Design proposal of a DNA biosensor by means of relative bioimpedance measurements and genetic amplification: preliminary results

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Abstract. In this work, we propose the instrumentation of a DNA biosensor whose detection technique is based on relative bioimpedance measurements. The scope of this work is to report the preliminary results of an initial functional test using two samples: milli-q water and saline solution.

1. Introduction
Molecular biology was revolutionized by the discovery of the Polymerase Chain Reaction (PCR), a technique that multiplies the number of Deoxyribonucleic Acid (DNA) fragments in a logarithmic and controlled way [1]. PCR became a common and important method of DNA detection and it’s been widely used by medicine to diagnose viral and bacterial infections, genetic diseases, several types of cancer, and other diseases [2, 3]. The reaction consists of a PCR mix containing the DNA template; a pair of primers that determine the region to be amplified; DNA polymerase, the enzyme that makes the copies of the region to be amplified; nucleotides, which serves as the building blocks from which the polymerase create new DNA copies; and, the buffer, which provides the optimal conditions for the reaction to occur [3, 4]. The PCR mix should be thermically cycled in order to achieve the genetic amplification, consisting of 3 main thermal stages: the first one is called Denaturalization, in this stage the PCR mix is heated at approximately 95 °C to split the hydrogen bonds that hold the double-strand of the template DNA molecule; the second stage is called Annealing, the PCR mix is cooled around 60 °C so that the primers can bind themselves to the single-strand DNA at a specific position delimited by the primers’ nucleotide sequence; and, the third stage is called Extension, which is the stage where the polymerase creates the complimentary copy of the DNA strand where the primers are bound, using the nucleotides as building blocks; this stage occurs at around 70 °C [1, 3, 4]. These 3 stages correspond to one cycle. Normally, the thermal cycle is repeated around 20-35 times, obtaining billions of copies of the target DNA fragment [4].
The amplified PCR products are conventionally separated by molecular weight and observed in an agarose gel by means of electrophoresis, followed by ultraviolet illumination in use of a DNA-intercalating stain, normally Ethidium Bromide (EtBr), a potent mutagen [4] added to the PCR products prior to the electrophoresis [1, 4] making the sample handling and disposal more difficult. Even though EtBr has been replaced by non-mutagen dyes, the relatively simple method lacks sensitivity and should be performed by highly trained personnel [5-7]. These disadvantages have been overcome by the introduction of real-time quantitative PCR (qPCR). This method uses fluoroscopic dyes or probes to quantify the fluorescence emitted by the PCR sample as an indirect measure of the amplified DNA, by this method it is also possible to estimate the total initial amount of template DNA in a sample [3]. Unfortunately, this type of PCR requires expensive, complex and hardly accessible labelling procedures and technology [5, 8, 9], hindering the introduction and implementation of genomic medicine in developing countries. Thus, low cost, accessible and easy-to-use alternative technologies are needed.

Motivated by the limitations of conventional and quantitative PCR mentioned above, electrochemical DNA biosensors have been of great interest in the last decades [9, 10]. It is known that DNA is an electroactive molecule and that the electrical properties of DNA aqueous solutions are strongly dependent on DNA concentration [8, 11-14]. Electrochemical detection of PCR products relies on different biophysical DNA properties. According to Adriana Patterson et. al. (2013) [9], there are four different approaches for real-time electrochemical detection of nucleic acids amplification: solid-phase qPCR, solution-phase qPCR using electrochemical intercalators, solution-phase qPCR using sequence-specific reporters and electrochemical quantification methods using isothermal amplification. Although there are several approaches relying on the technologies mentioned in [9], other authors have tried a different approach trying to simplify the technique and minimize the reagents needed for the amplicons detection, such as label-free real-time electrochemical qPCR. Jeong et al. (2019) [15] developed an interdigitated electrode PCR chip for amplicon detection using Electrical Impedance Spectroscopy (EIS) at relatively low frequencies (from 100 to 10000 Hz), achieving lower sensitivity than other electrochemical approaches. However, at these frequencies it is common to obtain noise from the capacitive effect of the electrode-electrolyte interphase from the alpha dispersion phenomena, meaning that the measurement system should be very precise in order to reject false results, thus the instrumentation and measurement setup becomes less accessible.

In this work, we propose the instrumentation of a DNA biosensor based on relative bioimpedance measurements. The scope of this work is to document the instrumentation proposal of a system capable of monitoring changes in the bioimpedance of a sample, as well as the preliminary results of an initial functional test of the system proposed.

2. Methods
The genosensor design proposal consists of 4 main modules: 1) Thermocycler: based on thermoelectric cooling modules for genetic amplification; 2) Function generator: for injecting a sinusoidal electrical signal to the sample for bioimpedance measurement; 3) Gain and phase detector: for the relative bioimpedance measurement of the PCR sample compared to the voltage drop across a reference resistor; and, 4) Control: this module concentrates the general control of the modules, the acquisition of measurements and the processing and storage of the acquired data. Figure 1 shows a block diagram of the proposed system.

2.1. Control
The control block was built based on a Raspberry Pi 4 microcomputer (Raspberry Pi Foundation, United Kingdom) making use of its general input and output ports (GPIO). This module is used to control the frequency of the sine wave signal and to carry out the temperature control. The microcomputer is also used for data acquisition of the measured DC values from the gain and phase detector using an 8-channel 10-bit analog-to-digital converter (ADC) integrated circuit MCP3008 with a reference voltage of 2.2 V. The data is saved in a CSV format file and transferred to a personal computer for subsequent analysis via the built-in USB communication of the Raspberry Pi 4.
2.2. Thermocycler
A thermocycling system was designed using thermoelectric cooling technology (TEC). TEC modules (Peltiers) are devices with rectangular geometry that vary the coefficient of thermal transfer from one side of the device to the other, depending on the current injected. Furthermore, if the polarity of the current is reversed, the heat transfer will also change its direction, allowing the object in contact (in this case, an aluminium thermal block containing the PCR tubes) to cool it or heat it, according to the temperature profile required by the PCR.

![Block diagram of the proposed system.](image)

**Figure 1.** Block diagram of the proposed system.

2.2.1 Digital Proportional-Integral-Derivative Control. To control the current flowing through the Peltiers, a digital proportional-integral-derivative control (PID) was designed and implemented in the Raspberry Pi 4 microcomputer. The PID control was codified in Python 3 language using public-domain libraries and it is based on the difference between the temperature set point corresponding to the PCR thermocycling stage and the temperature of the thermal block measured by an LM35 type temperature sensor. The gain parameters of the PID control were adjusted by simply changing its value on the code determined by the empirical method.

2.2.2 Pulse Width Modulated signals (GPIO). The output of two of the general ports of the microcomputer (one for heating and the other one for cooling) are pulse width modulated (PWM) signals at a frequency of 50 kHz, the duty cycle of the PWM signal is proportional to the PID control output.

2.2.3 Power stage (H-bridge). The PWM signal is connected to an H-bridge type power stage using MOSFET type transistors to vary the power supplied to the Peltiers, in contact with the thermal block, as well as the current’s direction to cool or heat, as the case may be. In order to obtain a smoother behavior of the Peltiers, instead of having an ON/OFF behavior, the output of the H-Bridge was filtered using passive components to obtain a direct current signal whose amplitude is proportional to the duty cycle and, therefore, to the digital PID output.

2.3. Electrical injection
2.3.1 Signal generator. A multifrequency sine wave generator was designed using the integrated circuit MAX038 and external electronic components to set the frequency range. This integrated circuit outputs a sinusoidal signal of a specific frequency in function of the current that inputs one of its pins. We used the built-in 2.5 V voltage reference of the MAX038 connected in series to a digital potentiometer MAX5454 (100 kOhms) whose wiper position (resistance) is controlled by the control module, and therefore the output frequency of the MAX038. The bandwidth of the signal goes from 119 kHz to 2.2 MHz in 256 logarithmic steps with an amplitude of 1 V.
2.3.2 Voltage divider. A reference resistor of 1 kOhm \((Z_{ref})\) is connected in series to the PCR sample using two silver cylindric electrodes \((Z_{sample})\), configuring a voltage divider powered by the sine wave signal. In this sense, we obtain two signals: a reference signal \((V_B)\), which is the voltage drop in the reference resistor and, the voltage drop in the PCR sample \((V_A)\). Each signal is obtained by means of differential amplifiers to avoid currents drops. These two signals are fed to the gain and phase detector.

2.4. Measurement module

2.4.1 Gain and phase detector. For this module we make use of an evaluation board of the AD8302 integrated circuit. This module compares the reference and the PCR sample signals and delivers two DC voltages: \(v_{mag}\) and \(v_{phs}\), which are proportional to magnitude ratio and phase angle between both signals, respectively, and can be expressed as in \([16]\):

\[
\begin{align*}
 v_{mag} &= \alpha_1 \cdot \log \left( \frac{|V_A|}{|V_B|} \right) + 900 \text{ mV} \\
 v_{phs} &= \alpha_2 \cdot (|\theta_A - \theta_B| - 90^\circ) + 900 \text{ mV}
\end{align*}
\]  

(1)

Where \(\alpha_1\) and \(\alpha_2\) are coefficients obtained from the AD8302 data sheet \([17]\): \(\alpha_1 = 600 \text{ mV/decade}\), \(\alpha_2 = -10 \text{ mV/degree}\). Since the differential amplifiers are configured identically, the impedance magnitude ratio of \(Z_{sample}\) to \(Z_{ref}\) is the same as the ratio of \(V_A\) to \(V_B\). Thus, the impedance magnitude ratio \(|Z_{sample}/Z_{ref}|\) and phase differences \(\theta\) can be solved from (1):

\[
\begin{align*}
 |Z_{sample}/Z_{ref}| &= |V_A/V_B| = 10^{v_{mag}/600 \text{ mV/decade}} \\
 \theta &= \theta_A - \theta_B = \left( \frac{v_{mag} - 900 \text{ mV}}{10 \text{ mV/degree}} + 90^\circ \right)
\end{align*}
\]  

(2)

Note that the phase formula is negatively biased, meaning that all phase differences measured by our system will be noted as negative. This comes from the fact that AD8302 is not capable of distinguishing between positive and negative phase differences \([16][17]\), but such detail does not affect our system because in bioimpedance measurements a positive phase behavior (inductive effects) has never been reported and electrolytes’ impedance, responsible of tissue impedance, are always capacitive, as denoted by \([16]\).

Both outputs are acquired by the control module using an ADC connected to the Raspberry Pi by serial communication.

All of the modules are embedded and synchronized to work as one integrated system.

2.5 Thermocycler test

To evaluate the thermocycler module performance, we ran three thermal cycles to calculate the heating and cooling temperature ramp in \(\text{°C/s}\), as figure of merit. The temperature profile of the test was the same as the first three cycles of a standardized PCR assay, consisting of denaturalization at 95 \(\text{°C}\) for 30 seconds followed by 30 seconds at 62 \(\text{°C}\) corresponding to the annealing stage, and 30 seconds at the elongation stage at 72 \(\text{°C}\).

2.6 Bioimpedance measurements test

Bioimpedance measurements were carried out at the end of the elongation stage, corresponding to the temperature of 72\(\text{°C}\), since it is the moment at which the impedance measurements will be performed in a real-time electrochemical PCR assay, at the end of each cycle. By this, we evaluate the measurement range of the electrical injection in conjunction with the gain and phase detector. We measured the impedance magnitude ratio and phase angle of two samples with a volume of 25 microliters: Milli-Q water, as the maximal impedance sample since it is the vehicle of the PCR; and, NaCl Solution (0.9% w/v), as the minimal impedance sample.
3. Results

Figure 2 shows the temperature data of the thermocycler test, acquired from the LM35 sensor that is located in the middle of the thermal block. From this data, we obtained a heating ramp of 1.84 °C/s (from 72 to 95 °C) and a cooling ramp of 1.27 °C/s (from 95 to 62 °C). Table 1 shows the figures of merit of other commercial and experimental systems as a comparison with our results. Also, we calculated the time it would take to complete a 30-cycle assay, which resulted in 01:15:11.2 (hh:mm:ss.0). This result shows that our system is capable of controlling and accurately maintaining the temperatures that are needed for a PCR assay.

![Temperature Profile](image)

**Figure 2.** Temperature data of the thermocycling test obtained from the LM35 sensor.

| System            | Heating ramp (°C/s) | Cooling ramp (°C/s) |
|-------------------|---------------------|---------------------|
| Proposed system   | 1.84                | 1.27                |
| Sailaja et al 2019| 1.8                 | 1.4                 |
| Eppendorf         | 1.1                 | 1.2                 |
| Master Cycler     |                     |                     |
| GENEAMP 2700      | 1.0                 | 2.2                 |

**Table 1.** Comparison of heating and cooling ramps of the system proposed with those reported in [18].

Figure 3 shows the magnitude ratio and phase of the two samples from the impedance measurement system proposed, in relation to the reference resistance of 1 kOhm, acquired at the final of the elongation step at 72 °C, which will be the moment of measuring in an electrochemical PCR assay. From this, the synchronization of the measurement module with the thermocycling module is proven. Also, the system’s capability to perform coherent bioimpedance measurements at the time it was design for is confirmed.
4. Discussion
The figures of merit resulted from the thermocycling test are similar to those reported by Sailaja et al. (2019) [18] which is a system based also on thermoelectric cooling technology with the difference that we used two peltier modules while Sailaja et al used six; in that sense, our proposal is simpler but equally effective. According to [18], the figures of merit of our system proposal even overcome those of two commercial thermocyclers systems (Eppendorf Master cycler from Eppendorf AG, and GeneAmp from ThermoFisher Scientific) by a few decimals (see Table 1). Also, from the temperature graphs published in [18], we can observe (although not measure) that our system has less oscillation in temperature when temperature should be hold in each thermocycling stage then the proposed system by Sailaja et al. In that sense, we believe our system proposal meets the performance needs for genetic amplification by PCR thermocycling.

On the other hand, from the bioimpedance data we believe that our system proposal has enough measurement range to detect changes in electrical impedance of PCR samples due to DNA amplification, although comparison with other published works is not possible since our proposal is based on relative bioimpedance changes, not on absolute measurements, which makes our proposal simpler. Figure 3 shows coherent magnitude and phase bioimpedance relative changes for Milli-Q and Saline Solution samples, values are in dynamic ranges as theoretically expected. Experiments with actual PCR samples are needed to confirm observations and viability of the system to detect DNA, but those are out of the scope of the present work.

5. Conclusion
In this work, we have presented an instrumentation proposal of a DNA biosensor whose detection method is based on relative bioimpedance measurements; and reported the preliminary results of an initial functional test using Milli-Q and NaCl solution as samples. Our system presents figures of merit comparable to commercial and experimental systems reported in the literature. Experiments with actual DNA samples are needed to confirm the viability of the system to detect specific genes, but those are out of the scope of the present work.

Figure 3. Magnitude ratio and phase angle of Milli-Q water and saline solution in relation with the reference resistance.
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