Software for Gene-Biosensor based on PCR and Electrical Bioimpedance

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Abstract. Gene detection by the use of bioimpedance measurements is an emerging technical proposal from the last two decades. Our recent studies have shown the feasibility to use multifrequency bioimpedance to detect specific label-free Deoxyribonucleic Acids (DNA) sequence in the final product of Polymerase Chain Reaction (PCR). We have developed a gene-biosensor integrated by a thermocycler block for final point PCR process and a relative bioimpedance meter at every PCR cycle in the sample. The system demands a dynamic software for all gene-biosensor modules control. This work reports a control program design for a gene-biosensor based on PCR product and bioimpedance measurements, the general structural philosophy, operating routines and subroutines as well as its interaction with the hardware in every module are described. The program was designed on the basis of Python language version 3.8.3 with the support of the Visual Studio Code as Integrated Development Environment (IDE), and using Windows 10 as the operating system. Results indicate the control program allows a suitable governing of the bioimpedance meter and PCR thermocycler block, both as a well-integrated system. Bioimpedance and temperature measurements are in agreement with the control operating structural design. Additional amendments regarding an on-line monitoring system are warranted.

1. Introduction
In the last two decades gene detection by the use of bioimpedance measurements has been proposed as an emerging technique, some research groups seem to agree that Deoxyribonucleic Acids (DNA) could work as an electronegative charge carrier given its electrical properties associated with conductivity [1]. Such properties may be a function of volumetric Electrical Bioimpedance (EBI). EBI measurements may be useful for the detection of specific label-free DNA-sequences in Polymerase Chain Reaction (PCR) final point product. In a recent study, we have proposed the use of EBI measurements to detect
the dipole effect of the water molecule, and its interaction with the electronegative charge of the DNA molecule, where this interaction was shown to be a function of DNA concentration and correlated with changes in volumetric electrical properties and EBI of DNA [2, 3].

Biosensors on the basis of EBI have significant potential to be used as simple and inexpensive detection devices due to their size, speed, and potential label-free operation. Currently they are used in water analysis and others applications [4]. We have developed a gene-biosensor integrated by a thermocycler block for PCR final point process and a relative bioimpedance meter at every PCR cycle in the sample. The system demands a dynamic software for all gene-biosensor modules control. This work reports the design of a control program for a gene-biosensor based on PCR product and bioimpedance measurements. The general structural philosophy, operating routines and subroutines as well as its interaction with the hardware system in every module are described.

2. Methods

2.1. General description of the gene-biosensor hardware governed

The general configuration of the gene-biosensor hardware to be controlled consists of 4 main modules. 1) Thermocycler that uses a thermo-electric cooling technology (TEC) for genetic amplification by PCR process. 2) Function generator uses an MAX038 integrated circuit and external electronic components to set the frequency range of signals in the EBI measurements. 3) Gain and phase detector that uses an AD8302 integrated circuit to perform relative EBI measurements on the PCR sample. 4) The controls that handles the acquisition, processing and storage of the acquired data. A detailed description of the operation of all hardware components together has already been documented [5].

2.2. General control program structure

The program was designed on the basis of a Python language version 3.8.3 with the support of the Visual Studio Code IDE, using Windows 10 as the operating system. Numerical methods and libraries available for Integral and Derivative Proportional Control (PID) system were used. The program interacts with hardware gene-sensor through a Raspberry Pi 4-B (Raspberry Pi Foundation, United Kingdom) by the use of its General Purpose I/O (GPIO) ports and the Serial Peripheral Interface (SPI) communication protocol.

The general operating principle of the control program is illustrated in the flowchart in figure 1. Input/output parameters are considered in the user section for control configuration and outcome parameters for processing. The general structure is designed in accordance with two main functions Bioimpedance and Thermocycle, both interacting in three sequential subsections: Basal condition, Initial denaturation and PCR process as showed in figure 1.

2.3. General control process description

A brief description of the general control process for interaction of three sequential subroutines (Basal condition, Initial denaturation and PCR process) showed in figure 1 is as follows: First, the control program starts with a graphic window through which the user enters the configuration variables for thermocycle function (temperatures, times and number of cycles (nc), as defined in subsection 2.3.1). Second, initial Bioimpedance measurements of the DNA sample are developed at preprogrammed parameters (defined in subsection 2.3.2) for basal conditions (room temperature and zero amplification cycles). Then, the program starts the first thermocycle with the configuration variables corresponding to the initial denaturation of DNA and subsequently performs the DNA sample bioimpedance measurement. Finally, an iterative control structure begins that allows the thermocycle and bioimpedance measurements through the whole PCR process to be carried out a total of nc times configured by the user. The general process concludes with the transition of the sample to room temperature and obtaining the data acquired in the bioimpedance registry.
2.3.1. The Thermocycle function allows the user to control the temperatures and times of the PCR process applied to the DNA sample. It was designed in two essential tasks: 1. - Save the PCR configuration variables defined by the user in the graphic window. 2. - Control the temperature of the DNA sample to the desired values and times in each PCR step. First, the function arrives to the desired temperature and reads the real temperature of the DNA sample through a temperature sensor positioned physically close to the sample. Then, it compares the desired temperature value and that of the temperature sensor to perform a transition process towards the desired temperature by means of a control of the power supply to a pair of Peltiers (Thermoelectric Cooling Technology-TEC) that are in the thermal block enclosing the DNA sample. Finally, the function keeps the sample at the desired temperature for the indicated time. The temperature sensor reading is developed by SPI communication protocol with a 8-channel 10-bit analog to digital converter (ADC), the desired temperature is controlled with the implementation of an external library for a digital proportional, integral and derivative control (PID) and the use of the pulse width modulated (PWM) signal through the GPIO Raspberry Pi ports (H-bridge type power stage: turn-on or off according to the required case: heating or cooling the temperature in the sample).

2.3.2. Bioimpedance function was designed for two specific tasks: 1. - Programming a function generator in the bandwidth of 119 KHz to 2.2 MHz at 256 steps lineally spaced and injection of the
current in the DNA sample, and 2. - Measuring relative bioimpedance in magnitude and phase in the DNA sample at every frequency. First, frequency of the function generator is commanded by the use of a digital potentiometer. Second, current injection at the programmed frequency is applied in DNA sample. Then, bioimpedance magnitude and the phase is determined by a comparison of a reference resistor in series with the DNA sample using two silver cylindrical electrodes, and outputs two DC voltages proportional to magnitude ratio (Gain) and phase angle between both signals, respectively. Last, data acquisition of the measured DC values from the gain and phase detector by means of an 8-channel 10-bit ADC is developed, storing DC values obtained in two data vectors, one for gain and one for phase for each cycle.

In this function, the digital potentiometer configuration and the acquisition process of the gain and phase data are controlled through the GPIO Raspberry Pi ports and SPI communication commands.

2.4. Evaluation of the control program structure

The control program was evaluated directly on the gene-sensor hardware system. The outcome parameters Bioimpedance measurements and PCR process were analyzed through a DNA amplification sample by PCR final point. Three thermal cycles to calculate the heating and cooling temperature ramp in °C/s were developed 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 °C for 30 seconds followed by 30 seconds at 62 °C corresponding to the annealing stage, and 30 seconds at the elongation stage at 72 °C. In addition, DNA sequence amplification was developed by a whole thermocycle PCR process at 29 cycles. The PCR assay was designed to evaluate the operating control program and it is not intended to evaluate the effectiveness of the hardware in DNA sequence detection. Thus bioimpedance meter and DNA sample preparation details are not in the scope of the present report.

3. Results
3.1. User interface

Figure 2 shows a screenshot of the user interface window for configuration parameters designed in Python and implemented in Raspberry Pi OS. Parameters related to initial temperature, ramp temperatures, times and number of cycles for PCR process are defined by the user and works as control configuration of the gene-biosensor system.

![Figure 2. Screenshot of the graphic configuration window.](image-url)
3.2. Thermocycle

Figure 3 shows the temporal course of the thermocycler block temperature for the three main subroutines: basal condition, initial denaturation and two initial cycles as representative PCR process. Temperature data digitized by the hardware system was correctly collected and stored in suitable vectors. Said vectors were recovered out of line (CSV file) for its analysis and graphical representation.

![Figure 3](image3.png)

**Figure 3.** Temporal course of the thermal block temperature (basal condition, initial denaturation and two initial PCR cycles).

3.3. Bioimpedance

Figure 4 shows bioimpedance measurements of the three main subroutines: basal condition, initial denaturation and PCR process at the 29th cycle. Bioimpedance data digitized by the hardware system was correctly collected and stored in suitable vectors and recovered out of line (CSV file) for graphical representation.

![Figure 4](image4.png)

**Figure 4.** Bioimpedance curves: Gain and phase for basal condition, initial denaturation and PCR process at 29th cycle.
4. Discussion
The algorithm of the control program allows for a correct and well-organized storage and collection of the bioimpedance and temperature data, using data storage vectors that are converted to CSV files for the graphical representation of the data in some analysis and visualization software (Microsoft Excel in this case). Bioimpedance measurements were coordinated through the three main subroutines: basal condition, initial denaturation and all cycles of the PCR process. Temporal course of the temperature ramps at every cycle for the whole PCR process seems coherent with characteristics time constants of commercial PCR thermal cycler. Bioimpedance curves for Gain and Phase seem to have a differentiation pattern of approximately 0.5dB, which allows the initial denaturation curve to be distinguished from the final PCR product curve at cycle number 29th, such observations seem evident below 1MHz and match with a coherent PCR process, thus in principle the bioimpedance observations should correspond to a DNA sequence amplification process. Biophysical interpretation of the findings are not in the scope of the present report. The main disadvantage of the program so far is the absence of a real time monitoring of the temperature PCR process and bioimpedance measurements at every PCR cycle through an interactive user interface.

5. Conclusions
The implemented program allows a correct and well-organized control operation of a gene-biosensor designed on the basis of label-free DNA amplification by final point PCR product and bioimpedance measurements. Graphical user interface programming is necessary for a real time graphical representation.

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