with a finger length of 2 mm and width of 200 um, and the active area surrounded by oxide.

Figures 20 and 21 show some results from the investigations on these sensors. The sensor after a cleaning step is set by depositing a single strand of DNA (reference probe) and connecting to one read-out channel of the board. Afterwards, it is covered by a solution where, first, the non-complementary and, later after a washing step, the complementary sequence of DNA is introduced. A reduction of the sensor capacitance value is detected when the complementary strand is present inside the solution (hybridization occurrence). The detected capacitance variation is around 40%.
The same topology of sensor has been used without the reference probe. In this case (Figure 20), the impact of the complementary or the non-complementary strand inside the solution is almost ineffective. The third curve at the bottom of Figure 21 shows the capacitance of the electrodes when they are immersed in a TE NaCl solution (no DNA). If only one channel is used to measure the absolute value of the capacitance, a resolution of 0.1% is demonstrated.

9 Conclusion

The potential benefits of molecular medicine are enormous. The foremost is the promise of early disease detection and treatments optimized for each patient. In fact, clinical molecular diagnostics is revolutionizing how laboratory medicine and clinical diagnostics are conducted.

Many efforts are spent to identify viable technology, through a comprehensive look at available technologies, for molecular diagnostics, including probe-based nucleic acid assays, microarrays, and sequencing; obtaining a complete understanding of the chief molecular diagnostics tests, i.e., predictive, screening, prognostic, monitoring, pharmacogenomic, and theranostic, from their basic principles to their
applications. The technology is devoted discovering feasible market opportunities by identifying high-growth applications in different clinical diagnostic areas and by focusing on expanding markets, such as communicable diseases, cardiology, and oncology; and focusing on global industry development through an in-depth analysis of the major world markets for molecular diagnostics.

About the DNA investigations, the detection sensitivity is still a challenge especially in label-free detection systems. In this paper, read-out electronics for a label-less, capacitive-based DNA sensor have been described, and experimental results from a preliminary characterization have been presented. The systems have been proven to be capable of achieving a resolution of 0.01% for the capacitive shift using a differential reading of the signals detected by two sensors: a dummy sensor and an active sensor.

Measurements connected to the sensor prototypes confirm the high sensitivity of the system, showing a relative error of less than 0.01% when an accurate calibration of the set-up parameters is used.

The solutions investigated are all suitable for a possible integration with a micro-fabricated sensors. Future work should focus on array of cells with respective processing electronics for the fast identification of diseases on a genetic base.

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**References**


