
Jimma University

School of Graduate Studies

Jimma Institute of Technology

School of Biomedical Engineering

# Ex-Vivo Breast Tissues Characterization Using Bioimpedance Spectroscopy

By: Shimelis Nigusu Hordofa

A Thesis Submitted To School Of Graduate Studies of Jimma University, In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biomedical Engineering (Bioinstrumentation Stream)

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Advisors: - Dr.Timothy Kwa (PhD)

Eng. Wasihun Alemayehu (M.Sc.)

November, 2019

# DECLARATION

I would like to declare that this thesis research is my original work and has not been submitted for a degree in any other university and I assure it with my signature.

Name: Shimelis Nigusu

Signature_____ Date _____ On behalf of the school of Biomedical Engineering at Jimma University, Jimma Institute of Technology, we the advisors of this research with the title of **Ex-Vivo Breast Tissues Characterization Using Bioimpedance Spectroscopy: A Novel Approach for Breast Cancer Detection** and I, the evaluator, confirm that this research proposal is approved as MSc thesis for the student.

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# *ABSTRACT*

*Breast cancer incidence and mortality are drastically increasing globally. The factor behind these problems are complex but reflect both aging and growth of the population within a given country. On top of this, regardless of Country's economic profiles, breast cancer incidence is dramatically spreading both in developed and developing regions. In order to tackle this problems, breast cancer detection and screening techniques have been introduced to both national and global market. X-ray mammography, ultrasound and magnetic resonance imaging (MRI) are the most commonly being used techniques for the detection of cancerous tissues in breast. Furthermore, several researches have been done to characterize breast tissues even though they come up with their critical down falls which underscores the need for an improved technique for breast tissues characterization that can satisfies the need of wider community.*

*In this study the Bioelectric Impedance Spectroscopy was used to characterize the breast tissue samples in ex-vivo setup to identify and analysis the frequency response of malignant and normal breast tissues. The AFE (Analog Front End) circuitry was designed to achieve the four electrodes configuration of Ad5933evalboard.Breast tissues which were collected from 20 patients were characterized with excitation current 1 KHZ-100 KHZ frequency ranges. The primary results indicated that there was significant difference between the frequency response of normal and malignant breast tissues with their dielectric properties. The result from ANOVA (Analysis of Variance) test illustrated that there was significant statistical difference ($p=0.0002$) among the phase angle of three breast tissue samples .More importantly Turkey's Honestly Significant Difference Post Hoc test showed that there was significant difference ($p<0.001$) between two groups of breast tissue samples (normal tissues and high grade cancerous lesions) at $p=0.05$ level of significance. The results of bode and Nyquist plot also indicated that there was a significant differences in the frequency responses of malignant and normal breast tissues particularly in their magnitude and phase values. The results of this study strongly suggested that BIS (Bioimpedance Spectroscopy) technique can be used to identify breast cancer. However, large scale and in-vivo study required before applying as routine clinical instruments*.



Key Words: **Ad5933evalboard, AFE circuit, Bioelectric Impedance Spectroscopy, Breast Cancer, Excitation Current, Frequency Ranges**, **Significant Difference**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ADC** | Analog Digital Converter |
| **AFE** | Analog Front-End |
| **AVOVA** | Analysis of Variance |
| **BIS** | Bioimpedance Spectroscopy |
| **CMRR** | Common Mode Rejection Ratio |
| **CVC** | Current to Voltage Converter |
| **DSP** | Digital Signal Processor |
| **EC** | Extracellular |
| **EIS** | Electric Impedance Spectroscopy |
| **FNAC** | Fine Needle Aspiration Cytology |
| **GUI** | Graphical User Interface |
| **IC** | Intracellular |
| **$I^2C$** | Inter-Integrated Circuit |
| **IMIX** | Imaginary Index |
| **MIX** | Magnitude Index |
| **MRI** | Magnetic Resonance Imaging |
| **MSE** | Mean Square Error |
| **PGA** | Programmable Gain Amplifier |
| **PIX** | Phase Index |
| **RC** | Resistor and Capacitor |

| | |
|---|---|
| **RIX** | Real Index |
| **TUS** | Tissue Under Study |
| **USB** | Universal Serial Bus |
| **VIN** | Voltage In |
| **VNA** | Vector Network Analyzer |
| **VOUT** | Voltage Out |
| **WHO** | World Health Organization |

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# CHAPTER ONE

# INTRODUCTION

## 1. 1 Background of the Study

The breast is an organ whose main role is producing milk for breast feeding. Medically it recognized as mammary organ since it holds mammary gland used to produce milk. This mammary gland are have several structure which let the milk production. For example lobules are the special tissues that situated in the breast containing some mammary glands. In most females breast growth during puberty stages and particularly expands during lactation. Glandular tissues, connective tissue, and adipose tissue are the major tissues that construct breast in both females and males. Anatomically, the male breast is nearly identical to that of females but the male breast tissues is lacking specialized lobules since there is no physiological need for milk production in male breast [1].In female breast, the largest ducts are situated between lobules and nipple to connect the two structures. Nipples are the structure of breast which allows milk to come out during breast feeding. Human breast does not has muscular tissues. However, breast tissue is located on top of the muscles of the chest wall.

### 1.1.1 Breast Cancer Overview

Cancer is a diseases involving abnormal or unrestrained cell growth within the organ with the likely to propagate to rest parts of the body so called primary and secondary organs. Thus breast cancer is one type of cancer which involves the growth of malignant tumor within the breast that start at a particular parts of breast and then invades the rest part of breast structure through metastasis. In other way, breast cancer is the uncontrolled growth of breast cells, occurring in women but occasionally also in men. Generally speaking, cancer is defined as a mutation of gene that regulates cell growth of breast tissues. The genes act as manager in the cell's nucleus, directing all functions of the cell including growing and reproducing. Normally, healthy parent cells divide into daughter cells under the control of genes, doing so whenever a bunch of cells are dead, or to repair an injury when cells are worn out. Healthy new cells take over the tasks of the old ones. In the case of cell abnormalities, the genes are damaged and the cells preserve dividing and growing violently, causing in the formation of a tumor [2].

### 1.1.2 Breast Cancer Detection Techniques

In the past times several breast cancer detection systems have been designed to reduce the occurrence of breast cancer globally. Nowadays the most commonly used systems for the detection of cancerous tissues in breast includes X-ray mammography, ultrasound and magnetic resonance imaging (MRI). Mammography, designated as the "gold standard", is typically the first technique used on a patient. If the mammogram shows anomalies, it is then followed up with ultrasound, or a biopsy, to confirm results [3]. Mammography is the procedure of imaging breast tissue with x-rays for screening or diagnostic purposes in detecting or diagnosing cancer [4].

Ultrasound is commonly used after an irregularity is found in a mammogram to establish whether it is a non-threatening cyst or a malignant tumor [5]. Ultrasound images are made by sound wave reflections off the body. This technique has the benefits of being non-invasive, involves no ionizing radiation, has portable equipment and is comparatively cheap. However it cannot always decisively identify malignancies, therefore, biopsies are occasionally required as a follow-up procedure. Like ultrasound, MRIs are used after mammograms to regulate whether irregularities are destructive or not. MRI is high resolution and non-invasive, but it is very expensive. MRI machines are also large and thus not mobile, and scans can be time-consuming and are unreachable for consistent screening.

In spite of the existence of these systems in global market, several researches come up with their critical drawbacks which highlights the need for an enhanced technique for detection of breast cancer that satisfies the need of wider community. For example, X-ray mammography it is problematic to identify lesions when the breast tissue is very dense [3], [4] . Further, X-rays are a impose ionizing radiation, so there are health concerns related with undertaking mammograms too repeatedly. MRI is high resolution and non-invasive, but it is very expensive. MRI machines are also large and thus not mobile, and scans can be time-consuming and are unreachable for regular screening.

Nowadays, bioelectric impedance spectroscopy is one such technique that is being explored as an alternative way for identifying the presence of malignant tissue inside the breast. It is a well-established and non-invasive method which used to regulate the bioelectrical impedance, which is a measure of the resistance of tissue to the flow of an electrically applied current [6]. Electrical impedance spectroscopy (EIS) studies the frequency response of the electrical impedance of tissues

which allow us to identify the malignant tissues development in breast. The electrical impedance of the biological tissue be subject to the tissue structure, tissue illness and frequency of the applied signal. Similarly, as the capacitive reactance of the cell membrane changes with the signal frequency, the bio impedance also alters with the frequency of the applied electrical signal [7]. Therefore the current penetration and conduction paths considerably changes with the frequency of applied signal which allow us to discriminate malignant and normal tissues based their frequency responses.

Studies showed that electrical impedance examination of biological tissues provides the potential for a safe, simple, portable and low-cost method for a comprehensive range of bio-medical applications which includes the identification malignant tissues of breast through breast tissues characterizations [7] [8].The idea of Bioelectric Impedance spectroscopy is primarily grounded on the bulk electrical properties of tissues. These properties are used to govern the passage of current flow through the body. In the perspective of bioelectrical impedance, dielectric property of a biological tissue usually implies to its electrical resistance(R) and electrical reactance (X).

These two parameters are called the main intrinsic electrical properties of the tissues. Electrical resistance(R) of tissue is a measure of the tissue's ability to resist i.e. oppose the charge pass through it. Electrical reactance(X) of tissue provides a measure of polarizability of the tissue, i.e. its ability to store charge via electrical capacitance. Therefore, by theoretical definition, Bio impedance is a complex number (Z) which has a real part, represented by the resistance (R) and an imaginary part, represented by the reactance (X).

$$\boldsymbol{Z^2 = R^2 + jX^2} \qquad (1)$$

In other words, impedance can be defined as the frequency domain ratio of voltage to current [9]. The magnitude of the complex impedance is the ratio of the voltage amplitude to the current amplitude, while the phase represents the shift in phase of the current with respect to the voltage. Analytically, given an AC (Alternating Current) and voltage, defined as:

$$\boldsymbol{V = [V]e^{-(i\omega t+\varphi v)}} \qquad (2)$$

$$\boldsymbol{I = [I]e^{-(i\omega t+\varphi i)}} \qquad (3)$$

The ratio of current injected to biological tissues and voltage developed due to opposition to current gives bio impedance of biological tissues

$$\mathbf{Z} = \frac{[\mathbf{V}]\mathbf{e}^{-(\mathbf{i}\omega\mathbf{t}+\boldsymbol{\varphi}\mathbf{v})}}{[\mathbf{I}]\mathbf{e}^{-(\mathbf{i}\omega\mathbf{t}+\boldsymbol{\varphi}\mathbf{i})}} = \mathbf{Z} = \mathbf{e}^{(\boldsymbol{\varphi}\mathbf{v}-\boldsymbol{\varphi}\mathbf{i})} \quad (4)$$

Where $\frac{[V]}{[I]}$ is the impedance magnitude and $\boldsymbol{\varphi v} - \boldsymbol{\varphi i}$ the phase angle? [6]

Theoretically, the term bioimpedance or biological impedance manifest both the resistance of tissue's fluid and capacitance of cellular membrane at specific applied excitation signal frequency. The capacitive properties of living tissues shows noticeable changes when the excitation current frequency is changes from lower to higher ranges. At lower driving frequency, the capacitive behaviors of cellular does not have any role to the measured impedance data since cellular membrane performances as short circuit at lower frequency particular less than 1KHZ [9].Though, the cellular membrane starts to allow the passage of excitation signal as driving frequency increasing and thus it's possible to measure both the reactive and capacitive values as real part and imaginary part of impedance data. The reactive and resistive values of impedance data allow us to discriminate many biological tissue's pathological condition since they are conveys generic information about the fundamental physiological status of particular tissues. Four main specific dispersion (energy loss) windows have known to offer related information about most alterations of tissue's electrical properties, such as the accumulation of fluids or cellular elementary structural changes. These four dispersion windows includes, α, β, δ and γ. [10].

Therefore, in this study the Bioelectric Impedance Spectroscopy was used to characterize the breast tissue samples in ex-vivo setup to distinguish and analysis the frequency response of malignant and normal breast tissues. The four electrode configuration (on the right side of figure 1) was used since it considerably reduce skin to electrode polarization of soft tissue and lets the correct measurement of tissue's impedance. The AFE (Analog Front End) circuitry was designed to achieve this electrodes outline. The performance of the system was tested by using RC (Resistor and Capacitor) circuit through the variations of resistors and capacitors as it is discussed in chapter three materials and method section.

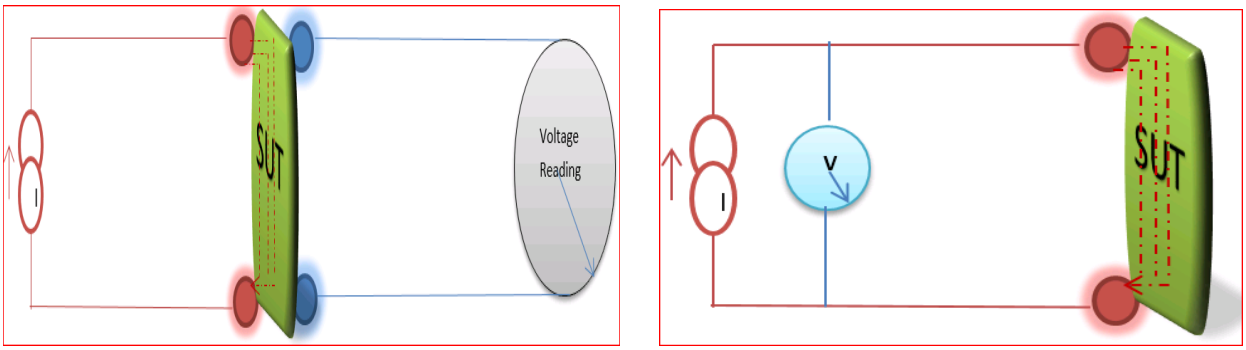



Figure 1 Two electrode (left) and four electrode (right) configuration

In bio impedance spectroscopy, a small amplitude electrical current is applied to the living tissues (cells, tissues or body parts) and the surface capacities are measured to estimate the bio impedance for their non-invasive characterization.AD5933 evaluation board was used to provide excitation signal with frequency spectrum ranging 1KHZ-100KHZ.The frequency response of breast tissues was logged on computer through interfacing the evaluation board with computer via USB(Universal Serial Bus) cable. The frequency response of those tissues contains impedance data which expressed in four parameters; magnitude, phase, real part and imaginary part of tissues impedance These parameters were obtained at each frequency sweep (points).AD5933 evaluation has user graphical interface which installed in computer and helps to program the board as well as assists in logging the impedance data on personal computer for further data analysis. Table 1 shows the important input parameters that were selected from the GUI (Graphical User Interface) part of AD5933 Evalboard software.

Table 1 Input parameters used to characterize the breast samples

| **Input Parameters** | **Values** |
|---|---|
| Peak to Peak Voltage | 2V p-p |
| Start Frequency | 1KHz |
| Frequency increment | 10KHz |
| Number of Settling Time | 15min |
| Excitation Frequency Range | 1KHz-100KHz |
| Internal Clock | 16 MHz |

## 1.2 Statement of the Problem

Breast cancer diseases are drastically increasing both in incidence and mortality globally. The factor behind these problems are difficult but reflect both aging and growth of the population within a given country. On top of this, irrespective of Country's economic profiles, breast cancer incidence is dramatically spreading both in developed and developing regions. Its reported that nearly 18.1 million incidence and 9.6 million cancer deaths were occurred globally [11].The Global Maps Presenting the most common type of Cancer Mortality by Country in 2018, showed that breast cancer is found to be the most highly happening cancer mortality as compared to other types of cancerous diseases.

There is noticeable increase of breast cancer mortality and incidence in most Sub-Saharan countries. It was indicated that an increasing public health problem in a continent with existing set-ups having been developed mainly for maternal, child health and infectious diseases were intensified the problems associated with breast cancer in African [12] [13].Moreover, Breast cancer is one of the predominant disease happening in women of Ethiopia .As reported by Addis Ababa Cancer registry office breast cancer holds about 34% of all female cancer cases, followed by cervical cancer at which is about 16% [14]. Even though breast cancer has a evidently higher incidence in developed countries, half of new breast cancer diagnosis and about 60% of breast cancer deaths is happening in the developing world .

As evidences show, many techniques have been developed to detect or diagnosis the development of breast cancer. However, they come up with their own drawbacks to fulfill the actual need on the ground. Currently, most existing technologies being used for cancer detection are costly, large in size, impose radiation to the users (x-ray) and unreachable to the wider community which is suffering from a delay in diagnosis of breast cancer which lets the treatment very difficult. Despite the fast spreading of breast cancer, there is a lack of a system that offers the potential for a safe, simple, portable and low-cost technique to determine and monitor tissue's physiological changes due to pathological conditions. Besides to this, several studies have been conducted to characterize the breast tissues and to identify malignant tissues from normal tissues for the purpose of detecting cancer in human breast. Even though they have contributed their own effort for the breast tissues characterization they come up with some critical gaps which highlights the need of other improved techniques

## 1.3 **Objectives and Hypothesis of the Study**

### 1.3.1 General Objective

The main objective of this study is to investigate the possibility of using bioelectric impedance spectroscopy for breast cancer detection through the characterization of normal and malignant breast tissues based on their frequency response.

### 1.3.2 Specific objectives

1. To design and test the AFE (Analog Front End)circuit for ad5933 impedance analyzer board
2. To evaluate the performance of electrical impedance spectroscopy on RC circuit
3. To analysis the effect of frequency change on the response of malignant and normal breast tissues
4. To determine the optimum frequency point at which malignant and normal tissues are more distinguished

### 1.3.3 Research Hypothesis

It is possible to distinguish malignant and normal breast tissues through breast tissues characterization based on their impedance parameters with frequency spectrum.

## 1.4 Significance of the Study

Electrical impedance analysis of biological tissues offers the potential for a safe, simple, portable and low-cost technique to determine and monitor tissue's physiological changes due to pathological conditions. Therefore, the motivation of this research is derived from the fact that bio-impedance spectroscopy technology has a great potential to be used as non-ionizing, easy and low-cost tool for breast tissue characterization for the purpose of distinguishing malignant breast tissues from the normal one. Furthermore, it is evident that once the system is developed based on this principle it has the following important advantages

- EIS equipment is easy to use, is noninvasive, and inexpensive, a fraction of the cost of ultrasound machines.
- EIS has been shown to detect cancerous lesions as small as 3 mm in diameter.
- Electrical impedance data may be obtained easily; acquisition takes about <15 min.

The outcomes of the study may very supportive to indicate the proper and timely intervention strategies and thus can provide the most reliable approach to address level of awareness community pertaining to breast cancer screening and diagnostics techniques. Currently Ethiopia has announced countrywide strategic action plan on non-communicable diseases control so, it is the proper time to conduct such a study to give an attention to the breast cancer and new way of breast cancer identification tool which is based on EIS (Electrical Impedance Spectroscopy). It also helpful to provide relevant information for future studies on breast cancer detection and biological tissues characterizations.

## 1.5 Scope of the Study

This study was particularly aimed at characterizing breast tissues by using bioelectric impedance spectroscopy in ex-vivo setup. Breast tissues were taken from patients through biopsy procedures and their frequency response were analyzed by using impedance data analyzing software such as MATLAB, AD5933EValboard and Microsoft excel. In this study the AFE circuit was designed to customize the AD5933 evaluation board for soft tissues application. Generally, this study is aimed to provide preliminary information to detect the existence of breast cancer based on the breast tissue's frequency response and in -vivo test was not performed due to ethical consideration.

## 1.6 Organization of the Study

This study is segregated into five chapters, in sequential order, to help the reader to grasp and understand the work efficiently. From Chapter 1, the reader will get the overall background of the study including the basic concepts bioelectric impedance spectroscopy followed by the base problem, the purpose and the significance, scope of the research work. Chapter 1 also includes the general and specific objectives to be achieved in this research. In Chapter 2, related works on the area of breast tissues characterization is reviewed. Chapter 3 will explain the methodology and materials used for this research. Chapter four is explain about results obtained from the study and discussion. Finally Chapter five concludes the findings of this study and recommends the future directions

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Basics of Dielectric Properties of Biological Tissues

The electrical properties of biological tissues have been the attention of study for a century for several reasons. In fact, they govern the paths of current flow through tissues and therefore it's very vital to study the widespread range of biological tissues frequency response. Biological tissues have the capability of conducting an electric current and thus have an impedance parameters which related with them. Electrical resistance and capacitance are the parameters which are highly carries information about dielectric properties of biological tissues. These parameters are frequency dependent i.e. their values are changes along with change of driving frequency. More importantly, the electrical capacitance and resistance are decreasing when the excitation current frequency meaningfully increasing. This due to different components of tissues (extracellular medium, cell membrane and intracellular medium) contribute to the impedance in changing amounts at each frequency points of excitation current [15].The previous studies of biological tissues and electricity played a significant role to the discovery and characterization of electrical properties of tissue. In the context of bioelectrical impedance, dielectric property of a biological tissue usually refers to its electrical resistance (R) and electrical Capacitance (X). These two parameters are known as the main inherent electrical properties of the tissues [16]. Electrical resistance (ohm) of tissue is a measure of the tissue's capability to oppose flowing current whereas Electrical capacitance of tissue provides a measure of polarizability of the tissue, i.e. its ability to store charge via electrical capacitance. A substantial alteration of dielectric properties in certain frequency range, by most often is known as a dielectric distribution. Any variations in tissue composition may causes a change in the tissue electrical properties [17]. This standard has been used to detect or monitor the incidence of several illnesses or conditions such as development of cancerous cells in biological tissues which includes breast tissues.

## 2.2 Related Work on Dielectric Measurements

A lot of techniques have been introduced to detect the development of cancerous tissues in human breast. For the purpose of breast tissue characterization the bio impedance analysis attracted the

attention of many researchers in past decades. Wide research has determined that there is a noteworthy difference between the values of these properties for healthy and malignant breast tissue. Since the late 1940s, several studies have been conducted to understand the dielectric properties of living tissues in terms of their permittivity and conductivity with various frequency ranges via varied measurement procedures.

The study conducted by Chauveau et al. [18] Have examined the need to analyze the tissues electrical parameters over a different frequency ranges. The measurement was performed in ex-vivo set for normal and pathological breast tissues with minimum current frequency range of 10 KHZ to 10 MHZ. Accordingly, from these measurements and a model that contains a constant phase components, the extracellular resistance, the intracellular resistance, and the membrane capacitance were calculated to model the equivalent tissues network. From the measurements and equivalent model circuit values, three indices are defined for categorizing tissue pathological conditions. Based on the results of this study, it was recommended that these parameters, and the indexes based on them, would let cancerous tissues to be distinguished from normal tissues and those with fibrocystic changes.

The study by Piperno, G et al. [19] used a parametric model to developed and define the variation of dielectric properties of tissues as in terms of excitation current frequency ranges. The experimental set up with signal frequency ranging from 10 HZ to 100 GHZ was used to model the four dispersion regions. The construction of this model was based on the data acquired from rigorous literature survey and parameters obtained from Cole-Cole model calculation techniques. The goal of this study was to allow the prediction of dielectric data that were in line with those enclosed in the vast body of literature on the subject. The analysis was carried out on a Microsoft Excel spreadsheet. Parameters are given for 17 tissue types. The outcomes of this study proposed that at lower frequencies, where the data values are not easily accessible and reliable, it's rigorously recommended to use the tissues equivalent model to analysis the frequency response of tissues with careful information extraction techniques. Those fundamental information that provides 'best estimate' based on present knowledge and important to be aware of the restrictions of the model mainly where there are no data to support its estimates.

In the past years, most of the study in the field of dielectric property measurements, have been conducted at high frequencies (20 MHZ-20 GHZ), by applying network vector analyzer (VNA)

and open-end coaxial cable. The VNA based method has continued the method of choice for the dielectric description of biological materials at high frequencies. This practice involves pressing the coaxial probe against the tissue while reflection coefficient or admittance of the probe sample boundary is measured and used to approximate the specimen's dielectric properties. In the study Trans Scan were used to study 100 suspicious breast lesions and compared their results with MRI and US scans [20]. According to the study findings, it's found that the Trans Scan 2000 system had 81% sensitivity and 63% specificity in detection and differentiation of the breast malignant and benign lesions. It is noteworthy that these values were higher than the sensitivity and specificity values of typical x-ray mammography systems as the sensitivity and specificity of such systems are about 40% .However [21] showed that the Trans Scan system is only capable of identifying highly conductive inclusions located close to the breast's surface and therefore the Trans Scan mammography system still suffers from limited detectability depth.

Another study was performed by C. Gabriel et al. [22], which aimed to characterize the electrical properties of normal (healthy) breast from 0.5 to 20 GHZ. This was the first time measurements were taken from such a large number of samples, a total of 354 from 93 patients. The tissue to be measured was taken from afresh removed tissue during reduction surgeries. The measurements happened within five minutes to five hours from the time the tissue was removed from the body. To acquire the measurements, a precision open-ended coaxial probe with a tiny sensing area (depth of 3 mm) was positioned on the sample. The probe was attached to either an Agilent 8720ES or Agilent 8722D vector network analyzer (VNA), which recorded the measured values. This study determined that the contrast in dielectric properties between malignant and mostly adipose tissue can be as high as 10:1. However, the difference for malignant and glandular tissues is only about 10%. The variability in dielectric properties due to other factors, such as patient age, sample temperature, and time from removal to measurements were all found to be insignificant. The variations from patient to patient and from sample to sample or breast to breast within patient were also determined to be statistically irrelevant.

In order to examine the variability of the dielectric properties of the breast tumor tissues, Sugitani et al [23]conducted large scale study on breast and surrounding tissues. The researcher used Agilent E5071C vector network analyzer (VNA) and an open-ended coaxial probe of Agilent 85070 dielectric probe kit to measure the Dielectric properties of breast tissues were measured in

the frequency range of 0.5–20 GHZ. The findings of this study indicated that both the conductivity and permittivity of cancerous tissues four times greater than its corresponding normal breast tissues and even though these dielectric properties difference was not found to be significant particularly for cancer and stroma tissues. At the frequency of point of 6GHZ the dielectric constants of the cancer tissues were distributed from 35 to 65, whereas those of stroma tissues were from 15 to 50.Thus the study concluded that the dielectric constants of the stroma tissues were close to those of cancer tissues.

Another study by S.M. Salvador et al [24] created a well-organized breast phantoms which mimic actual breast tissue as correctly as possible. In order for their phantoms to mimic actual breast tissue accurately, numerous tissue types were combined into the breast phantom in such a way that both their dielectric properties and physiological layout estimated those of a real breast. They select four tissue types that were incorporated in the breast phantoms: fat, gland, skin and tumor. These tissue phantoms are first mixed separately and their relative impedivity and conductivity measured and then they are combined into comprehensive breast phantoms. In this work the researchers noted that for the tumor and skin phantoms, the match between their measurements and real tissue measurements in both electrical resistance and capacitance was good, particularly when compared to the inherent distinctions in tissue properties from breast to breast and patient to patient.

The study conducted by Moqadam SM et al [25] was aimed at characterizing breast tissues based the time of their removal and ambient temperature effect on the dielectric properties of breast tissues in ex-vivo experimental setup. Therefore, this study examined the samples of breast tissues based on their delay of time during surgery to measurement. The study also investigated the effect of temperature alteration on the dielectric properties of tissues under study. Finally, this study concluded that there is direct relationship between temperature changes and dielectric properties of breast tissues .i.e. as the temperature of the ambient environment changes, the values of dielectric properties of breast tissues also changing. The dielectric properties of excised breast tissues were measured the dielectric properties at 27 °C, 20°C, 5°C, 10°C and −10°C. Even though in the study conducted by [26] it was assumed that variability in dielectric properties due to temperature of the sample is negligible.

Recently, numerous studies and investigation are being undertaken to understand the possibility of discriminating malignant and non-malignant tissues of breast and other biological tissues based on their dielectric property (impedance) variations. Remarkable evolution has been made in the enhancement of dielectric measurement apparatus and in the modification of the measurement methods which, aimed at further refining existing dielectric measuring tools. However, today, there is still a need for additional study and exploration to differentiate malignant and non-malignant tissues of breast so that timely revealing and management of cancerous disease is possible. Over the years, many *in vitro* and *in vivo* studies have been performed to study the dielectric properties of cancerous and normal breast tissues. The results of these studies are summarized in Table 2.

Table 2 summary of the studies conducted on dielectric properties breast tissues

| **Author** | **Frequency Ranges** | **Major findings of the Study** |
|---|---|---|
| Fricke *et al*. [27] | 20KHZ | From the samples of breast fat, gland, mastitis, fibro adenoma and carcinoma, the extracted the values of cellular fluids resistance and cellular membrane capacitance. It was found that, the permittivity of cancerous tissues was higher than the normal fat and gland tissues at 20KHZ frequency point. |
| Singh *et al*. **[**28] | 100HZ-100KHZ | In this study in-vivo dielectric properties measurement was conducted on female breast with and without tumors. The finding showed that, the tumor breast has lower resistance and higher permittivity as compared to the normal breast tissues. |
| Chaudhary *et al*. [29] | 3MHZ - 3 GHZ | They removed normal and cancerous breast tissues and studied their frequency parameters in terms conductivity and permittivity. It was indicated that, cancerous breast tissues have higher dielectric properties as compared to normal tissues. However, the difference was very minimum. |
| Surowiec *et al*. [30] | 20KHZ - 100 MHZ | They performed in *vitro* dielectric studies in three dissimilar samples of breast tissues They found higher conductivity of cancerous tissues than normal (2 to 4mS/cm that of the tissue surrounding the tumor). |

| | | |
|---|---|---|
| Jossinet [31] | 488HZ-1MHZ | In this work six groups of people were examined in-vivo setup for the measurement of dielectric properties of normal and cancerous breast tissues. They found that the variability of dielectric properties of breast tissues is much less above frequency ranges of 10KHZ. |
| Morimoto *et al*. [32] | 0 - 200 KHZ | They measured the dielectric parameters of breast tumors *in vivo.* It was determined that there are significant variances between normal and cancerous tissues. Nevertheless, it has been reported that malignant tumors have lowered capacitance compared to benign tumors. This is different from the findings of the study performed by Jossinet (31) and Fricke (27) which have documented higher capacitance values for malignant cancers. |

## 2.3 Summary of Literature Review

As briefly discussed above under literature review section, several studies have been conducted to characterize the breast tissues and to identify malignant tissues from normal tissues for the purpose of detecting cancer in human breast. Even though they have contributed a lot for the breast tissues characterization they come up with some critical gaps which underscores the need of other improved techniques.

For instance, most of the researchers used high frequency ranges (GHZ) to characterize the breast tissues. However, it is noteworthy that impedance data acquisition and characterization of biological tissues is highly advantageous at low rather than high frequencies. This is due to the higher contrast of capacitance and phase angle data at low frequencies. Moreover, dispersion (energy loss) in biological tissues decreases significantly at low frequencies, rendering impedance spectroscopy safer and accurate [33].In the study conducted by [26] it was assumed that variability in dielectric properties due to temperature of the sample is negligible. However, it is found that [25] the electrical properties of tissue also be determined by its temperature. The movement of the ions that carrying the current increases as the temperature extracellular fluid declines.

Most researchers were used vector network analyzer (VNA) to provide excitation current and sense voltage drop across the probe or electrodes which found to be very expensive and large in size for wider application developing countries like Ethiopia. Furthermore, vector network analyzer involves two electrodes measurement setups for alternating current. But it can cause signal noise

for direct current measurements because of the electrode polarization that subsequently contributes to inappropriate results for the conductivity of the sample between the electrodes [34].

The transmission line method (Trans Scan 2000) was used by several studies as it was mentioned in section 2.2 (literatures review) part. However, as it was showed in the research conducted by [35] the techniques of transmission line is not recommended for ex-vivo impedance measurements particularly for semisolid or solid breast tissues samples. In contrast to this it's very appropriate method for fluidic samples measurements. Currently, the open-ended coaxial cables are frequently used for dielectric properties measurements even though they considered the sample under study is homogenous sample which has uniform and well defined shape. In practical is very difficult to get sample such like shape and dimensions. Therefore, heterogeneous sample shape is very challenging to be measured by this techniques [36].

In this study, the aforementioned gaps were addressed through considering the critical factors that affects the accurate measurements of bioelectric impedance of breast tissues. Study conducted by [37] recommends beta and alpha excitation current frequency for biological tissues characterization. Beta ($f$ <1000 HZ) and alpha (1000 HZ < $f$ < 100 MHZ) areas are predominantly advantageous for tumor detection and measurements, since most changes between normal and pathological tissue seem to appear in this frequency range. Therefore 1 KHZ – 100 KHZ excitation current's frequency range was used in this study to characterize the breast tissue i.e. in the recommended ranges. In this study AD5933 EvalBoard was used as it comprises components of complete digital impedance meter. The AD5933 integrated circuit is an interesting solution for impedance measurements.AD5933 board is less expensive as compared to VNA and Trans Scan TS2000 which enable us for wider application in low resource setting areas. In order to reduce tissue electrode polarization effects, tetra polar electrodes method was employed by designing Analog Front End circuitry for AD5933Evalboard. The advantage of tetra polar electrodes method is that the polarization on the current electrodes has no effect on the voltage difference between the voltage electrodes [38].

# CHAPTER THREE

# MATERIALS AND METHOD

## 3.1 Study Area

This study was conducted at Jimma University Medical Center particularly at pathology department under the supervision of clinical collaborator Dr.Solomon Kebede. The former Jimma University University Specialized Hospital currently named as Jimma University Medical Center is of the well-known University Hospital serving many population of South West part of Ethiopia. Geographically, it is situated in Jimma city 352km southwest of Addis Ababa. This Medical Center is serving the society both as referral and teaching hospital so that it provide the inpatient and outpatient services for the community of Jimma Zone and Jimma University. On top of this, the center provides tremendous delivery cases for the population coming from its catchment areas which accounts around 17 million each year [39].

## 3.2 Study Design

In this study quantitative research approach was used to investigate the frequency response of breast tissues removed from patient during surgical biopsy procedures. Bioimpedance Spectroscopy method was used to characterize the frequency response of each patent's breast tissues in ex-vivo experimental setup. EIS (Electrical Impedance Spectroscopy) method selected since it is a well-established and non-invasive techniques which used to determine the bioelectrical impedance which enable ones to detect the malignant tissues development in breast and other biological tissues. A low amplitude alternating electrical signal with 1 KHZ-100 KHZ frequency range was injected to the tissues under investigation to characterize the sample in terms of its bioimpedance (reactance and resistance). The excitation current (sine wave) generator was AD5933 Evaluation board version 1.5 manufactured by Analog device Inc. in June 2018. This study was conducted in pathology department of Jimma University Medical Center from June 2019 to November 2019 G.C (Gregorian calendar).

## 3.3 List of Material Used

Table 3 List of Materials used for the Study

| S.No | Item Name | Function/role | Category |
|---|---|---|---|
| **1** | AD533Evaboard | A high precision impedance converter and frequency generator. Provides frequency sweep for breast tissues under test | Hardware |
| **2** | AD5933EVZ | A graphical user interface used for data logging and programming the evaluation board. | Software |
| **3** | Electrodes | 3M Silver Chloride (Ag/Agcl) electrodes used for data acquisitions from breast tissues | Hardware |
| **4** | MATLAB | Used for impedance data analysis via bode and Nyquist plot | Software |
| **5** | Microsoft Excel | It was used for statistical data analysis for impedance tissues | Software |
| **6** | AFE Circuit | Transforms the two electrodes of AD5933Evalborad to four electrodes setup | |
| **7** | Protues | It was used to design and test the analog front-end circuit | Software |

## 3.4 Ad5933 Evaluation Board Overview.

The AD5933 EvalBoard is impedance analyzer board with high processing capacity and good precision. It contains an on board frequency generator oscillator with 12bits MSPS and ADC (Analog to Digital Converter).The frequency generator allows the tissues under investigation to be excited with well-known amount of excitation signal. Furthermore, the frequency response of tissues under study is sampled with another on board digital signal processor component at each frequency points.AD5933 EvalBoard is also equipped with the DFT (Discrete Fourier Transforms) which transforms the time domain to its frequency domain. The AD5933Evalbaord also comprises of an internal temperature sensor with 13bits resolution. The AD5933 from Analog Devices Inc.

allows the carrying out of impedance measurements using a 2 electrodes measurement outline with a frequency range from 1 KHZ to 100 KHZ, and a system average error of 0.5%. The chip is equipped with a serial $I^2C$ (Inter-Integrated Circuit) interface to communicate with external devices using application software to control the internal functions of the chip and to regain the measured impedance data. The AD5933evalboard is a very small size impedance analyzer instrument (see figure 2) which allow the frequency response of biological tissues [40].

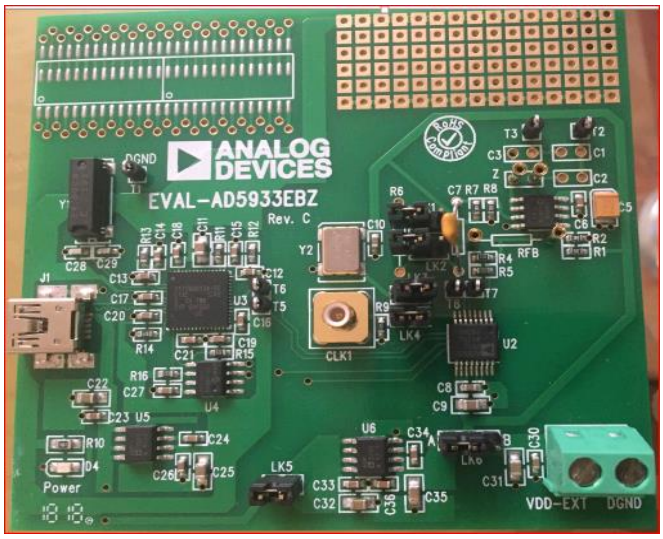



Figure 2 AD5933evalbaord manufactured by Analog device, 2018

The main functional blocks of AD5933Evalboard are categorized and explained below:

- **The transmitter stage:** consists of a direct digital synthesizer (DDS), which generates the digital sine waves, which can be configured for a specific impedance excitation frequency, a 12-bit digital-to-analog converter (DAC) and a programmable gain amplifier (PGA), which adapts the generated voltage applied to the impedance Z (w).

- **The receiver stage**: comprises a current-to-voltage converter (CVC), a voltage amplifier, an antialiasing low pass filter (LPF) and a 12-bit analog-to-digital converter (ADC). The receiver stage will adapt and convert the flowing current through Z (w) into a voltage that will be fed to the following stage.

- **The impedance estimation stage**: obtains 1024 sample points of the signals generated by the DDS module and the digitalized input signal x[n], in order to estimate the real and imaginary impedance values using DFT.

- **The chip control stage**: this comprises the $I^2C$ core module responsible for controlling all the chip functions, communicate with application software and storing the measured results.

## 3.5 Limitation AD5933Evalbaord

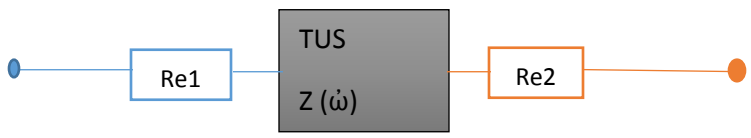
AD5933Evalbaord is the first impedance analyzer board which commercialized by analog device company and completely incorporated electrical impedance analyzer components, which might let the application of minimum-size instrumentation for electrical bioimpedance measurements. However, AD5933Evalbaord is not fit for most soft tissues electrical bioimpedance measurement applications for the following limitation.

### 3.5.1. DC Cancellation

Even though the AD5933 has a good DDS core that can provide very accurate excitation signals at different frequencies, this excitation signal has a DC-bias which is different for each excitation amplitude. A DC voltage difference across biological cells under test might cause polarization which disturbs measured impedance value .Therefore, a high pass filter is important to cancel the DC component of the excitation before reaching the sample under investigation. Thus, passive RC high pass filter should be employed with a specific cutoff frequency to avoid the interfering of dc signal and which is appropriate solution for dc signal cancellation

### 3.5.2 Two Electrodes Configuration

AD5933Evalboard is a two electrodes method impedance measuring device. In the 2 electrode technique, the same electrodes are used to provide current and sense the voltage developed inside the tissues under study. Therefore, such set up was found that it introduces unwanted impedance into the measurement which more probably considered as noise. If the same electrodes are employed to sense voltage and provide current as indicted below, there is probability that the current of excitation signal interfere into the measured voltage causing another impedance *Re1* and *R*e2 into the TUS (Tissue under Study).This impedance contributes a substantial value of impedance to the actual measured value of tissues impedance under test.



Thus, the 4 electrode method is a vigorous electrode arrangement that reduces the effect of the electrode impedance and the skin electrode contact impedance. This technique uses a couple of electrodes to excite the TUS and a different couple of electrodes to measure the impedance. In the case of current excitation, electrical current does not flow through the sensing electrodes and thus the sensed voltage does not contain any voltage drop caused by *Re1*and *R*e2.

## 3.6 Analog Front End Design

In order to solve the aforementioned gaps the AFE (Analog Front End) circuit is designed as it illustrated in the following sections .The functionality of this Analog front end circuit is to adapt the AD5933 Evalboard electrodes configuration from a 2 Electrode measurement system to a 4 Electrode measurement system. This way the polarization effect that introduced during two electrode measurement of AD5933 Evalboard is removed and possible for measuring the dielectric properties of soft tissues like breast. All these need be provided by the AFE while maintaining the signal input and output signals of theAD5933 Evalboard circuit within working levels, i.e. avoiding current or voltage saturation.

### 3.6.1 High Pass Filter Design

Filter is an electronic filter that permits signals with a frequency higher than a definite cutoff frequency and diminishes signals with frequencies lower than the cutoff frequency. The amount of attenuation for each frequency be subject to the filter design. In this study an excitation signal having a frequency range of 1 KHZ-100 KHZ was used to characterize the frequency response of breast tissues in ex-vivo setup. Therefore, a high pass filter with less than 1 KHZ cut off frequency was designed .The excitation signal having frequency less than 1 KHZ was blocked by this filter to avoid the electrode- tissues polarization effect which contributes noise during impedance measurements as it explained under section 3.5.1 of this chapter. The desired cut off frequency of 1 KHZ is selected based the general formula given equation 5 below.

$$\mathbf{Fc} = {}^{\mathbf{1}}\!/_{\mathbf{2\pi RC}} \qquad (5)$$

Where R is resistor, C is capacitor and Fc is cut off of frequency. In order to obtain less than 1 KHZ cut off frequency, R=22KΩ and C=7.2nF values of resistor and capacitor was used respectively.Ad5933evalbaord has low pass filter which attenuates any signal having higher

frequency greater than 100KHZ.Therefore , environmental frequency greater than 100KHZ can be blocked by this internal filter on the receiver stages.

### 3.6.2 Impedance buffering Stage

The main purpose of this stage is to provide electrical impedance transformation from high pass filter to current resistor with 1 KΩ to prevent impedance loading this resistor causes to high pass filter with low impedance value. This stage begins with the current setting resistor. In this stage, 1 KΩ resistor was used to obtain a constant current of 200uA .This amount of current was used as excitation current to characterize breast tissues. The amount of current flowing the sample is 200µA which is below threshold of perception (<1mA) which indicates the safe amount of excitation current. Trans-conductance amplifier is acting as buffer to match the impedance of high pass filter and current setting resistor since it has high input resistance(around 1Mohms) and low output resistance (typically tens of ohms).Unity gain was achieved by making a negative feedback from input terminal to output terminal. The input voltage and output voltage of buffer is the same (there is no a significant amplification).

### 3.6.3 Differential Amplifier

By definition, differential amplifier is an electronic circuit that amplifies the difference of two inputs. LM741 differential amplifier was used to design the amplifier circuit of the AFE.LM741 differential is capable of providing high gain and can support higher voltages at its input. It also much less sensitive to external noise due to its good Common Mode Rejection Ratio (CMRR>>1).The conceptual diagram of this differential amplifier is illustrated in figure 3.

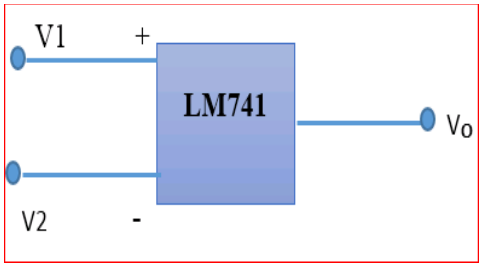



Figure 3 LM741 conceptual diagram

As it illustrated in figure 3, the output of differential is given in equation 6 and the differential gain is also given in equation 7. The Ad is the gain which differential amplifier amplifies the difference between two points.

$$Vd = v1 - v2 \quad (6)$$

Where $Vd$ output voltage, $v1$ and $v2$ are inputs to the differential amplifier.

Differential gain (Ad) is given by:

$$vo = Ad(v1 - v2) \quad (7)$$

From equation 6 and 7, the differential gain of the amplifier is:

$$Ad = vo/Vd \quad (8)$$

To understand the basic amplification characteristics of LM741 differential amplifier, simulation circuit was designed on protues software as indicated in figure 4 and the amplification performance was tested.

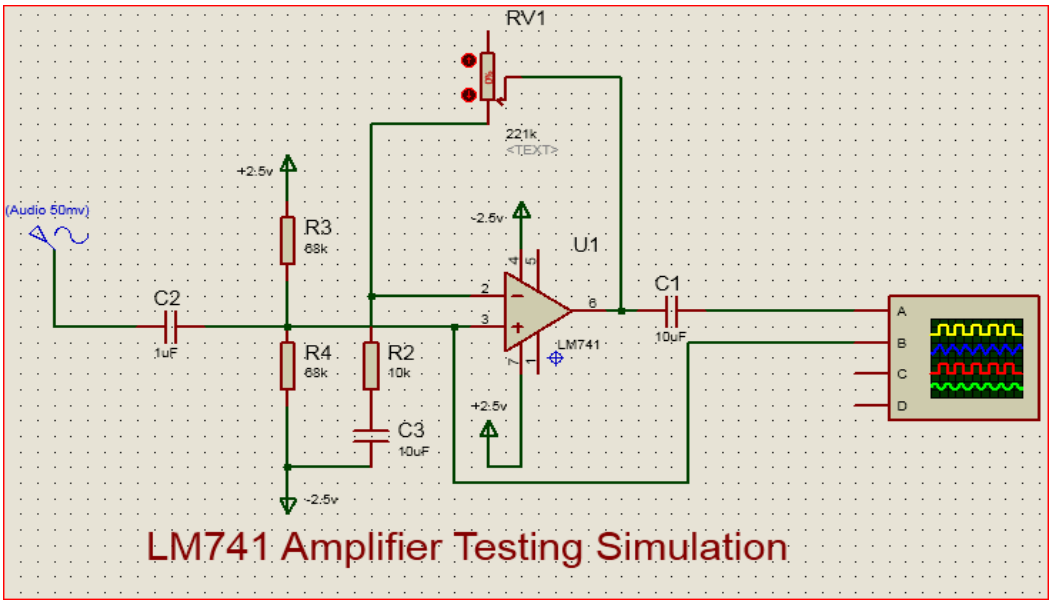



Figure 4 Circuit Design for LM741 simulation

After each components of the circuit were tested, the analog front end circuit was made on mesh board and connected to AD5933 Evalboard as indicated in the figure 5 .The components used on this mesh board were exactly the same as that of specified on protues simulation to maintain circuit characteristics. The functionality of each components were checked by using multimeter and digital oscilloscope and the results was the same as obtained from protues simulation. The

performance of AD5933evalbaord was tested with AFE and without AFE to observe the effects of AFE on the board performance particularly during lower driving frequency.

The AFE the removed the dc bias component from the voltage output of AD5933 with a high-pass filter at the first part of AFE. The AC(Alternating Current0 voltage from AD5933 Evalboard drives the circuit of AFE then injects an AC current into the breast sample with two electrodes. The amount current goes to samples is determined by current setting resistor found immediately after the first Trans conductance amplifiers. The amount of current excites the tissues is 200µA since Voltage coming from AD5933 is approximately 2V and the value of current setting resistor is 10 KΩ (ohms law). The AFE enabled the AD5933 Evalboard to avoid the phase shift problem which is more probably caused while measured soft tissues impedance with two electrode configuration i.e. it's a problem of signal cross talk when the same electrode injects current and measure voltage developed in tissues.

In general, the AFE proposed in this paper solved with basic limitation of AD5933 listed under section 3.5. It implements a comprehensive 4 electrode measurement arrangement, and fully adjusts AD5933evalbaord for electrical bioimpedance measurements of breast tissues. This accomplishment is attained by the addition of very a small number of ICs (Integrated Circuits), in principle only two, and a few passive components. This simple AFE, in combination with the AD5933 Evalboard impedance spectrometer, decreases the size and complexity of the electronics of electrical impedance measurement system. Figure 5 illustrates Ad5933evalboard connected AFE circuit.

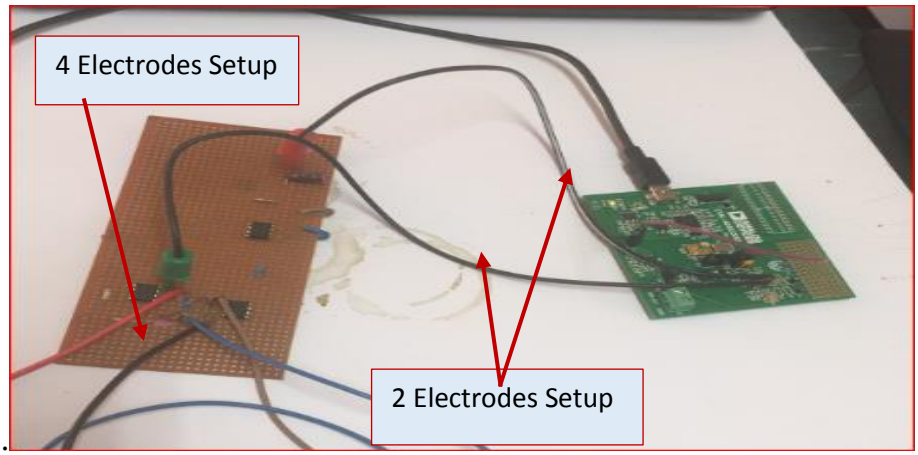



Figure 5 AD5933Evalboard connected to AFE circuit

## 3.7 Conceptual Diagram of Proposed System

As it is indicated in figure 6, AD5933 evaluation board was used as frequency generator which excites the external breast tissues samples with known frequency spectrum (1 KHZ-100 KHZ). The tissues frequency responses (impedance data) also sampled by on board ADC and the DFT is processed by on board DSP engine at each frequency points. The Ad5933 Evalboard contains fully featured graphical user interface software which allows impedance data logging on computer. By interfacing to the AD5933 board via a USB (Universal Serial Bus) cable, it easy to download the frequency responses of breast tissues under test on personal computer so that it has eased the impedance data analysis by MATLAB and Microsoft excel. AFE (Analog Front End) circuit was designed and integrated to ad5933 to transform the two electrode configuration of AD5933 evaluation board to tetra polar electrodes configuration. The functionality of this Analog front end circuit is to adapt the AD5933 electrodes configuration from a 2 Electrode measurement system to a 4 Electrode measurement system. This way the polarization effect that introduced during two electrode measurement of AD5933 Evalboard is removed for measuring the dielectric properties of soft tissues like breast tissues. The design process of analog front end circuit is discussed in result and discussion part of this document.

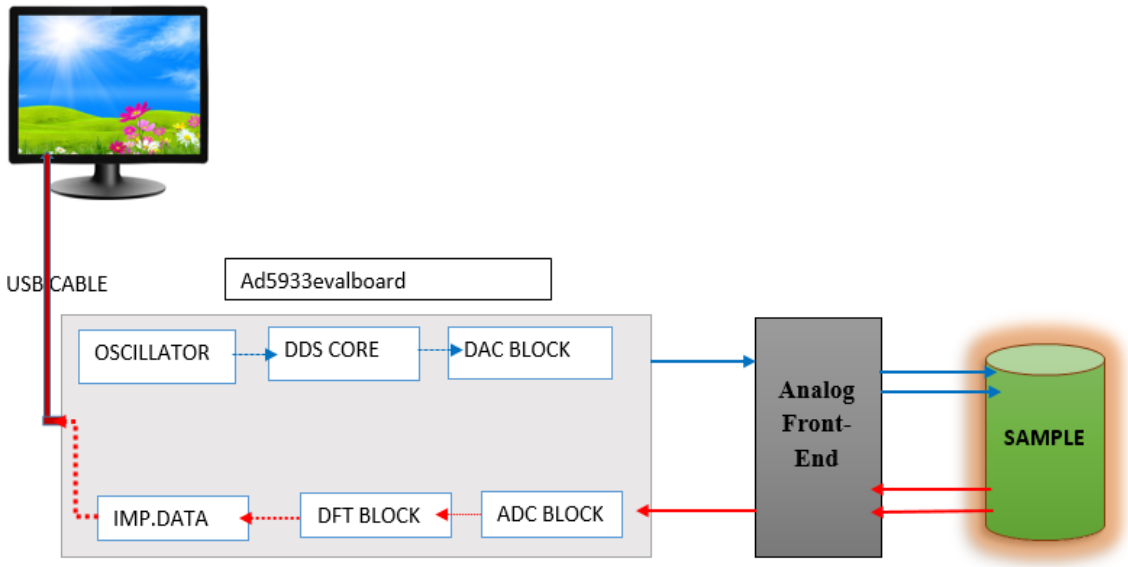
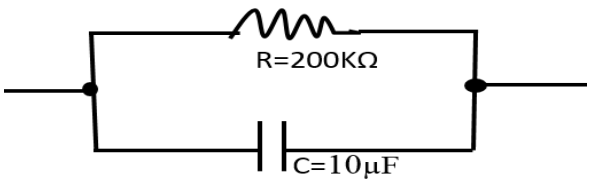


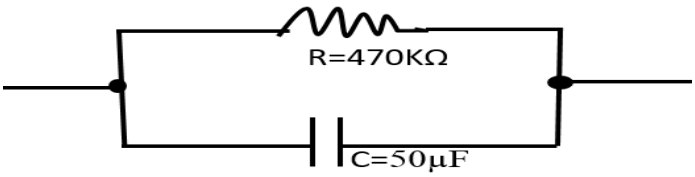
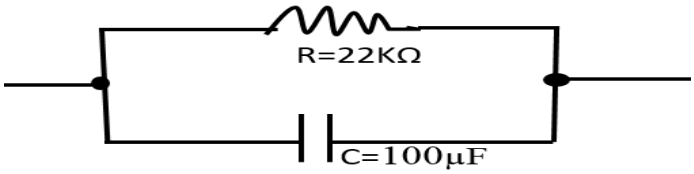

Figure 6 Schematic block Diagram of proposed method

## 3.8 System Calibration

AD5933 Evalboard was calibrated with different sets of capacitors and resistors (RC circuit) to evaluate its performance before measuring tissues dielectrics properties. The overall system was calibrated with analog front end and without analog front to measure the improvement made because of incorporating AFE to AD5933 Evalboard. The values of capacitors and resistors used to create RC are indicated in table 4. In the calibration a load with a known impedance value is placed between the exciting electrodes. Since the calibration, load (capacitor and resistor value) is known, the calibration factor can be calculated to compensate the error occurred. The AD5933 is specified to a typical system average error of 0.5 % within the frequency range of 1 KHZ up to 100 KHz.

Table 4 Comparison of actual impedance data and the data obtained from Ad5933evalboard for RC circuit

| **Values** | **Resistors(*KΩ*)** | **Capacitors(*μF*)** |
|---|---|---|
| Original | 200 | 10 |
| Measured | 200.123 | 9.955 |
| Error (percentage) | 0.061% | 0.449% |
|  | | |
| **Values** | **Resistors(*KΩ*)** | **Capacitors(*μF*)** |
| Original | 470 | 50 |
| Measured | 469.562 | 50.105 |
| Error (percentage) | 0.093% | 0.209% |

<table>
<tr><td colspan="3"> 
</td></tr>
<tr><th>Values</th><th>Resistors(KΩ)</th><th>Capacitors(µF)</th></tr>
<tr><td>Original</td><td>22</td><td>100</td></tr>
<tr><td>Measured</td><td>22.115</td><td>100.215</td></tr>
<tr><td>Error (percentage)</td><td>0.522%</td><td>0.215%</td></tr>
<tr><td colspan="3"> 
</td></tr>
<tr><td>Average error</td><td>0.225%</td><td>0.291%</td></tr>
</table>

The formula given in equation 9 was used to calculate the percentage errors.

$$\text{percentage error} = \left|\frac{\text{measured value} - \text{actual value}}{\text{actual value}}\right| * 100\% \qquad (9)$$

Figure 7 Ad5933Evalboard calibration Setup

### 3.8.1 Two-Point Gain Factor Calculation

According AD5933 manufacture guideline, AD5933 system must be calibrated for a known impedance range to determine the gain factor before any valid measurement can take place [49]. The gain factor is simply determined by placing known impedance between the input/output of the AD5933 and measuring the resulting magnitude of the code with parameters given in table 5 as input parameters.

Table 5 Input parameters used to calibrate the system with two-point gain factor calculation.

| Input Parameters | Values |
|---|---|
| Output excitation voltage | 2 V (p-p) |
| Calibration impedance value | 200KΩ |
| PGA gain | ×1 |
| Supply voltage | 3.3 V |
| Feedback gain resistor | 200 KΩ |
| Calibration frequencies kHz | 55 and 65 kHz |
| Excitation current | 200µF |

## 3.9 Description of Study Participants

The participants of this study were females having invasive ductal carcinoma breast cancer and treated at Jimma University Medical Center. The patient's clinical reports shows that all the participants' cancerous breast section shows mixed inflammatory tissues with predominance of neutrophils and lymphocytes a long with fibroblasts and bland glands ducts and stratified squamous epithelia. The pathology examination report of the study participants is summarized in table 6.

Table 6 description of study participants

| Subject Code | level of Cancerous | Pathological Descriptions |
|---|---|---|

| | | |
|---|---|---|
| **1060** | High Grade | Gray white well circumscribed upper outer measuring 3*2cm gray white with multiple brownish and darkish foci |
| **1167** | Low Grade | ovoid gray white firm ,encapsulated tissues specimen measuring 2.5 *2.5 cm |
| **1283** | Low Grade | ovoid gray white firm ,encapsulated tissues specimen measuring 2.5 *2.5 cm |
| **1283** | Low grade | ovoid gray white firm ,encapsulated tissues specimen measuring 2.5 *2.5 cm |
| **1312** | High grade | Solid white tumoral growth with high metastasis stages |
| **1347** | Low grade | Shows sheets, nets and branched malignant epithelia tissues |
| **1420** | High grade | Solid white tumoral growth ,delicate fibrous stroma separating the tumor |
| **1484** | High grade | Solid white tumoral growth ,delicate fibrous stroma separating the tumor |
| **1523** | High grade | Solid white tumoral growth with high metastasis stages |
| **1524** | High grade | Circumscribed soft tissues to firm tumoral growth measuring 2.3*3*1.5 |
| **1583** | High grade | Solid white mass which is 1cm near to nipple with tumor with lateral margin |
| **1112** | Low grade | Ductal carcinoma with feature graded irregular nuclear membrane |
| **1594** | Low grade | Circular mastectomy with firm covered with tumoral sheet |
| **1632** | Low grade | Section shows cords sheets and tubule round tissues with deep surgical margin |
| **1638** | High grade | Invasive ductal carcinoma grade 3 stage IIIA(T3N2aMX) |
| **1678** | High grade | 3*32*2cm grayish white firm mass with irregular radiating border |
| **1245** | High grade | Ductal carcinoma firms A2x including deep surgical margin |
| **1261** | Low grade | A2-2x superficial surgical margin with solid mass in left upper quadrant |
| **1388** | High grade | Invasive ductal carcinoma NOS(gradeIII stage PT2 No Mx) |
| **1486** | High grade | Ductal carcinoma NOS grade III deep surgical margin involved |

### 3.9.1 Histological Description of Cancer Grades

In this study, all cancer types were invasive ductal carcinoma which begins in the milk duct but has grown into the surrounding normal tissues in the breast. However, all breast tissue samples were labeled as low grade and high grade lesion with pathologist. Grading indicates how the cancerous tissues are differentiated and growing inside the breast as compared to the structure of normal breast tissues. Grading is the histologic description of how closely the cancer cells resemble their normal cells of origin. Low grade lesion are look a little bit like the normal breast tissues and

they are growing a little faster than normal tissues. As it shown in figure 8B, the microscopic section of low grade lesion shows cords sheets and tubule of round cells with vesicular chromatin and prominent irregular membrane and scanty glands infiltrating into desmoplastic adipose tissues. The glandular formation was around 75% and nuclear pleomorphic were larger than normal with open vascular nuclei. In this low grade lesion it was found that moderate variability in size and shape with mitotic count of 8-15 mitoses per 10 high power fields. On the other hand, high grade lesions are looks like very different in structures and anatomical morphology as compared to normal breast tissues. They are characterized by fast growing and associated with more aggressive behaviors .The compound light microscopic image of high grade lesion is presented in figure 8A.The pathological report of these cancerous lesions were indicate sheets nests and tubule like structures composed of malignant epithelia cells with scant cytoplasm and nuclei having prominent nucleoli which infiltrates to surrounding adipose tissues. In this category a high proliferative rate and areas of tumor necrosis are common. The mitotic account of this category was around 16 mitoses per 10 high power fields with less than 10 % tubular differentiation. The microscopic image of normal breast cancer also indicated in figure 8C which were used as control group.

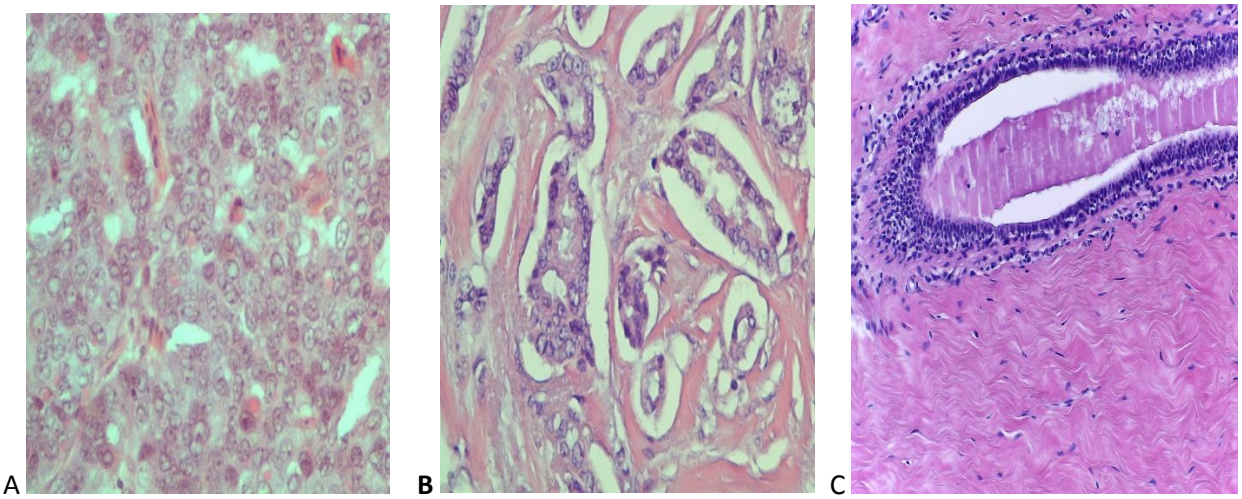



Figure 8 Microscopic image of high grade (A), low grade (B) breast cancer tissues and (C) normal tissue

## 3.10 Breast Tissue Collection Process

The breast tissues samples used in this study were collected from surgery resection during biopsy procedures. After surgeons removed the entire cancerous lesion from patients, they were labeled as low grade lesions, high grade lesion and normal tissues by the clinical collaborator of this study.

One malignant (either low grade or high grade lesions) tissues samples were taken from each 20 patient's breast and FNAC(Fine Needle Aspiration Cytology) procedures was used to determine the margin between cancerous and normal surrounding tissues .Since the healthy tissues surrounding cancerous was also removed during surgical procedures, a normal sample tissues was also collected from each 20 patients. Sample were placed in different plastic container closed tightly and then transported to the data measuring room. The time elapsed between tissues removal and measurement was approximately 20-25minutes.

## 3.11 Experimental Setup and Data Acquisition

In this work, healthy and malignant breast tissues of 20 patients have been gathered and characterized. Before measuring the dielectric properties of these tissues the distance between sets of electrodes were measured as indicated in figure 9. Study conducted by [41] stated that the distance between each electrodes i.e. distance between current and sensing electrodes and distance among sensing and current electrodes influences the measurement accuracy of dielectric properties of biological tissues. Therefore, the distance between similar electrodes was fixed as to be 25mm whereas the distance between opposite electrodes was fixed as to be 1.5cm as shown in figure 9. Furthermore, the thickness of each tissues were also measured so that they were fixed at 1.5cm to avoid tissue's thickness variation on excitation signal penetration. However, the size of samples were varying from 3*2.5 to 1.5*0.5 with arbitrary shape.

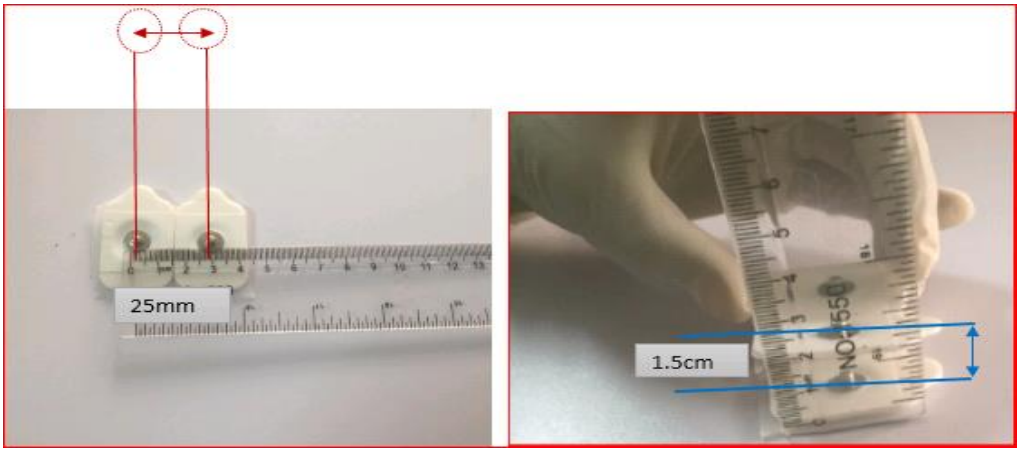



Figure 9 picture showing the distance between sets of electrodes.

On top of this, the study conducted by [25] found that the electrical properties of tissue depends on its temperature. The mobility of the ions that transport the current is increases as temperature

increase .Therefore, the temperature of the measuring system was maintained at 36.875 and 37.6875 throughout the data acquisition process.

### 3.11.1 Experimental Setup

As explained in previous sections, bioimpedance spectroscopy techniques was used to excite the breast tissues at different frequency points. The breast tissues samples were placed between four electrodes (two current electrodes and two sensing electrodes) as shown in figure 10 below. The tissues were placed inside measuring and current electrodes as tight as possible to avoid electrode movement which actually causes baseline wandering. Sensing electrodes were placed under the breast tissue sample while current electrodes were placed on top side of the samples. Every sample was measured four times and then the averaged values were taken.

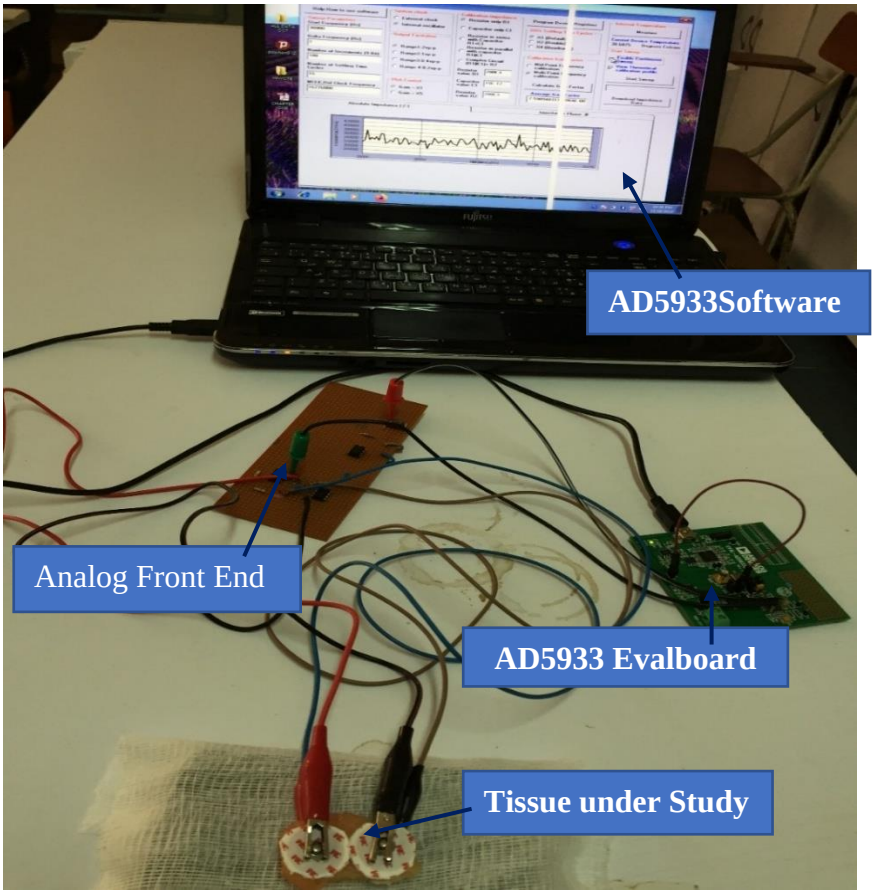



Figure 10 system experimental setup during breast sample measurement

Dielectric properties of breast tissues were measured in the frequency range of 1 KHZ–100 KHZ using AD5933 impedance analyzer evaluation board and 3M silver chloride (Ag/Agcl) electrodes. Breast tissues were excited at different frequency sweep with 2p-p voltage and 200μA alternating current for about 10 -15 minutes.

## 3.12 Data Analysis Techniques

After tissues impedance was measured as explained in section 3.11.1 above, impedance data was downloaded from AD5933 evaluation board to personal computer for data analysis. The impedance data of 20 samples were averaged at each frequency points i.e. 1KHZ,10 KHZ ,20 KHZ, 30 KHZ,40 KHZ ,50 KHZ,60 KHZ,70 KHZ,80 KHZ,90 KHZ and100 KHZ and then the standard deviation was calculated and included as error bar .The tissues impedance data containing four important parameters: Phase, magnitude, real part and imaginary part of tissues frequency response was analyzed using MATLAB (for Nyquist and bode plot) and Microsoft excel (for statistical analysis).Bode and Nyquist plots are a very useful way to represent the frequency response of biological tissues. Bode plot indicates gain and phase of a system as a function of frequency. This is referred to as the frequency domain behavior of a system whereas a Nyquist plot shows the complex plane plot of real part (resistance) and imaginary part (reactance) with frequency as an implicit variable. Therefore, these two plots were used to analysis the frequency responses of breast tissues measured in ex-vivo setup. Beside this, on the phase angle obtained from 20 patients' inferential statistical analysis were performed to investigate the significance differences among the three sets of breast tissues (low grade lesion, high grade lesion and normal breast tissues). Differences among samples were assessed using, Single factor one-way ANOVA(Analysis of Variance) followed by Turkey's honestly Significant Difference Post Hoc Test .The level of significance was set at P=0.05 for all statistical analyses as it discussed in results and discussion part of this document. Generally, the following flow chart (figure11) illustrates the overall steps (work flow) and activities performed during data acquisition process.

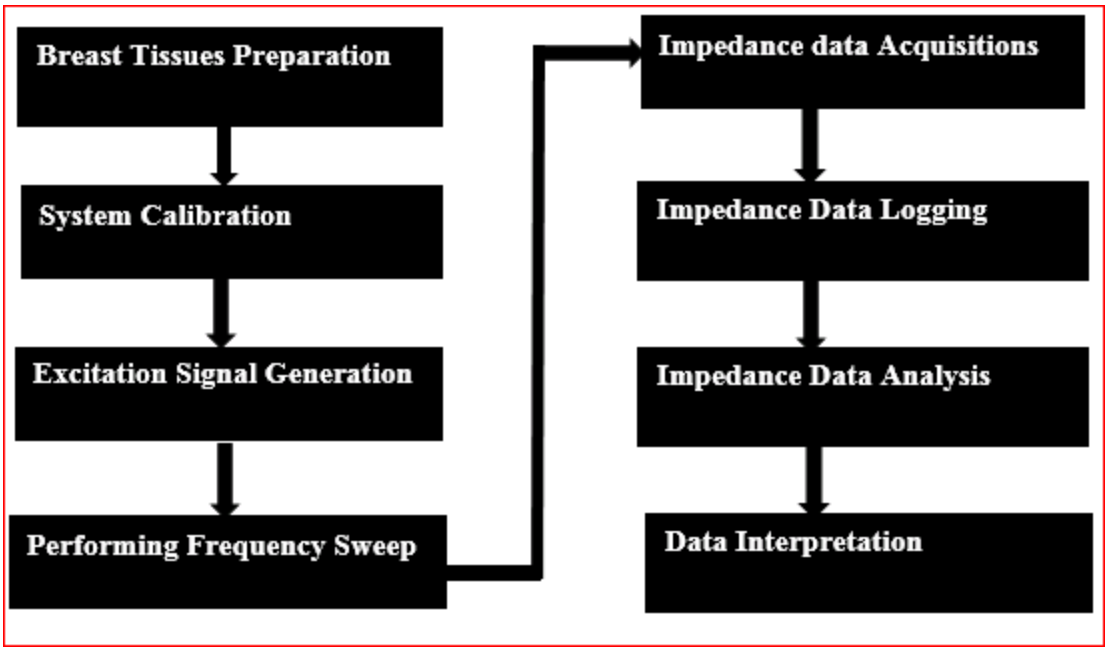



Figure 11 Impedance Data Acquisition process basic steps (work flow)

# CHAPTER FOUR

# RESULT AND DISCUSSIONS

## 4.1 Study Results

### 4.1.1 Nyquist Plot Analysis

The equivalent circuit of biological tissues models tissues as electrical circuit which includes one resistor in series with one capacitor, and both in parallel with one resistor as illustrated in figure 12. The resistor and capacitor which are in series mimic the extracellular resistance (Rint) and cellular membrane capacitance (Cm) and the resistor in parallel with the two components mimics the extracellular resistance of tissues (Rext).

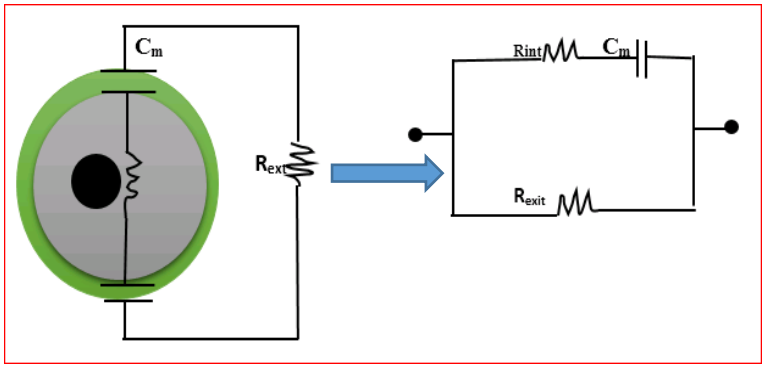



Figure 12 Breast tissues as electrical circuit and equivalent dumped RC circuit

Therefore in this study, all these Nyquist parameters were extracted from impedance data measured for three breast tissue samples by using equations suggested by [42].By inserting the values into equations given in 10, 11 and 12 $R_{ext}$, $R_{int}$ and $C_m$ of all the three breast tissue samples were calculated and the corresponding equivalent tissue's electrical circuit was modeled as illustrated in table 8.

$$Rext = Ro \quad (10)$$

$$Rint = {Ro * R\infty}/{Ro - R\infty} \quad (11)$$

$$Cm = \frac{1}{2\pi fc(Rint+Rexit)} \quad (12)$$

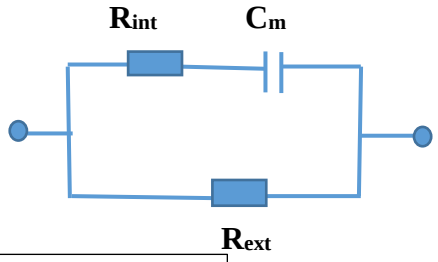
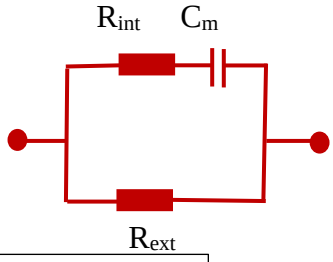
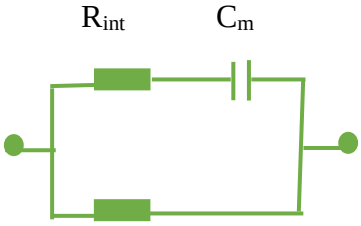
Where Ro is impedance modulus at lower driving frequency, $R\infty$ impedance modulus at maximum driving frequency and fc is the frequency at which the imaginary part of the impedance reaches its maximum. Therefore, from impedance data acquired from three breast tissues samples, the Nyquist impedance parameters were calculated. The impedance data obtained from each breast tissues are presented in table 7. Impedance modulus both at minimum and maximum driving frequency are taken from the average of all 20 breast cancer patients.

Table 7 impedance data parameters taken from measured data

| Impedance Parameters | Normal Tissues | High grade Lesions | Low Grade Lesions |
|---|---|---|---|
| **fc(KHz)** | 60 | 40 | 40 |
| **Ro(Ω)** | 903.141 | 878.421 | 895.341 |
| **R∞( Ω)** | 182.090 | 101.697 | 134.678 |

To model the equivalent RC (Resistor and Capacitor) circuit of each breast tissues, the values in table 7 were substituted in equation 10, 11 &12 to calculate the corresponding $R_{ext}$, $R_{int}$ and $C_m$ of the tissues. The equivalent RC circuit of each tissues are modeled as indicated in table 8.

Table 8 Tissues equivalent RC circuit modeled from impedance data

| **Normal Tissues** | **High Grade Lesions** | **Low Grade Lesions** |
|---|---|---|
|  |  |  |
| *$R_{int}$=228.073Ω*<br>*$R_{ext}$=903.141Ω*<br>*Cm=5.888nF* | *$R_{int}$=115.012Ω*<br>*$R_{ext}$=878.421Ω*<br>*Cm=8.014nF* | *$R_{int}$=158.523Ω*<br>*$R_{ext}$=895.341Ω*<br>*Cm=5.034nF* |

Nyquist plots are parametric plots of a system response. It is often used in a feedback system to assess stability. Each point on the plot is a complex impedance measured at each frequency points. The Nyquist plot in figure 13 is obtained from impedance data of three tissues by plotting imaginary part (reactance) against real part (resistance) of tissues frequency response

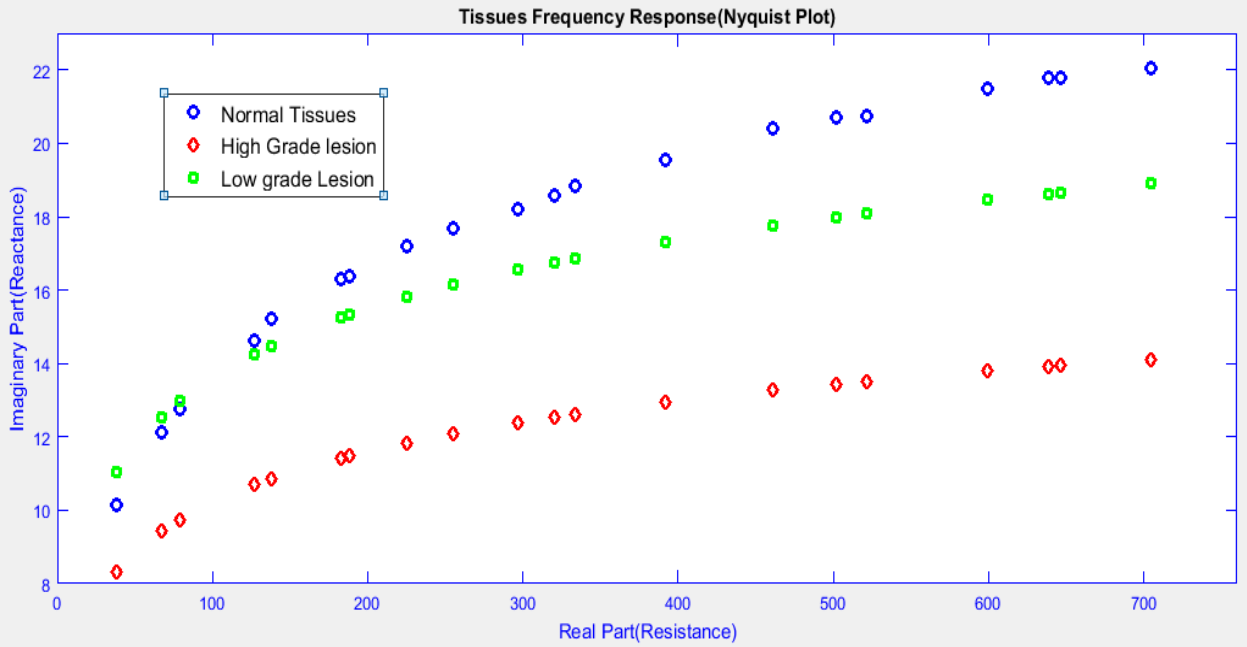



Figure 13 Nyquist plot of three breast tissues obtained from Matlab

### 4.1.2 Bode Plot Analysis

Bode plot is popular presentation method for impedance data. The impedance is plotted with log frequency (log on the X-axis and both the absolute value of the impedance (|Z| =Z0) and phase-shift on the Y-axis and it is explicitly shows frequency information of sample under test. In this study bode plot of impedance data of three breast tissues samples were plotted using Matlab in order to understand their frequency responses in terms of magnitude and phase. The results of bode plots of normal tisseus and high grade cancerous lesion is presented in figure 14.

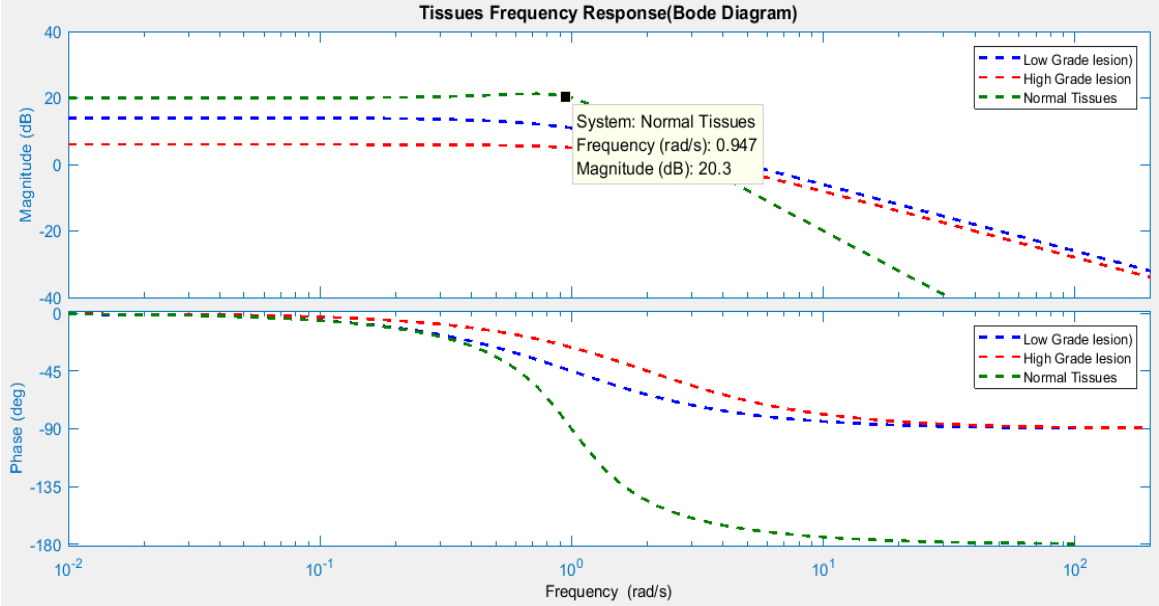



Figure 14 Frequency Response of Breast tissues Samples in Bode Plot (Normal Tissues)

### 4.1.3 Impedance Index Analysis

According to [43], two frequencies (Lowest and highest) are sufficient in approximating the main features of the impedance spectrum of biological tissues.1Khz (lowest) and 100KHZ (highest) frequencies are used to parameterize the impedance spectrum of three breast tissues categories into four impedance indices .The formula used to calculate those indices: Magnitudes index (MIX), Phase index (PIX), Real part Index (RIX) and Imaginary part Index (IMIX) are presented below.

$$\mathrm{MIX} = \mathrm{abs}\left(\frac{\mathrm{Z1KHZ}}{\mathrm{Z100KHZ}}\right) \quad (13)$$

$$\mathrm{PIX} = \arg(1\mathrm{KHZ}) - \arg(100\mathrm{KHZ}) \quad (14)$$

$$\mathrm{RIX} = \mathrm{Re}\left(\frac{\mathrm{R1KHZ}}{\mathrm{Z100KHZ}}\right) \quad (15)$$

$$\mathrm{MIX} = \mathrm{Im}\left(\frac{\mathrm{Z1KHZ}}{\mathrm{Z100KHZ}}\right) \quad (16)$$

Where abs ($\mathbf{Z}_1$ KHZ) and abs ($\mathbf{Z}_{100}$ KHZ) are the magnitude impedance of the complex impedance at driving frequency of 1 KHZ and 100 KHZ respectively. Re(Z1 KHZ) and Re(Z100 KHZ) are real part values at 1 KHZ and 100 KHZ frequencies whereas arg(1 KHZ) and arg(100 KHZ) are

phase angle values at 1 KHZ and 100 KHZ frequency points. Im (1 KHZ) and Im (100 KHZ) indicates the imaginary values at 1 kHz and 100 KHz frequency points.

The real part (RIX) values indicates the changes in resistivity of breast tissues while the imaginary part (IMIX) reflects the reactance changes which measures the capacitive nature of cellular membrane. The magnitude index (MIX) and Phase angle index (PIX) reflects changes along the cumulative effects of resistance and reactance. The impedance index of each breast and their total impedance index were calculated as shown in table 9.

Table 9 Impedance Index of Breast Tissue Samples

| Impedance Index | Normal Tissues | High Grade Lesion | Low Grade lesion |
|---|---|---|---|
| **PIX** | 36.4 | 19 | 36.33 |
| **IMIX** | 2.316 | 14.844 | 5.282 |
| **MIX** | 4.962 | 8.637 | 6.648 |
| **RIX** | 4.102 | 7.131 | 4.653 |
| **Index Sum** | **40.502** | **26.131** | **40.983** |

### 4.1.4 Linera Regression Analysis

This Regression analysis gives information on the relationship between dielectric properties of breast tissues and excitation current frequency. The regression plots indicates the functional relationship between independent variable (Frequency of excitation signal) and dependent variables (dielectric properties as impedance).Therefore, the linear regression plot of the normal and high grade cancerous breast tissue samples were plotted as indicated in figure 15.Furthermore, the impedance data was fitted with best line fit equation and the correction coefficients were also calculated for each plots. The square correction coefficient values of each breast tissues were also calculated to indicate the relationship status of the independent and dependent variables.

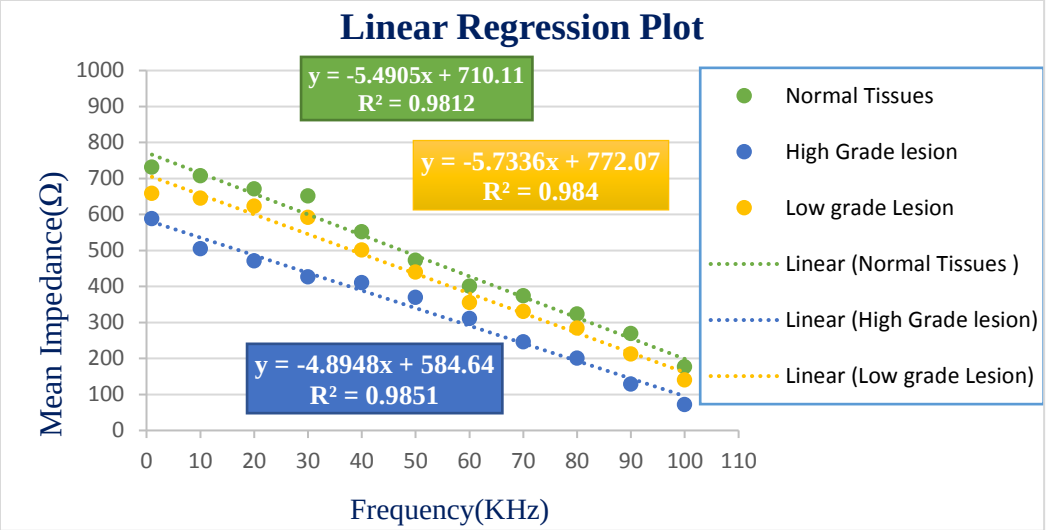



Figure 15 linear Regression Plot of Breast Tissue Samples

The measurement error values were included to each impedance data to show the degree of dispersion during data measurements. As it was explained chapter three section 3.11.1, the impedance data of each breast tissue samples were measured four times to avoid measurement error during data acquisition process. The standard deviation errors of each sample tissues were included to the bar graph as it indicated in the figure 16. Its was found that the average standard deviation was ±2.067 normal tissues , ±2.192 for low grade and ±3.167 for high grade cancerious lesion .

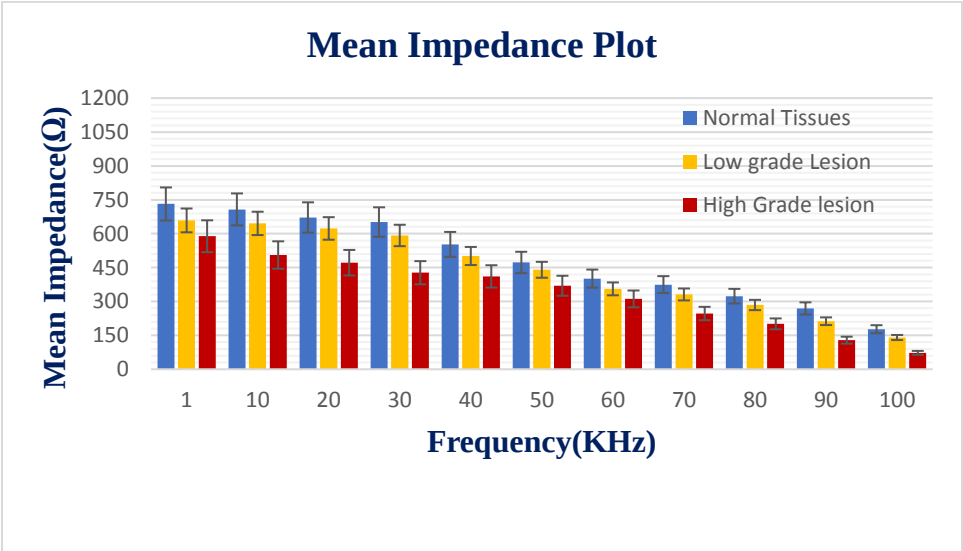



Figure 16 the impedance mean and standard deviation of the tumors and normal tissues.

### 4.1.5 Phase Angle Analysis

Phase angle is the time delay between incoming excitation signal and voltage developed inside breast tissues samples. Phase angle reflects the relative contribution of cellular fluids (Resistance) and cellular membrane (reactance).The mean phase angle data of three breast tissue sample at each frequency points are presented in table 10. By definition, phase angle is positively associated with resistance and negatively associated with reactance.

Table 10 Mean Phase angle data of three breast tissue samples

| **Mean Phase Angle Data of Three Breast Tissue Samples** | | | |
|---|---|---|---|
| **Frequency(KHz)** | Normal Tissues(θ) | Low Grade Lesion(θ) | High Grade Lesion(θ) |
| **1** | 55.6 | 48.34 | 27.6 |
| **10** | 56.9 | 41.71 | 22.9 |
| **20** | 57.5 | 36.12 | 19.2 |
| **30** | 57.4 | 31.51 | 15.6 |
| **40** | 56.3 | 27.58 | 11.5 |
| **50** | 52.3 | 24.31 | 7.4 |
| **60** | 44.1 | 21.5 | 3.4 |
| **70** | 35.1 | 19.06 | -0.2 |
| **80** | 27.6 | 16.94 | -3.4 |
| **90** | 22.9 | 15.05 | -6.1 |
| **100** | 19.2 | 12.01 | -8.6 |

On the phase angle obtained from 20 patients' (table 11) inferential statistical analysis were performed to investigate the significance of the differences among the three sets of breast tissues (low grade lesion, high grade lesion and normal breast tissues). Differences among samples were assessed using, Single factor one-way ANOVA followed by Turkey's honestly Significant Difference Post Hoc Test .The level of significance was set at P=0.05 for all statistical analyses.

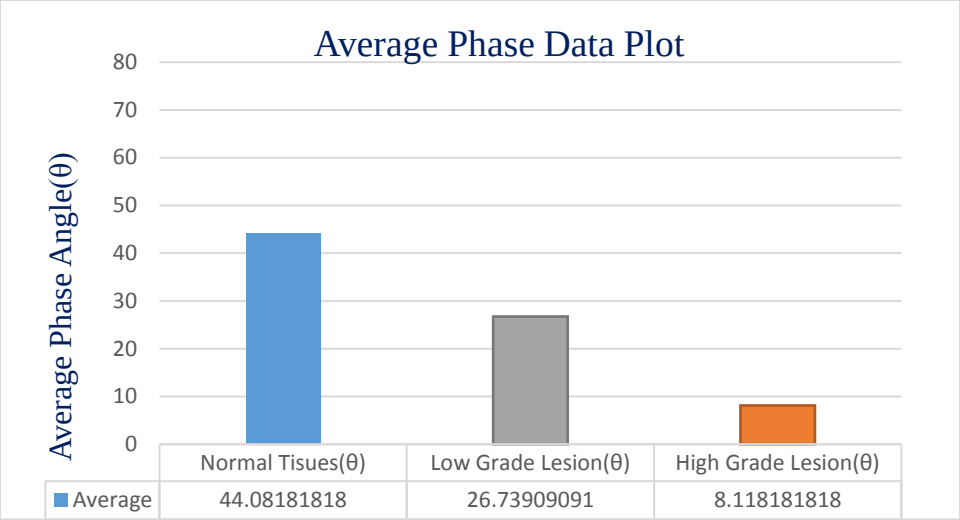



Figure 17 Average Phase Angle obtained from Single factor ANOVA analysis.

Single factor one –way ANOVA test was performed to test the significance of statistical difference of three breast tissues samples based their phase angle. The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of three different breast tissues.

Table 11 Results of Single Factor ANOVA test on phase angle

| **ANOVA** | | | | | | |
|---|---|---|---|---|---|---|
| ***Source of Variation*** | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| **Between Groups** | 7116.60248 | 2 | 3558.3 | 20.809 | 2.15E-06 | 3.31583 |
| **Within Groups** | 5129.99042 | 30 | 171 | | | |
| **Total** | 12246.5929 | 32 | | | | |

## 4.2 Discussion

In this study, breast tissues samples were characterized by bioimpedance spectroscopy techniques. The main purpose of the study was to understand the significant difference between normal and cancerous breast tissues based on their frequency response (dielectric properties).The results

obtained from different breast tissue samples illustrated that there is a significant difference between normal and cancerous breast tissue's frequency responses. The results obtained from the analysis employed in this study showed that normal breast tissues appear to have significantly higher dielectric properties both in magnitude and phase angle than their counterpart cancerous tissues. For instance table 8 shows that the impedance parameters of normal tissues are significantly greater than that of high grade and low grade cancerous lesions. The capacitance of cell membrane $C_m$ and the cellular fluids resistance $R_{ext}$ and $R_{int}$ of cancerous lesions are lower than the corresponding normal tissues. The normal tissues exhibits higher resistance and capacitance due to high content of adipose tissues and low content of cellular fluids.

From the plot obtained in figure 13, we can observe that because of the high impedance of the cell membrane in lower driving frequency, the impedance is only influenced by extracellular fluids resistance since the excitation signal cannot penetrate the tissues .However, as frequency increases the excitation signal starts to penetrate the tissues and thus we can find both capacitive (imaginary part) and resistive (real part). On top of this, the Nyquist plot of cancerous tissues are have lower imaginary part and real part magnitude due to its lower capacitance and resistance .Therefore, the frequency responses of normal and cancerous breast tissues can be differentiated by Nyquist plot.

As indicated in figure 14, the bode plot indicate both magnitude and phase of three breast tissues samples on the same graph. From the graph of bode plot ,we can notice that, the magnitude of normal breast tissues reaches 20.3 dB at frequency 0.947 rad/s whereas others are still below this peak magnitude. Even though it's difficult to quantify the difference of three breast tissues samples, the bode plot indicates that the frequency response information of these tissues are different in magnitude and phase. The normal tissues magnitudes is much greater than the corresponding cancerous tissues particularly high grade cancer lesion. This due to high adipose tissues and low water contents of normal breast tissues. The amount of water found in tissues played significant role for its electrical conductivity and resistivity. The less water content of breast tissues, the more resistivity and low conductivity which results in high impedance magnitude

As we can see from table 9,the impedance index sum of three breast tissues were presented. The total impedance index values of normal and high grade canceroius lesion were significantly different from each other. The High grade cancerous lesions found to be the lowest impedance index sum as compared to nromal tissues and low grade cancrous lesion . Therefore the total impedance index reflects whether normal and cancerous tissues are the different or not based their

impedance content. Furthermore, as we can observe from the linear regression plot (figure 15), the dielectric properties (breast tissues impedance) have negative relationship(y=-ax+b) with excitation signal frequency. On the other hand, the dielectric properties of all three breast tissues are decreasing as excitation frequency increases. Besides to this, the square correlation coefficients ($R^2$) of the three breast tissue samples indicates that, there is strong relationship($R^2$=0.984) between dielectric properties of breast tissues and excitation signal frequency, i.e. dielectric properties of breast tissues are frequency dependent parameters.

From result presented in table 10, the phase angle of cancerous breast tissues are lower than counterpart normal breast tissues. Lower phase angle suggests tissues death, cell membrane poor integrity while higher phase angle suggests large quantity of intact cell membrane and high resistivity of tissues cellular fluids [44].More importantly, figure 17 is evidence that the average phase angle values of normal breast tissues is much greater than the rest cancerous lesion tissues. Phase angle is the indication of tissues integrity and compactness .Therefore, normal tissues are more compact and well integrated than counterpart cancerous lesions.

An analysis of variance showed that the statistical difference of the three breast tissue samples was significant, F (2, 30) =20.809, p=0.0002 as it indicated in table 11.Furthermore, The Post-hoc Tukey's HSD(Honestly Significant Difference) test also showed that there is significant difference between two groups of breast tissue samples (normal tissues and high grade cancerous lesions) with F (2, 30) =15.526, MSE=171, $p<0.001$ at p=0.05 level of significance .All other comparisons were not significant. In this study it was found that, the frequency response difference of these tissues found to be maximum at excitation signal frequency 30 KHZ as it illustrated in figure 18.

Generally, a very good discrimination among the values of impedance data of different breast tissues samples was found .This result suggested that each breast tissue sample can be characterized by specific values of electrical impedance for each frequency points, and its frequency response is a sort of "electrical fingerprint" which characterizes every tissue in relation to its structure and tissue's integrity. More importantly, the amount of adipose tissues, water and ions contents of tissues and cell membrane strength determines the amount of dielectric properties of these breast tissues.

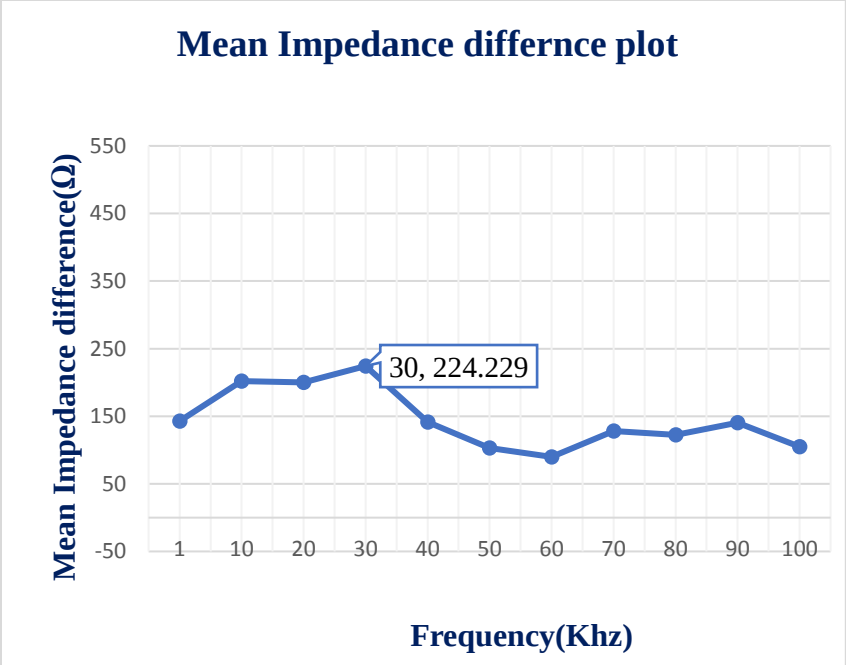



Figure 18 Mean Impedance Difference Plot of Normal and High grade lesion tissue

## 4.3 Comparison Analysis with Previous Works

In this study we performed breast tissues characterization with tetra polar electrodes setup and electrical impedance spectroscopy techniques .It was found that there is significant statistical difference among frequency response of three breast tissues samples over a frequency ranges of 1 KHZ-100KHZ. Lazebnik et al. [45] used high frequency ranges to characterize breast tissues sample and found that the dielectric contrast between fibro glandular tissue and cancerous tissue to be as little as 1.1:1 in the range between 0.5 GHZ and 20 GHZ. They found lower dielectric properties difference due to high frequency ranges application to characterize the tissues. At high frequency ranges it was found that there is high energy loss (tissues dispersion) due to low contrast of capacitance and phase angle. The findings of our study proofed that, using low frequency is advantageous to discriminate normal and malignant breast tissues due to higher contrast of dielectric properties of breast tissues at low frequency ranges.

Other studies conducted by Surowiec *et al.* [30] used cavity perturbation method consists of a resonant cavity that resonates at specific frequencies to characterize breast tissues samples. The dielectric measurement of breast tissues performed by this method requires the sample size should be very small to be fitted in cavity of the measuring device which lets the sample preparation process too difficult and complicated. Furthermore, this process may introduce air pockets within

the sample or between the sample and cavity which would affects the tissues properties or increase the density from pushing the tissues into the cavity. However, our study used tetra polar electrodes method to measure the impedance of breast tissues samples which overcome the problems and challenges mentioned in the study by [30]. Unlike the mentioned method the tetra electrodes is non-destructive and allows good contact between tissues and sets of electrodes. This method does not requires intensive tissues processing and also the tissues dielectric properties are easily evaluated from the measured impedance with knowledge of the sample dimension. On top of this, the tetra electrodes set up allows the independent current injection process so that the resulting measured voltages more sensitive to local area and less sensitive to other regions i.e. less signal cross talk and unwanted signal noise. Therefore, the method we followed was good in providing improved bioimpedance measurement with arbitrary samples shape.

C. Gabriel et al. [22] employed Vector Network analyzer device for breast tissues characterization which found to be very expensive and large in size for wider application in developing countries like Ethiopia. Furthermore, vector network analyzer involves two electrodes measurement setups for alternating current injection and voltage sensing and thus it can cause signal noise for direct current measurements because of the electrode polarization that consequently gives incorrect results for the conductivity of the sample between the electrodes. However, we employed AD5933 evalboard along with its software to characterize breast tissues and acquire tissues frequency responses.AD5933evaloard is less expensive as compared to other impedance analyzer device used in the previous works and thus we followed a very economical way of breast tissues characterization techniques as compared to other techniques employed so far. The summary of comparison analysis with previous works are presented in table 12.It indicated that, the impedance difference between normal breast tissues and malignant cancerous lesion significantly maximum at 30KHZ frequency point which indeed higher than previous works achievements.

Table 12 Summary of comparison analysis with previous works

| Author(s) | Frequency | Method | Major Findings |
|---|---|---|---|
| Lazebnik et al. | 0.5 GHZ - 20 GHZ | Probe connected to Agilent 8722D vector network analyzer (VNA) | 1.1:1 dielectric contrast between fibro glandular tissue and cancerous tissue |
| Surowiec *et al.* | <100 GHZ | cavity perturbation method consists of a resonant cavity | They found higher conductivity of cancerous tissues than normal (2 to 4mS/cm that of the tissue surrounding the tumor). |
| C. Gabriel et al. | 0.5 to 20 GHZ | open-ended coaxial probe connected to VNA | 10:1 dielectric contrast for malignant and normal breast tissues |
| This study | 1 KHZ -100 KHZ | Tetra polar electrodes with ad5933evalbaord | 224.229Ω mean impedance difference at 30 KHZ between normal and malignant breast tissues |

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

In this study, breast tissue sampled were removed from 20 patients were characterized by bioimpedance spectroscopy techniques. The primary results indicated that there is significant difference between the frequency response of normal and malignant breast tissues particularly with their dielectric properties such impedance modulus and phase angle. The result from single factor ANOVA (Analysis Of Variance) test illustrated that there are significant statistical difference ($p=0.0002$) among the phase angle of three breast tissue samples .More importantly Turkey's honestly Significant Difference Post Hoc Test indicated that there was significant difference ($p<0.001$) between two groups of breast tissue samples (normal tissues and high grade cancerous lesions) at $p=0.05$ level of significance .However, all other comparisons were not significant.

The results of this study was very promising to use the bioimpedance spectroscopy techniques for cancerous disease detection since cancerous tissues frequency response are significantly different from their counterpart normal tissues .The frequency response (dielectric properties) are the intrinsic properties which depends on tissues structure and compositions. Any changes in tissue physiology should produce alterations in the tissue electrical properties .Biologically cancer disease causes noticeable physiological changes on breast tissues and its surrounding once it starts growing aggressively. These changes includes increment tissues water content (necrosis) and disintegration of cellular membrane bipolar structure. Therefore, these physiological changes are critically observed in dielectric properties of those cancerous tissues. From the results of this study, the dielectric properties of cancerous tissues were found to be lower than the corresponding normal tissues. The results of this study also indicated that impedance difference of normal and cancerous breast tissue was very high at 30 KHZ excitation frequency signal and thus it is possible to use this particular frequency points to identify cancerous lesions from normal tissues .On top of this ,it was found that the dielectric properties ( impedance ) both malignant and normal breast tissues were frequency dependent ,i.e. there is strong correlation between excitation signal frequency and

bioimpedance of breast tissues. Therefore, the selection of appropriate frequency ranges of excitation signal is very crucial to characterize breast tissues.

A lots of activities have been done in order to fill the gaps of previous studies particularly those mention under literature review part of this document. The potential effect of temperature was controlled by fixing the measuring temperature at optimum value. Excitation signal frequency range within B-dispersion was used to avoid critical energy loss during data acquisition process. AFE was designed to avoid tissues polarizations which more probably caused during two electrode configuration tissues measurements. The AFE circuit enabled Ad5933evalbaord to be more accurate and tetra polar electrodes configuration.

## 5.2 Recommendations

In this study we tried to address the frequency response difference of normal and cancerous tissues of 20 patient's breast which were obtained from surgical biopsy procedures. It was very successful in providing a preliminary information regarding the dielectric properties of different tissues under temperature monitored environment. However, we would like to recommend researchers to conduct large scale study to establish impedance threshold values for both normal and cancerous breast tissues under different circumstance so that cancerous detection at early stages will be possible .

A potential constraint to this study was that breast tissues data were acquired from *ex-vivo* experimental setup and *in-vivo* studies are necessary to evaluate the real feasibility of the technique proposed. Impedance also changes as a function of time after death, due to cell membrane permeability changes occurring within hours after cell death .The reported results were obtained from 20 patients which cannot enable us to determine the impedance threshold values for both normal and malignant breast tissues. However, critical attention was given for tissues collection, measurement and data acquisition process to avoid the potential error. Therefore, we would like to recommend the other researchers to conduct study of breast tissues characterization in in-vivo setup so that the aforementioned factors will be controlled.

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# 7. APPENDIXES

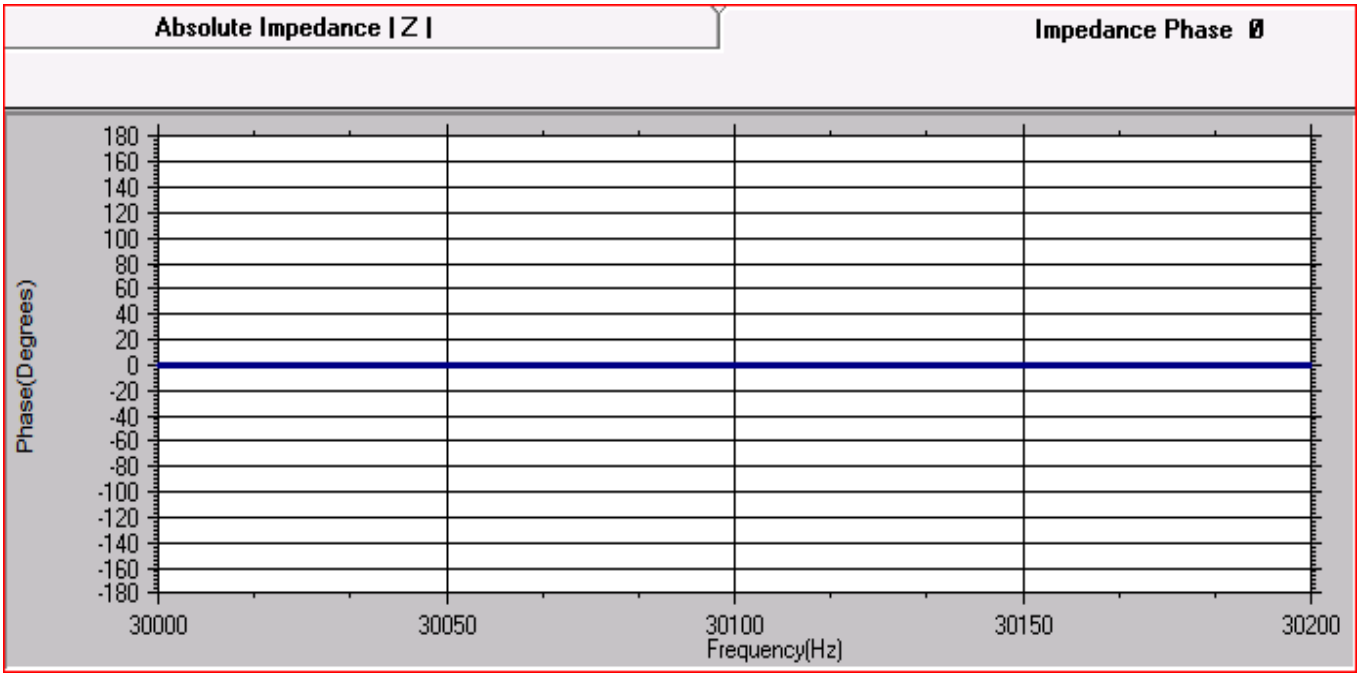
| **Mean Impedance data collected from 20 patients (Normal Tissues)** | | | | | |
|---|---|---|---|---|---|
| Frequency(KHz) | Impedance (Ω) | Real Part (Ω) | Imaginary Part (Ω) | Magnitude (Ω) | Phase Angle(θ) |
| 1 | 731.642 | 711.786 | -4.456 | 903.141 | 55.6 |
| 10 | 707.523 | 693.914 | -3.689 | 783.456 | 56.9 |
| 20 | 671.667 | 662.291 | -3.092 | 700.092 | 57.5 |
| 30 | 651.542 | 648.341 | -1.789 | 700.001 | 57.4 |
| 40 | 552.567 | 548.634 | 1.983 | 673.987 | 56.3 |
| 50 | 472.705 | 460.761 | 3.456 | 671.543 | 52.3 |
| 60 | 401.211 | 368.481 | 5.721 | 489.293 | 44.1 |
| 70 | 374.715 | 355.74 | 4.356 | 389.012 | 35.1 |
| 80 | 323.491 | 307.483 | 4.001 | 305.555 | 27.6 |
| 90 | 269.254 | 261.508 | 2.783 | 285.723 | 22.9 |
| 100 | 177.209 | 173.511 | 1.9234 | 182.09 | 19.2 |

| Mean Impedance data collected from 20 patients (High grade cancer) | | | | | | |
|---|---|---|---|---|---|---|
| Frequency(KHz) | Impedance (Ω) | Real Part (Ω) | Imaginary Part (Ω) | Magnitude (Ω) | Phase Angle(θ) | |
| 1 | 588.615 | 514.482 | -8.61 | 878.421 | 27.6 | |
| 10 | 505.552 | 467.235 | -6.19 | 834.561 | 22.9 | |
| 20 | 471.595 | 459.83 | -3.43 | 832.091 | 19.2 | |
| 30 | 427.313 | 426.952 | -0.22 | 765.001 | 15.6 | |
| 40 | 411.125 | 406.416 | 2.17 | 587.127 | 11.5 | |
| 50 | 369.615 | 366.958 | 1.63 | 374.43 | 7.4 | |
| 60 | 311.295 | 310.254 | 1.02 | 334.012 | 3.4 | |
| 70 | 246.708 | 245.915 | 0.89 | 272.121 | -0.2 | |
| 80 | 200.925 | 200.552 | 0.61 | 169.239 | -3.4 | |
| 90 | 128.795 | 128.627 | 0.41 | 143.944 | -6.1 | |
| 100 | 72.488 | 72.151 | 0.58 | 101.697 | -8.6 | |

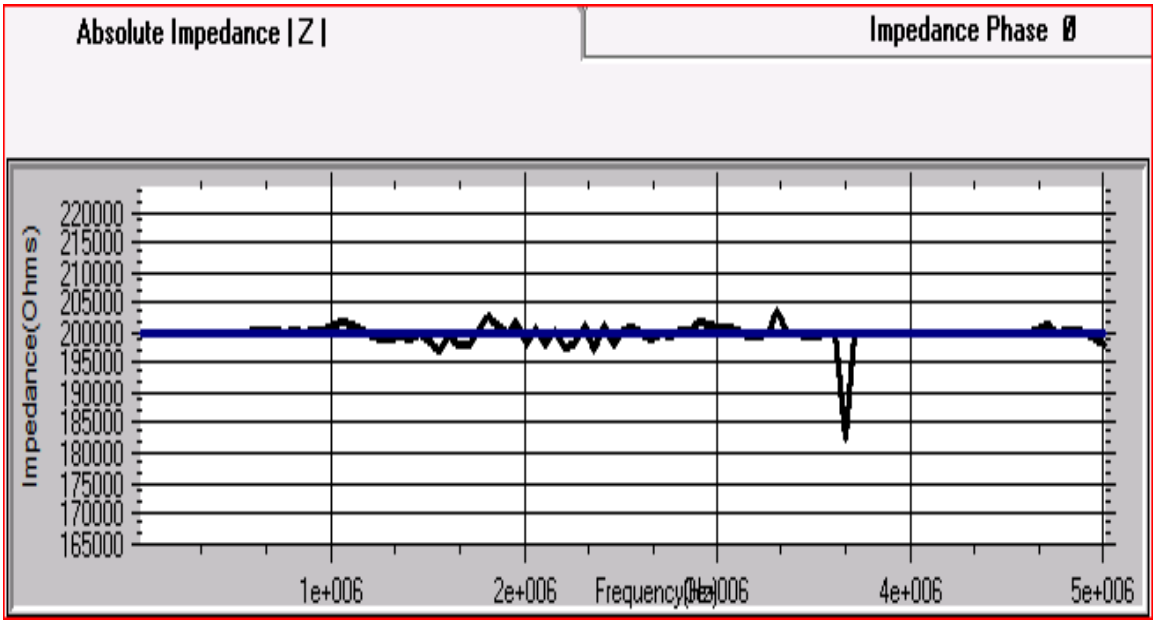
**Mean Impedance data collected from 20 patients (Low grade cancer)**

| Frequency(KHz) | Impedance (Ω) | Real Part (Ω) | Imaginary Part (Ω) | Magnitude (Ω) | Phase Angle(θ) | |
|---|---|---|---|---|---|---|
| 1 | 658.811 | 652.906 | -2.43 | 895.341 | 48.34 | |
| 10 | 645.621 | 642.198 | -1.85 | 789.345 | 41.71 | |
| 20 | 623.245 | 621.707 | -1.24 | 746.586 | 36.12 | |
| 30 | 592.151 | 592.136 | 0.12 | 745.121 | 31.51 | |
| 40 | 501.321 | 498.153 | 1.78 | 623.445 | 27.58 | |
| 50 | 440.322 | 439.281 | 1.021 | 637.475 | 24.31 | |
| 60 | 355.814 | 354.514 | 1.14 | 541.327 | 21.5 | |
| 70 | 331.091 | 330.528 | 0.75 | 453.605 | 19.06 | |
| 80 | 284.413 | 283.964 | 0.67 | 364.111 | 16.94 | |
| 90 | 212.641 | 212.268 | 0.61 | 237.495 | 15.05 | |
| 100 | 140.523 | 140.311 | 0.46 | 134.678 | 12.01 | |

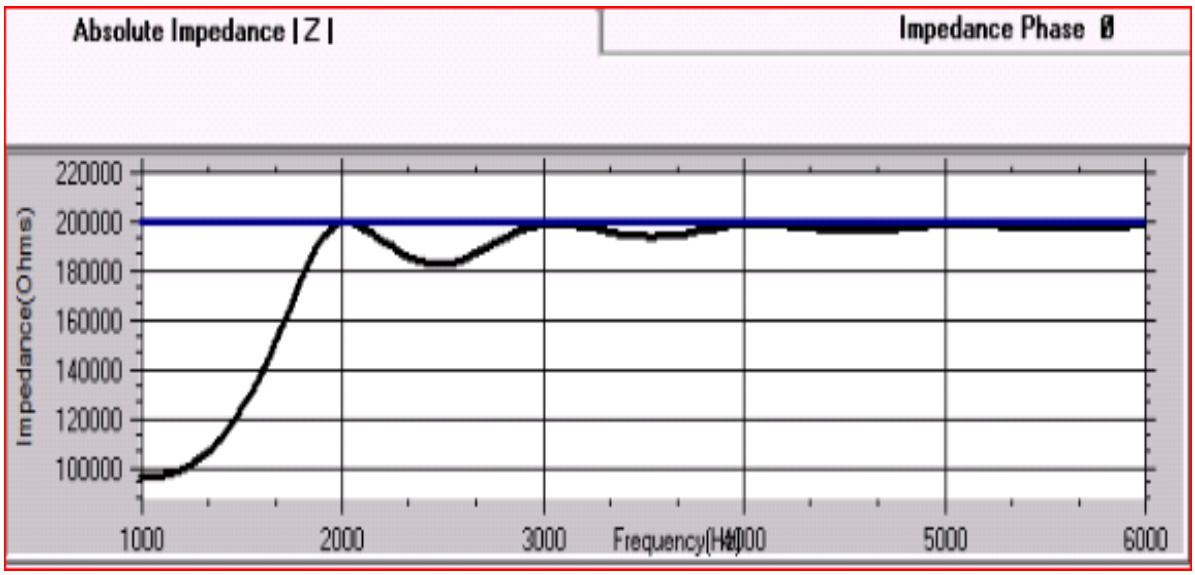
Ad5933 phase output with AFE. The phase shift is removed due tetra polar electrodes configuration.



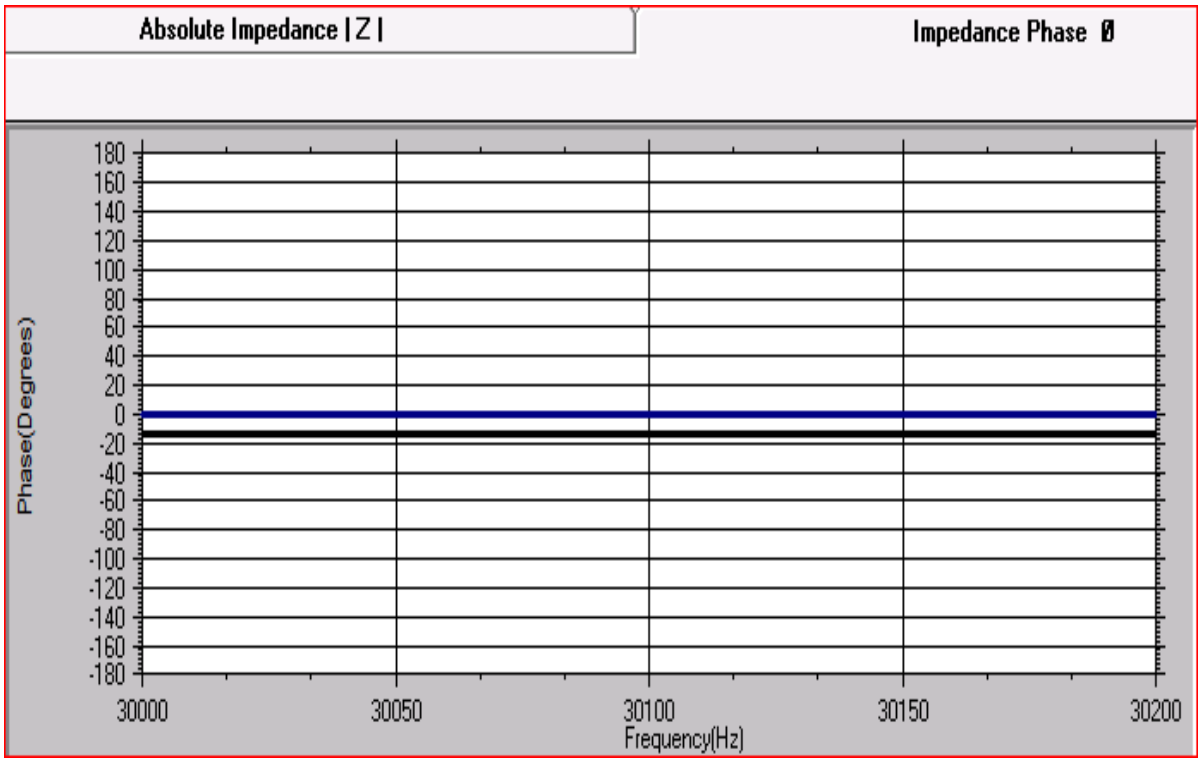

Ad5933 test output without AFE. The output is not stable due to the interface of dc power



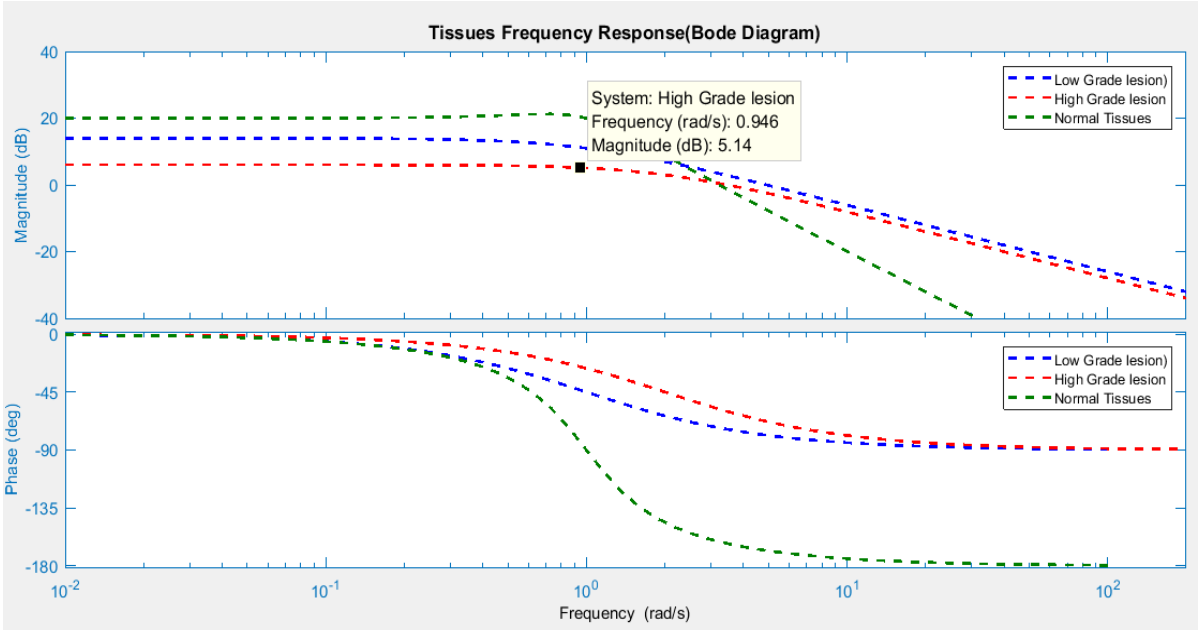
AD5933 test output with AFE. The output signal is gradually stabilized as AFE removes DC bias signal



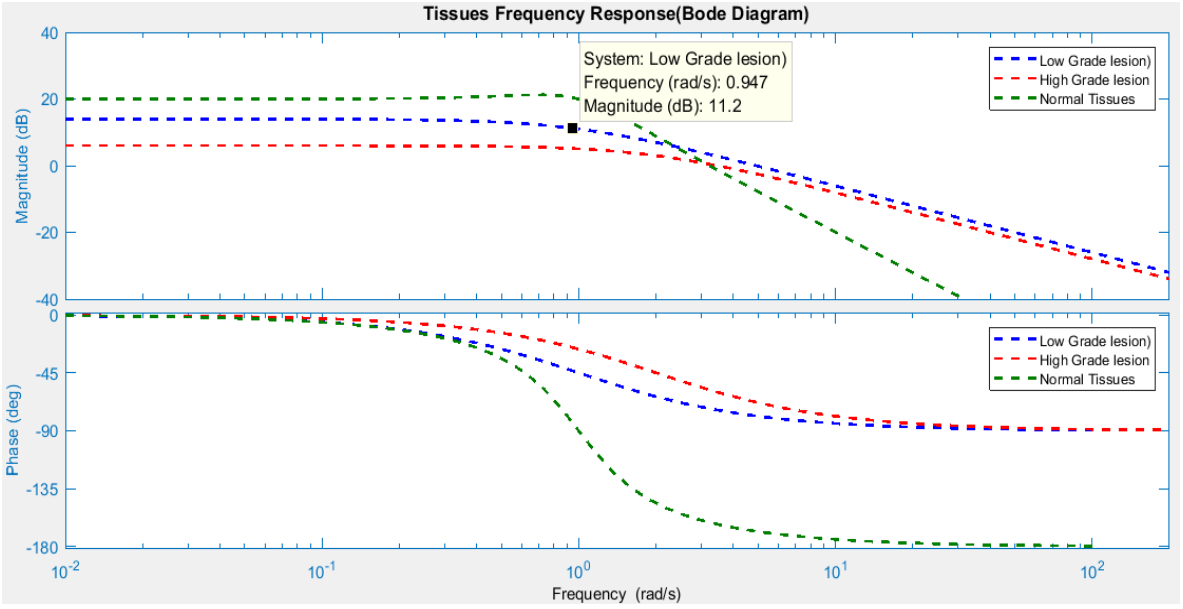

Ad5933 phase output without AFE. Phase is shifted due signal cross talk



Frequency Response of Breast tissues Samples in Bode Plot (High Grade Lesion)



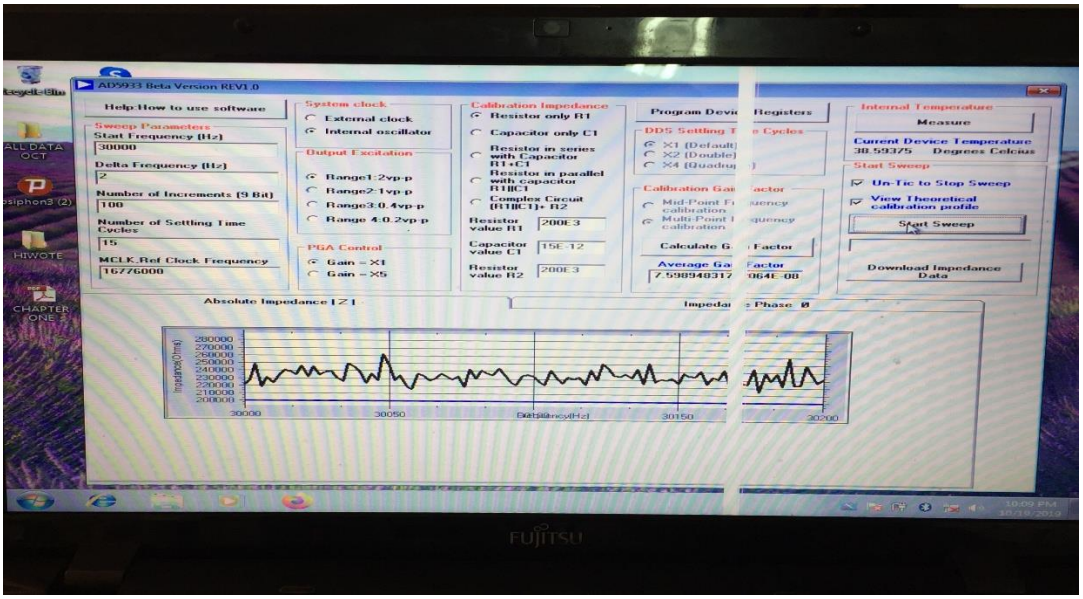

Frequency Response of Breast tissues Samples in Bode Plot (Low Grade Lesion)



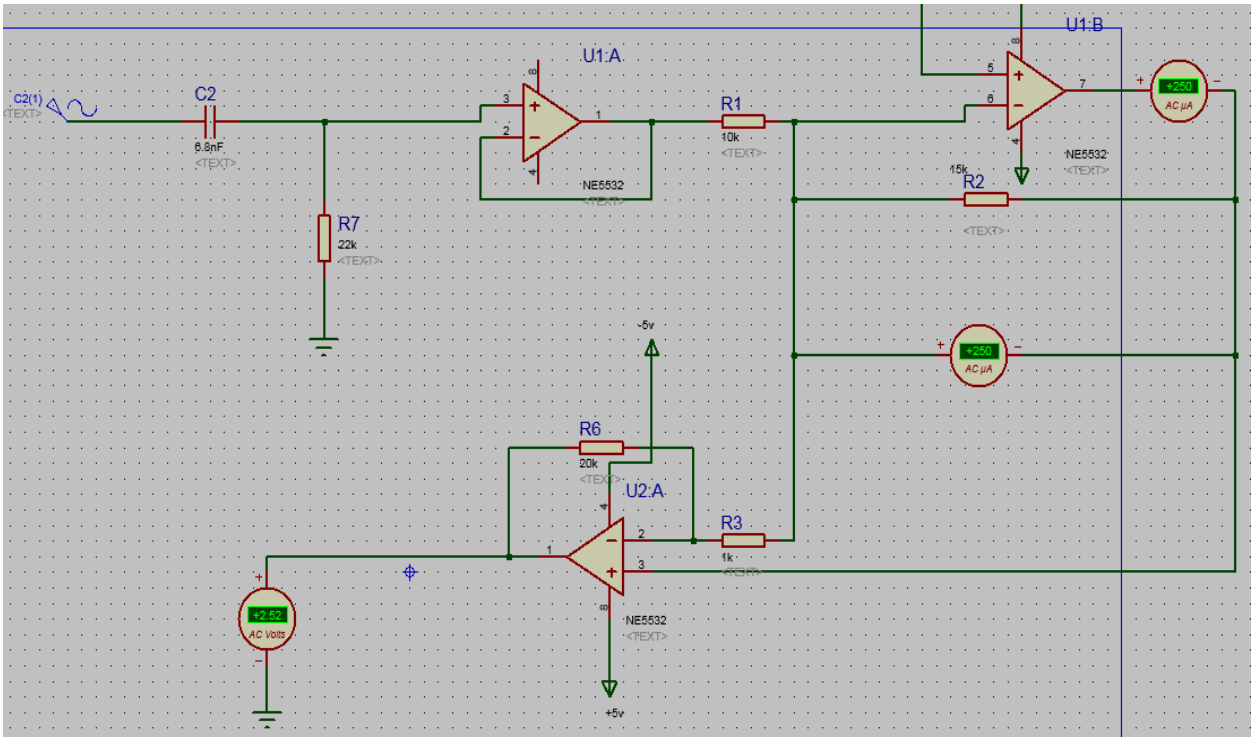
The mean impedance and SD of three breast tissue sample

| Frequency(KHz) | Normal tissues(Ω) | High Grade lesions(Ω) | Low grad Lesion (Ω) | Difference |
|---|---|---|---|---|
| **1** | 731.642±3.45 | 588.615±5.12 | 658.811±3.65 | 143.027 |
| **10** | 707.523±1.42 | 505.552±3.15 | 645.621±1.35 | 201.971 |
| **20** | 671.667±5.45 | 471.595±5.41 | 623.245±0.85 | 200.072 |
| **30** | 651.542±0.95 | 427.313±6.45 | 592.151±1.05 | 224.229 |
| **40** | 552.567±1.81 | 411.125±1.75 | 501.321±4.85 | 141.442 |
| **50** | 472.705±2.45 | 369.615±0.48 | 440.322±0.12 | 103.09 |
| **60** | 401.211±1.05 | 311.295±5.52 | 355.814±4.05 | 89.916 |
| **70** | 374.715±0.55 | 246.708±3.35 | 331.091±5.01 | 128.007 |
| **80** | 323.491±1.15 | 200.925±1.09 | 284.413±2.03 | 122.566 |
| **90** | 269.254±3.6 | 128.795±0.45 | 212.641±0.15 | 140.459 |
| **100** | 177.209±0.81 | 72.488±0.97 | 140.523±1.01 | 104.721 |

AD5933evalbaord Software used for data logging

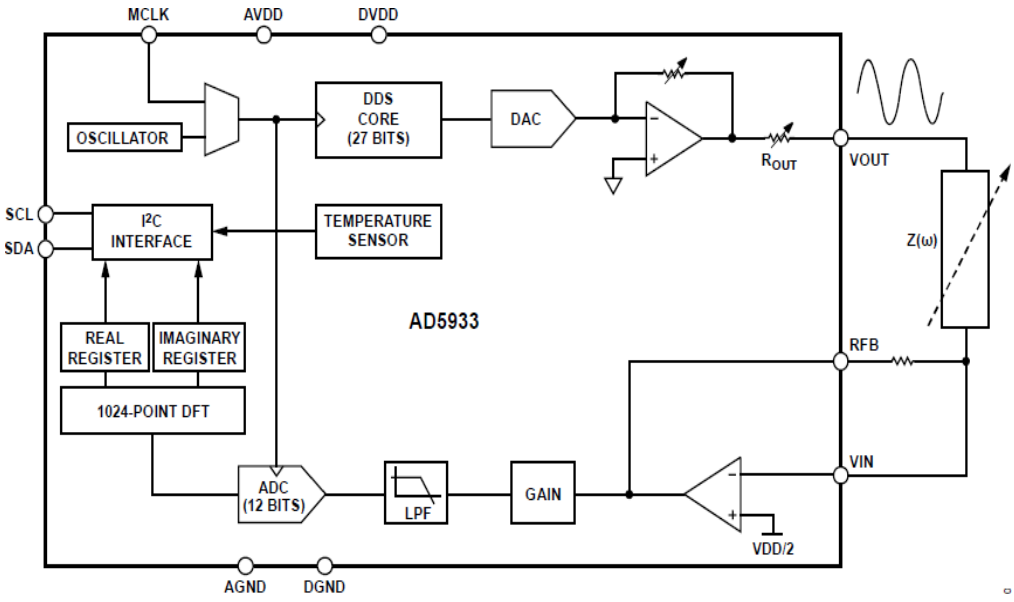



AFE circuit designed on protues software

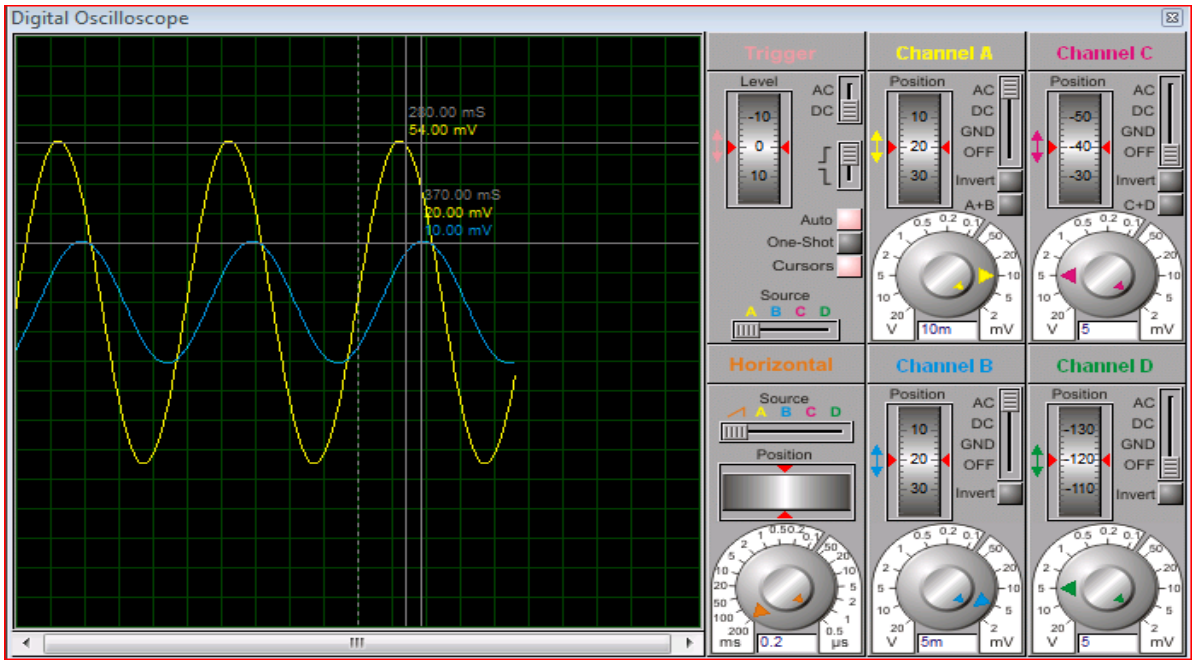



Photos showing the breast tissues samples hermetically closed in plastic containers

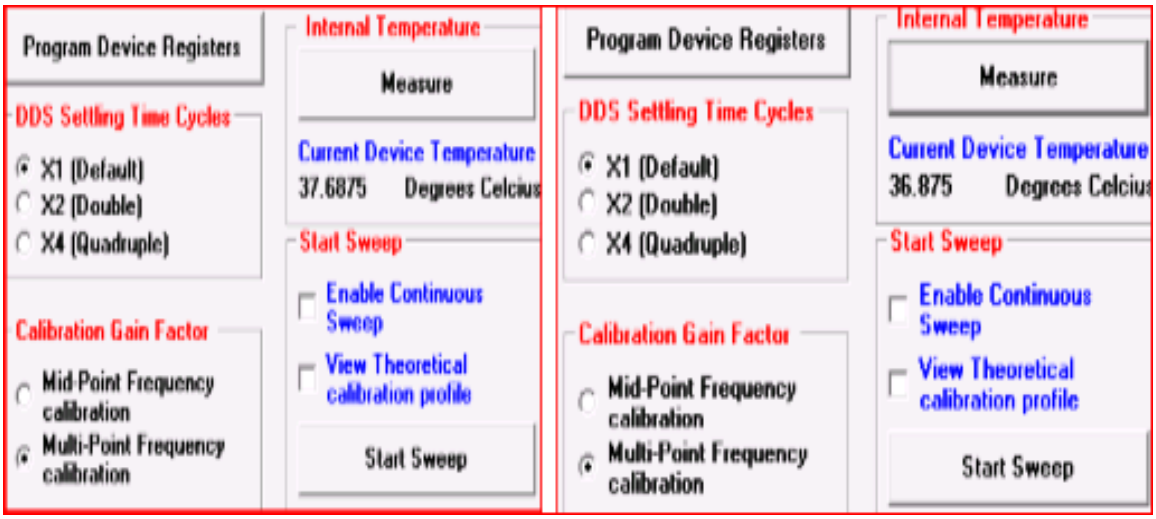
AD5933 impedance network analyzer general block diagram [33].



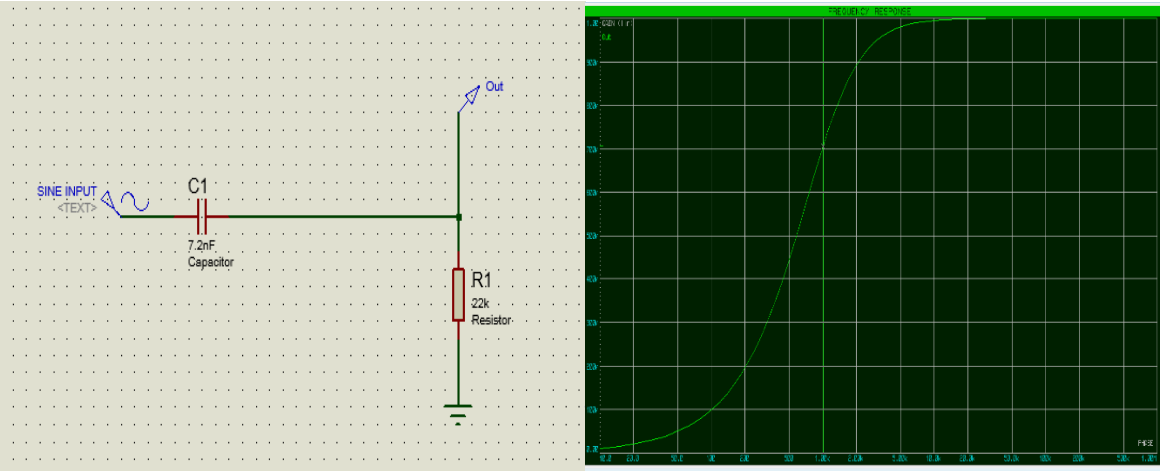
Simulation output of differential Amplifier indicating amplified input (Yellow)




System temperature indicator during data acquisition process. All data were taken at this temperature setting in order to avoid temperature variation effect



High pass filter designed on protues software and its frequency response




Buffer circuit which balance the load between current resistor and high pass filter