frontiers | Frontiers in Electronics

ORIGINAL RESEARCH
published: 22 July 2022
doi: 10.3389/felec.2022.836669

Oscillation-Based Spectroscopy for Cell-Culture Monitorization

Pablo Pérez[1,2], *Juan A. Serrano-Viseas*[1,2], *Santiago Fernández-Scagliusi*[1,2], *Daniel Martín-Fernández*[1,2], *Gloria Huertas*[2,3] *and Alberto Yúfera*[1,2]*

[1]*Instituto de Microelectrónica de Sevilla (IMSE-CSIC), Universidad de Sevilla, Sevilla, Spain,* [2]*Dto. Tecnología Electrónica, Escuela Técnica Superior de Ingeniería Informática, Universidad de Sevilla, Sevilla, Spain,* [3]*Dto. Electrónica y Electromagnetismo, Facultad de Física, Universidad de Sevilla, Sevilla, Spain*

Biological Impedance is a physical property related to the state and inherent evolution of biological samples. Among the existing impedance measurement methods, Oscillation-Based (OB) tests are a simple and smart solution to indirectly measure impedance correlated with the amplitude and frequency of the generated oscillation which are proportional to the sample under test. An OB test requires tuning of the system blocks to specifications derived from every measurement problem. The OB setup must be done to obtain the optimum measurement sensitivity for the specific constraints imposed by the system under test, electronic interfaces, and electrodes employed for test. This work proposes the extension of OB measurement systems to spectroscopy test, enabling a completely new range of applications for this technology without the restrictions imposed by setting a fixed frequency on the electrical oscillator. Some examples will be presented to the measurement of cell cultures samples, considering the corresponding circuit interfaces and electric models for the electrode-cell system. The proposed analysis method allows the selection of the best oscillator elements for optimum sensitivity range in amplitude and frequency oscillation values, when a specific cell culture is monitored for the OB system.

Keywords: oscillation based test, bioimpedance, microelectrode, spectroscopy, ECIS

OPEN ACCESS

Edited by:
Pedro Bertemes-Filho,
Santa Catarina State University, Brazil

Reviewed by:
Karabi Biswas,
Indian Institute of Technology Kharagpur, India
Anna-Maria Pappa,
Khalifa University, United Arab Emirates

***Correspondence:**
Alberto Yúfera
yufera@imse-cnm.csic.es

Specialty section:
This article was submitted to Bioelectronics, a section of the journal Frontiers in Electronics

Received: *15 December 2021*
Accepted: *31 May 2022*
Published: *22 July 2022*

Citation:
Pérez P, Serrano-Viseas JA, Fernández-Scagliusi S, Martín-Fernández D, Huertas G and Yúfera A (2022) Oscillation-Based Spectroscopy for Cell-Culture Monitorization. Front. Electron. 3:836669. doi: 10.3389/felec.2022.836669

INTRODUCTION

Biological Impedance or Bio-Impedance (BI) is a very relevant biomarker among different biological and medical processes in live matter. Impedance is a physical property related to the opposition of an entity (or matter) to allow the electric current to flow between two separated points. This opposition to the free electric charge flow is characterized by two general parameters: the conductivity (σ) and the permittivity (ε). These properties reflect the physical state and underlying biological processes and is largely dependent on frequency (Schwan, 1957). BI is employed as a biomarker in many biological fields, such as in cell culture applications in cell biology ("ECIS Cell,), genetics (Schmidt et al., 2019), biochemistry (Jun et al., 2018), pharmacology (Alexander et al., 2013), tissue engineering (Nordberg et al., 2017), immunology (Hsu et al., 2018), and food control (Rego et al., 2015). To solve the main drawback of classical end-point protocols, Giaever et al. (Giaever and Keese, 1986) proposed the so-called Electrical Cell-Substrate Sensing (ECIS) technique as an alternative procedure to perform real-time monitoring of CCs, thereby avoiding multiple samples, and performing all measurements over the same culture. The ECIS method is based on placing the culture between two electrodes. When an AC voltage (the most common is sinusoidal, at 1 kHz frequency) is applied between the electrodes, the cells in the current line's path cause a lower or higher current depending

on their higher or lower resistance. A low-density cell culture means a large current due to its lower resistance, and vice versa.

This real-time monitoring procedure for cell culture requires the bioimpedance test between the 2-electrodes system, using the corresponding circuits for that. Also, an adequate electric model for the electrode-medium-cell system is required to decode the bioimpedance measurements, and to know approximately how many cells are present in the culture at a given instant.

In addition, to have a full and exact bioimpedance description of a biological material, in most cases it is required to test it in a wide frequency range. Bioinstrumentation must be properly designed to work in the frequencies of interest. Electronic systems for a BI test apply algorithms and strategies to pick-up the BI value of every biological sample under test (BSUT), requiring specific circuits with strong specifications, making the required instrumentation circuit difficult to design and implement.

BI techniques have been widely developed by many authors. A classical reference describing the general methods and applications can be found in (Grimnes and Martinsen, 2008), while (Pérez García, 2019) provides a more detailed review on the cell culture field. Most of them consider the need of using a sinusoidal generator circuit as a voltage generator to be applied at the system under test. This is difficult for electronic designers and increases the cost for spectroscopy applications.

The Oscillation-Based (OB) technique (Huertas et al., 2015) has been proposed as a useful approach for BI tests. Advantages over different design strategies include reduction of the number of electronic building blocks and avoidance of the sinusoidal signal generator. The OBT is fundamentally based on designing an electronic oscillator with the BSUT located in the electrical oscillation path. Such implementation forms an electronic bio-oscillator with specific oscillation amplitudes and frequencies directly related to the BSUT.

For the second important point, the electrical model to decode the bioimpedance measurements, several proposals have been delivered, with two being the most relevant. In (Giaever and Keese, 1991) the cell culture is considered as a monolayer and the solution of Maxwell's equations is obtained for the electrical field in the electrode-cell interface. To find the solution, parameter values such as R_b (barrier resistance between cells), h (electrode-cell interface distance), and r (cell radius) were proposed and selected to fulfil the equations derived. In a subsequent study, Huang et al. (Huang et al., 2004), considered a single cell on top of an electrode and extracted the electrical performance of the system by simulating its electrical response to an AC signal with finite-element simulation tools. A new resistance R_{gap}–the so-called gap resistance–was incorporated to the electrode-cell electrical model that represented the obstruction to the electrical current flowing along the electrode-cell interface, which usually extends several nanometres. They also included a geometrical parameter *ff*–the so-called *fill factor*–that described the percentage of electrode area covered by the cells. In this work, the second model was chosen, because in a general cell culture, the monolayer phase is only of application in the confluence phase, with the ff parameter being an adequate parameter for real-time monitoring of the cell culture.

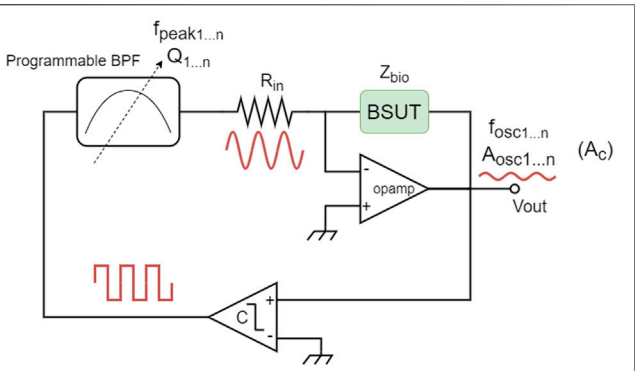

FIGURE 1 | Simplified schematic for the programmable Oscillation-Based (OB) approach for bioimpedance measurements and spectroscopy analysis.

This work presents an extension of the OB technique to spectroscopy analysis by introducing programmable electronic bio-oscillator parameters. The Bio-Oscillator will be described in more detail in the paper. First, the main components of the OB programmable system will be defined, together with the frequency programming procedure, system constraints, and specifications. Then, mathematical simulations and results for several study cases will be described. In parallel, the Figures-of-Merit (FOM) for design optimization and spectroscopy will be defined. Finally, discussion and conclusions will be highlighted.

MATERIALS AND METHODS

The Programmable OB System Design

The Oscillation-Based (OB) method is an established sensing circuit approach for BI assessment (Huertas et al., 2015) which reuses the methodology of the Oscillation Based Test (OBT) as described in (Pérez et al., 2017). The major advantage of this technique is the avoidance in designing a sinusoidal circuit generator for signal injection. OBT simplifies the hardware resources required to perform the impedance measurement. The main blocks involved in OB technique are a Band Pass Filter (BPF) and a non-linear element closing the feedback loop, usually a comparator (C) (Huertas et al., 2015).

A block diagram for a programmable OB circuit is proposed in **Figure 1**. The voltage inverter amplifier introduces the biological sample under test (BSUT) into the oscillation feedback loop. A programmable BPF is used to implement the suggested spectroscopy analysis. This block provides different peak frequencies (f_{peak}) and quality factor (Q) which will define the different electrical oscillations related with the biological impedance under test (BSUT).

The whole OB system can be modelled by the transfer functions which govern the individual blocks in the closed-loop oscillator. The transfer function corresponding to BSUT (and its electrical interface, Z_{bio}) will be denoted as Hz(s) in what follows. Oscillations are formed and stable if the Barkhausen criteria is accomplished. This mathematical condition implies

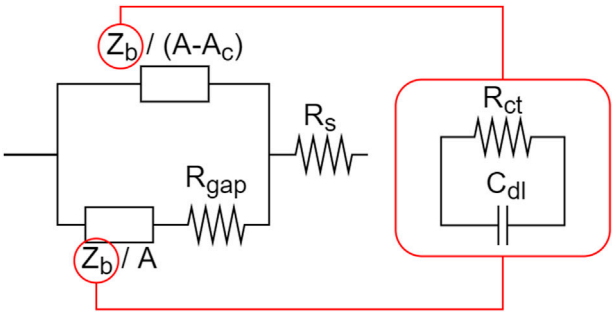

FIGURE 2 | Circuit model for an electrode of area A. Electrode area is partially covered (Ac) with cells. Impedance Zb represents the contributions of the Charge transfer resistance and the double layer capacitance (Pérez García, 2019), (Borkholder, 1998).

that an OB loop is formed and a couple of oscillation parameters, namely frequency (f_{osc}) and amplitude (a_{osc}), will be achieved, which contain relevant information about the BSUT. Moreover, in this case, a spectroscopy analysis will be performed with different f_{peak} and Q programmed values. Biological impedance information is implicit in the oscillation parameters (f_{osc}, a_{osc}) and, therefore, such information can be derived by measuring the amplitude and frequency of the output signal (V_{out}).

System Constrains and Specifications

The scheme proposed in **Figure 1** is a powerful tool proposed for bioimpedance spectroscopy analysis. As a major governing block in the system (**Figure 1**), the Band Pass Filter (BPF) parameters strongly define performance and electrical behavior of the formed oscillation loop. These parameters are quality factor (Q) and central frequency (f_{peak}). Medium and high Q filters are required to decrease distortion and increase signal-to-noise ratio (SNR). Central frequency modifies the oscillation frequency by establishing a maximum frequency and adapting sensitivity and dynamic ranges to the set value. A direct implication of this is that specific applications of OB, such as OBT (Huertas et al., 2015), requires a certain knowledge of the impedance values for the samples under test, so that central frequency could be established to a relevant value for the process under analysis (Pérez et al., 2016), (Pérez et al., 2018).

Existing OB methods in the literature report a constant central frequency f_{peak} for the specific target application. This design limits its application on a general BI test problem, where the frequency range may be an open specification of the BI frequency range of interest which is not previously defined or known. The Oscillation-Based Spectroscopy (OBS) is defined as a method for searching the optimum frequency range of OB systems and analyzing the sample under test for a wide range of frequencies, by programming its central frequency. Previous works manage to describe the design process for culture applications and a fine tuning of the OB parameters (Pérez et al., 2020).

BI spectroscopy analysis applications are widely described in the literature (Stupin et al., 2021) for very different applications such as cell growth, cell characterization, tissue analysis, medical diagnosis, and many more. A technique extensively employed to observe cell culture growth (Pérez et al., 2018) is based on the Electrical Cell-substrate Impedance Sensing (ECIS) technique (Giaever and Keese, 1986), (Wegener et al., 2000), (Borkholder, 1998). The electrode model for the underlying process is described in **Figure 2**.

The above impedance model along with the interface, presented in **Figure 1**, is described by a transfer function with the following form:

$$Hz(s) = \frac{R_s d_2 \cdot s^2 - (R_s d_1 + n_1) \cdot s - (R_s d_0 + n_0)}{R_{in} d_2 \cdot s^2 + R_{in} d_1 \cdot s + \frac{d_0}{d_2}}$$

Where:

$$n_1 = \frac{R_{ct} R_{gap} R_{ct} C_{dl}}{A - A_c}$$

$$n_0 = \frac{R_{ct}}{A - A_c} \cdot \left(\frac{R_{ct}}{A_c} + R_{gap} \right)$$

$$d_2 = R_{gap} \cdot R_{ct}^2 C_{dl}^2$$

$$d_1 = R_{gap} \cdot R_{ct} \cdot C_{dl} + \left(\frac{R_{ct}}{A_c} + R_{gap} \right) \cdot R_{ct} C_{dl}$$

$$d_0 = \left(\frac{R_{ct}}{A_c} + R_{gap} \right) + \frac{R_{ct}}{A - A_c}$$

The Hz(s) transfer function represents the gain of a standard opamp-based inverter amplifier with R_{in} input resistance, with the equivalent impedance in **Figure 2** at the feedback path. The parameters R_{ct} , and C_{dl} correspond to the charge transfer resistance and double layer capacitance, respectively (Borkholder, 1998), connected in parallel, which forms impedance Z_b. The R_s value corresponds to spreading resistance through cell culture medium, and finally the resistance R_{gap} models the current flowing laterally through the electrode cell interface (Huang et al., 2004), (Pérez et al., 2018). **Figure 3** and **Figure 4** illustrate how magnitude and phase

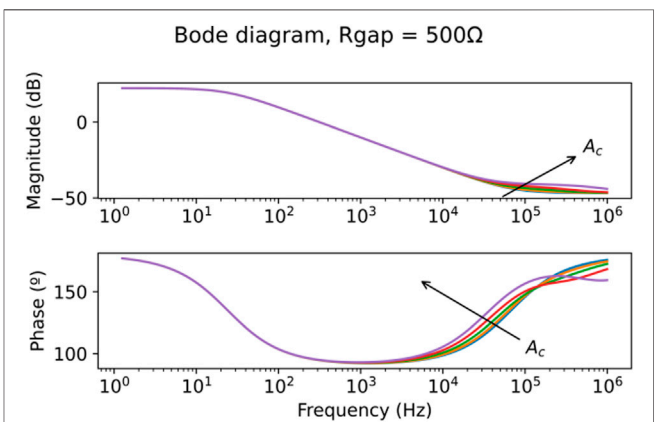

FIGURE 3 | Electrical impedance spectrometry for Hz(s) with R_{ct} = 1.3**MΩ**, C_{dl} = 3.2 ***nF***, R_s = 455**Ω**, R_{gap} = 500**Ω** and variations for $A_c \in (0, A)$.

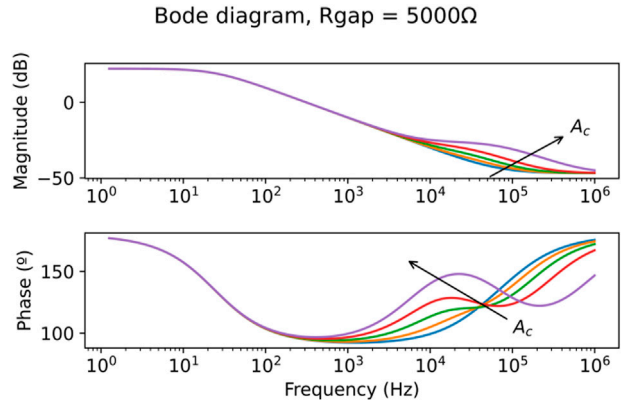

FIGURE 4 | Electrical impedance spectrometry for Hz(s) with R_{ct} = 1.3MΩ, C_{dl} = 3.2 *nF*, R_s = 455Ω, R_{gap} = 5000Ω and variations for $A_c \in (0, A)$.

of the cell electrode bioimpedance change with the area covered by cells (A_c).

The R_{gap} factor is dependent on the cell line under study (Huang et al., 2004), (Serrano et al., 2018), (Yúfera et al., 2011). This factor significantly affects the electrical response of the system and continuous monitoring systems for cell culture devices should consider its effect when selecting appropriates frequencies for measuring the impedance. Continuous sensing of the biological underlying process (cell growth over the substrate) requires the analysis of this impedance. The ECIS technique works under the basis that impedance seen by the applied electric field over the membrane capacitance (C_{mem}) is very large and can be considered as infinite for any applied frequency. This means that the electric current lines do not penetrate inside the cells. Most ECIS applications work between 100 Hz and 100 kHz AC signals-for this reason (Pérez et al., 2018), (Wegener et al., 2000), (Keese et al., 2004; Bagnaninchi and Drummond, 2011; Holland et al., 2018; Parekh et al., 2018).

The Oscillation-Based Test (OBT) (Huertas et al., 2015) technique approaches this process by establishing an electrical oscillator at a fixed frequency and implementing the bio-sample under test within the sample loop. This type of sensor would require a certain tuning for optimization of the operating frequency so that the best test is performed. Furthermore, since frequency is typically fixed, the operation is constrained to specific applications and may not perform the best operation frequency when exposed to processes of different natures or magnitudes.

The variation observed with respect to R_{gap} introduces a significant difference on the biological sample electrical response and the OBT sensor system (see **Figure 3**, **Figure 4**). Extending OBT to a wide range of frequencies removes the limitation imposed by setting a fixed frequency and allows navigation of the whole range and performance of a full spectroscopy of the system under test.

As was commented, the OB system is based upon a BPF, the bioimpedance interface, and a non-linear element (a comparator in our case). The scheme was illustrated in **Figure 1**. BPF transfer function $(H_{BPF}(s))$ and open loop system $(H(s))$ response is described by the following equations:

$$H_{BPF}(s) = \frac{G_0 \cdot \frac{f_{peak}}{Q_f} \cdot s}{s^2 + \frac{f_{peak}}{Q_f} \cdot s + f_{peak}^2}$$

$$H(s) = H_{BPF}(S) \times H_z(S)$$

Design of the band pass filter parameters Q factor (Q_f), Central frequency (f_{peak}), and Gain (G_0) will define the generated oscillations. The following oscillation condition must be met (Sánchez, 2006) for the system to be stable:

$$1 + N(a_{osc}) \times H(S) = 0$$

Where $N(a_{osc}) = \frac{4 \cdot V_{ref}}{\pi \cdot a_{osc}}$ corresponds to the comparator non-linear transfer function. Validation of this equation implies the existence of a complex pole pair:

$$1 + N(a_{osc}) \times H(s) = \left(s^2 + f_{osc}^2\right) \cdot \left(s^2 + B \cdot s + A\right) = 0$$

Where f_{osc} and a_{osc} correspond to the oscillation signal V_{out} (**Figure 1**) parameters (OB frequency and OB amplitude). Finding a solution to this system allows to study the behavior of the proposed oscillator and analyze performance over different tuning parameters. This will be evaluated in the following section. The maximum oscillation frequency or $f_{peak,max}$ will depend on the speed of each building block (BPF, bioimpedance converter, and comparator). For each of these blocks, the maximum operation frequency must be as high as possible in order to guarantee that the frequency range of interest correctly tests the BSUT. So it can be established that $f_{peak,max}$ represents the maximum frequency of interest of BI. In our application (Cell Culture Monitoring), frequency of interest goes from 0.5 kHz to 100 kHz, which is the frequency range where most differences are observed with respect to the cell covered area (A_c).

RESULTS

As was explained, the Oscillation-Based system is defined by the BPF parameters, namely Q factor (Q_f), Central frequency (f_{peak}), and Gain (G_0). The system is tuned at specific frequencies by establishing f_{peak} value. The Q_f, meanwhile, will affect the frequency ranges due to its influence over the BPF block and in the OB system (Pérez García, 2019). The solution of the oscillation equation provides f_{osc} and a_{osc} which ultimately are defined by the electrical blocks of the system (BPF, Interface, Comparator) and the sample under test.

Spectroscopy extension requires modification of the BPF parameters to establish oscillations in different tuning points. Oscillation amplitude a_{osc} ranges are consistent across all the f_{peak} range but oscillation frequency f_{osc} is highly dependent on the f_{peak} value and a certain figure is required to compare across different operation points. Due to this, normalized frequency is established as a method to observe frequency deviation from the

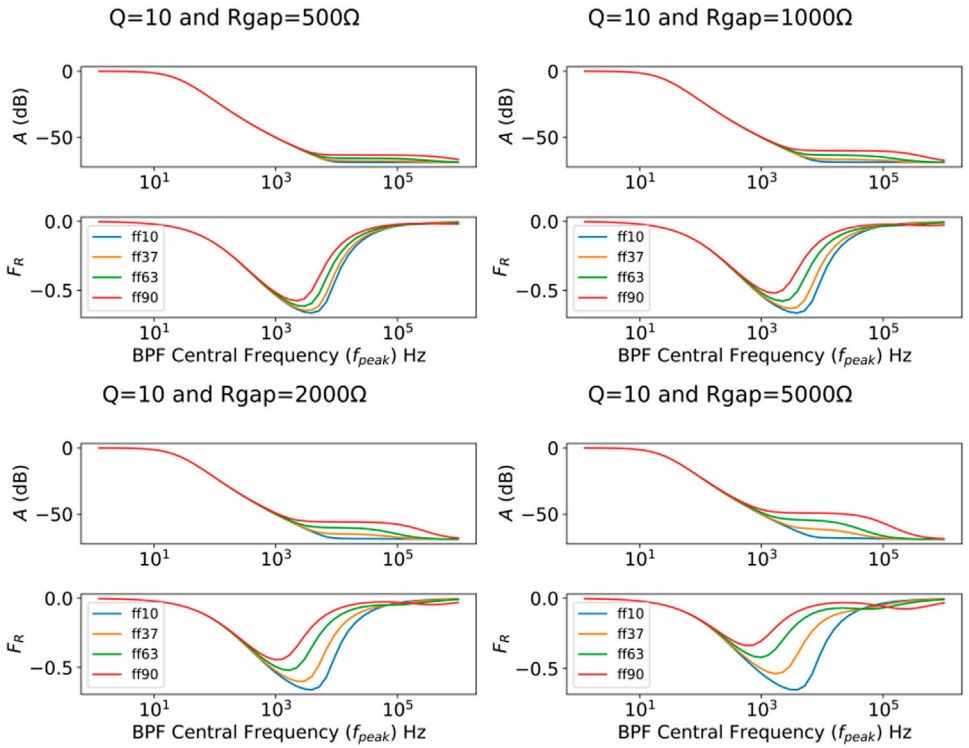

FIGURE 5 | Oscillation-Based (OB) parameters for different values of R_{gap}, and Q_f = 10. The simulations are performed over different fill factor levels (10%, 37%, 63%, and 90%).

central filter frequency. Proposed expression for amplitudes (A) and relative frequency factor (F_R) are:

$$A = 20 \cdot log_{10}\left(\frac{a_{osc}}{1}\right)$$

$$F_R = \frac{f_{osc} - \mathrm{f}_{peak}}{\mathrm{f}_{peak}}$$

Using the above expressions and the oscillation equations described, the OB system is solved to evaluate the oscillation parameters for different biological samples with discrete R_{gap} values. Considering a specific Q_f = 10 value, such results are presented in **Figure 5**, **Figure 6**, and illustrate the variations in the OB system parameters for each case explored. Oscillation amplitude is denoted as A and scaled in dB. Whereas F_R is an expression accounting for the rate between f_{peak} which is the central peak frequency of the oscillator and f_{osc}, the resulting oscillation frequency including the effect of the BSUT. This magnitude allows us to compare responses across the f_{peak} spectrum range.

Response variation is significant for a single order of magnitude range over R_{gap} parameter and directly translates into measurement sensitivity since the dynamic ranges of both A and F_R as an expression of the underlying biological impedance variations are crucial for the estimation of A_c (cell covered area).

An even better way of comparing these dynamic ranges is proposed by applying a normalization with respect to the model of the empty electrode.

$$A_N = 20 \cdot log_{10}\left(\frac{a_{osc}}{A_0}\right)$$

$$F_N = log_{10}\left(\frac{\mathrm{f}_{osc}}{f_0}\right)$$

Where A_0 and f_0 correspond to the values obtained for the oscillation at every different fpeak value with the empty electrode (no cells attached). The following curves in **Figure 6** illustrate spectroscopy normalized curves for Q_f = 10.

The R_{gap} factor serves as an example of the limitation existing in classic OBT cell culture sensor. Design procedures (Pérez et al., 2020) describe strategically tuned band pass filter blocks to maximize dynamic ranges and optimize information retrieval from sensor curves; this is performed by adjusting the filter central frequency of the BPF block to an operating point where the variation in both parameters is maximal across the biological process of a cell culture growing in a substrate. Cells adhered to the substrate (A_c) represent a portion of total substrate Area (A). Spectroscopy extension for OB cell culture sensing would enable the sensing device to operate on a wide range of frequencies, thus increasing the amount of information gathered in a single acquisition for any cell culture monitoring task. Furthermore, R_{gap} model intrinsic physical properties of the cell sample under test are not negligible and may reduce sensor sensitivity by reducing oscillator effective dynamic ranges for the oscillation parameters. An OB system implementing live tuning

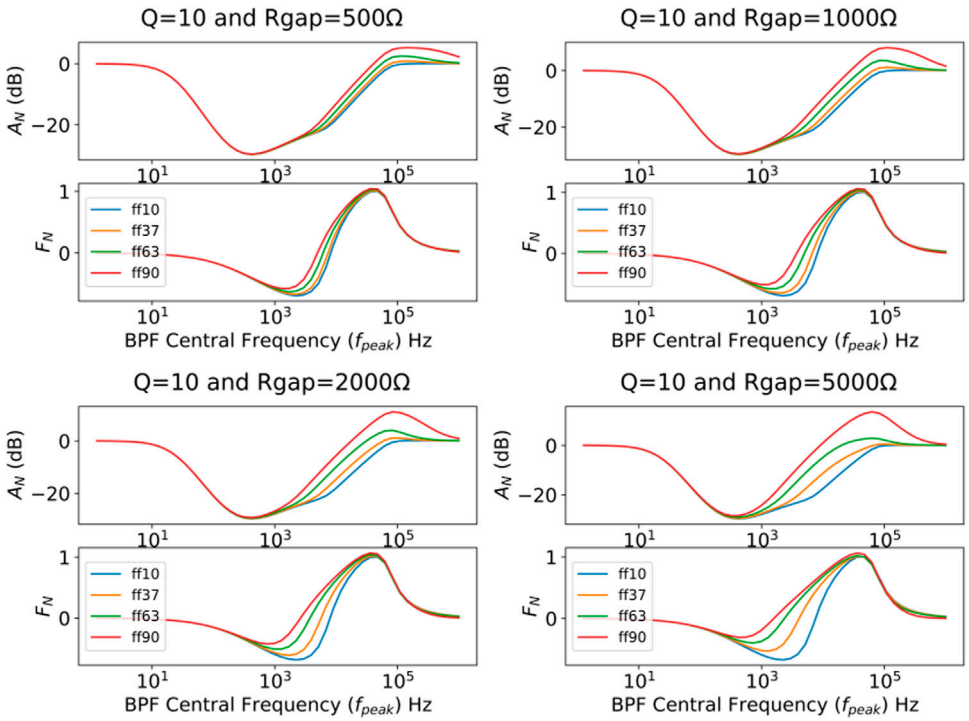

FIGURE 6 | Oscillation Based Spectroscopy (OBS) normalized response for different values of R_{gap}, and Q_f = 10. The simulations are performed over different fill factor levels (10%, 37%, 63%, and 90%).

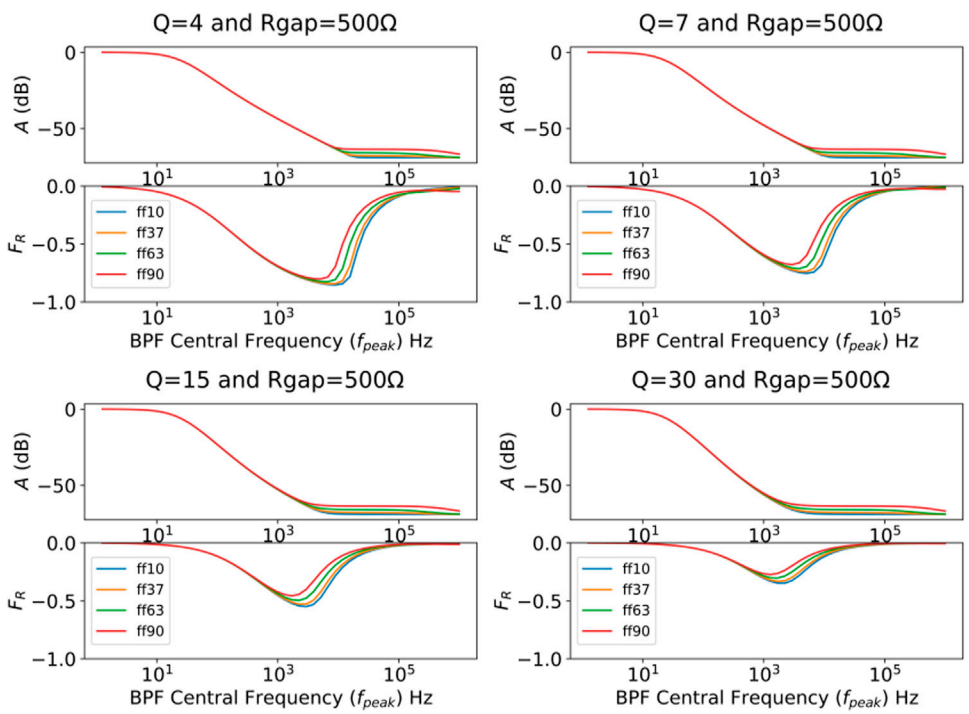

FIGURE 7 | Oscillation-Based (OB) Spectroscopy system for different values of Q_f parameter, with a fixed R_{gap} = 500. The simulations are performed over different fill factor levels (10%, 37%, 63%, and 90%).

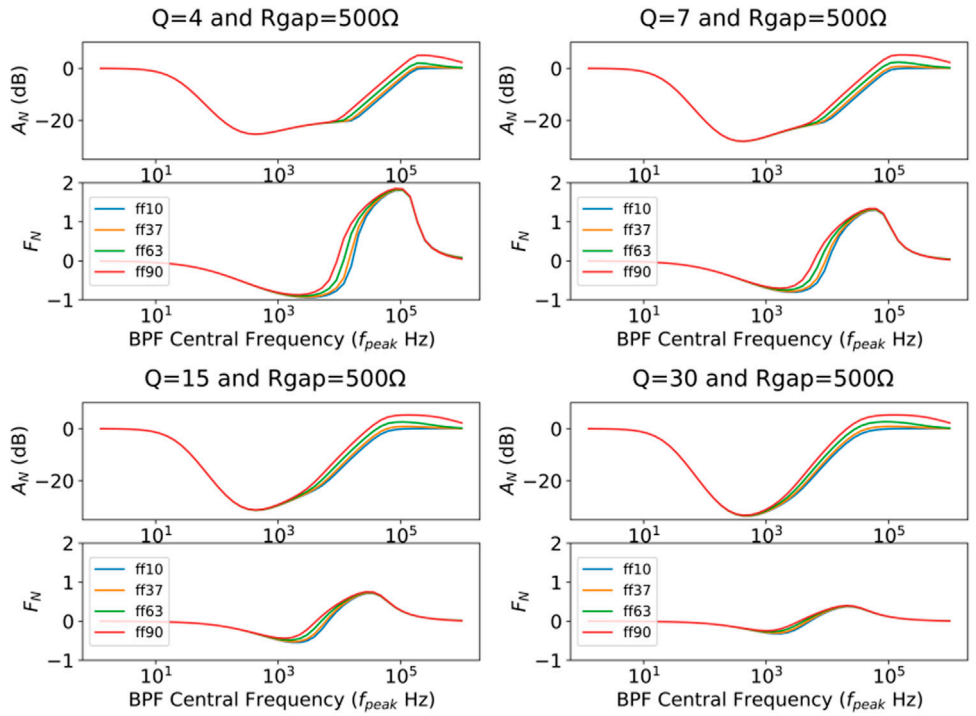

FIGURE 8 | Oscillation-Based (OB) Spectroscopy normalized response for different values of Q_f parameter, with a fixed R_{gap} = 500. The simulations are performed over different fill factor levels (10%, 37%, 63%, and 90%).

capacities or wide range analysis would not be affected or compensated for by the R_{gap} value.

OB Spectroscopy features an additional parameter, Q_f, as the quality factor of the band pass filter (BPF) block. Influence of this parameter has been previously reported for single tuning of the OBT sensor (Pérez et al., 2020). However, exploration of different values of Q_f may be of interest for acquiring additional information of the system under test. **Figure 7** describes the system curves for different Q_f values over a cell culture with a R_{gap} = 500Ω.

Amplitude curves for the different Q_f describe very different system responses for this parameter. Q-factor influences both frequency and amplitude. Frequency dynamic range is highly influenced by this value, while relation is inversely proportional, with the lowest Q_f value resulting in the greater dynamic range. The amplitude is affected by a small frequency shift along the frequency axis. There is a limit upon which the system is not oscillating anymore due to the BPF not being selective enough and hence the system does not meet the oscillation conditions. Normalization of these sensor curves for different values of Q_f are provided in **Figure 8**.

Spectroscopy extension of the OB sensor is oriented toward maximization of the cell culture sensor sensitivity in terms of both oscillation parameters: amplitude and frequency. **Figure 8** normalized the results from **Figure 7** with respect to the empty electrode model and describes the effect Q_f imposes over both oscillation parameters. This parameter is capable of imposing stricter or more relaxed oscillation frequencies with respect to the central frequency of the BPF. This feature enables the OBS sensor to control the oscillation with greater precision.

The curves above illustrate a potential to perform a multi- Q_f analysis over the sample. This second dimension adds more information which may be employed to gain further sensitivity over impedance samples under test.

DISCUSSION

This article proposes a design strategy to implement an Oscillation Based Spectroscopy (OBS) cell culture monitoring sensor. Advantages over the single frequency OBT approach were described and illustrated with the mathematical modeling of the Electrode Cell-substrate Impedance Sensing (ECIS) interface, the system blocks, and the oscillator behavior. Major contributions or conclusions of this work are:

- The system constraints and design parameters have been identified and modeled in the transfer function equations. Using such a design, an extensive symbolic mathematical solving method was applied by a computer program combining Wolfram Mathematica and *Python*. The implemented code in the form of Jupyter Notebook to replicate or evaluate this work is published and accessible at (Pérez, 2021).
- Spectroscopy extension of the OBT sensor can improve the performance of this kind of system in both sensitivity and

adaptability with respect to different biological impedance samples, specifically cell culture samples, and different types of electrodes. Optimum sensitivities can be found by maximizing the dynamic range of amplitudes and frequencies of system oscillations. This fact will increase the signal to noise ratio (SNR), improving the measurement quality.

- The proposed technique enables people to work with electrodes with different geometries or alternative specifications, for example, materials, since it is possible to program the final central frequency of the BPF for the best performance, considering the actual electrodes employed.
- Along with the spectroscopy frequency sweep for the impedance under test, a second dimension is explored with the Q_f, quality factor of the filter which affects the oscillation behavior and sensing dynamic ranges. Multi- Q_f acquisition may be worth exploring as a viable tool to also maximize sensor sensitivity by making the band pass filter quality factor programmable.
- The main electrode-cell parameter employed in our work is Rgap. In several works we have demonstrated that this parameter is dependent on the cell line, so it can be used for cell identification tasks also. Alternative electrical models (Giaever and Keese, 1991) deliver other parameters, such as barrier resistance, Rb, that it is a well-accepted biomarker for cell cultures for biology researchers. However, Rb is not able to be introduced into the flow design because of the complexity of its math modeling.

CONCLUSION

In this work, we have described the potential of an Oscillation Based spectroscopy analysis system to observe the electrical response of a biological system under test, and consequently how to incorporate this information into the system test procedure. The system under test considered in this paper is a biological sample of cell cultures, the impedance of which would be obtained by fine tuning an OB system at an adequate central frequency and quality factor to optimize the dynamic range for OB oscillation and hence achieve greater sensitivity.

The results presented in this work are useful for real-time OB sensing systems. The extension of these systems to perform spectroscopy analysis would remove the constraints imposed by tuning the oscillator at a fixed frequency for maximizing ranges in a particular sample (with a specific measurement electrode set). This method isolates the OB measurement system from the sample and electrical interface physical since the process is stepping up from a single frequency acquisition to a full spectrum data analysis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This work was supported by the project, Sistema de medida y electroestimulación para aplicaciones de diferenciación y motilidad celular, funded by: "Fondo Europeo de Desarrollo Regional (FEDER) y a la Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía, dentro del Programa Operativo FEDER 2014-2020. Referencia: US-1380661. Universidad de Sevilla. We also thanks to the Andalucía Government's, PAIDI 2017-2020, P18-FR-2308. Proyectos I+D+i Junta de Andalucía, Proyectos de Excelencia (Frontera): Sistema de medida y electroestimulación para aplicaciones de diferenciación y motilidad celular. US-1380661.

REFERENCES

Alexander, F. A., Price, D. T., and Bhansali, S. (2013). From cellular cultures to cellular spheroids: Is impedance spectroscopy a viable tool for monitoring multicellular spheroid (MCS) drug models? *IEEE Rev. Biomed. Eng.* 6, 63–76. doi:10.1109/RBME.2012.2222023

Bagnaninchi, P. O., and Drummond, N. (2011). Real-time label-free monitoring of adipose-derived stem cell differentiation with electric cell-substrate impedance sensing. *Proc. Natl. Acad. Sci. U. S. A.* 108 (16), 6462–6467. doi:10.1073/pnas.1018260108

Borkholder, D. (1998). *Cell based biosensors using microelectrodes*. Stanford, CA: Stanford University.

ECIS cell-based assays from applied BioPhysics."Available at: http://www.biophysics.com/(Accessed Feb 04 2018).

Giaever, I., and Keese, C. R. (1991). Micromotion of mammalian cells measured electrically. *Proc. Natl. Acad. Sci. U. S. A.* 88, 7896–7900. doi:10.1073/pnas.88.17.7896

Giaever, I., and Keese, C. R. (1986). Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture. *IEEE Trans. Biomed. Eng.* 33 (2), 242–247. BME-. doi:10.1109/TBME.1986.325896

Grimnes, S., and Martinsen, Ø. G. (2008). *Bioimpedance and bioelectricity basics*. Massachusetts: Academic.

Holland, I., McCormick, C., and Connolly, P. (2018). Towards non-invasive characterisation of coronary stent re-endothelialisation – an *in-vitro*, electrical impedance study. *PLoS One* 13 (11), e0206758. doi:10.1371/journal.pone.0206758

Hsu, C.-L., Sun, A., Zhao, Y., Aronoff-Spencer, E., and Hall, D. A. (2018). "A 16×20 electrochemical CMOS biosensor array with in-pixel averaging using polar modulation," in *2018 IEEE Custom Integrated Circuits Conference (CICC)* (San Diego, CA, USA: IEEE), 1–4. doi:10.1109/CICC.2018.8357044

Huang, X., Nguyen, D., Greve, D. W., and Domach, M. M. (2004). Simulation of microelectrode impedance changes due to cell growth. *IEEE Sens. J.* 4 (5), 576–583. doi:10.1109/JSEN.2004.831302

Huertas, G., Maldonado, A., Yúfera, A., Rueda, A., and Huertas, J. L. (2015). The bio-oscillator: A circuit for cell-culture assays. *IEEE Trans. Circuits Syst. Ii.* 62 (2), 164–168. doi:10.1109/TCSII.2014.2386233

Jun, M.-H., Kim, S., Ku, B., Cho, J., Kim, K., Yoo, H. R., et al. (2018). Glucose-independent segmental phase angles from multi-frequency bioimpedance analysis to discriminate diabetes mellitus. *Sci. Rep.* 8 (1), 648. doi:10.1038/s41598-017-18913-7

Keese, C. R., Wegener, J., Walker, S. R., and Giaever, I. (2004). Electrical wound-healing assay for cells *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 101 (6), 1554–1559. doi:10.1073/pnas.0307588100

Nordberg, R. C., Zhang, J., Griffith, E. H., Frank, M. W., Starly, B., Loboa, E. G., et al. (2017). Electrical cell-substrate impedance spectroscopy can monitor age-grouped human adipose stem cell variability during osteogenic differentiation. *Stem Cells Transl. Med.* 6 (2), 502–511. doi:10.5966/sctm.2015-0404

Parekh, A., Das, D., Das, S., Dhara, S., Biswas, K., Mandal, M., et al. (2018). Bioimpedimetric analysis in conjunction with growth dynamics to differentiate aggressiveness of cancer cells. *Sci. Rep.* 8 (1), 783. doi:10.1038/s41598-017-18965-9

Pérez García, P. (2019). *Circuit design for biomedical laboratories based on impedance measurement*. [Online]. Available: https://idus.us.es/handle/11441/88997 (Accessed Jun 14, 2021).

Pérez, P. Research/FIE2021 at main · pablopgus/Research, Available: https://github.com/pablopgus/Research/tree/main/FIE2021 (accessed Dec. 15, 2021).

Pérez, P., Huertas, G., Maldonado-Jacobi, A., Martin, M., Serrano, J. A., Olmo, A., et al. (2018). Sensing cell-culture assays with low-cost circuitry. *Sci. Rep.* 8 (1), 8841. doi:10.1038/s41598-018-27295-3

Pérez, P., Jacobi, J. A. M., López, A. J., and Martínez, C. (2017). "Remote sensing of cell-culture assays," in *New insights into cell culture technology*. Editors j. Sivakumar and T. Gowder (India: InTech).

Pérez, P., Maldonado, A., Yúfera, A., Huertas, G., Rueda, A., Huertas, J. L., et al. (2016). Towards bio-impedance based labs: A review. *J. Electr. Eng.* 4, 116–127. doi:10.17265/2328-2223/2016.03.002

Pérez, P., Serrano, J. A., Martín, M. E., Daza, P., Huertas, G., and Yúfera, A. (2020). A computer-aided design tool for biomedical OBT sensor tuning in cell-culture assays. *Comput. Methods Programs Biomed.* 200, 105840. doi:10.1016/j.cmpb.2020.105840

Rego, D., Costa, L., Pereira, M. T., and Redondo, L. M. (2015). Cell membrane permeabilization studies of chlorella sp. by pulsed electric fields. *IEEE Trans. Plasma Sci.* 43 (10), 3483–3488. doi:10.1109/TPS.2015.2448660

Sánchez, G. H. (2006). *Oscillation-based test in mixed-signal circuits*. New York: Springer.

Schmidt, S., Schubler, M., Hessinger, C., Schuster, C., Bertulat, B., Kithil, M., et al. (2019). Microwave induced electroporation of adherent mammalian cells at 18 GHz. *IEEE Access* 7, 78698–78705. doi:10.1109/ACCESS.2019.2923073

Schwan, H. P. (1957). Electrical properties of tissue and cell suspensions. *Adv. Biol. Med. Phys.* 5, 147–209. doi:10.1016/B978-1-4832-3111-2.50008-0

Serrano, J. A., Huertas, G., Maldonado-Jacobi, A., Olmo, A., Perez, P., Martin, M., et al. (2018). An empirical-mathematical approach for calibration and fitting cell-electrode electrical models in bioimpedance tests. *Sensors* 18 (7), 2354. doi:10.3390/s18072354

Stupin, D. D., Kuzina, E. A., Abelit, A. A., Emelyanov, A. K., Nikolaev, M. D., Koniakhin, S. V., et al. (2021). Bioimpedance spectroscopy: Basics and applications. *ACS Biomater. Sci. Eng.* 7 (6), 1962–1986. GIF. doi:10.1021/ACSBIOMATERIALS.0C01570/ASSET/IMAGES/MEDIUM/AB0C01570_0017

Wegener, J., Keese, C. R., and Giaever, I. (2000). Electric cell–substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. *Exp. Cell. Res.* 259 (1), 158–166. doi:10.1006/excr.2000.4919

Yúfera, A., Daza, P., and Canete, D. (2011). "Using microelectrode models for real time cell-culture monitoring, " in 2011 Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. (Boston, MA, USA: IEEE), 3983–3986. doi:10.1109/IEMBS.2011.6090989

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.