Assisted laser impedance spectroscopy to probe breast cancer cells

F Mesa\(^1\), B A Paez-Sierra\(^2\), A Romero\(^3\), P Botero\(^4\) and S Ramírez-Clavijo\(^1\)

\(^1\) Faculty of Natural Sciences, Department of Biology, Universidad del Rosario, Bogotá, Colombia
\(^2\) Physics Department, Universidad Militar Nueva Granada, Campus Nueva Granada, Cajicá 250247, Colombia
\(^3\) Master of Business and Science Program, Keck Graduate Institute, 535 Watson Drive, Claremont, CA 91711, United States of America
\(^4\) Universidad del Rosario, School of Medicine and Health Sciences, Biomedical Engineering Program, Bogotá, Colombia

E-mail: fredy.mesa@urosario.edu.co

Received 3 August 2020, revised 14 October 2020
Accepted for publication 21 October 2020
Published 27 November 2020

Abstract

Confidence and integrity are critical in the physical and chemical analyses of tissues and living cells. However, many of the probes targeting biological markers for confocal spectroscopy affect cells' molecular identity. Hence, we combined photonics with electrical analysis in an assisted laser impedance spectroscopy facility and applied it to characterize two breast cancer cell lines (BT-474 and MCF-7) and lymphocytes (as a normal control). The setup comprised a sample holder with a \(\sim 15000\) cell capacity fitted with two isolated conducting electrodes arranged concentrically and connected to an impedance analyser with a 20 Hz–1 MHz sweeping frequency. Capacitive transconductance measurements showed bands at 3491, 3494 and 3470 Hz corresponding to the BT-474, MCF-7, and lymphocytes, respectively. Under photonic stimulation by a 532 nm laser, these dark reference bands shifted to 3518, 3566 and 3674 Hz, respectively, reflecting optical transitions favouring ionic transport in the cells. Based on the experimental Nyquist diagrams and taking into account the roughness nature of the cell membrane, a constant phase element (CPE) was introduced in the circuit. The CPE was explained through a fractional parameter, \(\alpha\), based on fractional calculus. Results showed that, under photonic stimulation, \(\alpha\) is less than \(1/2\), and the minimum change of series and membrane resistances are about 28.95\% and 58.88\%, respectively.

Supplementary material for this article is available online

Keywords: ALIS, MCF-7, BT-474, cancer, fractional model

(Some figures may appear in colour only in the online journal)

1. Introduction

Eukaryotic cells have measurable electrical properties, including the capacitance of the cell membrane and the conductivity of the cytoplasm \([1–4]\). The cell membrane is the outer barrier of a cell and is composed of a lipid bilayer, proteins and carbohydrates in varying compositions depending on the degree of specialization and the functions it serves. Some of these proteins form channels \([5]\) that control the material flow between the intracellular and extracellular media and are key to the production and detection of cellular electrical activity.
[6, 7]. In this way, the cell membrane can be considered as a parallel-plate system and represented as a capacitor acting as a dielectric interface, generating electrical signals in response to stimuli (i.e. applied electrical DC or AC voltage with a fixed or variable frequency) [8, 9]. It has been shown that ions move and accumulate on both sides of the membrane and generate an electric field, blocking the ion dynamics. The total or partial blockage of ions is modelled as impedance; in biological materials, such as living cells, it is referred to as bioimpedance [10].

Electrical impedance spectroscopy (EIS) is based on the use of electrical contacts (macro- and micro-electrodes) [11–13] and can be used to measure the bioimpedance of biological systems [14–17] in a non-invasive manner. EIS is widely used to discriminate between normal and tumour cell types [18, 19]. The electrical differences between the cells are attributed to differences in the ionic concentration, water content, membrane permeability, mass density and electrical resistivity [20, 21]. It has been reported that healthy tissues have higher impedance than tumour tissues [22]. Various devices connected to systems, with different types of design have been developed to measure impedance in vitro of breast cancer cell lines or in vivo in patients. One of them consists of a nanochannel system, which manages to trap a cell [23], another uses magnetic nanoparticles functionalized with antibodies that recognize proteins present in breast cancer tumor cell lines (proteins as EpCAM, MUC-1 and HER-2), both systems could be applied to detect tumor cells in human fluids such as blood [24]. Further, improved detection of mammary gland tumors has been achieved with imaging EIS techniques [15, 25], electrical impedance mapping [20], multi-frequency systems [26] and procedures to simultaneously collect data from EIS and tomosynthesis [27]. Recently, the changes at the molecular level responsible for the chemoresistance of cancer cells have been monitored by EIS [25].

Laser stimulation combined with EIS recognizes proteins present in tumor tissue of the head and neck [28, 29] to produce surface-enhanced Raman scattering signal spectroscopy (SERS) [30]. SERS was previously tested in squamous cell carcinoma cell lines [28] and subsequently in breast cancer cell lines [31]. Several studies show that EIS is a promising tool for the diagnosis and treatment of breast cancer [32], but few of them have used assisted laser impedance spectroscopy (ALIS).

EIS-based sensors have been manufactured to detect interactions between cells and their surroundings, such as cell spreading, growth, death, adhesion and motility, being parameters close linked to their electrical responses in culture [25, 32–36]. The cytotoxicity of drugs and toxins can also be detected based on impedance measurements [33, 37]. It has been shown that the metabolic changes related to obesity are associated with intracellular lipid accumulation, leading to changes in adipocyte impedance that can be monitored in real-time in three-dimensional adipocyte cultures [38]. Usually, electrodes can be made of various materials: the most commonly used is gold metal because it enables high reproducibility for most applications [39], and others are made with polymers based on poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) [40, 41].

Since several EIS probes have matured to demonstrate the ability to recognize viral growth and cellular response to infections [42–44], ALIS involves an additional parameter, photostimuli, which can induce reversible changes at the molecular level and reflected in the electrical properties of a specimen, namely resistance or capacitance [45]. The unique selectivity of ALIS as a label-free probe makes it a well-suited technique for high accuracy and fast screening, especially cancer cells or viral particles [43, 44, 46] including those that cause respiratory disease, such as influenza virus and COVID-19 and also EIS microfluidic devices for virus identification and quantitative titer of recombinant baculovirus [47].

The development of new photonic biosensors and methods for ALIS is crucial for label-free strategies modulated by low power photostimuli [45] acting as artificial biomarkers that can generate a new fingerprint of tumor cells applicable in the diagnosis and treatment of diseases such as cancer [48].

The development of new photonic biosensors and ALIS methods is crucial for label-free strategies. Especially, combining impedance spectroscopy with low power photostimuli [45], where photons act as artificial biomarkers and generate a unique fingerprint of tumor cells applicable in the diagnosis and treatment of cancer [46].

Here, we present an ALIS measurement facility (figure 1) consisting of a sample holder with two isolated, concentric stainless-steel electrodes coupled to an inductance, capacitance, resistance (LCR) meter with an AC voltage signal with sweeping frequency from 20 Hz to 1 MHz. The holder is designed to have a maximum capacity of about 500 µl. The holder is coupled to a monochromatic laser of 532 nm and the measurements are taken both in the dark and under the light. ALIS allows cells to be interrogated with high specificity and reproducibility based on the artificial marker of the photonic response by comparing the unique and complementary cell signatures in darkness and under photonic excitation. ALIS analyses were performed on three cell types in solution—two breast cancer cell lines with different molecular profiles and lymphocytes from a blood sample of a healthy person as a control. The differences between the photon–cell interactions in cell lines can be measured using this system. At the same time, the resistance, capacitance, impedance and phase (R, C, Z, θµ respectively) can be measured from the AC electric current and voltage as a function of frequency. Thus, equivalent circuits with similar configurations are set for both darkness and photonic excitation. Considering the linearity of the experimentally measured Nyquist plots, a constant phase element (CPE) is set for all circuits and described in terms of a fractional parameter, α, based on fractional calculus.

2. Materials and methods
2.1. Cell culture and load samples for ALIS analysis

The MCF-7 and BT-474 cell lines were acquired from the American Type Culture Collection. Both cell lines were grown in vitro in Dulbecco’s Modified Eagle Medium (DMEM;
Gibco 11965092) supplemented with 10% serum fetal bovine-SFB (Gibco 16000036) and 1% antibiotic-antifungal agent (Gibco 15240062) in either 25 cm$^2$ or 75 cm$^2$ culture flasks and kept in an incubator at 37 °C and 5% CO$_2$. When the cells reached 85% confluence, they were trypsinised by applying trypsin EDTA (Gibco 25200056) for 3 min, adding 5 ml of DMEM, then centrifuging at 700 $\times$ g for 5 min. The supernatant was immediately discarded, 5 ml of phosphate buffer saline (PBS) was added, and the cell solution was again centrifuged at 700 $\times$ g for 5 min. The recovered pellet was finally resuspended in 1 ml of DMEM and the cell concentration was quantified in a Neubauer chamber (10 $\mu$l of the cell suspension was mixed with 90 $\mu$l of trypan blue).

Lymphocytes were obtained from a 10 ml sample of blood donated by a healthy volunteer who provided informed written consent which was reviewed and approved by the Institutional Ethics Committee, as recorded in act No. DVG0005682-CV866. Whole blood was collected in a tube containing heparin, transferred to a 15 ml tube, and centrifuged at 700 $\times$ g for 5 min. The recovered buffy coat and transferred to 2.5 ml of Ficoll Histopaque (SIGMA Ref 1077) then centrifuged for 30 min at 1008 $\times$ g without acceleration or braking. The white layer was aspirated and transferred to a 15 ml tube with 10 ml of PBS, then again centrifuged at 700 $\times$ g for 5 min. The supernatant was subsequently removed, and the pellet was resuspended in 5 ml of PB-MAX Karyotyping Medium (Gibco 12557013), which is an optimized RPMI 1640 medium, supplemented with fetal bovine serum at confidential proportion of the manufacturer, 2.05 mM of L-glutamine, and phytohemagglutinin M (PHA) at confidential proportion of the manufacturer. The cells were then cultured in the incubator for 2 d, then were quantified in a Neubauer under the same conditions as for cell lines.

To set the optimal cell number for ALIS measurements, different cell concentrations were prepared in the respective culture media. A stock concentration was used to prepare dilutions with the desired cell number (between 2 500 to 30 000 cells) in a total volume of 50 $\mu$l, although the holder capacity is 500 $\mu$l.

Experimental trials revealed that a load volume of 50 $\mu$l composed of 15 000 cells and culture media was appropriate to create substantial measurable changes such that ALIS could differentiate each cell type.

2.2. Assisted laser impedance measurements

The ALIS measurement setup, shown in figure 1, comprises a cylindrical sample holder (where the cells are loaded) with two isolated stainless-steel electrodes separated by a polytetrafluoroethylene O-ring. The holder is electrically connected via a homemade Bayonet Neill–Concelman connector (BNC)-based breakout box to a Keysight E4980AL-precision LCR meter. Each connector consists of one BNC-type terminal and another terminal fixed with a clamp clip; both sample and probes are coated with an antioxidant alloy (Kelvin probe) to block parasitic signals. All measurements were achieved with an AC signal of 100 mV amplitude over a frequency range from 20 Hz to 1 MHz and with zero DC bias. Each point measurement was an average of 20 trials.

Measurements were carried out at 20 °C in a dark and electromagnetic isolated room. Each experiment was achieved for 3 min. either in darkness or illumination. Sample temperature was monitored during all measurements. Before all ALIS experiments, a compensation protocol was performed, first with an open-loop configuration and then with a short-circuit connection, to correct for thermal noise and wiring, capacitance and impedance leakage signals. Photonic excitation was realized with an unpolarized TEM 00 solid-state laser of wavelength $\lambda = 532$ nm and power of 50 mW. To prevent damage to the cells, the laser beam intensity was attenuated with neutral-density filters ranging from 0.3 to 3.0. The beam was aligned with a dielectric mirror and passed through a beamsplitter (40/60). One beam was aligned to a semiconductor detector to monitor the photon power during

![Figure 1. Assisted laser impedance spectroscopy (ALIS) facility used for characterizing cells (MCF-7 and BT-474 breast cancer cells and lymphocytes as a normal control).](image-url)
the experiments, and the second beam was aligned to a beam expander and focused onto the sample holder. The power density of the laser was varied from 0.05 to 0.8 mW cm\(^{-2}\), and set to 0.1 mW cm\(^{-2}\), which assures sample reversibility and verified with the subsequent dark measurement. In addition, the power of the photons does not induce local heating of the cells, and it was much lower than that of 10 mW cm\(^{-2}\) for typical Raman spectroscopy experiments [46]. All optics were assembled on a vibration-isolated breadboard. The compatible elements were acquired from Thorlabs. Moreover, in order to avoid parasitic signals, all ALIS facility is attached to an independent ground pole. The impedance noise for darkness or illumination is in the order of 10\(^{-8}\) \(\Omega\), which is low in comparison to the smallest impedance signal of 2.8 \(\Omega\) (see supplementary information figure S1 (available online at https://stacks.iop.org/JPJ/54/075401/mmedia)). All data were collected using a LabView-based interface software from National Instruments. All measurements with suspended cells were performed in triplicate; the error was less than 5.5% in all measures.

2.3. Ethics approval and consent to participate

The entire study, including the methodology for obtaining the biological material and its manipulation was approved by the ethics committee of the University of Rosario (CEI-UR) in act No. DVGO005 682-CV866. All methods were performed in accordance with the relevant guidelines and regulations and a healthy volunteer provided written informed consent for participation in this study.

3. Results

3.1. Experimental results

The advantage of combining photonic-based analysis with electrical characterization is that it offers unique capabilities to detect subtle features, such as ion transport, that are difficult to evaluate by other techniques. The light-cell interaction gates additional free and polarized charges and are further driven by the alternating field and contribute to the global impedance. Therefore, we added the laser-stimuli to the impedance characterization, acting as an artificial marker, thus bypassing additional sample preparation, namely using physical and chemical biomarkers or functionalizing electrodes. Consequently, combined laser excitation with electrical characteristics are particularly useful for distinguishing cancer cell lines. Figure 2 shows the spectrum of electrical impedance and phase angle as functions of the frequency between 20 Hz and 1 MHz for the breast cancer cell lines (MCF-7 and BT-474) and the control cell line (lymphocytes) in darkness and under illumination. The curves (called Bode diagrams) for all cell lines (figures 2(a) and (b)) exhibit the typical behaviour of capacitive circuits. When a voltage source is connected to the system, the positive terminal of the source attracts negative charges from the electrode to which it is connected, leaving an excess of positive charges on it and giving rise to a polarization effect and forming an electric field. Because the voltage is AC, the electrode connected to the positive terminal varies from one half-cycle to the next, generating a charge variation on each electrode. The rate of this load variation is directly proportional to the frequency of the power supply. The magnitude of the current flowing through the sample depends on the rate of charge transfer and generally increases with increasing frequency, which is interpreted as a decrease in the impedance or an increase in the conductance of the system. Therefore, the impedance is inversely related to the frequency of the AC signal, as shown in figure 2. The impedance values of the BT-474 and MCF-7 cell lines differed from those of the lymphocytes. Since all measurements were carried out under similar conditions (i.e. number of cells and total volume \((V)\), alternating current (AC) stimulus and photonic excitation), differences in these measures can be attributed to differences in their molecular profiles. Considering the cylindrical geometry of the sample holder with loaded cell volume of height, \(L\), internal and external radii, \(r_1 = 2\) mm and \(r_2 = 4\) mm, respectively, the impedance, \(Z\), at each AC frequency, \(f = \omega/2\pi\), with or without illumination by a laser source, \(\omega' = \frac{2\pi}{\lambda}\), can be expressed as

\[
Z(\omega, \omega') = \frac{1}{\sigma(\omega, \omega')} \frac{\ln (r_2/r_1)}{2\pi L} = \frac{1}{\sigma(\omega, \omega')} \frac{(r_2^2 - r_1^2)}{2V} \ln (r_2/r_1),
\]

where \(\sigma(\omega, \omega')\), is the electrical conductivity of the sample in response to the external electric AC and photonic electric fields, \(E = E_0 \exp (j\omega t)\) and \(E' = E_0' \exp (j\omega' t)\), respectively. Hence, the impedance and phase angle deviations of BT474 and MCF7 relative to those of lymphocytes have intrinsic \(\sigma(\omega, \omega')\) values that are inherent to the molecular properties of breast cancer tumours. The hormone receptor status, such as estrogen receptor (ER) and progesterone receptor (PR), as well as the human epidermal growth factor receptor 2 (HER2) are routinely assessed by immunohistochemistry in a breast cancer tumour. Those are a prognosis marker that help to determine the patient’s better treatment. The breast cancer cell lines used in this study have a different molecular profile MCF7: PR+, ER+, HER2− and BT474: PR+, ER+, amplified HER2 [49]. In addition, \(\sigma(\omega, \omega')\) takes into account the structure and composition of the cell, including the amount and types of proteins present in the membrane. However, the phase plots exhibit behaviour that differs from a purely parallel capacitor \((C_p)\) and resistor \((R_p)\) characteristic.

Figure 2 shows that the phase is negative and approaches zero for all cell types in darkness or under photonic excitation for frequencies above about 80 Hz. This difference implies that the electrolyte (culture medium/cells) does not exhibit perfect dielectric behaviour, instead allowing charges to flow from one electrode to another. Thus, the equivalent circuit of this system must include resistive elements that ultimately dominate its behaviour as the frequency increases indefinitely.

A first approach for the impedance analysis is to macroscopically assess the interface region and bulk of the
electrolyte solution at different [50] or single frequencies [51]. Therefore, the external measurements using the ALIS facility of the parallel resistance ($R_p$) and parallel capacitance ($C_p$) in all samples were achieved. Results are depicted in figure 3. Interestingly, both $R_p$ and $C_p$ faces a frequency dependency that differs from their ideal behaviour. It can be observed that the resistance, $R_p$, decays with increasing frequency, indicating that the behaviour is not purely resistive. This may be because the biological tissue constituted by the respective cell lines, is a dielectric material composed of intracellular and extracellular parts immersed in an ionic solution. For each measurement, a consistent total sample volume composed of culture medium and a consistent number of cells was used.

The investigated cells have spherical morphology, the diameters of MCF-7, BT-474 and lymphocytes are approximately 15 [52], 10 [53] and 7.5 μm [54]. The volume of MCF-7 and BT-474 respect to lymphocytes are 8 and 2.4 times larger, respectively. Therefore, based on geometrical features, the highest impedance is observed for lymphocytes, followed by BT-474 and MCF-7. Consequently, the geometrical cell features and membrane signatures were considered in the analysis of ALIS measurements. Therefore, the electrical conductivity, $\sigma(\omega,\omega')$, shown in equation (1) is specific for each external AC and photonic excitation fields over the frequencies $\omega$ and $\omega'$, respectively. Hence, the relative lipid/carbohydrate level is a factor related to $\sigma(\omega,\omega')$ over the capacitive and resistive behaviour of the cell membrane. Therefore, based on the published Raman spectra for BT-474, MCF-7 and lymphocytes [55–57], the approximate lipid-to-carbohydrate ratios were extracted, as summarized in table 1. The overall capacitance, $C_p$, is proportional to the lipid-to-carbohydrate integrated Raman vibrational ratios. In contrast, the $R_p$ values are inversely proportional.

In figure 3, capacitance in dark conditions scales with cells geometry. While under illumination, C–N bonds from proteins are closer in energy to that of the photonic field, being at resonance in comparison with C–C bonds from lipids. Therefore, considering the concept of transconductance ($g$) [58] (susceptance) for the capacitive part, gives the relation between the extracted current and the applied external voltage, and given by $g = \omega C_p$ [59]. In addition, $g$ is useful since it only accomplishes the frequency-dependent part of the admittance ($Y = 1/Z$). Therefore, the emerging $g$ curves are straightforward to deconvolute and extract the resonance frequencies, useful to unravel the drastic changes in the electrical behaviour of the cell lines. In order to have a better comparison between $g$ curves of the different cells, the normalized transconductance is defined as $\frac{\omega C_p}{C_{\text{ref}}\omega}$. The curves are deconvoluted with Gaussian like functions and shown in equation (2)

$$g = \sum_{i=1}^{n} \frac{A_i}{W_i \sqrt{\pi/2}} e^{-\left(\frac{2(f-f_i)}{W_i}\right)^2},$$

being $A$, the area of the curve, $W$ the full width at half maximum, $F = \log(f)$ with $f$ and $F_c = \log(f_c)$ the center of the peak.

The resonance frequencies of the involved ion distributions were determined as shown in figure 4. The resonance frequency values obtained in darkness were 3062, 4014 and 8560 Hz for the BT-474, MCF-7 and lymphocytes, respectively. A blue shift was observed under lighting conditions for all cells: the peaks of the cancer cell lines shifted to 4472 Hz while those of the lymphocytes shifted to 9538 Hz. Thus, the overall conductivity is further modulated by the photonic source frequency, $\omega'$.

Next, the peaks were deconvoluted from the normalized capacitive transconductance curves over frequencies from 20 Hz to 1 MHz (see figure 4) to identify the ionic dynamics involved and the resonance of the ions with the external AC and photonic excitation fields. The driving AC frequency is inversely proportional to the ion mass: heavy ions are activated at low frequencies, while lighter ions are activated at high frequencies (see figure 5(a), red and light blue curves, respectively). The deconvoluted band located at 3491 Hz or at 3518 Hz (dark blue) in darkness or under illumination, respectively, can be considered as references for the three types of cells since they are consistent between cancer and normal cells. Therefore, cell changes can be monitored by measuring the ratio of the area of the reference band to the other

Figure 2. ALIS characteristics of electric impedance and phase spectra of MCF-7, BT-474 and control (lymphocyte) cell lines under (a) darkness and (b) photonic excitation.
Figure 3. Capacitance ($C_p$) and resistance ($R_p$) curves in parallel of cell lines BT-474, MCF-7 and lymphocytes (control) (a) in darkness and (b) with photonic excitation.

Table 1. Molecular fingerprints of BT-474, MCF-7 and lymphocytes.

| Cell type      | Raman bands (cm$^{-1}$) | Ratio of integrated intensity: lipid/carbohydrate levels | Molecular vibration mode assignment (L/P, L/C, P/C) |
|----------------|--------------------------|---------------------------------------------------------|--------------------------------------------------|
| BT-474         | 1081/1125                | 0.9                                                     | C$-\text{C}$ (Lipid), C$-\text{N}$ (Protein), C$-\text{N}$ (Protein) or C$-\text{O}$ (Carbohydrates) |
| MCF-7          | 1081/1125                | 1.1                                                     |                                                  |
| Lymphocytes    | 1070/1100                | 0.5                                                     |                                                  |

deconvoluted bands. However, a stimulus with a DC source ($\neq 0$ V) can shift the reference level of the alternating signal from the ALIS setup, which allows sub-levels of the electronic transitions of the cells to be activated or inhibited by shifting the Fermi level.

Under lighting conditions, the reference bands (dark blue) of the BT-474, MCF-7 and lymphocyte cells located at 3491, 3494 and 3470 Hz, respectively, shifted to 3518, 3566 and 3674 Hz, respectively. The lighting effect induces optical transitions in the cells, which favour electronic transitions; cells have a higher absorption at the laser wavelength ($\lambda = 532$ nm) than at other wavelengths [52]. The resonant times, $\tau$ defined by $f = 1/\tau$, associated with the corresponding maximum of the transconductance peak (figure 5(a)) obtained with different cell concentrations (between 2500 and 30 000 cells per 50 $\mu$l) are shown in figure 5(b). Even though, $\tau$ values are between 13 and 23 $\mu$s, the maximum capacitive transconductance signal was observed for 15 000 cells.

The electrical behaviour of BT-474, MCF-7 and lymphocytes were also reported using Nyquist diagrams ($Z' = Z_{re}$, $Z'' = Z_{im}$), and Kramers–Kronig consistency of the real and imaginary parts were addressed.
the expression for fractional derivatives of Caputo’s formula
\[
_f^\alpha\! [f(t)] := \begin{cases} 
\frac{1}{\Gamma(n - \alpha)} \int_0^t \frac{f^\prime(\tau)}{(t - \tau)^{n-\alpha}} \, d\tau, & n - 1 < \alpha \leq n \\
\frac{d^\alpha f(t)}{dt^\alpha}, & \alpha \in \mathbb{N}
\end{cases}
\]
where \( f^\prime \) is the fractional operator applied to the function \( f(t) \), \( t > \gamma \), \( \alpha \) is the fractional index of the derivative, \( \Gamma \) is the gamma function given by \( \Gamma(n - \alpha) = \int_0^\infty \, e^{-u}u^{n-\alpha} \, du \) with \( n - \alpha > 0 \), and \( f^\prime(\tau) = \frac{d^\alpha f}{d\tau^\alpha} \) denotes the \( n-\alpha \) integer derivative of function \( f \) with argument \( \tau \).

The impedance can be derived by applying equation (5) to the electric current signal in equation (4) and the driving signals, \( V \) and \( V' \). Since the LCR meter maintains the signal, \( V \), with the same amplitude of 100 mV independently of the measurement condition (i.e. darkness or photonic excitation), then the relationship between the impedance and capacitive effect can be expressed as follows:
\[
Z_\alpha = \frac{1}{\eta C[j(\omega')^\alpha + (j\omega')^\alpha u(\omega', \alpha, V_{\text{ind}}, V_o)]}
\]
where \( u(\omega', \alpha, V_{\text{ind}}, V_o) \) is a function that involves the dependence with the photon frequency \( (\omega') \), the fractional parameter \( \alpha \), and the ratio between the induced potential \( (V') \) and the amplitude \( V_o \).

When \( \alpha \) takes a value of +1 or −1, it indicates a capacitive or inductive reactive impedance, respectively. Equation (6) can be extended in the form of the CPE:
\[
Z_\alpha = \frac{1}{P(j\omega')^\alpha + \eta P(j\omega')^\alpha u(\omega', \alpha, V_{\text{ind}}, V_o)}.
\]

where \( \eta = \frac{d^{\alpha-1}Q}{dt^{\alpha-1}} \) is a calibration function and is given in \([s^{1-\alpha}]\) to maintain the impedance units as Ohms for \( \alpha \neq 1 \). To compute the fractional derivative for the electric current, the expression for fractional derivatives of Caputo’s formula [52] was considered and expressed in the following form:

Since the cell membrane resembles a porous surface contact, its capacitance is modulated by charge transfer and differs from that of an ideal capacitor. Thus, in this description, a CPE is considered to enhance the impedance circuit.

\subsection{3.2. Theoretical model}
\subsubsection{3.2.1. Photonic effect on the constant phase element: a fractional calculus approach.}
An adjustment to the Nyquist experimental curves must be adequate and as precise as possible. For this reason, we now consider an interpretation of the CPE on the basis of fractional calculus [52]. Let the stimulus voltage signal be \( V = V_o e^{\omega t} \), where \( V_o \) is the amplitude of the impulse signal (100 mV), \( \omega \) is the frequency and \( t \) is time. Assuming that the photonic excitation potential is \( V' = V'_o e^{(j\omega')t} \), the total potential difference supplied by both sources, LCR analyser and photonic radiation, to a capacitor with constant plates separation can be expressed as \( V_T = V + V_{\text{ind}} = Q/C \), where \( V_{\text{ind}} \) is the induced potential due to the photonic excitation, \( Q \) is the electric charge and \( C \) is the capacitance, and the electric current intensity, \( I \), is given as
\[
\frac{dV_T}{dt} = \frac{1}{C} \frac{dQ}{dt} \quad I = C \frac{dV_T}{dt}
\]

Additionally, taking into account the advantages of the fractional calculus (i.e. describing anomalous transport and memory effects), equation (3) can be extended in the form of a fractional derivative:
\[
\frac{d^\alpha Q}{dt^\alpha} = \eta l = C \frac{d^\alpha V_T}{d\tau^\alpha} \quad \text{with} \quad 0 < \alpha \leq 1
\]

where \( \eta = \frac{d^{\alpha-1}Q}{dt^{\alpha-1}} \) is a calibration function and is given in \([s^{1-\alpha}]\) to maintain the impedance units as Ohms for \( \alpha \neq 1 \). To compute the fractional derivative for the electric current, the expression for fractional derivatives of Caputo’s formula [52] was considered and expressed in the following form:

Figure 5. (a) Deconvoluted transconductance curves and (b) maximum resonance period (\( \tau \)) for different cell concentrations.
well diluted within the culture media, in this situation, charge transfer is promoted by the external electric field \( (\varepsilon = E + E') \) and not by thermal activation. \( \varepsilon \) induces energetic states of different localization lengths which are mixed at long distances favouring this charge transport [60].

Figure 6 shows the electrical circuit obtained from the electrical photoimpedance system under dark and lighting conditions; the series resistance \( (R_s) \), membrane resistance \( (R_m) \), capacitance in the metal/electrolyte interface or double-layer capacitance \( (C_{DL}) \), membrane capacitance \( (C_m) \), \( P \), \( W \) and \( \alpha \) are adjustable parameters, which are reported in table 2. Particularly in dark conditions and considering an uncertainty of 0.05%, MCF-7 and lymphocytes cells behave similar to a Warburg element, while BT-474 cells exhibit dominantly CPE-type behaviour. Under photonic excitation, the fractional parameter, \( \alpha \), for all cell lines was less than \( \frac{1}{2} \). The Warburg element \( \left( \frac{A_j}{\sqrt{T_j}} + \frac{A_{\omega}}{\sqrt{\omega}} \right) \), accomplishes two frequency-dependent elements, namely, a real resistive part and an imaginary capacitive part. The membrane resistance is independent of the frequency; thus, it is more appropriate to couple the Warburg with the resistive element.

The equivalent impedance reads
\[
Z = \frac{2}{j\omega C_{DL}} + 2R_s + \frac{1}{\left( \frac{1}{C_m} + \frac{1}{\left( C_m + \frac{1}{R_m} \right)} \right)} + \frac{R_s}{\sqrt{1 + \frac{1}{R_m^2}}}
\]
(8)

where
\[
\|Z\| = \sqrt{Z_{\text{re}}^2 + Z_{\text{im}}^2}.
\]
(9)

The Nyquist diagrams show that, regardless of the darkness or lighting conditions, the impedance behaviour differs drastically from purely resistive, capacitive, inductive or a combination thereof. Thus, it is important to consider a fractional CPE within the analysis. In addition, the differences between the Nyquist graphs in darkness and under photonic excitation for all cell lines can be explained by the effects of the photon–cell interactions (figure 6), which can influence the molecular reactivity.

This reactivity is determined by the energetic molecular orbital levels, where the highest occupied molecular orbital (HOMO) can donate an electron and the lowest unoccupied molecular orbital (LUMO) can receive an electron [62, 63]. In particular, HOMO–LUMO transitions and intermediate trap states near the LUMO are favoured by photonic interactions. All circuit elements addressed in figure 6 are affected by the photonic stimulation, especially \( R_s \); however, \( R_m \), \( C_{DL} \) and the fractional parameter, \( \alpha \), are direct evidence of these interactions (table 2). Under illumination, \( \alpha \) is less than \( \frac{1}{2} \) and the change of series and membrane resistances are significantly modified with a minimum of about 28.95% and 58.88%, respectively, (see table 3).

The other parameters (i.e. \( C_m \), \( W \) and \( P \)) involve more complex charge transfer dynamics modulated by the cell membrane.

The fitting results elucidate the relationship between the differences in \( W \), \( P \) and \( \alpha \) in the dark and under illumination; the differences in \( R_s \), \( R_m \), \( C_{DL} \), \( C_m \), \( W \), \( P \) and \( \alpha \) are expressed as percentages in table 3. Notably, the fractional parameter, \( \alpha \), is in the range of 0.48–0.74 in the dark and 0.43–0.48 under photonic excitation.

3.2.2. Normalized differential dielectric permittivity. Since the MCF-7 and BT-474 share DMEM as the measurement medium for ALIS analysis, and while Lymphocytes were measured in PB-MAX, we considered a further analysis of the impedance results.

The sample loaded for ALIS analysis is a 50 \( \mu \)l volume, accomplishing of cells, and medium. Hence, the cells volume ratio to medium volume for MCF-7, BT-474, and lymphocytes are \( 5.30 \times 10^{-4} \), \( 1.57 \times 10^{-4} \), and \( 6.63 \times 10^{-5} \), respectively. Therefore, it is appropriate to elucidate how the medium contributes on the overall impedance measurement. Accordingly, taking into account the relation between the complex impedance of the mixture (\( Z_{\text{mix}} = Z \)), i.e. cells and medium, with the complex dielectric permittivity \( (\varepsilon_{\text{mix}}^*) \) by the relation [64, 65].

\[
Z = \frac{1}{j\omega \varepsilon_{\text{mix}}^* G}
\]
(10)

being G a factor related with the sample holder geometry and the vacuum permittivity \( (\varepsilon_{\text{o}} = 8.54 \times 10^{-12} \text{ F m}^{-1}) \), and

\[
\varepsilon_{\text{mix}}^* = \varepsilon' - j\varepsilon''.
\]
(11)

Thus, the real and imaginary dielectric permittivity \( \varepsilon' \) and \( \varepsilon'' \) read

\[
\varepsilon' = -\frac{Z_{\text{im}}}{\omega G (Z_{\text{re}} + Z_{\text{im}}^2)}
\]
(12)

\[
\varepsilon'' = \frac{Z_{\text{re}}}{\omega G (Z_{\text{re}} + Z_{\text{im}}^2)}.
\]
(13)

Therefore, \( \varepsilon' \) and \( \varepsilon'' \) are computed from the impedance and phase measurements either under darkness or laser excitation conditions. To reach the effect of the medium and the mixture of cells: medium on the impedance measurements, figure 7 illustrates the normalized dielectric permittivity computed with equations (12) and (13) by dividing \( \varepsilon' \) and \( \varepsilon'' \) into the corresponding maximum value.

For further analysis of data reported in figure 7, we defined the normalized differential permittivity through the relation

\[
\Delta \varepsilon_{\text{norm}}^{(n)} = \frac{\varepsilon_{\text{sample}}^{(n)} - \varepsilon_{\text{medium}}^{(n)}}{\varepsilon_{\text{max-sample}}^{(n)} - \varepsilon_{\text{max-medium}}^{(n)}}.
\]
(14)

Equation (14) and figure 8 summarizes the normalized differential permittivity under darkness and illumination conditions.

Although the cells are well diluted for the ALIS analysis, it is observed that the proposed normalized differential permittivity \( \Delta \varepsilon_{\text{norm}}^{(n)} \) and \( \Delta \varepsilon_{\text{norm}}^{(n)} \) are sensitive to the measurement situation for each cell type, i.e. darkness or illumination.
Table 2. Values obtained from the parameters of the electric circuit model.

| Mode           | Cells line | $R_s$ (Ω) ± Δ% | $R_m$ (Ω) ± Δ% | $C_{DL}$ (F) ± Δ% | $C_m$ (F) ± Δ% | $W$ ± Δ% | $P$ ± Δ% | α ± Δ% |
|----------------|------------|----------------|----------------|-------------------|---------------|----------|---------|-------|
| Darkness       | BT-474     | 60.31 ± 3.2    | 759.18 ± 3.1   | $15.41 \times 10^{-06}$ ± 3.0 | $5.58 \times 10^{-06}$ ± 1.9 | $22.534 \pm 2.25$ | $1.27 \times 10^{-05} \pm 0.7$ | 0.74 ± 0.16 |
|                | MCF-7      | 48.84 ± 3.0    | 520.96 ± 6.7   | $9.11 \times 10^{-6}$ ± 5.7 | $1.45 \times 10^{-6}$ ± 2.3 | $1.70 \times 10^{-4} \pm 4.6$ | $2.63 \times 10^{-4} \pm 8.4$ | 0.48 ± 1.9   |
|                | Lymphocytes | 51.34 ± 0.89  | 1919.6 ± 3.7   | $7.89 \times 10^{-6}$ ± 4.3 | $5.13 \times 10^{-6}$ ± 5.4 | $3.12 \times 3 \pm 1.2$ | $9.00 \times 10^{-5} \pm 1.37$ | 0.50 ± 0.25  |
| Illumination   | BT-474     | 28.90 ± 0.43   | 21.22 ± 2.8    | $3.36 \times 10^{-5}$ ± 3.7 | $1.01 \times 10^{-6}$ ± 0.47 | $1.23 \times 4 \pm 0.26$ | $1.90 \times 10^{-4} \pm 0.75$ | 0.46 ± 0.18  |
|                | MCF-7      | 34.70 ± 11     | 24.93 ± 7.1    | $2.94 \times 10^{-5}$ ± 4.4 | $1.51 \times 10^{-6}$ ± 1.0 | $1.15 \times 4 \pm 0.65$ | $1.86 \times 10^{-4} \pm 1.4$ | 0.48 ± 0.33  |
|                | Lymphocytes | 21.11 ± 0.8    | 39.73 ± 3.1    | $2.98 \times 10^{-5}$ ± 2.8 | $1.09 \times 10^{-6}$ ± 3.2 | $1.05 \times 4 \pm 0.82$ | $2.99 \times 10^{-4} \pm 1.0$ | 0.43 ± 0.20  |
Figure 6. Assisted laser impedance model in dark and light conditions. (Cell figure reproduced from [61].)

Table 3. Relative percentage changes due to the photonic effect.

| Mode       | Cell line | $\Delta R_p$ (%) | $\Delta R_m$ (%) | $\Delta C_{DL}$ (%) | $\Delta C_m$ (%) | $\Delta W$ (%) | $P$ (%) | $\alpha$ (%) |
|------------|-----------|------------------|------------------|---------------------|-----------------|--------------|---------|-------------|
| (Dark-)    | BT-474    | 52.08            | 97.20            | -118.21             | 81.84           | 45.41        | -1404.38| 38.03       |
| Illum.//   | MCF-7     | 28.95            | 95.21            | -222.38             | -3.78           | 32.01        | 29.36   | 1.03        |
| Dark       | Lymphocytes | 58.88            | 97.93            | -278.19             | 78.81           | -232.91      | -232.75 | 14.95       |

4. Discussion

The total parallel measurements of resistance and capacitance ($R_p$ and $C_p$, respectively) demonstrate very different behaviours of ideal resistive or capacitive elements. In particular, figure 2 shows that the impedance is inversely related to the frequency. Moreover, the phase behaviour differs from that of a standard resistor, capacitor, inductor or combination thereof. Therefore, the extrinsic conductivity, $\alpha(\omega, \omega')$ expressed in equation (1) is based on the experimental measurements of $Z(\omega, \omega')$ and the loaded sample volume in the sample holder. Hence, $\alpha(\omega, \omega')$ works as a measure of both darkness and photonic excitation phenomena through the measurement of $Z(\omega, \omega')$.

Figure 3 shows the decrease in $R_p$ with increasing frequency. In contrast, $C_p$ increased as the frequency increased from 10 Hz to approximately 26 Hz, which is consistent with charge-injection behaviour, and decreased as the frequency increased further. Under illumination conditions, the peak in $C_p$ shifted to 22 Hz. The external electric field $\varepsilon = E + E'$ polarizes and promotes induced charges at the cell membrane, which are further driven within the field. $\varepsilon$ yields energetic states of different localization lengths and mixed at long distances, thus boosting charge transport [60]. The photonic effect is manifested in the optical transition of charge states close to the LUMO of the cells (see figure 6). The normalized capacitive transconductance curves are shown in figure 4 to magnify the differences between those in darkness and under photonic excitation. These curves were obtained by applying the concept of transconductance through the relation $g = 2\pi f C_p$, where $f$ is the AC frequency [66]. Then, $g$ curves are deconvoluted by means of Gaussian distributions.

The capacitive transconductance was measured for different cell number, i.e. 2500 to 30 000 in a 50 µl volume to avoid additional features due to cell confinement within the sample holder. Even though, the resonant times, $\tau = 1/f_{\text{maximum}}$ are between 13 and 23 µs and summarized in figure 5(b), the maximum capacitive transconductance signal was established for 15 000 cells. Statistical analyses of obliquity and kurtosis and correlation matrices under different experimental configurations are provided in the supplementary information (tables S1 and S2 and figure S2).

Figure 5(a) shows that the photonic effects lead to a decrease in the integrated intensity of the band and a shift in the peak toward a lower resonance frequency. Since excitation frequency and ionic mass keeps an inverse relation, $f^2 \propto 1/m$ [67], the changes at lower or higher frequencies can be attributed to heavier or lighter ions, respectively. Similarly, the interaction of light with cells induces an ion release rate at trap states even without a polarizing field $E$ [68], which subsequently are driven by the local electric field. The meaning of ion release due to interaction with light is in a similar manner as that stated for charge injection. Since light-cell interaction induces or gates free and polarized charges (ions), they are released into the solution [69, 70].
Figure 7. Normalized dielectric permittivity of MCF-7, BT-474 and lymphocytes (a) in darkness and (b) under illumination.

The integrated intensity of the dark band at 3491 Hz decreased under lighting conditions, while those of the dark bands at 3780, 4531 and 4914 Hz increased. Given the frequency values, these dynamics can be attributed mainly to light ions and associated primarily with the contribution of intracellular charges [71]. At high frequencies, the impedance of the cell membrane behaves like a short circuit, allowing ion exchange between the extracellular and intracellular media. At low frequencies, this impedance obstructs the current flow and, accordingly, ion transport through the cell membrane [72].

Figure 9 shows the Nyquist plots obtained in darkness and under photonic excitation. The curves exhibit high curvature radii, and they are larger than those of the real and imaginary components of the impedance. Therefore, a CPE within the circuit was introduced. Since all analysed samples were complex in nature (i.e. living cells, anomalous transport, and memory effects), a fractional calculus model to describe the CPE was introduced. Interestingly, the CPE involves a fractional parameter, $\alpha$, ranging between 0.45 and 0.74 (table 2) and is related to the AC and photonic frequencies ($\omega$ and $\omega'$, respectively).

The decreases in $R_s$ and $R_m$ with illumination shown in table 3 can be attributed to an increase in the extracellular ionic charge arising from the photogenerated charge of the intracellular region. In particular, the decrease in $R_m$ is due to the formation of an electric field in the membrane due to the lighting effect and photogenerated charge flow. In addition, the $C_{DL}$ value increased upon illumination. These changes may be attributed to a narrowing of the bilayer in the electrodes or an increase in the ionic charge. In the second scenario, the phenomenon is associated with the photogenerated charge.
in the intracellular zone, since UV–vis absorption spectra indicate electronic transitions for a wavelength of 532 nm. In addition, given that the intracellular potential is negative, the photogenerated charge is driven by the electric field between the intracellular and extracellular part, which forces ions to flow to the outside of the cell, leading to an increase in $C_{\text{DL}}$. This effect was particularly apparent in the BT-474 cells and lymphocytes; there was photogenerated load in the MCF-7 cells, but it was of opposite polarity compared with the other two cell lines.

The results show that $\alpha$ is between approximately 0.48 and 0.74 in dark conditions, which is indicative of the fractional behaviour of the cells’ impedance. Interestingly, the cell behaviour under lighting conditions is also in the fractional range with $\alpha$ between 0.43 and 0.48. This finding indicates that the behaviour of the system cannot be described based on purely resistive and capacitive combinations, so it is important to incorporate the generalized CPE element.

Taking into account the area of each cell line for MCF-7, BT-474 and lymphocytes (707, 314, 176) and the
regressed parameters $C_m$ and $R_m$ in table 2, an estimate of the resistivity “thickness” $= R_m$ “area” (ranges between 7 and 627 kΩ µm$^2$), and permittivity “thickness” $= C_m/\text{area}$ (2.13 and 9.42 mF m$^{-2}$), respectively; the conductivity of the culture media (DMEM) obtained with equation (9.42). As the photonic source wavelength was set to 532 nm ($\pi$), with the frequency of the external AC electric field: $\omega$, this technique. Results showed that impedance varies inversely between the HOMO, LUMO and intermediate states of the cells is low. The global phenomenon can be considered as an assisted photonic process. In addition, the external AC electric field addresses the charge carriers either under darkness or illumination conditions. Therefore, this ALIS enables a more comprehensive cell characterization strategy, particularly for cancer cells, as it delivers unique, cell-type-specific information.

Experiments were conducted with two breast cancer cell lines, BT-474 and MCF-7 and human lymphocytes from a healthy person as control cells to demonstrate an application of this technique. Results showed that impedance varies inversely with the frequency of the external AC electric field: $f = \omega/2\pi$. As the photonic source wavelength was set to 532 nm ($\lambda = 2\pi c/\omega'$), which is close to the maximum visible absorption of cells, evidence of the effects of the AC and photonic fields on the impedance was observed. An intrinsic property of each cell type is the electrical conductivity, $\sigma(\omega, \omega')$, which is related to the frequencies of the external AC source and photonic excitation, $\omega$ and $\omega'$, respectively. The relationship between the measured $Z(\omega, \omega')$ values and $\sigma(\omega, \omega')$ was established. Since all cells have similar geometrical configuration and size, $Z(\omega, \omega')$ was quite different for all samples. Therefore, it was necessary to look for deeper evidence based on the membrane dynamics and based on lipid-to-carbohydrate levels either in darkness or under photonic excitation. Therefore, $Z(\omega, \omega')$ and $\sigma(\omega, \omega')$ both reflect intercellular and intracellular features. Thus, the total $C_p$ and $R_p$ are important measurements to refine the electric model. Thus, the microscopic electric picture accomplishes interfaces, ion dynamics, photonic effects, membrane features and intracellular and extracellular dynamics. Further data analysis was done to deconvolute the capacitive transconductance measurements, revealing the presence of three unique bands in each cell type, peaking at 2380, 3491, 3780 Hz for BT-474 cells, 2190, 3494 and 4531 Hz for MCF-7 and 2490, 3470 and 4914 Hz for lymphocytes. Interestingly, under photonic excitation, the dark reference bands exhibit a blue shift toward higher frequencies.

In addition, we introduced a generalized CPE descriptor based on the fractional calculus and on experimental findings: the high curvature of the Nyquist plots, the nature of the sample (i.e. living cells, anomalous transport and memory effects), the effects of the external electric AC field and the influence of the artificial photonic biomarker. Considering these four aspects, we proposed a CPE of a fractional nature, which takes into account a fractional parameter, $\alpha$, between 0.43 and 0.74, where $\alpha$ values of $-1$ or $+1$ indicate an inductive or capacitive system, respectively. However, for a system represented by a combination of resistors, capacitors and inductors, the description of the CPE is more complex, and $\alpha$ is dependent on the cell type and measurement conditions (i.e. darkness versus illumination).

In order to take into account the medium contribution on the impedance measurements, we defined a normalized differential dielectric permittivity. The description helps distinguish the measurement condition for all cells i.e. darkness or illumination.

## Acknowledgments

The Universidad del Rosario and COLCIENCIAS Contract: FP44842-07-2018 Code: 122277657905, supported this work. Authors acknowledge Hernan Bernal support from Clinical Engineering Laboratory at Universidad del Rosario. A R and P B developed the experimental part of the degree work of the Biomedical Engineering program at the Universidad del Rosario (UR) and the Colombian School of Engineering Julio Garavito (ECIJG).

## Ethical statement

The entire study, including the methodology for obtaining the biological material and its manipulation was approved by the ethics committee of the University of Rosario (CEI-UR) in act No. DVG0005 682-CV866. All methods were performed in accordance with the relevant guidelines and regulations and a healthy volunteer provided written informed consent for participation in this study.

## Author contributions

F M and S R carried out the design and approach of the investigation. A R and P B developed the experimental part under the supervision of S R. The data was taken by A R, P B and F M. Analysis and result discussion were performed by F M and B P. The manuscript was written by F M, B P and S R.

## Conflict of interest

The authors declare no competing interests.

## ORCID iDs

F Mesa https://orcid.org/0000-0003-0920-7748
B A Paez-Sierra https://orcid.org/0000-0001-8706-9913
References

[1] Geddes L A and Baker L E 1967 The specific resistance of biological material—A compendium of data for the biomedical engineer and physiologist Med. Biol. Eng. 5 271–93

[2] Eisenberg R 2018 Electrical structure of biological cells and tissues: impedance spectroscopy, stereology, and singular perturbation theory

[3] Bordi F, Cametti C, Rosi A and Calcabrini A 1993 Frequency domain electrical conductivity measurements of the passive electrical properties of human lymphocytes BBA - Biomembranes 1153 77–88

[4] Ehret Ret al 1997 Monitoring of cellular behaviour by impedance measurements on interdigitated electrode structures Biosens. Bioelectron. Vol. 12 29–41

[5] Dubyak G R 2004 Ion homeostasis, channels, and transporters: an update on cellular mechanisms Am. J. Physiol. Adv. Physiol. Educ. 28 143–54

[6] Nakajima K I et al 2015 KCNJ15/Kir4.2 couples with polyamines to sense weak extracellular electric fields in galvanotaxis Nat. Commun. 6 1–10

[7] Richard E and Miller C 1990 Steady-state coupling of ion-channel conformation to a transmembrane ion gradient Science 242 1208–10

[8] Barsoukov E, Kim J H, Hwang K S, Kim D H, Yoon C O and Lee H 2001 Parametric analysis of electrical storage materials: new concept and application Synth. Met. 117 53–59

[9] Wang P, Xu G, Qin L, Xu Y, Li Y and Li R 2005 Cell-based biosensors and its application in biomedicine Sensors Actuators B 108 576–84

[10] Rocha P R F et al 2016 Extracellular electrical recording of pH-triggered bursts in C6 glioma cell populations Sci. Adv. 2 1–9

[11] Heileman K, Daoud J and Tabrizian M 2013 Dielectric spectroscopy as a viable biosensing tool for cell and tissue characterization and analysis Biosens. Bioelectron. 49 348–59

[12] Thein M, Asphahani F, Cheng A, Buckmaster R, Zhang M and Xu J 2010 Response characteristics of single-cell impedance sensors employed with surface-modified microelectrodes Biosens. Bioelectron. 25 1963–9

[13] Rocha P R F, Schlent P, Schneider L, Dröge M, Mailänder V, Gomes H L, Blom P W M and De Leeuw D M 2015 Low frequency electric current noise in glioma cell populations J. Mater. Chem. B 3 5035–9

[14] Medeiros M C R et al 2016 An electrical method to measure low-frequency collective and synchronized cell activity using extracellular electrodes Sens. Bio-Sens. Res. 10 1–8

[15] Malich A, Böhm T, Facius M, Kleintech I, Fleck M, Sauner D, Anderson R and Kaiser W A 2003 Electrical impedance scanning as a new imaging modality in breast cancer detection—a short review of clinical value on breast application, limitations and perspectives Nucl. Instrum. Methods Phys. Res. A 497 75–81

[16] Mansoorifar A, Koklu A, Ma S, Raj G V and Besok A 2018 Electrical impedance measurements of biological cells in response to external stimuli Anal. Chem. 90 4320–7

[17] Kang G, Kim Y, Moon H, Lee J-W, Yoo T-K, Park K and Lee J-H 2013 Discrimination between the human prostate normal cell and cancer cell by using a novel electrical impedance spectroscopy controlling the cross-sectional area of a microfluidic channel Biomicrofluidics 7 044126

[18] Halter R J, Schned A, Heaney J, Hartung A, Schutz S and Paulsen K D 2008 Electrical impedance spectroscopy of benign and malignant prostatic tissues J. Urol. 179 1580–6

[19] Dean D A, Ramanathan T, Machado D and Sundararajan R 2008 Electrical impedance spectroscopy study of biological tissues J. Electrost. 66 165–77

[20] Zou Y and Guo Z 2003 A review of electrical impedance techniques for breast cancer detection Med. Eng. Phys. 25 79–90

[21] Hong J L, Lan K C and Jang L S 2012 Electrical characteristics analysis of various cancer cells using a microfluidic device based on single-cell impedance measurement Sensors Actuators B 173 927–34

[22] Zhang F, Jin T, Hu Q and Pe P 2018 Distinguishing skin cancer cells and normal cells using electrical impedance spectroscopy J. Electroanal. Chem. 823 531–6

[23] Han A, Yang L and Frazier A B 2007 Quantification of the heterogeneity in breast cancer cell lines using whole-cell impedance spectroscopy Clin. Cancer Res. 13 139–43

[24] Le F-N, Gutierrez-Iglesias G, Martinez-Cuaclit A, Mata-Miranda M M, Alvarez-Jiménez V D, Sánchez-Monroy V, Golberg A and González-Díaz C A 2019 A biosensor capable of identifying low quantities of breast cancer cells by electrical impedance spectroscopy Sci. Rep. 9 6419

[25] Crowell L L, Yakisch J S, Außerheide B and Adams T N G 2020 Electrical impedance spectroscopy for monitoring chemoresponse of cancer cells Micromachines 11 832

[26] Kerner T E, Paulsen K D, Hartov A, Soho S K and Poplack S P 2002 Electrical impedance spectroscopy of the breast: clinical imaging results in 26 subjects IEEE Trans. Med. Imaging 21 638–45

[27] Kim B S, Isaacsen D, Xia H, Kao T-J, Newell J C and Saulnier G J 2007 A method for analyzing electrical impedance spectroscopy data from breast cancer patients Physiol. Meas. 28 S237–46

[28] Vohra P, Ngo H T, Lee W T and Vo-Dinh T 2017 Squamous cell carcinoma DNA detection using ultrabright SERS nanorattles and magnetic beads for head and neck cancer molecular diagnostics Anal. Methods 9 5550–6

[29] Vohra P, Strobbia P, Ngo H T, Lee W T and Vo-Dinh T 2018 Rapid nanophotonics assay for head and neck cancer diagnosis Sci. Rep. 8 11410

[30] Donnelly T, Smith W E, Faulks K and Graham D 2014 Silver and magnetic nanoparticles for sensitive DNA detection by SERS Chem. Commun. 50 12907–10

[31] Reza K K, Dey S, Wuethrich A, Sina A A A, Korbie D, Wang Y and Trau M 2018 Parallel profiling of cancer cells and proteins using a graphene oxide functionalized ac-EHD SERS immunosassay Nanoscale 10 18482–91

[32] Mohammadpour Z and Majdzadeh A K 2020 Applications of two-dimensional nanomaterials in breast cancer theranostics Actuators 10 9

[33] Asphahani F, Thein M, Veiseh O, Edmondson D, Kosai R, Veiseh M, Xu J and Zhang M 2008 Influence of cell adhesion and spreading on impedance characteristics of cell-based sensors Biosens. Bioelectron. 23 1307–13

[34] Lo C-M, Lo J-C, Sató P Y, Yeung T-L, Mok S C and Yip K-P 2016 Monitoring of ovarian cancer cell invasion in real time with frequency-dependent impedance measurement Am. J. Physiol. Cell Physiol. 311 C1040

[35] El Hasni A, Schmitz C, Bui-Göbbels K, Bräunig P, Jahnen-Dechent W and Schnakenberg U 2017 Electrical impedance spectroscopy of single cells in hydrodynamic traps Sensors Actuators B 248 419–29

[36] Gomes H L, Leite R B, Afonso R, Stallinga P and Mata-Miranda M M 2016 Monitoring of ovarian cancer cell invasion in real time with frequency-dependent impedance measurement Am. J. Physiol. Cell Physiol. 311 C1040

[37] El Hasni A, Schmitz C, Bui-Göbbels K, Bräunig P, Jahnen-Dechent W and Schnakenberg U 2017 Electrical impedance spectroscopy of single cells in hydrodynamic traps Sensors Actuators B 248 419–29

[38] Gomes H L, Leite R B, Afonso R, Stallinga P and Mata-Miranda M M 2016 Monitoring of ovarian cancer cell invasion in real time with frequency-dependent impedance measurement Am. J. Physiol. Cell Physiol. 311 C1040

[39] El Hasni A, Schmitz C, Bui-Göbbels K, Bräunig P, Jahnen-Dechent W and Schnakenberg U 2017 Electrical impedance spectroscopy of single cells in hydrodynamic traps Sensors Actuators B 248 419–29
cells in vitro by use of polarized light spectroscopy Appl. Opt. 43 1296

[55] Chaturvedi D, Balaji S, Bn V, Ariese F, Umamaheswaran S and Rangarajan A 2016 Different phases of breast cancer cells: Raman study of immortalized, transformed, and invasive cells Biosens. Nano 6 57

[56] Yue J, Shen Y, Liang L, Cong L, Guan X, Lu S, Liang C. Shi W and Xu W 2020 In situ and ex situ surface-enhanced Raman spectroscopy (SERS) analysis of cell mitochondria J. Raman Spectrosc. 51 602–10

[57] Hobro A J, Kumagai Y, Akira S and Smith N I 2016 Raman spectroscopy as a tool for label-free lymphocyte cell line discrimination Analyst 141 3756–64

[58] Razavi B 2014 Fundamentals of Microelectronics (Hoboken, NJ: Wiley)

[59] Romano S D and Sorichetti P A 2011 Dielectric Spectroscopy in Biodiesel Production and Characterization (London: Springer)

[60] Michaeli K, Beratan D N, Waldeck D H and Naaman R 2019 Voltage-induced long-range coherent electron transfer through organic molecules Proc. Natl Acad. Sci. USA 116 5931–6

[61] Anonymous 2020 Cancer survivor - the journey (https://thyroidcancersurvivor.wordpress.com/2010/10/13/what-is-cancer/)

[62] Suvitha A, Periandey S, Boonmade S and Govindarajan M 2014 Vibrational frequency analysis, FT-IR, FT-Raman, ab initio, and DFT studies, NBO, HOMO-LUMO and electronic structure calculations on pycolinaldehyde oxime Spectrochim. Acta A 117 216–24

[63] Choudhary N, Bee S, Gupta A and Tandon P 2013 Comparative vibrational spectroscopic studies, HOMO–LUMO and NBO analysis of N-(phenyl)-2,2-dichloroacetamide, N-(2-chlorophenyl)-2,2-dichloroacetamide and N-(4-chlorophenyl)-2,2-dichloroacetamide based on density functional theory Comput. Theor. Chem. 1016 8–21

[64] Xu Y, Xie X, Duan Y, Wang L, Cheng Z and Cheng J 2016 A review of impedance measurements of whole cells Biosens. Bioelectron. 77 824–36

[65] Sun T, Green N G and Morgan H 2008 Analytical and numerical modeling methods for impedance analysis of single cells on-chip Nano 3 55–63

[66] Grodd D and Ionescu A M 2011 The vibrating body transistor IEEE Sens. J. 17 86–21

[67] Hu H and Liu F 2014 Density-functional-theory formulation of classical and quantum Hooke’s law Sci. China Technol. Sci. 57 692–8

[68] Obraztsov P A, Kaplas T, Garnov S V, Kuwata-Gonokami M, Wang L, Cheng Z and Cheng J 2016 A review of impedance measurements of whole cells Biosens. Bioelectron. 77 824–36

[69] Ma Y, Poole K, Goyette J and Gaus K 2017 Introducing membrane charge and membrane potential to T cell signaling Front. Immunol. 8 1513

[70] Isom L, Patino G, Lopez-Santiago L and Yuan Y 2013 Voltage-gated ion channels Neuroscience in the 21st Century, ed D W Pfaff (New York: Springer) pp 29–58

[71] Preedy V R 2012 Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease (New York: Springer)

[72] Ibrahim B, Hall D A and Jafari R 2017 Bio-impedance spectroscopy (BIS) measurement system for wearable devices. 2017 IEEE Biomedical Circuits and Systems Conf. (BioCAS) 2017 IEEE Biomedical Circuits and Systems Conf. (BioCAS) (Torino: IEEE) pp 1–4

[73] Chen M-T, Jiang C, Vernier P T, Wu Y-H and Gunderson M A 2009 Two-dimensional nanoscale electric field mapping based on cell electropheromeabiliation PMC Biophys. 2 9

[38] Zemmyo D and Miyata S 2019 Evaluation of lipid accumulation using electrical impedance measurement under three-dimensional culture condition Micromachines 10 455

[39] Elamine Y, Pmc I, Lyousy B, Anjos O, Estevesinh V L M, da Graça Miguel M and Gomes H L 2019 Insight into the sensing mechanism of an impedance based electronic tongue for honey botanic origin discrimination Sensors Actuators B 285 24–33

[40] Inacio P M C et al 2017 Bioelectrical signal detection using conducting polymer electrodes and the displacement current method IEEE Sens. J. 17 3961–6

[41] Asgarifar S, Pmc I, Mestre A L G and Gomes H L 2018 Ultrasensitive bioelectronic devices based on conducting polymers for electrophysiology studies Chem. Pap. 72 1597–603

[42] Nidzwoński D et al 2017 A rapid-response ultra-sensitive biosensor for influenza virus detection using antibody modified boron-doped diamond Sci. Rep. 7 15707

[43] Pennington M R and Van de Walle G R 2017 Electric cell-substrate impedance sensing to monitor viral growth and study cellular responses to infection with alphaherpesviruses in real time ed GA Smith mSphere 2 e00039-17

[44] Siuzdak K, Niedzielański P, Sobaszek M, Lega T, Sawczak M, Czacyk E, Dzibajowska K, Ossowski T, Nidzwoński D and Bogdanowicz R 2019 Biomolecular influenza virus detection based on the electrochemical impedance spectroscopy using the nanocrystalline boron-doped diamond electrodes with covalently bound antibodies Sensors Actuators B 280 263–71

[45] Harrison M G, Grüner J and Spencer G C W 1996 Investigations of organic electroluminescent devices by impedance spectroscopy, photo-impedance spectroscopy and modulated photovoltage spectroscopy Synth. Met. 76 71–75

[46] Lashkaryov V and Nikolenko A S 2013 Laser heating effect on Raman spectra of Si nanocrystals embedded into SiOx matrix Semicond. Phys. Quantum Electron. Optoelectron. 16 86–90

[47] Poenar D P, Iliescu C, Boulare J and Yu H 2014 Label-free virus identification and characterization using electrochemical impedance spectroscopy Electrophoresis 35 433–40

[48] Conde J, Rosa J, Lima J C and Baptista P V 2011 Nanophotonics for molecular diagnostics and therapy applications Int. J. Photoenergy 2012 619530

[49] Naholtz J-M 2002 Breast Cancer Management ed Wiseman L R vol 624 (Philadelphia, PA: Lippincott Williams & Wilkins) 624

[50] Medeiros M C R, Mestre A L G, Inácio PMC, Santos J M L, Araujo I M, Bragança J, Biscarini F and Gomes H L 2016 Performance assessment of polymer based electrodes for in vitro electrophysiological sensing: the role the electrode impedance Proc. SPIE 9944 994404

[51] White E A, Orazem M E and Bunge A L 2011 A critical analysis of single-frequency LCR databridge impedance measurements of human skin Toxicol. In Vitro 25 774–84

[52] Matliff M A and Jamali Y 2019 The concepts and applications of fractional order differential calculus in modeling of viscoelastic systems: a primer Crit. Rev. Biomed. Eng. 47 249–76

[53] Salakij C, Salakij J, Narkong N-A, Pitakkgingthong D and Poothong S 2003 Hematology, morphology, cytochemistry and ultrastructure of blood cells in painted stork (Mycteria leucocephala) Nat. Sci. 37 506–513

[54] Bartlett M, Huang G, Larcom L and Jiang H 2004 Measurement of particle size distribution in mammalian