A Practical Approach to Compare Time Domain and Frequency Domain Bioimpedance Measurements

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Abstract

Bioimpedance spectroscopy is widely used in clinical and biotechnological applications for electrical characterization of biomaterials in a non-invasive fashion. States of the living organs dynamically change during impedance measurement. Therefore, fast acquisition of the impedance spectrum is desired. Unlike a conventional frequency-domain method, the time-domain method can facilitate fast spectroscopy.

In order to investigate the feasibility of using time-domain as a faster alternative to the frequency-domain, the result of both measurement techniques shall be compared. Hence, a measurement device is instrumented with the capability of generating signals using both techniques.

An impedance measurement system, including front-end analog circuit based on the three-electrode system, data acquisition, and control unit has been developed. The system excites a dummy cell by generating a customized binary wideband signal and measures the response signal. The process is repeated once again using the frequency-domain method. Finally, experimental results are compared with simulation and theoretical results.

Comparison between the experimental and simulation results reveals a deviation of less than 10 percent for both measurement techniques. The time-domain method can be used as a faster alternative to the frequency-domain method within acceptable accuracy.
Acknowledgements

I would first like to thank my thesis advisor, Ørjan Grøttem Martinsen of the Department of Physics at University of Oslo. The door to Prof. Martinsen office was always open whenever I ran into a trouble spot or had a question about my research or writing. He consistently allowed this thesis to be my own work but steered me in the right direction whenever he thought I needed it.

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Finally, I must express my very profound gratitude to my parents and my wife, Azar Sanaei, for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Author

Pouya Afsharian
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## Abbreviation

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<tr>
<td>AC</td>
<td>Alternative Current</td>
</tr>
<tr>
<td>ADC</td>
<td>Analog-to-Digital Converter</td>
</tr>
<tr>
<td>AWG</td>
<td>Arbitrary Waveform signal Generator</td>
</tr>
<tr>
<td>BMS</td>
<td>Binary Multi-Frequency Signal</td>
</tr>
<tr>
<td>CC</td>
<td>Current-Carrying Electrode</td>
</tr>
<tr>
<td>CF</td>
<td>Crest Factor</td>
</tr>
<tr>
<td>DAC</td>
<td>Digital-to-Analog Converter</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data Acquisition</td>
</tr>
<tr>
<td>dB</td>
<td>Decibel</td>
</tr>
<tr>
<td>DC</td>
<td>Direct Current</td>
</tr>
<tr>
<td>DDS</td>
<td>Direct Digital Synthesizer</td>
</tr>
<tr>
<td>DFT</td>
<td>Discrete Fourier Transform</td>
</tr>
<tr>
<td>DIBS</td>
<td>Discrete Interval Binary Sequences</td>
</tr>
<tr>
<td>ENOB</td>
<td>Effective Number Of Bits</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>GA</td>
<td>Genetic Algorithms</td>
</tr>
<tr>
<td>IDFT</td>
<td>Inverse Discrete Fourier Transform</td>
</tr>
<tr>
<td>IEC</td>
<td>International Electrotechnical Commission</td>
</tr>
<tr>
<td>kHZ</td>
<td>Kilo Hertz</td>
</tr>
<tr>
<td>M</td>
<td>Measuring Electrode</td>
</tr>
<tr>
<td>MLSB</td>
<td>Maximum Length Binary Sequence</td>
</tr>
<tr>
<td>Op-amp</td>
<td>Operational Amplifier</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed Circuit Boards</td>
</tr>
<tr>
<td>PM</td>
<td>Phase Modulated</td>
</tr>
<tr>
<td>PRBS</td>
<td>Pseudo-Random Binary Sequence</td>
</tr>
<tr>
<td>R</td>
<td>Reference Electrode</td>
</tr>
<tr>
<td>RISO</td>
<td>Isolation Resistor</td>
</tr>
<tr>
<td>RMS</td>
<td>Root Mean Square</td>
</tr>
<tr>
<td>SCPI</td>
<td>Standard Command For Programmable Instrumentation</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>SMA</td>
<td>SubMiniature version A connectors</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-To-Noise Ratio</td>
</tr>
<tr>
<td>UBC</td>
<td>Unipolar-To-Bipolar Converter</td>
</tr>
<tr>
<td>VCCS</td>
<td>Voltage-Controlled Current Source</td>
</tr>
<tr>
<td>VCO</td>
<td>Voltage-Controlled Oscillator</td>
</tr>
<tr>
<td>XOR</td>
<td>Exclusive OR</td>
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Study of passive electrical properties of the tissue is generally carried out by measuring and analysis of electrical impedance of the biomaterials (Grimnes and Martinsen 2008a). The frequency response of biological tissue is highly influenced by their physiological and physiochemical status, such as disease, swelling, and infection. Complex impedance also varies with the changes in the health status of the biological cells. For instance, blood is a good conductor of electricity. Since the cancerous tissue is containing more blood, then the cells show a less impedance path for electrical current. Analyzing electrical property of the tissue by bioimpedance technique is found to be an efficient tool for noninvasive investigation of physiological and pathological states (Bera 2014).

Due to dynamic changes in states of living organs, the impedance of biomaterial is time-variant. Conventionally, the electrical bioimpedance is considered time-invariant, and therefore dynamic changes are essentially ignored and treated as noise or interference (Abtahi et al. 2014). To achieve a consistent result, the electrical properties of the biomaterial shall be relatively stable during performing Bioimpedance measurement. Therefore, quick scanning of frequencies that introduce less disturbance to bioimpedance measurement and follow the temporal behavior of the biomaterial is desired.

A conventional method to obtain complex frequency response within a range of arbitrary frequencies is called frequency-sweep or frequency-domain method. Frequency of the applied sine waveform changes over time by this method. For many years, this approach has been adapted to in-vivo and ex-vivo tissue experiences. The main drawback of the frequency-sweep technique is the total measuring time to acquire the complete impedance spectrum. Moreover, the accuracy of measurement by this method depends on the number of measurements at each frequency that can potentially extend the measuring time. In 1994, Steendijk (Steendijk et al. 1994) scanned 8 frequencies in 30 seconds while Warren in 2000 (Warren et al. 2000) managed to acquire a complete spectrum of 20 frequencies in 20 seconds.

On the other hand, the time-domain measurement technique can facilitate
the fast acquisition of the impedance spectrum. Instead of applying single frequencies at each time, a complex wideband signal can be constructed that comprise a range of arbitrary frequencies. Most recent electrical bioimpedance approaches are based on simultaneous multi-frequency measurement. Multi sine, chirp, and binary excitation signals are utilized for applications such as impedance tomography, myocardium, and lung tissue characteristics. Thanks to the time-domain approach, the total measuring time to acquire a complete bioimpedance spectrum is drastically reduced (Sanchez et al. 2013). Besides, generating binary wideband excitation signals are cost-effective too. Unlike the analog multi-frequency signals, binary wideband signals do not require digital-to-analog circuits.

The Bioimpedance measurement is performed by connecting the electrodes to bio material. The simplest method is to use two electrode arrangement. In two-electrode system, estimating the possible contribution of neutral electrode is difficult. Moreover, in some situations, there are difficulties to work with large area of neutral electrode. Controlling the measured tissue zone becomes easier by adding the third electrode (Grimnes and Martinsen 2008c).

This thesis aims to instrument an efficient and cost-effective portable device which measures bioimpedance in a fast acquisition fashion within acceptable accuracy. The goal is to use the device as a tool to compare time-domain and frequency-domain bioimpedance measurements.

The objective of the current thesis is to instrument a prototype for rapid bioimpedance measurement. The device adopted a three-electrode system as a front-end circuit. A signal generator composes a binary time-domain signal with concentrated power at arbitrary frequencies within the range of 1 Hz to 500 kHz. This device shall be able to communicate with a remote computer for analysis and plotting complete impedance spectra. The designed system should be able to utilize both frequency-sweep and wideband excitation signals for impedance measurement and shall be tested on circuits with lumped elements. Eventually, the quality of the two measurement techniques should be compared.

This thesis is structured in six chapters. Chapter 2 is an introduction to main concepts, definitions, a background of bioimpedance spectroscopy, and literature review of binary excitation signals. Chapter 3 provides methods and tools to design, verify, and validate the prototype system. Results are presented in Chapter 4 and discussed in detail. Chapter 5 summarizes the complete work and address achievements. Chapter 6 suggests further works.
Chapter 2

Theoretical Framework

Electrical impedance spectroscopy is used to characterize biomaterial. Variety of the applications using this technology. Biological materials are time-varying systems. The conventional method of exciting the biomaterial is based on frequency sweep, which is called frequency-domain measurement. In this method, single sinusoid signals with specific frequencies within a desirable frequency range stimulate the system one at the time, and the corresponding responses are measured. Then an averaging over the frequency responses provides the complete measurement result. The time-averaging method removes the effects that are induced by the electrical and mechanical properties of the biomaterial during measurements. In absence of using a complex averaging technique, useful information may potentially be lost. The main advantage of using this method is its high SNR (Signal-to-Noise Ratio). Wideband impedance spectroscopy or time-domain measurement is an alternative to overcome this limitation and reducing measuring time drastically. In this technique, there is a trade-off between measuring time and accuracy. The spectrum accuracy is essentially lost in price of increasing impedance measuring time. The objective is to compose an excitation signal which minimizes measuring time and providing accurate impedance spectrum.

Biomaterials are extremely sensitive to the applied electrical field, and this means that amplitude of excitation signal shall be limited in order to guarantee a linear response of the system under test and avoid causing the death of cells if a living organ is investigated. Safety regulations, according to the norm IEC/UL 60601-1 shall be followed in such circumstances.

The first measurement of full impedance spectra is reported in the 1970s with publication(Creason and Smith 1972). The work was aimed to generate a mixed ac signal with several harmonics superimposed on a desired dc bias potential for an electrochemical system. The ac voltage and current were decomposed by Fast Fourier Transform (FFT) to calculate the impedance. Maximum Length Binary Sequence (MLSB) generator is suggested to characterize an RC circuit in (Ichise et al. 1974) and later in 1996, the same signal is utilized for bone fractures investigation by(Schneider 1996). Other signals have been
already described in (Ojarand 2012) and (Ojarand et al. 2013).

This chapter comprises three main sections. Section 2.1 briefly explains the terms and concepts of biomaterial and bioimpedance that are used throughout the thesis. Section 2.2 introduces the idea and a proper method to measure the impedance of a biomaterial. Conventional and modern methods and techniques to generate wideband binary signals are comprehensively reviewed in Section 2.3.

2.1 Basic Definitions

In this section, the general concepts related to dielectric material properties and their frequency response is introduced. Then the biological cell compartments are explained, and application of electrical bioimpedance in order to the characterization of biomaterial is described. Ultimately, a simplified electrical model of a single cell is represented. Later this model is used for simulation purpose, test, and evaluation of the final design.

2.1.1 Dielectric and Permittivity

The electrical bioimpedance theory originated in the dielectric spectroscopy field. In dielectric spectroscopy, dielectric property of a material is measured as a function of frequency. An external electrical stimulus causes momentum in dipole orientation of a biological sample that typically expressed as permittivity or conductivity. Permittivity is also described as a measure of capacitance when an electric field is formed in a particular medium. Moreover, cell membrane behaves as a dielectric while inner and outer cellular medium conduct ions through the membrane. This leads to an electrical phenomenon when electrical impedance spectroscopy field is applied.

Dielectric is a martial that can be polarized by applying an electrical field around it. When the electrical field is applied to the dielectric, charges do not flow through the martial as it happens typically in a conductor, but the field shifts the equilibrium position of the dipoles inside the dielectric. This shift causes a phenomenon that is so-called dielectric polarization.

The Eq.2.1.1 expresses a displacement vector for an isotropic and homogenous material.

\[ \vec{D} = \epsilon_0 \epsilon_r \vec{E} \]  

(2.1.1)

Where \( \vec{D} \) is the electrical displacement vector and \( \vec{E} \) is the electric field vector. \( \epsilon_r \) represents material’s relative permittivity and \( \epsilon_r \) is vacuum’s dielectric permittivity (Grimnes and Martinsen 2008b).
2.1.2 Dielectric Relaxation and Dispersion

Relaxation and dispersion of dielectric material are interchangeable properties that one defined in time-domain and another in frequency-domain. Assume that a system is disturbed by a step input function, then the system is allowed to be relaxed. The time that takes until the system reaches its equilibrium is called relaxation time. The delay in molecular polarization with respect to the applied electric field in a dielectric material is known as dielectric relaxation. Dielectric response of the material is delayed because the dipoles require time for rearrangement. Dielectric response to a sinusoidal excitation signal depends on its frequency as a step function can be synthesized by sinusoidal function according to Fourier analysis. Dispersion is described as a dependency of the permittivity of a dielectric material on the frequency of the excitation electric field and is correspondent frequency domain concept of relaxation (Grimnes and Martinsen 2008b).

2.1.3 Biological Material and Electrical Bioimpedance

In the circuit theory, impedance is defined as a ratio between voltage and current. It describes a capacity of a medium to prevent the flow of current, or flow of electric charge in an electrical circuit. In addition to resistance in DC circuits, the impedance in AC circuits comprises inductive and capacitive behavior of the circuit. Hence, electrical bioimpedance refers to opposition of biological material to the electrical current that passes through the material.

Biological materials consist of millions of biological cells. Each cell in a simple form is constructed by a plasma membrane, a nucleus and several organelles that are submerged in internal watery solution known as cytoplasm. The membrane prevents the free flow of molecules inside and outside the cell. The membrane controls the cell’s communication.

The cell membrane structure consists of two layers of phospholipid molecules that form a double layer that separates the cellular and non-cellular portion of tissue. The double-layer membrane is characterized by its strength, permeability, flexibility, and contains cholesterols and protein channels.

The cell membrane has a high capacitive response; then, it represents an electrical impedance that depends on frequency. A wideband excitation signal includes both low and high frequencies. Lower frequency current flows mostly through the non-cellular portion of tissue since the membrane’s impedance is too big. On the other hand, higher frequencies pass through the cellular and non-cellular liquid. Therefore, the impedance is bigger in lower frequencies. In reality, there are more factors involved. In tissue, there are a lot of cells exist that every single one contributes to the electrical impedance measurement.

According to the Schwan’s (Grimnes and Martinsen 2008a) electrical examination of a biomaterial, dispersion data is frequency-dependent. The relaxation mechanism associated with the electrical bioimpedance is divided into three
main groups of $\alpha$, $\beta$ and $\gamma$. Figure 2.2 shows how the permittivity and conductivity of the electrical bioimpedance alter concerning the frequency of the excitation signal.

$\alpha$ represents the intracellular structure influence, ionic dissemination, and dielectric losses of the material. $\beta$ is influenced by the interfacial polarization effect and includes a response from intracellular organelle membranes and protein molecules. $\gamma$ illustrates information regarding the dipolar relaxation, i.e., water molecules and proteins (Grimnes and Martinsen 2008a) (See Table 2.1).

2.1.4 Simplified Electrical Model of A Single Cell

A simplified electrical model of a cell in suspension clamped by two metal electrodes are shown in 2.3.
<table>
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<th>Type</th>
<th>Characteristic frequency</th>
<th>Mechanism</th>
</tr>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>mHz-kHz</td>
<td>Counterion effects (perpendicular or lateral) near the membrane surfaces, active cell membrane effects and gated channels, intracellular structures (e.g. sarcotubular system.), ionic diffusion, dielectric losses (at lower frequencies the lower the conductivity).</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.001-100 MHz</td>
<td>Maxwell–Wagner effects, passive cell membrane capacitance, intracellular organelle membranes, protein molecule response.</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>0.1-100 GHz</td>
<td>Dipolar mechanisms in polar media such as water, salts and proteins.</td>
</tr>
</tbody>
</table>

Table 2.1: Dielectric Dispersion. (Grimnes and Martinsen [2008a])

$C_s$ represents a parasitic or stray capacitance between front-end of measuring circuit and the electrodes. $R_{in}$ is the input resistance of the bioimpedance measurement circuit. The resistance and capacitance of the membrane are modeled as $R_m$ and $C_m$, respectively. The resistance of membrane is typically much greater than its reactance; then it is ignored in the model. Similarly, the reactance of cytoplasm is ignored due to much larger cytoplasm resistance.

In addition to the cell model, there are bulk resistance $R_{so}$ and capacitance $C_{so}$ that characterize the suspension between the electrodes.

Interfacial impedance $Z_e$ of the electrode system is an important element of the model where metal meets the electrolyte. When the contact between electrolyte and the metal surface has happened, atoms are ionized and produce a double layer on the surface of the electrode (Helmholtz-layer) that represents a capacitor $C_H$. By increasing the distance of ions to the metal, the electrostatic forces disappear, but there are adequate forces to keep a diffuse layer (Gouy-Chapman layer). The layer can be interpreted as a capacitor $C_G$ (Ojarand [2012]).

The simplified electrical model of the cell with attached electrodes can be illustrated as Figure 2.5. The model is used in simulations, implementation, test, and measurements as a dummy cell.
Figure 2.3: Simplified electrical model of a single cell in suspension between the electrodes (a), and its equivalent circuit (b) (Ojarand 2012)

Figure 2.4: Simplified electrical model of a electrode-electrolyte interface (Ojarand 2012)
Figure 2.5: The electrical model of the cell with attached electrodes. (Ojarand 2012)
2.2 Electrical Bioimpedance Spectroscopy

Electrical bioimpedance spectroscopy is one of the techniques to measure electrical bioimpedance and has been broadly used in physiological research, medical diagnostics, and imaging. There are some examples as body composition determination (Kyle et al. 2004), skin cancer detection (Aberg et al. 2004), and Electrical Impedance Tomography (EIT) (Brown 2003) among others. This technology has become popular in the past few decades so that it has been also utilized in other industries like food processing (Pliquett 2010), electrochemical (Chang and Park 2010) and biotechnological field.

Historically, bioimpedance spectroscopy has been performed by the frequency-sweep technique where the frequency of alternative current (AC) signals is changed in a range of desired frequencies and voltage signal is measured across the system.

2.2.1 Impedance Representation

Assume that an AC excitation current with a magnitude of \( I(\omega) \) and phase of \( \phi \) is applied to a linear system and potential of the system is measured with a magnitude of \( V(\omega) \) and phase of \( \theta \) (See Figure 2.6).

\[
\text{Figure 2.6: Schematic explanation of impedance.}
\]

The impedance of the system is expressed as a ratio of the measured potential over applied current, as follows:

\[
Z(\omega) = \frac{V(\omega)}{I(\omega)} = \frac{V \cos(\omega t + \theta)}{I \cos(\omega t + \phi)} \quad (2.2.1)
\]
And in complex form of

\[ Z(\omega) = |Z|e^{j\psi} = |Z|(\cos \psi + j \sin \psi) \]  \hspace{1cm} (2.2.2)

Where

\[ |Z| = \frac{V}{I} \]  \hspace{1cm} (2.2.3)

\[ \psi = \theta - \phi \]  \hspace{1cm} (2.2.4)

The magnitude and phase of the impedance \( Z(\omega) \) can be represented in a two-dimensional reference system with imaginary \( Z_{Im} \) and real \( Z_{Re} \) vectors. See Figure 2.7.

![Figure 2.7: Magnitude and phase of the impedance \( Z(\omega) \)](image)

\[ Z_{Re} = |Z| \cos(\psi) \]  \hspace{1cm} (2.2.5)

\[ Z_{Im} = |Z| \sin(\psi) \]  \hspace{1cm} (2.2.6)

And corresponding phase and magnitude is expressed by:

\[ \psi = \arctan \frac{Z_{Im}}{Z_{Re}} \]  \hspace{1cm} (2.2.7)

\[ |Z| = \sqrt{Z_{Re}^2 + Z_{Im}^2} \]  \hspace{1cm} (2.2.8)
The real part of impedance is the resistance $R$ and the imaginary part is the reactance $X$:

\[ Z_{Re} = R \]  \hspace{1cm} (2.2.9)

\[ Z_{Im} = X = X_C + X_L \]  \hspace{1cm} (2.2.10)

The reactance $X$ can be decomposed into two elements of capacitance and inductance:

\[ X_C = \frac{1}{jC\omega} \]  \hspace{1cm} (2.2.11)

\[ X_L = jL\omega \]  \hspace{1cm} (2.2.12)

### 2.2.2 Current Source Versus Voltage Source

Usually, in bioimpedance measurement, the current source is considered over voltage source. Biomaterials are sensitive to the electric field. Applying the voltage source causes non-linearity in current response when the current is high. Using a current source decrease this non-linearity. Besides, according to the impedance spectrum, the voltage can be anticipated.

Moreover, current density is well-controlled on the electrodes. Challenges with the real current source shall be considered as a higher noise level in active sources and performance degradation in higher frequency when stray capacitance causes current instability. In order to protect biosample and avoiding possible overvoltage at lower frequency, current spectral shape shall be designed following the impedance spectral shape. The current shall be increased with respect to the impedance as frequency increases (Ojarand et al. 2013). See Figure 2.8.

In practice, a voltage source is preferred. In case of using voltage as an excitation source, the current automatically increases by frequency as the impedance of the biomaterial decreases. Furthermore, the rise of impedance in lower frequency does not introduce excessive voltage (Ojarand et al. 2013).

### 2.2.3 Three-Electrode System

The bioimpedance measurement is performed by connecting the electrodes to the biomaterial. The simplest method is to use a two-electrode arrangement. In two-electrode system, estimating the possible contribution of the neutral electrode is difficult. Moreover, in some situations, there are difficulties in working
with a large area of neutral electrode. By adding the third electrode, controlling the measured tissue zone becomes easier (Grimnes and Martinsen 2008b).

Three-electrode system comprises two subsystems and three-terminal network. Figure 2.9 demonstrate the generalized schematic of the circuitry with the main elements. The first subsystem provides an excitation signal. The signal is applied to the non-inverting input of an operational amplifier (Op-amp). The potential between reference electrode (R) and current-carrying electrode (CC) is controlled by the Op-amp and a relative corresponding current flows through the material. Finally, measuring electrode (M) captured the current and the second subsystem measures its amount. Sensitivity field of the electrode system depends on the position of the reference electrode (Grimnes and Martinsen 2008b).

The topology of the system features external ground reference noise cancellation in circumstances when noise signals are capacitively connected to the biomaterial. The noise will be canceled through the current reading channel (Grimnes et al. 2009) (See Figure 2.10).
Figure 2.10: The three-electrode system with noise cancellation. (Grimnes et al. 2009)

Figure 2.11 shows the first compartment of the system is called the non-inverting operational amplifier.

![Non-inverting operational amplifier circuit](image)

Non-inverting operational amplifier benefits high input impedance of the Op-amp, then it can draw very little current from the excitation source. In principle, it acts as an isolation circuit that causes less disturbance of the excitation source when the impedance of the material under test is high.

A particular form of a non-inverting amplifier is so-called voltage follower. The voltage follower is a circuit that also known as unity-gain amplifier, buffer amplifier, or isolation amplifier. The output voltage directly follows the input voltage. Figure 2.12 shows a voltage follower circuit. In case of measuring the impedance of a two-terminal component by using the three-electrode system, the non-inverting operational amplifier circuit will be reconstructed as a voltage follower.
The second subsystem is a current meter that utilizes a transimpedance amplifier. Figure 2.13 shows a simple Transimpedance amplifier circuit.

Transimpedance amplifier converts current to voltage. The output voltage corresponds to the input current times the feedback resistor value if the amplifier is ideal. It means that the input impedance of the amplifier is assumed infinity, as a result total amount of the input current passes through the feedback resistor. This is not a case in the real electronic world.

Hence, the non-inverting operational amplifier circuit provides the excitation voltage signal with less disturbance to the load under test, and the Transimpedance amplifier picks up the current signal and converts it to the voltage signal. More detail about instrumenting the complete system and its challenges come further in chapter 3.

2.3 Wideband Impedance Spectroscopy

The time-domain signal composition and the technique to generate such a signal is the heart of our desired system for fast impedance spectroscopy. The motivation behind using a wideband time-domain signal is already explained, and various method of constructing wideband excitation signals and their pros and cons are discussed in brief. This section particularly reviews the literature regarding generating binary wideband excitation signals and looks close to their properties and privileges.
2.3.1 Binary Wideband Excitation Signals

There exist several types of wideband excitation signal that each is synthesized by a specific method. This thesis is concentrated on an application utilizing binary wideband signal to stimulate biomaterial. Therefore, further literature review covers only binary signals. The motivation behind using binary excitation signals can be considered as the following facts:

- Full-band Crest Factor of 1.
- Cheaper implementation compares to analog wideband signals. There is no need to shape the binary excitation signal using DAC.
- According to Fourier analysis, a pulse signal can be decomposed to a series of sine and cosine signal with a wide range of frequencies. Further, it is explained how a binary signal can be simply synthesis by several arbitrary frequencies.

Several metrics are proposed in the literature (Godfrey et al. 1999), but the most desired one is the Crest Factor (CF). Consider the waveform function of $x$. Crest factor is defined as the ratio of peak amplitude $L_\infty$ norm to RMS (Root Mean Square) that corresponds to $L_2$ norm of a waveform and is expressed as follows:

$$CF = \frac{||x||_\infty}{||x||_2} = \frac{|x|_{\text{peak}}}{|x|_{\text{rms}}} \quad (2.3.1)$$

Crest Factor can be inspected and interpreted visually. Below, Crest Factors of example, normalized waveforms are shown:

$$CF_{\text{SineWave}} = \sqrt{2} \quad (2.3.2)$$

$$CF_{N \text{ Superimposed Sine Wave}} = \sqrt{2N} \quad (2.3.3)$$

$$CF_{\text{Square Wave}} = 1 \quad (2.3.4)$$

$$CF_{PWM \text{ Signal With Period of } T} = \sqrt{\frac{T}{T_{ON}}} \quad (2.3.5)$$

A smaller Crest Factor shows how well desired magnitudes are distributed between maximum and minimum values of the signal; in other words, how compact is the signal.
Analog-digital converter (ADC) is used to capture the response voltage of impedance measurement. Considering the absence of distortion in a multi-harmonic signal, the ideal N-bit ADC SNR is reduced due to Crest Factor and Number of harmonics of the signal ($N_h$).

$$SNR = 6.02N + 1.76 - 20\log_{10}\left(\frac{CF}{\sqrt{2}}\right) - 10\log_{10}N_h$$ \hspace{1cm} (2.3.6)

Then Effective Number of Bits is given by:

$$ENOB = \frac{SNR - 1.76 + 20\log_{10}\left(\frac{CF}{\sqrt{2}}\right) + 10\log_{10}N_h}{6.02}$$ \hspace{1cm} (2.3.7)

That means the smaller the Crest Factor, less number of the bits are required in order to obtain the specified measurement. Excitation signals with Lower Crest Factor use the maximum dynamic range of ADCs, by this SNR improves.

Besides Crest Factor, another important metric is impedance spectrum accuracy that obtains if both current and voltage are measured in maximum SNR.

Selection of binary excitation signals are reviewed in the following section and strength and weakness of those signals are discussed. The studied signals are Maximum Length Binary Sequences (Ai Hui and Godfrey 2002) (Godfrey et al. 2005) (Ojarand and Min 2013) (Rees et al. 1992) (Sun et al. 2007), Discrete Interval Binary Sequences (Bos and Krol 1979).

### 2.3.2 General Purpose Signals: Pulse

The short pulse excitation signal is expressed in the form of:

$$u(t) = \begin{cases} A & 0 \leq t \leq T_1 \\ 0 & T_1 \leq t \leq T \end{cases} \hspace{1cm} (2.3.8)$$

With $T_1$ the pulse width and $T$ the measurement period generates an impulse response with an equivalent frequency resolution of $\frac{1}{T}$. The excitation is deterministic, and if response becomes negligible before measurement window ends, no leakage occurs. The shape of the power spectrum can be easily modified by the pulse shape and minimum Crest Factor of $\sqrt{\frac{T}{T_1}}$ is yield. Amplitude spectra of the pulse and MLBS signal are the same. Although more sophisticated impulse techniques are generated by Halvorsen and Brown (1977), the general characteristics remain the same (Pintelon and Schoukens 2012).
2.3.3 General Purpose Signals: Maximum Length Binary Sequence (MLBS)

MLSB is a pseudo-random binary sequence (PRBS) and provides a uniform spectral density similar to the white noise over a wide range of the frequencies. MLSB is a repeatable and deterministic signal that delivers more energy than pulse signal over the measurement period.

By using digital shift registers with feedback coefficient control, MLBS generate a series of zeroes and ones recursively. In practice, MLBS works based on D flip-flops and XOR as feedback (See 2.14). It conforms to the linear recurrence:

\[ a_n = \left( \sum_{i=1}^{n} c_i a_i \right) \mod 2 \]  
(2.3.9)

The output signal is generated out of \( a_n \) register and recently generated signal imported to the \( a_1 \) register. Concurrently, every element in its register shifts to the right. XOR performs \( \mod 2 \) operation. The feedback coefficient of \( c_i \) are expressed as the following function:

\[ f(x) = 1 + \sum_{i=1}^{n} c_i x^i \]  
(2.3.10)

The n-stage registers generate a periodic sequence with a length of \( L = 2^n - 1 \) in one period. Comparing to the AC sweep technique, hardware cost is significantly less since no signal generator and lock-in amplifier are required.

Figure 2.14: Maximum Length Binary Sequence (MLBS). The n-stage registers generate a periodic sequence with length of \( L = 2^n - 1 \) in one period. (Sun et al. 2007)
The MLBS gives minimized hardware and simplified system architecture. The binary signal provides high-frequency resolution in a short period. The spectral power spectrum of MLBS is relatively flat; however, the power magnitude components decrease inversely proportional to the frequency. Since the power is distributed over the whole range of frequencies, then SNR is lower in comparison to the single or frequency sweep technique. The MLBS signals are binary, then they have an optimum Crest Factor for full-band and are robust to noisy environments, but are sensitive to cross-talk. The crest factor of MLBS signals vary with respect to their spectral band in use; the Crest factor of one is expected at infinity spectral band. It means part of the signal power is wasted in unwanted measuring frequency range (Pintelon and Schoukens 2012) (Sun et al. 2007).

![Figure 2.15: Maximum Length Binary Sequence (MLBS). Time-domain signal and its power spectral density (PSD).](Pintelon and Schoukens 2012)

2.3.4 General Purpose Signals: Nonlinearly Modulated Chirp Excitation

Chirp excitation signal or linear chirp is a sine sweep wave within range of frequencies that can be implemented by Direct Digital Synthesizer (DDS) or Arbitrary Waveform Generator (AWG). Time-domain chirp function is given by:

\[
V_{Ch}(t) = \sin \left( 2\pi \left( f_{init}t + \frac{k_{Ch}t^{n+1}}{n+1} \right) \right)
\] (2.3.11)
Where $T$ is the sweep period, $k_{ch}$ is the chirp rate and expressed by $k_{ch} = (f_{final} - f_{init}) / T$ and $f_{init}$ is start frequency. Chirps signal rises linearly (up-chirp) or falls linearly (down-chirp). Chirp excitation signal has a low Crest Factor of about 1.45 (Pintelon and Schoukens 2012) and can be generated easily. The disadvantage of chirp signal is the lack of freedom in selecting arbitrary power spectrum magnitudes in the signal. The power spectrum is neither flat nor within the desired frequency band. Practically, generating a discrete chirp is more suitable (Ojarand 2012).

The chirp signal is called Nonlinearly Modulated if the instantaneous frequency $f_i$ changes nonlinearly. To simplify realization, the change is normally considered exponential, then:

$$f_i(t) = k't_{init}$$ (2.3.12)

In this case, the output of Voltage-Controlled Oscillator (VCO) can be expressed as:

$$V_{Ch}(t) = V_p \sin \left( 2\pi \left( f_{init}t + \int_0^t K_f V_{mod}dt \right) \right)$$ (2.3.13)

Where $V_p$ is the peak amplitude of the signal and $K_f$ is the frequency sensitivity of VCO in $Hz/V$.
Figure 2.17: Chirp signal generator by using voltage-Controlled Oscillator (VCO). (Ojarand et al. 2009)

Figure 2.18: Titled waveforms and its normalized power density spectra. (Ojarand et al. 2009)

Using exponential instead of the linear version of the excitation chirp signal can, by more than 20%, enhance one cycle’s chip energy content in the desired frequency range (Ojarand et al. 2009).

2.3.5 Optimized Signals: Discrete Interval Binary Sequences (DIBS)

In the previous sections, the excitation signals are studied that could be applied directly into the system, but there are excitation signals where an iterative algorithm is needed to optimize the design and tailor-made it for a specific purpose. One of these signals is called Discrete Interval Binary Sequences (DIBS) that is known as Binary Multi-Frequency Signal (BMS) (Ojarand 2012).

DIBS is a periodic binary excitation signal the amplitude sign can only change at an equidistance discrete set of points in time (Bos and Krol 1979). Power spectrum magnitude of the signal can be optimized by selecting a proper switching sequence as the energy of the signal is concentrated within the desired frequency band. Since not all of the power is concentrated at frequencies of interest, the crest factor depends on the complexity of the signal, but rather small.

(Paehlike and Rake 1979) Suggested an iterative scheme to put more power of the signal into weakest spectral lines and improve the SNR. DIBS in compare with MLBS can be generated in any sequence length with an arbitrary power
Besides, DIBS signal can be realized based on analog multi-sine excitation signal. Eq.2.3.14 defines a periodic multi-sine waveform:

\[ f(t) = \sum_{i=1}^{n} A_i \cos(2\pi f_i t + \psi_i) \]  (2.3.14)

Where \( A_i \) is the amplitude of the \( i \)-th component and \( \psi_i \) is the initial phase of the \( i \)-th component.

Minimization of the Crest Factor of multi sine is complicated and there are several algorithms are proposed to overcome the challenge, e.g. Synthesis of low-peak-factor signals and binary sequences with low autocorrelation (Schroeder 1970), A new method for synthesis of low-peak-factor signals (Bos 1987), peak-factor Minimization using a time-frequency domain swapping algorithm (Bos, 1987), nonlinear Chebyshev approximation method (Guillaume et al. 1991), and geometric discrepancy criteria (Rivera et al. 2006).

The method described by (Bos 1987) uses an iterative approach to find the particular phase angle vector to maximize the similarity of the signal to a two-level signal. If \( f_n \) for \( n = 0, \ldots, N - 1 \) represents the signal having the specified power spectrum and phase angles of \( \phi = (\phi_1, \ldots, \phi_L) \), then the problem is:

\[ \min_{\psi, A} \sum_{n} (f_n + AS_n) \]  (2.3.15)

Where \( A \) is the amplitude of a two-level signal and \( S_n \) is either -1 or +1.

The suggested algorithm by Van der Bos could result in achieving a better Crest Factor than Schroder phase.

Previously Schroder drives a formula to adjust the phase angles as it follows. He claims that a signal with arbitrary spectrum magnitude can be constructed in the form of a periodic Phase Modulated (PM) signal whose instantaneous frequencies are equal to the desired harmonics and change during an interval of the fundamental period proportional to their power (Bos 1987). Eq.2.3.16 is the desired formula for the phase angles of a given relative power spectrum of \( p_l \) (Schroeder 1970):

\[ \psi_n = \psi_1 - \sum_{i=1}^{n-1} (n - l) p_l \]  (2.3.16)

An iterative clipping algorithm has been developed by Van der Ouderaa (Ouderaa et al. 1988) to optimize phases of multi-sine signal to concentrate the energy of the signal at arbitrary frequencies. The method is very similar to the algorithm presented by Van den Bos (Bos 1987). The iterative procedure
is started by a given set of specified spectrum magnitude and arbitrary phase values. Inverse Fourier of the signal is calculated, and the discrete-time signal is generated. Then the signal is clipped by given level values, and new modified magnitude and phase vectors are calculated using the Fourier Transform. The new phase vector is maintained as a first approximation to the solution, but the new calculated amplitudes of the signal is rejected, and the original ones are kept. The cycle is repeated until no improvement in lower Crest factor is observed. The clipping level values begin with a lower value (e.g., 0.7 \( A_{\text{max}} \)) as iteration is started to almost no clipping (e.g., 0.999 \( A_{\text{max}} \)) at the end of the process. Normally algorithm optimizes the Crest factor after a few hundreds of iterations. This algorithm called Clipping Algorithm.

![Clipping Algorithm Diagram](image)

**Figure 2.19:** Clipping algorithm. (Pintelon and Schoukens 2012)

A more complicated algorithm is suggested by Guillaume (Guillaume et al. 1991) where the Gauss-Newton algorithm, in combination with Levenberg-Marquardt, is employed to minimize the multivariate non-linear infinity-norm function. In practice, the algorithm is turned out to be a better algorithm than the clipping algorithm and a significant reduction in calculation time when the number of points is considered significantly large. Figure 2.20 shows the evolution of the Crest Factor versus the time for swapping algorithm and the infinity-norm algorithm (Guillaume et al. 1991).

The algorithms, as mentioned above, are among many others that proposed methods to optimize a non-linear and non-convex function. However, these computed solutions can only guarantee local optima. Genetic algorithms (GA) (Horner and Beauchamp 1996) can give a better approximation for a large number for frequency components. Converging to a global minimum has not been guaranteed yet since exploring all local optima is time-consuming.

A novel empirical method for optimization of optimization the Crest factor of a multi sine excitation signal is introduced by Ojarand (Ojarand et al. 2014). According to the experiment, if the combination of initial phases of a multi sine excitation signal changes within the full range from 0 to 360, then several equal
minimums can be found. The observation shows that search for smallest Crest Factor can be found using the sequential search with a limited number of phase steps. Figure 2.21 shows the flowchart of the algorithm:

The algorithm search for the optimum initial phase components of the multi sine signal within larger to smaller phase intervals with a resolution of $\Delta \phi$. Once a minimum is found, the signal with the corresponding phases is saved, until the iteration reaches the minimum given resolution. Then the signal with the lowest calculated Crest factor is selected.

The new method for Crest Factor optimization is fast and give the same result as other exhaustive search methods while the frequency components are counts below 6. For a higher number of frequencies, the result is better or the
same as other methods (Ojarand et al. 2013).

Synthesizing of wideband binary excitation signal based on the Walsh functions is introduced first by Yuxiang Yang (Yang et al. 2009). An FPGA generates a signal, regulated by a Unipolar-to-Bipolar converter (UBC), driven using a voltage-controlled current source (VCCS) and finally, a wideband binary excitation signal is injected to a biomaterial model. The experiment shows that the VCCS has a good performance on the excitation signal so that the practical waveform on the load matches well the theoretical analysis (Yang et al. 2010).

The multi sine signals are decomposed by Fourier series in a set of sine and cosine orthogonal signals. Joseph L Walsh introduced the Walsh function in 1923. The function includes orthogonal sets in the time interval $[0, 1)$ taking values of -1 and +1 (Beauchamp 1975) and expresses as $WAL(n, t)$ where $n$ denoted the order and $t$ the normalized time. Similar to Fourier series, Walsh function can also expressed as the even function $CAL(n, t)$ and the odd function $SAL(n, t)$ and defined by the following equations:

\[
SAL(k, t) = WAL(2k - 1, t) \tag{2.3.17}
\]

\[
CAL(k, t) = WAL(2k, t) \tag{2.3.18}
\]

Where $k$ is the sequency of the Walsh functions which is defined as one half the average number of zero-crossing over the unit interval $[0, 1)$ and is used to measure of signal frequency.

![The Walsh functions $SAL(2^0, t)$ to $SAL(2^6, t)$ in a period (Yang et al. 2009).](image)

Figure 2.22: The Walsh functions $SAL(2^0, t)$ to $SAL(2^6, t)$ in a period (Yang et al. 2009).
Walsh function can be easily generated as the product of Rademacher functions (Yang et al. 2009) and Rademacher functions can be driven from Sinusoidal function as:

\[ SAL(2^{k-1}) = R(k, t) = Sgn(\sin(2^k \pi t)) \]  

(2.3.19)

Where \( Sgn(x) \) denotes the signum function:

\[
Sgn(x) = \begin{cases} 
1 & x > 0 \\
0 & x = 0 \\
-1 & x < 0 
\end{cases}
\]  

(2.3.20)

\( SAL(2^{k-1}) \) is a series of square function and in the time-domain comply the same symmetry as \( \sin(2^k \pi t) \) function and closest to it.

Finally, the excitation signal can be synthesized based on the superposition of the Walsh functions \( SAL(2^{k-1}) \) according to the following equation:

\[
f(p, t) = Sgn \left( \sum_{k=1}^{p} SAL(2^{k-1}, t) \right)
\]  

(2.3.21)

In which \( p \) is the number of mixed frequencies ranging from \( 2^0 \) to \( 2^{p-1} \). The synthesized excitation signal for \( p = 7 \) and its magnitude and power spectrum are depicted in Figures 2.23 and 2.24.

![Figure 2.23: The synthesized MFM signal \( f(7, t) \) in a period (Yang et al. 2009).](image.png)
Comparison with MLBS and Pulse signals, the SNR may be improved by Walsh based excitation signal and as other binary signals take advantage of Crest factor of 1. In addition, the signal can be realized by FPGA, and FPGA's operating clock can alter the bandwidth of its harmonics.
Chapter 3

Methodology

In this chapter, a practical method is developed in order to measure and compare time-domain and frequency-domain bio-spectroscopy techniques. The method comprises instrumentation and simulation. A design specification is written according to the criteria provided by the research proposal and the theoretical framework in chapter 2. The Red pitaya development board is employed as a data acquisition unit. Prototyping the device also includes coding in C and python programming languages and a front-end analog circuit which is based on the three-electrode system. Besides, the front-end circuit connected to the dummy cell is modeled in OrCAD PSpice simulation software. To obtain a satisfactory final design, the outcomes from the simulation and testing of the physical device are compared. Once the outcomes meet our expectation in the design specification, the design is locked. Figure 3.1 presents the workflow of the design and development of the prototyped device.

Section 3.1 presents the requirement in the design specification. Prototyping comprises two parts: 1) Hardware realization, 2) Software development. Hardware realization divided into two sub-sections. Data acquisition platform introduces in section 3.2 and its hardware characteristics discuss in detail. Section 3.3 describes Front-end analog circuit and design challenges and preferences. Following, Section 3.4 explains the step-by-step recipe to generate a
binary wideband signal. Finally, Section 3.5 and 3.6 focuses on implementation and verification of the prototype.

### 3.1 Design Specification

A set of requirements are concluded in Table 3.1. The requirements with (T) defined as threshold or expectations of the thesis and the ones with (O) defined as objectives that may be achievable or can be postponed to further work.

<table>
<thead>
<tr>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Signal generator shall generate a binary sequence that distributes the power of the signal uniformly and optimally in arbitrary frequencies within the bandwidth of 500 kHz (T) or 1MHz (O).</td>
</tr>
<tr>
<td>The hardware shall be able to generate both time-domain and Frequency-domain excitation signal.</td>
</tr>
<tr>
<td>The hardware shall be able to communicate with computers for post-processing and visualization of scientific data.</td>
</tr>
<tr>
<td>The software shall be able to measure and visualize the excitation signal and the magnitude (T) and phase (O) spectrum of the response signal in off-line (T) or online mode (O).</td>
</tr>
<tr>
<td>The device shall be able to measure the impedance of an electrical model of a single cell with RC passive components (T) or biological cell (O) within ±15% accuracy.</td>
</tr>
<tr>
<td>Front-end electronics shall utilize the three-electrode topology, including operation amplifiers with high input impedance and low bias current to prevent current leakage that leads to inaccurate response signal measurement.</td>
</tr>
<tr>
<td>The front-end electronics shall provide sufficient bandwidth and introduce low noise characteristics to guide the signal through this stage towards DAQ analog input port.</td>
</tr>
<tr>
<td>The Data Acquisition (DAQ) device shall provide minimum one input and one output analog channel with a sampling rate of minimum 10 times more than input signal’s Bandwidth to avoid aliasing and provide more accurate measurement for further processing of data.</td>
</tr>
<tr>
<td>The DAQ device shall provide ADC of minimum 10-bit resolution to retrieve acceptable information from the response signal for the post-processing stage.</td>
</tr>
<tr>
<td>The DAQ device shall generate and acquire a wideband binary signal with a bandwidth of 500KHz (T) or 1MHz (O).</td>
</tr>
<tr>
<td>The DAQ device shall be able to provide near-flat analog input bandwidth of at least 500KHz (T) or 1MHz (O) for the minimum power attenuation and maximum accuracy during measurement.</td>
</tr>
</tbody>
</table>

Table 3.1: Design specification

### 3.2 Data Acquisition Platform

To fulfill requirements of the design specification regarding the DAQ, two development boards were candidate, Red Pitaya and Analog Discovery 2. The Red Pitaya is selected because of its BNC connectors and higher sampling rate on
its channels. Advantage of BNC connectors over twisted pair wires are shielding; shielding prevents cross talk between measuring channels. Red Pitaya is an open-source measurement and control tool that features the following capabilities:

Two fast analog inputs with a sample rate of 125 Msps and 14-bit resolution. Input is DC coupled with an absolute maximum input voltage range of 30V (1500V ESD protected). The inputs are provided with Ω terminated SMA connectors. These inputs can be configured for two input voltage ranges ±1 V and ±20 V (STEMLab 2019a). See Red Pitaya board layout in Figure 3.2.

Figure 3.2: Red Pitaya STEMLab Board layout (STEMLab 2019b)

The impedance of the fast analog input channels at DC-coupled mode is given by graph 3.3.

Analog input measures the signals with -3dB frequency bandwidth of 50 MHz. The frequency response is shown in Figure 3.4.

Low noise voltage signal at input.

Figures 3.5 and 3.6 show the noise amplitude and power spectrum of Red pitaya input channel. Measurements are performed on 16k continuous samples at full rate (125MS/s).

In addition to analog inputs, the Red Pitaya development board features two fast analog outputs that can generate arbitrary waveforms. In this thesis, the binary signal is ultimately generated by using the fast analog output and the arbitrary waveform signal Generator (AWG) to take advantage of BNC connectors.

Prior to the use of the fast inputs and outputs, the device shall be calibrated.
The calibration process can be performed using Oscilloscope and Signal generator application and the given instruction by the application. In addition, a power supply is required to generate a relatively accurate voltage level to calibrate inputs.
Figure 3.4: Bandwidth of fast analog inputs (STEMLab 2019a)

Figure 3.5: Acquired signal at terminated IN1 input (STEMLab 2019a)
Figure 3.6: Noise Level (STEMLab 2019a)
3.3 Front-End Hardware Development

Front-end hardware depicted in Figure 3.7 consolidates two different circuits: non-inverting operational amplifier and transimpedance amplifier. The non-inverting operational amplifier isolates the measuring load from the excitation signal generator circuitry. While the transimpedance amplifier converts the current flowing through the load to a voltage signal with a known proportional factor that is defined by the feedback resistor.

![Figure 3.7: Front-end circuit based-on Three-electrode topology](image)

Using negative feedback in the non-inverting operational amplifier causes the load draws very little current from the excitation source and leads to less disturbance in impedance measurement. In Figure 3.7, the excitation signal is applied to the non-inverting input. The output of non-inverting operational amplifier, op-amp mirrors the same voltage level as its input voltage. Since voltages on input pins on transimpedance op-amp are in the same levels, the total voltage drop on load is equal to the excitation voltage level. Theoretically, the input impedance of an Op-amp is considered infinite. Therefore, it is assumed that the amount of input current passing through the Op-amp inputs are close to zero. Inevitably, the current of the load passes through the feedback resistor of the transimpedance Op-amp. This simple topology provides a full compact solution to measure the impedance of the load precisely.

Accuracy of the response voltage determines the measurement accuracy. The accuracy depends on the characteristics of the op-amps. Ideally, the total amount of the current passes through the feedback resistor of the transimpedance circuit. However, in the real world, a small amount of the current flows into both inverting and non-inverting inputs of Op-amps. This leads to disturbances in impedance measurement by creating a bias voltage across the measuring impedance. Many op-amps take advantage of a built-in input bias cancellation circuit.

Moreover, use of an input bias current resistor, specified as a value of impedance and feedback resistor in parallel, ensure the same level of input bias current on both inputs where may eliminate output error. The same principle applies to the non-inverting operational amplifier stage. The supply current can leak back into inverting input of the Op-amp. A better Op-amp features the less possible input bias current in the range of few nano amps to some femto amps.
The wideband binary excitation signal is a pulse shaped signal which is composed of several frequency components. Gain bandwidth product and Small-signal bandwidth parameters of an Op-amp controls the shape of a square waveform. All frequency components of the input signal shall pass through the flat frequency response of the Op-amp with minimum attenuation.

The specification of the front-end electronics suggests a 5 MHz unity-gain bandwidth for impedance measurement. By this, three Op-amps are candidates for prototyping. Op-amp characteristics are listed in Table 3.2.

According to the Table 3.2, the premium choice will be LTC6268-10 from Linear technology that offers overestimated and generous bandwidth option with pico-Ampere input bias current and therefore achieving an optimum accuracy of the current measurement. OPA1S2385 combines high bandwidth, FET-input operational amplifiers with a fast SPST CMOS switch that specifically designed for application for capturing fast signals. Low input bias current and voltage noise make it possible to amplify extremely low-level input signals for maximum signal-to-noise ratio. Both LM7171 and OPA1S2385 products from Texas Instruments provide sufficient bandwidth, but OPA1S2385 offers around 1000 times lower bias current. LTC6268-10 and OPA1S2385 are optimized for low-voltage operations.

Although low input bias current is an essential parameter to be considered in the ultimate design of the impedance measurement circuit, high operating voltage range, sufficient bandwidth and PDIP package of LM7171 make it a perfect choice for prototyping on a breadboard. The Lm7171 provides a very high slew rate at 4100V/µs and unity-gain bandwidth of 200 MHz while consuming 6.5mA in typical mode. Operating voltage of ±15V allows for large signal swings and provide greater dynamic range and signal-to-noise ratio. These inte-
Step Response

**Small-Signal**

<table>
<thead>
<tr>
<th>OUTPUT (V)</th>
<th>TIME (20 ns/ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Large-Signal**

<table>
<thead>
<tr>
<th>OUTPUT (V)</th>
<th>TIME (20 ns/ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LM7171

$A_v = -1$

$V_s = \pm 5V$

OPA1S2385

$R_L = 1K\Omega$

connected to

$V_s/2$

$V_o = V_{CM} = V_s/2$

LTC6268-10

See image captions for more details.

Table 3.3: Operation amplifier comparison.
grated circuits are cross-compared with respect to small-signal and large-signal step response.

The output voltage of the transimpedance amplifier in Figure 3.7 is formulated as:

\[ V_{TI,O} = -R_f (I_Z - I_{TI,Ib}) \]  \hspace{1cm} (3.3.1)

\[ I_Z = \frac{V_{Exc} - V_{TI,Iov}}{Z} \]  \hspace{1cm} (3.3.2)

Where,

\( V_{TI,O} \): Transimpedance output voltage

\( R_f \): Transimpedance feedback resistor

\( I_Z \): The amount of the current passes the impedance

\( I_{TI,Ib} \): Transimpedance input bias current

\( V_{Exc} \): Excitation voltage signal

\( V_{TI,Iov} \): Transimpedance input offset voltage

\( Z \): Measuring impedance

From 3.3.1 and 3.3.2, the compact formula can be gives as:

\[ V_{TI,O} = -R_f \left( \frac{V_{Exc} - V_{TI,Iov}}{Z} - I_{TI,Ib} \right) \]  \hspace{1cm} (3.3.3)

Considering negligible input bias current and input offset voltage for the operational amplifier, the formula can be written in short form of:

\[ V_{TI,O} = -R_f \left( \frac{V_{Exc}}{Z} \right) \]  \hspace{1cm} (3.3.4)

Hence, feedback resistor is calculated as:

\[ R_f = Z \left( \frac{V_{TI,O}}{V_{Exc}} \right) \]  \hspace{1cm} (3.3.5)

And impedance is given as:

\[ Z = -R_f \left( \frac{V_{Exc}}{V_{TI,O}} \right) \]  \hspace{1cm} (3.3.6)
Value of the feedback resistor in transimpedance circuit should be determined according to three factors: 1) Excitation signal voltage range, 2) Impedance value range within the desired frequency range and 3) Desired output voltage range. Although the fast analog input can be configured for small signals $\pm 1V$ to achieve better reading accuracy, in order to use full output swing range of LM7171, the analog input is switched to $\pm 20V$ range. In practice, an embedded voltage limiter in Red Pitaya board carries out this operation. Factors 1 and 2, basically define the expected range of current passes through the measuring impedance.

### 3.4 Excitation Signal Generator

The binary excitation signal generator is described as a function with two inputs and one output. The inputs to the function are arbitrary pairs of magnitudes and frequencies to construct a signal which its power concentrated in the given frequencies. This generator is aimed to distribute the power of the signal uniformly across desired frequencies. An ideal generator can allocate total power of the signal within given frequencies.

![Figure 3.8: Power spectrum density of bipolar and polar signals, normalized for equal powers. Half-width rectangular pulses are used.](Lathi 2009)

Unipolar and bipolar signals with their corresponding Fourier transform functions are demonstrated in Figure 3.9. By definition, a bipolar signal passes the zero line, while the unipolar signal does not. Advantage of using bipolar signal is to suppress the power of the signal in even frequencies and more significantly zero or DC frequency. Although a bipolar signal oscillating with a single frequency, the total power cannot be allocated only to that specific frequency. An ideal square signal comprises infinite number of frequencies. The rising and falling edges and generally the fast transitions between maximum and minimum values requires contribution of more frequencies. Besides frequency, duty cycle is another factor that influences power spectrum of a signal. Duty cycle is defined as the amount of time the signal is at its maximum level as a percentage of the total time it takes to complete one cycle. A bipolar signal with 50 percent duty cycle suppresses even frequencies while increasing or
decreasing the duty cycle leads to leak power into even frequencies.

![Frequency Spectrum Image](image)

Figure 3.9: The frequency spectrum of a bipolar signal with 49% and 50% duty cycle. (Bogdan[2017])

Moreover, the phase of a bipolar signal plays a significant role in its frequency representation. The challenge for an excitation bipolar signal generator is to find a combination of bipolar signals with matching frequency, duty cycles and phases as the maximum power of the signal is concentrated in arbitrary frequencies. This optimization can be done by different methods which are already described in the chapter 1.

The method used in this thesis is inspired by the clipping algorithm, whereby each iteration, an analog signal is constructed, clipped, and quantized into bipolar levels. After each iteration, total distributed signal power at the desired frequencies are measured and monitored. Finally, the best signal with maximum performance is selected. The procedure to generate such signal is described in details in the following section.

### 3.4.1 Procedure

The generator takes two sets of arbitrary magnitudes and frequencies. An example set of magnitude and phases are considered as follows. The results of the calculations in the following stages are according to the given example.

\[
\text{Arbitrary Magnitude Vector} = [1, 1, 1, 1, 1, 1, 1, 1, 1, 1]
\]

\[
\text{Arbitrary frequency Vector} = [50,100,150,200,250,300,350,400,450,500]
\]

Initially, a set of phases is required to construct a frequency domain signal. Phases can be either generated optimally by Schroder algorithm (Schroder 1970) or selected randomly. The algorithm with an optimum phase array converges faster.
Schroeder considered a periodic signal $r(t)$ with a period of $T$ and finite bandwidth as its Fourier series represented as:

$$r(t) = \sum_{k=1}^{N} \sqrt{P_k} \cos \left( \frac{2\pi kt}{T} + \theta_k \right)$$  \hspace{1cm} (3.4.1)$$

Where $P_k$ is the relative power of the $k$th harmonic $\sum_{1}^{N} P_k = 1$ and $\theta_k$ its phase angle.

The problem defined as how to choose the $\theta_k$ to minimize $r_{\text{max}} - r_{\text{min}}$. For solving the problem, consider the following periodic phase-modulated signal with piece-wise linear phase:

$$s(t) = \cos \left[ \psi(t) \right]$$ \hspace{1cm} (3.4.2)$$

Where

$$\psi(t) = \int_{0}^{t} \psi(\tau) \, d\tau$$ \hspace{1cm} (3.4.3)$$

And

$$\psi(t) = \frac{2\pi k}{T}, \quad t_{k-1} \leq t \leq t_k, \quad k = 1, 2, ..., N.$$ \hspace{1cm} (3.4.4)$$

Here, the time epochs $t_k$ at which the instantaneous frequency changes its
value are given by:

\[ t_k = T \sum_{l=1}^{k} p_l \]  \hspace{1cm} (3.4.5)

Where

\( T \): Period of the signal

\( N \): Number of harmonics

\( p_l \): Relative power of the \( l \)th harmonic of the prescribed power spectrum.

The time interval during which the instantaneous frequency of the signal equals \( k/T \) is proportional to the relative power of the \( k \)th harmonic:

\[ t_k - t_{k-1} = T \left( \sum_{l=1}^{k} p_l - \sum_{l=1}^{k-1} p_l \right) = T p_k \]  \hspace{1cm} (3.4.6)

The approximation seems to be much better for a continuous signal having smooth power spectra than for discontinuous signal with irregular spectra. The instantaneous phase of \( s(t) \) in the \( n \)th time interval is given by:

\[ \psi(t) = \phi_n + \frac{2\pi n}{T} t, \quad t_{n-1} < t < t_n \]  \hspace{1cm} (3.4.7)

Where \( \phi_n \) is a phase constant that, in the limiting case of \( N \gg 1 \), corresponds to the phase angle of the \( n \)th Fourier component of \( s(t) \).

For \( \psi(t) \) to be continuous at \( t = t_{n-1} \), the following relation must obtain:

\[ \phi_n = \phi_{n-1} \]  \hspace{1cm} (3.4.8)

\[ \phi_n + \frac{2\pi n}{T} t_{n-1} = \phi_{n-1} + \frac{2\pi (n-1)}{T} t_{n-1} \]  \hspace{1cm} (3.4.9)

Or

\[ \phi_n = \phi_{n-1} + \frac{2\pi}{T} t_{n-1} \]  \hspace{1cm} (3.4.10)
Hence,

$$\phi_n = \phi_1 + \frac{2\pi}{T} \sum_{k=1}^{n-1} t_k$$  \hspace{1cm} (3.4.11)

Where,

$$\phi_1 = \phi_0 + \frac{2\pi}{T} t_0 = \phi_0 + \frac{2\pi}{T} (0) = \phi_0$$  \hspace{1cm} (3.4.12)

With Eq[3.4.5] one has,

$$\phi_n = \phi_1 + 2\pi \sum_{k=1}^{n-1} \sum_{l=1}^{k} p_l$$  \hspace{1cm} (3.4.13)

Or the recursive method to produce phase vector can be compacted in the following formula that suits for programming purpose (Schroeder [1970]):

$$\phi_n = \phi_{n-1} + 2\pi \sum_{l=1}^{n-1} p_l$$  \hspace{1cm} (3.4.14)

The set of generated phase vector that is shown in Figures 3.11 and 3.12 are the result of the calculation of the given example vectors.

![Figure 3.11: Magnitude of the initial constructed signal.](image)

A frequency-domain signal is reconstructed by mixing selected magnitude
and phases.

$$X_d(k) = \sum_{k=0}^{N-1} a_k e^{j\phi_k}$$ (3.4.15)

The zero-order hold (ZOH) describes the effect of converting a discrete-time signal to a continuous-time signal by holding each sample value for one sample interval (Wikipedia [2019]). In order to simulate this effect, the discrete representation of the signal in the frequency domain is convolved with the reversed transform function of the zero-order hold filter.

$$X(k) = X_d(k)|X_{\text{ZOH}}^{-1}(k)|$$ (3.4.16)
Where

\[ |X_{SH}(k)| = \text{Sinc}\left(\frac{\pi k}{N}\right) \] (3.4.17)

Using Inverse Discrete Fourier transform (IDFT) the signal is presented as a time series.

\[ x(n) = \frac{1}{N} \sum_{k=0}^{N-1} X(f) e^{j2\pi kn/N} \] (3.4.18)

In this stage, the signal is clipped and quantized into a two-level bipolar...
signal.

\[ x_q(n) = Sgn(x(n)) \]  
(3.4.19)

\[ x(n) = \sum_{n=0}^{N-1} x_q(n)e^{-j2\pi kn/N} \]  
(3.4.20)

Figure 3.16: Clipped time-domain signal

So far, the first bipolar signal is initiated. In order to iterate the steps, new phases are yield by calculating Discrete Fourier transform (DFT) of the recently obtained bipolar signal.

The procedure will continue using the initially defined amplitude set and the recently generated phase set. Again the amplitude and phase arrays are mixed.
to construct a new frequency domain signal, and then a new set of the bipolar signal is reproduced. The procedure can be repeated by arbitrary number of iteration. This number can be determined according to best practices. In each loop, two main processes are carried out. One is the process of self-adjusting magnitude of power spectra so that the power is distributed as uniform as possible among desired harmonics. The other one is to calculate ratio of the actual to the specified harmonic magnitudes using Parseval’s theorem as is described below:

$$Power\ Factor = \frac{P_{\text{specified}}}{P_{\text{actual}}} = \frac{\frac{1}{M} \sum_{k=0}^{M-1} |X(k)|^2_{\text{specified}}}{\frac{1}{N} \sum_{k=0}^{N-1} |X(k)|^2} \quad (3.4.21)$$

Figure 3.18: Magnitude and phase representation of the optimized signal with concentrated power on arbitrary frequencies using developed clipped algorithm.

The final bipolar signal representation in the frequency domain for the given example set of arbitrary magnitude and frequency vectors are shown in Figure 3.18. The algorithm attempt to achieve the best result by distributing the power uniformly among the given frequencies by alternating the phase vector values, suppressing the desired harmonics with higher magnitudes and empowering the ones with lower magnitudes. By comparing the phase representation of the initial generated bipolar signal at the start of the algorithm and the output of the algorithm, one can see the phase vector includes less zero values. The phase values of the final signal are alternating much faster with respect to the Schroder phase vector to achieve more optimum power spectra. See Algorithms in Appendix A.
3.5 Implementation

The pre-defined procedure of generating a binary excitation signal is implemented by C programming language. Then the program is hardcoded in Red Pitaya board. The initial idea was to use an advanced web programming tools provided by Red Pitaya team and creating a web-based application which interacts with the user simply. Although the web-based application still seems the best solution, the software kit and documentation is not properly released for developers at the time of implementation and there are some difficulties in use of the current documentation. As an alternative solution to study and verify the result of the algorithm, the results are obtained by Oscilloscope and spectrum analyzer web applications. The applications are freely available in Red Pitaya Marketplace. The front-end circuit is tested on Breadboard. The test setup is shown in Figure 3.19.

![Figure 3.19: Prototyping: Test setup](image)

3.6 Simulation

The OrCAD PSpice software is used mainly by electronic design engineers and electronic technicians to create electronic schematics and perform the mixed-signal simulation. Schematic model of the exact front-end analog circuit with impedance model is drawn in OrCAD capture. Schematic designs of the circuits for time- and frequency-domain measurement of the modeled dummy cell are shown in Figure 3.20 and Figure 3.21, respectively.

After running a transient analysis by the simulator, the output and input results are presented in the Fourier series in the software. The results are exported to a personal computer in excel format for post-processing and visualization purposes.
Figure 3.20: Time-domain simulation. Data point from the signal generator is given as a text file to the power supply.

Figure 3.21: Frequency-domain measurement. In every single simulation, a new frequency value is assigned to AC power source.
3.7 Test and Verification

Test bench architecture diagram is illustrated in Figure 3.22 where the test and verification process is performed. Client access remotely or wired via a PC to the Red Pitaya development board. Desired frequency and magnitude vectors are hardcoded in the C program that generates the binary signal. Then the C program is compiled and executed. The constructed binary signal will be transferred to the PC as data points file. Python code runs on PC and retrieves the data points from the file and execute a routine to push data back to Red Pitaya into the digital-to-analog converter buffer. This leads to generating an arbitrary waveform based on the data point values at output channel 1. The excitation signal passes through Front-end circuit and the dummy cell. Further, the excitation and response signals are captured via fast analog inputs channel 1 and 2. Both channels are visualized in Oscilloscope and spectrum analyzer applications that can be accessed via Red Pitaya web interface on PC web browser. Figures 3.23 and 3.24 show the example of the Red Pitaya web applications. The results of measurements can be exported as excel file for post-processing and final analysis. Then impedance curve versus frequency is presented as a result of the test.

The Red Pitaya Development Board can be controlled remotely over a LAN or wireless interface using Python via SCIP (Standard Command for Programmable Instrumentation) list of commands. SCPI interface/environment is commonly used to control test and measurement instruments for development, research, or test automation purposes.

Moreover, the written application in Python can generate single tone signals by user-defined frequency. The results of the experience in both scenarios of single tone and wideband measurements are cross-compared with theoretical and simulation reference values.
Figure 3.23: Oscilloscope and spectrum analyzer web application, developed by STEM Lab

Figure 3.24: Oscilloscope and spectrum analyser web application, developed by STEM Lab
Chapter 4

Result and Discussion

Following several trials, tests, and verifications of different designs, the final design which realize the threshold requirements is locked and validated. This has achieved after that the results of the final implemented design have been compared with simulation results and requirements at test and verification stage.

The final test is carried out on the testing workbench that described in Section 3.7. The time-domain excitation signal and the cell model response are shown in the Figure[4.1]. The unstable overshoot and ringing are observed at the output of transimpedance. The reason is that the transimpedance amplifier is driving capacitive load at analog input connection of Red Pitaya board. This leads oscillations at the output of the amplifier. To eliminate oscillations or reduce ringing, isolation resistor (RISO) can be optimally calculated according to the capacitive load value. Although fine-tuning of the analog design is out of scope of this thesis, its further improvement is suggested for product development.

After capturing both input channels, Discrete Fourier Transform (DFT) of signal and its corresponding response are calculated by spectrum analyzer web application. The results of the measurement in the frequency domain are exported and processed for both channels. The outcome is represented as a magnitude of the impedance in Figure [4.2].

The impedance magnitude representation of a single cell in saline suspension, see in subsection 2.1.4 Figure[2.3] and simplified equivalent circuit with passive element values of $C_{dl} = 2.2nF$, $C_s = 30pF$, $C_m = 6pF$, $R_{cy} = 99.5k\Omega$, $R_s = 62.8k\Omega$ are shown in Figure(below). The presented graph compares the exact transfer function of the model with simulation and test results of the final design. The measurements are performed for an example input excitation signal with a bandwidth of 450kHz. The frequency range is considered between 50kHz and 500kHz with linear space of 50kHz. A solid line highlights the transfer function of the area of interest. As is evident from Figure[4.3] the time- and frequency-domain simulation relatively fit the theoretical curve. The differences between the transfer function and the simulation results are negligible and are
Figure 4.1: Excitation signal measurement and the response to the cell model on Oscilloscope web application, developed by STEM Lab

counted as processing error during simulations. The simulation outcomes are finally post-processed in Excel.

Accuracy is addressed quantitatively by a relative error that is a so-called deviation. The accuracy is utilized as a tool to measure the uncertainty of our measurements. Deviation values can be found in Table 4.1. Maximum error of frequency-domain and time-domain measurements are calculated as $-8.13\%$ and $9.94\%$, respectively. The negative sign indicates that the measured value is less than the simulation value. The accuracy is within $\pm 10\%$ of the expected value, and from specification perceptive, the requirement is fulfilled. Result of the table is visualized as a bar graph in Figure 4.4. The graph shows the relative error in percentage. The formula to calculate the relative error is given as follows:

$$
\text{Relative Error} \ [%] = 100 \times \frac{\text{Measured Value} - \text{Expected Value}}{\text{Expected Value}} \tag{4.0.1}
$$

Error bar in Figure 4.5 indicates the uncertainty in a reported measurement given by both methods of generating excitation signals. The error is calculated with respect to the obtained simulation results from both methods. In general, the trend suggests that error is gradually increasing on higher frequencies.

Ultimately, a PCB board of the front-end circuit is constructed in collaboration with UiO E-LAB. The schematic of the PCB board is presented in Appendix B. In process of developing PCB board some features are enhanced such as proper grounding so that analog circuit is isolated properly from the supply ground. In addition, the PCB is placed in metal enclosure in order to block electromagnetic fields from other sources that may disturb small signals. Figure 4.6 visualizes the result of an experiment with PCB board by using the same set of
Figure 4.2: Excitation signal measurement and the response to the cell model on spectrum analyzer web application, developed by STEM Lab.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Relative Error %</th>
<th>Time-domain Meas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>-0.678</td>
<td>9.323</td>
</tr>
<tr>
<td>100</td>
<td>-4.718</td>
<td>8.532</td>
</tr>
<tr>
<td>150</td>
<td>-5.848</td>
<td>3.983</td>
</tr>
<tr>
<td>200</td>
<td>-6.284</td>
<td>-0.155</td>
</tr>
<tr>
<td>250</td>
<td>-6.404</td>
<td>1.676</td>
</tr>
<tr>
<td>300</td>
<td>-7.230</td>
<td>9.468</td>
</tr>
<tr>
<td>350</td>
<td>-7.383</td>
<td>8.015</td>
</tr>
<tr>
<td>400</td>
<td>-7.614</td>
<td>4.558</td>
</tr>
<tr>
<td>450</td>
<td>-7.916</td>
<td><strong>9.941</strong></td>
</tr>
<tr>
<td>500</td>
<td>-8.137</td>
<td>6.589</td>
</tr>
</tbody>
</table>

Table 4.1: Relative Error.

example arbitrary frequency and magnitudes as used for previous tests.

It should be noted that the values of passive elements in the cell model on PCB board are not precisely identical with the prototype version. Therefore, the relative errors in Figure 4.7 for three sets of measurements are compared with transfer function of the cell model. The graph suggests an improvement in measurement after the PCB design, although small deviation can be seen in lower frequency. The uncertainties shall be investigated in further work.

The impedance formula is given by Eq. 3.3.6 in Section 3.3 shows that the impedance value is directly proportional to the value of the feedback resistor $R_f$. Consequently, tolerance of the resistor has a direct impact on the accuracy of final measurement. The feedback resistor used in this experiment has 5% tolerance. The other root causes of uncertainty are summarized in cause and effect diagram known as Fishbone diagram shown in Figure 4.8. The cumulative
effect known as tolerance stack-up leads to total measurement uncertainty.

Furthermore, calibration of DAQ channels and considering input bias of Op-amp in calculations reduce offset error. Although breadboard configuration is adequate for prototyping purpose, the results are still within the specification range. However, considerations in designed PCB version such as proper grounding and using metal enclosure reduces error. Also, using the full dynamic range of ADC, as it is explained in Section 2.3.2 could be beneficial. An optimized response signal with a low crest factor can efficiently take advantage of full dynamic range of ADC. Other factors to improve the design are left to further work.
Figure 4.4: The relative error of frequency-domain and time-domain measurements versus their simulation results.

Figure 4.5: Comparison of measurement errors from frequency-domain and time-domain methods with respect to the theoretical result.
Figure 4.6: Time-domain measurement on PCB design board versus its corresponding transfer function.

Figure 4.7: The relative error of three measurements done by Frequency-domain, time-domain, and time-domain on PCB board with respect to the transfer function. Dash lines show relative error of ±10%.
Figure 4.8: Cause and effect (Fishbone) diagram
Chapter 5

Conclusion

A prototype device for impedance measurement is designed and instrumented. The device is capable of measuring impedance spectra up to 500kHz by using both time-domain and frequency-domain methods. However, the device covers 90 percent of the specified target bandwidth in the design specification. A personal computer controls the device and analysis data. Red Pitaya development board is utilized to generate both time-domain and frequency-domain excitation signals. The unit is also responsible for signal processing. A front-end analog circuit with the three-electrode system is instrumented to excite voltage signal and capture response current. A dummy cell which is modeled with the lumped circuit is used as a sample under test. The sample is excited by both time- and frequency-domain signals. Test outcome is used for comparison of the two measurement techniques.

OrCAD PSpice software is utilized to simulate the front-end circuit and the cell model. The generated time-domain signal is imported to the software for simulation purpose. AC sweep simulation is used for reproducing frequency-domain excitation signal. The results of both simulations are used for test and verification purpose.

Ultimately, the outputs of measurements on the physical device and the results of simulations are presented and cross-compared. Accuracy is employed as a measure to compare the results. Comparison between the experimental and simulation results reveals a deviation of less than 10 percent for both measurement techniques. Hence, time-domain method can be used as a faster alternative to frequency-domain method within acceptable accuracy.

Moreover, a PCB version of the prototype is developed in collaboration with UiO E-LAB. The preliminary results out of PCB designed version were promising as the PCB design could reproduce the result with better accuracy than the early prototype.
Chapter 6

Further Work

Many adaptations, optimizations, and tests have been left for the further due to lack of time. Further work concerns improvements of electronics, process automation, deeper analysis and covering wider frequency ranges. There are some ideas that I would have liked to try during developing prototype in Chapter 3.

Although the system is tested for a limited range of frequencies from 50kHz to 500kHz and it could cover 90% of the target bandwidth in the design specification, the device is potentially capable of generating wideband signals including lower and higher frequencies than 50kHz and 500kHz, respectively.

In addition, the process of analyzing the excitation response and plotting impedance spectra could be automated. Attempts have been made to migrate from several computing stages to an automated and user-friendly interface. But due to time constraint and lack of proper documentation for developing the user interface tool, this process is left for further work.

Several potential causes have been identified and presented in the fishbone diagram to improve the performance of the system. For improving accuracy of the measurement, it is recommended to minimize the crest factor of the response signal in the interest of using maximum dynamic range of ADC.


Creason, Sam C. and Donald E. Smith (1972). “Fourier transform faradaic admittance measurements II. Ultra-rapid, high precision acquisition of the frequency response profile”. In: *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* 40.1, A1–A5. ISSN: 0022-0728. DOI: [https://doi.org/10.1016/S0022-0728(72)80146-3](https://doi.org/10.1016/S0022-0728(72)80146-3).


**Guillaume, P. et al. (1991). “Crest-factor minimization using nonlinear Chebyshev approximation methods”. In: *IEEE Transactions on Instrumentation and Measurement* 40.6, pp. 982–989. ISSN: 0018-9456. DOI: [10.1109/19.119778](https://doi.org/10.1109/19.119778).**


Ojarand, Jaan et al. (2009). “Nonlinear Chirp Pulse Excitation for the Fast Impedance Spectroscopy”. In:


Pinterest (2019). Plasma structure, Web Page. URL: https://i.pinimg.com/originals/ac/98/c5/ac98c5f6fb9fa676f2877b29b5543f2b.png

Pliquett, Uwe (2010). “Bioimpedance: A Review for Food Processing”. In: Food Engineering Reviews 2.2, pp. 74-94. ISSN: 1866-7929. DOI: 10.1007/s12393-010-9019-z URL: https://doi.org/10.1007/s12393-010-9019-z


Appendix A

Algorithms
Algorithm 1 Wideband Binary Signal Generator

1: procedure WIDEBAND_BINARY_SIGNAL_GENERATOR(\vec{A}_I, \vec{K}, N, Opt, Loop Count)
2: if Opt = Schroeder then
3: \vec{\Phi}_I = PhaseGen(Schroeder, \vec{K})
4: else if Opt = Random then
5: \vec{\Phi}_I = PhaseGen(Random, \vec{K})
6: else
7: \vec{\Phi}_I = PhaseGen(Schroeder, \vec{K})
8: end if
9: \vec{A}, \vec{\Phi}, \vec{K} = Initialize(\vec{A}_I, \vec{\Phi}_I, \vec{K}, N)
10: \epsilon = C_I
11: for M = 0 : (Loop Count − 1) do
12: \vec{A}, \vec{\Phi} = TFSwap(\vec{A}, \vec{\Phi})
13: PF = PFCalc(\vec{A}, \vec{K})
14: if M > 0 then
15: Compute: \rho = CorrelationCoefficient(\vec{\Phi}, \vec{\Phi}_Old)
16: end if
17: if (\rho > \rho_{Old} and M > 1) then
18: for all k \in \vec{K}_{Specified} do
19: Compute: Av = Average(|A(k)|)
20: end for
21: if PF = PF_{Old} then
22: \epsilon = \epsilon + C
23: end if
24: for all k \in \vec{K}_{Specified} do
25: if |A(k)| < Av then
26: |A(k)| = |A(k)| + \epsilon
27: end if
28: \rho_{Old} = \rho
29: end for
30: PF_{Old} = PF
31: \vec{\Phi}_{Old} = \vec{\Phi}
32: end if
33: end for
34: end procedure
Algorithm 2 Phase Generator

1: procedure PHASEGEN(Opt, K)
2: for all $k \in (\vec{K}_{\text{specified}})$ do
3: if Opt = Schroeder then
4: $\phi_k = \phi_{k-1} + 2\pi \sum_{l=1}^{k-1} P_l$
5: return $\vec{\Phi}$
6: else if Opt = Random then
7: $\vec{\Phi}_I = \text{rand}(-1, 1)$
8: return $\vec{\Phi}_I$
9: end if
10: end for
11: end procedure

Algorithm 3 Initializer

1: procedure INITIALIZE($\vec{A}_I, \vec{\Phi}_I, \vec{K}_I, N$)
2: for all $k \in \vec{K}_I$ do
3: Compute: $X_d(k) = \sum_{k=0}^{N-1} a_k e^{j\phi_k}$
4: end for
5: for all $k \in \vec{K}_I$ do
6: Compute: $|X_{SH}(k)| = \text{Sinc} \left( \frac{\pi k}{N} \right)$
7: end for
8: for $k = 0 : (N - 1)$ do
9: Compute: $X(k) = X_d(k)|X_{SH}^{-1}(k)|$
10: end for
11: for $n = 0 : (N - 1)$ do
12: Compute: $x(n) = \frac{1}{N} \sum_{k=0}^{N-1} X(k)e^{-j2\pi kn/N}$
13: end for
14: for $n = 0 : (N - 1)$ do
15: Compute: $x_q(n) = \text{Sgn} \{ \text{Re} \{ x(n) \} \}$
16: end for
17: for $n = 0 : (N - 1)$ do
18: Compute: $X_q(k) = \frac{1}{N} \sum_{n=0}^{N-1} x_q(n)e^{-j2\pi kn/N}$
19: end for
20: for $k = 0 : (N - 1)$ do
21: Compute: $\phi_k = \angle X_q(k)$
22: end for
23: return $\vec{A}_I, \vec{\Phi}, \vec{K}_I$
24: end procedure
Algorithm 4 Time-domain Frequency-domain swap

1: procedure TFSWAP(⃗A, ⃗Φ)
2:     for k = 0 : (N − 1) do ▷ Frequency-domain signal construction
3:         Compute: \( X(k) = \sum_{k=0}^{N-1} a_k e^{j\phi_k} \)
4:     end for
5:     for n = 0 : (N − 1) do ▷ Time-domain signal reconstruction
6:         Compute: \( x(n) = \frac{1}{N} \sum_{k=0}^{N-1} X(k)e^{j2\pi kn/N} \)
7:     end for
8:     for n = 0 : (N − 1) do ▷ Quantization: converting to binary signal
9:         Compute: \( x_q(n) = Sgn(Re\{x(n)\}) \)
10:    end for
11:    for k = 0 : (N − 1) do ▷ Phase extraction, Stage 1
12:         Compute: \( X_q(k) = \frac{1}{N} \sum_{n=0}^{N-1} x_q(n)e^{-j2\pi kn/N} \)
13:    end for
14:    for k = 0 : (N − 1) do ▷ Phase extraction, Stage 2
15:         Compute: \( \phi_k = \angle X_q(k) \) and \( a_k = |X_q(k)| \)
16:    end for
17:    return ⃗A, ⃗Φ
18: end procedure

Algorithm 5 Power Factor Calculator

1: procedure PFCALC(⃗A, ⃗K)
2:     for all \( k \in (⃗K_{\text{specified}}, \⃗K) \) do ▷ Phase extraction, Stage 1
3:         Compute: Power Factor = \( \frac{\sum_{k=0}^{M-1} |X(k)|^2}{\sum_{k=0}^{M-1} |X(k)|^2} \)
4:     end for
5: end procedure
Appendix B

PCB Design

B.1 Schematics
Figure B.1: Schematic of PCB design.
B.2 Image
Figure B.2: PCB board version of the prototype is developed in collaboration with UiO E-LAB